# Unit 3 Notes

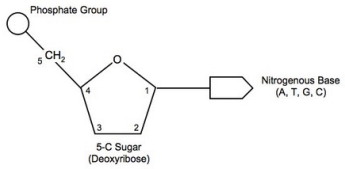
- Red dot points are syllabus points
- Black information is notes

# Heredity

- Structural properties of the DNA molecule, including nucleotide composition and pairing and the hydrogen bonds between DNA, allow for replication

**DNA STRUCTURE**

- DNA = deoxyribonucleic acid
- Composed of subunits called nucleotides that are arranged in a double helix
- DNA holds inheritable information
- Very long molecule, 10,000's of base pairs
- Each DNA strand runs anti-parallel
- Ladder like structure: Sides made of alternating phosphate and sugar molecules
    - Rungs made of nitrogenous bases held together by weak hydrogen bonds that can be broken easily during replication




**NUCLEOTIDE**

- Has 3 components: 5 carbon sugar, negatively charged phosphate group + a nitrogen base
- Complimentary base pairing: Adenine - Thymine, Guanine - Cytosine
- Guanine + cytosine have 3 hydrogen bonds
- Adenine + thymine have 2 hydrogen bonds
- Purine (single sugar structure) = adenine and guanine
- Pyrimidines (double sugar structure) = thymine and cytosine

**DNA REPLICATION PROCESS**

1. Enzyme called helicase unwinds parent DNA.
    - Double stranded DNA unwinds at replication fork
    - Strands coated in a protein (SSB) to stop them from re-annealing. (Single stranded binding proteins)

2. The nucleotides of template strand are exposed and unpaired

3. Primers (short DNA segments) are needed to start replication process
    - Primers synthesised by the enzyme RNA primase
    - DNA polymerase extends nucleic acid chain
    - Free nucleotides attached to complementary bases from nucleoplasm
    - Synthesis occurs in 5' to 3' direction, this creates a leading and lagging strand
    - Lagging strand made of small fragments called Okazaki fragments

4. Ligase joins DNA strands together
    - DNA rezips and 2 identical daughter molecules are created

- DNA is a helical double-stranded molecule that occurs bound to proteins in chromosomes in nucleus, and as unbound circular DNA in the cytosol of prokaryotes, and in the mitochondria and chloroplasts of eukaryotic cells

Nucleosome: structural unit of a eukaryotic chromosome, consisting of a length of DNA coiled around a core of histones.

**RELATIONSHIP BETWEEN DNA, GENES AND CHROMOSOMES**
- Chromosomes and genes are made of DNA
- Chromosome is a long DNA strand which is wrapped around histones
- Gene is a short segment of DNA which encodes for specific characteristics, allele is a specific form of gene which is responsible for variation of how trait is expressed

**GENES**
- Instructions for proteins, most genes have 2 alleles
- Position on chromosome is locus

**DNA IN PRO/EU KATYOTES**
- In prokaryotes DNA is found in plasmids (extra DNA) and in nucleoid
- DNA condenses into chromosomes in humans
- Eukaryotes: DNA is in mitochondria, chloroplasts and nucleus
- Prokaryote: DNA is in the cytosol of the cytoplasm

- Table below shows differences/similarities of DNA packaging/structure in chromosomes of...

| | Prokaryote | Eukaryote |
|---|---|---|
| Nucleus | No nucleus | Has a nucleus |
| Organelles | No membrane bound organelles | Has membrane bound organelles |
| Single/Double Stranded | Single stranded chromatid molecule | Double stranded chromatid molecule |
| Shape | Circular | Linear |
| Plasmids | Has plasmids | No plasmids |
| Location | Occur in nucleoid region | Occur inside nucleus |
| # of Chromosomes | 1 chromosome | Multiple chromosomes |

DNA in nucleus compared to DNA in mitochondria

| | Nucleus DNA | Mitochondria DNA |
|---|---|---|
| Inheritance | Both parents | Maternal lineage |
| Shape | Linear | Circular |
| # of Copies | Low # | High # + random segregation |

- Proteins, including enzymes and structural protein, are essential to cell structure and functioning

- Proteins are essential to cell/organism function and structure
- Structural proteins are used in the cell wall to maintain shape
- Enzyme Examples: Helicase (unwinds DNA)
    - Amylase (breaks down glucose into simple sugars)
    - Protein Carriers (required in active transport)

- Protein synthesis involves transcription and translation of a gene into messenger RNA in the nucleus, and translation into amino acid sequence at the ribosome

**TRANSCRIPTION**

- Only anti-sense (lagging) strand is transcribed
- Takes place in nucleus of eukaryotes

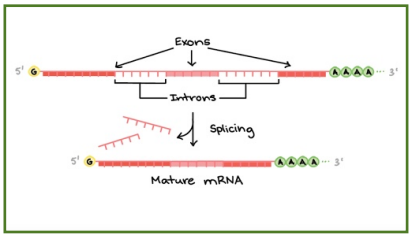
Initiation: - A protein (RNA polymerase) attaches to promoter region
- DNA is unzipped to expose nucleotides

Elongation: - Messenger RNA is complimentary transcribed using DNA as template
- Only 1 DNA strand is transcribed (anti-sense strand, 3' end), mRNA is single stranded

Termination: - Transcription stops when termination sequence is reached
- RNA polymerase detaches from DNA, mRNA released

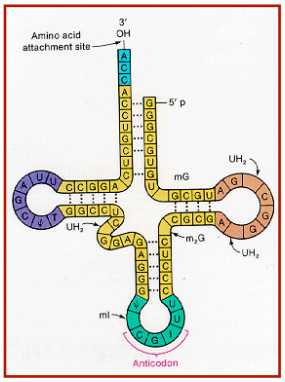
**POST TRANSCRIPTION CHANGES**

- mRNA is initially pre mRNA until spliceosome removes non coding introns and leaves coding extrons.
- Methylated cap is added to 5' end
- Adenine tail added to 3' end
- mRNA is now mature → ready to leave nucleus




**TRANSLATION**

- mRNA moves from nucleus to cytoplasm + binds to ribosome
- tRNA brings amino acids to ribosome
- Starts with start codon AUG/Methionine
- Codon has 3 bases, 1 codon codes from 1 amino acid
- tRNA reads mRNA codon
- tRNA contains anti-codon
- tRNA bind to site on ribosome + bring amino acid until stop codon reached




**AMINO ACID**

- Joined by polypeptide bonds to form polypeptide chain
- Polypeptide chain travels to endoplasmic reticulum in vesicle for final folding to become functioning protein

