A3.1 Diversity of Organisms

Learning Outcomes

1SL **Variation between organisms as a defining feature of life**
Students should understand that no two individuals are identical in all their traits. The patterns of variation are complex and are the basis for naming and classifying organisms.

2SL **Species as groups of organisms with shared traits**
This is the original morphological concept of the species as used by Linnaeus.

3SL **Binomial system for naming organisms**
Students should know that the first part of the name identifies the genus, with the second part of the name distinguishing the species. Species in the same genus have similar traits. The genus name is given an initial capital letter but the species name is lowercase.

4SL **Biological species concept**
According to the biological species concept, a species is a group of organisms that can breed and produce fertile offspring. Include possible challenges associated with this definition of a species and that competing species definitions exist.

5SL **Difficulties distinguishing between populations and species due to divergence of noninterbreeding populations during speciation**
Students should understand that speciation is the splitting of one species into two or more. It usually happens gradually rather than by a single act, with populations becoming more and more different in their traits. It can therefore be an arbitrary decision whether two populations are regarded as the same or different species.

6SL **Diversity in chromosome numbers of plant and animal species**
Students should know in general that diversity exists. As an example, students should know that humans have 46 chromosomes and chimpanzees have 48. Students are not required to know other specific chromosome numbers but should appreciate that diploid cells have an even number of chromosomes.

7SL **Karyotyping and karyograms**
Application of skills: Students should be able to classify chromosomes by banding patterns, length and centromere position. Students should evaluate the evidence for the hypothesis that chromosome 2 in humans arose from the fusion of chromosomes 12 and 13 with a shared primate ancestor.
NOS: Students should be able to distinguish between testable hypotheses such as the origin of chromosome 2 and non-testable statements.

8SL **Unity and diversity of genomes within species**
Students should understand that the genome is all the genetic information of an organism. Organisms in the same species share most of their genome but variations such as single-nucleotide polymorphisms give some diversity.

9SL **Diversity of eukaryote genomes**
Genomes vary in overall size, which is determined by the total amount of DNA. Genomes also vary in base sequence. Variation between species is much larger than variation within a species.

10SL **Comparison of genome sizes**
Application of skills: Students should extract information about genome size for different taxonomic groups from a database to compare genome size to organism complexity.

11SL **Current and potential future uses of whole genome sequencing**
Include the increasing speed and decreasing costs. For current uses, include research into evolutionary relationships and for potential future uses, include personalized medicine.

12HL **Difficulties applying the biological species concept to asexually reproducing species and to bacteria that have horizontal gene transfer**
The biological species concept does not work well with groups of organisms that do not breed sexually or where genes can be transferred from one species to another.

13HL **Chromosome number as a shared trait within a species**
Cross-breeding between closely related species is unlikely to produce fertile offspring if parent chromosome numbers are different.

14HL **Engagement with local plant or animal species to develop a dichotomous key**
Application of skills: Students should use local plant or animal species to develop a dichotomous key.

15HL **Identification of species from environmental DNA in a habitat using barcodes**
Using barcodes and environmental DNA allows the biodiversity of habitats to be investigated rapidly.

Guiding Questions

What is a species?
What patterns are seen in the diversity of genomes within and between species?

Linking Questions

What might cause a species to persist or go extinct?
How do species exemplify both continuous and discontinuous patterns of variation?

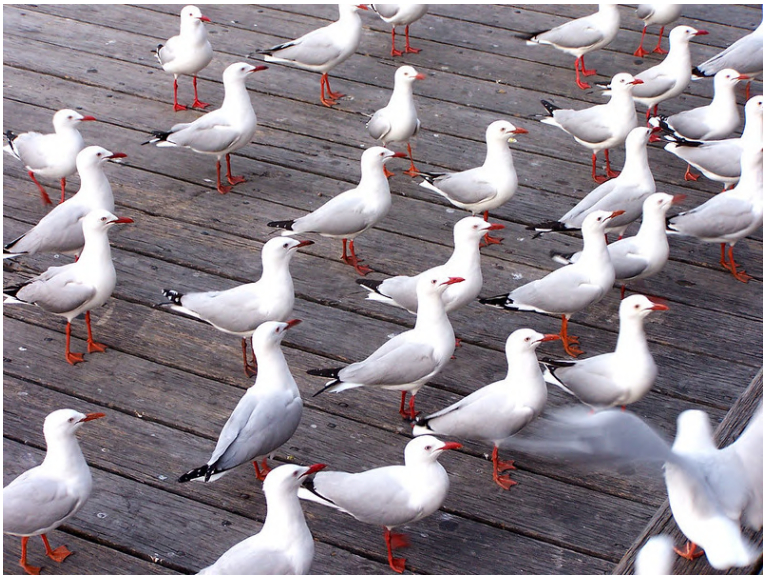
In memory of Gunter Bechly

1SL Variation between organisms as a defining feature of life

organism – an individual life form

variation – differences between individuals

- intraspecific variation – differences between individuals within the same species
- interspecific variation – differences between individuals across different species



Trish Flickr CC BY-NC-ND 2.0

These seagulls appear to be the same however, careful measurements will reveal each bird varies slightly in beak length, body mass and other traits.

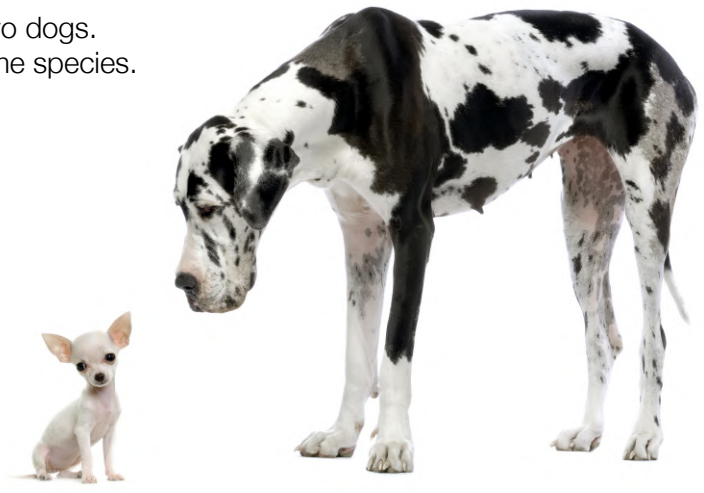
No two individuals in a population are identical in their traits.

Even identical twins begin to pick up unique mutations in their DNA over their lifespan.

Identical twins also have unique fingerprints. The same genes controlling their fingerprints add a random component to the pattern.

Members of the same species can also show great variation in features.

Imagine digging up fossil bones of these two dogs.
It would not be clear that they were the same species.

