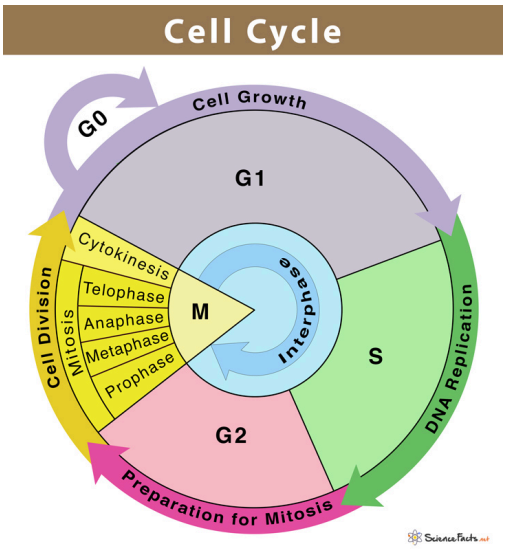
Chapter 2 – Cell Biology

The cell cycle:



Interphase:
3 phases of interphase:

- G1 phase (gap)
  - metabolic activity and growth
- S phase (synthesis)
  - duplication of chromosomes (DNA replication)
  - duplications of centrosomes (contain 2 centrioles each)
- G2
  - Further cell growth
  - Error checking of newly synthesized DNA

Meiosis:
(M phase of cell cycle)

- Prophase
  - chromatin threads condense to form chromosomes (since DNA is replicated, chromosomes will consist of two sister chromatids joined at the centromere)
  - the nuclear membrane disintegrates and the nucleolus disappears
  - the mitotic spindle begins to form and is completed by the end of prophase.
  - the two centrosomes (containing 2 centrioles each) move to opposite poles of the cell
- metaphase
  - the chromosomes move to the center of the cell and line up along the equator (metaphase plate)
  - the spindle fibers attach to the chromosomes at the centromere.
  - the centromeres of the chromosomes are alined on the equator
  - the centrosomes are now at opposite poles of the cell
- anaphase
  - the spindle microtubules shorten and pull on the centromere causing the sister chromatids to separate.
  - the centromere gets pulled first and the arms of the chromosome follow
  - at the end of this phase each pole has a set of identical maternal and paternal chromosomes.
  - the sister chromatids are not referred to as chromosomes
- telophase
  - the chromosomes decadence to form chromatin
  - the two new nuclear membranes (sometimes called nuclear envelopes) form for each of the new daughter cells
  - the nucleoli reapear and the spindle disappears
  - the cell elongates and a cleavage furrow forms to get ready for cytokinesis

cell division (C phase of cell cycle):

- cytokinesis

  plant
  - the cytoplasm of plant cells divide with the formation of a structure called a cell plate
  - cellulose is deposited at this site, forming a wall that divided the parent cell into two daughter cells, each one with a cell membrane

  animal (no cell wall)
  - cytoplasm divides by a precess called cleavage
  - the cell membrane around the middle of the cell draws together to form a cleavage furrow
  - the cleavage furrow continues to develop until the cell membrane eventually meets at a point
  - then the cell is cleaved, or split, into two daughter cells

Meiosis:

- Prophase I
  - chromatin threads condense and form chromosomes (DNA has already replicated so 2 sister chromatids)
  - maternal and paternal homologous chromosomes are attracted to each other and pair up, this is when **crossing over** occurs
  - the nuclear membrane disintegrates and nucleolus disappears
  - centrosomes move to opposite poles
- Metaphase I
  - maternal and paternal chromosomes line up in pairs along the equator
  - this is called **independent assortmen**t because each pair is lined up on one side or the other, independent of every pair. this results in random assortment of chromosomes in the 4 daughter cells
  - the spindle fibres attach to centromeres
- Anaphase I
  - spindle fibres shorten pulling on the centromere of each chromosomes
  - the pair of homologous chromosomes split up where one member moves to one ends and the other moves to the other end
- Telophase I
  - a new nuclear membrane forms and the chromosome uncoil
  - the spindle fibres disintegrate
- Cytokinesis I
  - the cell splits into two cells
- Prophase II
  - chromatin condenses to form chromosomes
  - new spindle fibres are produces
  - nuclear membrane disintegrates
- Metaphase II
  - individual chromosomes line up on equator in random order
  - the spindle attaches to sister chromatids at centromeres
- Anaphase II
  - the centromeres of each chromosome disconnect allowing sister chromatids to separate

    - the spindle fibres shorten pulling sister chromatids to opposite poles of cell
- Telophase II
    - chromosomes unwind and reform into chromatin
    - four new nuclear membranes form around the nuclei
- cytokinesis II
    - the cells separate into four new non-identical, haploid daughter cells

Binary fission:

1. single chromosomes is tightly coiled
2. genetic material in chromosome and plasmids replicates and separates
3. the original and replicate chromosomes attach to the cell membrane and are pulled to separate poles as the cell elongates.
4. the new cell wall starts to grow. As this process commences, a cleavage furrow develops in the cell membrane.
5. the new cell wall fully develops
6. the two cells separate(cytokinesis), forming two identical daughter cells. The chromosome become tightly coiled again.

Chapter 3 – DNA

DNA in Prokaryotes vs Eukaryotes:

Eukaryotic cells: DNA is bound to proteins in chromosomes in the nucleus, which is enclosed in a nuclear membrane. DNA is also found in the mitochondria and chloroplasts.
Prokaryotic cells: DNA is unbound circular DNA in the nucleoid region of the cytosol. The nucleoid region is not bound by a nuclear membrane.

Structure of a nucleotide:

3 parts:

- Pentose (5 carbon) sugar - Deoxyribose
    - The carbon atoms in each molecule are numbered 1-5. One of the ester bonds is formed with the 3' carbon and the other side is formed with the 5' carbon.
- Nitrogenous base
    - Guanine and cytosine have 3 hydrogen bonds and thymine and adenine have 2 hydrogen bonds. (AT2, CG3)
    - Purines (2 rings) : Adenosine and Guanine
    - Pyrimidine (1 ring) : Cytosine and Thymine
- Phosphate group (the phosphate is attached to the sugar molecules by an ester bond and is then called phosphodiester (covalent) bond.

Structure of a DNA molecule:

- Double helix
- Strands joined together by hydrogen bonds between complimentary nitrogenous bases.
- Backbone is a chain of alternating sugar and phosphate groups. (hence sugar-phosphate backbone)

- DNA molecules have an antiparallel structure - that is, the two strands of the helix run in opposite directions of one another. Each strand has a 5' end and a 3' end.

Functions of DNA:

Heritability:	Genetic material can be passed onto offspring
DNA comparisons:	DNA very similar in different organisms and less similar in different species
Code storage:	The sequence of nucleotides contains a code in segments called genes
Replication:	Complimentary bases enable DNA molecules to be replicated.

DNA replication:

DNA replication occurs in the preparation for all cell division, Mitosis or Meiosis. It occurs during the S phase of the interphase of the cell cycle.

1. DNA helicase unzips/ unwinds the double strand by breaking the hydrogen bonds between the nitrogenous bases.
2. Primase enzyme attaches a short sequence of RNA called a primer to show the DNA polymerase where to start adding nucleotides.
3. Complimentary nucleotides are added by the enzyme DNA polymerase. Synthesis of the new daughter strand is in a 5' to 3' direction adenine pairs with thymine, and cytosine pairs with guanine.
4. Ligase is used as a glue to make sure all the nucleotides are joined together. The result is the production of two identical DNA molecules thar are each made of one parent strand and one new daughter strand. Therefore it is described as semi-conservative.
5. In eucaryotic organisms, two sister chromatids are now ready for division. In prokaryotes, two circular chromosomes are now ready for binary fission.

Leading and Lagging strand:

The leading strand, runs 5' to 3' towards the fork this means as Helicase is unzipping DNA polymerase is creating the new strand in that direction so it can run continuously

The lagging strand, runs 5' to 3' away from the fork and is made in small pieces called Okazaki fragments. Helicase keeps unzipping in one direction but DNA polymerase runs the other direction. Primase will keep making primers starting near the replication fork and DNA polymerase will run the opposite direction in short Okazaki fragments.

Coding & Non-coding DNA:

Some sections of genomes will code for protein (Coding DNA) and other sections don't code for any proteins (non-coding DNA)

Introns and Exons: Within genes introns are the parts of the gene that are removed and don't code for anything. Exons are the sections of the gene that code for amino acids.

Protein Synthesis:

Transcription:

1. Occurs in the Nucleus. One gene becomes unzipped and RNA polymerase binds to the strand.

2. Template strand (also known as non-coding strand or antisense strand, runs 3’- 5’) is used for transcription. The coding strand (also known as sense strand) is the opposite strand. (RNA polymerase is NOT bind to this stand. The RNA codons will be the same as this strand, run 5’-3’). RNA polymerase binds to a promoter region after transcription is initiated.
3. RNA polymerase adds free-floating nucleotides according to the complimentary base pair rules. (T replaced with U). New strand of mRNA is synthesised in 5' to 3' direction.
4. DNA bases are read in triplets and complimentary mRNA triplets are called codons. This continues until there is a termination signal (stop codon) this is then called pre-mRNA.
5. Pre-mRNA consists of introns and exons. Introns are removed and exons are joined together to create mature mRNA. This mRNA strand leaves through the nuclear pore.

Translation:

1. Initiation: Ribosome binds to a molecule of mRNA. A start codon (usually AUG) signals start of translation. Only one codon enters and is translated at a time.
2. Elongation: A tRNA molecule, which includes an anticodon is attracted to the corresponding codon due to complimentary base pairing. On the other side of the tRNA is the amino acid specified by the codon. As one codon is read and the other exits another's slides into the ribosome to be read. TRNAs transfer the amino acids to the mRNA-ribosomal complex in the order specified by the codons of the mRNA.
3. Termination: Elongation continues until a stop codon in the mRNA enters the ribosome. The polypeptide chain formed folds to form the protein.