**DNA v RNA**

- Nucleic acids are genetic material of all organisms
- DNA carries blue print to assemble proteins
- RNA plays role in making proteins

| | DNA | RNA |
|---|---|---|
| Nucleic Acid | Deoxyribonucleic | Ribonucleic |
| Location In Cell | Nucleus, mitochondria, chloroplast | Ribosome |
| Structure | Double stranded | Single stranded |
| Bases | Thymine - Adenine<br>Cytosine - Gaunine | Uracil - Adenine<br>Cytosine - Gaunine |
| Size | Long | Short |

- The genetic code is a triplet base code: including coding and non-coding DNA, and many genes contain information for protein production

- Only a small amount of DNA strand is used for RNA template → This region is a gene
- A triplet base has 3 nitrogen bases → This is a codon
- tRNA has an anti codon → This is complementary to the mRNA section

DNA STRAND: AGC TAT CGA GTC AAA
mRNA: UCG AUA GCU CAG UUU
Anti codon (tRNA): AGC UAU CGA GUC AAA

**RIBOSOME DIFFERENCES IN EU/PRO KARYOTE**

| | PROKARYOTE RIBOSOME | EUKARYOTE RIBOSOME |
|---|---|---|
| Structure | 2 unequal sub units | 2 sub units as well (40s and 60s) |
| Location | Mitochondria + chloroplast | Bound to ER and lose in cytoplasm |
| Size | Smaller than eukaryote ones | Bigger then prokaryote ones |

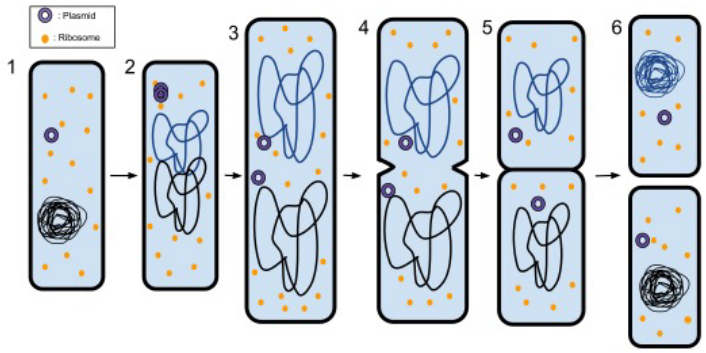
- Continuity of life requires the replication of genetic material and it's transfer to the next generation through processes including binary fission, mitosis, meiosis and fertilisation

**BINARY FISSION**
**PROKARYOTES:**

- Use binary fission to reproduce
- Single circular chromosome

**STEPS**

1. Replication of DNA
2. Each DNA copy attaches to opposite ends of the cell membrane
3. Cell increases in size
4. Each copy of duplicated chromosome attaches to a different part of the cell
5. Cell eventually begins to pull apart separating chromosome (cytokinesis)
6. Wall forms across the cell and divides into 2 identical cells




**MITOSIS**

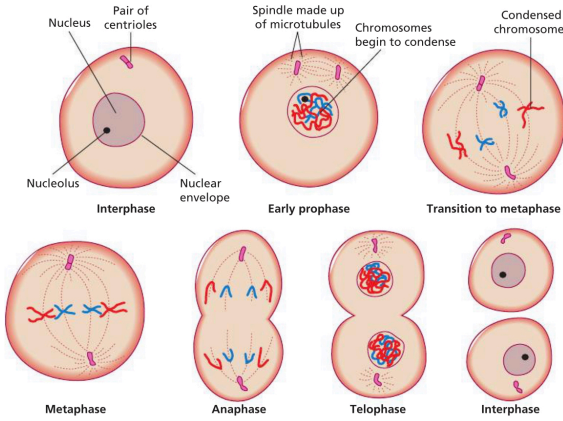
Interphase: DNA replication occurs
DNA not condensed into chromosomes

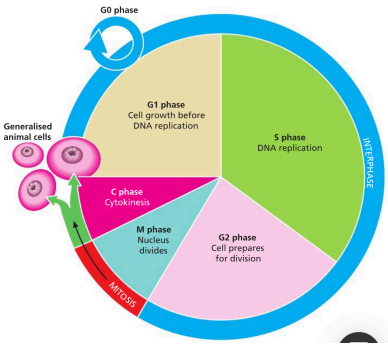
Prophase: DNA condensed into visable chromosomes
Spindle fibres appear

Metaphase: Chromosomes line up at equator

Anaphase: Chromosomes are pulled apart to either side of cell

Telophase: Cytokinesis occurs
Nuclear membrane reforms
2 identical daughter cells produced (diploid 2n)






**CELL CYCLE**

- Cell spends majority of time in interphase
- During S phase DNA replicated before cell division
- Mitosis starts in M phase + ends in C phase

**MEIOSIS**

Prophase I: DNA condenses into chromosomes
Homologous chromosomes pair up and crossing over occurs

Metaphase I: Homologous chromosomes line up at equator

Anaphase I: Microtubules (spindle fibres) pull homologous chromosomes apart and move to opposite ends of cell

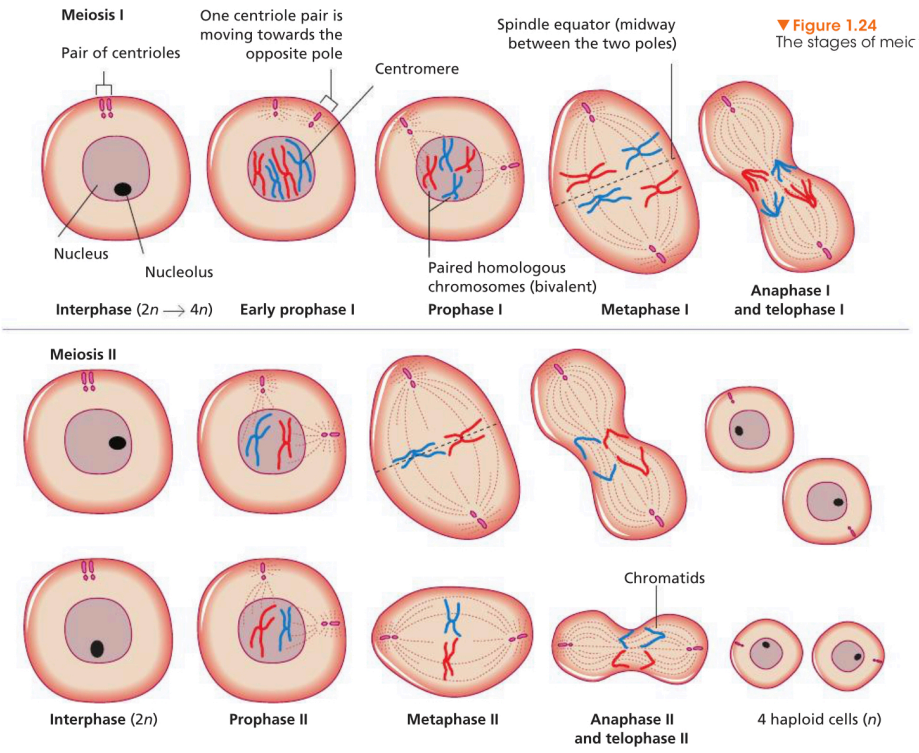
Telophase I: Spindle fibres broken up, new nuclear membrane forms
Chromosomes uncoil and cell divides