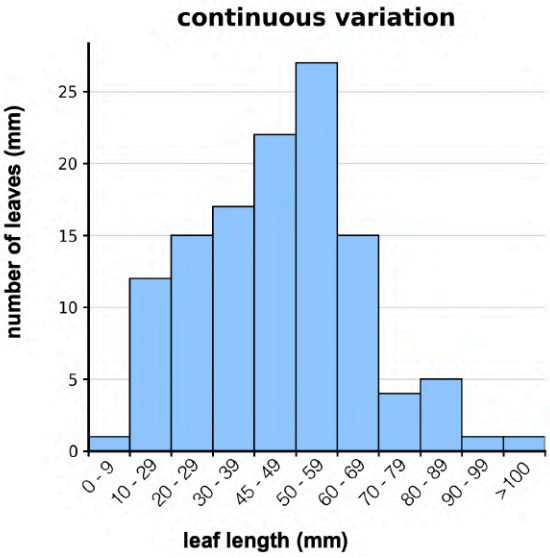


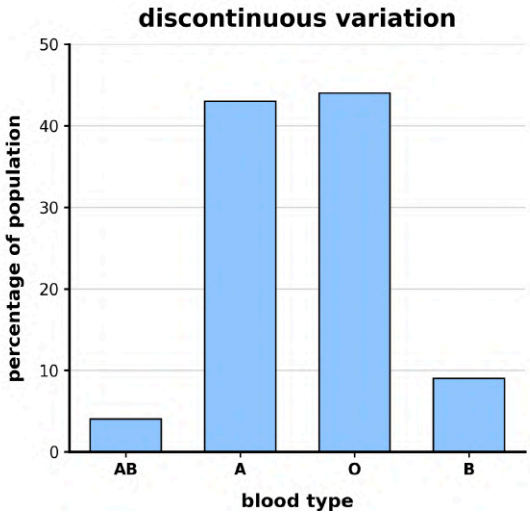
shutterstock

Patterns of variation are complex and determined by genes and the environment.
Variation is the main way that living things are named and classified.

continuous variation – characteristic that changes gradually between a range of quantitative values
- example beak length, seed mass, shoot dry mass
- often fits a normal distribution or bell curve
- involve many genes (polygenic), often influenced by environment

discontinuous variation – phenotype characteristic that falls into discrete qualitative categories
- example blood type, biological sex, pea flower colour
- usually one gene with different alleles, less influenced by environment





2SL Species as groups of organisms with shared traits

morphology – visible internal and external structures of an organism

physiology – study of the function of internal parts

Carl Linnaeus (1707-1778) was a Swedish botanist who named thousands of plants and animals. He is known as the Father of Taxonomy.

He grouped living things according to their morphology.

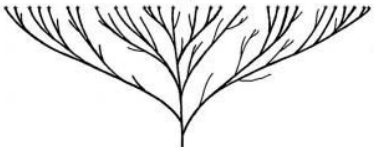
morphological species concept – species are a group of organisms with shared traits

Optional Extension (not required for the IB exam)

The idea that species were unchanging (fixity of species) arose from Greek thinkers such as Aristotle.

lawn model

Darwinian evolutionists believe that species can change across major groups from bacteria to mankind.



tree model

Linnaeus was a creationist. At first, he thought that species did not change, however, when he later observed hybridization, he realized they do change. He understood there is adaptation within major groups.

orchard model

The Hebrew word for *kind* in the Bible was translated as *genus* or *species* in the Latin.
The meaning of a *kind* or *species* used in the bible is different to the Linnaean *species*.
The bible teaches a fixity of *kinds* not a fixity of *species*.
An example is the cat *kind* and the great diversity of cat *species* within it.

"Finis creationis telluris est gloria Dei
ex opere Naturae per Hominem solum."
(The end of the Earth's creation is the glory of God,
as seen from the works of Nature by man alone.)
Carl Linnaeus

3SL Binomial system for naming organisms

In the time of Linnaeus, plants had long descriptive names that often included their human uses.
The tomato was called: *Solanum caule inermi herbaceo, folis pinnatis incisis, racemis simplicibus*.
Translated: herbaceous nightshade with smooth stem, incised pinnate leaves and a simple raceme

istock

Linnaeus introduced a two-part name.

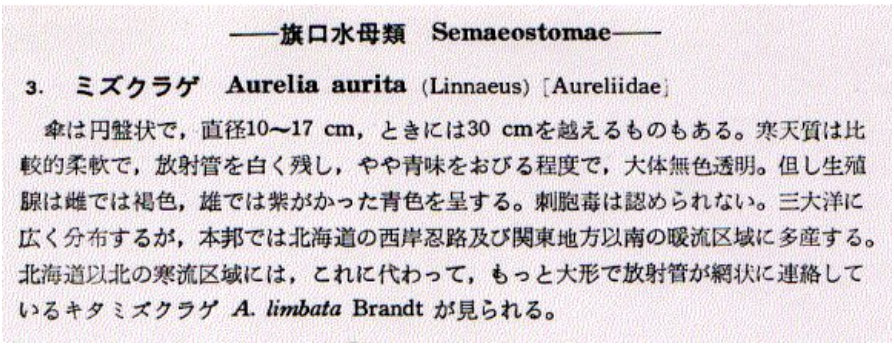
binomial system – international system that uses a unique two-part Latin name for each organism

Common name:
dog / gǒu / perro / chien / calb / kukura / hund / inu / anjing / subaka

Scientific name:
Canis familiaris L.
Genus species

It is written in *italics* or underlined.
The genus name is capitalized. The species name is not capitalized.
The author who named the species can be included eg. Linnaeus.
First mention in full then later it can be abbreviated to *C. familiaris*

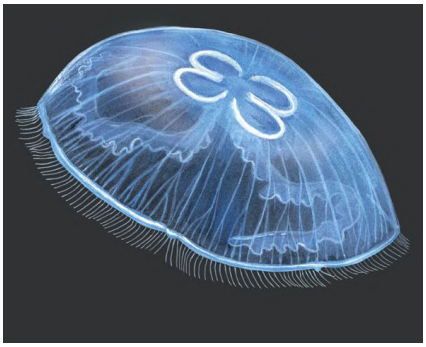
Here is a description of a common jellyfish in a Japanese guide to marine life.



——旗口水母類　Semaeostomae——

3.　ミズクラゲ　**Aurelia aurita** (Linnaeus)［Aureliidae］

傘は円盤状で，直径10～17 cm，ときには30 cmを越えるものもある。寒天質は比較的柔軟で，放射管を白く残し，やや青味をおびる程度で，大体無色透明。但し生殖腺は雌では褐色，雄では紫がかった青色を呈する。刺胞毒は認められない。三大洋に広く分布するが，本邦では北海道の西岸忍路及び関東地方以南の暖流区域に多産する。北海道以北の寒流区域には，これに代わって，もっと大形で放射管が網状に連絡しているキタミズクラゲ *A. limbata* Brandt が見られる。

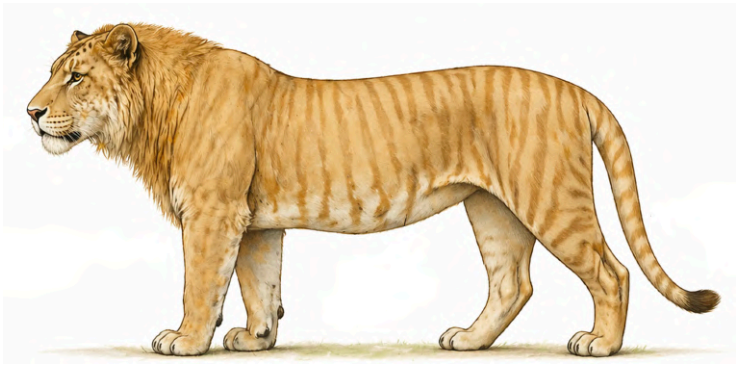


4SL Biological species concept

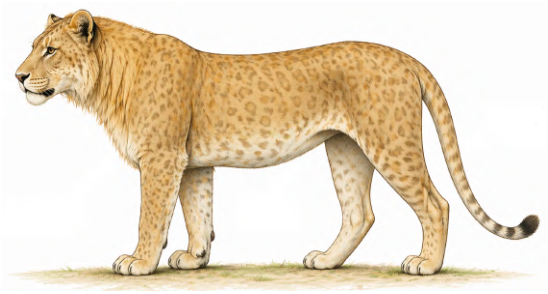
biological species - group of organisms able to produce fertile offspring

The biological species grouping is not aways clear:
- o different species sometimes produce hybrids – many are infertile but some are fertile
  - often this occurs outside their normal habitat for example in zoos or by artificial selection
- o many organisms reproduce asexually
- o it is not always clear which fossil species could breed together
- o it is not always possible to bring isolated species together to check if they can breed
- o distant populations of a *ring species* do not breed together but they are connected by interbreeding populations – where does one species start and end?