Chapter 4 – Variation

- A gene carrier a set of instructions for how to make a protein. then the gene goes through protein synthesis it is expressed. By coding for proteins genes can determine important facets of biological structure and function. the observable traits and known as the phenotype
- Heredity is the transmission of traits from one generation to the next, traits can be passed on but in sexually reproducing organisms offspring are not identical to their parents
- The diversity of genetic and phenotypic traits within and between populations is called variation

Genotype and phenotype:

- Phenotype is shaped by the presence or absence of particular proteins and the activity of them
- Phenotypic expression of genes depends on interaction of genes and environmental factors
- The alleles of a particular gene are found on homologous chromosomes and form the genotype
- The genotype strongly influences the phenotype but environmental factors can influence the phenotype as-well.
- Genotypes for height, size, skin colour or flower colour can have a range of phenotypes given the same genotype
- Genotypes such as ABO blood group and strictly defined by genotype.

External environmental factors:

- temperature, pH, availability of food, light exposure and wind exposure.

- these do not change the genome. it is the interaction with the environment with either the gene or protein that it codes that determines the changes.
- e.g. hydrangea flower colours depend on the pH of the soil.

Internal environmental factors:

- Action of hormones, some hormones are driving forces for growth, low levels can result in small birth weights and slow development.

Mutations:

Termed mutation: permanent change to an organism's DNA sequence.
Spontaneous mutation: mutations may arise spontaneously during DNA replication or cell division. occur during the synthesis phase. mistakes can occur when DNA polymerase inserts the wrong nucleotide e.g. adenine pairs with thymine but may spontaneously undergo a chemical change that makes it resemble guanine. Repair mechanisms can correct mistakes in G2 phase but in some cases they may not be corrected.

Somatic mutations: A mutation in a somatic cell only affects the body cell in which it occurs and the daughter cells produced from it by mitosis. all other cells of that organism lack the mutation.
Germline mutations: Mutations that occur in gametes (sex cells), they have the potential to be inherited and be incorporated into every cell of the offspring. normally germline mutations result in development of abnormalities that cause the embryo to be aborted. it carried through to birth, it may result in congenital disorders in the offspring.
Recessive and dominant mutations:

- recessive mutations can be masked if a normal copy of the gene is present, for the mutant phenotype to occur, both recessive alleles must contain the mutation
- dominant mutations lead to a mutant phenotype even in the presence of a normal copy of the gene. may be loss or gain function

Mutagens:

Physical or chemical environmental factors that cause mutations.

Physical mutagens

- various types of high energy radiation that cases DNA damage
- they affect the nitrogen bases casing distortions in the double helix
- can also cause double strand breaks
  - sometimes the broken ends leave sticky ends so they can repair easily
  - other times there are no sticky ends and during repair mistakes can be made
- e.g. UV light - structural distortion by cross-linking neighbouring nucleotides
- e.g. X-rays - gene and chromosome aberrations

Chemical mutagens

- common outcome is substitution of one nucleotide for another
- e.g. mustard gas (sulfur mustard) - affects guanine, causing a substitution mutation.
- e.g. Colchicine - prevents spindle formation in mitosis and so doubles chromosome number

Biological agents

- action of invasive pathogens such a bacteria and viruses may cause mutations
- the DNA from these pathogens becomes permanently integrated into the host cells DNA

Types of mutations:

Point mutations: single nucleotide within the original DNA sequence is affected by substitution, addition or deletion.

Substitution

- one nucleotide is replaced by another
- differences between sequences of just one nucleotide are called single nucleotide polymorphisms (SNP's)
- silent (synonymous) mutations occur when the substitution still codes for the same amino acid as the original codon.
- missense mutations occur when SNP changes the amino acid
- nonsense mutations occre when a SNP creates a stop codon and leads to early termination of translation.

Insertion and Deletions

- insertion is the addition of one or more nucleotides at a site within the original gene sequence
- a deletion is the loss of nucleotides from a site within the original gene
- the effect of this is frameshift.
- the consequence of this is that the translated protein has no resemblance to the original polypeptide.

Effects of mutations on survival:

Neutral mutation

- the protein product is unchanged compared with original → organism survival is unchanged
- missense substitution are sometimes also neutral, provided the original amino acid is swapped with another that has similar properties

Deleterious mutation

- random mutations may distrupt the function of the encoded protein, undermining the organisms overall ability to carry out its basic processes and survive.
- nonsense mutations are typically deleterious because the result is an incomplete protein that is non functional.

Beneficial mutations

- gene mutations produce new allele that benefits the survival of the organism
- could be missense mutation that changes the function of the original protein or a nonsense mutation that eliminates a harmful protein

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Chromosome mutations: alterations to chromosomes differ from point mutations because they can affect many genes simultaneously. Can be mutation of chromosome number or chromosome structure.

Variations in chromosome number:

- in many eukaryotic organisms, the somatic cells are diploid (2n) (they contain 2 sets of chromosomes, one form each parent). gametes are haploid. (n)

Monoploidy

- Haploid number instead of a diploid number.
- in colonial insects, males of species are monoploid (1n) in contrast females are diploid.

- males do not need to become diploid to function (like haploid gametes of diploid animals)
- there chromosomes represent a single complete and operational set.
- the advantage of diploid organisms is that any defective alleles can be compensated for by a function allele on the corresponding chromosomes
- in monoploid organisms, a defective allele is the only allel available and the consequences are likely to be deleterious.

Polyploidy

- cell divisions that give rise to haploid gametes fail so that half the gametes contain two copies of each chromosome (2n) and the rest have none.
- if a diploid gamete fuses with a haploid gamete the resulting individual is triploid (3n). if two diploid gametes fuse, a tetraploid (4n) individual will be produced.
- Polyploidy is common in flowering plants, ferns and algae. it can be beneficial in plants e..g inc. fruit size, hardiness and infertility
- it is lethal in human. the pregnancy will most likely stop or if it continues the baby will die within 1 month.

Aneuploidy

- there is an addition of loss of 1 chromosome (or a few) from a cell
- reproductive failure by miscarriages is common
- how it happens:
- in meiosis, identical chromosomes come together and then segregate into seperate cells so gametes finish with only one of each pair of chromosomes. sometimes two identical chromosomes (homologous chromosomes in first meiotic division, 2 sister chromatids in second meiotic division) do not separate but go into the same cell. This is called non-disjunction.
- if a gamete containing both homologous chromosomes fuses with a normal gamete, it will produce a zygote with 3 such chromosomes. this condition is called trisomy.
- if a gamete that has non of the homologous chromosomes fuses with a normal gamete, it will produce a zygote with only 1 such chromosome. this condition is called monosomy.
- these conditions can happen on the sex chromosome or autosomal chromosomes to have different results.

Variation in chromosome structure:

- change in chromosome structure largely come about due to the occurence of two or more double-strand breaks in chromosomes and the rearrangement of the broken segments of the chromosomes
- may occur natural in meiosis as the chromosomes entangle around one another for crossing over or because of exposure to mutagens. the breaks are usually repaired but sometimes mistakes can be made.
- classes of chromosomal rearrangements:
    - deletions
    - inversions
    - translocations
    - duplications
- changes to chromosome structure are another cause of genetic variation

Deletions

- a chromosome may undergo double-strand breaks at two positions and the section may drop out.
- if the two ends rejoin the chromosome will be shorter with a segment missing
- leads to absense of genes and have big effect on development of organism
- e.g. williams syndrome

Inversions

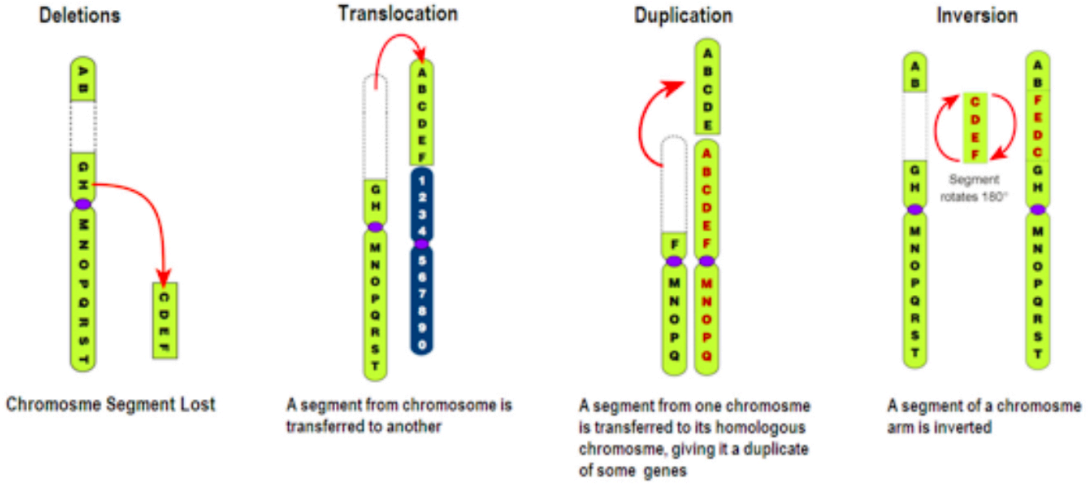
- if a chromosome breaks in two places and the segment rotates before it rejoins again.
- the effects are less dramatic because no genes have been gained or lost.
- it may distrust a gene in which it occurs or causes two genes to become fused together.
- if chromosomes do not align properly for meiosis, it may reduce fertility

Translocation

- a section of one chromosome breaks off and reattaches to another chromosome.
- normal control of genes in that segment are lost, often resulting in cancer

Duplications

- an extra copy is made of a section of a chromosome and inserted either into the same chromosome or another chromosome.
- gene sequences can be replicated several times.
- These are very harmful.



Meiosis causes variation:

- crossing over
  - the swapping of alleles that occurs during meiosis prophase I.
  - paired maternal and paternal homologous chromosomes align so that corresponding DNA sequences from the paired chromosome are able to cross over on another
  - the chiasma is the points of contact between the two (non sister) chromatids.
- independent assortment
  - the random orientation of maternal and paternal homologous chromosomes at the equator during metaphase I.

