Revision Guide

Biology - Unit 4

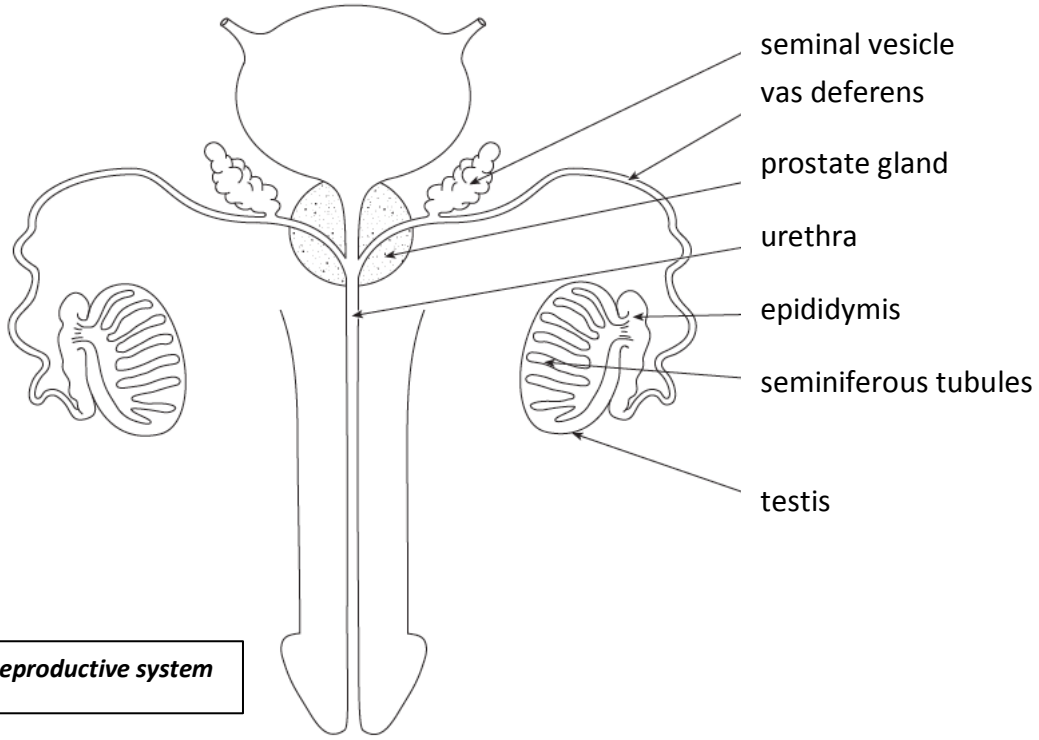
GCE A Level WJEC

These notes have been authored by experienced teachers and are provided as support to students revising for their GCE A level exams. Though the resources are comprehensive, they may not cover every aspect of the specification and do not represent the depth of knowledge required for each unit of work.

Content

Section 4.1 - Sexual Reproduction in Humans

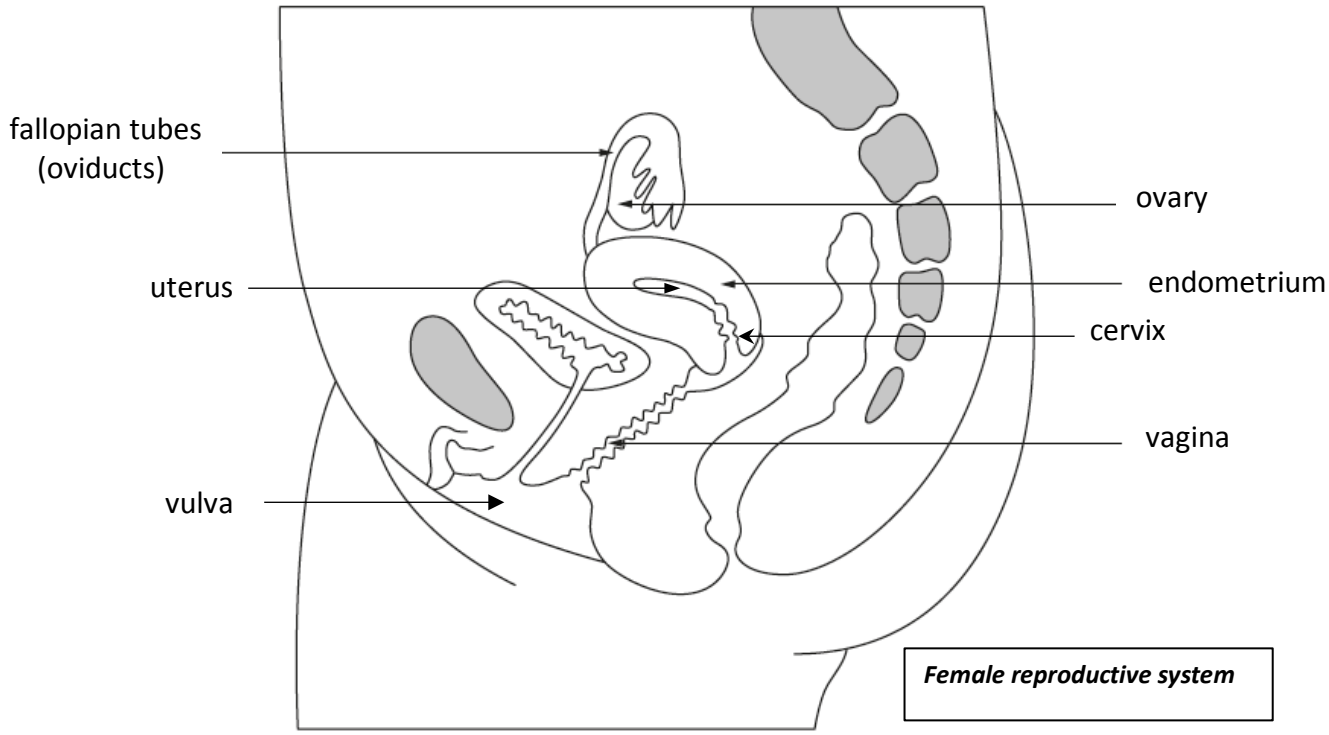
The Male Reproductive System



Male reproductive system

Structure	Function
Scrotum	External sac of skin containing the testes.
Testes	• Produce gametes (sperm formed by spermatogenesis), • Produce testosterone.
Epididymis	Sperm are stored here and mature to become fully mobile.
Vas deferens	Carries sperm towards the penis during ejaculation.
Seminal vesicle	Secretes a fluid into the vas deferens that contains a mixture of chemicals which make up approximately 60% of semen. Seminal fluid provides nutrients for sperm such as fructose for respiration and amino acids. Seminal fluid is alkaline which helps to neutralise the acidity of any urine remaining in the urethra and the acidity of the vaginal tract.
Prostate gland	Secretes a fluid into the vas deferens that contains a mixture of chemicals which make up approximately 30% of semen. Prostate fluid contains zinc ions and is also alkaline which helps to neutralise the acidity of any urine remaining in the urethra and the acidity of the vaginal tract.
Urethra	• Carries semen (a mixture of spermatozoa, seminal and prostate fluids) through the penis and out of the body. • Carries urine from the bladder through the penis and out of the body.
Penis	Specialised organ adapted to transfer semen to the vagina during sexual intercourse.

The Female Reproductive System



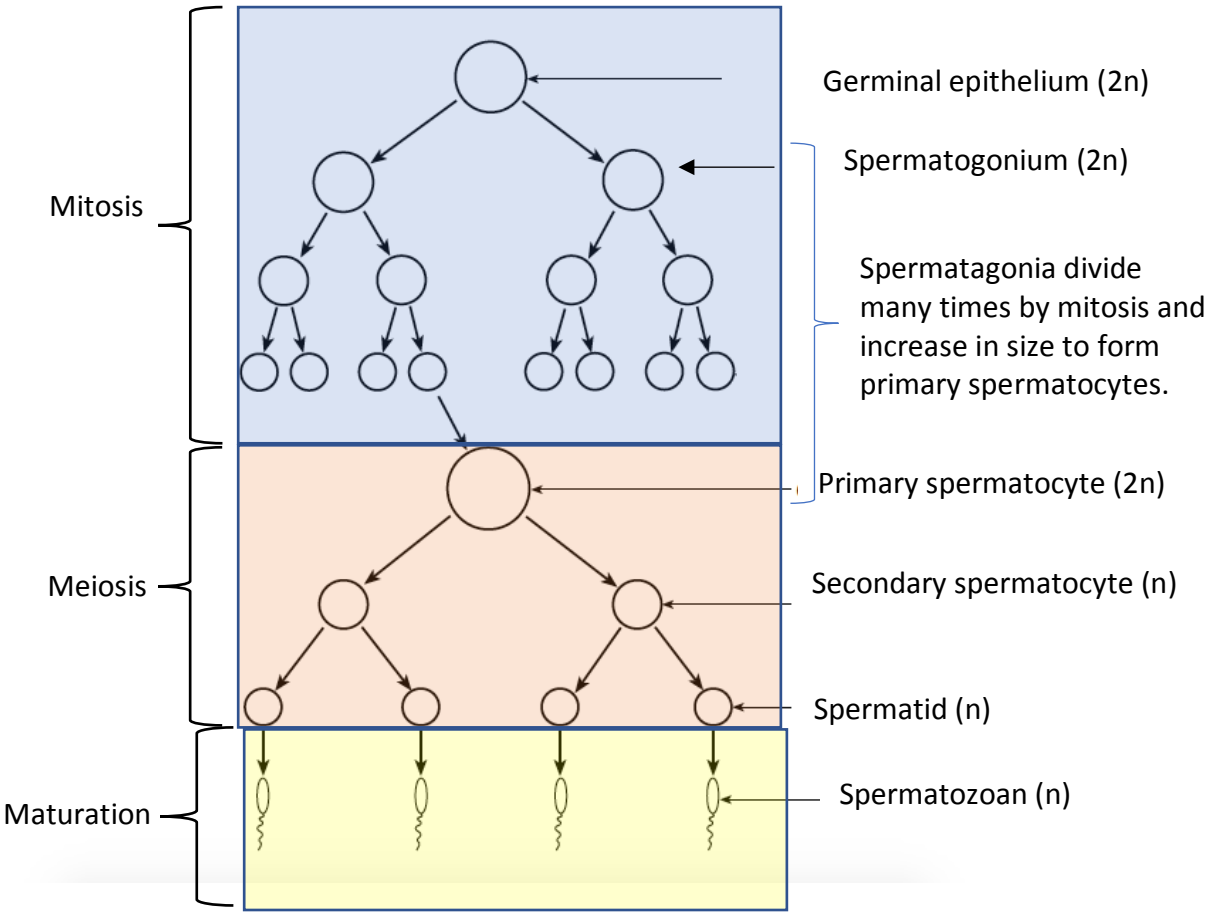
Female reproductive system

Structure	Function
Ovary	• Production of gametes (secondary oocytes formed during oogenesis), • Produce oestrogen and progesterone.
Fallopian tubes (oviducts)	They have a lining of ciliated epithelial cells which move the secondary oocyte to the uterus.
Uterus	Holds the developing foetus until birth.
Endometrium	The inner most layer of the uterus wall. It has a good blood supply and builds up every month during the menstrual cycle. If implantation of an embryo does not happen then the endometrium is shed during menstruation.
Cervix	A narrow ring of connective tissue and muscle, it acts as a barrier between the uterus and the outside environment during pregnancy. During pregnancy, a mucous plug forms in the cervix which helps prevent entry of pathogens.
Vagina	It has muscular walls and opens at the vulva. Semen is deposited in the vagina during sexual intercourse and the foetus is able to pass out from the uterus through the vagina during birth.

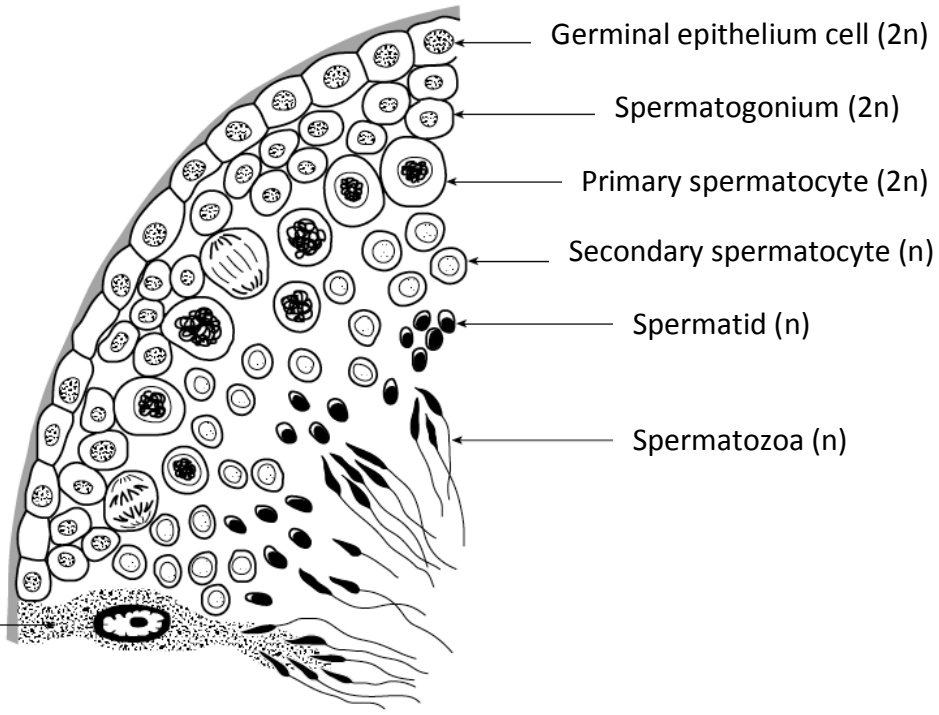
Spermatogenesis

- Spermatogenesis takes place in the seminiferous tubules.

Spermatogenesis is the formation of sperm in the testis

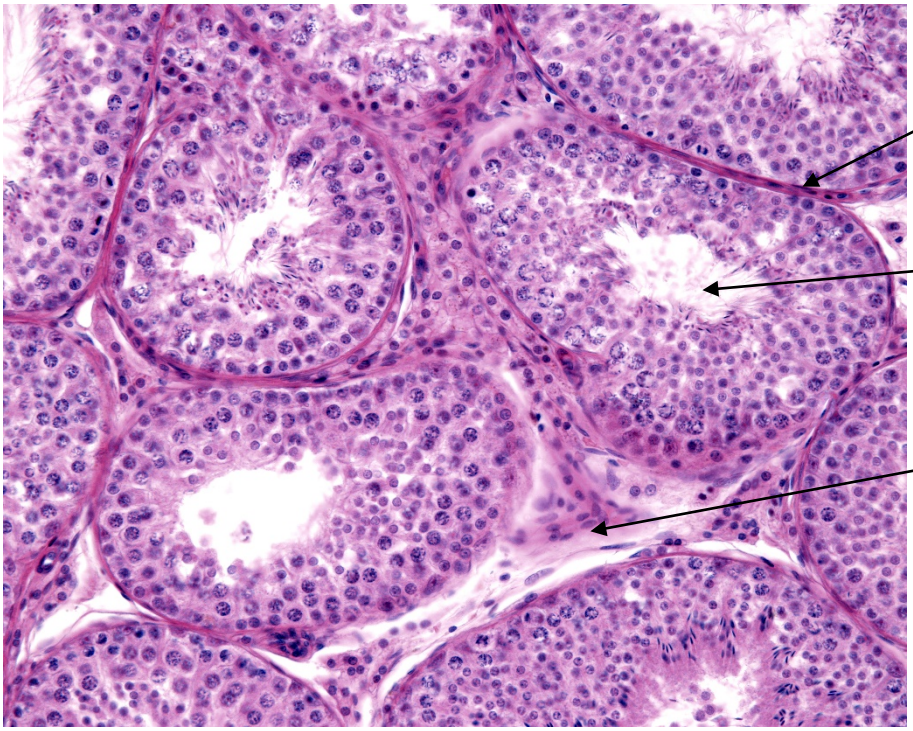


T.S. drawing of seminiferous tubule illustrating spermatogenesis



Sertoli cell
They provide nourishment for spermatids and protection against the male's immune system.

T.S. Testis showing several seminiferous tubules



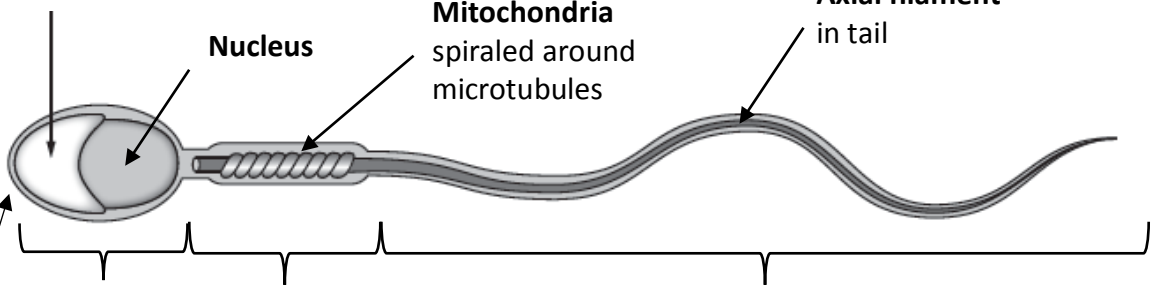
Basement membrane of connective tissue surrounding seminiferous tubules

Lumen of seminiferous tubule

Interstitial cells (Leydig cells) Secrete testosterone which stimulates spermatogenesis

High Power Structure and function of sperm cell

Acrosome containing protease enzymes *(see acrosome reaction)*



Nucleus

Mitochondria spiraled around microtubules

Axial filament in tail

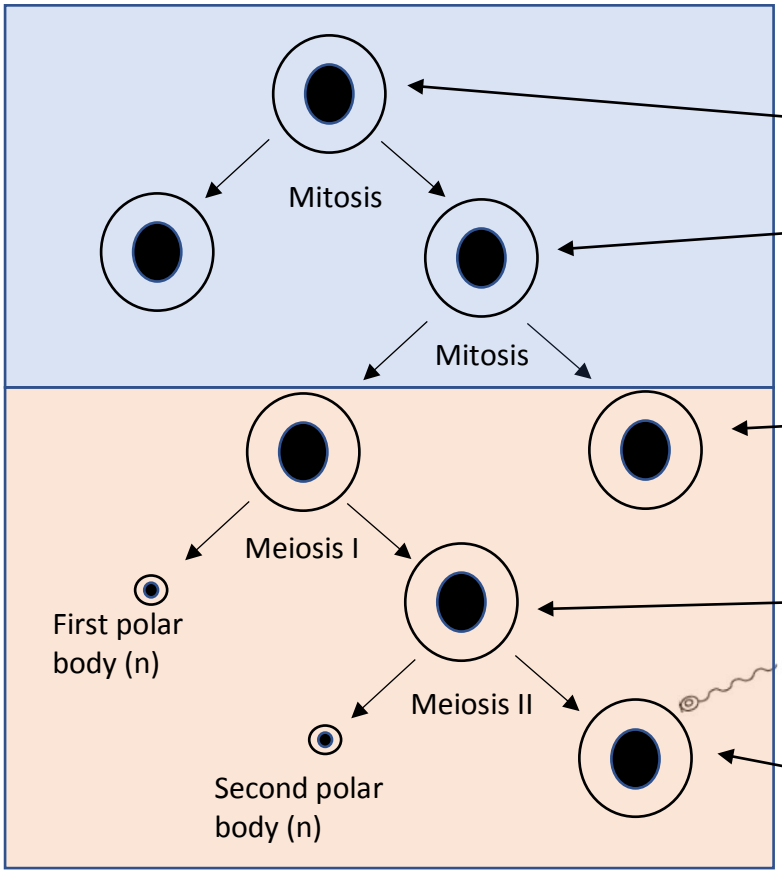
Cell membrane becomes more permeable during capacitation.

Head
5µm

Mid-section
8µm
Contains microtubules that are responsible for movement of tail.
Contains large numbers of mitochondria, spiraled around microtubules, to provide ATP for microtubules.

Tail
50µm
Microtubules from mid-section extend into axial filament in the tail. Whiplash movement of tail propel the spermatozoa.

Oogenesis



Oogenesis is the production of ova

Germinal epithelium (2n)

Oogonium (2n)
Divide many times and enlarge to produce large number of primary oocytes

Primary oocyte (2n)
Present at birth, but development delayed at Prophase I

Secondary oocyte (n)
Meiosis I competed just before ovulation.
Meiosis II stops at metaphase II

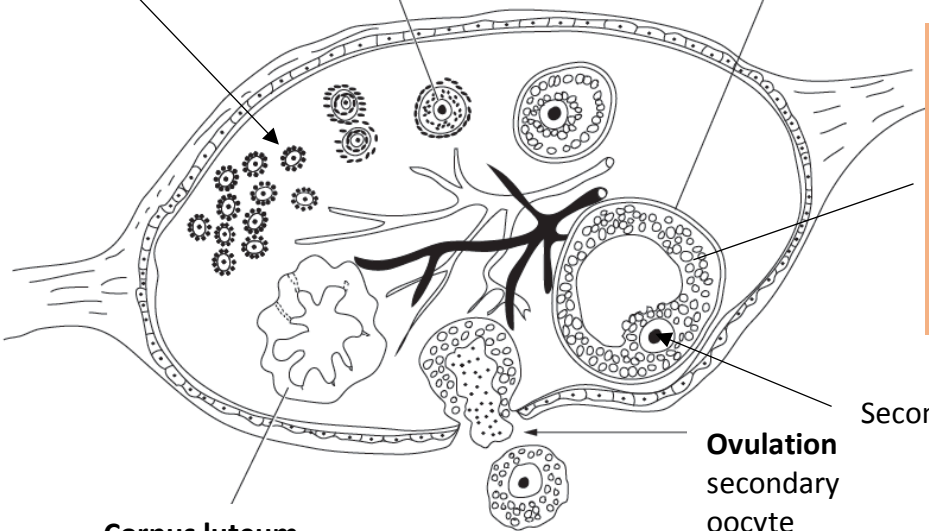
Ovum (2n)
Meiosis II completed if fertilisation occurs.

T.S. drawing of ovary

Primary follicle containing primary oocyte (2n)

Secondary follicle developing in response to FSH

Graafian follicle (mature secondary follicle) containing secondary oocyte ready for ovulation.