Liger (male lion x female tiger)
- faint stripes
- often larger than parents



Leopon (male leopard x female lion)
- spots are brown not black
- size of a lioness



AI generated biocode.co

Other species definitions include:
morphological species – organisms grouped by shared traits
Challenges: a species may be highly variable. Also different species can appear very similar.

ecological species – organisms grouped by niche similarity including abiotic and biotic factors
Each niche requires unique adaptations.
Helpful to tell microbes apart such as two species of *Paramecium.*

evolutionary species – organisms grouped by a recent common ancestor
A single lineage of populations descended from a common ancestor that remain separate from other lineages and have their own unique selective pressures for example the dingo.
Challenges, hybridization between separate lineages can sometimes occur.

Each definition is helpful. The biological species definition remains the most widely used.

Other ways to identify a species are to check:
- o genome – chromosome number and DNA sequences show how closely related species are
- o proteome – proteins/biomolecules involved in metabolism can help group different organisms
- o courtship behaviour which prevents interbreeding

5SL Difficulties distinguishing between populations and species

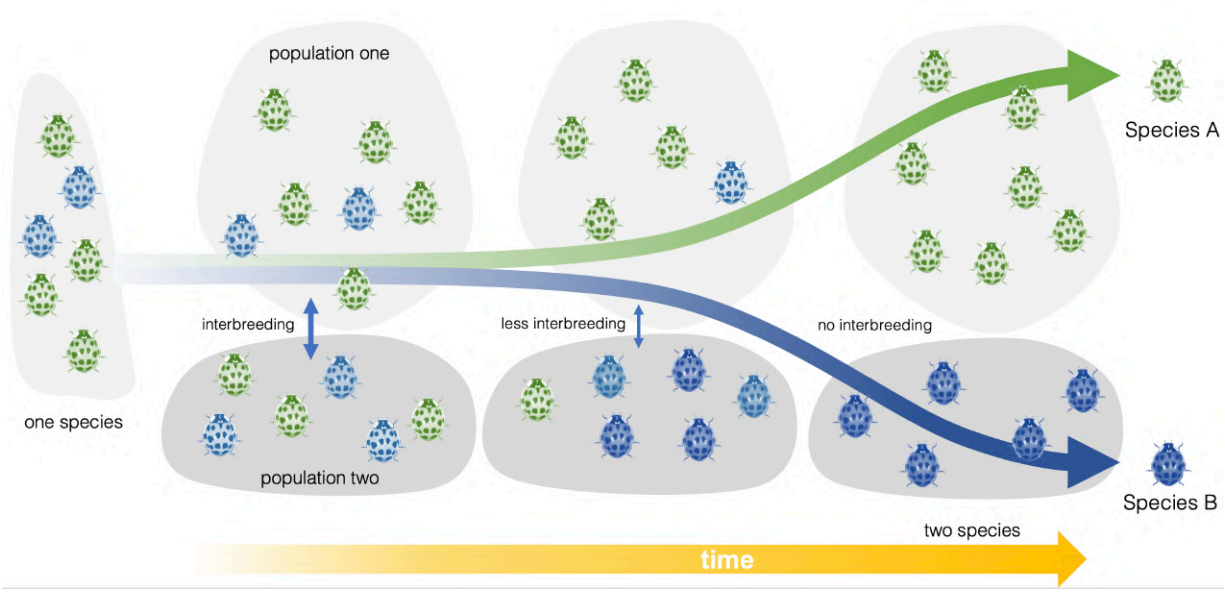
population- group of individuals of the same species living close enough to interbreed

speciation – process where one species splits into two or more new species

A population is the smallest unit of evolution.
New species often arise when two populations become reproductively isolated (see A4.1).
Each population adapts to their unique environment until eventually they can no longer interbreed.

This process usually occurs gradually as traits and behaviour change.
Thus, the decision to regard two populations as the same species or as different species is arbitrary.

At what point do the two populations form truly separate species?



The woolly mammoth went extinct thousands of years ago.
At one time it was considered to be the same species as the Asian elephant *Elephas maximus*
*R*esearchers are unable to tell it if they could breed together, therefore, a fairly arbitrary decision was made to make it a different genus *Mammuthus primigenius*.

6SL Diversity in chromosome numbers of plant and animal species

Members of the same species have the same number of chromosomes.
Organisms with a different number of chromosomes are unlikely to breed successfully.
Although rare, chromosomes can fuse together or split apart to change chromosome number.

diploid - having two sets of chromosomes (giving an even number)
Polyploidy in plants through non-disjunction of a whole set of chromosomes during meiosis can cause chromosome numbers to instantly double.

Chromosome number is not a valid indicator of genetic complexity of an organism.
Less complex organisms may have high chromosome number due to having:

- many short chromosomes
- multiple sets of the same chromosomes

scientific name	common name	diploid chromosome number	protein coding genes	million base pairs (Mb)
Caenorhabditis elegans	nematode	6	≈19 983	100
Oryza sativa	rice	24	≈40 000	3 850
Myrmecia pilosula	jumper jack ant	2 (male ants have 1)		215
Polyommatus atlanticus	blue butterfly	452		
Canis familiaris	dogs	78	≈19 000	2 500
Pan troglodytes	chimpanzee	48	≈19 000	3 200
Homo sapiens	humans	46	≈19 433	3 100

We have a similar number of protein coding genes as a nematode – how does that make you feel? Many animal cell organelles and their cell proteins are similar in different animals – therefore humans share about 60-80% of genes with nematodes. However, there is greater gene splicing in humans - so we can produce more proteins than a nematode.

Chimpanzee and human DNA is said to be 98% similar. Banding patterns also align very well. This agrees with Darwinian evolution which predicts a common ancestor. However, we have 46 chromosomes and chimpanzees have 48? If we share a common ancestor then either:

- an ancestor leading to humans lost a chromosome pair – unlikely as this would be fatal
  OR
- an ancestor leading to humans decreased chromosome number by fusion of chromosomes

Evidence for the fusion hypothesis is found on page 11.

We humans like to think of ourselves as special, set apart from the rest of the animal kingdom by our ability to talk, write, build complex structures, and make moral distinctions. But when it comes to genes, humans are so similar to the two species of chimpanzee that physiologist Jared Diamond has called us "the third chimpanzee."
Ann Gibbons 1998

7SL Karyotyping and karyograms

karyotype - number and type of chromosomes an organism has in its nucleus

karyogram - image of the chromosomes of an organism arranged in homologous pairs by length

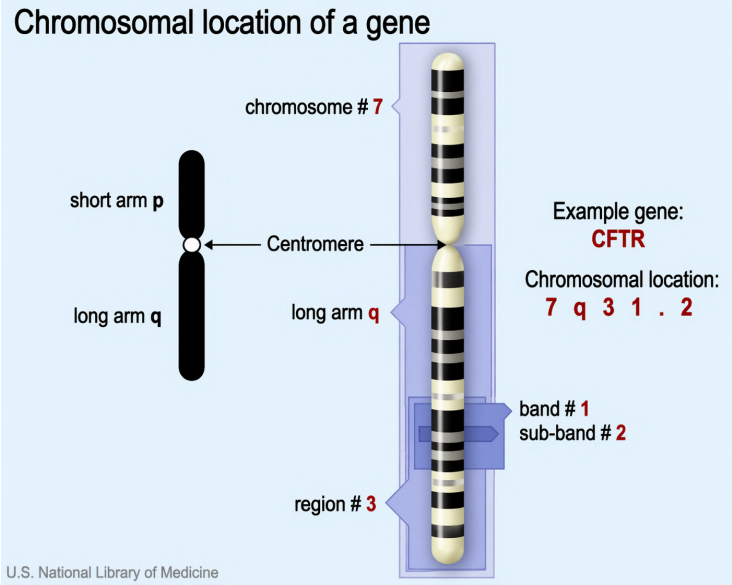
Human females have two X chromosomes and males have an X and a Y.