    - an allele on one chromosome has an equal chance of being paired with, or separated from, any allel on another chromosome (inheritance is independent)
    - Maternal and paternal chromosomes can orientate towards either pole. the number of possible orientations is equal to 2 raised to the power of the amount of chromosome pairs. in humans 2 to the power of 23, this gives us the possible outcomes.
- random segregation
    - during anaphase I, the randomly lines up maternal and paternal homologous chromosomes move to opposite poles of the cell.
    - this random separation of a pair is called random segregation
- random fertilization
    - the random union of gametes is called random fertilisation.
    - each gamete has a unique set of alleles. fertilisation promotes variation because a male gamete can fertilise any of the female gametes, resulting in a unique combination.
    - the random selection of a mate leads to variation.

Chapter 5 – Genetics

Allele: a gene is the stored set of instructions for a protein. alleles are different forms of a gene. pairs of alleles are found on a set of maternal and paternal chromosomes. A set of alleles is called a genotype

Dominant: when present (even only once) it will be expressed in the phenotype. it masks a recessive allele

Recessive: only expressed in the phenotype when present with the same allele. is masks by the dominant allele

Pure breed/homozygous: when the pair of alleles is identical. either both dominant or both recessive (e.g. AA or aa)

Hybrid /heterozygous: two different alleles of a gene are present. e.g. Aa

Autosomal trait: a trait inherited on an autosome (a chromosome that is not sex chromosome)

Sex- linked trait: a trait inherited on a sex chromosome (X or Y). The Y chromosome is short and contains relatively few genes. X chromosomes are longer and contain more genes

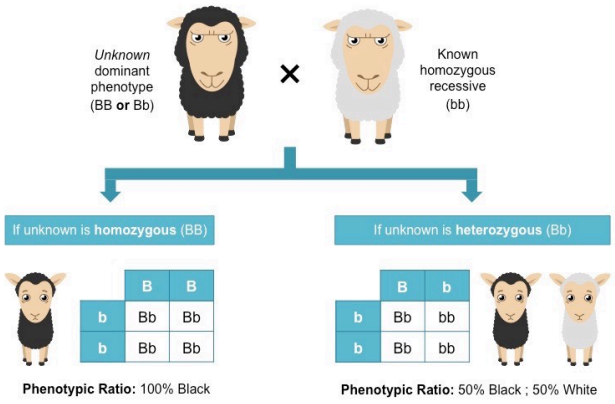
Monohybrids: an organism that is heterozygous with respect to one gene. monohybrids are the offspring from a cross between parents who are both homozygous with different alleles. e.g. parents AA and aa have a possibility of 100% Aa (the offspring (Aa) are the monohybrids)

Monohybrid cross: the cross between 2 monohybrids. the result is a 3:1 ratio in phenotypes.

Test cross: If an organism's genotype is unknown and is displaying dominant phenotype (their phenotype is either Aa or AA) , it is possible to predict the genotype by performing a test cross.

- the test is done by crossing the individual whose genotype is unknown with an organism that is homozygous recessive (aa).
- if the result of this cross all have the dominant trait (all be Aa) then we know that the unknown parents genotype was AA (homozygous dominant)

- if the result of the cross is a mix between individuals who show the dominant trait and individuals with the recessive trait. we know that the unknown genotype was Aa (heterozygous)



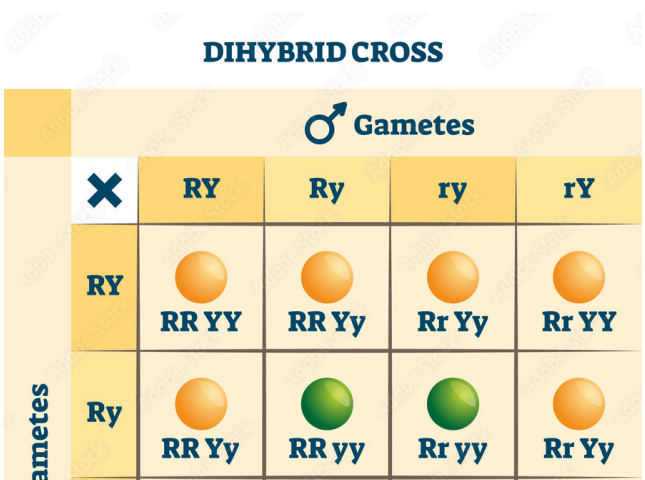
Multiple alleles for one gene:

- when there are 3 or more alleles for one gene.
- e.g. in humans the ABO blood system has 3 alleles. Ia, Ib and i which make 4 possible phenotypes (A, A, AB and O).
- Ia and Ib are both dominant, this means when are paired together they are co-dominant and make the blood type AB.
- Blood type A or blood type B is made when an Ia or Ib is paired with i (heterozygous) , since it is recessive the dominant Ia or Ib will be expressed. Another way is when Ia is paired with another Ia (same with Ib) (homozygous)
- Blood type O is the recessive trait, meaning 2 i's must be paired together to get this trait. (homozygous)
- In the population, there are four possible phenotypes with no one showing variation that is in-between each blood group, this produces discontinuous variation (variation in characteristics that shows only a few clearly distinct phenotypes.)

Independent assortment:

- a parent has 2 copies of every gene (one paternal copy and one maternal copy)
- however gametes will only take 1 copy.
- The observation of characteristics that are inherited independently of one another is know as independent assortment. (when the genes are carried on different chromosomes)
- Alleles for e.g. heigh and flower colour segregate and assort independently because they are carried on seperate chromosomes, which themselves segregate and assort independently.
- If a parent is heterozygous for colour (Pp) and heigh (Hh), since the alleles are independently assorted (Which allele a gamete receives for gene P has no bearing on which allele a gamete receives for gene H)
- the possible gametes are PH, Ph, pH, ph.

Dihybrid cross:



- A dihybrid cross is a cross between two organisms, with both being heterozygous for two different traits.
- the possible gametes for two parents are crossed in a table to see the possible offsprings genotype and phenotype.

Polygenic inheritance:

- is the inheritance of more than one gene that affects the inheritance of a single characteristic. For one characteristic, two or more genes and therefore two or more sets of alleles contribute to this phenotype.
- e.g. human height. humans have a range of heights
- the condition of showing a range of phenotypes is called continuous variation. (always polygenes)

Dominance inheritance patterns:

- Incomplete dominance
  - when two different alleles are present, but neither allele is completely dominant. Both alleles partially contribute to the phenotype, a third intermediary phenotype is observes
  - e.g. a Red snapdragon crosses with a White snapdragon, both red and white are dominant so the offspring may be a pink colour.
  - in scenarios like this the alleles should be represented with a special notation. e.g. C(for colour) and either Cw or Cr.
- Codominance
  - when two alleles are completely dominant, both alleles are expressed and observed in the phenotype.
  - e.g. Shorthorn cattle, alleles for coat colour are red coat (Cr) and white coat (Cw). the offspring have roan coats (Cw, Cr) which are a mixture pf red and white hair.

Modes of inheritance:

- Autosomal recessive
  - alleles come in pair (one gene in each pair comes from mum the other comes from dad)
  - recessive inheritance means both genes in pair must be the abnormal in order to cause the disease
  - people with only one defective allele are called carriers
- Autosomal dominant
  - a single dominant allele is responsible for the occurence of a phenotype
  - an affected person will usually have an affected parent
  - phenotype occurs in every generation
- X-linked recessive
  - males who have the recessive allele on their X chromosome will always express the phenotype. females will only express it when they have the recessive allele on both their X chromosomes.
  - For a female offspring to be affected, her father MUST be affected and her mother is either affected or a carrier.
  - X linked inheritance can be eliminated as a possibility if a father passes a trait to their son. (they only give their Y chromosome to their sons)

- X-linked dominant
    - Similar to recessive but heterozygous females will always show the phenotype and any individuals with the phenotype must have a parent with the phenotype.
    - males that show the phenotype will not pass the affected allele to their son, but they will pass it to their daughter.
    - affected daughters from affected fathers
- Y -linked
    - a trait carried on the Y chromosomes.
    - only males are affected

Chapter 6 – Biotechnology tools and techniques

Tools:

Restriction enzymes:

- Enzymes that cut DNA molecules at recognition sites (specific nucleotide sequence).
- Usually 4-8 bases long.
- The main source of restriction enzymes are bacteria.
- Naturally occurring restriction enzymes protect bacteria by cutting foreign DNA and then removing invading organisms.

Ligase:

- Seals/ reassembles DNA fragments in the process of ligation.
- It sticks the backbone together. (polymerase makes weaker hydrogen-bonds joining together complimentary base pairs) Ligase catalyses a more permanent covalent bond closing up the sugar-phosphate backbone forming a phosphodiester bond and sealing the backbone.

Polymerase:

- Are a class of enzymes that synthesise new strands of DNA/RNA based on a template strand and according to complimentary base pair rules.
- DNA polymerase are vital tool in biotechnology, enabling efficient and accurate amplification of DNA templates. (dNTPs – free nucleotide)

Primers:

- Short fragments of single stranded nucleic acid.
- They are made in laboratory, are about 20 nucleotides in length and are usually labelled with an enzyme, or radioactive or fluorescent dye tag.
- They are attracted to a target DNA strand by complimentary base pairing, and they demarcate (identify) on a strand of DNA where elongation/ synthesis should start.

Amplification:

- Used to greatly increase the number of copies of a DNA sequence for further laboratory use.
- This can be achieved either by vivo (in cell), by inserting the sequence into a cloning vector that replicates within a host cell or in Virto (in test tube), by polymerase chain reaction.

Annealing:

- The process of joining two pieces of DNA by complimentary base pairing (joining of overhanging sticky ends). The two pieces are joined by weak hydrogen bonds only, and therefore only temporarily.

Techniques:

Polymerase chain reaction:

PCR is a cyclic method used to rapidly amplify the amount of a particular DNA sequence.