Prophase II: Chromosomes condense, nuclear membrane breaks down
New spindles form at right angles

Metaphase II: Spindle fibres line attach to chromosomes and line at equator

Anaphase II: Sister chromatids pulled to opposite ends, cell elongates
Chromatid becomes daughter cell chromosome

Telophase II: Sister chromosomes uncoil, new nuclear membrane forms
2 cells divides again to form new haploid cells (gametes (n))

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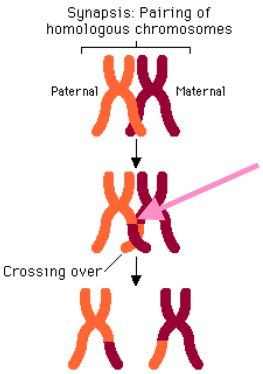



Chiasmata

• Phenotypic expression of genes depend on interaction of genes and environment

**GENE EXPRESSION**
- Cells do not express all genes in their genome at same time
- Some genes active (produced via protein synthesis)
- Others inactive or switched off

**EUKARYOTIC**
- Only 2% of human genome contains genes that are transcribed
- Coding proteins: exons
- No coding proteins: introns

**DNA METHYLATION**
- Attachment of methyl group to nucleotide/histone protein
- DNA methylation prevents genes from being transcribed

**EPIGENETICS**
- Study of chemical modifications to gene function that aren't due to DNA sequence change

**ENVIRONMENTAL INFLUENCE ON GENE EXPRESSION**
- Gene expression can be influenced by environment
- Himalayan Rabbits: temperature effects fur colour, in warm areas 'C gene' is switched on but in colder extremities fur is black
- Hydrangea: pH of soil, flowers are blue in acidic soil and red in neutral/basic soil

• Mutations in genes and chromosomes can result from errors in DNA replication or cell division, or from damage by physical or chemical factors in environment
- Mutation: permanent change in DNA structure, can be caused by environment factors

- Physical Mutagen: produce energy which damages DNA
  - Radiation (X-Ray): cause loss of adenine/guanine bases and creates gaps, incorrect bases inserted during DNA replication
  - UV: hydrogen bonds along DNA strand are broken causing adjacent thymine bases to bonds
  - Nuclear Radiation: causes breaks in DNA strand

- Chemical Mutagen: causes substitution, addition or deletion of a base
  - Alcohol: causes DNA breaks which permanently alters it
  - Ciggies
  - Mustard Gas: causes guanine to be replaced by other bases

- Biological Mutagen: damages cells and changes DNA, some insert fragment of their own vaccinations
  - Virus
  - Bacteria: no actual DNA changes but causes genes to switch on/off
  - Micro-organisms

**POINT MUTATIONS**
- Change in a DNA sequence at one point or base
- Difference between sequences in nucleotides at 1 position called: single nucleotide polymorphism (SNP/snips)

**SUBSTITUTION**
- Occurs when one nucleotide is replaced by another

**INSERTION/ADDITION**
- Addition of one or more nucleotide(s)

**DELETION**
- Loss of nucleotide(s)

**Frameshift Mutation**
Codons downstream from mutation are all affected

**SILENT MUTATION**
- Occurs when a base change results in the same codon coding for the same codon as the original codon

**MIS-SENSE MUTATION**
- Arises when single nucleotide changes the codon and therefore the amino acid

**NONSENSE MUTATION**
- Occurs when a single nucleotide mutation results in a stop codon, incomplete polypeptide

**EFFECTS OF MUTATION ON SURVIVAL**
- Mutations change protein structures, which has an affect on protein function

**NEUTRAL, DELETERIOUS, BENEFICIAL MUTATIONS**

| | NEUTRAL | DELETERIOUS | BENEFICIAL |
|---|---|---|---|
| What Happens | • Protein product is unchanged<br>• Same amino acid is used anyway despite base change | • Disrupts function of protein | • Gene mutation leads to new allele |
| Result | • Correct protein made | • Incomplete or non functional protein | • New allele is beneficial to survival |
| Survival Effect | • No effect on survival | • Effects ability to carry out process and survive | • Increased chance of survival |

| | NEUTRAL | DELETERIOUS | BENEFICIAL |
|---|---|---|---|
| Examples | • Synonymous<br>• Missense | • Nonsense | • Missense: original protein is changed<br>• Nonsense: eliminates protein that harmed survuval |

**CHROMOSOMAL MUTATIONS**

- Changes structure of chromosome
- Double strands, re-arrangement of broken segments, loss of part of chromosome
- Naturally occurring (meiosis), exposure to mutagen

DELETION

- Chromosome undergoes double stranded breaks at 2 locations
- Results in a lost segments

Effect: fatal, survivors have adverse effects

DUPLICATION

- Extra copy is made of a section of chromosome + inserted into same/other chromosome
- Causes gene sequences to be repeated many times

Effect: usually harmful, sometimes advantageous

INVERSION

- Chromosome breaks in 2 places, middle segment rotates 180* before rejoining
- Reserves gene sequence

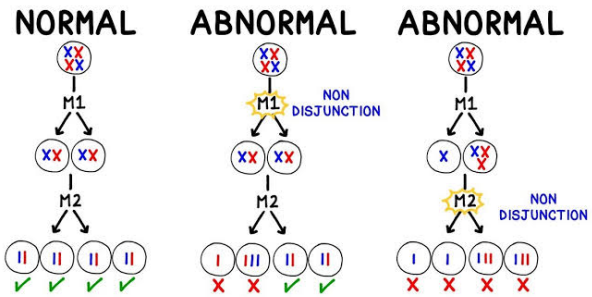
Effect: less dramatic than other mutations, often causes non-disjunction in meiosis