Cells are stained and placed under a microscope.
Chromosomes condense and become visible during cell division.
DNA readily absorbs stains. Chromosome means coloured (*chromo*) bodies (*some*).

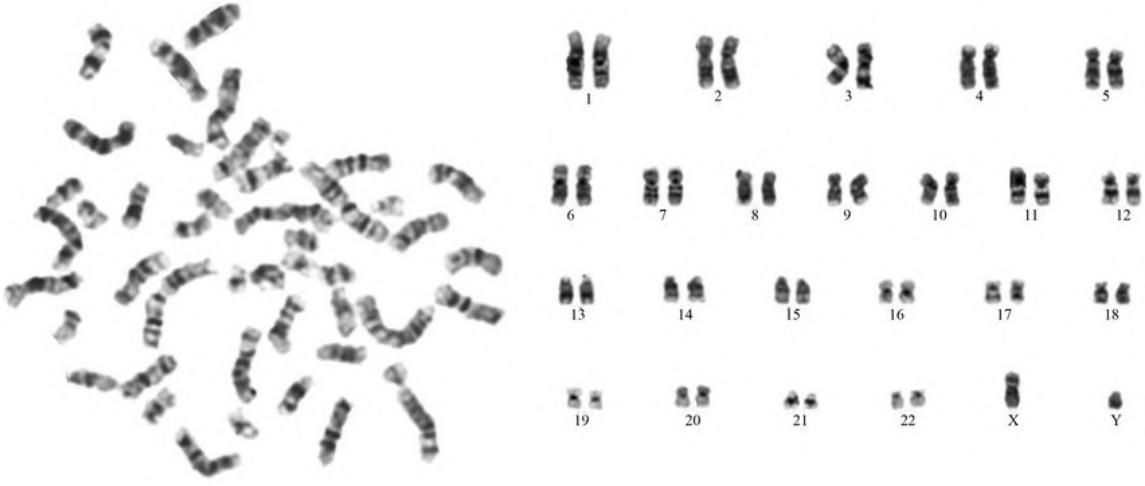
During metaphase the cell is lysed (burst) and a picture taken of the unsorted chromosomes.

Each replicated chromosome is digitally cut and pasted:

- in order of length
- centromere location
- banding patterns



unsorted sorted



Yan et al 2019

The chromosomes appear thick as they are actually composed of two chromatids closely stuck together because anaphase has not yet occurred.

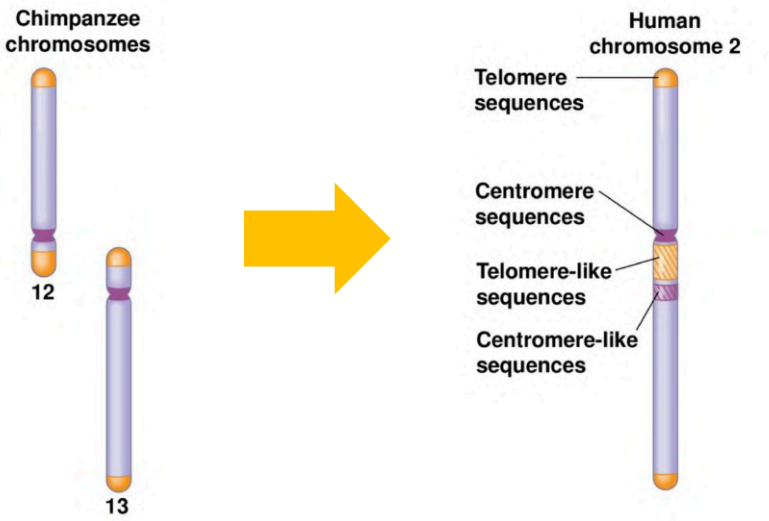
NOS

A good testable hypothesis:

a) has measurable data
b) occurs under well-defined conditions
c) is predictive and limited to one concept at a time – if x occurs, then y will happen
d) states what will happen if x occurs
e) can be falsified
f) can be replicated

Fusion of chromosome 2 from chromosomes 12 and 13 of a primate ancestor is a testable hypothesis because:

a) it has measurable data in the form of genetic coding
b) there are reasonably clear definitions describing evidence for chromosome fusion
c) there is one cause (a fusion) and clear outcome (two chromosomes fused together)
d) it predicts what should be found on human chromosome 2
e) it can be falsified if the expected features are missing
f) cannot be easily replicated



Chromosome 2 matches chromosomes 12 and 13 by:

- length
- centromere location
- centromere-like sequences location
- alignment of banding patterns
- telomere-like sequences location

Optional Extension (not required for the IB exam)

Is the evidence for the chromosome 2 fusion theory unmistakable?

A few geneticists question the fusion hypothesis and raise the following points:

- all documented fusions in living mammals involve satellite DNA but it is missing in chromosome 2
- telomere to telomere fusions have only been seen in cancer cells
- the double telomere region unexpectedly has few repeats – it is said to be highly degenerate
- joining two telomere ends should form two sections that run in opposite directions, however, the telomeric repeats are scattered and run in both directions
- the telomere region may be an *interstitial telomere* – these are found scattered over the genome
- the predicted join location is in the middle of a functional promoter of an RNA helicase gene
- the extra 'cryptic' centromere region is very small, occurs in the middle of a transport gene and codes for amino acids in the protein of that gene
- this is an example of forensic science – possible steps that led to fusion are not fully repeatable
- it can be hard to falsify if the expected pattern is badly erased by genetic changes over time

Tomkins 2020

These unexpected findings show the evidence is unclear and faces a number of challenges.

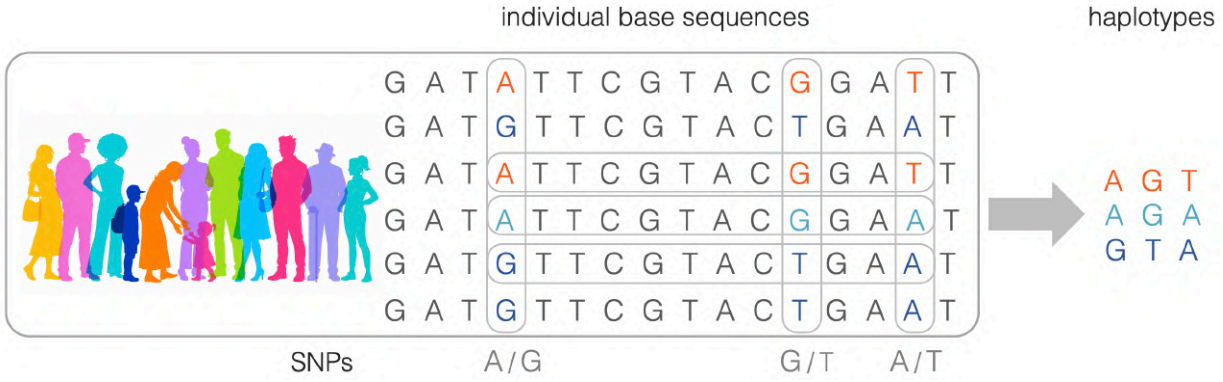
8SL Unity and diversity of genomes within species

genome - all the genetic information of an organism or species

Includes the nucleus, mitochondria, chloroplasts in plants and plasmids in bacteria.