Items necessary for PCR:

- the DNA strand that needs to be copied (template)
- a specific enzyme called taq DNA polymerase which catalysis the formation of new DNA from free nucleotides (taq is used because of its ability to resist denaturing at high temps)
- a buffer solution that contains salts and other chemicals to maintain correct pH.
- a supply of the 4 nucleotides (deoxynucleotide triphosphates or dNTP's) to build the new strand
- two sets of single-stranded DNA primers. they are about 20 nucleotides long and complimentary to the nucleotides at either end of template DNA.
- a thermocycler to put DNA through temperature cycles.

Denaturation:	Annealing:	Extension:	Repeat:
- double stranded DNA heated to 95°C - this breaks weak hydrogen bonds between bases and two template strands separate (denature).	- the temp is reduced to 50-60°C. (to allow formation of hydrogen bonds) - primers anneal (join via hydrogen bond) to complimentary sequence at opposite ends on each strand.	- temp raised to 72°C (optimal temp for taq) - starting from primers new DNA strands are synthesised using polymerase from free nucleotides. result is 2 copies of the DNA strand	- each cycle doubles number of DNA strands. - cycle is repeated until sufficient quantities of DNA are obtained

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Gel Electrophoresis:

- Gel electrophoresis is a technique that can seperate large, charged molecules (such as dyed fragments of DNA) according to size and charge so they can be visualised and identified with a comparison.
- DNA has an overall negative charge due to the phosphate groups in the backbone.
- An agarose gel is poured into a mould and wells are created on one side.
- the DNA samples and a DNA ladder (a collection of DNA fragments of known base pair lengths used as a standard to compare samples) and pipetted into seperate wells.
- the gel is then placed in a tray and filled with buffer solution with a positive electrode on one side and negative on the other.
- When the electric current runs the fragments are repelled by the negative electrode and more towards the positive side (opposites attract)
- the gel acts as a sieve. the smaller strands of DNA can move through faster and further than large strands.
- This therefore separates DNA strands based on their size
- DNA is not visible to a fluorescent DNA binding dye (ethidum bromide) is added. The Dye binds to the DNA and fluoresces under a UV light showing a pattern of bands.

- each band contains many thousands of pieces of DNA of the same length.
- the bands in each lane are compared with the standard.
- the ladders can be used to determine how many base pairs are in each band. the ladder is made of pieces of DNA with a known number of bp. by comparing the location along the gel of DNA with the ladder the size of each fragment can be determined.

1. Set up apparatus	2. Pipette samples	3. Turn on current	4. Visualises and compare
- set up gel and make wells with a comb. - cut DNA into fragments with restriction enzymes - dye them with fluorescent dye	- pipette samples and the DNA ladder in wells - make sure neg charged samples are at the neg electrode side of apparatus.	- neg molecules are repelled by neg electrode and migrate to pos electrode - smaller fragments more faster (go far), larger molecules slower (no far)	- shine UV light and photograph results - bands in each lane can be compared to ladder to determine no. of bp

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DNA profiling:

- also known as DNA fingerprinting
- used by scientists to identify an individual by comparing an unknown sample of DNA with known profiles by looking for matches in non coding regions of a genome.
- Every humans DNA is almost identical
- no coding regions have satellite DNA (long stretches of DNA made of repeating noncoding units called short tandem repeats (STRs)) individuals of the same species have unique banding patters of DNA that make up their STRs which reflect their unique genetic information (differentiate an individual of another in the same species)
- the banding patterns are visualised by gel electrophoresis and are compared to known DNA profiles to distinguish between individuals.
- Repeating units of STRs are 2-5 bp long (e.g. AGAGAG). larger repeating sequences are called variable nucleotide tandem repeats (VNTRs) which are 5< bp long.
- STRs are different from individual to individual. e.g. one organism may have sequence ATGC repeated 12 times which another may have it repeated 18 times.
- the repeated sequences can be cut (restriction enzymes), amplified (PCR) and fluorescently tagged so the number of repeats can be determined and visualised with gel electrophoresis.

Technique:

1. starts with isolating DNA sample from any somatic (body) cell. a specific fragment is cut at a recognition site using restriction enzymes
2. PRC amplifies the DNA fragment
3. the fragments are separated and the length and number of repeats is determined by gel electrophoresis. smaller fragment have that fewer STRs go further through the gel.
4. the DNA is visualised under UV light
5. the profile is the unique set of patterns in the bands of gel electrophoresis. they are different to other individuals because we are all genetically unique (except for identical twins) we can then compare the differences in length of the STRs with different people.

Uses:

- can be used to identify the origin of a DNA sample at a crime scene and compare suspects profiles to DNA evidence.
- reveal family relationships
- Identify disaster victims.

Recombinant DNA technology:

- Recombinant DNA: DNA composed of one or more genes from two different organisms (usually two diff species). it is when foreign DNA is transferred into the genome of the host organism and then expressed (phenotype shown) in the host. the host is a transgenic organism or genetically modified organism.
- the trait (from the foreign gene) is shown through gene expression and can be passed on to future generations.
- Recombinant DNA tech uses tools such as restriction enzymes, ligase, vectors and cloning. a Vector (a vehicle that transports foreign DNA into host cells) can be introduced into the host and will grow and produce multiple copies of the incorporated DNA fragment (gene cloning). the clones that contain the relevant DNA can be harvested, (insertion of DNA fragments that have a desirable gene sequence into a target organism)
- Transgenic organisms are given desirable traits. e.g. disease resistance, faster growth, greater product quality and yield and tolerance to adverse environmental conditions
- To insert DNA into organisms (plant or animal) a plasmid is a common tool used. they are highly engineered vectors for molecular cloning for large scale production of molecules (e.g. insulin). plasmids are used to insert DNA into bacteria, it is a circular piece of DNA found in bacteria that reproduces independently of the bacterial chromosome.

Transferring genes (Vectors):

- gene guns can be used to deliver genes. a gene can be inserted into a vector to carry the gene to a target organism.
- Plasmid Vector: Agrobacterium tumefaciens is a common bacterium used as a vector. Plasmids (in the bacterium) can be copied (replicated) many times with a foreign inserted gene. Purified recombinant plasmids can be inserted into a new organism directly. However this is not an efficient method of gene delivery because plasmid DNA is not very stable in body cells in this form.
- Viral Vector: Viruses word by injecting their nucleic acid into the host cell. This means it is possible to insert desired genes into viral DNA/RNA and use the virus to insert the new gene into the target cells. Viruses are pathogens so it is necessary to remove or disable genes that cause disease symptoms. Viruses being used are adenovirus and retrovirus. The problem is that the human immune system will attack the virus which decrease the chance of survival within the new host. It can also disrupt normal gene regulation and result in the development of cancer.
    1. Identify an isolate the desired gene:
        a. DNA sequencing and mapping help locate the desired gene.
    2. Extract using restriction enzymes:
        a. cut the gene of interest out of a donor organism.
        b. a specific restriction enzyme is used (specific nucleotide sequence)
    3. Use the same restriction enzymes to cut the plasmid/vector:

    a. plasmid extracted from bacteria by rupturing cell wall.
    b. Plasmid is cut open using same restriction enzyme.
4. Annealing and ligation:
    a. weak attractive forces (hydrogen bonds) draw complimentary nucleotides together. (annealing)
    b. ligase binds the foreign DNA into the plasmid by catalysing the two covalent phosphodiester bonds. (ligation). this makes it permanent.
5. Place recombinant plasmid into bacteria for cloning:
    a. recombinant plasmids function as vectors and are added to bacterial culture. (most common bacteria used is E.Coli)
    b. some bacteria will take then up and they replicate many times by binary fission. (so many copies of the incorporated foreign DNA are made)
6. Transformation and expression:
    a. The process where bacteria takes in the plasmid into its genome is called transformation.
    b. If the gene has been expressed it is transcribed and translated into a protein to be used by the host organism. (gene will cause protein synthesis and express the desired trait)
7. Vector for another host organism:
    a. the bacteria vector is inserted into the host organism by a gene gun or other method (e.g. mixing it with embryos)
    b. Viral vector or Plasmid Vector.
8. New phenotype observed
    a. the desired gene expression is the phenotype that was originally observed in the donating organism.

DNA sequencing:
Sanger sequencing:

- also referred to as deoxynucleotide sequencing or chain-termination sequencing.
- items necessary:
    - dideoxynucleotide triphosphate (ddNTP's) in addition to normal nucleotide triphosphates (dNTPs). ddNTPs are the same as normal nucleotides but have a hydrogen group instead of hydroxyl group and will prevent the addition of any more nucleotides. (stoping the elongation of chain)
    - primer
    - template single stranded DNA
    - all four types of dNTP's (ACGT)
    - one type of dyed ddNTP (one of each A,T,C,G)
    - DNA polymerase

Technique:

1. the region of DNA to be sequenced is identified, cut and amplified (using PCR). it is then heated and denatured to be a single stranded template DNA.
2. everything is added into the reaction mixture. there are 4 seperate mixtures one with ddTTP, one with ddATP, ddCTP and ddGTP.

3. the mixture is cooled so that the DNA primer is annealed to the single-stranded DNA at the 3' end of template strand
4. the temp is raised again and DNA polymerase extends the new strand by attaching complimentary dNTPs in the 5' to 3' direction
5. DNA polymerase will randomly attach a ddNTP (coloured with fluorescent dye) instead of a normal dNTP , it terminates the synthesis of the strand by preventing formation of phosphodiester bond.
6. this is repeated. after enough cycles it is almost guaranteed that a ddNTP will have been incorporated at every single position of the target DNA in at least one reaction.
7. The mixture is heated again to denature the partially complete (not fully complete because of ddNTP) double strand to release the single stranded chain termination molecules from their templates.
8. They are separated with gel electrophoresis (smallest travel further, long dont travel as far) where each mixture is at a different well.
9. as gel electrophoresis proceeds, a laser scans across the bottom of the gel, detecting the different dyes and revealing the base sequence.
10. the base sequence which is read on the gel electrophoresis machine is the complimentary sequence to the template strand. so we must use complimentary base pair rules to find the template strand sequence.
    Uses:

- In medicine DNA profiling can be used to diagnose or treat diseases. Scientists can see is a gene contains changes (mutations) that link to a disorder.