A follicle consists of a ball of diploid cells surrounding an oocyte.
The cells of the follicle develop and increase in numbers as the oocyte develops.

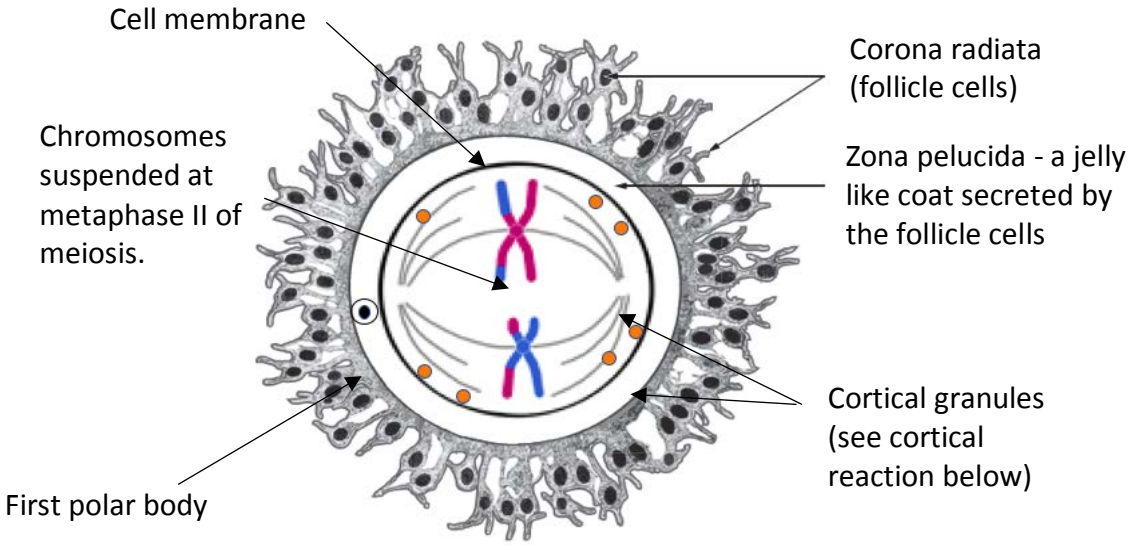
Secondary oocyte

Ovulation secondary oocyte being released

Corpus luteum

- Development stimulated by LH.
- If fertilisation occurs, it starts secreting progesterone and oestrogen.

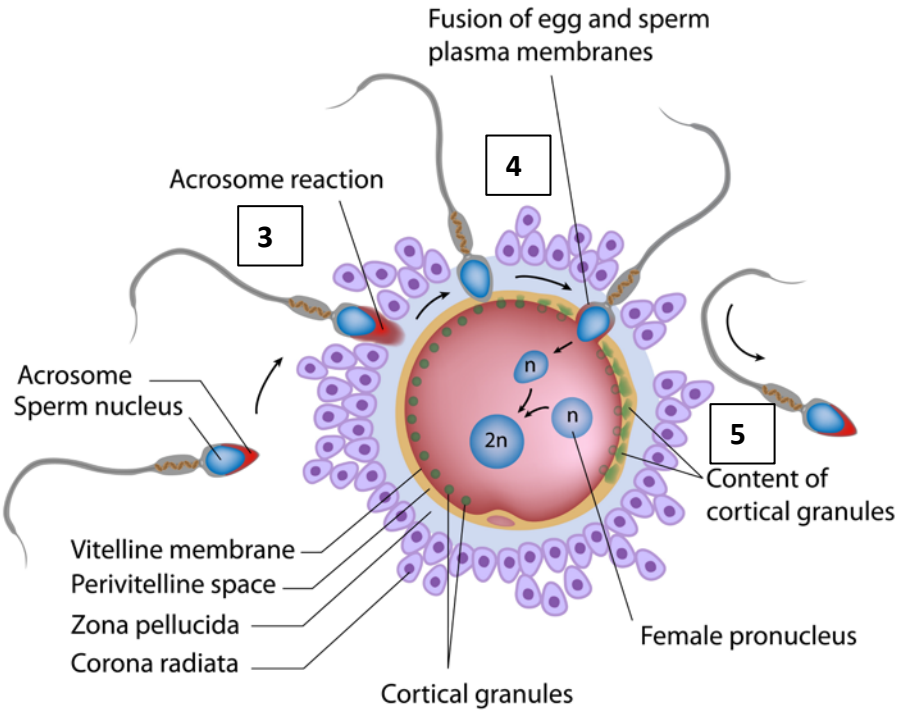
Structure of a secondary oocyte



Fertilisation

1. Following sexual intercourse, spermatozoa move into the fallopian tubes.

2. **Capacitation** increases the permeability of the cell membrane in the head of the sperm above the acrosome.



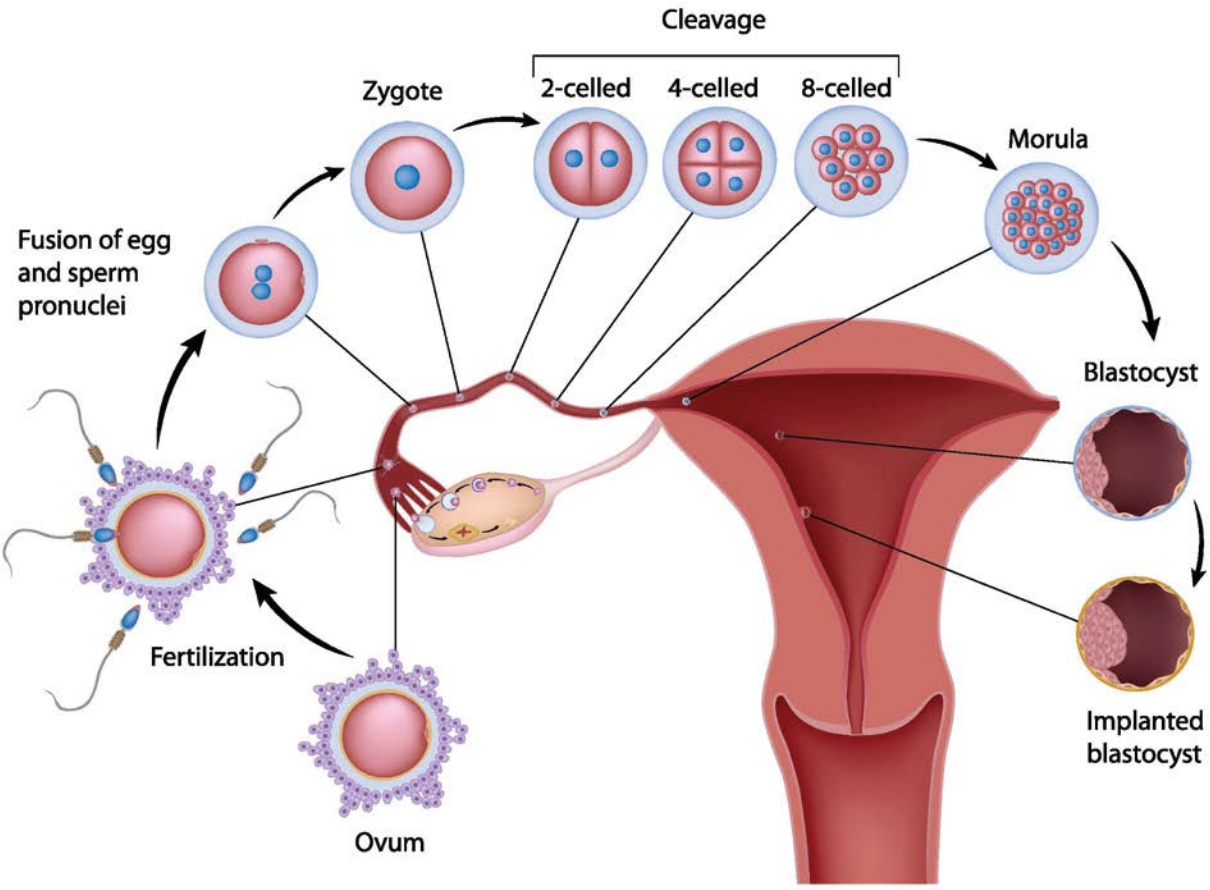
3. **Acrosome reaction** releases hydrolase enzymes which digest the zona pellucida.

4. **Fusion** of sperm and secondary oocyte membranes; genetic material of sperm cell enters the secondary oocyte **triggering completion of meiosis II** and formation of ovum and second polar body.

5. **Cortical reaction** in which cortical granules fuse with the cell membrane and modify the zona pellucida to form the fertilisation membrane; this prevents polyspermy.

6. Nuclei of the sperm and ovum fuse to form a **zygotic nucleus**.

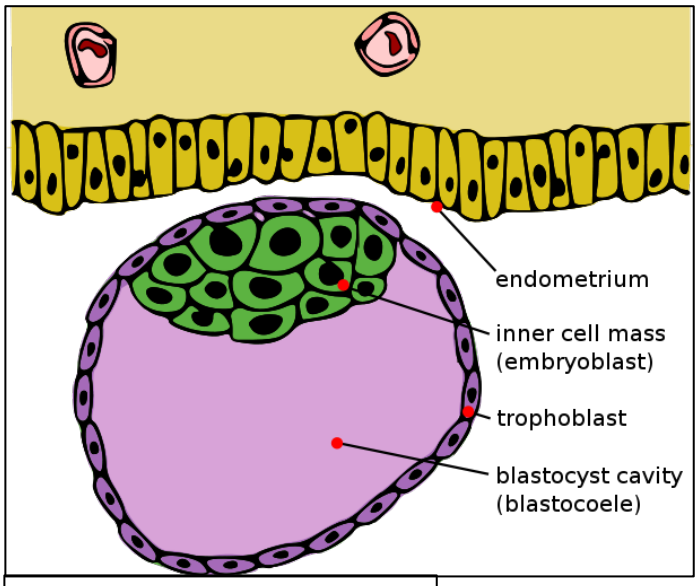
Implantation



1. **Cleavage**
The zygote undergoes repeated mitotic divisions as it moves down the oviduct to form a ball of cells called the **blastocyst**.

2. **Implantation**
The blastocyst moves into the uterus where it attaches and sinks into the endometrium. Cells on the outside of the blastocyst, the trophoblast cells, form trophoblastic villi that penetrate the endometrium. The villi increase the surface area for the absorption of nutrients from the endometrium.

3. **Formation of the placenta**
The placenta begins to develop from the trophoblast cells.



A blastocyst prior to implantation

https://commons.wikimedia.org/wiki/File:Blastocyst_English.svg

Role of the Placenta and Amniotic Fluid

1. Exchange of gases and nutrients

- nutrients
- waste products
- oxygen and carbon dioxide

2. Providing barrier between maternal and foetal blood

- protects foetal capillaries from higher blood pressure and changes in blood pressure of the mother.
- cells of the chorionic villi fuse together thus preventing the mother's phagocytes from passing into the foetus. The mother's antibodies are small enough to cross into the foetal blood and provide passive immunity to the foetus.

3. Secretion of hormones

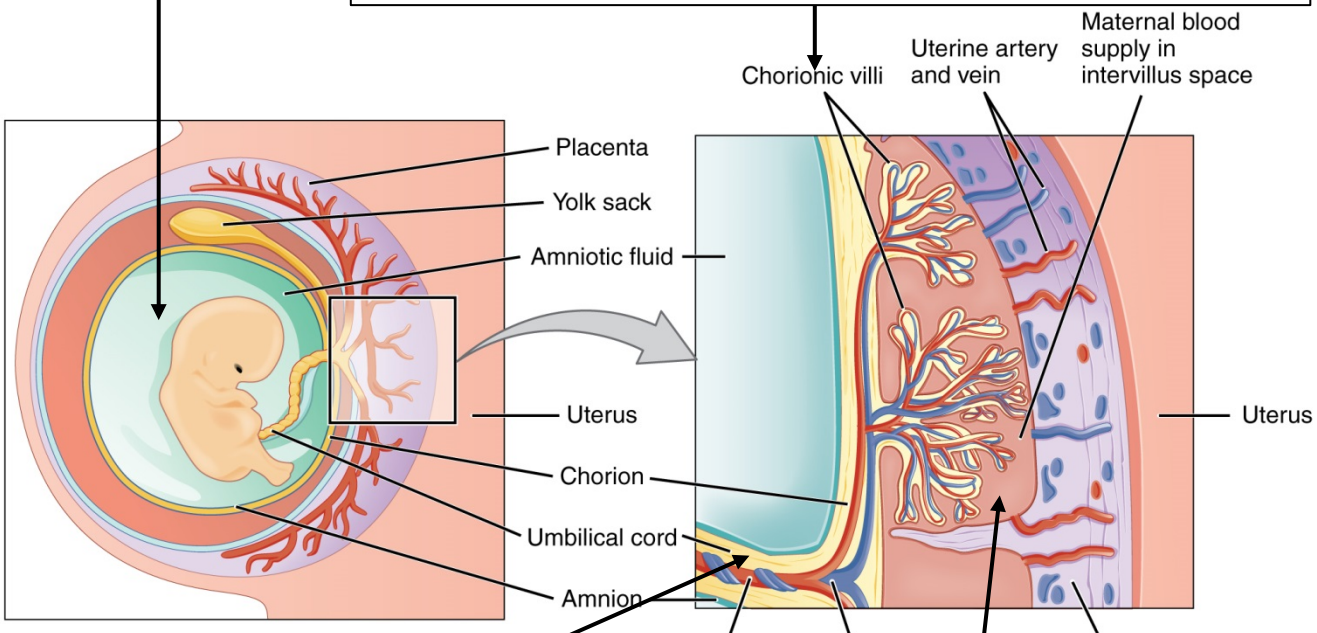
The placenta acts as an endocrine gland:

- Following implantation the placenta takes over secretions of **human chorionic gonadotrophin** from the blastocyst. This maintains the corpus luteum and its secretions of progesterone and oestrogen for the first 16 weeks of pregnancy.
- As the placenta develops it takes over secretions of **progesterone and oestrogen** from the corpus luteum .

Amniotic fluid
acts as shock absorber thus protecting the foetus from injury during development.

Chorionic villi
They are adapted for **increased efficiency of exchange** due to:

- **microvilli** that increase surface area.
- **thin walls** - approximately 5µm therefore distance for diffusion is short.
- **a counter-current flow** of blood between foetal and maternal blood that maintains the concentration gradient.

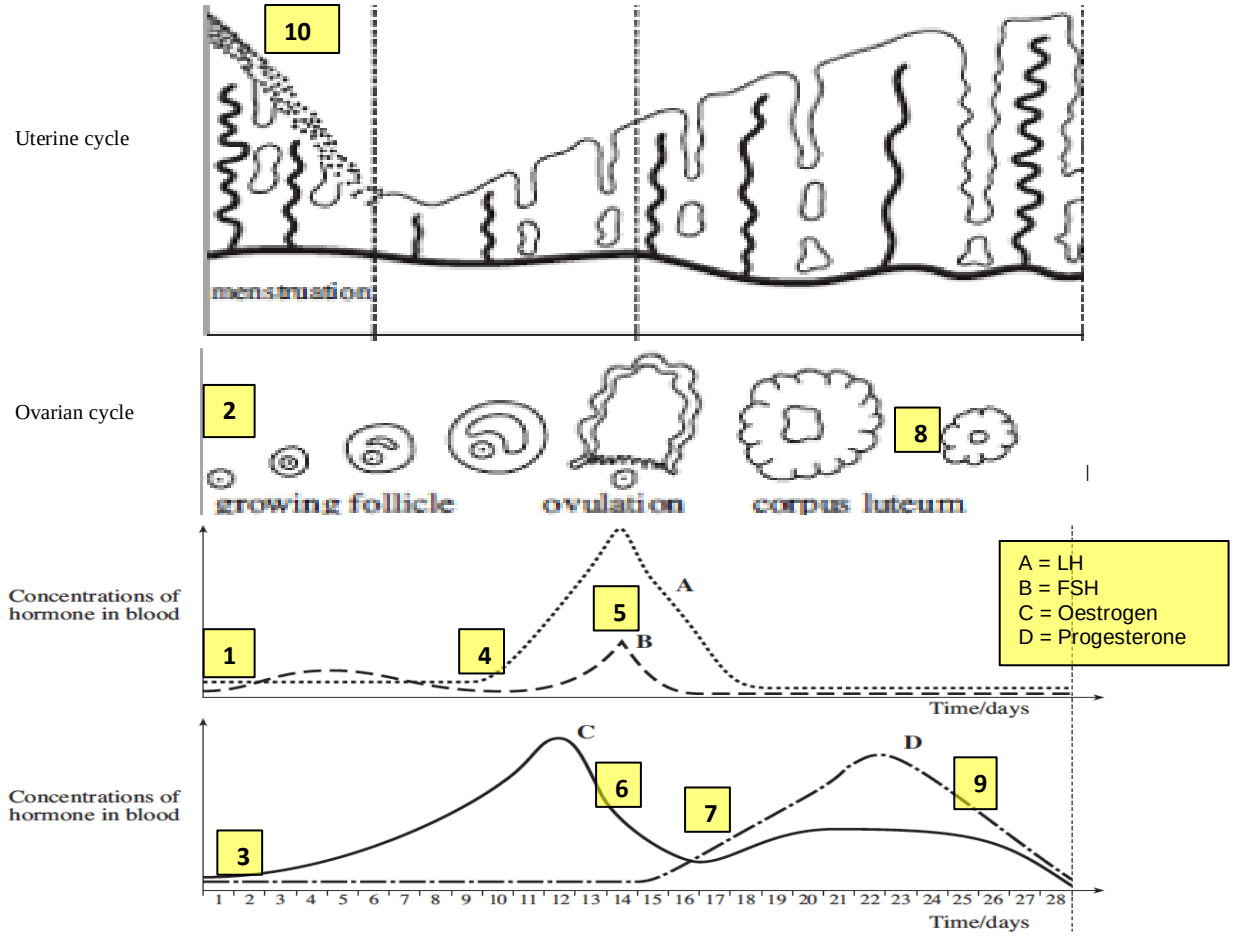


https://upload.wikimedia.org/wikipedia/commons/2/2d/2910_The_Placenta-02.jpg

Umbilical arteries and veins
transport materials between the foetus and mother.

Intervillious spaces
Contain mother's blood surrounding chorionic villi. Maternal and foetal blood do not mix.

Role of Hormones in the Menstrual Cycle



1. Anterior pituitary gland secretes follicle stimulating hormone (FSH).
2. FSH stimulates maturation of primary follicle to secondary follicle.
3. Maturing follicle secretes Oestrogen.
4. Oestrogen:
   - stimulates Lutenising hormone (LH) production by anterior pituitary gland.
   - Inhibits secretion of FSH.
   - triggers rebuilding of endometrium.
5. LH:
   - stimulates secretion of FSH,
   - Induces ovulation on day 14,
   - stimulates conversion of Graafian follicle into corpus luteum.
6. FSH inhibits oestrogen production.
7. Corpus luteum secretes progesterone that:
   - maintains endometrium,
   - Inhibits secretion of FSH,
   - Inhibits secretion of LH.

   (The corpus luteum also releases oestrogen. However, during the latter half of the menstrual cycle oestrogen does not stimulate LH secretions because of the effects of progesterone).
8. Falling levels of FSH and LH cause corpus luteum to degenerate.
9. Progesterone levels decrease as secretions form degenerating corpus luteum decline.
10. Low levels of progesterone and oestrogen cause endometrium to breakdown and be shed during menstruation.

Role of Hormones in Pregnancy

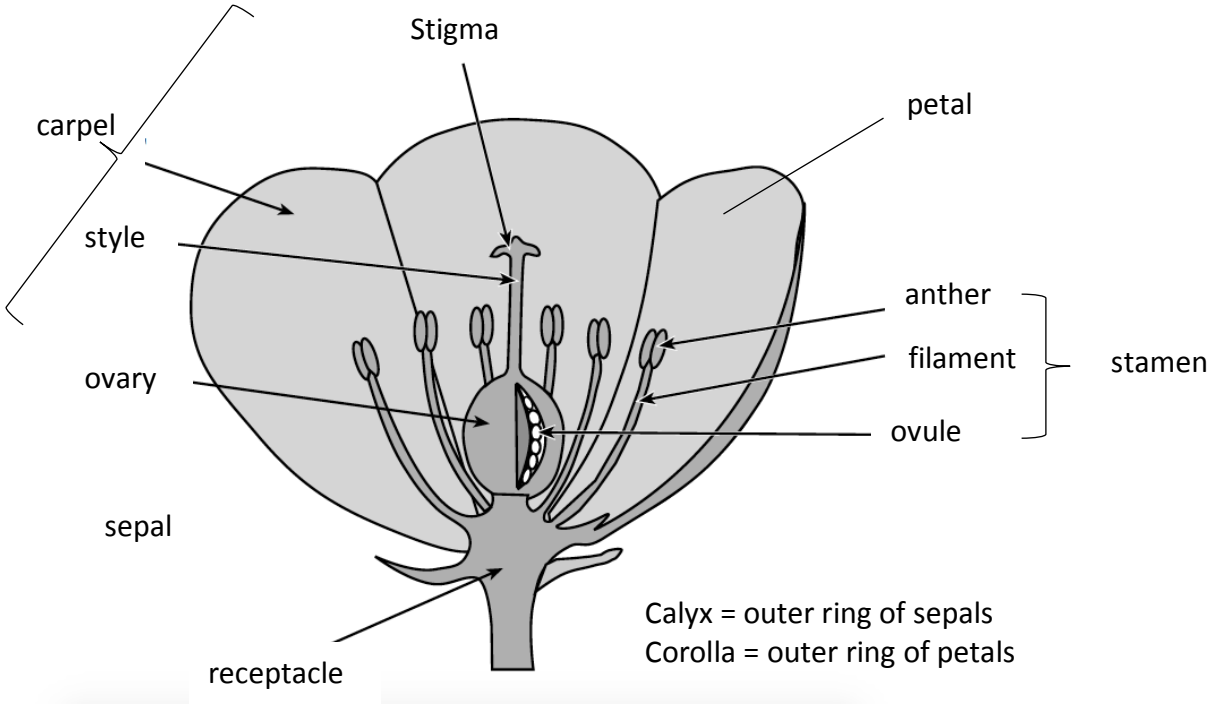
1. Following fertilisation (about 6 days), the developing embryo (blastocyst) begins to secrete human chorionic gonadotrophin (hCG).
2. If the implantation is successful, the developing placenta will take over secretions of hCG. The importance of hCG is:
   - maintenance of the corpus luteum for the first 16 weeks of pregnancy.
3. The corpus luteum secretes oestrogen and progesterone which:
   - inhibit FSH, preventing development of any follicles,
   - inhibit LH, preventing ovulation,
   - (oestrogen) stimulates growth of the uterus to accommodate the growing foetus,
   - (oestrogen) stimulates growth and development of mammary glands,
   - (progesterone) maintains the wall of the endometrium,
   - (progesterone) suppresses the uterine wall's ability to contract by inhibiting secretions of oxytocin.
4. During pregnancy, as the corpus luteum degenerates the placenta will take over responsibility for the secretions of progesterone and oestrogen.