Genes come in alternative forms called alleles. Most genes have two alleles.
The alleles may only be a few bases different and yet code for a different phenotype.
Human variation (diversity) is due to the unique combination of alleles each person has.
Each person also has a unique number of random base mutations scattered throughout their genome.

single nucleotide polymorphism SNPs – locus where more than one base may be present (SNPs - pronounced snips)



There are >600 million SNPs in the human population – most are rare and others are more common.
An individual may carry >5 million SNPs in their DNA and 300-500 of the more common ones.
They can be used to determine ancestry, risk of genetic disease, in forensics or to improve agriculture.
About 5% of SNPs make a change to phenotype, the rest are silent or neutral point mutations.

The Human Genome Project database:

- from 1990 to 2003 mapped the 3 billion base pairs of human DNA
- at first estimated there were 23 000 protein coding genes (now closer to 19 000 - 20 000)
- protein coding genes only make up about 2% of the genome
- genes were found to be almost identical (99.9%) from one person to another

ENCODE Project database see nature video:

- aims to find the function of each gene and regulatory non-protein coding regions
- focused on RNA transcription and DNA binding sites
- found at least 80% of DNA has a function
- will help identify genetic diseases caused by regulatory non-protein coding regions

Gencode Release 47 2024 for the human genome found:

protein coding genes	19 433	- drugs target a small proportion of proteins
non-coding RNA genes	43 499	- RNA can be used as a drug or drug target = new careers
pseudogenes	14 703	
total number of genes	78 724	
total RNA transcripts	385 659	

The main difference between a worm, chimpanzee or human is not due to the 'islands' of protein coding genes but to the 'sea' of regulatory genes.

Optional Extension (not required for the IB exam)

Comparisons that state human and chimpanzee genomes are 98% similar do not include all of the non-coding DNA or insertions and deletions. As researchers begin comparing this non-coding DNA the similarity may drop closer to 95% or less. If human and chimpanzee genomes are not 98% similar then the evolutionary timeline of 6.5 million years since a shared ancestor becomes problematic.

The non-coding DNA was once thought of as junk.
Some textbooks still state that much of the human genome is junk DNA (Long Story Short) with no known function. Predictions that purposeless mutations randomly formed the genetic code and accumulated a record of chaotic waste and dead ends have hindered genomics research.
Intelligent design theorists predicted that DNA would be highly functional.

'the failure to recognize the implications of the non-coding DNA will go down as the biggest mistake in the history of molecular biology'
John Mattick(2003)

"it is a remarkable fact that the greater part of the genome might as well not be there, for all the difference it makes."
Richard Dawkins (2009)

"The human genome is littered with pseudogenes, gene fragments, 'orphaned' genes, 'junk' DNA, and so many … pointless DNA sequences that it cannot be attributed to anything that resembles intelligent design."
Kenneth Miller (1994)

9SL Diversity of eukaryote genomes

Eukaryote genomes vary in *size* and are measured in millions of base pairs (Mb).
Genome size is similar in members of the same species and varies greatly between different species.

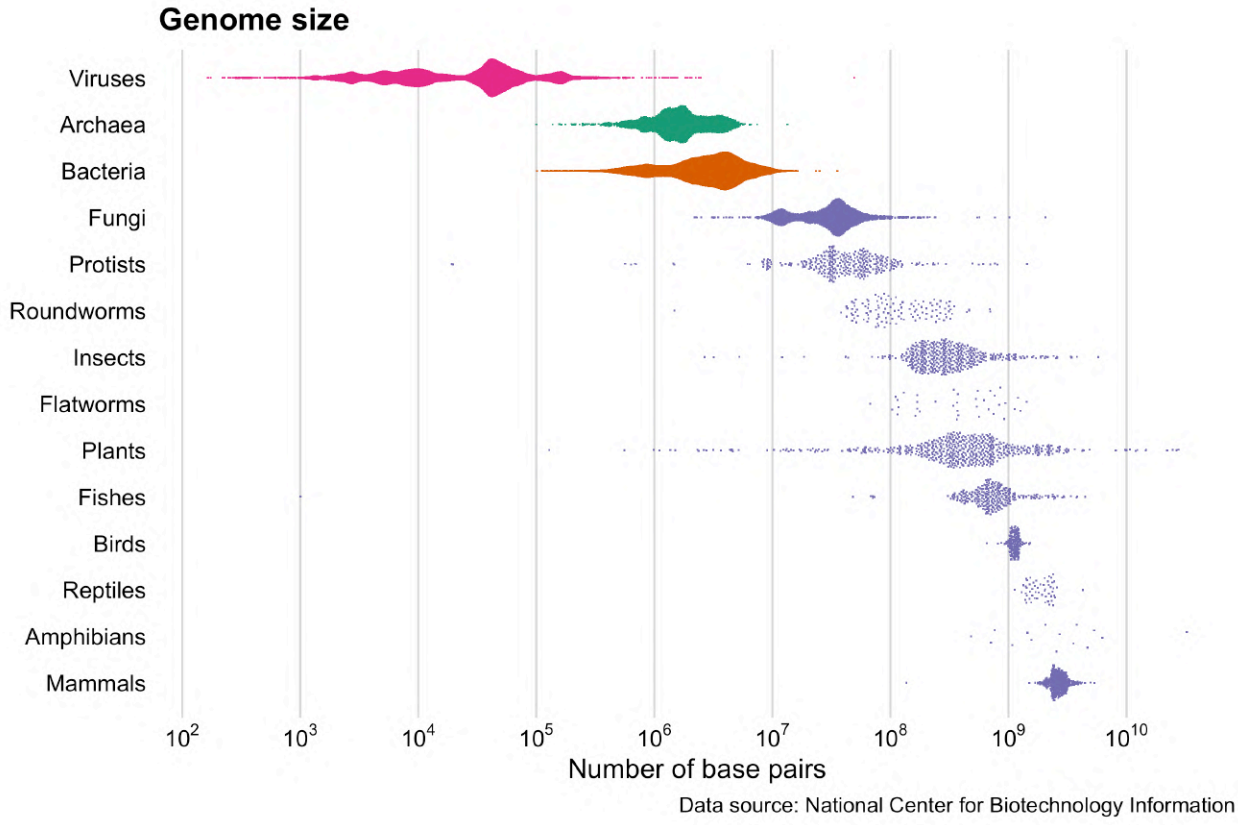
Genomes also vary in their *base sequence* with some variation within the same species. Base sequence variation increases when comparing different species that are more distantly related.

Some genes with a vital function such as cytochrome c involved in respiration have few changes in base sequence even between distantly related separate species.

10SL Comparison of genome sizes

Genome size, gene number and chromosome number all tend to increase with increasing complexity but there are many exceptions.

Reasons for the large variation in chromosome number and gene number include:
- chromosome lengths can vary (many small vs a few large chromosomes)
- RNA splicing can form many proteins from one gene
- more complex organisms tend to have more regulatory DNA
- some species are polyploid and have multiple sets of chromosomes



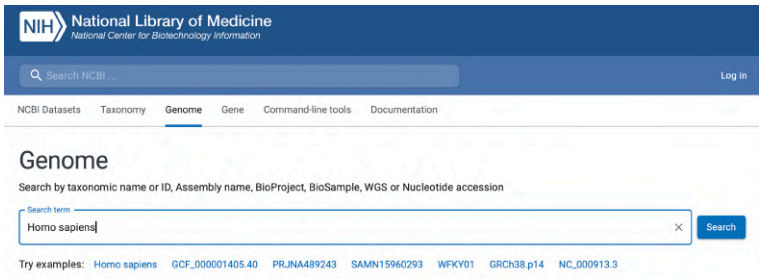
Tom White

Tmesipteris oblanceolata - a fern has the largest genome - 50x bigger than the human genome.

