Unit 4

SAC 1 and 2

Resource Book

Key Words

The Species Concept

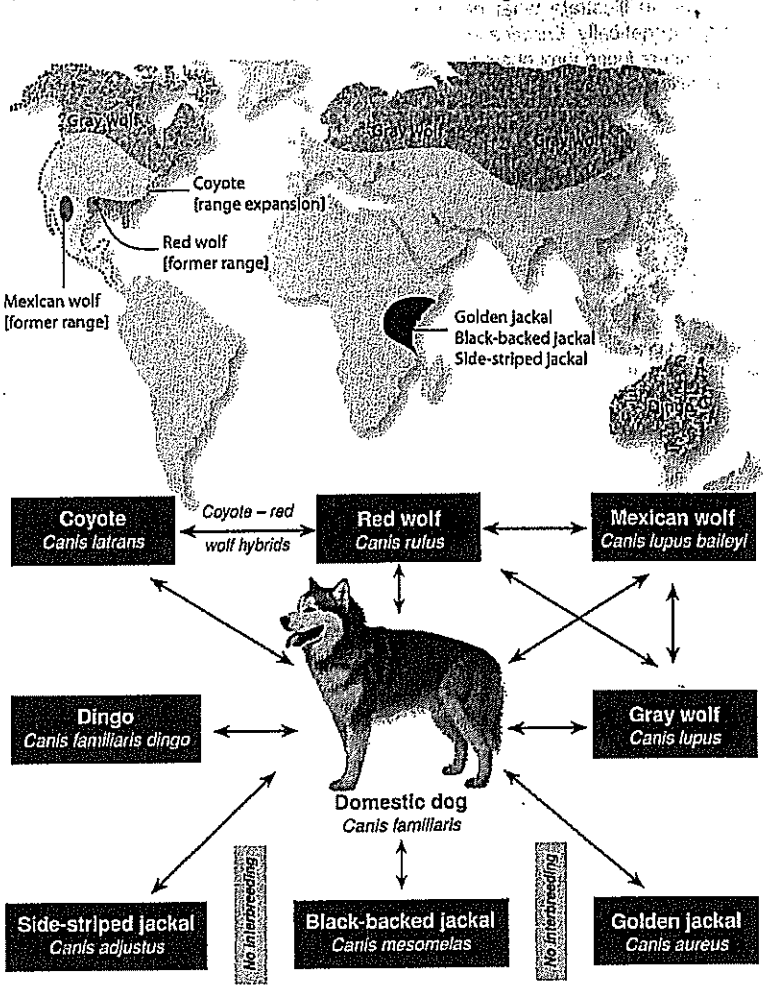
One of the best recognized definitions of a biological species is as "*a group of actually or potentially interbreeding natural populations that is reproductively isolated from other such groups*" (Ernst Mayr). However, the concept of a species is not as simple as it may first appear. The occurrence of cryptic species and closely related species that interbreed to produce fertile hybrids (e.g. species of *Canis*), indicate that the boundaries of a species gene pool can be unclear. Increasingly, biologists are turning to molecular analyses to help clarify relationships between the closely related populations that we regard as one species.

Geographical distribution of selected *Canis* species

The global distribution of most of the species of *Canis* (dogs and wolves) is shown on the map, right. The **gray wolf** inhabits the forests of North America, northern Europe, and Siberia. The **red wolf** and **Mexican wolf** (original distributions shown) were once distributed more widely, but are now extinct in the wild except for reintroduction efforts. In contrast, the **coyote** has expanded its original range and is now found throughout North and Central America. The range of the three jackal species overlap in the open savannah of Eastern Africa. The **dingo** is distributed throughout the Australian continent. Distribution of the domesticated dog is global as a result of the spread of human culture. The dog has been able to interbreed with all other members of the genus listed here to form fertile hybrids.