TRANSLOCATION

- Section of one chromosome breaks off, reattached to another one

Effect: normal control over genes as section is lost

**VARIATIONS IN CHROMOSOME NUMBER**




MONOPLOIDY

- +: more economical
- -: mutations more harmful as usually deleterious, diploid masks this better
- Example: Often in colonial insects, males are monoploid (1n), gametes made by mitosis
- Females diploids, gametes made by meiosis
- Males produced by parthenogenesis: entire organism regenerated by single cell

POLYPLOIDY

- +: common for flowering plants to have increased size, better hardiness
- -: lethal in humans, reduced fertility
-
- When gametes aren't haploid (some diploid, some none)
- Diploid fuse with haploid to form (3n) or 2 diploid fuse (4n)

- Organisms can have extra sets of chromosomes

ANEUPLOIDY
- Addition or loss of chromosome from cell, causes miscarriage
- Instead of chromosome in meiosis separating into each cell, they go into 1 cell
- 2 types of gametes produced: 1 has 2 copies of a chromosome, other has 0 (disjunction)
- Disjunction can occur in 1st/2nd meiotic division

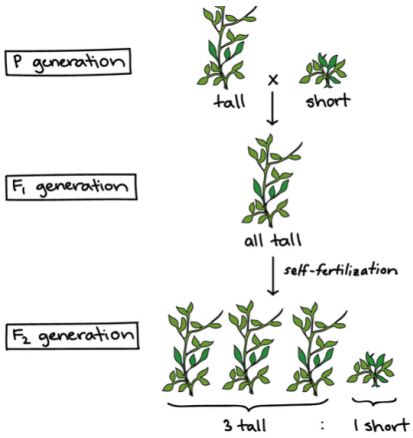
• Variations in the genotype of offspring as rise as a result of the processes of meiosis, including crossing over and random assortment if chromosomes and fertilisation, as well as result from mutations

| | Description of Process | How it Creates Variation |
|---|---|---|
| Meiosis - Independent Assortment of Alleles | • Homologous chromosomes seperate independently of each other during meiosis I | • Forms random combinations of chromosomes, creating a novel combo of chromosomes which creates novel genotypes |
| Meiosis - Crossing Over | • Exchange of alleles between homologous chromosomes | • Chromosomes then have a new combo of alleles<br>• Different alleles create new genotypes |
| Fertilisation | • Fusion of gametes from maternal and paternal parents | • Combines genetic material from 2 individuals<br>• New genetic combinations (m/paternal alleles) result in new genotype |
| Mutatiuon | • Permanent change in DNA sequence | • Variation created by many point/chromosomal mutations<br>• Ultimate source of new alleles as other processes only shuffle/change combinations of new alleles |

- Frequencies of genotypes and phenotypes of offspring are determined by patterns of inheritance, including dominance, autosomal and sex-linked alleles, multiple alleles and polygenes

**ALLELES**
- Each gene usually has 2 alleles
- 2 identical alleles (GG/gg): homozygous
- 2 different alleles (Gg): heterozygous
- Individuals receive one gene from each parent that are then paired

**MENDELIAN INHERITANCE, PUREBREEDING, + HIS PEAS**
- Chose peas as model organism and created purebreeding tall/short plants
- Peas are good as have quick growth + high seed rate
- Then he breed them with others to see how they inherited traits
- Found that tall peas always had a hidden recessive trait and in the second self fertilisation, the hidden recessive form appeared in some peas in F2 (3 tall pease to 1 short)
- Also found inheritance of different traits were independent
- Theory that traits are controlled by single genes, each organism has 2 copies of each gene
- Purebreeding: 2 pure parents will always have offspring the same as themselves




- Law of dominance: mating between 2 organisms of different traits, offspring exhibits only 1 parent (dominant alleles)
    - Recessive traits only show when both alleles are recessive
- Law of independent assortment: alleles of 2 or more genes get sorted into gametes independently of one another. The allele a gamete receives for 1 gene doesn't influence the allele received for another gene
- Law of segregation: when gametes are formed, each one receives 1 gene copy which is selected randomly.
- Heterozygous: produces G and g gametes
- Cross test: used to determine if organism with dominant phenotype is homo/hetero
- Mono hybrid: when parents of 2 different genotypes at a locus breed to create hybrids like (Gg)

**INCOMPLETE DOMINANCE**
- When one trait is not fully dominant over its partner
- Expressed by a mixing of phenotypes
- Red flower x white flower = pink flower

**BLENDED DOMINANCE**
- Both alleles in a genotype are fully expressed in the heterozygote
- Similar to incomplete <u>but</u> it has bits of both phenotypes
- Red cattle x white cattle = roan cattle

**POLYGENES**

- Characteristics controlled by more than one gene are poly genetic and transmitted through poly genetic inheritance
- Happens when a single characteristic is controlled by the alleles of 2 or more genes interacting with one another
- Results in an expression of multiple genes -> range of phenotypes
- Continuous variation: showing a range for phenotypes, variations controlled by 2 or more genes (skin colour)
- Example: human skin colour, 4 genes control melanin, the specific combination results in the range of pigments in skin
- Discontinuous variation: only 1 gene involved, results in small # of phenotype (hair line)

**MULTIPLE ALLELES**

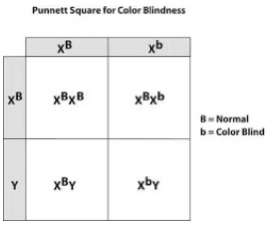
- Usually only 2 alleles present for 1 gene, multiple alleles is 3+ in a gene within a population
- When more than 2 genes types can occupy a locus
- Human blood type: alleles $I^A$, $I^B$, i \\ $I^A$, $I^B$ are codominant and ii is recessive
  2 O parents must have O child
  1 A parent + 1 O parent can have a couple of types of children but depends on if A parent is homo/heterozygous. If its hetero they can have O blood group kiddies

**SEX LINKED**

- Genes located on X or Y chromosome: X/Y linked
- Genes on sex chromosome don't appear equally in both sexes
- Hemizygous: only having a single copy of a gene instead of 2 (all genes on male X chromosome are hemizygous)
- Gametes have 22 autosomal chromosomes and one X and X/Y (males), females have XX