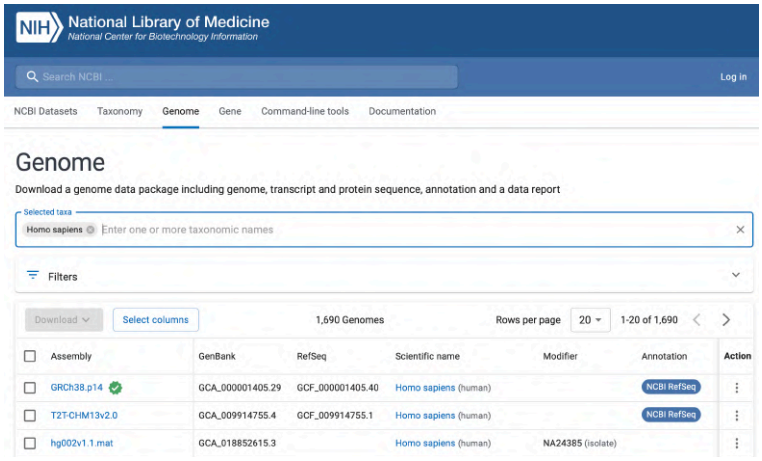
Use of a Database

NIH NCBI genome database

1. type in a species name and click search



2. then click on the first assembly name (blue highlighted link) in the assembly column



3. Record the genome length in Gb or Mb, chromosome number and gene number.

Also try the comparative genome viewer for a visual comparison of genes between species.

11SL Current and potential future uses of whole genome sequencing

Whole genome sequencing can now be done in a matter of days depending on the size of a genome.
The cost of genome sequencing is also decreasing.
This information can allow for *personalized medicine* where drug treatments take into account the unique genetic makeup of a person.

A polygenic risk score can help show how many genes and mutations an individual has that may increase the risk of a heart attack, diabetes or cancer.
This allows changes to be made in lifestyle and diet.
It can also help doctors prescribe the most appropriate medication.

Whole genome sequencing of organisms:
- allows proteins and biochemical pathways to be targeting by pharmaceutical drugs and vaccines
- is used in phylogenetics to classify species and determine their evolutionary ancestry

Evolutionary relationships can be deduced.
Similar sequences suggest more closely related species and more recent divergence.

A whole genome sequencing in birds has found that:
- there was a rapid 'big bang' of bird evolution
- sequences can produce different phylogenetic family trees – as more genomes are sequenced a clearer picture will emerge
- many genes and a sex chromosome may have evolved independently
- vocal learning genes arose independently multiple times in birds and again in humans

Nahmavis grandei Eocene Green River Formation of Wyoming. Matt Mechtley Wikimedia

Optional Extension (not required for the IB exam)

Darwinian evolution predicts gradual increases in complexity of living things over time.
However, the fossil sequence shows sudden appearance of major groups in the geologic column.

Avalon Explosion of Ediacara fauna
- sudden appearance of marine life including cells that carry out photosynthesis.

Cambrian Explosion
- sudden appearance of almost all the main animal groups

Great Ordovician Biodiversification Event
- sudden appearance of many varieties of marine invertebrates

Silurian Terrestrial Explosion
- sudden appearance of many land plants

Devonian Nekton Revolution
- sudden appearance of fish and other active swimmers

Odonotode Teeth
- sudden appearance of teeth

Devonian Terrestrial Explosion
- sudden appearance of walking land animals (arrive before the oldest walking fish)

WorthPoint

Carboniferous Insect Explosion
- sudden appearance of insects, dragonflies, caterpillars, beetles

Triassic Explosions
- sudden appearance of mammals, dinosaurs, gliding reptiles, ichthyosaurs

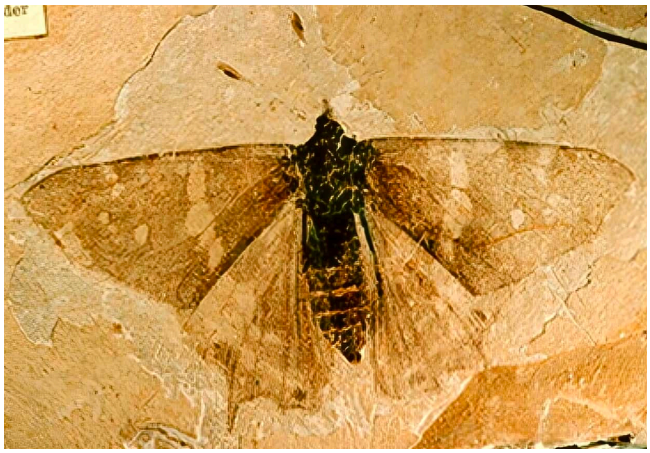
Darwin's Abominable Mystery
- sudden appearance of flowering plants

Avian Explosion or Big Bang of Bird Evolution
- sudden appearance of many kinds of birds

Early Tertiary
- sudden appearance of mammals including bats, primates and rodents

These life explosions were not predicted by the Theory of Evolution.

Kuban

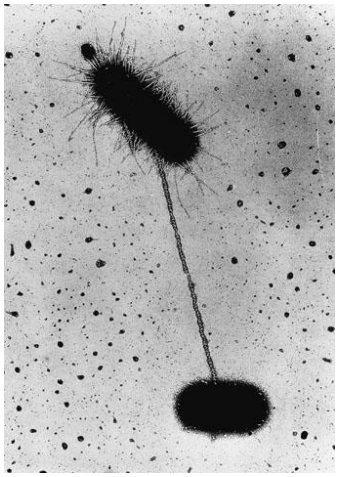


"The fossil record is the most obvious and serious threat to my theory."
Charles Darwin.

12HL Difficulties applying the biological species concept

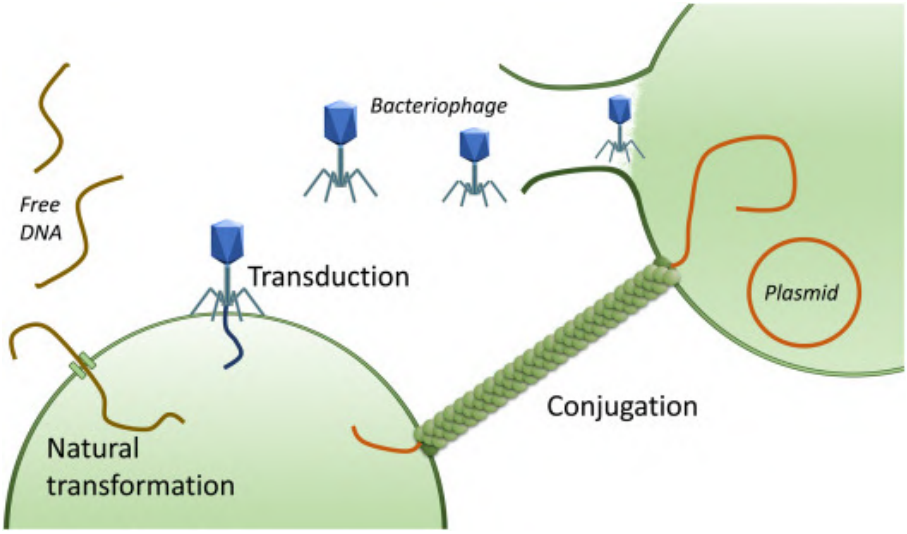
There are many exceptions to the biological species concept.

Asexual Reproduction
Dandelions reproduce asexually by mitosis and do not use their flowers for sexual reproduction.
They form identical clones that do not breed together.
Some clones have slightly different phenotypes from other clones.
Should these various clones be considered separate species as they no longer breed together?