Interbreeding between *Canis* species

The *Canis* species illustrate problems with the traditional species concept. The domesticated dog is able to breed with other members of the same genus to produce fertile hybrids. Red wolves, gray wolves, Mexican wolves, and coyotes are all capable of interbreeding to produce fertile hybrids. Red wolves are very rare, and it is possible that hybridization with coyotes has been a factor in their decline. By contrast, the ranges of the three distinct species of jackal overlap in the Serengeti of Eastern Africa. These animals are highly territorial, but simply ignore members of the other jackal species and no interbreeding takes place.

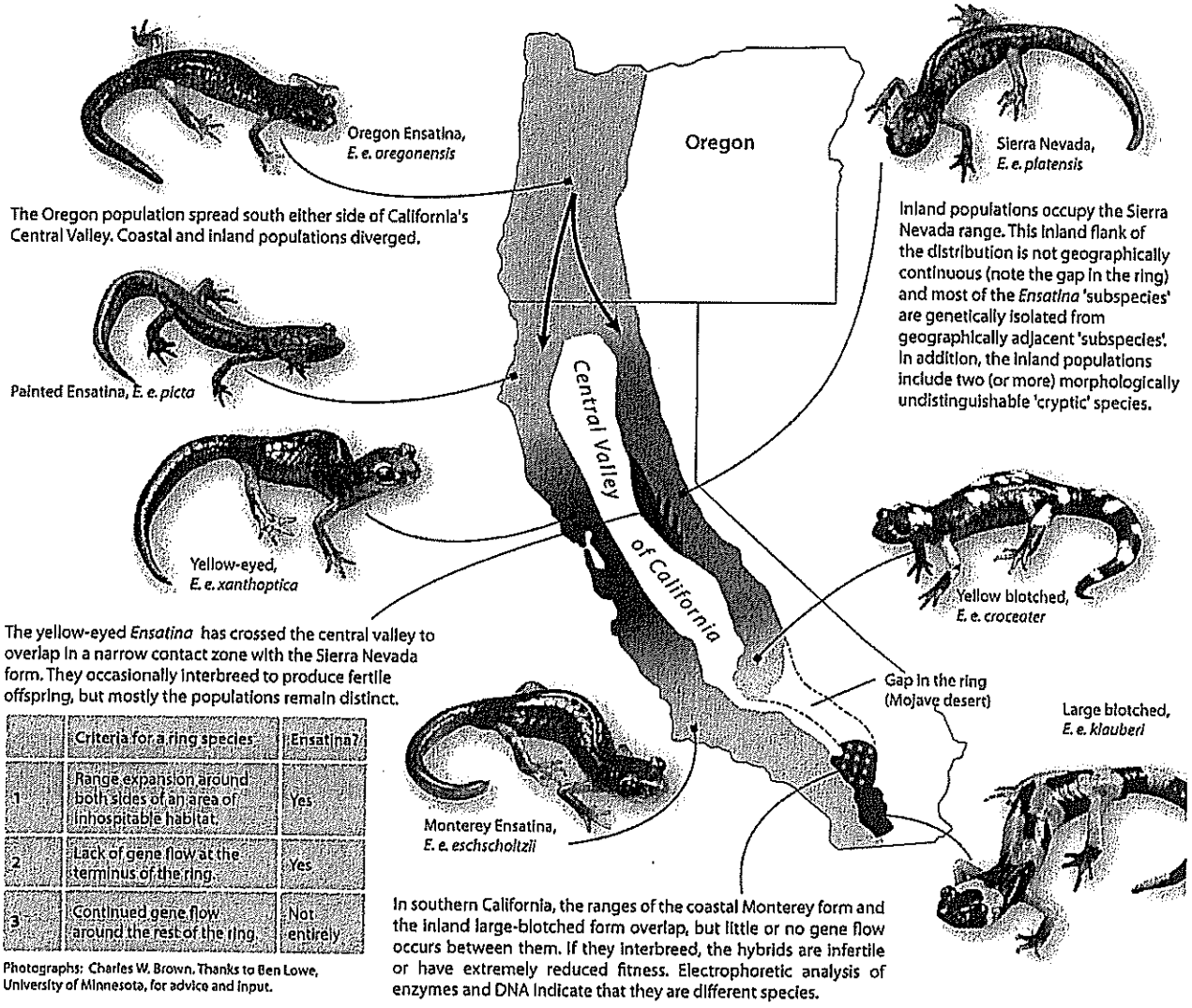


1. Describe the type of barrier that prevents the three species of jackal from interbreeding:
   POSSIBLY A BEHAVIOURAL BARRIER. (could be others)
2. Describe the factor that has prevented the dingo from interbreeding with other *Canis* species (apart from the dog):
   GEOGRAPHY
3. Describe a possible contributing factor to the occurrence of interbreeding between the coyote and red wolf:
   RANGE STARTED TO OVERLAP
4. The gray wolf is a widely distributed species. Explain why the North American population is considered to be part of the same species as the northern European and Siberian populations:
   GENE FLOW IS STILL ABLE TO OCCUR (LAND/ICE BRIDGE) B/W ALASKA/RUSSIA.
5. Explain what you understand by the term species, identifying examples where the definition is problematic:
   REPRODUCE TO PRODUCE FERTILE OFFSPRING.
   * HOW DO WE KNOW OF THE CLASSIFICATION OF EXTINCT SPECIES

Speciation

Ensatina in the Pacific North-West: Ring Species or Cryptic Species?

A **ring species** is a connected series of closely related populations, distributed around a geographical barrier, in which the adjacent populations in the ring are able to interbreed, but those at the extremes of the ring are reproductively isolated. Such ring species are regarded as important because they are seen to illustrate what happens over time as populations diverge genetically. *Ensatina eschscholtzii* is a species of lungless salamanders found throughout the Pacific North-West of the USA to Baja California in Mexico. *E. eschscholtzii* has long been