Next Generation sequencing:

- NGS applies same principles as the sanger method with more advanced technology.
- The technology is used to determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA.
- NGS is massively parallel, sequencing millions of fragments simultaneously per run. This process translates into sequencing hundreds to thousands of genes at one time.

Highly parallel: many sequencing reactions take place at the same time
Micro scale: reactions are tiny and many can be done at once on a chip
Fast: because reactions are done in parallel, results are ready much faster
Low-cost: sequencing a genome is cheaper than with Sanger sequencing
Shorter length: reads typically range from 50-700 nucleotides in length

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Genome Mapping:

- the genome is the entire set of genetic instructions. (includes genes and non coding sequences)
- Genetic mapping is identifying and recording the positions of genes on a chromosome. when it is known (called its locus) it can be shown on a diagram.
- A genetic marker (gene with a known location of chromosome) and a DNA marker (a DNA sequence with a known location of a chromosome) can be associated with a specific trait or

phenotype. These can be short DNA sequences, such as short tandem repeats (STR) or longer sequences such as genes.
- Variation in a sequence can be identified as being associated with disorders or beneficial phenotypes.

Technique:
- GENETIC / LINKAGE MAPPING
- Gene mapping is supported by the theory of crossing over (when the same chromosome segments of mum and dads chromosome cross over during meiosis)
- when crossing over occurs, genes that are close together are likely to be swapped together. (the one segment of the chromosome that swaps during crossing over has both the genes on it because the genes are close together)
- two genes on the same chromosome are ‘linked’. the distance between the genes is the ‘linkage distance’. the shorter the distance the more likely they will be inherited together.
- If several generations of offspring inherit the same disease or beneficial phenotype and the same genetic marker (known sequence of DNA), it is probable that there is a gene with an allele associated with the disease located near the marker. (the disease and DNA marker are near each other (short linkage distance) so when crossing over occurs, they swap together)
- this allows scientists to map a phenotypes relative position (cytogenetic location) on a chromosome. these are illustrated in diagrams by using distinctive patterns of bands created when the chromosome is stained with chemicals.
- DNA tools can be used to extract DNA fragments from samples and detect subtle differences in the patterns (such as STRs) that distinguish one individual from another.
- An individual with a disease may have a slight variation in DNA patterns than an individual who is not affected by the disease.
- this DOES NOT identify the actual DNA sequence of the gene responsible for the disease but only indicate that the allel associated with the disease is present and approx. where the relevant gene is located on the chromosome carrying that marker.
- PHYSICAL MAPPING
- this determines the precise molecular location of the genes on a chromosome
- scientists are able to calculate the physical distance (bp) between known DNA sequences (gene markers) by working out the bp between them.
- it can help indicate the size of genes and improve accuracy of genetic mapping

Uses:
- to determine which genes are present on each chromosome and the approximate location on that chromosome.

Chapter 7 -Biotech in Agriculture and Environmental conservation

Recombinant DNA in agriculture:
- the process most commonly used is transformation (taking a gene from one species are inserting it into another to obtain a desired characteristic). the technology used i recombinant DNA technology.

- Transgenic organisms have been engineered for desirable traits. Bioengineering is the combination of biology and engineering to create usable transgenic organisms.
- The genes can be inserted into the genome with a gene gun, viral vectors or plasmid vectors (e.g. Agrobacterium tumefaciens, It has a Ti (Tumor inducing) plasmid that has the ability to penetrate cell walls and insert specific genes into the genome of the host plant cell). A desired gene is cut from a foreign source and inserted into the Ti plasmid to make a recombinant plasmid and then returned to Agrobacterium tumefaciens for cloning. It is then cultured with plant cells susceptible to penetration by Ti plasmid. Once the plasmid penetrates the host the genes are inserted, transforming the host plant into a genetically modified crop.

Herbicide resistance:

- herbicides are substances used to control weeds. they are sprayed on the crops to ideally only kill the weeds leaving the crops unharmed. however it usually damages the crop.
- herbicide tolerant crops are developed.
- e.g. Glyphosate- resistant roundup ready soybeans are modified and have a gene from a bacterium that provides resistance to glyphosate (a herbicide).
- Glyphosate inhibits biochemical pathways in plants preventing them from producing essential amino acids causing them to die.
- Roundup is a name from a glyphosate herbicide that kills weeds (but also kills crops).

Disease resistance:

- diseases that damage and kill crops are a big problem in farming. disease resistant genes from other plants or organisms that are resistant to the diseases are are put into crops to make them disease resistant as well
- e.g. Stem rust is a common disease in wheat. It can be treated with fungicides, however the stem rust pathogen can become resistant to fungicides and there are many new strains of stem rust that frequently appear. (fungicides is not a good solution).
- genes from rust resistant flax plants have been taken and put into rust susceptible wheat plants to make them stem rust resistant.

Faster growth rate:

- Animals and plants genetically modified to grow faster than normal for benefits of human consumption.
- e.g. AquAdvantage Salmon is capable of growing double the rate of normal Atlantic Salmon.
- a better hormone -regulating gene found in Pacific Chinook Salmon is added to the Atlantic salmon as well as a promot gene from ocean pout. which both work to increase speed of growth of the fish. (not affecting its size or other qualities)
- the fish now fully grows in 16-18 months rather than 3 years.

Greater product quality and yield:

- increase in quality (nutrition) in rice –
- Many people (especially children) in developing countries suffer from a Vitamin A deficiency that may lead to death. In India 57% of children under 6 suffer from vitamin A deficiency.

- Some programs to help already exist but cost millions per year to keep them going. they are not sustainable. Having a more varied diet is unachievable as foods with vitamin A are not available throughout the year and people in the developing countries cannot afford to buy a varied diet. Crops which are rich in Provitamin A are perishable (die quickly) so farmers shouldn't use their resources to grow the crops.
- Beta-carotene is known as provitamin A because it is the precursor for Vitamin A. it naturally occurs in corn, mangos and daffodils (yellow pigment).
- Scientists inserted the gene for b- carotene into rice (making it golden). It is called golden-rice.
- Genes from a daffodil and a gene from a soil bacterium were extracted and put into a plasmid which was then inserted into Agrobacterium which reproduced and was mixed with rice plant embryos and the genes were expressed in the plant.

    Concerns:

    . concerns about cost (may be cheaper to just use supplements)
    . the nutrition level in golden rice is not significant enough to alleviate Vitamin A deficiency.
    . Fears of cross breed and contamination to wild rice.
    . concerns that is may harm people and be controlled by large corporations more concerned with profit than people.
    . Making a new GM plant in a lab might cost a few hundred thousand dollars, but following the rules can cost millions. And this has to happen in every country that wants to use it. Developing countries, where farming could improve a lot, feel the loss the most.
    . Sometimes, countries make rules that stop any new GM farming. More money goes into safety research than making new things that could help people.
    . there are fears of loosing the export market due to the perceived contamination with transgenic crops.

Tolerance to adverse conditions:

- crops may face many abiotic stresses in their environment. may be due to climate change. some of these stresses are :
    - § extreme temps
    - § drought
    - § flooding
    - § high salinity
    - § deficient soil nutrient
- adverse conditions affect the survival of an organism.
- E.g. genetically modified wheat and barley to tolerate high saline conditions.
- Two genes were introduced into the plant, an OAT gene from a plant species ‘A thaliana’ that codes for and enzyme that assists growth in high salt conditions, and the cyanamide hydratase (CAH) gene from the soil fungus ‘Myrothecium verrucaria’ that codes for an enzyme that is used as a marker to assist in selection of the GM plants in the laboratory.

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DNA technology in Environmental conservation:

The importance of conservation:

Biogeography: The study of the distributions of animals and plant species and how throes distributions relate to the environment, to the origins of the species and to the changes that have occurred over time. The geographical size of an ecosystem, the habitats it contains and the changes it has undergone all have impacts on biodiversity. Knowing this can help scientists decide weather or not an area needs active protection, restoration or management.

Reproductive behaviors: Is behavior related to the production and care of offspring, including the establishment of mating systems, courtship, sexual behavior, fertilization and raising of young. They need to be considered when planning conservation strategies to prevent inbreeding and loss of advantageous alleles, gene pool diversity and reproductive fitness.

Population Dynamics: Study of the number, gender, age and relatedness of individuals in a population. Pop. size is directly affected by the numbers of births, deaths, immigrations and emigrations. All of these changes can cause a shift in dynamics. Population growth, density, urbanization and migration are factors to be considered in population dynamics. Small populations has a smaller gene pool and therefore have higher risk of losing genetic diversity (especially due to genetic drift), population size is a key consideration when planning conservation strategies.

Monitoring endangered species:

- monitoring endangered species is a crutial part of conservation. it helps scientists identify species threatened with extinction and provide evidence of the effectiveness of conservation strategies. monitoring data can be used to diagnose the cause of population declines and measure management effectiveness.
- factors monitored are:
    - behavior
    - geographical movement
    - reproduction
    - diversity
    - population size
    - population growth.
- Environmental DNA (eDNA) is DNA left behind in an environment by an organism. this can collect data about the changing distribution of an animal over time.
- Animal droppings (scats) can be collected and DNA sequencing can be conducted. Some individuals (animals) can be identified using DNA profiling. This makes it possible to asses the genetic health and diversity of the population which can help guide conservation management.
- E.g. The northern quoll is a carnivorous marsupial and can be identified by its distinct white spots. The populations have declined with land clearing, the inc. in cane toads and predation from other feral animals. Long term-monitoring is being conducted in the Pilbara. Scats were collected for DNA analysis and sequencing was conducted. DNA profiling was used to identify individuals. This made it possible to assess the genetic health of the populations.