**X-LINKED RECESSIVE**

- When recessive phenotype is determined by and alleles on the X chromosome
- Males receive 1 X from mother, inheriting 1 affected gene will result in them being affected
- <u>Males</u> with recessive allele: - Always express phenotype (only have 1 X chromosome)
  - Males show X-linked recessive more than females
  - Males with X-linked disease can't pass it onto sons as they inherit the Y chromosome only and its uneffected
  - Affected males can have affected daughters if mother passes the recessive X-linked gene as well
  - Example: haemophilia, colour blindness



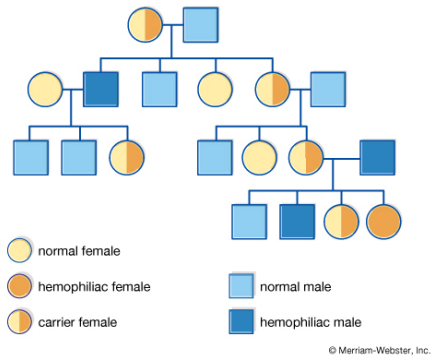

- <u>Females</u> with recessive allele: only express when both X chromosomes have affected allele. If the female if heterozygous (Gg) they are a carrier

**X-LINKED DOMINANT**

- Similar to recessive but heterozygous females always show the phenotype
- Affected males don't pass affected alleles to sons but will pass them to all daughters

- Carrier females (hetero) expected to pass affected alleles onto 50% of offspring regardless of sex
- Every affected individual must have an affected parent

**PEDIGREES**
- Graphic representation showing pattern of occurrence in a family




**AUTOSOMAL DOMINANT**
- Both males + females affected equally
- All affected individuals have 1 affected parent
- 2 affected parents can have unaffected children

**AUTOSOMAL RECESSIVE**
- Both males + females affected equally
- 2 unaffected heterozygous parents can have affected offspring
- 2 recessive parents only have recessive offspring

- DNA sequencing enables mapping of species genomes; DNA profiling identifies the unique genetic makeup of individuals

**DNA SEQUENCING VS DNA PROFILING**

- Sequencing determines the order of base pairing or nucleic acid sequence in DNA to help identify specific genes
- Sequencing uses Gene probes
- Profiling is the analysis of the DNA and identify s species or individuals
- Profiling uses gel electrophoresis

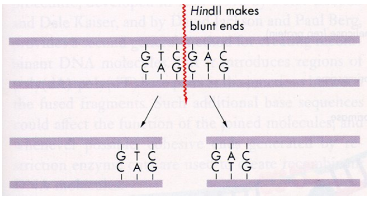
**GENETIC ENGINEERING**

**PLASMIDS (VECTORS)**

- Found in bacteria
- Extra chromosomal DNA
- Often carry useful genes for traits like antibiotic resistance
- Easily copied and transferred between bacteria in conjunction
- Can be picked up from environment if they are encountered by the bacteria in transformation
- Vector: an instrument/organism used to transfer genes between organisms

**RESTRICTION ENZYMES (ENDONUCLEASES)**

- How do they get the enzymes? Isolate them from bacteria
- They are a natural defence against viruses in bacteria, they act as molecular scissors ✂️
- Cut DNA at specific sites known as restriction sites (no longer than 6 bases)
- Each restriction enzyme has 1 specific recognition sequence

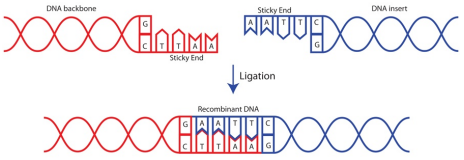



**BLUNT ENDS**

- Created when the cleaved fragments have no overhanging bases
- Rarely used in biotech as DNA bonds are weak

**STICKY ENDS**

- Cuts are asymmetrical + create over hanging bases on fragments
- Useful in biotech as DNA bonds are strong




**NAMING RESTRICTION ENZYME**

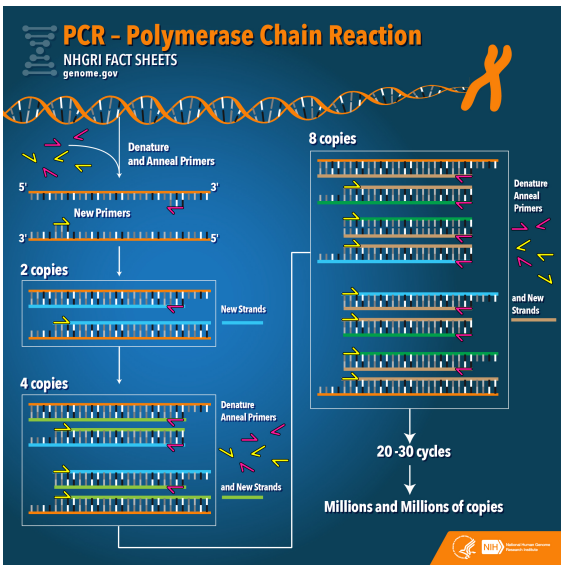
EcoR1 eco:bacteria isolated from, R1:place its cut from

**LIGATION**

- Complementary DNA fragments joined with DNA Ligase with is a molecular glue

## POLYMERASE CHAIN REACTION

- Technique used to amplify (copy) large quantities of DNA, copies DNA at exponential rate
- Each cycle takes 1-3 mins
- Uses taq polymerase
- Taq polymerase was isolated from thermophyllic bacteria ⟶ can function at high temps without denaturing
- PCR uses: DNA, nucleotides, primers, taq polymerase




## PCR PROCESS (Remember D-A-E)

| Process | Temperature | Description |
|---|---|---|
| Denaturation | 95°C | - Mixture heated to 95°C<br>- DNA denatures and seperate to be single stranded |
| Annealing | 50°C-60°C | - Temperature adjusted<br>- Allows primers to attach to single stranded DNA |
| Extension | 72°C | - Temperature changed to 72°C<br>- Initiates taq polymerase to start adding free nucleotides to single stranded template DNA |
| Repeat | - | - Process repeated about 30 times<br>- Creates millions of copies of DNA sample |

- How to calculate amount of DNA in copies → 1 strand produces 2 copies, so do 2, to the power of number of cycles done. 10 cycles = $2^{10}$ = 1024 DNAs