considered a ring species, which probably expanded southwards from an ancestral population in Oregon along either side of California's Central Valley. However, molecular analyses are now indicating that the story of *Ensatina* is more complicated than first supposed. Geographically adjacent populations within the ring may be genetically isolated or comprise morphologically identical but genetically distinct **cryptic species.** Regardless of the conclusions drawn from the evidence (below), species such as *Ensatina* give us reason to reevaluate how we define species and quantify biodiversity.



	Criteria for a ring species	Ensatina?
1	Range expansion around both sides of an area of inhospitable habitat.	Yes
2	Lack of gene flow at the terminus of the ring.	Yes
3	Continued gene flow around the rest of the ring.	Not entirely

Photographs: Charles W. Brown. Thanks to Ben Lowe, University of Minnesota, for advice and input.

6. The Ensatina species complex fulfils two of the three criteria necessary to define a ring species (table, above left) yet does not fit comfortably with Mayr's definition of a biological species. Describe the aspects of Ensatina that:

   (a) Supports the idea that they are a single species: ALL ADJACENT POPULATIONS ARE ABLE TO SUCCESSFULLY BREED

   (b) Does not agree with the standard definition of a biological species: SOME POPULATIONS ARE UNABLE TO SUCCESSFULLY BREED.

7. Yellow-eyed *Ensatina* is a mimic of the toxic California newt. What might this suggest about the selection pressures on this subspecies and their influence on the rate at which the population becomes genetically distinct?

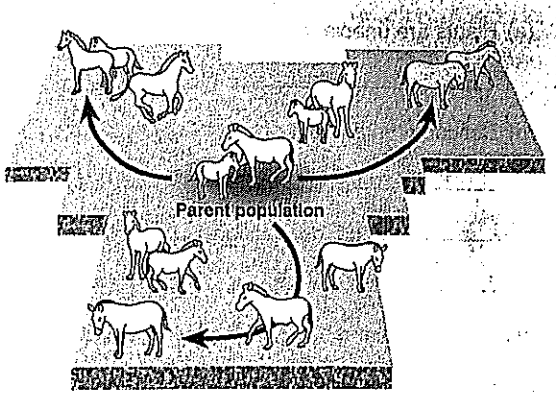
   SIMILAR SELECTION PRESSURES TO NEWT MAY DIVERGE FROM OTHER SALAMANDERS

Allopatric Speciation

Allopatric speciation is a process thought to have been responsible for a great many instances of species formation. It has certainly been important in countries which have had a number of cycles of geographical fragmentation. Such cycles can occur as the result of glacial and interglacial periods, where ice expands and then retreats over a land mass. Such events are also accompanied by sea level changes which can isolate populations within relatively small geographical regions.