Assessing gene pools for breeding programs

- Inbreeding depression: In small populations of animals and plants, there is a risk that closely related (genetically similar) individuals will breed together. the offspring will have an increased risk of deleterious recessive alleles becoming homozygous making them vulnerable to genetic diseases.
- Biotechnologists can use techniques (such as DNA profiling) to selectively breed individuals.
- E.g. Tasmanian devil: Feral animals and farmer culling reduced their numbers greatly. The devil facial tumor disease killed tens of thousands of them. Genetic methods have been used in breeding programs to preserve genetic diversity and minimize inbreeding. Pedigrees, PCR techniques, sequencing and mapping of genomes have helped scientists determine the genetic variation remaining and the relatedness between individuals. Restriction enzymes are used to cut fragments of the genome and sequence the sections, they can them compare the sequences of individuals to determine how closely related they are. In 2012 a disease free population was released onto Maria Island, they are an insurance population (a population brought in from the wild as a safeguard against extinction).

Quarantine to prevent the translocation of exotic species and spread of diseases

- Quarantine is the isolation of organisms that have arrived from elsewhere or been exposed to an infectious or contagious disease. They are monitored until scientists can confirm the possibility of disease in no longer present.
- Quarantine plays an important role in preventing the entry of exotic pests and diseases that could affect plant, animal and human health and the environment.
- e.g. The khapra bettle is a quarantine status pest in Australia. They are currently absent in Australia, if they became established here would affect international trade. They are considered to be the most serious pest of stored grain in the world.
- The beetles can survive for up to 6 years without food and remain undetected under floors in cracks and crevices in sea containers. Through container history tracing they found out the contamination is likely from when the container previously carried high risk goods from countries know to have khapra beetles (may be years before the detection.) They can also enter via contaminated goods from countries known to have Khapra beetles. they enter on plant products known as hosts.
- The Khapra beetles can be confused with a less detrimental pest called warehouse beetle so identification through observation is less accurate. Australia also receives 3 million container per year so it is not possible to inspect every single container.
- DNA fingerprinting is used. in involves the extraction of random fragments of beetle DNA that are cut and amplified and tested to see if they are unique to that species. This enables biosecurity officers to quickly and accurately identify Khapra beetles. The department of Biosecurity is testing eDNA technology. eDNA can be used to determine if insects are present in important goods and sea containers. (eDNA is DNA that is left behind by an insect in the environment. can be parts of skin, urine, hair or other secretions) By testing samples of

dirt or dist vacumed up in sea containers. eDNA technology and DNA fingerprinting could rapidly detect whether khapra beeltes have been present in the sea containers. the eDNA device is no bigger than a mobile phone and can return results in about 20 mins.

Ethical issues associated with transgenic organisms:
Effects on non-target organisms:

- Some TO are engineered to produce deadly toxins to target organisms. (plants releasing toxins to prevent pests). there are concerns that non target organisms will be affected.
- There are concerns that gene transfer from GM foods to cells of the body of bacteria in gastrointestinal tract that will adversely affect human health. (especially in antibiotic-resistant genes used as markers when created GMO's were to be transfered. (this is a low probability). the use of gene transfer technology without antibiotic resistant genes is encoraged.
- there are concerns about outcrossing. this is the migration of genes from GM plants into conventional crops or related species in the wild. this may have an indirect effect on food safety and security. e.g. case have been reported where GM crops approved for animal consumption were detected in products intended from human consumption. Countries have adopted strategies to reduce mixing.

Rapid evolution of pesticide resistant species:

- roundup ready crops that can resist glyphosate (herbicide) are popular. after decades of use some detrimental affects have been observed. some invasive weeds have developed resistance through natural selection. This has led farmers to using more of the chemical to kill weeds to protect the crops. study found the GM crop farming uses 25% more herbicide than non GM farming. This speeds up evolution of resistant weeds by natural selection and increased pollution into the ecosystem.
- Transgenic organisms themselves may evolve quickly with limiting factors for growth removed. If an adverse condition was removed from the environment of a herbicide resistant crop the transgenic crop would grow a lot more resulting in the transgenic organism becoming a pest that could outcompete native plants or other crops.

Gene flow of crop species resulting in emergence of 'super weeds':

- plants that are wind pollinated and are transgenic organisms could spread and pollinate nearby crops of farmers who may not want to use transgenic organisms. (e.g. cotton is wind pollinated). This means introduced genes may be transferred. If a gene for herbicide resistance or other trait is transferred to another crop the growth rate for that crop may increase until it may become a pest or a weed. The farmers may not be able to control the growth of the weed making it a super weed.

Chapter 8 – Evidence for evolution

*life has existed on earth for approximately 3.5 billion years and has changed and diverse overtime. *
Evolution: Is the process of cumulative, inheritable change in a population over many generations. the word 'evolve' means to gradually develop.
Theory of evolution by natural selection:

- theory by Alfred Russel Wallace and Charles Darwin

- All species are linked to a common **ancestor**. (a species from which other species have evolved, a common ansestor refers to an ansestor that is shared by different species)
- Individuals within a population showed a range of variation in their characteristics. Those with traits most suited to their environment would have na advantage making them more likely to survive and pass traits to next generation and accumulate over time.
- A new species arises when populations become so different from other populations of the same species that they could no longer interbreed.

Biogeography:

Is the study of the distribution of organisms and ecosystems acorss the world and through geologic time. Australia and other landmasses in the southern hemisphere share many plant and animal groups, by looking at patterns of these distributions and fossils today, we are able to reconstruct evolutionary history. This provides evidence that these countries were once connected as gondwana.

Comparative genomics:

Genomics: the study of the whole set of genes of a species and the interactions of the genes within a genome.

- some organisms share molecular homologies with one another, as well as the observed structure (morphological) ones.

Relatedness: measure of evolutionary distance. Is reflected in the similarity of their DNA sequences. Two species are more related if they have a more recent common ancestor and less related if they have a less recent common ancestor.

Homology: similarities between a pair of structures, or genes in the case of molecular homology, due to shared ancestry.

Taxon: the named group of organisms, such as Beatles or reptiles

Clade: a group of organisms that includes all the descendants of a common ancestor and the ancestry species itself.

Comparative genomics: a field of biological research in which researchers use a variety of tools to compare the genome sequence of different species. the more similar the sequence, the more closely related those species are in their evolutionary history.

- features shared by very different species (e.g. humans and fish), can be encoded by identical gene sequences that have been conserved in both. These indicate that they share a common ancestor.
- genomes of closely related species (e.g. humans and chimpanzees) have been studied, certain sequences differed.
- genetic relatedness can be measured with particular genes are compared, repeated intron sequences (STR's) or all of the differences between DNA sequences in the genome are compared.
- DNA-DNA hybridisation methods are used to analyse the relatedness of pairs of species. This is when DNA is extracted from two organisms, purified and cut into fragments. It is unwound and the hydrogen bonds joining the two sugar phosphate backbones are broken. The single strands from the two organisms are mixed, some of the double-stranded DNA that forms contains DNA from each of the two species is known as hybrid DNA. Some lengths of DNA

dont pair up because the bases dont match. The molecules are then heated, greater similarity in the hybridised sequences means there will be more complimentary bonds (the hybrid strands bind stronger, more resistance against separation). the separation resistance can be measured to work out evolutionary relatedness.

- Ribosomal RNA is sometimes used because across different species the code is very similar and there are fewer differences. (highly conserved code)

Bioinformatics:

The cumulating of advancements in engineering, computer science, maths and biology. Is the digital storage, retrieval, organisation and analysis of an enormous volumes of biological data such as nucleotide and amino acid sequences from different species. It has increased the size, accuracy and scope of data sets (such as those needed for comparative geonomics)

Phytogenic trees:

Diagrams that show how organisms are related to each other, is hypothetical. can be built using physical info (such as body shape, bone structure, behaviour) or molecular info (genetic sequences)

Mutation rate:

- The frequency of a new mutation in a single gene or organism over time is fairly constant within a species.
- when comparing genomes of two species, mutation rate can be used as molecular clock to estimate at what point in time those species diverged from a common ancestor.
- e.g. for humans mutation rate is estimated to be 10(-8) changed nucleotides per nucleotide base pair per generation.

Comparative biochemistry and protein conservation:

Comparative biochemistry: the study of different kinds of protein (including enzymes), their fundamental units (amino acids) and cell machinery. involves analysis of similarities and differences and results enable evolutionary biologists to estimate relatedness between species.

- a protein that is well suited to its function will be conserved while other traits around it may evolve. (two distantly related species may share similar protein sequences for protein while function is the same in both species)
- the number of amino acid differences in the same protein in different species is used to determine the relationship between the species. (small no. of changes → recent divergence from common ancestor, large no. of changes → more distant evolutionary relationship)

The fossil record:

fossil: preserved remains and traces that provide evidence of past life. (can be hard, such as teeth, bones and shells, or impressions in rock where the organisms tissue has decayed, can also include footprints, burrows or preserved waste) The study of fossils is called palaeontology.

- Only a small percentage of animals leave fossilised remains, most fossils are destroyed by natural processes (weathering or erosion).

- Fossils show that there has been a clear change over time from simple to complex organisms, which is evidence for evolution.

Fossilisation:

Requires very specific and rare conditions to become a fossil:

- organic matter needs to quickly be deposited and covered in sediments, creating environment that **lacks oxygen**, preventing decomposition. Plant and animal remains can be preserved if they are covered in waterborne mud, sand or clay (can happen in beds of lakes, rivers or calcium rich seabeds)
- in many fossils minerals from the sediment have replaced natural bone or shell material (makes remains harder and more likely to fossilise) This process is called **mineralisation**
- organisms covered in sedimentary material (consolidate to form sedimentary rock), this protects organic matter from scavengers and slows its decay for long enough to fossilise.
- this tissue (leaves and muscle) can be preserved in films or impressions left in rock. soft materials (volcanic ash) fills and impression, or minerals form in impression left in sedimentary rock by and organism. result in opal fossil.
- fossils can be formed from freezing and dehydration.
- plant material may be partly dissolved and some tissue is replaced with dissolved salts that petrify the material (replace it with rock) called petrification

principle of superposition:

- fossils found lower down in the earth and older than fossils found closer to the surface.
- the layers of rock in an area being surveyed form a profile. each later of rock in a profile is called a stratum (plural strata)

Transitional forms and pace of evolution:

Intermediate states are called transitional forms. they exhibit traits from both ancestral form and the more modern species. they give evidence for evolution in major groups, documenting change over time on a broad scale.