Role of Hormones at Birth

1. Just before birth oestrogen levels increase and progesterone levels decrease.
2. Oxytocin secretion by posterior pituitary gland is no longer inhibited. Secretions of oxytocin stimulates contraction of the uterine wall which stimulates the secretion of more oxytocin (this is an example of positive feedback).
3. Prolactin secreted by the anterior pituitary gland during and after birth stimulates production of milk by mammary glands.

Section 4.2 - Sexual Reproduction in Plants

Generalised structure of a dicotyledonous, insect-pollinated flower

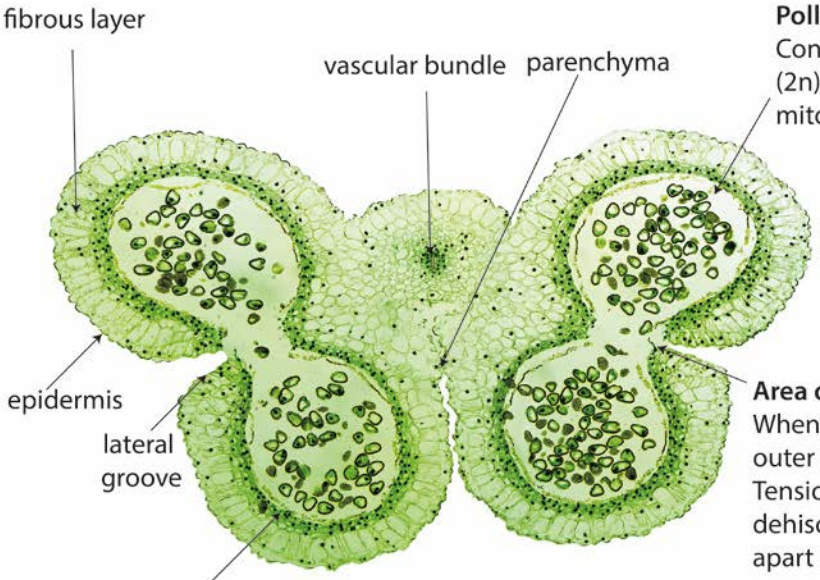


Comparison of insect and wind-pollinated flowers

Wind-pollinated flower	Insect-pollinated flower
Petals small and green or no petals at all. The flowers are located above the leaves, or produced in early spring to give maximum advantage from wind currents, without interference from leaves.	Large and brightly coloured petals, often with guidelines to the nectaries to attract the insect pollinators.
The stigmas are large and feathery and hang outside the flower to catch airborne pollen in wind currents.	The stigma is sticky to trap pollen grains and is inside the flower, where insects rub against it thus causing pollen to be deposited.
Large anthers suspended outside the flower to release pollen grains into wind currents.	Anthers on rigid filaments, inside the flower: where insect must rub against them. This increases chance of pollen being deposited on insect body.
Vast numbers of small, light, smooth-walled pollen grains are produced which are easily carried by the wind.	The pollen grains are large with spiky walls which stick to insect bodies.

Development of Pollen

- The anther consists of four pollen sacs arranged in two pairs, side by side.
- Pollen grains contain the male gametes and are formed inside the pollen sacs**.**

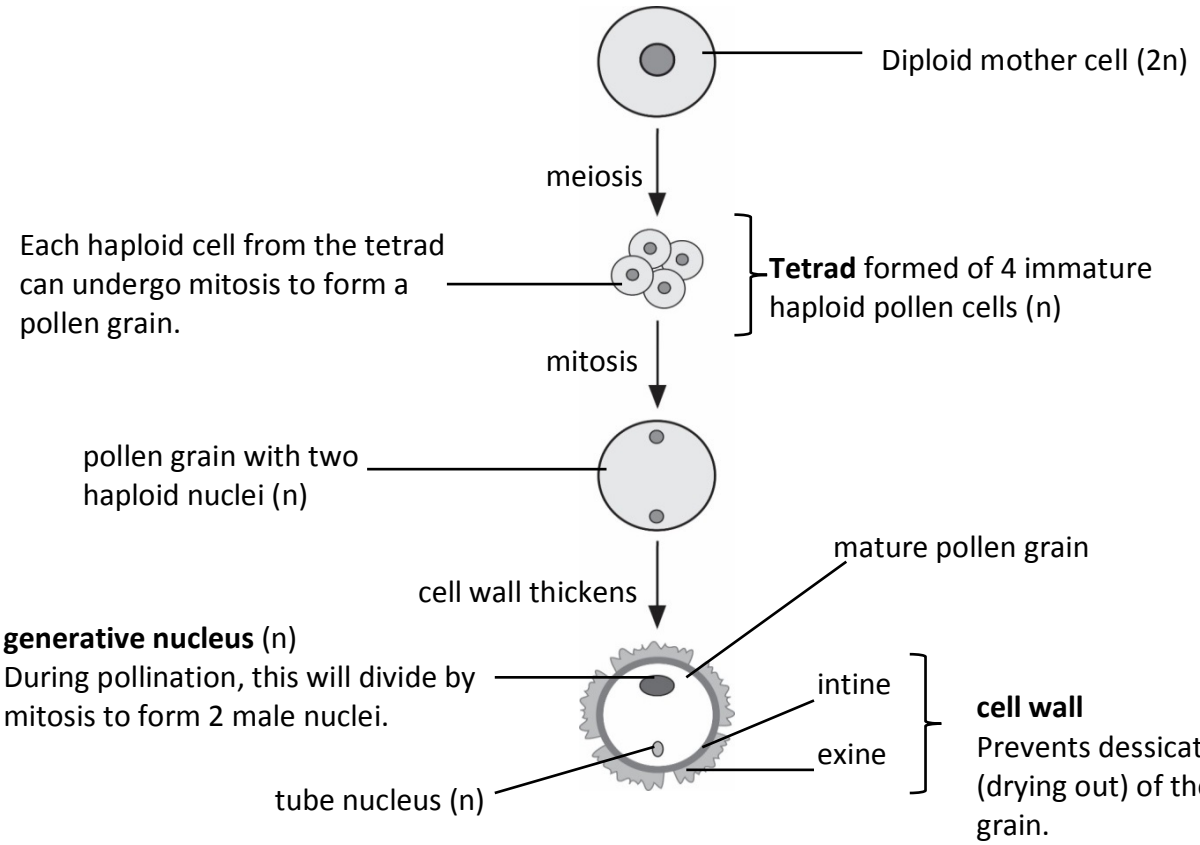


Pollen Sac
Contains a large number of diploid (2n) mother cells produced by mitosis.

Area of dehiscence (Stomium)
When pollen grains are mature, outer layers of the anther dry out. Tension in the lateral grooves causes dehiscence - walls of the anther pull apart releasing the pollen grains.

Tapetum
Inner wall of cells that provide nutrients to the developing pollen grains.

Formation of Pollen



Development of an Ovule

1. The ovule contains a mass of cells called the **nucellus** surrounded by two protective **integuments**.
   The ovule is carried on a short stalk called the **funicle**.
   One cell in the nucellus enlarges and develops into the **megaspore mother cell (2n).**

2. **Meiosis** of the mother cell produces **four haploid megaspores (n).**

3. Three of the haploid megaspores degenerate

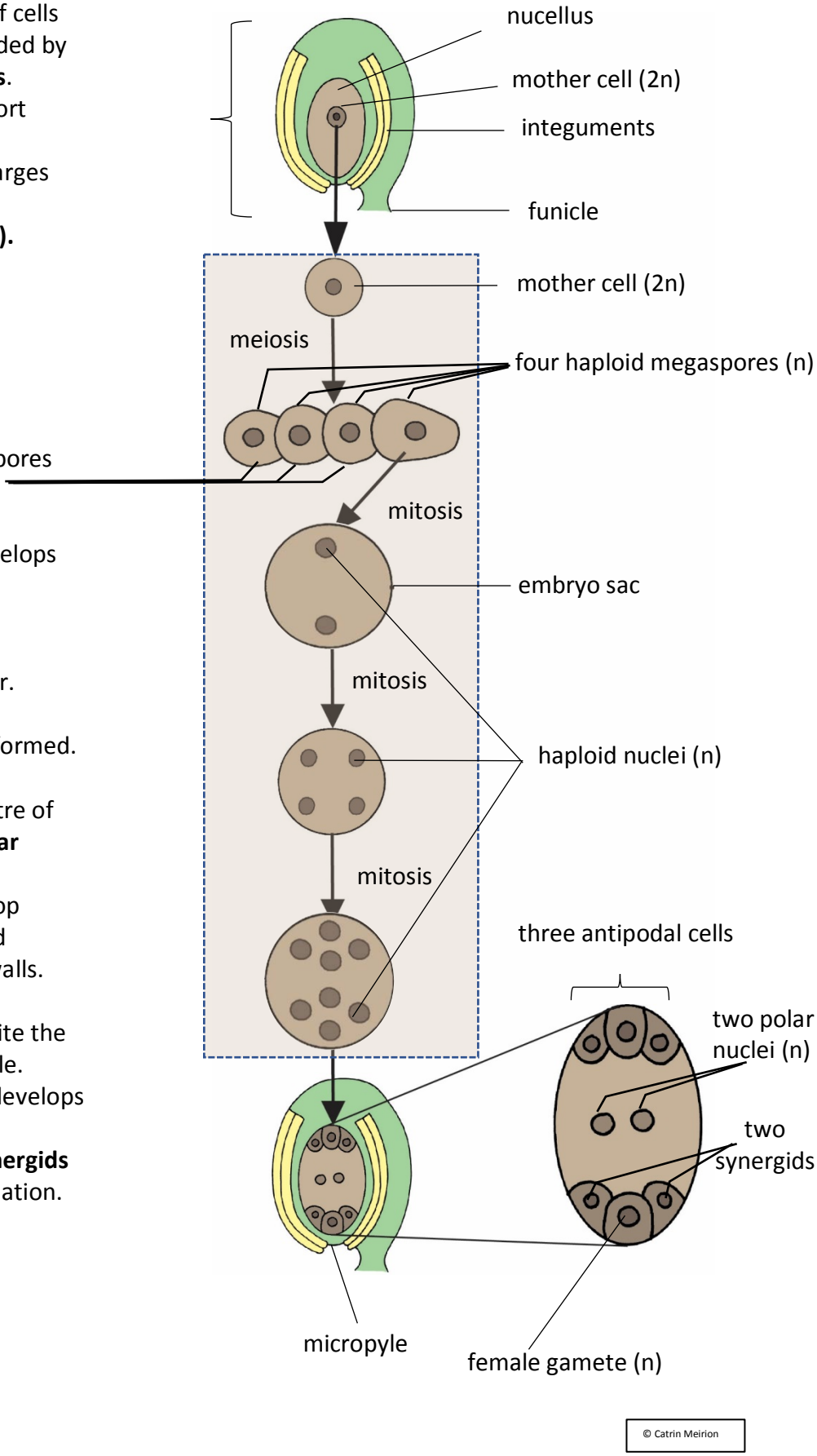
4. One haploid megaspore develops into the embryo sac.

5. Three **mitotic** divisions occur.

6. Eight haploid nuclei (n) are formed.

7. Two nuclei move to the centre of the embryo sac to form **polar nuclei (n)**.
   The remaining nuclei develop cytoplasm around them and become separated by cell walls.

8. Three **antipodal cells** opposite the micropyle play no further role.
   One cell nearest micropyle develops into **female gamete (n).**
   The other two cells form **synergids** that degenerate after fertilisation.



Pollination

The transfer of pollen grains from the anther to the mature stigma of the same species.

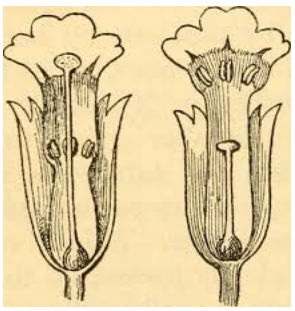
Self-pollination	**Cross-pollination**
Transfer of pollen from the anther of a flower to the mature stigma of the same flower or another flower on the same plant of the same species.	Transfer of pollen from the anther of a flower to the mature stigma of another flower on another plant of the same species.
Leads to self-fertilisation which leads to inbreeding.	Leads to cross-fertilisation which leads to outbreeding.
Genetic variation dependent on: • crossing over during prophase I of meiosis, • independent assortment during metaphase I of meiosis, • mutation.	Genetic variation dependent on: • crossing over during prophase I of meiosis, • independent assortment during metaphase I of meiosis, • mutation, AND • Combining genotypes of gametes from two different individuals.
Less genetic variation.	More genetic variation.
Greater chance of two potentially harmful recessive alleles combining.	Reduced chance of producing harmful combinations of alleles.
Successful genomes are preserved, which is an advantage in a stable environment, but a disadvantage if the environment changes suddenly.	Advantageous in terms of evolution, because if the environment changes suddenly it is likely that there are individuals within the population with a combination of alleles that will allow the species to survive.

Adaptations of Flowers to Promote Cross-Pollination

1. Chemical self-incompatibility - gametes from the same parent plant are unable to fuse and form a zygote or, if the zygote forms, then it fails to develop.

2. **Irregular flower structure** - e.g. primrose *(Primula vulgaris)*

https://c1.staticflickr.com/4/3801/20388518981_b33a41ae01_z.jpg



Pin-eyed variety Thrum variety

The diagram shows two varieties of primrose. The stigma of the pin-eyed variety is above the anthers therefore pollen grains will not fall onto it. Insects tend to pollinate a stigma on the same level as anthers that they collected the pollen from. Therefore, pollen tends to be transferred from pin-eyed flowers to thrum flowers and *vice versa*.

3. **Dichogamy** - anthers and stigmas mature at different times.

4. **Monoecious plants** - have separate female and male flowers on the same plant, e.g. hazel (*Corylus avellana*).

5. **Dioecious plants** - have separate male and female plants, e.g. willow (*Salix sp.*)

The Process of Double Fertilisation

Double fertilisation involves two male gametes in **two** separate fertilisation events.

- Fertilisation of female gamete to form a diploid zygote,
- Fertilisation of the polar nuclei to form a triploid endosperm nucleus.

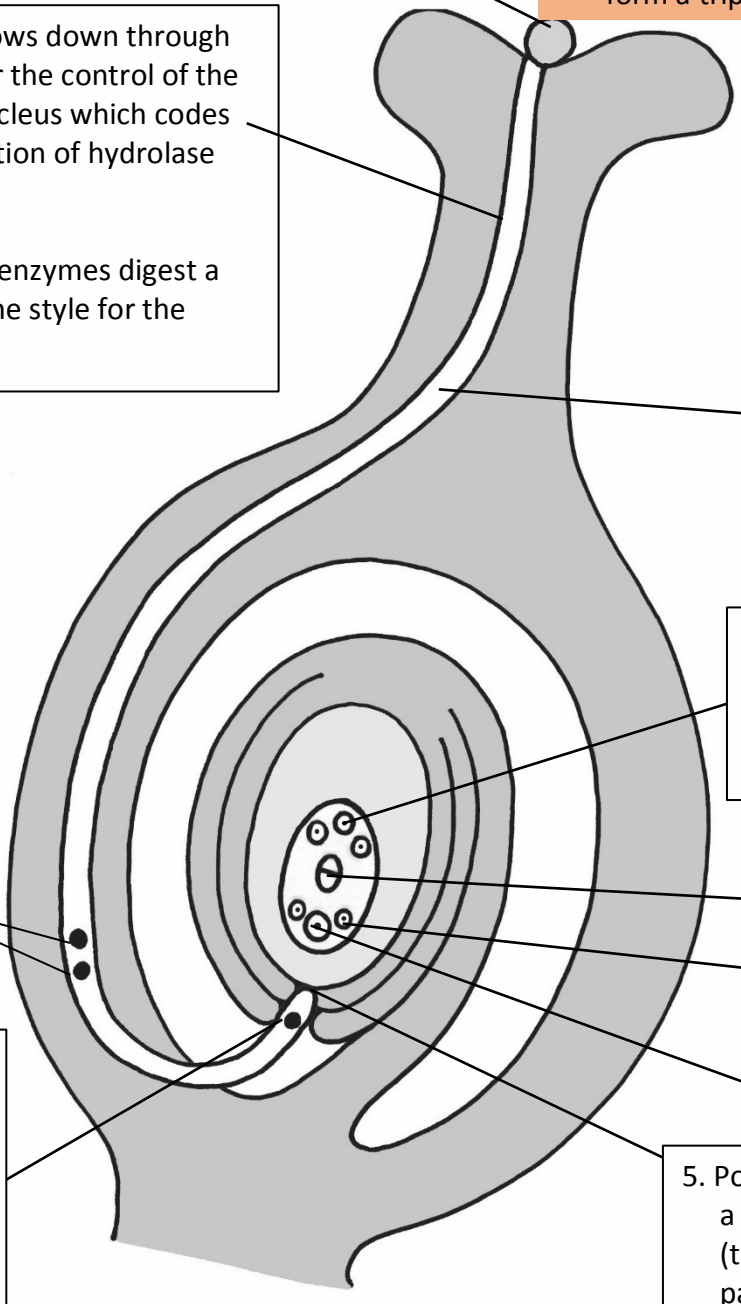
1. **Pollen grain** lands on stigma and absorbs water. If pollen grain and stigma are compatible, the pollen tube will germinate.

2. **Pollen tube** grows down through the style under the control of the pollen tube nucleus which codes for the production of hydrolase enzymes.

   The hydrolase enzymes digest a way through the style for the pollen tube.

3. During germination and growth of the pollen tube, the generative nucleus (n) divides by mitosis to form **2 male nuclei (n);** the **gametes**.

4. Growth of the pollen tube is a positive chemotrophic response. The pollen tube grows towards chemicals secreted by the embryo sac.



5. Pollen tube grows through a **gap in the integuments** (the **micropyle**) and passes into the embryo sac.

6. Once the pollen tube has entered the embryo sac, the pollen **tube nucleus** disintegrates.

7. **Double fertilisation**:
   - one male gamete fuses with the female gamete to produce a **diploid zygote (2n)**.
   - the second male gamete fuses with the two polar nuclei to form a **triploid primary endosperm nucleus (3n)**.

8. After fertilisation, the **antipodal** cells and the synergids play no further role.

Formation and Structure of Seed and Fruit

Following fertilisation:

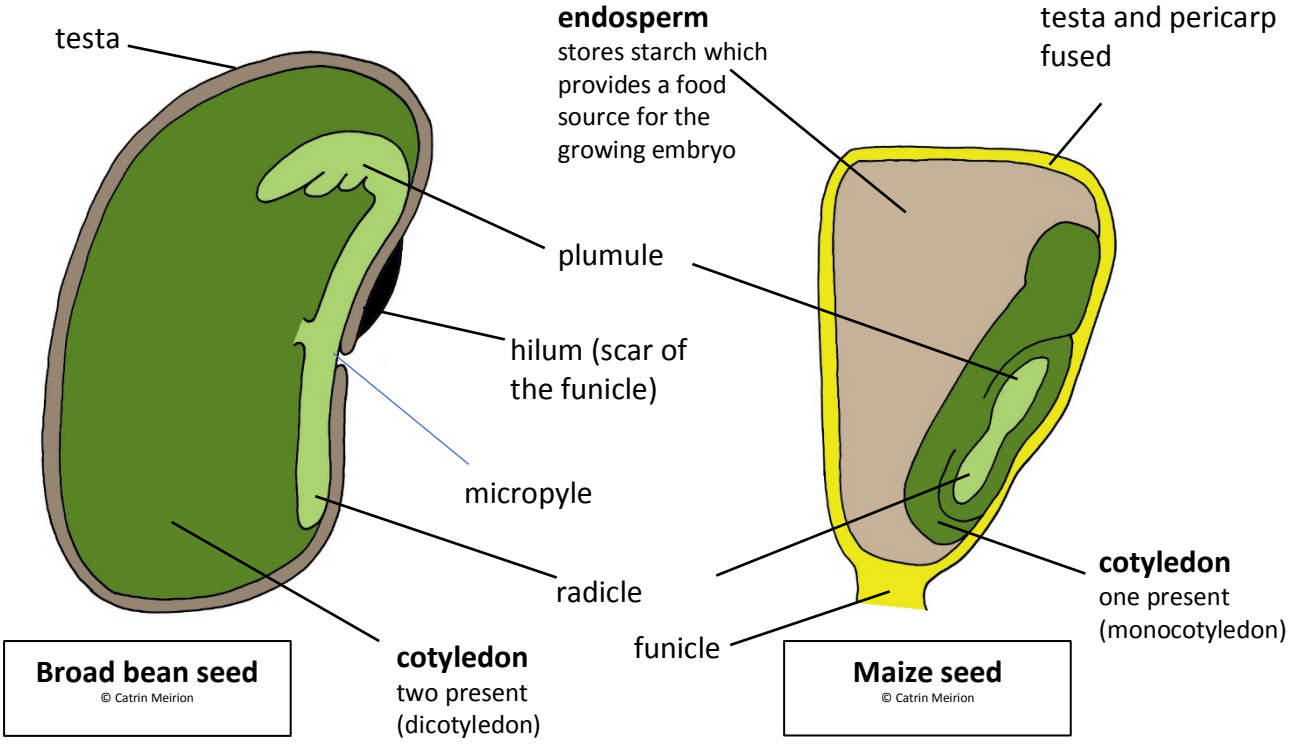
1. The diploid zygote divides by mitosis to form the diploid embryo which then differentiates into a young shoot (the plumule), young root (the radicle) and one or two seed leaves (the cotyledons).