Stage 1: Moving into new environments

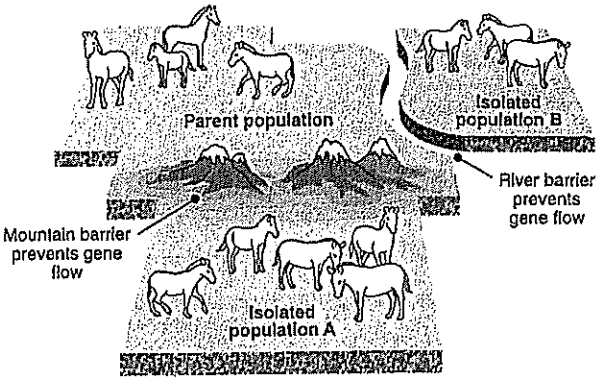
There are times when the range of a species expands for a variety of different reasons. A single population in a relatively homogeneous environment will move into new regions of their environment when they are subjected to intense competition (whether it is interspecific or intraspecific). The most severe form of competition is between members of the same species since they are competing for identical resources in the habitat. In the diagram on the right there is a 'parent population' of a single species with a common gene pool with regular 'gene flow' (theoretically any individual has access to all members of the opposite sex for mating purposes).



Stage 2: Geographical Isolation

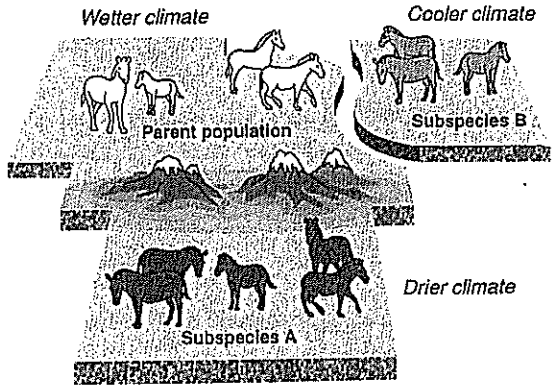
Isolation of parts of the population may occur due to the formation of **physical barriers**. These barriers may cut off those parts of the population that are at the extremes of the species range and gene flow is prevented or rare. The rise and fall of the sea level has been particularly important in functioning as an isolating mechanism. Climatic change can leave 'islands' of habitat separated by large inhospitable zones that the species cannot traverse.

Example: In mountainous regions, alpine species are free to range widely over extensive habitat during cool climatic periods. During warmer periods, however, they may become isolated because their habitat is reduced to 'islands' of high ground surrounded by inhospitable lowland habitat.



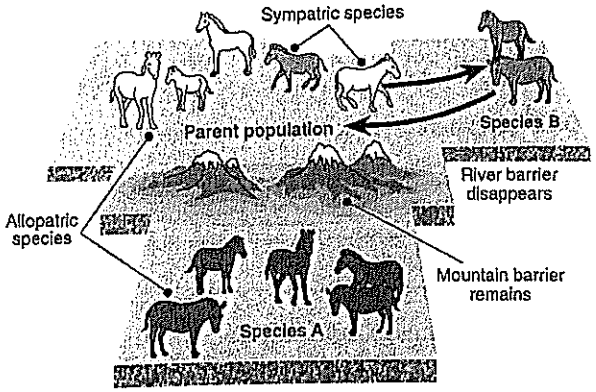
Stage 3: Different selection pressures

The isolated populations (A and B) may be subjected to quite different selection pressures. These will favor individuals with traits that suit each particular environment. For example, population A will be subjected to selection pressures that relate to drier conditions. This will favor those individuals with phenotypes (and therefore genotypes) that are better suited to dry conditions. They may for instance have a better ability to conserve water. This would result in improved health, allowing better disease resistance and greater reproductive performance (i.e. more of their offspring survive). Finally, as allele frequencies for certain genes change, the population takes on the status of a **subspecies**. Reproductive isolation is not yet established but the subspecies are significantly different genetically from other related populations.