## GEL ELECTROPHORESIS

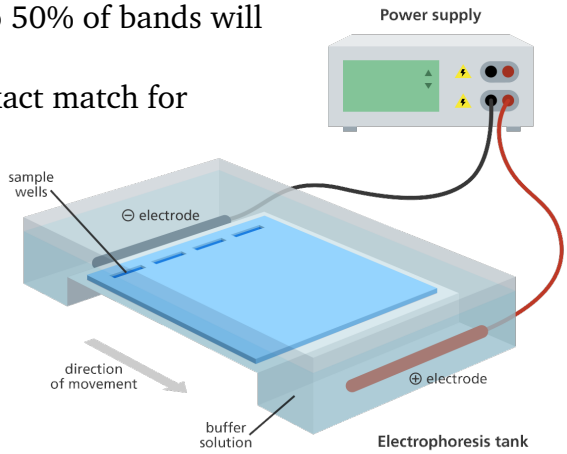
- Uses gel to seperate molecules by moving them in an electric field
- Molecules seperate due to size

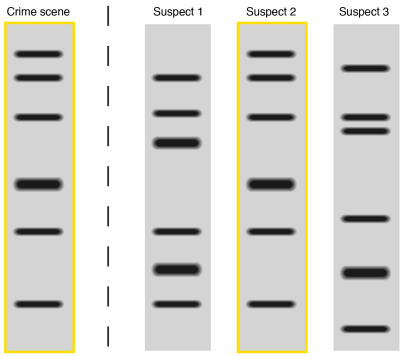
## STEPS

- DNA prepared (cut with restriction enzyme + amplified with PCR)
- Agarose gel covered in buffer solution
- DNA mixed with a loading dye to make it visible for pipetting
- DNA is loaded into wells of the gel
- Negative electrode is placed near wells, positive electrode placed at other end
- DNA fragments have a negative charge so they flow to positive end
- Big fragments move slowly + less, small fragments move quickly + further
- DNA fragments seperate producing a DNA profile
- Banding patterns are then analysed

**USES**

- Paternity tests (child is a mix of maternal/paternal DNA so 50% of bands will be shared with mother/father)
- Forensic appliances (crime scenes, banding has to be an exact match for suspect + DNA sample)
- Conservation (identification of endangered species + them from illegal trade)
- Identification of mutations
- Identification of specific alleles




**DNA MICROARRAYS**

- Purpose: determine gene expression (if gene is switched on and transcribed or switched off)
- Gene probe: tool that searches for for a specific region within a genome, for example it finds the HD gene in DNA that causes Huntington's disease
  Gene probe is single stranded so it can bind to the target DNA sequence and Identify the mutation

**Advantages**

- Allows large # of genes to be screened simultaneously
- Helps identify mutations by determining nucleotide sequence to see where difference are

**Uses**

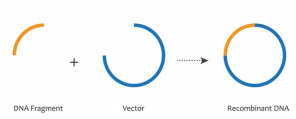
- Can identify diseases like breast cancer genes or interesting DNA and mutations

**Process**

- DNA array is a collection of microscopic DNA sequences attached to a solid surface
- If a gene is active within a cell → mRNA is transcribed
- mRNA can be converted (using reverse transcription) into cDNA (Copy DNA)
- cDNA binds (hybridises) to complementary DNA (gene probe) on the DNA chip
- If the cDNA is fluorescently labeled, then the complementary DNA (probe) can be identified
- Each probe is known as a gene sequence
- Greater the genes expression → more fluorescence is seen in microarray well

- Recombinant DNA technology and DNA identification technologies are applied in agriculture and environmental conservation

**GENE CLONING**




- Purpose: clone/create large copies of a gene
  Used to create/harvest biopharmaceuticals (insulin)

**Process**

1. Cutting + pasting DNA: Target gene is isolated, then cut out using restriction enzymes
   Bacterial plasmid is cut open using same restriction enzyme
   DNA ligase ligates the target gene into the plasmid
   Resulting plasmid is the recombinant plasmid

2. Bacterial transformation: R.P is inserted into bacteria by transformation
   Some bacteria will pick it up, other won't

3. Bacterial selection: Plasmid selected will contain antibiotic resistance genes
   Bacteria is grown on an agar plate containing antibiotics
   Only bacteria with the R.P will survive + growth

4. Harvesting amplified DNA/protein: Bacteria then placed in optimal growth conditions
   Allows bacteria to replicate lots
   Bacteria then listed (cut open) + plasmids extracted
   Or the proteins produced can be harvested for use

| Applications | Agriculture | Conservation |
|---|---|---|
| Uses | - Modify plants to have increased nutrients for consumption<br>- Build fungicides/insecticides into plant to make it resistant to pests<br>- Herbicide resistant stops the crop from getting killed, less environment pollution | - Small populations of vulnerable species are more likely to inbreed (more mutations and increased chance of alleles becoming homozygous)<br>- DNA profiles allow appropriate individuals to reproduce (no inbreeding)<br>- Allows study of genetic diversity in a species<br>- Preserve the genetics and improve long term survival rate |
| Bad Effects | - Pests can come resistant to new plants as they eat them all the time<br>- Over use of herbicides<br>- Cross pollination can wipe out the original plant strain<br>- May effect other plants/species in an unpredicted way | |

**GMO & TRANSGENICS**

- GMO organism: has been genetically modified by addition/deletion of genes or insertion of DNA from another individual/species
- Transgenic organism: has been modified by incorporating DNA from another species into its genome

TRANSGENIC PLANTS: AGROBACTERIUM + TI PLASMIDS

- Agrobacterium cause crown gall disease in plants
- They insert a Ti plasmid into the cells of the host
- Ti plasmids codes for genes, which code for enzymes that cut host DNA + integrate a segment of Ti plasmid
- Host plants cells are modified via horizontal gene transfer
- This vector method used instead of gene gun as its more effective

TRANSGENIC ANIMALS: MICRO-INJECTION OF DNA

Micro-injection

- Desired genes injected directly into embryo using a needle
- Causes genes to be distributed randomly
- If it works, only 1 copy of the target cell will be in each cell

Embryonic stem cells

- Desired genes introduced into an embryonic stem cell
- Stem cells can develop into any tissue type later
- Used to introduce desired gene in a specific location in genome, therefore certain cells can be picked which will turn into desired cell types

SELECTIVE BREEDING VS GMO

Selective breeding: used to enhance desirable characteristics in organisms

| | GMO | Selective Breeding |
|---|---|---|
| Differences | - Quick + effective<br>- Allows specific/precise modifications | - Takes generations to achieve<br>- Can affect other genes inadvertently |