2. The triploid endosperm tissue divides by mitosis to form endosperm tissue which forms the food source for the growing embryo. In some species of plant called monocotyledons, e.g. maize (*Zea mays*), the endosperm remains and only one cotyledon is present. In other plants called dicotyledons, e.g. broad beans (*Vicia faba*), the endosperm is quickly absorbed and stored in two cotyledons.

Development of the fruit after double fertilisation

Before fertilisation	After fertilisation
Ovule	seed
integuments	testa (seed coat)
mycropyle	pore
ovary wall	fruit wall (pericarp)
content of ovary	fruit
Attachment point of funicle to ovule	hilum

Comparing the structure of a broad bean and maize seed



Seeds have evolved as a survival strategy for a terrestrial mode of life. Plants have developed different mechanisms to enable the dispersal of seeds. This reduces competition following germination and increases the chance of growth into mature plants.

Dormancy and Germination

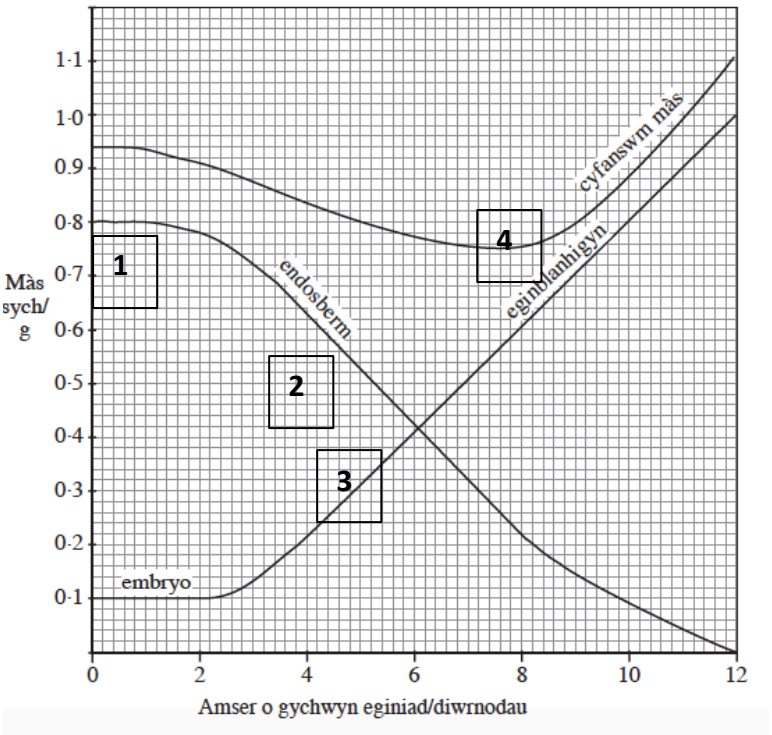
The water content of seeds is very low and it is the major factor that prevents germination. Seeds will remain dormant until suitable conditions are present:

- water to mobilise enzymes; for transport; and to vacuolate cells to make them turgid,
- oxygen for aerobic respiration,
- suitable temperature for enzymes to operate

Germination involves the rapid onset of biochemical activity and growth of a seedling until the plant can carry out photosynthesis and become independent of the food stores contained in the cotyledons or endosperm.

Mobilisation of Food Reserves During Germination

The graph below shows the relative changes in dry mass of the embryo, seed and endosperm/cotyledon.



1. Hydrolysis of food reserves in the endosperm/cotyledon and starch is converted to sugars.
2. The dry mass of the endosperm/cotyledon decreases because:
   - CO_2 is lost when the sugars are used in aerobic respiration,
   - sugars are sent to the embryo.
3. Mass of embryo increases as it receives sugars from the endosperm/cotyledon.

4. Total mass:

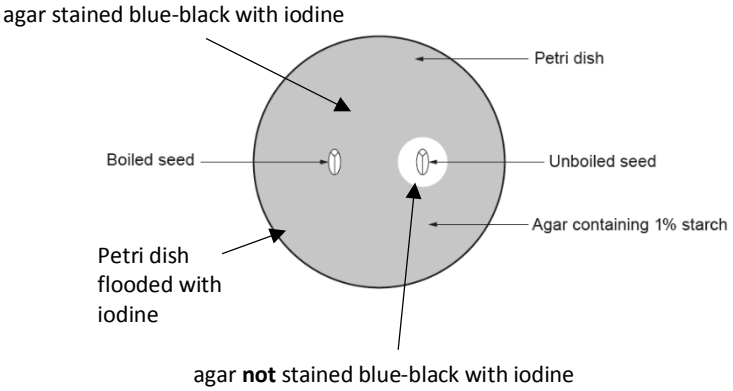
- decreases at first as CO_2 is lost in aerobic respiration
- then increases as the first leaves produced (plumule) begin producing biomass during photosynthesis.

Investigating the digestion of starch agar using germinating seeds

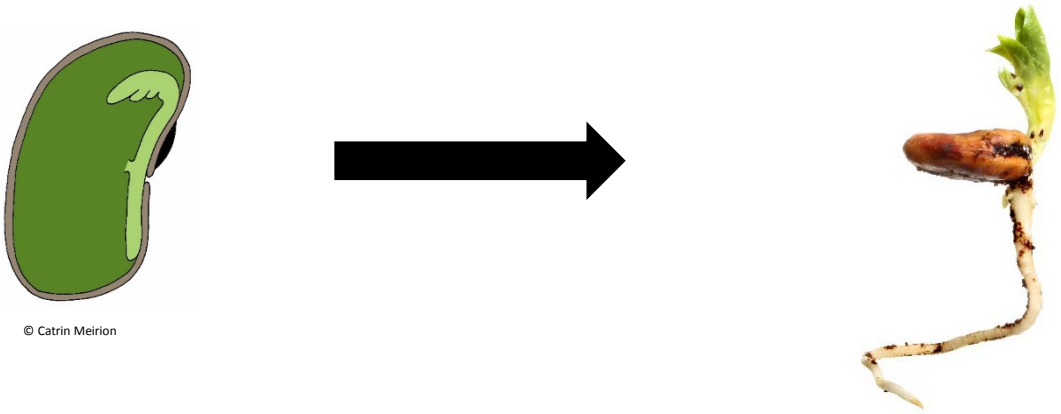
Explanation

Un-boiled seed - amylase produced by the germinating seed has diffused out of the seed and converted starch to maltose. The agar surrounding the seed does not stain blue-black with iodine as there is no starch present.

Boiled seed - boiling the seed has denatured the enzymes in the germinating seed. The agar surrounding the seed stains blue-black with iodine as the starch has not been digested.

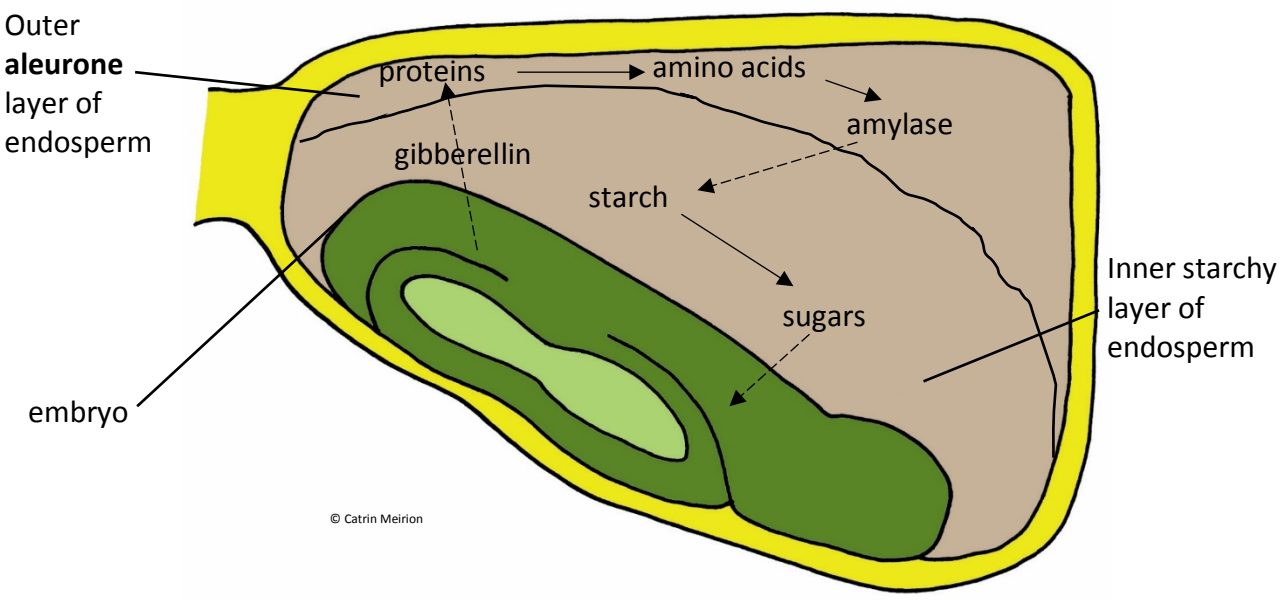


Germination in a non-endospermic seed, e.g. *Vicia faba* (broad bean)



1. Water is imbibed through the micropyle.
2. The cotyledons swell and the testa splits allowing entry of more water and oxygen for aerobic respiration.
3. Starch and protein reserves in the cotyledons are hydrolysed.
4. Products of hydrolysis are used as:
    - a source of energy for respiration,
    - growth of the plumule and radicle.

Germination in an endospermic seed, e.g. *Zea mays* (maize)



1. Following the imbibing of water, gibberellin (gibberellic acid) is released by the embryo.
2. The gibberellin diffuses to the aleurone layer (the outer layer of the endosperm) which contains protein.
3. Gibberellin induces the production of hydrolytic enzymes, e.g. amylase.
4. The hydrolytic enzymes diffuse into the inner layer of the endosperm and catalyse the breakdown of stored nutrients, e.g. starch.
5. Glucose and other breakdown products of the stored nutrients diffuse into the embryo where they are used for aerobic respiration and growth.

Section 4.3 - Inheritance

Genetic terms

You need to know and understand the following genetic terms:

Gene	A length of DNA on a chromosome normally coding for a specific polypeptide.
Locus	A specific position on a chromosome where a gene is located.
Alleles	Different forms of the same gene. *(This is always a **single** letter).*
Dominant	A dominant allele will always be expressed in the phenotype when present. *(It is represented with a CAPITAL letter).*
Recessive	A recessive allele will be 'hidden' when a dominant allele is present in a heterozygote. A recessive allele will only be expressed when it is homozygous. *(It is represented with a small case letter).*
Codominant	Alleles that are equally expressed in a heterozygote.
Phenotype	The characteristics of an organism resulting from both its genotype and the effects of the environment.
Genotype	The genetic make-up of an organism i.e. its alleles. *(This is always a **pair** of letters).*
Homozygous	Both alleles for a gene are identical.
Heterozygous	Both alleles for a gene are different.
F_1	The **first filial generation** - the offspring of the parents in a genetic cross.
F_2	The second filial generation - the offspring of an F_1 plant that is self-fertilised or a cross between two members of the F_1 generation.
Autosomes	Chromosome which are not sex chromosomes.
Sex Chromosome	Chromosomes which determine the sex of an individual organism.

The Principles of Mendelian Monohybrid Inheritance

Gregor Mendel (1822 –84) was an Austrian monk. He studied the inheritance of characteristics in the garden pea.

He chose the pea plant because they are easy to grow and have many easily distinguishable characteristics.

How to solve genetic problems

Mendel crossed a pea plant which was true-breeding for tall plants with a pea plant which was true-breeding for short plants. The F_1 generation were all tall. When the F_1 generation were crossed the F_2 generation showed a mix of tall and short plants.

Example – Mendel's Peas

Let **T** = tall allele
Let **t** = short allele

1. *Choose a letter to represent the alleles.* ***Capital = dominant. small case = recessive***

Parent **phenotype** Tall x Short

Parent **genotype** **T T** x t t

2. *The **phenotype** is the characteristic you see.*

Gametes **T** and **T** **t** and **t**

3. *The **genotype** is always a **pair of alleles (two letters)***

4. *The **gamete** is always a **single allele (one letter)***

F1 cross (First generation)

gametes	T	T
t	Tt	Tt
t	Tt	Tt

All F1 offspring have the **genotype** Tt.
They are heterozygous.
The recessive allele (short) is hidden by the dominant allele (tall.)

An F2 cross can happen when an F1 plant is self-pollinated (selfing)

F2 cross (second generation)

gametes	T	t
T	TT	Tt
t	Tt	tt

*Always check to see if you are asked to describe the **probability** or the **ratio** of results.*

The **ratio** of tall to short plants = 3 tall : 1 short

The **probability** of a tall plant = 75%
The **probability** of a short plant = 25%

The Monohybrid ratio
3 dominant phenotypes: 1 recessive phenotype

Top Tip - The dominant phenotype is the one which is expressed in the F_1 gneration.

Monohybrid inheritance is the inheritance of a single gene.

Top Tip - All genetic crosses must be set out to clearly explain all the steps, and to reduce the chances of error especially under exam pressure!

Heredity and Genetics is the study of inheritable characteristics.

Mendel's First Law of Heredity - The Law of Segregation.
The characteristics of an organism are determined by factors (*genes*) which occur in pairs. Only one member of a pair of factors (*genes*) can be represented in a single gamete.

The Principles of Dihybrid Mendelian Inheritance

Mendel studied the inheritance of two characteristics, seed colour and seed shape.
He knew from previous experiments that round seed is dominant to wrinkled and yellow seed is dominant to green.
Mendel crossed a true-breeding parent plant producing round and yellow seed with a true-breeding parent plant producing wrinkled and green seed.

Let R = allele for round seed Y = allele for yellow allele
r = allele for wrinkled seed y = allele for green allele

Parental generation

Phenotype:	Round Yellow seed	x	Wrinkled green seed
Genotype:	RRYY	x	rryy
Gametes:	RY	x	ry

Punnett square to show production of the F1.

Gametes	RY
ry	RrYy

All offspring are heterozygous plants,
Genotype = RrYy
Phenotype = Round and yellow seed

Top tip
If a plant is **true-breeding** it has been self-fertilised for many generations, always producing the same phenotype.
It has a homozygous genotype.

When the F1 produce gametes, one allele for seed shape and one allele for seed colour must pass into each gamete.
Remember any combination of gametes can be produced
In this way there are 4 possible gametes.
RY Ry rY ry

F1 generation

Phenotype:	Round Yellow seed	x	Round Yellow seed
Genotype:	RrYy	x	RrYy
Gametes:	RY, Ry, rY, ry	x	RY, Ry, rY, ry

Gametes	RY	Ry	rY	ry
RY	RRYY	RRYy	RrYY	RrYy
Ry	RRYy	RRyy	RrYy	Rryy
rY	RrYY	RrYy	rrYY	rrYy
ry	RrYy	Rryy	rrYy	rryy

Phenotype	Ratio
Round yellow seed	9
Round green seed	3
Wrinkled yellow seed	3
Wrinkled green seed	1

The Dihybrid ratio:
9 showing two dominant phenotypes
3 showing one dominant and one recessive phenotype
3 showing one dominant and one recessive phenotype
1 showing two recessive phenotypes

When these results are analysed it can be seen that the ratio of dominant to recessive for each characteristic still demonstrate the 3: 1 ratio

Shape of seed — Round: Wrinkled
Dominant: Recessive

Colour of seed — Yellow: Green
Dominant: Recessive

Remember – Mendel's laws only apply if the genes are on different chromosomes, i.e. not **linked.**

The significance of this is that **the two characteristics had behaved completely independently of one another**.

From the results of his dihybrid crosses, Mendel formulated his second law.

Mendel's Second Law – The Law of Independent Assortment.
Each of a pair of contrasted characters may be combined with either of another pair.

Why are Test Crosses Carried Out?

The only genotype that we can be certain of is that of an organism showing a recessive phenotype. An organism showing the dominant phenotype can either have a homozygous or heterozygous genotype.
A test cross or back cross is used to determine the genotype of the organisms with the dominant phenotype.

How are Test Crosses Carried Out?

The Monohybrid Test Cross
The organism whose genotype is unknown is crossed with a **homozygous recessive** organism.

1. If the organism with the unknown genotype is homozygous dominant then all the offspring will show the dominant phenotype. This is because they will have inherited one copy of the dominant allele form the homozygous dominant parent.
2. If the organism with the unknown genotype is heterozygous then the ratio of dominant to recessive phenotypes will be 1 : 1.
   E.g. If a tall pea plant (heterozygous) is crossed with a short pea plant (homozygous),
   Let T = tall
   t = short

Phenotype: Tall x short
Genotype: Tt x tt
Alleles: T, t x t, t

Gametes	T	t
t	Tt	tt
t	Tt	tt

The Monohybrid test cross ratio
1 dominant phenotype: 1 recessive phenotype

The Dihybrid Test Cross

This method is used to determine the genotype of an organism showing two dominant phenotypes. The organism can either be homozygous or heterozygous.

Example

In the summer squash plant, white fruit colour is dominant over yellow fruit colour.
Disc shape fruit is dominant over sphere shape.

Let W = allele for white colour D = allele for disc shape fruit
w = allele for yellow colour d = allele for spherical fruit

1. If the white disc plant is homozygous dominant:

Phenotype:	White Disc fruit	x	Yellow spherical fruits
Genotype:	WWDD	x	wwdd
Gametes:	WD	x	wd

Gametes	WD
wd	WwDw

Only one type of phenotype will be shown.

2. If the white disc plant is heterozygous.

Phenotype:	White Disc fruit	x	Yellow spherical fruits
Genotype:	WwDd	x	wwdd
Gametes:	WD, Wd, wD, wd	x	wd

Gametes	WD	Wd	wD	wd
wd	WwDd	Wwdd	wwDd	wwdd

The Dihybrid Test Cross Ratio
1 showing two dominant phenotypes
1 showing one dominant and one recessive phenotype
1 showing one dominant and one recessive phenotype
1 showing two recessive phenotypes

Top tip
It's worth learning the different ratios from the various genetic crosses because they can give you clues as to which type of inheritance is occurring.
If the observed ratio doesn't fit any of the Mendelian ratios, test-cross ratios or ratios for codominance, then there may be gene linkage involved.

Codominance

Codominance refers to inheritance patterns when **both** alleles in a heterozygous organism are equally expressed.

Where co-dominance is involved different letters are used to represent the alleles.

Example1
Coat colour in horses and cattle.
Red coat colour is co-dominant to white coat colour.
Animals that are heterozygous are roan-coloured.
(The animals have red hair interspaced with white hair; they don't have pink hair!)

E.g. If a white bull is crossed with a red cow
Let W = White hair
R = Red hair

Phenotype: White hair x Red hair
Genotype: WW x RR
Alleles: W x R

Gametes	W	W
R	RW	RW
R	RW	RW

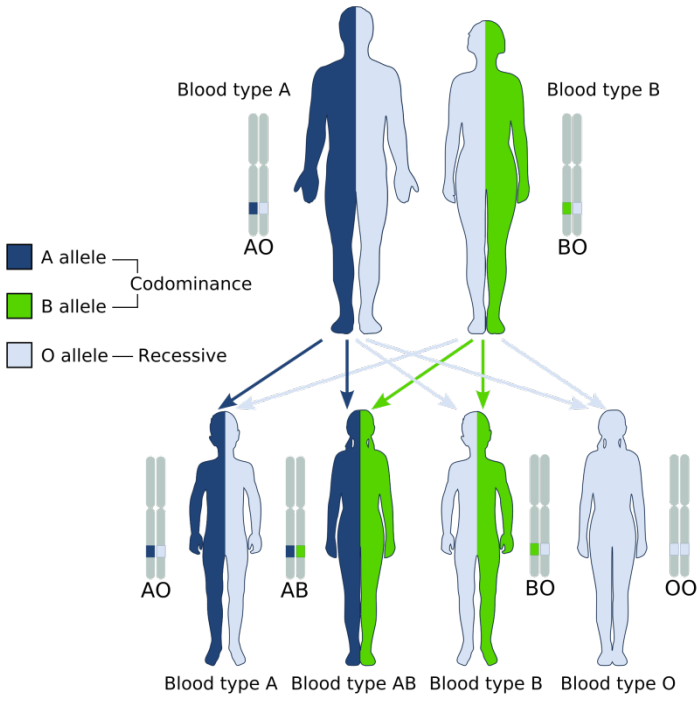
If a roan bull is crossed with a roan cow

Phenotype: Roan x Roan
Genotype: RW x RW
Alleles: R, W x R, W

Gametes	R	W
R	RR	RW
W	RW	WW

The ratio of phenotypes is 1 white : 2 roan : 1 red

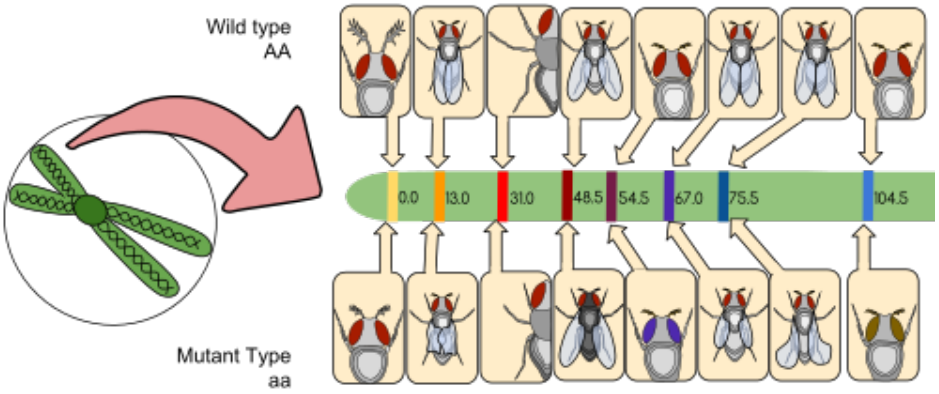
Example 2
Blood groups in humans
Another example of codominance is the human blood group AB, which is the result of two alleles A and B, both being equally expressed in the phenotype, neither being dominant to the other. However, blood group O is recessive to both blood group A and B.



Top tip
When alleles are codominant, the heterozygote always has a different phenotype from both homozygous parents

Linkage

If each chromosome only had one gene locus then Mendel's Principle of Independent assortment would hold true for all dihybrid crosses.
However, each chromosome carries many different genes and these are inherited together during meiosis. These genes are therefore linked.