Stage 4: Reproductive Isolation

The separated populations (isolated subspecies) will often undergo changes in their genetic makeup as well as their behavior patterns. These ensure that the gene pool of each population remains isolated and 'undiluted' by genes from other populations, even if the two populations should be able to remix (due to the removal of the geographical barrier). Gene flow does not occur. The arrows (in the diagram to the right) indicate the zone of overlap between two species after the new Species B has moved back into the range inhabited by the parent population. Closely-related species whose distribution overlaps are said to be **sympatric species**. Those that remain geographically isolated are called **allopatric species**.



Related activities: Reproductive Isolation, Stages in Species Development
Web links: Species and Speciation, Mechanisms of Speciation, Allopatric Speciation

1. Describe why some animals, given the opportunity, move into new environments:
FOOD, SHELTER, CLIMATE

2. (a) Plants are unable to move. Explain how plants might disperse to new environments:
SEED DISPERSAL

(b) Describe the amount of **gene flow** within the parent population prior to and during this range expansion:
WHILE POPULATION IS INTACT GENE FLOW IS HIGH.

3. Identify the **process** that causes the formation of new **mountain ranges**: EARTH MOVEMENT

4. Identify the event that can cause large changes in **sea level** (up to 200 metres): CLIMATE CHANGE

5. Describe six **physical barriers** that could isolate different parts of the same population:
MOUNTAINS, VALLEY, DESERT, RIVER, LAKE, TERRAIN

6. Describe the effect that physical barriers have on **gene flow**:
REDUCES

7. (a) Describe four different types of **selection pressure** that could have an effect on a gene pool:
PREDATORS, FOOD, CLIMATE, HUMANS

(b) Describe briefly how these selection pressures affect the isolated gene pool in terms of **allele frequencies**:
ONE: PREDATORS KILL SLOWEST SO THE ALLELES CONTRIBUTING TO SPEED INCREASE IN GENE POOL.

8. Describe two types of **prezygotic** and two types of **postzygotic** reproductive isolating mechanisms (see previous pages):

(a) Prezygotic: BEHAVIOUR, MECHANICAL

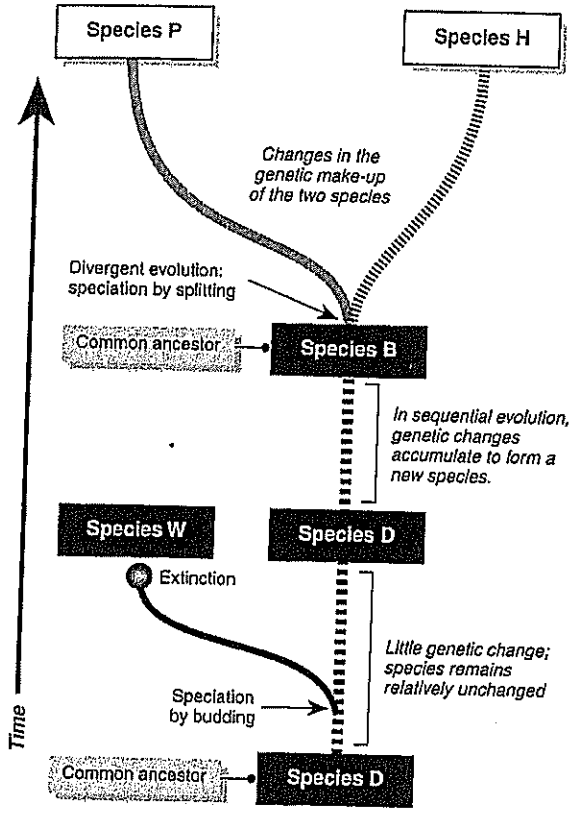
(b) Postzygotic: GAMETE INVIABILITY, OFFSPRING INFERTILITY