Horizontal Gene Transfer
A number of species that were considered separate and on different branches of an evolutionary tree have actually bred and transferred genes 'horizontally'.
This is especially common among prokaryotes.

Prokaryotes reproduce asexually but form varied strains by:
- mutation
- conjugation - the transfer of plasmids
- transformation - taking up naked DNA from the environment
- transduction – viral DNA can include DNA from a previous bacterial host



These processes mix up DNA in a gene pool and blur species boundaries.

Horizontal gene transfer can occur between: bacteria, viruses, archaea and eukaryotes

Although there are a number of repair mechanisms to remove viral DNA if genes enter eukaryote reproductive cells they can become part of the inherited genome.
Therefore, species that have the same genes may not always have a shared ancestor.

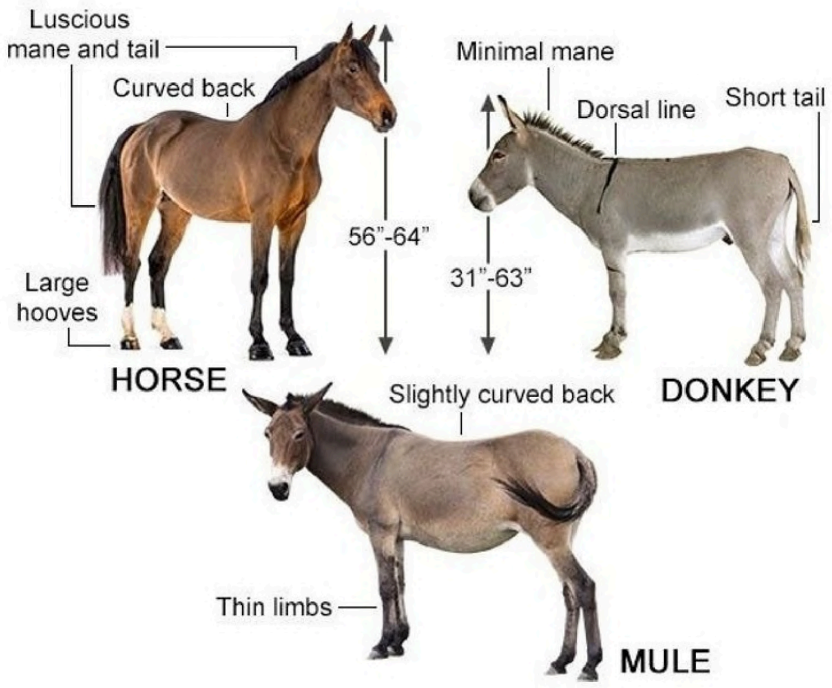
13HL Chromosome number as a shared trait within a species

Members of the same species have the same chromosome number.
Non-disjunction of chromosomes can cause chromosome numbers to vary in some individuals.

Closely related species may have the same or slightly different chromosome number.
Hybridisation can occur among closely related species, but not between unrelated species that lack a common ancestor.

Different species may be able to breed successfully and form a hybrid however, normally the hybrid will be sterile.
This is due to the hybrid having an odd number of chromosomes.
During meiosis II any extra chromosomes pair up randomly with other chromosomes which can result in a failure to complete meiosis.

Horses and donkeys are different species.
Horses have 64 chromosomes and donkeys have 62 chromosomes.
Mules have 63 which makes them sterile.
Male mules cannot mate with other mules successfully.
This shows that a species requires the same number of chromosomes to reproduce.

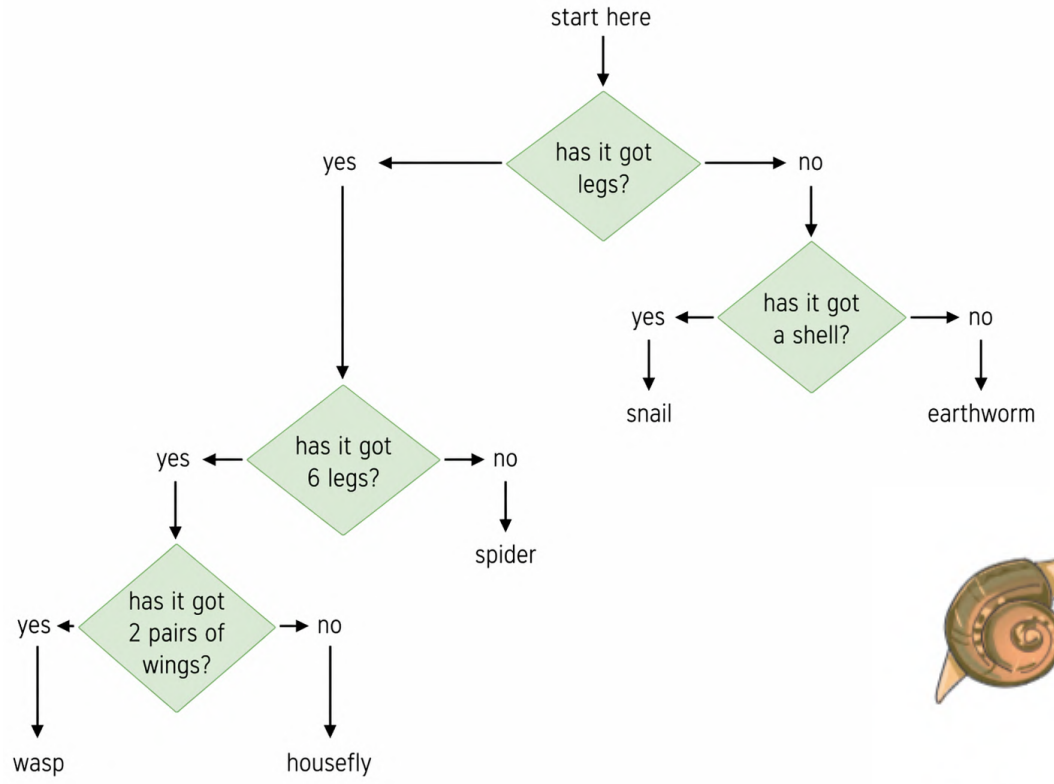


British Mule Society

14HL Engagement with local plant or animal species to develop a dichotomous key

Dichotomous means dividing into two.
A dichotomous key can be used to identify an organism by the features it has.
The key takes a group of living things and divides them into two groups according to their features.
This is repeated until groups are split into individual species.

Each step in a key presents a yes or no question.
Start at the beginning and follow the steps to identify each organism.



A numbered key works in the same way.

1 Has legs	Go to 2
Does not have legs	Go to 4
2 Has 6 legs	Go to 3
Does not have 6 legs	spider
3 Has 2 pairs of wings	……?
Does not have 2 pair of wings	housefly
4 Has a shell	snail
Does not have a shell	earthworm

Design your own dichotomous key to identify the organisms below.
Use visible features but do not use colour or patterns as these may be highly variable in a species.
Instead choose features that are unique to their genus or species group.