Gradualism: assumes that evolution occurs as a steady, slow divergence of lineages at an even pace.
Punctuated equilibrium: states that the apparent bursts of evolution are real, species remain fairly stable for long periods of time, but may swiftly change to a new species.

Fossil dating methods:

Comparative dating (relative)

- determining the age of a rock or fossil relative to other rocks or fossils found nearby.
- sedimentary rock is composed of sediment (weathered material from earths surface transported by water. Sediments are deposited to form defined layers of sedimentary rock called strata.
- Relative ages are assigned to fossils based on the strata in which they are found in.

Absolute dating

- assigns a numerical age in years to a fossil or rock. is based on chemical properties of minerals in the rock.
- radiometric dating uses known rates of decay of naturally occurring isotopes present in fossil, is the basis of carbon dating.
- electron spin resonance and luminescence are other techniques.

Comparative embryology and anatomy:
Close examination of physical characteristics of a species at embryonic and adult stages, can reveal evidence for evolution. Comparative anatomy is used to establish evolutionary relationships on the basis of structural similarities and differences, including the comparative study of **embryos**.
Comparative anatomy: the study of the similarities and differences in structure between different organisms. (structural features are called morphological features)

- Embryology
  Is the study of the anatomy of embryos and how they develop over time until the adult stage.
  Early in development , embryos show conserved homologous features that are not obvious in adults.
  e.g. all members of phylum chordata at some stage in development have, a dorsal notochord, pharyngeal slipts, a dorsal nerve cord and a tail.
- Homologous structures
  Anatomical structures that are common to more than one species and were inherited from a common ancestor but have different functions. Have same structural plan but perform different functions due to the different environments and selection pressures.
  When adaptive radiation (process of species rapidly diversifying into many taxa with different adaptations) occurs, organisms retain same basic structures.
  e.g. Wing of bat, wing of bird, let of crocodile, flipper of whale and arm of human all have same basic structure (pentadactyl limb). It has been modified to suit different functions.
  The environment can influence the form and use of homologous structures.
- Vestigial homologous structures
  homologous structures stemming from a common descent can eventually cease to have any functional use for an organism
  Vestigial structures: biological structures that have lost most/all of their original function from evolution.
  are evidence for evolution because it is hypothesised that they were once present and functional in their ancestors.
  e.g. pelvic bone in whale. vermiform appendix in humans.
- Analogous structures
  Are features of organisms that have the same function but a different basic structure that evolved independently.
  e.g. eye of octopus and vertebrates are similar. However in vertebrate eye, nerve fibres lie infront of the sensory cells of retina, in octopus they lie behind them. (vertebrates have blind spot, octopus dont) The development is different therefore products of two distinct lines of evolution.

Types of evolution:
Divergent evolution:

- Differences between groups of organisms accumulate to a critical point that leads to speciation (the development of a new species)
- Divergent evolution is a process by which related species evolve new traits over time spent living in different habitats

    - They become increasingly different from the common ancestor and from one another giving rise to a new species.
    - This pattern is usually the result of the dispersal of a single species to different environments; that is, groups from the same species become isolated from one another, stopping gene flow. The sub-populations are subjected to different environmental pressures, suited to certain structures that can perform functions specific to surviving their unique environment.

Adaptive radiation: A rapid increase in the number of species with a common ancestor, characterized by great ecological and morphological diversity. The driving force behind it is the adaptation of organisms to new environments/food sources. as members of population develop adaptations by natural selection favouring certain mutations over successive generations, they may diverge enough to become a new species. Can occur when environmental changes trigger availability of new resources and environmental niches.

e.g. Koalas , Tasmanian devils and marsupial moles are related because they have a common marsupial ancestor. However, they show quite different feeding structures that enable them to adapt to different diets.

- Homologous structures support Divergent evolution
    - Anatomical features that are similar in different species because they are inherited from a common ancestor.
    - Indicate that the species that possess them are related to each other and share a common ancestry.
    - Support the theory of divergent evolution, as they indicate that different species have evolved from a common ancestor and have diverged over time.

Convergent evolution:

- related organisms evolve similar adaptations in responce to living in the same environment.
- Is a process whereby unrelated organisms evolve similar adaptations in response to a similarity in their environments
- Organisms evolve very different types of structures that solve a problem in a similar way. The structures are genetically relatively different, but their functionality is very similar.

e.g. Modern anteaters include echidnas , numbats, and pangolins . All of these species have an elongated snout that functions as a smelling and digging device, and a long, extendible tongue that can extract ants from crevices. Analogous structures such as elongated snouts, are similar due to environmental pressures, not because they share a recent common ancestor. Instead, they would share a very distant common ancestor.

- Analogous structures support Convergent evolution
    - Anatomical features that have similar functions but are not derived from a common ancestor.
    - Have evolved independently in different species as a result of convergent evolution.
    - Indicate that the species that possess them are not necessarily related to each other and do not share a common ancestry.
    - Support the theory of convergent evolution, as they indicate that different species have independently evolved similar adaptations in response to similar environmental pressures.

Chapter 9 – Mechanisms of evolution

Mutation:

Mutation: a source of new alleles in a population gene pool, is a perminant change in the DNA sequence of a gene.

- can change one allele to another, has the net effect of a change in the frequency of an existing allele. (small change in allele frequency from mutation → insignificant effect on evolution, unless provides beneficial trait with respect to a selection pressure in environment)

selection pressure: Abiotic or Biotic environmental factor that enhances the survival and reproduction of those individuals in a population who possess a beneficial trait, and reduces survival and reproduction of individuals without that trait.

- when individuals in a population possess alleles or traits better suited to survive selection pressures, reproduce and pass on advantageous alleles. This is known as 'survival of the fittest'
- Mutations produce alleles that are selected against, selected for or selectively neutral.
- Harmful mutations are removed from population by selection (be found in low frequencies)
- Beneficial mutations will spread through population over generations through selection. (beneficial classified by whether is helps an organism survive to sexual maturity and reproduce) beneficial mutations are the source of genetic variation in all populations.

variation in populations

- variations are the basis of evolution
- Members of population have variation in genotype that causes variation in phenotype.
- Evolution relies on genetic variation that is inheritable.

gene pools

- genes are the means of transmitting phenotypes from one generation to another.
- gene pool: the total collection of alleles within a population.

Natural Selection:

Through mechanisms, favourable traits are selected for and inherited, and become more common in subsequent generations.

Accumulation: process of certain traits gradually becoming more common over generations.

Adaptive evolution: Natural selection selects for beneficial traits increasing their frequency in the population, and selects against deleterious alleles which decrease their frequency. (changes in a population of organisms that make that population better adapted to its environment)

- Natural selection occurs when selection pressures in environment confer and advantage on a specific phenotype and enhance its survival and reproduction, this results in changes in allele frequencies in the gene pool of a population. Through this process, individuals that have certain inherited traits are more likely to survive and reproduce at a higher rate than other individuals. This causes changes in the populations allele frequencies and therefore is a mechanism of evolution.

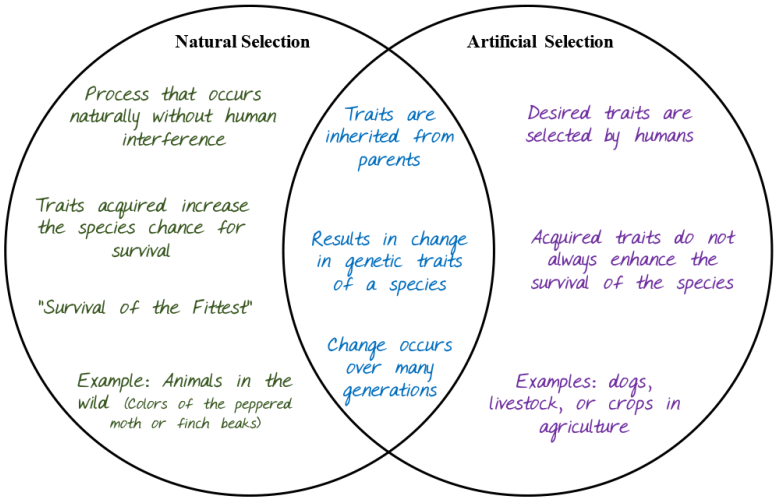
Principles of natural selection:

1. Variation: Individuals in a population differ from each other, due to mutations in alleles and meiosis/sexual reproductive processes. (crossing over, independent assortment, random fertilisation and random mating)
2. Overproduction: There are more individuals produced in a population than the environment can support. (environmental resources limited)
3. Competition and survival of the fittest: Environmental selection pressures favour those with advantageous traits. Leads to competition between individuals in population (those with advantageous trait outcompete others). 'Survival of the fittest'
4. Higher reproductive rates: Individuals with inheritable advantageous trait more likely to survive, reproduce and have higher reproductive rate that those without trait.
5. Heritability: Advantageous trait is passed to offspring.
6. Allele frequencies change over generations: Over consecutive generations, frequency of advantageous allele increases. frequency of disadvantageous allele decreases. After many gens. advantageous allele can become fixed (100%), disadvantageous allel can become extinct (0%)

Artificial selection: the intentional breeding or reproduction by humans of individuals with desirable traits, resulting in changes in allele frequencies in gene pools over time; the traits are beneficial to humans.

- Parental stock with certain desirable traits were selected and mated, and it was understood that these traits were often passed on to the offspring. Overtime these traits could be established in populations.
- The breeding of particular traits result in changes in allele frequencies over generations, and therefore is a mechanism for evolution.
- Cows have been bred for meat quality or quantity or quality of milk they produced. E.g. Jersey cows have been breed for both quantity and quality of milk. The Belgian Blue is a breed of cattle that has been bred for the meat industry, it has an unusual physique that comes from a naturally occurring 'double muscling' mutation. This results in huge muscles.
- The same goes with plant breeding. Breeding new crops is important for ensuring food security by developing new varieties that are higher-yielding, resistant to pests and diseases, drought resistant or regionally adapted to different environments and growing conditions.
- One major plant breeding technique is selection. (selectively propagating plants with desirable characteristics and eliminant those with less desirable characteristics.)
- another is deliberate interbreeding (crossing of closely or distant related individuals to produce new crop varieties (hybrids) with desirable properties)
- Traits breeders try to incorporate are:
    - increased yield, tolerance to environmental pressures, resistance to viruses, tolerance to insect pests, tolerance to herbicides, longer storage period.