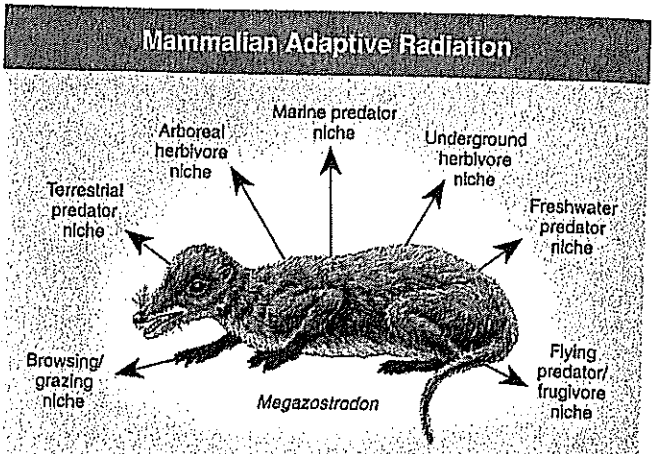
9. Distinguish between **allopatry** and **sympatry** in populations:
JUST NEED TO KNOW ALLOPATRIC

VARIATION
GEOGRAPHIC BARRIER
NATURAL SELECTION
SPECIATION

Patterns of Evolution

The diversification of an ancestral group into two or more species in different habitats is called **divergent evolution**. This process is shown below, where two species have diverged from a **common ancestor**. Note that another species budded off, only to become extinct. Divergence is common in evolution. When divergent evolution involves the formation of a large number of species to occupy different niches, this is called an **adaptive radiation**. The example below (right) describes the radiation of the mammals that occurred after the extinction of the dinosaurs; an event that made niches available. Note that the evolution of species may not necessarily involve branching: a species may accumulate genetic changes that, over time, result in the emergence of what can be recognized as a different species. This is known as **sequential evolution** (also called anagensis or phyletic gradualism).





***Megazostrodon*: one of the first mammals**

Megazostrodon (above) is known from fossil remains in South Africa. This shrew-like animal first appeared in the Early Jurassic period (about 195 million years ago) and probably had an insectivorous diet.

The earliest true mammals evolved about 195 million years ago, long before they underwent their major adaptive radiation some 65-50 million years ago. These ancestors to the modern forms were very small (12 cm), many were nocturnal and fed on insects and other invertebrate prey. It was climatic change as well as the extinction of the dinosaurs (and their related forms) that suddenly left many niches vacant for exploitation by such an adaptable 'generalist'. All modern mammal orders developed very quickly and early.

1. In the hypothetical example of divergent evolution illustrated above, left:

   (a) Classify the type of evolution that produced species B from species D: SEQUENTIAL *

   (b) Classify the type of evolution that produced species P and H from species B: DIVERGENT

   (c) Name all species that evolved from: **Common ancestor D:** B P H **Common ancestor B:** P H

   (d) Suggest why species B, P, and H all possess a physical trait not found in species D or W: EVOLVED BETWEEN D + B

2. (a) Explain the distinction between **divergence** and **adaptive radiation**:

   DIVERGE IS 1 INTO 2.

   ADAPTIVE IS 1 INTO MANY

   (b) Discuss the differences between **sequential evolution** and **divergent evolution**:

   SEQUENTIAL - OVER TIME ASPECTS CHANGES SO IT IS CLASSIFIED DIFFERENTLY

   DIVERGENT - OVER TIME 2 SPECIES EVOLVE FROM A SINGLE COMMON ANCESTOR.