GMO EXAMPLES

| | How GMO is made | How the GMO is useful |
|---|---|---|
| Golden Rice | - Biofortified to produce beta carotene which is converted into vitamin A<br>- Transformed with genes from a bacteria and a daffodil plant | - Helps reduce vitamin A deficiency in humans (effective in developing countries)<br>- Increases nutritional value of crops |
| Super Salmon | - A new growth hormone replaced in salmon<br>- Transgenic modification of a single gene in the 1st salmon | - Allows salmon to grow in cold winter conditions when they used to stop<br>- Harvested quicker for consumption, 16-18 months instead of 3 yrs |
| BT Cotton | - Gene coding for BT toxin inserted into cotton as a transgene<br>- It produces natural insecticide in it's tissue | - Kills the bollworm pests that eat the cotton<br>- Reduced use of insecticide increases the population of beneficial insects |

# Continuity of Life on Earth

- Life has existed on Earth for approximately 3.5 billion years and has changed and diversified over time

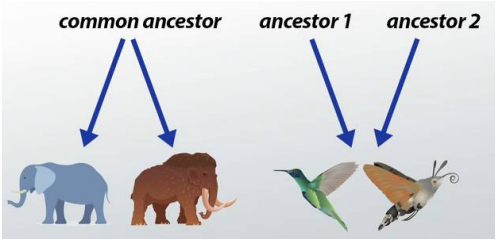
- 1st organisms were prokaryotes (archaea then bacteria) that are anaerobic
- Eukaryotes evolved when 1 prokaryote engulfed another and didn't digest it and it provided it with energy
- Photosynthesis from eukaryotes created oxygen

## DIVERGENT EVOLUTION

- 1 species diverges into 2 or more different species
- Driven by environmental pressures (food sources/predation)
- Adaptive radiation: occurs when 1 species becomes many in a relatively short time period
- Example: Australian marsupials have a common ancestor but have diverged into new species like possums + bandicoots

## CONVERGENT EVOLUTION

- When unrelated species become more alike by developing similar characteristics
- Become progressively more alike due to exposure to the same environmental pressures
- Example: Australian wombats share characteristics with American ground hog




- Evolutionary relationships between groups can be represented using phylogenetic trees

## PHYLOGENETIC TREE

- Shows evolutionary relationships through branches
- Created using morphology + more recently, DNA evidence
- Change when new evidence is found or species are recognised

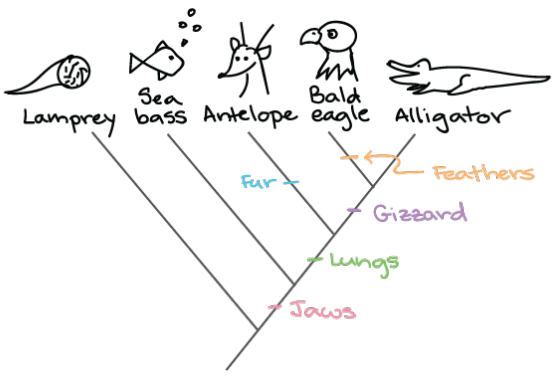
## STRUCTURE

- Recent descendants located at tips
- Common ancestor located at base
- Nodes demonstrate a common ancestor of 2 species
- Branch length indicates time (longer = more yrs)
- Closely related species are close, distantly related species are further away in diagram

## HOW TO MAKE PHYLOGENETIC TREE

- Identify oldest species and the logical order of the development of traits and graph the

| Feature | Lamprey | Antelope | Bald eagle | Alligator | Sea bass |
|---|---|---|---|---|---|
| Lungs | 0 | + | + | + | 0 |
| Jaws | 0 | + | + | + | + |
| Feathers | 0 | 0 | + | 0 | 0 |
| Gizzard | 0 | 0 | + | + | 0 |
| Fur | 0 | + | 0 | 0 | 0 |



- Evidence for the theory of evolution includes
- Comparative genomics (molecular evidence)
- Includes comparing DNA sequences or proteins (amino acid sequences) of various species
- More shared DNA = closer relation
- Closely related species will share higher level of similarities in proteins like cytochrome and haemoglobin

DNA Hybridisation: technique used to determine the amount of similarity between 2 species

Steps
1. Extract DNA from species
2. Heat DNA until it's single stranded (92)
3. Mix and cool hybridised DNA
4. Complementary bonds form between matching base pairs creating hybridised DNA
5. Level of hybridisation tested by heating the DNA
6. Stability of hybridised DNA measures how similar species DNA is

- More bonds → more heat to seperate → more closely related species are

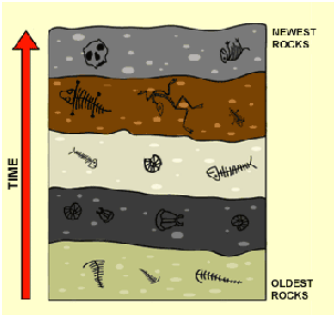
- The fossil record
- Fossil: Traces of a past life showing previously existing species that may be extinct now
  Can demonstrate how species have changed
- Types: bone, mineralisation, imprints
- Fossils don't show all species over time, holes exist due to incorrect fossilisation conditions
- Example: equine fossils show how leg length has increased over time and hoof development
- Archaeopteryx: a transitional species between feathered dinosaurs and modern birds
  Possesses non-avian dino similarities like long feathered tail + small teeth
  Possesses features non-avian dinos don't have like flight feathered + wings
  Furcula (fused clavicle) bone confirms birds + dino relation as they only have this anatomical feature

Formation
- Requires specific conditions: Low oxygen
  Covered in sediments (mud/silt/sand), often in lakes
  Bones mineralise when sediments replace bones/shell
  Sediments prevent decomposition and scavengers
  Sedimentary rocks tend to have fossils, metaphoric/igneous don't

Absolute Dating: Radiometric dating
- Relies on the predictable decay of isotopes
- Half life: time taken for half of isotope to decay
- Carbon-14: Isotope that breaks down into nitrogen-14
  Accurate for samples up to 12,000 yrs, relies on presence of organic carbon

- Potassium-40: Isotope breaks down into argon
  Can date items up to 4 billion yrs old
  Used to date rock surrounding the fossil

Relative/Comparative Dating




- Determines relative date of rocks/fossils
- Strata are layered deposited sediment layers in a progressive age order
- Law of superposition: states each rock layer is older than the one above it
- Fossils lower in strata are older
- Index fossils: fossils commonly widely distributed from a specific time frame. If found with a fossil of interest, it can help date the fossil. (Ammonites from the Mesozoic Era)

- Comparative anatomy and embryology

**Comparative Anatomy:** involves comparing anatomical structures of species

- Homologous structures: Common anatomical structures shared by different species
  Evidence of common ancestor
  Ex: pentadactyl limbs (humans/bats/whales) have same bones