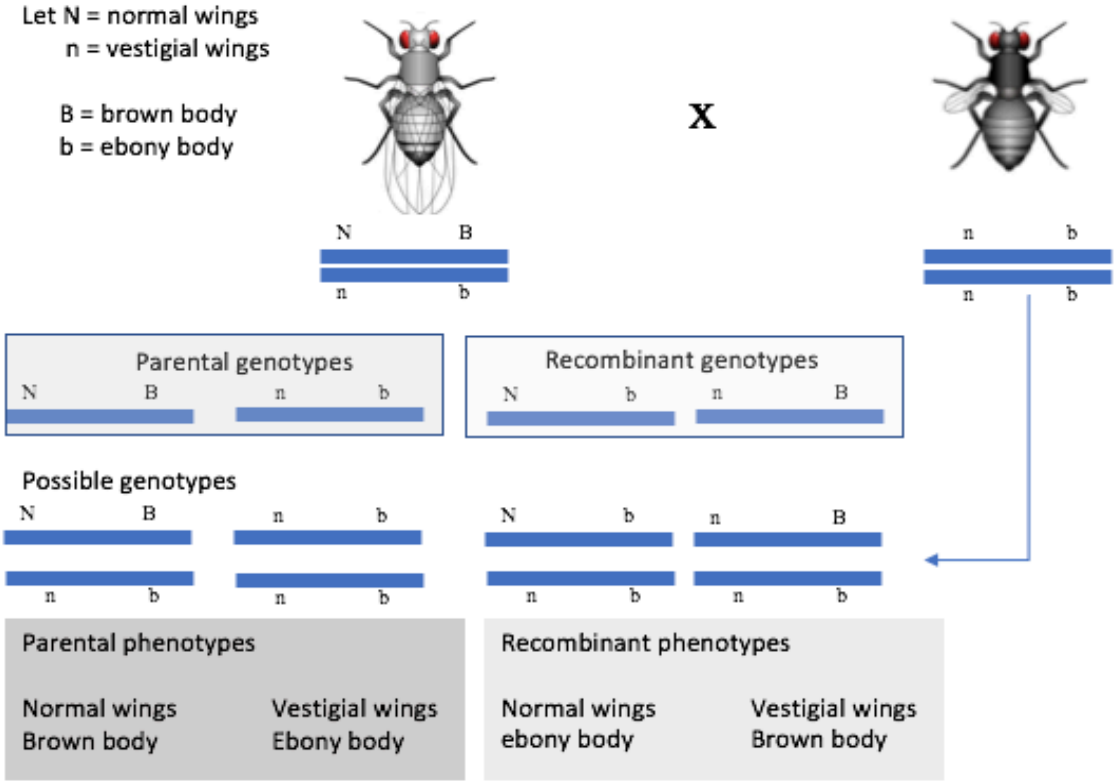


Diag. 1 – The relative positions of alleles on the second chromosome of a fruit fly (*Drosphilla*). These alleles will always be inherited together

https://en.wikipedia.org/wiki/Genetic_linkage#/media/File:Drosophila_Gene_Linkage_Map.svg

The Importance of Crossing Over

During meiosis when homologous pairs of chromosomes come together during prophase I of meiosis, **crossing over** may occur between chromatids at points called **chiasmata**.
If crossing over occurs between two genes, this separates alleles that were previously linked and allows them to combine in new recombinant genotypes.
This is important as a source of variation.



Diag. 2 - The gene for wing shape and body colour of a fruit fly are on the same chromosome; they are linked. Crossing over can provide recombinant genotypes that can lead to recombinant phenotypes.

The Chi-squared (χ^2) Test

The chi-squared test can be used to test the statistical significance of **discontinuous (discrete) variables**. For example, it can be used to determine if the results of a genetic cross are significantly different to expected results or whether the differences are due to chance alone.
It is how the statistical validity of results, such as those observed in genetic crosses, can be tested.

Example - Inheritance of seed shape in Mendel's peas

Below is a step by step method of how to use a chi-squared test.

In Pea plants, round seeds are dominant to wrinkled seeds.
Two parent pea plants both believed to be heterozygous for seed shape were crossed.
Out of 7324 seeds produced, 5474 were round and 1850 were wrinkled.
This is an observed ratio of 2.96 : 1 compared to the expected ratio of 3 : 1. Is this significant?

1. Formulate a null hypothesis

The null hypothesis always states that any deviation between observed and expected results is due to chance alone.
E.g. Inheritance of seed shape in peas is due to Mendelian inheritance and any deviation between the observed and expected result is due to chance.

2. Calculate the expected numbers from Mendelian ratios.

The monohybrid ratio for a cross between two heterozygotes is

3 dominant phenotype : 1 recessive phenotype
3 round : 1 wrinkled.

Out of 7324 peas the expected values would be

= (0.75 x 7324) round : (0.25 x 7324) wrinkled
= 5493 round : 1831 wrinkled

3. Calculate the degrees of freedom

The **degrees of freedom** are always the **number of categories (phenotypes) – 1**.
In this example there are two categories (phenotypes), round and wrinkled.
Degrees of freedom would therefore = 2 -1 = 1

4. Choose a suitable probability level
The probability level (P) is always 5%, therefore p = 0.05

Biologists consider that if the probability of any deviation between observed and expected values is equal to or greater than 5%, the deviation is said to be non-significant, i.e. the deviation is due to chance alone.
If the probability of any deviation between observed and expected values is less than 5%, the deviation is said to be significant. That is, some factor other than chance is influencing the results.

5. Calculate the chi-squared Value

The value for chi-squared is calculated using the formula:

$$\chi^2 = \sum \frac{(\text{observed value} - \text{expected value})^2}{\text{expected value}}$$

Normally, a table is used to help us calculate this value:

Phenotype	Observed (O)	Expected (E)	Difference (O-E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
Round seed	5474	5493	-19	$-19^2 = 361$	$361 \div 5493 = 0.07$
Wrinkled seed	1850	1831	19	$19^2 = 361$	$361 \div 1831 = 0.20$
				$\sum \frac{(O-E)^2}{E} =$	$0.07 + 0.20 = 0.27$

$\chi^2 = 0.27$

6. Find the critical value for χ^2

Knowing that,

- Degrees of freedom = 1
- P = 0.05

Using the probability table

Degrees of freedom	Probability								
	0.9	0.8	0.7	0.5	0.2	0.1	0.05	0.02	0.01
1	0.016	0.064	0.15	0.46	1.64	2.71	3.84	5.41	6.64
2	0.21	0.45	0.71	1.39	3.22	4.60	5.99	7.82	9.21
3	0.58	1.00	1.42	2.37	4.64	6.25	7.82	9.84	11.34
4	1.06	1.65	2.20	3.36	5.99	7.78	9.49	11.67	13.28

The critical value for chi-squared = 3.84

6. Formulate a conclusion

The conclusion should:

- **Compare calculated value for χ^2 and the critical value for χ^2.**
- **State the level of significance**.

This is always 0.05

- **Accept or reject the null hypothesis**

If the calculated value for χ^2 < the critical value for χ^2 then null hypothesis is accepted.
If the calculated value for χ^2 > the critical value for χ^2 then null hypothesis is rejected.

- **Say what it all means.**

If the null hypothesis is accepted then any deviation between the observed and expected result is due to chance alone.
If the null hypothesis is rejected then any deviation between the observed and expected result is due to some other factor than chance alone.

Exemplar Conclusion

The calculated value for χ^2 = 0.27. This is less than the critical value for χ^2 = 3.84 on a probability of p = 0.05 and one degree of freedom.
The null hypothesis is therefore accepted. Inheritance of pea shape is due to Mendelian inheritance and any deviation between the observed and expected results is due chance alone.

The Importance of Meiosis and Fertilisation in Sexual Reproduction

In the long term, if a species is to survive in a constantly changing environment and colonise new environments variation is essential.
Meiosis brings about **variation** in offspring produced by sexual reproduction.

There are three ways that Meiosis results in variety:

- Mixing the genotype of one parent with that of another increases variety of offspring. This is the basis of sexual reproduction in organisms. Two **haploid gametes** (sex cells) must fuse during **fertilisation**; as each contains half the genetic information (chromosomes) of the parent, their joining brings together chromosomes from two different sources resulting in a unique new combination of genetic information.

- **The random distribution and independent assortment of homologous chromosomes** on the equator of the spindle during **metaphase I** of meiosis. When the homologous chromosomes separate, the daughter cells contain different combinations of genetic information.

- **Crossing over during chiasmata** formation during **prophase I** of meiosis. Equivalent parts of homologous chromosomes may be exchanged thus producing new genetic combinations and the separation of linked genes.

The variety that meiosis and fertilisation brings about is essential to the process of evolution. By providing a variety of individuals, meiosis and fertilisation permits the natural selection of those best suited to the existing conditions and ensures that some will have characteristics that allow survival even if the environment changes.

Sex Linkage

Although certain genes on the sex chromosomes play a role in determining the gender of an individual, these chromosomes also contain genes for traits unrelated to femaleness or maleness. **A characteristic is said to be sex-linked if the gene which determines it are found on the sex chromosomes.**

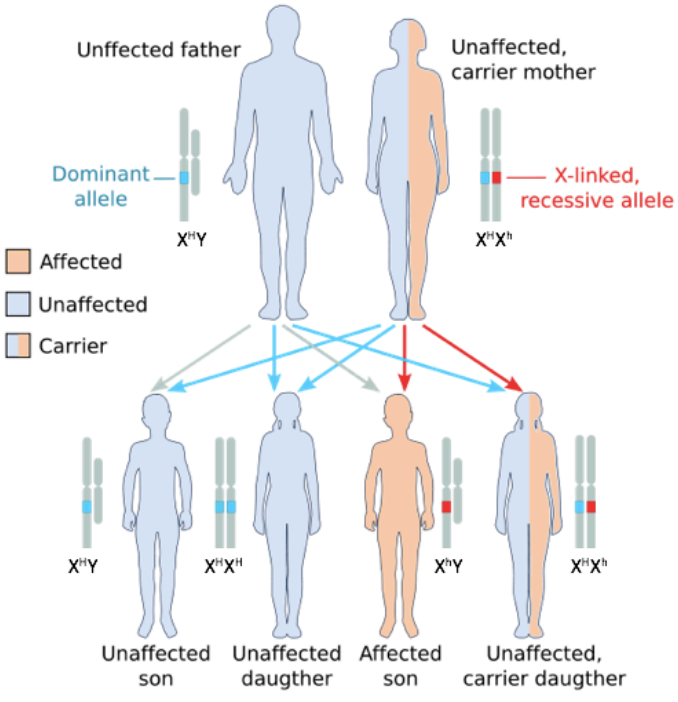
Since the human X chromosome is much larger than the Y there are more X-linked traits than Y-linked traits, and most of the X-linked genes have no homologous loci on the Y chromosome. Fathers pass X-linked traits to their daughters. Traits linked on the Y chromosome will only occur in males.
Mothers can pass X-linked traits to both sons and daughters. Traits linked on the X chromosome can arise in males and females.

To represent sex-linked alleles the same rules as all genetic crosses are followed, however the allele letter is attached to the X chromosome e.g. X^H or X^h.
The Y chromosome has no corresponding allele and therefore doesn't have an attached allele.

Example 1 - Haemophilia

Haemophilia is the inability of the blood to clot since blood-clotting factor 8 cannot be produced, leading to slow and persistent bleeding, especially in the joints. This is a potentially lethal inherited disease, and therefore the recessive allele that causes it is very rare in the population.

There are two alleles for the Factor 8 gene.
The allele H (dominant) codes for normal factor 8
The allele h (recessive) does not allow the production of factor 8.



Adapted from
https://commons.wikimedia.org/wiki/File:X_recessive_carrier_mother.svg

A genetic **trait** is a characteristic of an organism that is a result of gene expression and/or the environment, i.e. the phenotype.

Gametes	X^H	Y
X^H	X^HX^H	x^HY
X^h	X^HX^h	X^hY

Top tip
Do not confuse sex linkage and gene linkage.
They are both two different concepts.

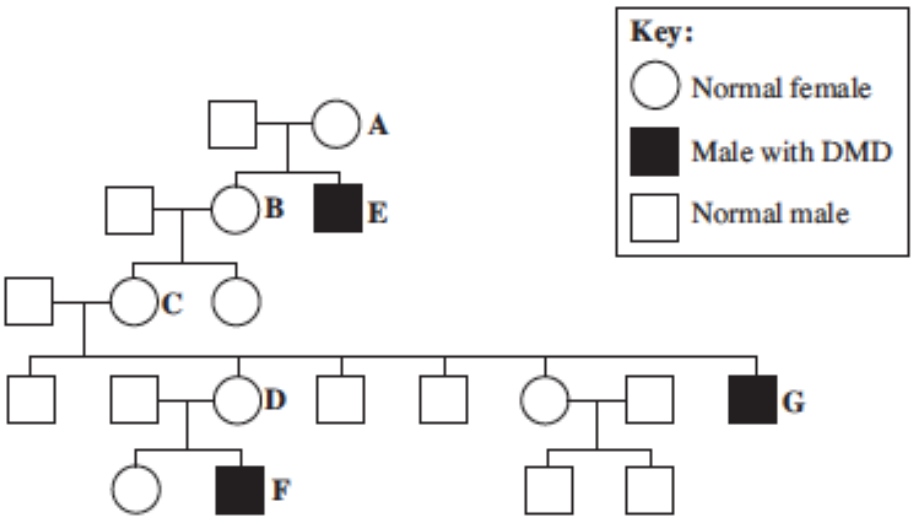
Example 2 - Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy (DMD) is caused by an sex linked recessive allele of a gene that codes for the protein dystrophin. Dystrophin is a component of a glycoprotein that stabilises the cell membranes of muscle fibres. Symptoms of DMD begin at around 2 - 3 years, and include loss of muscle mass and progressive muscle weakness.

Pedigree diagrams can be used to indicate whether inherited conditions are sex-linked or the result of dominant or recessive alleles.

Sex linked recessive alleles

- are carried on the X sex chromosome;
- are only expressed in females if both X chromosomes carry the allele (homozygous recessive);
- are always expressed in the male, since the Y chromosome does not have a homologous locus for the gene.



KEY:

X^N	Normal allele
X^n	Muscular dystrophy allele
Y	Male chromosome

Fig. 1 - Pedigree chart showing inheritance of DMD.

Females A, C and D must carry X^N because they are unaffected. But they also must have passed X^n on to males **E, F and G**. Their genotype must be X^NX^n.

Males E, F and G Males **E, F and G** could not have inherited the recessive allele from their father because the Y chromosome does not carry a gene for dystrophin (it does not have a homologous locus for the gene). They therefore must be X^nY because they are affected by DMD.

Gene Mutations

A mutation is a change in the amount or the arrangement of the genetic material (DNA or RNA) in a cell.

- Mutations are spontaneous random events and mutation rates are normally very low, but in organisms with short life cycles and more frequent cell division, the rate of mutation is higher.
- Mutations are the source of genetic variation which can result in evolution through natural selection.
- Most mutations occur during crossing over during prophase-I of meiosis or as a result of non-disjunction during anaphase-I or anaphase-II of meiosis.

The Effect of Mutagens, Carcinogens and Oncogenes

Mutations can affect protein synthesis and can change the phenotype of an organism, but some mutations have no effect on the phenotype.
Gene (point) mutations affect a single base in a gene and chromosomal mutations affect many genes.

The rate of mutation may be increased by mutagens, including:

- ionising radiations
  - gamma radiation,
  - UV
  - X-rays
- certain chemicals
  - polycyclic hydrocarbons in cigarette smoke

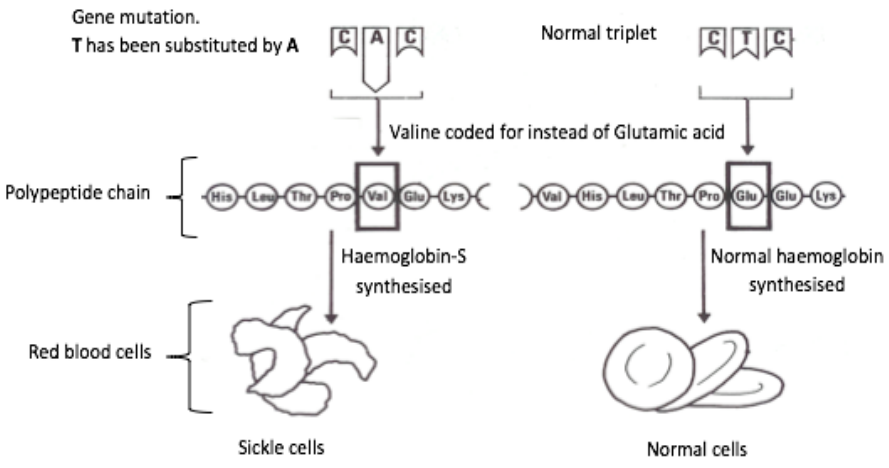
A mutagen which causes cancer is called a carcinogen.

Top tip
It's worth revisiting your year 12 notes on 'The Cell Cycle and Cell Division' (section 1.6). Many of the concepts from that section appear in the work on inheritance and evolution.

Some genes called proto-oncogenes can mutate to become oncogenes which are involved causing uncontrolled cell division to form a cancer.

Sickle cell anaemia - An example of a gene mutation

A change in the structure of DNA that occurs at a single locus is called a **gene mutation** or a **point mutation.**
Sickle cell anaemia is caused by a single mutation in the gene coding for haemoglobin**.**
he substitution of one base in the DNA molecule results in the wrong amino acid being incorporated into one of the polypeptide chains that make up the haemoglobin molecule. The mutant haemoglobin, Haemoglobin-S, causes the shape of the red blood cells to become sickle shaped. Haemoglobin-S is less efficient at carrying oxygen. The person suffers from anaemia.



Down's Syndrome - An example of a chromosome mutation

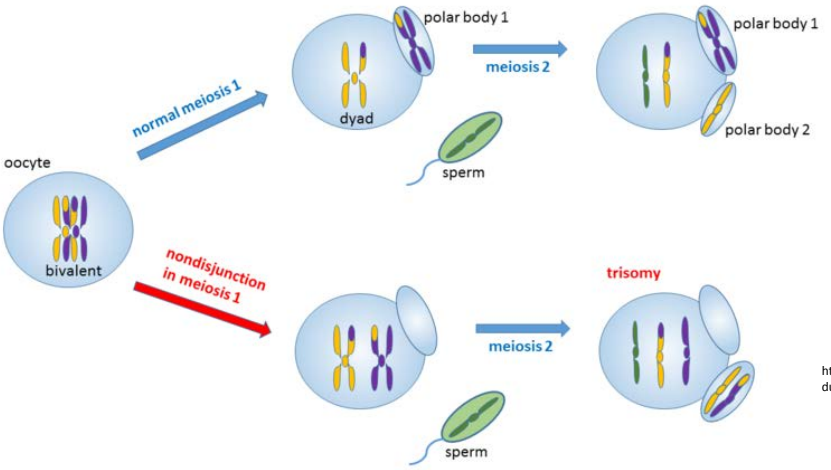
A **Chromosome mutation** is a change in the number of chromosomes in an organism

1. **Polyploidy**

   Some organisms have additional complete sets of chromosomes, e.g. three sets of chromosomes = triploid (3n). Modern wheat varieties show polyploidy (see section 4.4. - Sympatric Speciation).

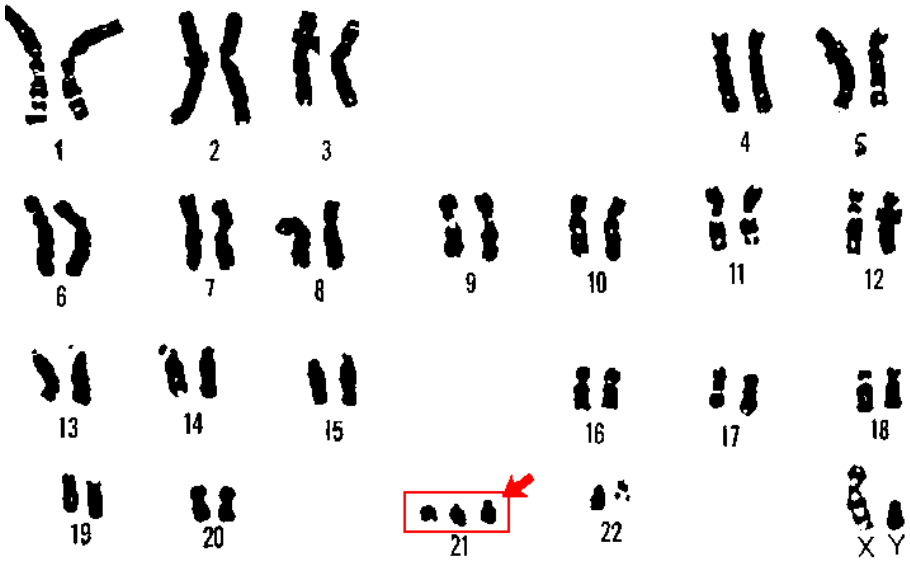
2. **Additional chromosomes**

   Sometimes during **anaphase I** of meiosis a pair of homologous chromosomes fail to separate. This is called **non-disjunction** and is often lethal.



https://commons.wikimedia.org/wiki/File:Trisomy_due_to_nondisjunction_in_maternal_meiosis_1.png

Down's syndrome is a frequent consequence of non-disjunction in humans. In this case, the 21st homologous chromosomes fail to segregate and a gamete can contain 24 chromosomes.
The fusion of the gamete with a normal one with 23 chromosomes results in the zygote having 47 chromosomes (2n + 1).



https://commons.wikimedia.org/wiki/File:21_trisomy_-_Down_syndrome.png

Non-disjunction can occur with other chromosomes but these normally result in the miscarriage of the foetus.
The 21st chromosome is relatively small carrying only around 200 to 300 genes out of an estimated total of 20 000 - 25 000 for the human genome, therefore, the offspring can survive.

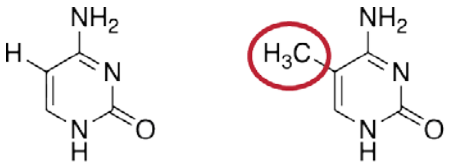
Epigenetics

Epigenetics refers to the control of gene expression by factors other than changes in the DNA sequence.
DNA can be modified post-replication; however, this does not change the DNA base sequence but changes the ability of a gene to be transcribed during protein synthesis.
There is a growing evidence that the environment can bring about these **epigenetic changes**.