Artificial selection vs natural selection:



Advantages of Natural selection:

- Slower growth rate, therefore time to adapt to changes in the environment, such as poor soil quality
- Higher genetic variation, less susceptible to changes in the environment. (AS breeds for one trait, this reduces variation, I.e. less chance of suitable alleles existing and more chance of extinction.)

Advantages of Artificial selection:

- Usually faster growth rate
- Increased nutritional value, yield, pest resistance, disease resistance, drought resistance

Sexual selection: a selection process that occurs between males or females in a population for an inherited trait that assists in copulation or the winning of a mate.

- individuals with certain inherited characteristics or behaviours are more likely than others to obtain mates and pass on their genes.
- over many generation frequencies of advantageous alleles increase (may become fixed) and frequencies of disadvantageous alleles decrease (may become extinct). this is why is it a mechanism of evolution.
- the advantageous allele does not necessarily assist the individual to survive its environmental selection pressures, it only helps it to win a mate. in some cases the trait can be a threat to survival e.g. moose grow big new antlers every year (takes a lot of energy)

Genetic Drift:

Genetic drift: a change in the gene pool of a population as a result of chance; usually occurs more noticeably in small populations.

- genetic drift occurs when a random, non - representative sample from a population produces the next generation. (it is not due to any advantage or disadvantage associated with the allele)
- genetic drift may result in the extinction of some alleles and fixation of others.
- Each individual inherits half their alleles from their mother and half from father, which half is a matter of chance (random fertilisation). Each gamete also has randomly selected chromosomes from meiosis (random assortment).
    - in large populations the randomness of inheritance of alleles is not noticeable overall. (proportion of alleles that are affected is low)
    - In a small population, there is a chance that some alleles present in a parental group will not be passed on at all, the same small number of alleles affected in a small population will be representative of a much higher proportion, there alleles may become lost from the gene pool.

Bottleneck effect:

- It occurs when a previously large population suffers a dramatic fall in numbers.
- A major environmental event like a natural disaster can greatly reduce the number of individuals in a population, leaving behind a small random assortment of individuals which in turn reduces the genetic diversity in the population as alleles are lost
- The surviving individuals end up breeding and reproducing with close relatives.
    - e.g. cheetas are an endangered species that survive genetic bottleneck effect. the surviving parents had to mate with their own offspring resulting in low variation of alleles.

Founder effect:

- The founder effect occurs when a small number of individuals from a large parent population start a new population
- The founder effect can come about as the result of chance
    - E.g. a chance event such as a storm may separate a small group of individuals from the main population
- As the new population is made up of only a few individuals from the original population only some of the total alleles from the parent population will be present. In other words, not all of the gene pool is present in the smaller population
- Because the population that results from the founder effect is very small it is more susceptible to the effects of genetic drift.

Gene Flow:

Gene flow: The transfer of alleles that results from emigration, immigration and migration of individuals between populations.

- Populations are defined by their reproductive and genetic isolation, generally there can be migration between populations. gene flow occurs when the migrants breed.
- Immigrants may add new alleles to a population. Emigrants may remove some alleles from their original population. This changes gene/allele frequencies in populations and it is therefore a mechanism of evolution.

Counting allele frequencies in a population:

- Allele frequency refers to the proportion of one allele relative to the sum of all the alleles for one gene.
- We express allele frequency as a decimal because the sum of all allele of one gene is 1.
    - If an allele frequency is 1, this indicates that all other alleles are extinct.

e.g. Albinism in kangaroos is recessive. There were 55 homozygous normal, 15 heterozygous and 5 homozygous albinos. (so 55 AA, 15 Aa and 5 aa)

A= normal

a= albino

Frequency of a allele = number of a alleles in population/total number of alleles for the gene in the population

= 25/150

=0.17

Microevolution:

Is the change in the gene pool below species level; any small-scale change in the gene pool of a population (change within a species)

- natural selection pressures that change the frequencies of various alleles within a population.
- small scale changes in allele frequencies occur from:
    - mutations
    - natural selection
    - selective breeding
    - genetic drift
    - gene flow

Macroevolution:

Is the evolution of new groups of organisms comprising many related species through multiple speciation events ;includes adaptive radiations.

- major evolutionary changes above species level, changes result from an accumulation of micro-evolutionary changes over many generations.
- large changes in a gene pool can be significant enough to lead to the production of a new species. (speciation)
- macroevolution is the result of a series of speciation events.
- e.g. the origin of mammals, separation between aquatic and terrestrial animals.

Macro vs Micro evolution

Microevolution	Macroevolution
A change in the frequencies of various alleles within a population	Changes in allele frequencies in more than one population/species
Change below the species level	Major evolutionary changes above the species level
Small-scale change in the gene pool of a population due to the mechanisms of mutation, natural selection, genetic drift and gene flow	Large scale change resulting from an accumulation of micro-evolutionary changes over many generations and a very long time

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Speciation:

Species: group of organisms that can interbreed to produce viable, fertile offspring and cannot breed with individuals of another species to produce fertile offspring.

Biological species concept: genetically isolated group with its own gene pool

Morphological species concept: defines species by structural features.

Speciation: is the formation of a new species. It is the process of one species splitting into two or more species.

> e.g. Galápagos tortoises are similar to the much smaller Chaco tortoise , found in South America, but are completely separate species. Darwin hypothesized that the tortoises on the islands originally came from the mainland population, but had changed over time to become better suited to the environment of the Galápagos.

3-processes that work towards macro-evolution

1. natural selection favour phenotypes that make population better adapted to its environment. Population change overtime, gene pools accumulate small changes in responce to NS (microevolution)
2. Population accumulates so many changes that new species is identified, this leads to speciations
3. sometimes a rapid series of speciation events leads to development of whole collection of new species (genes, family and maybe higher order) (macroevolution)

Mechanisms of speciation:

Reproductive isolation: speciation occurs when single population becomes two seperate populations that are unable to interbreed due to changes that produce physical, biological or behavioural barriers.

Isolating mechanisms: Separates two groups and prevent them from producing fertile offspring

- Pre-reproductive isolating mechanisms

Isolating biological or ecological mechanisms prevent organisms from being able to interact and reproduce.

- Temporal (time) mechanisms: individuals breed during different seasons of the year or times of the day
- Behavioural mechanisms: individuals have different reproductive courtship patterns.
- Morphological mechanisms: individuals have different reproductive structures (e.g. genital of different size, shape or location)
- geographic barrier: individuals separated by distance or barrier (river, mountain), depends on size/mobility. (small organisms can be easily transported across barriers by being carried on other animals; parts of plants like seeds and stems can float; small rodents can cling to floating vegetation; winds can carry insects over water.)

- Post-reproductive isolating mechanisms
  Mechanisms that prevent fertilization occurring or an embryo developing into viable offspring if fertilization does occur.
  - gamete morality: gametes dont survive
  - zygote morality: zygote forms but doesn't survive
  - hybrid sterility: adult offspring are formed but are infertile (unable to produce viable gametes), usually because have different numbers of chromosomes from each species. (acts in animals more than plants)

Allopatric speciation:
Allopatric speciation: speciation that occurs due to physical or geographic isolation.

- when populations become physically separated through geographic isolation, different selection pressures act on the populations or other random processes cause them to diverge.
- the divergence of populations into new species is known as divergent evolution.
- physical barriers that can seperate subpopulations form original species can include:
  - water
  - land
  - mountains
- new physical barriers can arise die to:
  - continental drift
  - rising sea levels
  - climate change

Process:

1. subpopulations - parents population divides into two ore more subpopulations
2. isolated by physical barrier - physical barrier separates and isolates subpopulations.
3. No gene flow - the populations are genetically isolates, no migration between populations
4. different selection pressures - different environments apply selection pressures, the populations evolve independently
5. natural selection - different advantageous alleles are selected for survival of the fittest, results in diff. allele frequencies.
6. genetic drift - occurs independently, causing alleles to be passed to offspring randomly

7. two different species - micro-evolutionary changes accumulate until the two species are no longer able to produce viable, fertile offspring. (they are reproductively isolated)

Sympatric speciation:
Sympatric speciation: evolution of two or more new species from a single population within the same place. (speciation that occurs without geographic isolation).

- Groups within same population feed on different things, or choose mates based on different characteristics, or choose mates at separate times.
- genetic separation occurs due to various pre-zygotic and post- zygotic processes.

Extinction:
The fossil record shows that nearly all the species that ever lived are no extinct.
Mass extinction: extinction of many species over a relatively short (geological) period of time.

- the most dramatic extinction even (sometimes called the 'Grey Dying' appears to have occurred at the end of the Permian period 250 mya. which was coincided with one of the most volcanic periods. One of the few survivors was the ancestor of dinosaurs, one of the most successful vertebrate groups ever to have evolved.

Australian bushfires:

- 1 million animals died during 2019-2020 bushfire season.
- High temp and drought caused higher intensity fire
- Some species entire distribution area was burned,
- ongoing mortalities after the fire from starvation, lack of shelter.
- Fast animals survive and slow animals perish → effects evolution of species and biodiversity.
- Sudden reduction in populations size can cause genetic bottlenecks that leads to inbreeding.

Preventing extinction:

- Populations with reduced diversity face increased risk of extinction, so conservation projects usually focus on maintaining genetic diversity.
- Rapid extinction events can lead to greater loss of large organisms than of small ones. A large distribution area is generally a big advantage, because it may allow some pockets of habitat to survive.
- Large population size can also be some protection, because the population is likely to have a more diverse gene pool and thus a greater variety of alleles and phenotype options as the pressures from natural selection change.