- Vestigial structures: Bones or organs which are present but have no apparent function
  Suggest evolution from an ancestor where they were functional
  Ex: appendix in humans no longer has function, coccyx (tail)

Analogous V Homologous Structures

- Analogous structures: Different structures in different animals with similar functions
  Not from a common ancestor
  Ex: insect wings and bird wings
- Homologous structures: similar structure in different animals with different functions
  From a common ancestor (homo=same)
  Ex: leg of mouse and wing of bat

**Comparative Embryology**

- Some vertebrates possess traits that are present in embryos but aren't obviously present in adult form
- The traits don't have a function but present because they were functional in their ancestors
- Provide evidence of evolution due to *ancestry*
- Ex: All vertebrate embryos have gills - evidence of fish like ancestor
  Whale embryos have limb buds - implying evolvement from land mammals

- Natural selection occurs when selection pressures in the environment confer a selective advantage on a specific phenotype to enhance its survival and reproduction; this results in changes in allele frequency in the gene pool of a population

Natural Selection
- The mechanism that drives the theory of all life descending from shared ancestors is natural selection
- Favoured traits are selected for and inherited → becoming more common in the next generations gene pool
- Acts on phenotypes

**Steps of Natural Selection**
1. Individuals differ and have variation (mutation, crossing over, random mating)
2. More offspring is born than can survive → struggle for survival and only some reproduce
3. Population exposed to a range of selection pressures
4. Some individuals have traits more suited to environment → more likely to survive and pass them on
5. Allele frequency in population changes
6. Individuals with less advantageous traits breed less and their alleles reduce

Adaptive Evolution: driven by natural selection so only organisms best suited to environment survive to reproduce

- In addition to environmental selection pressures, sexual selection, mutation, gene flow and genetic drift can contribute to changes in allele frequency in a population gene pool

**SEXUAL SELECTION**
- Often results in sexual dimorphism: males and females have significant difference in appearance + size
- Example: Peacocks → big tails, peahens → small tails
- Selection is driven by one sex, usually female
- Selected traits would often be selected against in natural selection

Process
- Driven by female choice or male competition
- Female choice: results in elaborate mating displays / vibrant appearances
- Male competition: large + muscular
- Females mate with individuals with desirable characteristics → offspring also has the trait
- Eventually the desirable traits frequency increases in population + undesirable ones lost from gene pool as they fail to breed

**GENE FLOW**

Variation

- All individuals have variation in genotype
- Some have different features/characteristics
- Variation in sexually reproducing organisms yields more variation than asexual organisms

Gene Pools: total number of alleles present in a population

- Characteristics determined by alleles present
- Population: group of individuals that live in same geographic area + reproduce to produce fertile offspring

Gene Flow:

- Most populations have some level of immigration/emigration
- Occurs when immigrants enter a population and breed
- Genes lost from populations when individuals emigrate + don't breed with population anymore
- Prevention of gene flow creates a new species/speciation

Genetic Drift: occurs by random-chance process when only small # of individuals reproduce. Usually occurs in small, isolated populations

- Means only a random subset of alleles passed onto next generation → allele frequency changes from 1 generation to next → alleles often lost
- Alleles can become fixed (recessive alleles are fixed)
- Offspring formed by small # of individuals aren't fully representative of full parent pop.

| | Bottleneck Effect | Founder Effect |
|---|---|---|
| Cause | - When a catastrophic event (fire or human intervention) drastically reduces population size | - When a few individuals move to a new area that have been isolated from bigger pop. |
| Effect | - Alleles lost through chance<br>- Expanded pop. only carries alleles of survivors | - Might not carry all alleles present in original population<br>- Less diversity and increased chance of deleterious recessive alleles coming together |
| Example | - Cheetahs breed with own offspring, mutations more likely | - Amish, have high chance of having Ellis-van Creveld syndrome (recessive trait) |

- Selective breeding (artificial selection) through the intentional reproduction of individuals with desirable characteristics results in changes in allele frequencies in the gene pools over time

**ARTIFICIAL SELECTION**

- Process where humans selected individuals that show a desirable trait to breed
- Selected individuals pass the desired alleles on → frequency of the alleles increase within population over generations

Examples: dog breeds, crop foods (carrot), cattle

- Traits selected wouldn't generally be selected in natural selection as they don't benefit the organism

| | Natural Selection | Artificial Selection |
|---|---|---|
| Reason | - Selection pressures are natural<br>- Organism must adapt to survive | - Humans are the selection pressure<br>- Choose specific animals to breed |
| Result | - Species being more suited to environment<br>- Increases survival chances<br>- Changed frequency in alleles | - Species develop increase in traits that benefits humans<br>- Changed frequency in alleles |
| Time | - Very slow<br>- Happens over many generations | - Still slow but quicker as breeding is controlled<br>- Happens over many generations |

- Speciation and macro-evolutionary changes result from an accumulation of micro-evolutionary changes over time

- Macro-evolutionary changes occur die to an accumulation of micro-evolutionary changes

<u>Micro-Evolution</u>

- Small scale variation of allele frequencies in the gene pool of a species
- New individuals are still the same species as their ancestors (same taxonomic group)

Examples: colour, size

<u>Macro-Evolution</u>

- Variation in allele frequencies at or above species level, produces a new species
- Large scale evolution a changes
- New individuals are a different species to ancestors

Examples: origin of mammals, evolution of humans, separation fo aquatic/terrestrial animals

- Differing selection pressures between geographically isolated populations may lead to allopatric speciation

Steps of Allopatric Speciation

1. Initially a single population exists
2. Physical barrier then divides the population (change in river movement, population spreads across island, lava flow seperate s a population)
3. Gene flow is prevented between populations
4. Selection pressures are different on each side of the barrier
5. Population on either side of the change die to natural selection
6. Differences also accumulate due to genetic drift +mutation
7. Differences increase/accumulate over many generations
8. Individuals no longer able to interbreed → new species is formed due to speciation

Example: Galapagos Island Finches

- Populations with reduced genetic diversity face increased risk of extinction
- Loss of genetic diversity → increases risk of extinction → due to reduced ability to adapt to changes in the environment as all individuals are similar (natural selection operates on differences)
- Diseases can spread quickly through the population
- Inbreeding may occur → increases chances of fixing of alleles (reducing diversity) and reduce fitness and creates abnormalities
- Genetic drift: also contributes to loss of diversity as some alleles die out