Epigenetic modifications - DNA methylation

The addition of methyl groups to bases prevents those bases being recognised and reduces the ability of that gene to be expressed.

Gene expression is the process by which information from a gene is used in the synthesis of a functional product,
e.g. proteins or functional RNA.

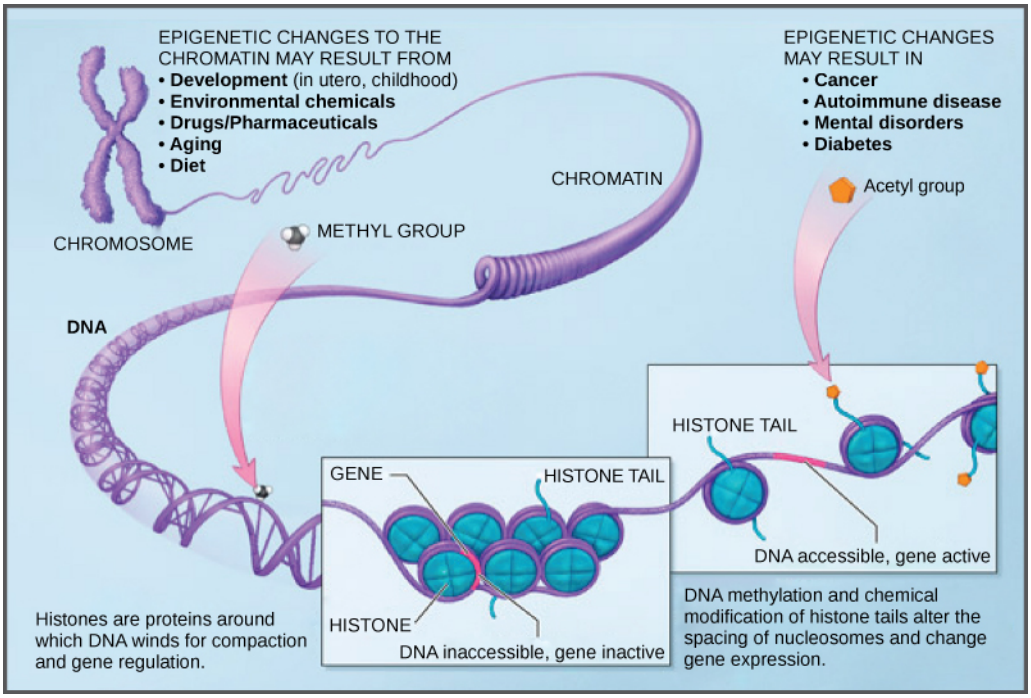


Cytosine methylated Cytosine

https://commons.wikimedia.org/wiki/File:DNA_methylation.png

Epigenetic modifications - Histone modification

The histone proteins used to organise the DNA in a chromosome can also be modified.
If the histone coils more tightly they can prevent gene expression, but if the histone coils loosely they can increase gene expression.



https://commons.wikimedia.org/wiki/File:Figure_16_03_03.jpg

Different epigenetic modifications can occur in cells of the same tissue and in different tissues resulting in different expression of the same gene in different parts of the same organism.
For example, the stem cells of the embryo progressively differentiate, switching off genes coding for enzymes that are not needed. Such changes mean that differentiated cells only express the genes that are necessary for their own activity, e.g. skin cells produce melanin but retinal cells produce rhodopsin.

Section 4.4 - Variation and Evolution

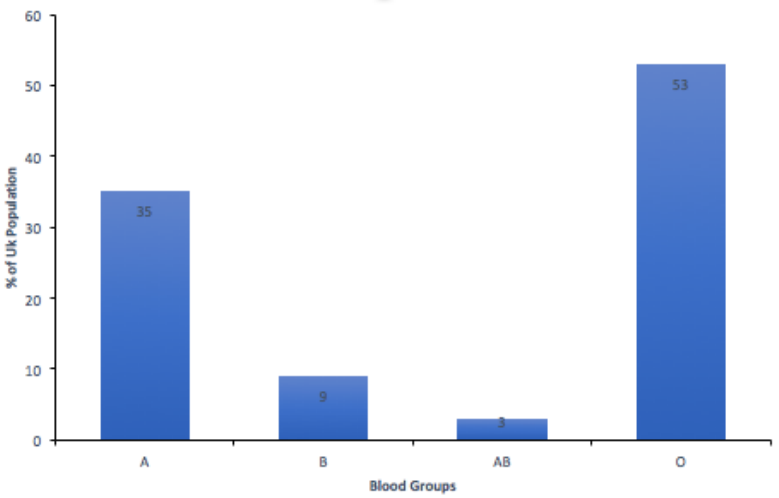
Genetic and Environmental Factors Produce Variation Between Individuals

Variation refers to the differences between organisms of the same species.
The total appearance of an organism is called its **phenotype.**
Phenotypic variation between people include:

- **Discontinuous** variation e.g. different blood groups.
- **Continuous** variation e.g. height and mass.

Discontinuous Variation

Discontinuous variation shows a limited number of phenotypes. There are no intermediate types.
It is normally controlled by one gene (monogenic).
The environment has no effect on the gene expression; the phenotype.
Phenotypes that are discontinuous are normally represented on bar charts or pie charts.

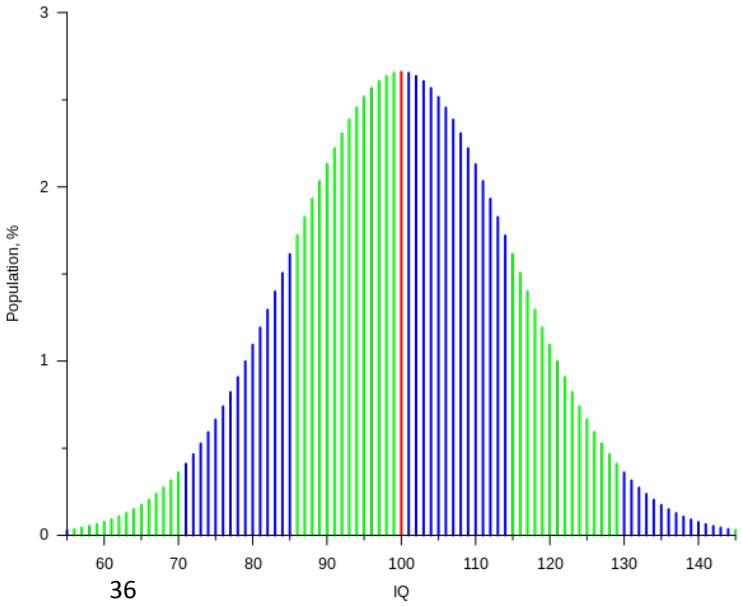


Heritable variation refers to the differences in phenotype due to genetic reasons.
This type of variation can be inherited.

Non-heritable variation refers to the differences in phenotype due to environmental reasons. This type of variation cannot be inherited.

Continuous Variation

With **continuous variation,** there is a gradation from one extreme to the other.
It is normally controlled by many genes (polygenic).
The phenotype is determined by the interaction of all the genes (polygenes) and the environment.
When a frequency distribution for such a phenotype is plotted a bell shaped curve is obtained.
This is called a **normal distribution curve.**



https://en.wikipedia.org/wiki/File:IQ_curve.svg

The Effect of Inter and Intra-Specific Competition on Breeding Success and Survival

Factors that affect populations of organisms.
Biotic factors affecting population size include:

- predation
- parasitism
- disease
- competition for resources.

Competition seems to be the major biotic factor affecting breeding success and survival and therefore determining the density and growth rate of populations.
The competition may be directly for resources such as sunlight, minerals and food, or it may be for nest sites or mates.

Intra-specific competition

Intra-specific competition is competition for resources between members of the same species.
In this competition, some of the population may not survive, or may not reproduce, so the growth of the population slows. Equally if the resource is plentiful with no competition then the population will increase.

Inter-specific competition

Inter-specific competition is competition for resources between members of different species.

Competition within a population may be very obvious, such as blackbirds fighting over territories in a garden. The winning competitor gains the resource. This is called **contest competition.**
In other cases, many individuals all compete for and gain some of the resource, e.g. lions squabbling over a carcase. This is known as **scramble competition.**
Many ecologists hypothesise that competition is the major factor in the maintenance of stable communities in natural equilibrium, especially for organisms higher up the food chain.

Top tip
Revisit your work on factors that control population size - section 3.5.

The Impact of Selective Agencies on the Survival of Organisms

Selection, in the context of evolution, is the process by which organisms that are better adapted to their environment survive and breed, while those less well adapted fail to do so.

New combinations of alleles produce unique genotypes, which when expressed in physical terms as phenotypes, undergo environmental selection that determines their suitability for the environment.

The better-adapted organisms are more likely to pass on their characteristics to succeeding generations.

Selective agencies are environmental factors that can alter the frequency of alleles in a population, when they are limiting.

Examples of selective agencies are:

- Supply of food
- Breeding sites
- Climate
- Human impact

Selection Pressure is the effect of the selective agencies acting on the population through Natural Selection.

Selective agencies increase the chances of some phenotypes and therefore some alleles being passed on to the next generation.

Selective agencies 'select for' a particular allele.

Remember, it is the phenotype that is suited, or not to a particular environment.
The phenotype is determined in part by the genotype.
If the phenotype provides an advantage, the alleles that produced it are transmitted to the next generation more successfully than other alleles.
The success of an allele depends on the extent to which they have contributed to a phenotype that is advantageous in a given environment.

If a phenotype gives a selective advantage, the alleles responsible for that phenotype will be **selected for** and it is more likely that they will be passed on to the next generation.

If a phenotype gives a selective disadvantage, the alleles responsible for that phenotype will be **selected against** and it is less likely that they will be passed on to the next generation.

The Concept of Gene Pool and Genetic Drift

A **gene pool** is a biological concept used to describe the large amount of genetic variation found in a population of organisms.
Each organism within that population contains just one of the many possible sets of genes that can be formed from the gene pool.
Population genetics is not concerned with the genotypes of individuals, but describes the proportions of the different alleles (the allele frequency) found in the whole gene pool.

Selection pressures can change the allele frequencies of the alleles present at a particular gene locus in a population and that allele frequency can be expressed either as a proportion or a percentage of the total number of copies of all alleles for that gene.

A gene pool will remain stable, i.e. the frequency of different alleles will not change, under the following conditions:

- the population is large,
- there is no selection pressure
- Mating is random.
- No mutations occur.
- All genotypes are equally fertile.
- No emigration or immigration (no gene flow)

A **gene pool** is the total of all the alleles for all the genes in a population.

Under these conditions the proportion of dominant and recessive alleles of a particular gene remains constant.
This would be a static gene pool and there would be no evolutionary change in the population.

In practice, the conditions above are rarely met and therefore the frequency of alleles within gene pools are constantly changing.

Genetic Drift

Sometimes, variations in allele frequencies in populations occur by chance.
This is known as **genetic drift.**
It may be an important evolutionary mechanism in small or isolated populations.
An example of genetic drift is when a few individuals become isolated from the rest of the species and start a new population, e.g. when a few individuals colonise an isolated island or some new habitat.

Genetic drift is a change in the frequency of an allele by chance.

These founder members of the new population are a small sample of the original gene pool from which they originated. By chance, the frequency of alleles will have changed.
While the founder population remains small it may undergo genetic drift and become even more different from the large parental population. This process is called the **founder effect**.

The Hardy-Weinberg Principle

The Hardy-Weinberg principle states that the frequency of alleles and genotypes within a population will remain constant from one generation to the next, if certain conditions remain true:

- The population is large;
- There is no selection for or against any phenotype;
- There is random mating throughout the population;
- There are no mutations;
- The population is isolated, i.e. there is no immigration or emigration.

The Hardy-Weinberg Equation

The Hardy-Weinberg equation allows us to estimate the frequencies of dominant or recessive alleles or of different genotypes within a population.

$$p^2 + 2pq + q^2 = 1$$

where,
p = frequency of the dominant allele (A)
q = frequency of the recessive allele (a)
$p + q = 1.0$

Top tip
In this equation, percentages are expressed as decimal numbers,
e.g. 20% = 0.2
100% = 1.0

The three terms of this equation indicate the frequencies of the three genotypes:

p^2 = frequency of AA (homozygous dominant)
$2pq$ = frequency of Aa (heterozygous)
q^2 = frequency of aa (homozygous recessive)

Under the conditions in which the Hardy-Weinberg principle operates, the frequencies of alleles p and q remain constant over generations.
The population is said to be in Hardy-Weinberg equilibrium.
The equation shows that a large proportion of recessive alleles exist in the heterozygotes.
Heterozygotes are therefore a reservoir of genetic variability.

Example 1 - Using the Hardy-Weinberg Equation to explain distribution of alleles and genotypes

If a recessive allele confers resistance to an insecticide for a particular insecticide, what would the frequency of alleles and distribution of genotypes be if 36% of the insect population were resistant to the insecticide?

If Insecticide resistance = q^2 = 36% = 0.36

Then q = $\sqrt{0.36}$

= 0.6

From, p + q = 1.0

p = 1.0 - q

p = 1.0 - 0.6

p = 0.4

Remember,
q^2 represents the percentage of homozygous recessive individuals.

Therefore, the distribution of alleles in the population is as follows:

Homozygous dominant = $p^2 = 0.4^2 = 0.16 = 16\%$

Heterozygotes = 2pq = 2 x 0.4 x 0.6 = 0.48 = 48%

Homozygous recessive = $q^2 = 0.6^2 = 0.36 = 36\%$

Example 2 - Using the Hardy-Weinberg Equation to calculate the frequency of an allele in a population

The absence of the skin pigment melanin is a condition called albinism. The condition is caused by a recessive allele. In a large population only one in 10 000 was albino. Calculate the proportion of the population who carry the allele for albinism.

If albinism is a homozygous recessive genotype, then:

q^2 = 1 in 10 000 = 1 ÷10 000 = 0.0001

Then q = $\sqrt{0.0001}$

= 0.01

From, p + q = 1.0

p = 1.0 – q

p = 1.0 - 0.01

p = 0.99

Remember,
a recessive allele can remain hidden in the heterozygous genotype and is therefore only selected against when it is in a homozygous recessive genotype.
This is how conditions caused by recessive alleles remain in a population even though they are selected against in the homozygote.

Therefore, the distribution of alleles in the population is as follows:

Homozygous dominant = $p^2 = 0.99^2 = 0.9801 = 98.01\%$

Heterozygotes = 2pq = 2 x 0.99 x 0.01 = 0.0198 = 1.98%

Homozygous recessive = $q^2 = 0.01^2 = 0.0001 = 0.01\%$

Proportion of population carrying the gene for albinism = 1.98% + 0.01% = 1.99%

Evolution through Natural Selection

Charles Darwin and Alfred Russel Wallace's theory of evolution states that existing species have arisen through modification of ancestral species by natural selection.

The theory is based on the following observations:

- In any population, there is **variation**.
- Individuals within a population have the potential to produce large numbers of offspring, yet the number of adults tends to stay the same from one generation to the next.

From these observations, two deductions can be made:

- There is a struggle for survival (**competition**) with only the 'fittest' phenotypes; surviving.
- The individuals that survive and reach reproductive age **reproduce** and pass on to their offspring alleles for the phenotypes that enable them to succeed (that is, a **selective advantage**), thus changing the allele frequencies.

In time, a group of individuals that once belonged to the same species may give rise to two different groups that are sufficiently distinct to belong to two separate species.

If the environment or conditions change, then the features needed to survive in it will change, so natural selection is a continuous process.

Isolation and Speciation

The process by which new species are formed is called **Speciation.**

Evolution, in terms of speciation, will **not** take place if the conditions under which the Hardy-Weinberg principle apply.

A species is a group of organisms that can interbreed to form fertile offspring.

Speciation can occur due to:

- genetic drift in isolated populations
- the founder effect of disproportionate allele frequencies in small populations
- natural selection

However, for new species to develop from a population, *some* form of **isolating mechanism** is essential.

The two forms of speciation and their isolating mechanisms are summarised in the following table:

Speciation	**Isolating mechanism**
Allopatric speciation	Geographical isolation
Sympatric speciation	Reproductive isolation

Allopatric Speciation

Allopatric speciation occurs as the result of two populations becoming **geographically isolated**. Any physical barrier that prevents two groups from meeting must also prevent them from interbreeding.
Such barriers include mountain ranges, deserts and oceans. Even a small stream can separate two groups of woodlice.

The isolating mechanism in allopatric speciation is **geographical isolation**.

A **deme** is a group of individuals within a population who breed with one another. It is possible for individuals from different demes to interbreed.

Gene pool Species X	Geographical barrier	Genetic drift	Two new species formed.
Species X inhabits a mountain range. Individuals form a single gene pool and freely interbreed.	The climate gets warmer. Species X moves higher up the mountains. Two separate **demes** of species X are geographically isolated. Distance between both populations prevent them from interbreeding. There is no gene flow between both gene pools.	Over time, genetic drift occurs in both gene pools. The frequency of alleles changes as both **demes** are subjected to different selection pressures.	The climate cools and both demes are no longer geographically isolated. If sufficient time has passed both gene pools may have changed. If both demes are no longer able to interbreed and produce fertile offspring then they are now considered two separate species, each with its own gene pool.

Adapted from https://commons.wikimedia.org/wiki/File:Topography-driven_Isolation_PEAK%26VALLEY_(allopatric_speciation).png

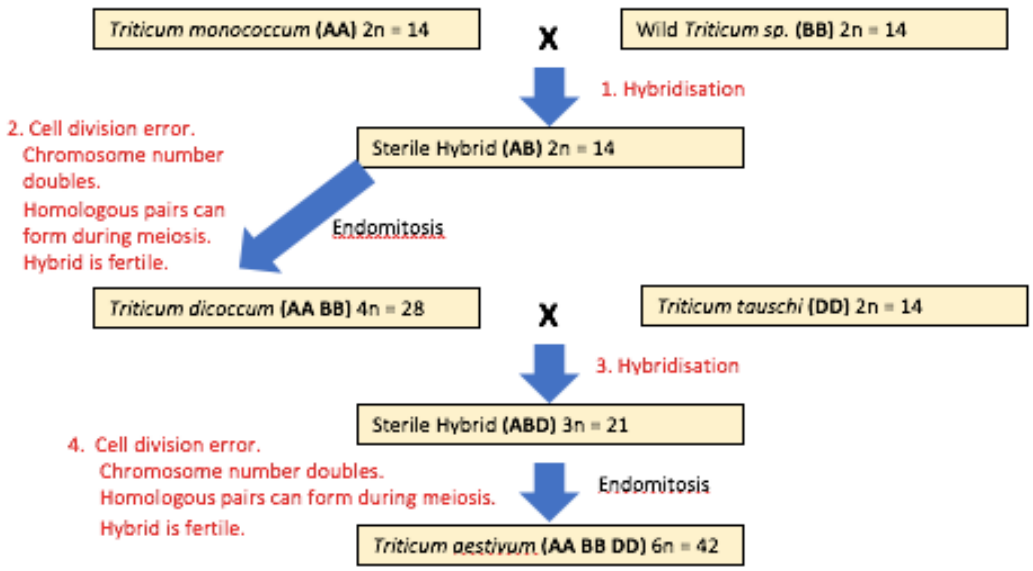
Sympatric Speciation

Sympatric speciation occurs when organisms inhabiting the same area become reproductively isolated into two groups for reasons other than geographical barriers.
The isolating mechanism in sympatric speciation is **reproductive isolation**.

The reasons for reproductive isolation include the following mechanisms:

- **Behavioural isolation** - in animals with elaborate courtship behaviour the steps in the display of one organism fails to attract the necessary response in a potential partner of another species.
- **Mechanical isolation** - the genitalia of the two groups may be incompatible.
- **Gametic isolation** - in flowering plants pollination may be prevented because the pollen grain fails to germinate on the stigma whereas in animals, sperm may fail to survive in the oviduct of the female.
- **Hybrid inviability** - despite fertilization taking place development of the *embryo* may not occur.
- **Seasonal isolation** – If the breeding season of two groups (demes) does not coincide, they cannot interbreed.
- **Hybrid sterility** - when individuals of different species breed, the set of chromosomes from each parent is different. These sets are unable to pair up during meiosis and so the offspring are unable to produce gametes.
  For example, a mule is a cross between a donkey and a horse. The mule is sterile.
  A horse has 64 chromosomes, with 32 in the gametes and a donkey has 62 chromosomes, with 31 in the gametes.
  A mule therefore has 63 chromosomes, and is therefore unable to form homologous pairs during prophase 1 of meiosis.

  Polyploidy is common in plants and sometimes sterile hybrids can double their chromosome numbers therefore become fertile. modern wheat (*Tricitum aestivum*) is a good example of this.



Investigation of Continuous Variation in a Species Using Student's t-test

If data shows **continuous (polygenic) variation** then it should be normally distributed.
Two sets of data showing normal distribution may be compared using Student's t test.

What does the *t*-test tell us?

It can help us decide whether differences between the mean of two groups of data is due to chance or some other factor by measuring the overlap between two sets of data.
Student's t-test uses a formula to calculate a value, 't'.

$$t = \frac{\left|\bar{x}^1 - \bar{x}^2\right|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

where,

$\left|\bar{x}^1 - \bar{x}^2\right|$ = the difference in mean values of sample 1 and sample 2

s_1^2 and s_2^2 are the squares of the standard deviation of the samples

n_1 and n_2 are the number of readings in each sample.

Top tip
this value should always be positive because it is the difference between two values.

If two sets of data have widely separated means and small variances (i.e. tightly clustered data), they will have little overlap and a big value of *t*.
If the two sets of data have means that are close together and large variances (i.e. widely spread data), they will have a lot of overlap and a small value *t*.

Example - Using the t-test to analyse variation in width of ivy leaves growing on a wall.

It was hypothesised that ivy leaves differ in size according to light levels. A sample of leaves were collected from a wall and the width of 15 leaves growing on the north side and 15 leaves growing on the south side were measured.

Maximum width of ivy leaf (mm)	
North facing	**South facing**
17	7
16	11
18	8
21	8
19	9
20	10
17	9
19	10
18	9
17	10
18	10
18	11
16	12
15	18
18	10

1. Formulate a null hypothesis

The null hypothesis always states that there is no significant difference between the means.
E.g. There is no significant difference between the mean maximum width of the ivy leaves on the north facing side of the wall and south facing side of the wall.