Garden snail

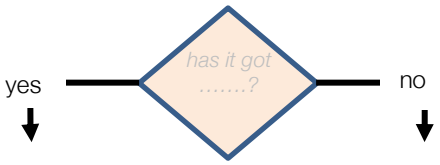
Pond snail

Gold-circle beetle

Woodlice

Fly

Mosquito



Salamander Dichotomous Key

1 a Hind limbs absent ..... *Siren intermedia*
b Hind limbs present ..... Go to 2

2 a External gills present in adults ..... *Necturus maculosus*
b External gills absent in adults ..... Go to 3

3 a Large size (over 7 cm long in Figure 1) ..... Go to 4
b Small size (under 7 cm long in Figure 1) ..... Go to 5

4 a Body background black, large white spots variable in size completely covering body and tail ..... *Ambystoma tigrinum*
b Body background black, small round white spots in a row along each side from eye to tip of tail ..... *Ambystoma maculatum*

5 a Body background black with white spots ..... Go to 6
b Body background light color with dark spots and/or lines on body.... Go to 7

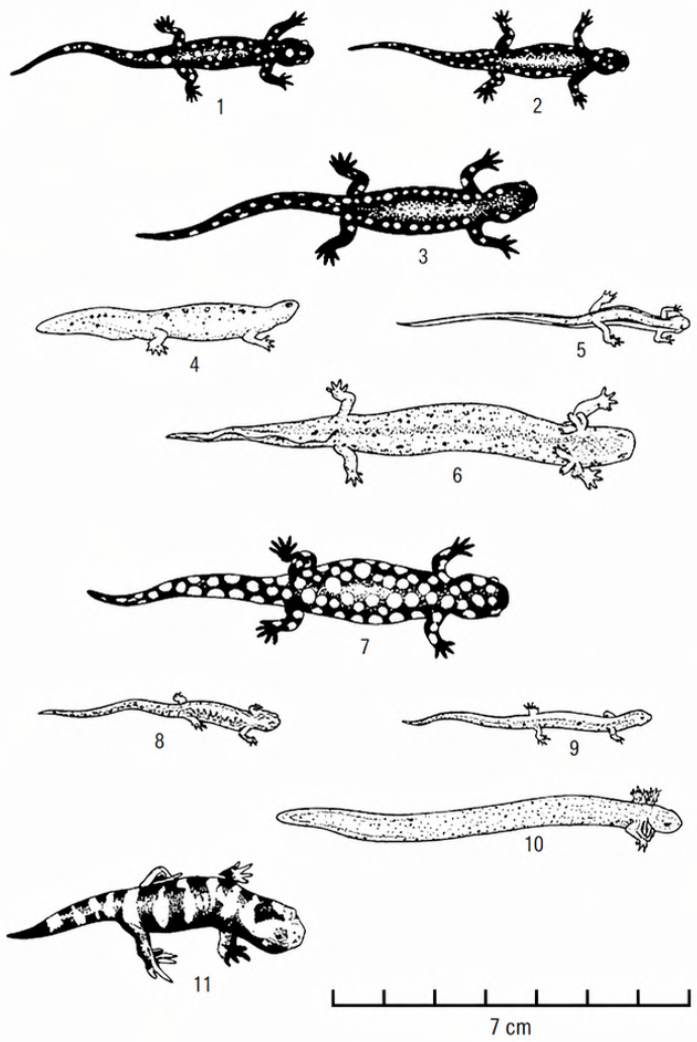
6 a Small white spots on black background in a row along each side from head to tip of tail ..... *Ambystoma jeffersonianum*
b Small white spots scattered throughout a black background from head to tip of tail ..... *Plethodon glutinosus*

7 a Large irregular white spots on a black background extending from head to tip of tail ..... *Ambystoma opacum*
b No large irregular black spots on a light background ..... Go to 8

8 a Round spots scattered along back and sides of body, tail flattened like a tadpole ..... *Triturus viridescens*
b Without round spots and tail not flattened like a tadpole ..... Go to 9

9 a Two dark lines bordering a broad light middorsal stripe with a narrow median dark line extending from the head onto the tail... *Eurycea bislineata*
b Without two dark lines running the length of the body ..... Go to 10

10 a A light stripe running the length of the body and bordered by dark pigment extending downward on the sides ..... *Plethodon cinereus*
b A light stripe extending the length of the body without dark pigment on the sides..... *Hemidactylium scutatum*



15HL Identification of species from environmental DNA in a habitat using barcodes

DNA barcodes - short length of DNA used to identify a species

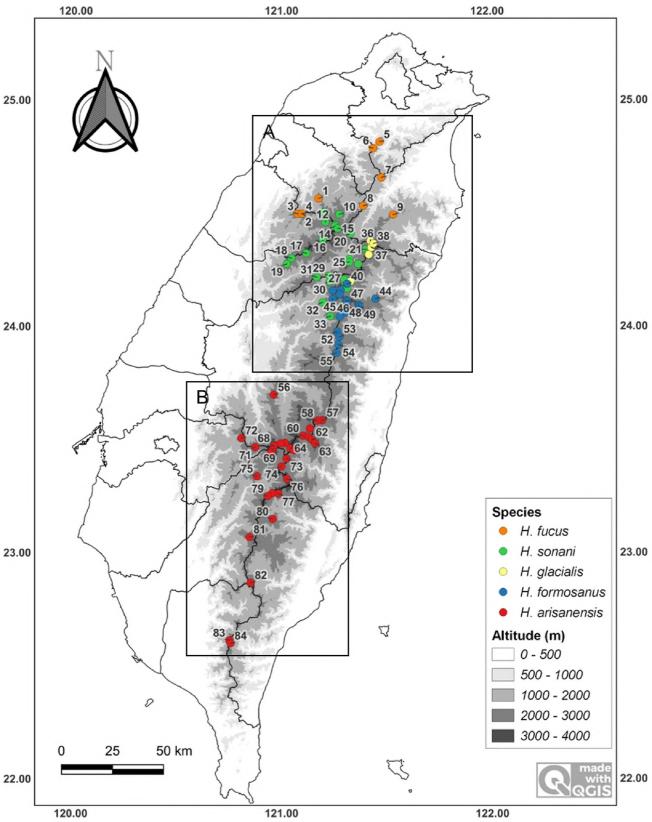
The mitochondrial gene for cytochrome oxidase subunit 1 (MT-CO1) is a good DNA barcode sequence as it is unique to each species of most eukaryotes. This is due to its high mutation rate producing a unique combination of mutations in different species. However, the mutations do not vary too much within one species.

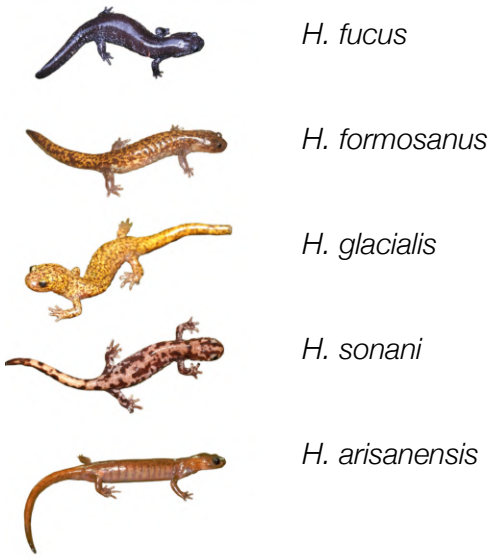
DNA barcodes can be used to rapidly identify:

- plants that may not be in flower or in fruit and so are harder to identify
- microbes in a water or soil sample that normally require a microscope to identify
- organisms from a small tissue sample such as a fish scale or hair follicle
- species without the need of expert taxonomists

Organisms leave DNA in their surroundings via dead cells and feces.
This environmental DNA (eDNA) can be used to measure species diversity in a certain location.
This is especially helpful for rare species which may not always be easy to observe or capture.
DNA samples are amplified using PCR before sequencing of a short target sequence.

Limitations
Sampling equipment must be sterilised to avoid contamination with DNA from a different location.
DNA barcodes show if a species is present or absent but not the population size of a species.
DNA databases must contain matching reference barcodes before a species can be detected.





H. fucus

H. formosanus

H. glacialis

H. sonani

H. arisanensis

Sampling sites of five Taiwanese salamanders. Chen et al 2024

Mitochondrial DNA analysis revealed they have a recent common ancestor.
H. arisanensis and *H. formosanus* were the most closely related and *H. fucus* the least similar.