2. Collect the data

3. Calculate the mean

4. Calculate the standard deviation for both samples

Standard deviation measures the spread of data about the mean. The smaller the standard deviation the more reliable the mean.

The following formula is used to calculate the standard deviation:

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

A table will be provided to scaffold your calculations:

North facing	Maximum leaf width (mm)	Deviation from the mean $(x - \bar{x})$	Deviation squared $(x - \bar{x})^2$
1	17	17 - 17.8 = -0.8	$-0.8^2 = 0.64$
2	16	16 - 17.8 = -1.8	$-1.8^2 = 3.24$
3	18	18 - 17.8 = 0.2	$0.2^2 = 0.04$
4	21	21 - 17.8 = 3.2	$3.2^2 = 10.24$
5	19	19- 17.8 = 1.2	$1.2^2 = 1.44$
6	20	20- 17.8 = 2.2	$2.2^2 = 4.84$
7	17	17- 17.8 = 0.8	$-0.8^2 = 0.64$
8	19	19- 17.8 = 1.2	$1.2^2 = 1.44$
9	18	18 - 17.8 = 0.2	$0.2^2 = 0.04$
10	17	17 - 17.8 = -0.8	$-0.8^2 = 0.64$
11	18	18 - 17.8 = 0.2	$0.2^2 = 0.04$
12	18	18 - 17.8 = 0.2	$0.2^2 = 0.04$
13	16	16 - 17.8 = -1.8	$-1.8^2 = 3.24$
14	15	15 - 17.8 = -2.8	$-2.8^2 = 7.84$
15	18	18 - 17.8 = 0.2	$0.2^2 = 0.04$
	Mean = 17.8		Σ = 34.4
South facing			
1	7	7 - 10.1 = -3.1	$-3.1^2 = 9.61$
2	11	11 - 10.1 = 0.9	$0.9^2 = 0.81$
3	8	8 - 10.1 = -2.1	$-2.1^2 = 4.41$
4	8	8 - 10.1 = -2.1	$-2.1^2 = 4.41$
5	9	9 - 10.1 = -1.1	$-1.1^2 = 1.21$
6	10	10 - 10.1 = -0.1	$-0.1^2 = 0.01$
7	9	9 - 10.1 = -1.1	$-1.1^2 = 1.21$
8	10	10 - 10.1 = -0.1	$-0.1^2 = 0.01$
9	9	9 - 10.1 = -1.1	$-1.1^2 = 1.21$
10	10	10 - 10.1 = -0.1	$-0.1^2 = 0.01$
11	10	10 - 10.1 = -0.1	$-0.1^2 = 0.01$
12	11	11 - 10.1 = 0.9	$0.9^2 = 0.81$
13	12	12 - 10.1 = 1.9	$1.9^2 = 3.61$
14	18	18 - 10.1 = 7.9	$7.9^2 = 62.41$
15	10	10 - 10.1 = -0.1	$-0.1^2 = 0.01$
	Mean = 10.1		Σ = 89.75

Using the formula:

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

Standard deviation for North side (sample 1)

$$s_1 = \sqrt{\frac{34.4}{15-1}} = 1.57$$

Top tip
By calculating formulae step by step, you are less likely to make a mistake.

Standard deviation for South side (sample 2)

$$s_2 = \sqrt{\frac{89.75}{15-1}} = 2.53$$

5. Calculate the value for t

Using the following formula:

$$t = \frac{\left|\bar{x}^1 - \bar{x}^2\right|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

where,

$\left|\bar{x}^1 - \bar{x}^2\right|$ = the difference in mean values of sample 1 and sample 2

s_1^2 and s_2^2 are the squares of the standard deviation of the samples

n_1 and n_2 are the number of readings in each sample.

$$t = \frac{17.8 - 10.1}{\sqrt{\frac{1.57^2}{15} + \frac{2.53^2}{15}}} = \frac{17.8 - 10.1}{\sqrt{\frac{2.46}{15} + \frac{6.40}{15}}} = \frac{7.7}{\sqrt{0.164 + 0.427}} = \frac{7.7}{\sqrt{0.591}} = \frac{7.7}{0.769}$$

t = 10.013

6. Calculate the number of degrees of freedom

Degrees of freedom = $(n_1 + n_2) - 2$

For this example = (15 + 15) - 2 = 30 -2
= 28

n_1 = number in sample 1
n_2 = number in sample 2

7. Choose a suitable probability level

The probability level (P) is always 5%., therefore p = 0.05

Biologists consider that if the probability of any difference between both means is greater than 5%, the deviation is said to be non-significant, i.e. the difference is due to chance alone.
If the probability of any difference between both means is less than 5%, the difference is said to be significant. That is, some factor other than chance is influencing the results.

8. Find the critical value for t

Knowing that,

- Degrees of freedom = 28
- P = 0.05

Using the probability table

Degrees of freedom	p = 0.1	p = 0.05	p = 0.02	p = 0.01	p = 0.002	p = 0.001
25	1.708	2.060	2.485	2.787	3.450	3.725
26	1.706	2.056	2.479	2.779	3.435	3.707
27	1.703	2.052	2.473	2.771	3.421	3.690
28	1.701	2.048	2.467	2.763	3.408	3.674
29	1.699	2.045	2.462	2.756	3.396	3.659

The critical value for t = 2.048

9. Formulate a conclusion

The conclusion should:

- **Compare calculated value for t and the critical value for t.**
- **State the level of significance**.

This is always p = 0.05

- **Accept or reject the null hypothesis**

If the calculated value for **t** < the critical value for **t** then null hypothesis is accepted.
If the calculated value for **t** > the critical value for **t** then null hypothesis is rejected.

- **Say what it all means.**

If the null hypothesis is accepted then any difference between the means of both samples is due to chance alone.
If the null hypothesis is rejected then any difference between the means of both samples is due to some factor other than chance alone.

Exemplar Conclusion

The calculated value for **t** = 10.013 is greater than the critical value for **t** = 2.048 on a probability of p = 0.05 and 28 degrees of freedom.
The null hypothesis is therefore rejected.
The difference between the mean maximum leaf width of both samples is due to some factor other than chance alone.

4.5 Application of Reproduction and Genetics

The Human Genome Project

The intended purpose of the Human Genome and 100K Projects is to improve knowledge and understanding of genetic disorders and improve their diagnosis and treatment.
The Human Genome Project used 'Sanger Sequencing' which sequences relatively small sections of DNA at a time (usually <1,000 bps). This process took a long time.

The Main Aims of the Human genome Project

- Identify all the genes in the human genome and identify which chromosome each is on.
- Determine the base sequences of the 3 billion base pairs in human DNA and store the information in databases.

The 100k Genome Project

New techniques e.g. Next Generation Sequencers (NGS) can sequence an entire genome in just a few hours.
NGS is enabling scientists to study variation within the human genome amongst 100 000 people in the U.K. This is known as the 100K genome project.

The Main Aims of the 100k Genome Project

- study variation within the human genome
- create a new genomic medicine service for the NHS
- enable new medical research to study the potential of new and more effective treatments
- study how best to use genomics in healthcare and how best to interpret the data to help patients
- kick-start a UK genomics industry.

Top tip
You are not required to know the details of how Sanger Sequencing or Next Generation Sequencing work.
You only need to appreciate that as technologies develop, DNA sequencing has become faster and cheaper thus making it a more accessible tool.

Genetics is a study of the functions of single genes.

Genomics can be defined as a study of the complete genetic material of an organism; their genome.

Ethical issues Surrounding Use of Knowledge from Genome Projects

A vast quantity of data has been produced by the Human Genome Project and the 100K Genome Project and its potential is profound. We do not know how this information might be used in future. Society is struggling to decide where the legal and moral responsibility for this information lies.

Ethical issues cover many areas, such as:

- ownership of genetic information, potential discrimination, social stigmatisation and misuse of the data.
- identification of allele sequences enabling scientists to scan a patient's DNA sample for mutated sequences and also to compare the sequence of DNA bases in a patient's gene to a normal version of the gene.
- screening of embryos to detect the presence of disorders such as cystic fibrosis, Huntington's disease and thalassaemia.
- concerns regarding the possibility of routine screening for adult onset disorders such as Alzheimer's disease and some cancers.
- screening of embryos has led to concerns over choosing alleles to ensure specific characteristics.
- concerns that the risks of discrimination and social stigmatisation could outweigh the benefits of testing.
- use of genetic screening and the value of genetic counselling.
- concerns regarding the storage of genetic information and its misuse.

Genome sequencing of other organisms

Genome projects have also been completed for a number of other species including chimpanzees and other primates.
This has allowed scientists to:

- look at evolutionary relationships. This provides true phylogenetic classification and can be used to correct mistakes made using classification based on phenotypic characteristics.
- consider how to conserve species in the future by targeting which species need particular protection.

Case Study – Malaria

Malaria is transmitted by the mosquito *Anopheles gambiae*. Rapid evolution of insecticide resistance in the species is hampering attempts to eradicate the disease which is responsible for over a million deaths per year. The malarial parasite, *Plasmodium sp*. has also developed multi-drug resistance.

- Sequencing of the *Anopheles gambiae* genome is allowing scientists to develop chemicals, which could render the mosquito susceptible again to insecticides.
- Sequencing of the *Plasmodium sp*. genome is allowing for the development of more effective drugs.

Top tip
Revisit your year 12 work 'Classification and Biodiversity' - section 2.1

DNA Profiling (Genetic Fingerprinting)

About 99.9% of the human genome is the same in every person. It is the remaining 0.1% that makes an individual's genetic profile unique.
A DNA profile (genetic fingerprint) represents only non-coding portions of DNA.
It is not the same as a DNA sequence which represents all the sequence of bases in a genome.

An individual's DNA profile is different from that of other individuals.
The human genome contains regions called exons (less than 2%). These are regions of DNA that code for proteins. Between exons are regions of non-coding DNA called introns which contain blocks of repeated nucleotides. It is the number of times that these blocks called Short Tandem Repeats (STRs) are repeated that produces the variation in individuals. A number of STRs are used to build up a unique fingerprint in the UK.

D7S280 is an example of an STR where 'GATA' bases repeat on human chromosome 7. Different alleles of this locus have from 6 to 15 tandem repeats of this sequence. The more times it repeats, the larger the of DNA will be.

Producing a DNA profile relies on two techniques:

1. The polymerase chain reaction (PCR)
2. Gel electrophoresis.

The Polymerase Chain Reaction (PCR)

To carry out numerous laboratory tests, large samples of DNA are required.
PCR rapidly produces many billions of molecules from a single DNA molecule. This enables tests to be carried out accurately and more rapidly regardless of the age of the sample.

PCR can be described as **semi-conservative replication** in a test tube. The sample of DNA is dissolved in a buffer and mixed with the enzyme **DNA polymerase**, **nucleotides** and short pieces of DNA called **primers**. The primers are single stranded DNA typically 6-25 base pairs in length which is complimentary to the start of the sequence. (They act as signals to the DNA polymerase to start copying).

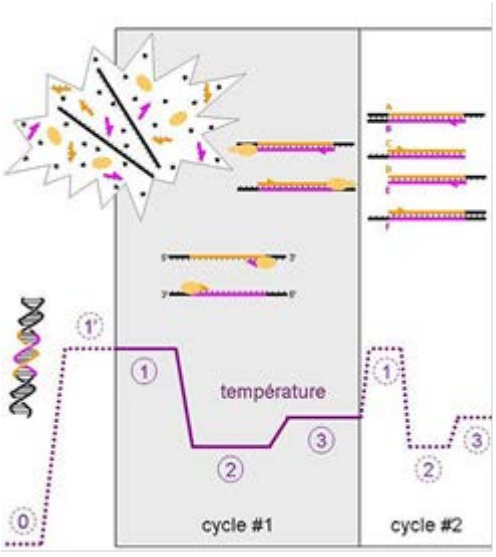
Method

1. The original DNA (target DNA) is denatured by heating to 95^{O}C and it separates into two single strands.

2. The solution is cooled to 50-60^{O}C triggering the primers to join the complimentary base sequences on each of the single strands of DNA. This in turn allows the primers to bind to the DNA strands (annealing), triggering DNA replication.

3. The solution is heated to 70^{O}C and the thermally stable DNA polymerase (which is not denatured at this temperature) catalyses the synthesis of a complementary strand for each of the single strands of DNA by forming the phosphodiester bonds in the sugar-phosphate backbone. This produces two identical double strands of DNA.

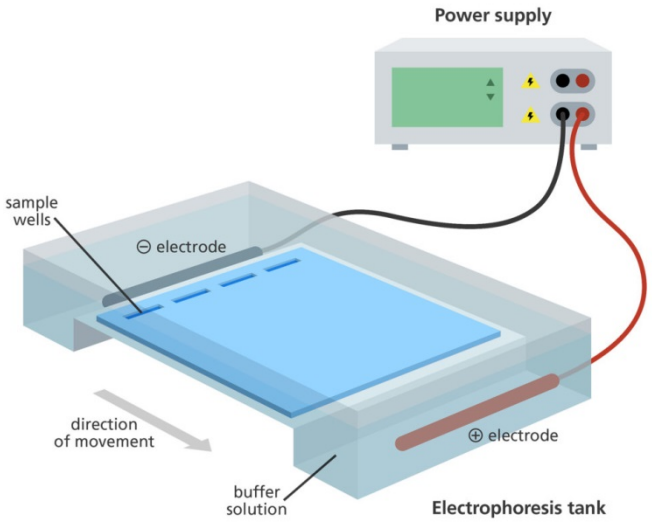
4. Steps 1-3 are repeated many times, doubling the quantity of DNA produced each time. After 40 cycles over a billion copies of the target sequence can be produced from just one piece of DNA.



https://commons.wikimedia.org/wiki/File:PCR_basic_principle1.jpg

Gel electrophoresis

Gel electrophoresis is a method of separating DNA fragments according to size. The gel is made from agarose (similar to agar), which contains pores in its matrix



https://www.flickr.com/photos/yourgenome/26344970413

Method

1. The DNA is extracted from the sample and cut into small fragments using **restriction endonucleases**.

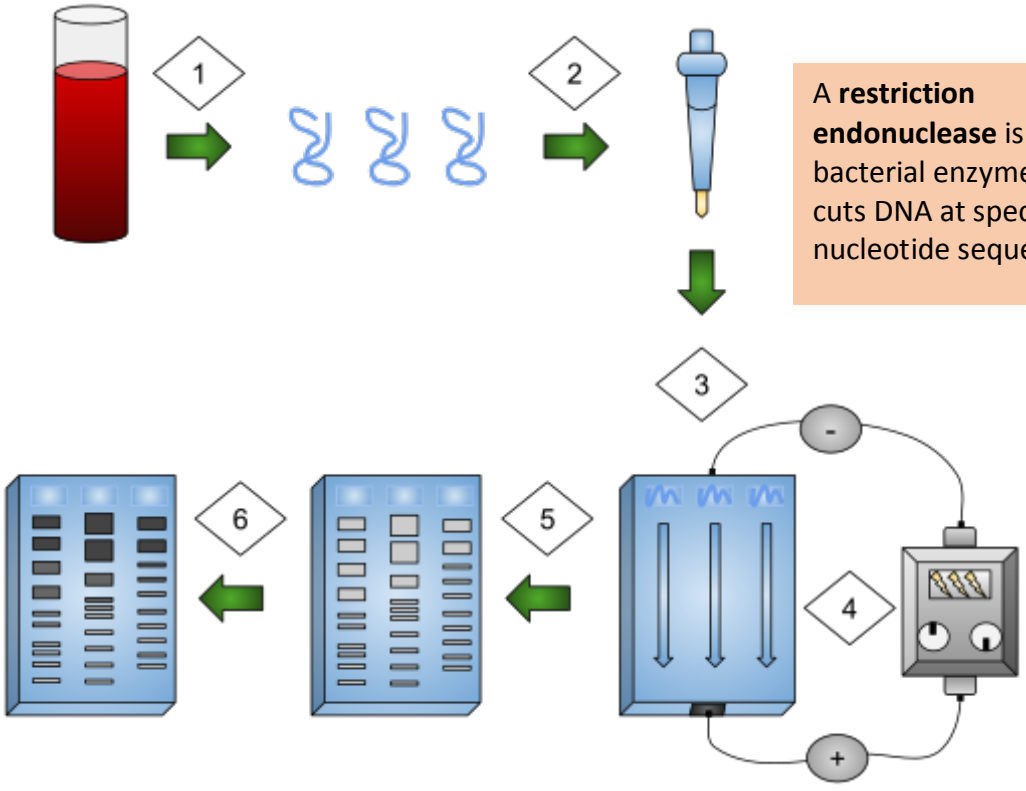
2. Fragments of DNA are loaded into wells at one end of a trough containing gel.

3. The gel is exposed to an electric current.

4. Since the fragments are negatively charged they move towards the positive terminal.

5. Smaller fragments find it easier to migrate through the pores in the gel and so travel further than large fragments in the same time.

6. The DNA becomes separated into bands according to the size of the fragments.



https://en.wikipedia.org/wiki/Gel_electrophoresis#/media/File:Gel_Electrophoresis_in_DNA_Fingerprinting.svg

A **restriction endonuclease** is a bacterial enzyme that cuts DNA at specific nucleotide sequences.

7. Fragment size can be estimated by running a DNA ladder (which contains fragments of known size) alongside.

Genetic Engineering

This is the **transfer of a gene from one organism into another**, so that the gene is expressed in its new host cell.
The new host cell is described as **transgenic.**

This method can be used to introduce genes from another species into a cell, e.g. human insulin gene introduced into a bacterial cell or a bacterial gene introduced into a plant.

The basic steps in genetic engineering are as follows:

1. Identify and obtain the gene.
2. Insertion of the gene into a vector, producing recombinant DNA.
3. Insertion of the vector into the host cell and identification of transgenic organism.
4. Production of protein by the host cell / separation and purification of the protein.

1. Identify and obtain the gene

A gene can be identified using a gene probe. This is a specific segment of single-strand DNA that is complementary to a section of the gene.

The identified and located gene can be isolated with either of two enzymes:

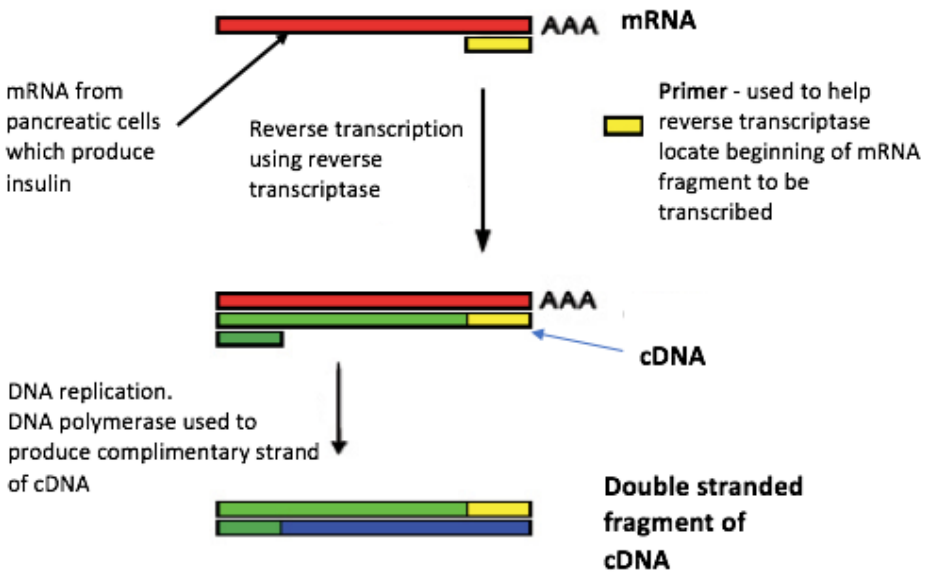
a) Reverse transcriptase
b) Restriction endonuclease

a) Using reverse transcriptase (and DNA polymerase)

Cells that produce a specific polypeptide will contain many copies of the functional mRNA transcribed from the target gene.

The mRNA can be isolated and complimentary single strands of copy DNA (cDNA) can be produced from the mRNA template using the enzyme **reverse transcriptase.**

DNA polymerase can then be used to make a double stranded DNA molecule. This will be an exact copy of the gene.



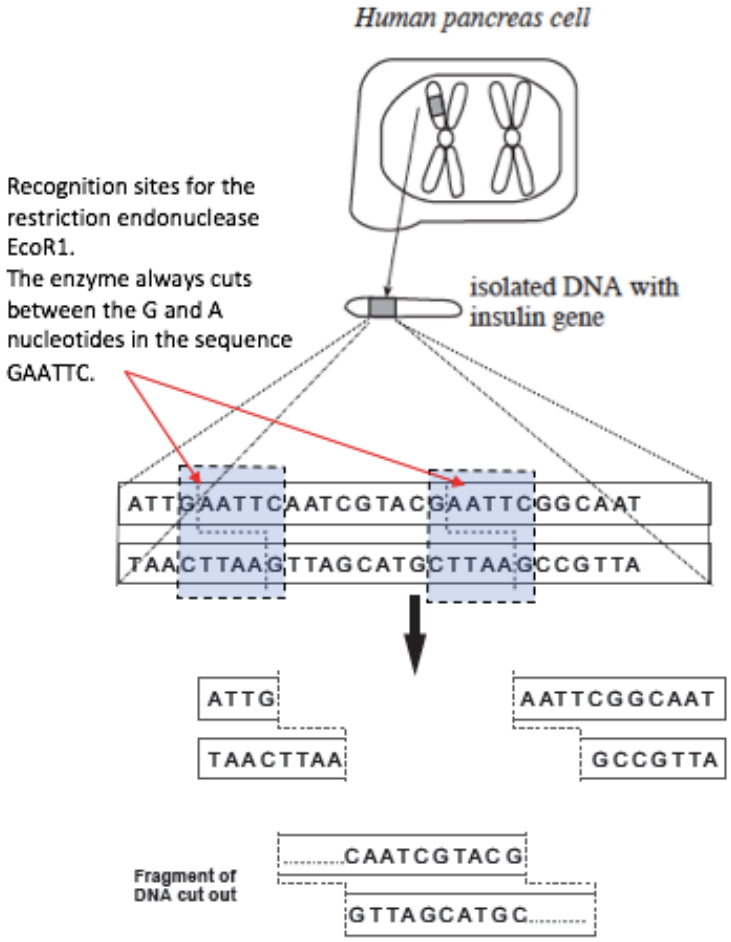
'Sticky ends' (unpaired bases) will need to be added at either end of the DNA strand.

Advantages of using reverse transcriptase

- This method avoids the need to locate the gene.
- The DNA produced does not include introns because the cDNA is copied from functional mRNA. (The pre-mRNA in the nucleus that has been transcribed from the DNA has been modified (post-transcriptional processing) to produce mRNA that does not contain introns).
- The DNA produced does not contain any non-functional fragments.

b) Using restriction endonuclease

Restriction endonucleases are bacterial enzymes that cut DNA at specific nucleotide sequences. The enzyme will cut the DNA into many small fragments and individual genes can be isolated.
Some restriction endonucleases cut straight across a DNA double strand, making a blunt cut. But many make a staggered cut, which leaves unpaired bases on both strands. These bases pair with complimentary sequences readily, so they are called **sticky ends**.



Disadvantages of using restriction endonuclease

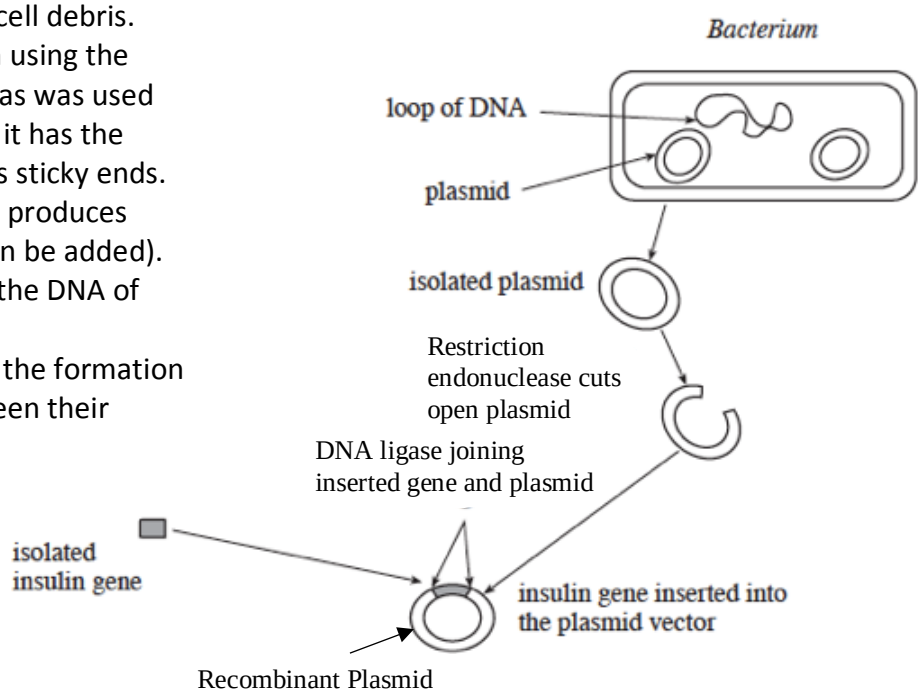
- If the recognition site occurs within the gene of interest, the gene will be broken into fragments that have no function.
- Eukaryotic genes contain introns, prokaryotic genes do not. If a eukaryotic gene was transferred into a bacterium it would not have the appropriate enzymes to process the pre-mRNA. The introns would not be removed after transcription and any proteins translated would therefore contain extra amino acids coded from the intron sequences. These proteins would be non-functional.

2. Insertion of the gene into a vector, producing recombinant DNA.

Cells are unlikely to take in a gene spontaneously, so the gene must be carried into the cell by a **vector**.
Viruses can be used as vectors as well as bacterial plasmids.
Plasmids are much smaller than the bacterial chromosome and contain only a few genes. Plasmids can move in and out of cells, which makes them useful for introducing genes into bacteria.

The method below describes how recombinant plasmids are produced.

1. Bacteria are treated to destabilise the cell walls and breakdown the cell membrane.
2. Plasmids are isolated from the cell debris.
3. The circular plasmid is cut open using the same restriction endonuclease as was used to isolate the gene. This means it has the same nucleotide sequence in its sticky ends. (If the restriction endonuclease produces 'blunt ends' then sticky ends can be added).
4. DNA ligase then joins together the DNA of the
5. plasmid and gene by catalysing the formation of phosphodiester bonds between their
6. sugar-phosphate backbones.



3. Insertion of the vector into the host cell and identification of transgenic organism

When plasmids are mixed with bacterial cells, as few as 1% of the bacteria take up the plasmid and become **transformed**.

To obtain transgenic bacteria which contain recombinant plasmids:

1. the plasmid must successfully incorporate the gene (become recombinant)
2. the bacteria must successfully take up the recombinant plasmids.

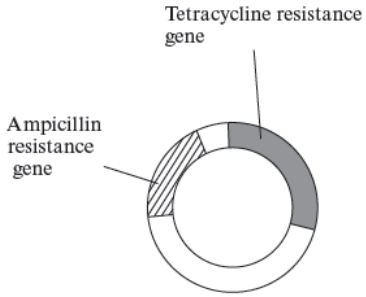
Successful transformation can be confirmed by:

- DNA sequencing
- Marker genes – these vary according the type of transgenic organism produced.

Use of antibiotic resistance genes in the selection of recombinant bacteria

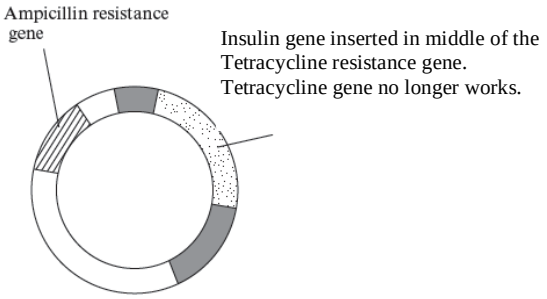
The method below describes how antibiotic resistant genes can be used in the selection of recombinant bacteria (be aware there are several variations to this method):

Special plasmids are used which already have two inserted genes, an ampicillin resistant gene and a tetracycline resistant gene, these will be used in identifying the transgenic cell later on.

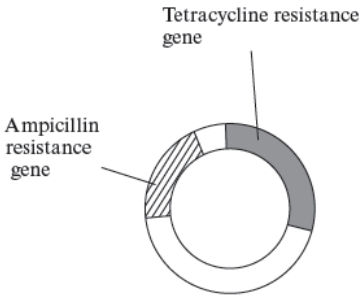


When bacterial plasmids are mixed with the gene fragments in the presence of restriction endonuclease and DNA ligase there are two possible outcomes:

a) The gene is included in the plasmid, disrupting the tetracycline resistant gene



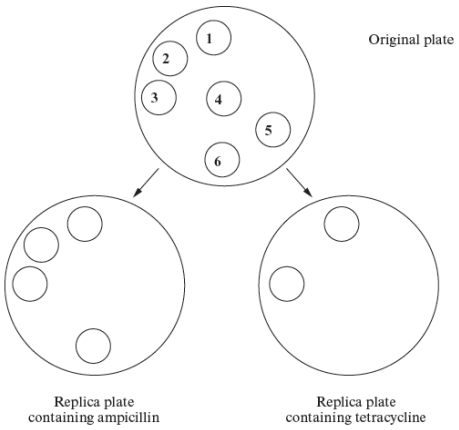
b) The plasmid is reformed without the inclusion of the gene



The plasmids are then mixed with the bacteria.
There are three possible outcomes:

1. Bacteria that have not taken up the plasmids
2. Bacteria that have taken up unaltered (non-recombinant) plasmids
3. Bacteria that have taken up recombinant plasmids.

The transgenic bacteria are identified using a technique called **replica plating**.
Bacteria are grown on agar in a petri dish.
A 'stamp' is used to transfer a sample of each bacterial colony onto agar plater containing either tetracycline or ampicillin.



Results

- The bacteria in **colonies 4 and 5** have not taken up any plasmids and therefore have no antibiotic resistance.
- the bacteria in **colonies 1 and 3** have taken up non-recombinant plasmids as they are able to grow in the presence of Tetracycline.
- the bacteria in **colonies 2 and 6** are transgenic; they have recombinant plasmids as they are able to survive in the presence of Ampicillin, but are unable to survive in the presence of Tetracycline.

4. Production of protein by the host cell / separation and purification of the protein.

Bacterial cells with recombinant plasmids are cultured in large volumes in fermenters. The bacteria divide over and over to form clones, each one containing copies of the recombinant plasmid.
The bacterial enzymes transcribe the inserted gene in the plasmid and translate the mRNA to produce the desired protein.

Concerns Over the Genetic Engineering of Bacteria

- Plasmids are easily transferred between bacteria. There are concerns that plasmids containing antibiotic resistance genes could be passed on to other bacteria. If plasmids with antibiotic resistance genes are passed on to pathogens, this could lead to infections that cannot be treated by using antibiotics.
- There are concerns that using fragments of human DNA could possibly transfer or activate oncogenes.

Issues surrounding the use of gene Technologies to produce genetically modified crops.

The development of GM crops brings many potential benefits. However, there are also many concerns. Both potential benefits and concerns should be evaluated when forming a judgment on the use of GM crops.

Benefits

- Superior keeping qualities;
- Higher yield
- A substantial reduction in pesticide use on crops engineered for resistance to fungal pathogens and insect attack.

Concerns

- Dispersal of pollen from crops engineered for herbicide resistance to wild relatives;
- Unknown effects of eating new protein produced in the crop;
- A reduction in biodiversity.

Gene Therapy

Gene therapy is a technique in which a defective allele is replaced with one cloned from a healthy individual, providing a treatment or cure.
The main challenge is in developing a gene delivery system, so that it is inserted correctly into the genome and functions correctly when there.

To introduce the DNA into the target cells, gene therapy uses:

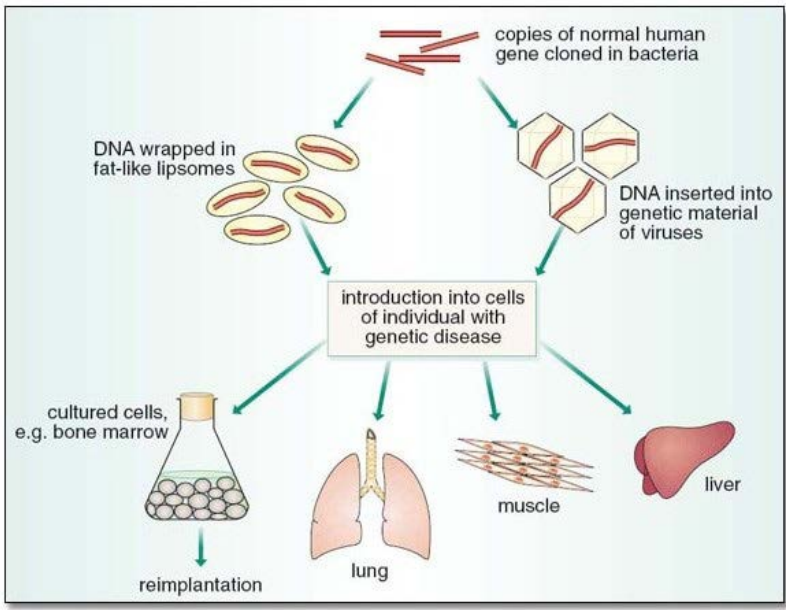
- A virus or plasmid as a vector
- or an injection of naked plasmid DNA

Genetic diseases can also be treated by replicating the function of genes using drugs.

There are two main approaches for gene therapy.

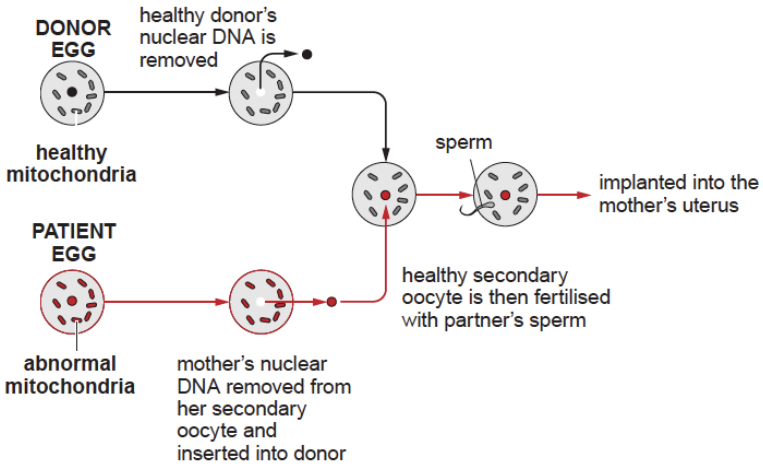
1. Somatic Cell Therapy

This method may be therapeutic, but the genetic changes are not inherited in daughter cells of the treated cells, and do not appear in future generations.
The treatment will have to be repeated regularly as the treated cells become worn out and are replaced by the body with new cells that don not contain a working copy of the gene.



http://drrajivdesaimd.com/2014/08/31/gene-therapy/

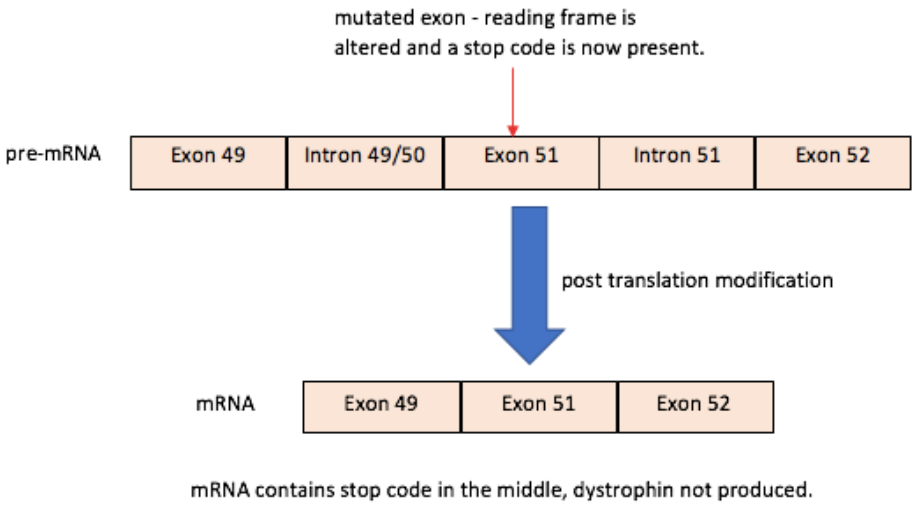
2. Somatic Cell Therapy



Germ-line therapy introduces the corrective genes into germ-line cells, the oocyte in this case, so the genetic correction is inherited. Germ-line therapy however is controversial. Genes interact with each other, e.g. some are switches that control other genes. Changing one gene or set of genes in the oocyte has the potential to cause unpredictable effects in future generations.

Duchenne Muscular Dystrophy (DMD)
A case study for treatment of genetic diseases by using drugs.

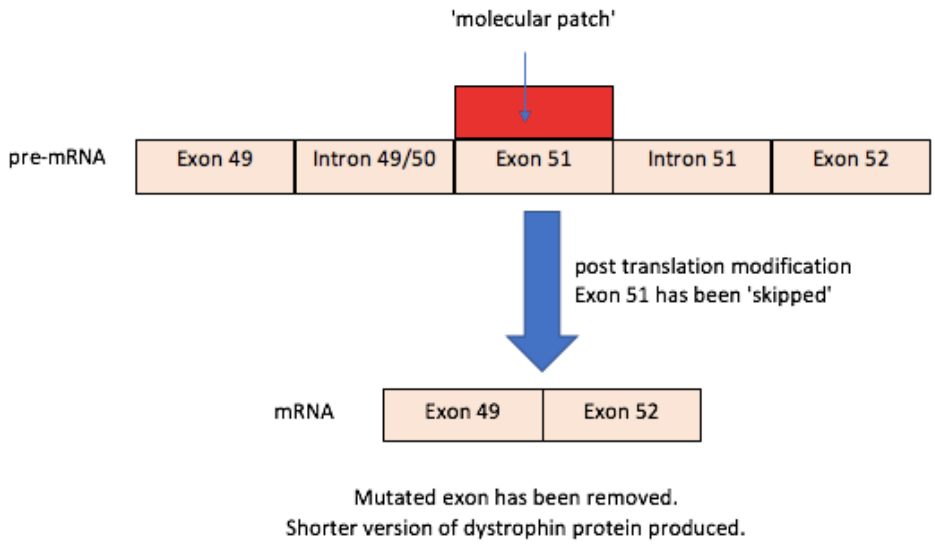
Duchenne muscular dystrophy (DMD) is a **recessive sex linked** form of Muscular Dystrophy affecting up to one in 3 500 live male births.
It is caused by one or more mutations in the **dystrophin gene.** The gene has 79 exons, and mutations in any of these alters how the gene is transcribed as mRNA. This results in the failure to produce dystrophin, which is an important structural component of muscle tissue. The result is severe wasting of the muscles and sufferers are often wheelchair bound by the time they reach teenage years.



A drug called **drisapersen** is in development which aims to treat DMD by introducing a **'molecular patch' over the exon(s)** with the mutation making the gene readable again. A shorter from of dystrophin is produced, but one thought to be more functional than the untreated version. This type of treatment is called exon skipping.

Mode of action of drisapersen

- Drisapersen is a 50-nucleotide sequence that is complementary to the mutated sequence.
- It binds to the mRNA over the exon with the deletion.
- That portion of mRNA becomes double-stranded.
- The ribosome is unable to translate that portion of the mRNA.
- The ribosome skips the mutation producing a shorter, partially functional dystrophin protein molecule.



Genomics and its possible impact on healthcare of the future

Genomics is the study of the structure, function, evolution and mapping of genomes as exemplified by the Human Genome and 100K Projects.

This should enable healthcare to be improved by;

- more accurate diagnosis,
- better prediction of the effect of drugs
- improved design of drugs
- Introduction of new and improved treatments for disease.

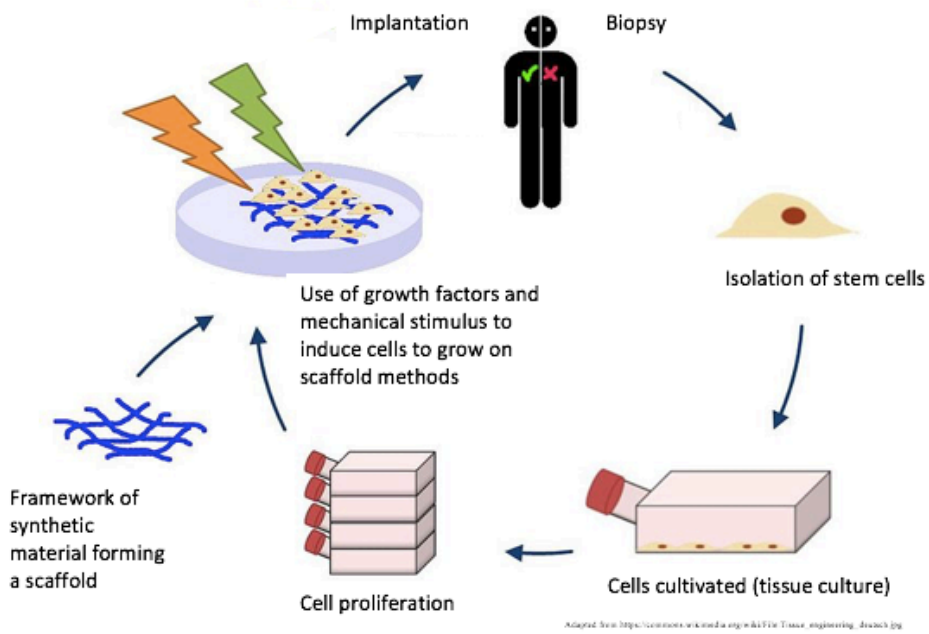
With the introduction of new generation sequencing (NGS) technology it may be possible to look at tailoring therapies to individual patients where an individual could have a unique treatment for a common disease.

Tissue Engineering

In theory, all cells can exist independently of the body provided they are supplied with the nutrients they require. Most cells, however, differentiate into cells which have specific functions, such as nerve or muscle cells, and most of these specialised cells do not normally divide again.

The technique of growing cells in a laboratory is called tissue culture. The medium, in which the cells are grown, has to be precisely controlled and conditions such as water potential and temperature have to be carefully monitored.
Cell cultures have been used for some time for medical and research purposes, e.g. in the culture of viruses for vaccine production and also in the production of monoclonal antibodies.

The aim of Tissue engineering is to repair, improve or replace biological functions by the replacement of tissues or organs.
Tissue engineering involves inducing living cells to grow on a framework of synthetic material to produce a tissue such as skin.
Other applications of tissue engineering include blood vessel replacement, bone and cartilage repair, and the treatment of degenerative nerve disease.



The Advantages and Disadvantages of Using Stem Cells

Central to tissue engineering is the use of **stem cells**.

A stem cell is an undifferentiated cell capable of dividing to give rise to cells which can develop into different types of specialized cells.

Sources of these cells are:

- from 3-5-day-old embryos; embryonic stem cell (ESCs). They are described as totipotent.
- adult tissues, such as bone marrow, that can be 'reprogrammed' to become induced pluripotent stem cells (iPSCs)

Cells that are totipotent have the ability to differentiate into all possible cell types for that organism.

Cells that are pluripotent have the ability to differentiate into many different cell types for that organism. They are not as versatile as totipotent cells.

The advantages of using stem cells are that large quantities of genetically identical cells can be produced quickly.

The disadvantages of using stem cells are:

- in mammals, the technique is very expensive and unreliable;
- in plants, disease or entry of pathogens may cause problems;
- inadvertent selection of disadvantageous alleles;
- long term or unforeseen effects such as premature aging of cells

Ethics of using stem cells and cloning human tissues and organs

The supply of embryos comes from the surplus of embryos which were not placed into a female's uterus during fertility treatment. Some think this is not an acceptable source of stem cells because they believe that using a human embryo represents the destruction of a potential human life. Opponents also argue that embryonic stem cell technologies are the first steps towards reproductive cloning, that is the possibility of cloning humans.
Research with human embryonic cells is banned in some countries.

Acknowledgements

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