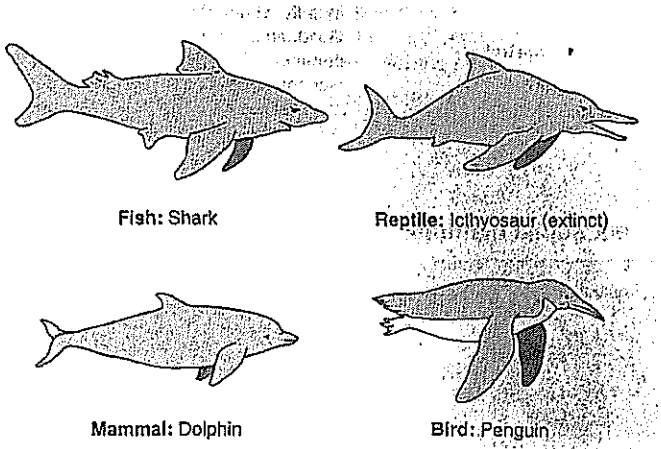
Related activities: The Species Concept, Adaptive Radiation in Mammals
Web links: Macroevolution
Periodicals: Evolution: five big

Convergent Evolution

Not all similarities between species are a result of common ancestry. Species from different evolutionary lines may come to resemble each other if they have similar ecological roles and natural selection has shaped similar adaptations. This is called **convergent evolution (convergence)**. Similarity of form due to convergence is called **analogy**.

Convergence in Swimming Form

Although similarities in body form and function can arise because of common ancestry, it may also be a result of **convergent evolution**. Selection pressures in a particular environment may bring about similar adaptations in unrelated species. These selection pressures require the solving of problems in particular ways, leading to the similarity of body form or function. The development of succulent forms in unrelated plant groups (*Euphorbia* and the cactus family) is an example of convergence in plants. In the example (right), the selection pressures of the aquatic environment have produced a similar streamlined body shape in unrelated vertebrate groups. Ichthyosaurs, penguins, and dolphins each evolved from terrestrial species that took up an aquatic lifestyle. Their general body form has evolved to become similar to that of the shark, which has always been aquatic. Note that flipper shape in mammals, birds, and reptiles is a result of convergence, but its origin from the pentadactyl limb is an example of **homology**.



Fish: Shark

Reptile: Ichyosaur (extinct)

Mammal: Dolphin

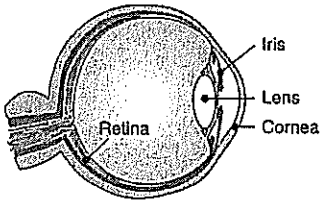
Bird: Penguin

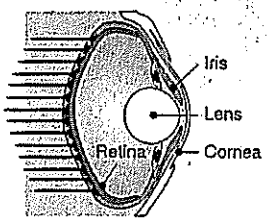
Analogous Structures

Analogous structures are those that have the same function and often the same basic external appearance, but quite different origins. The example on the right illustrates how a complex eye structure has developed independently in two unrelated groups. The appearance of the eye is similar, but there is no genetic relatedness between the two groups (mammals and cephalopod molluscs). The wings of birds and insects are also an example of analogy. The wings perform the same function, but the two groups share no common ancestor. *Longisquama*, a lizard-like creature that lived about 220 million years ago, also had 'wings' that probably allowed gliding between trees. These 'wings' were not a modification of the forearm (as in birds), but highly modified long scales or feathers extending from its back.

Mammalian eye

Octopus eye





1. In the example above illustrating convergence in swimming form, describe two ways in which the body form has evolved in response to the particular selection pressures of the aquatic environment:

   (a) STREAMLINED.

   (b) FLIPPERS

2. Describe two of the selection pressures that have influenced the body form of the swimming animals above:

   (a) WATER IS HARD TO MOVE THROUGH — SHAPE, FLIPPERS

   (b) _____

3. Early taxonomists, when encountering new species in the Pacific region and the Americas, were keen to assign them to existing taxonomic families based on their apparent similarity to European species. In recent times, many of the new species have been found to be quite unrelated to the European families they were assigned to. Explain why the traditional approach did not reveal the true evolutionary relationships of the new species:

   MORPHOLOGY ALONE IS NOT THE WHOLE PICTURE

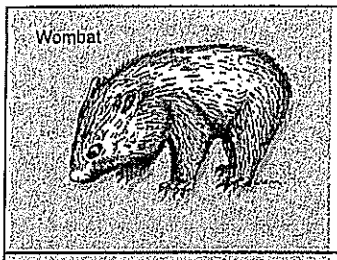
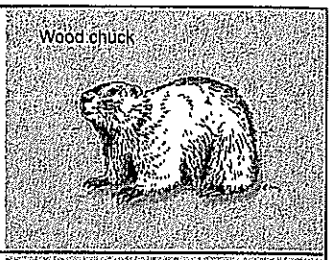
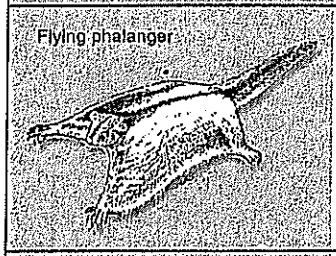
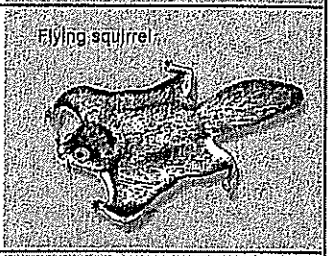
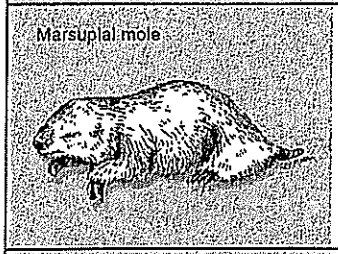
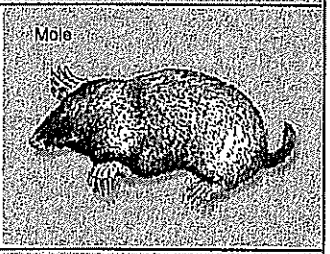
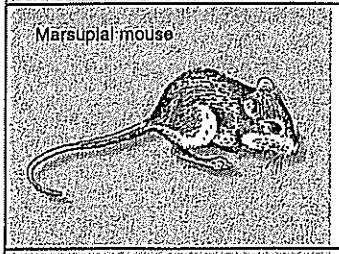
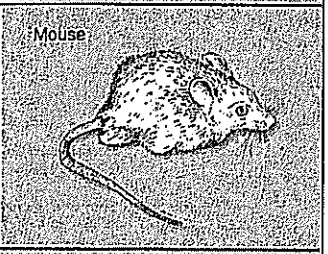
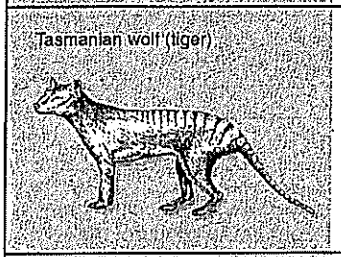
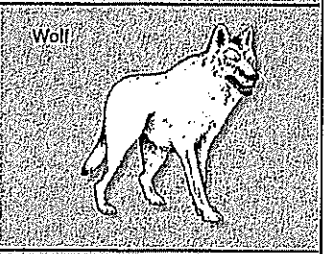
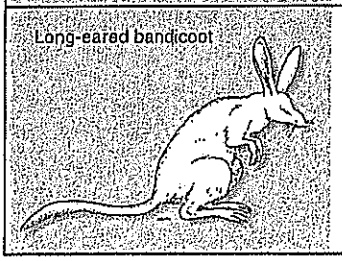
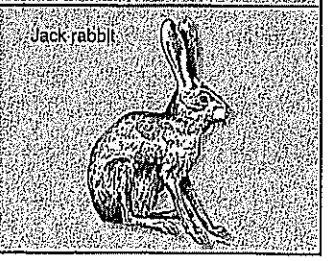
4. For each of the paired examples (b)-(f), briefly describe the adaptations of body shape, diet and locomotion that appear similar in both forms, and the likely selection pressures that are acting on these mammals to produce similar body forms:

Convergence Between Marsupials and Placentals

Marsupial mammals

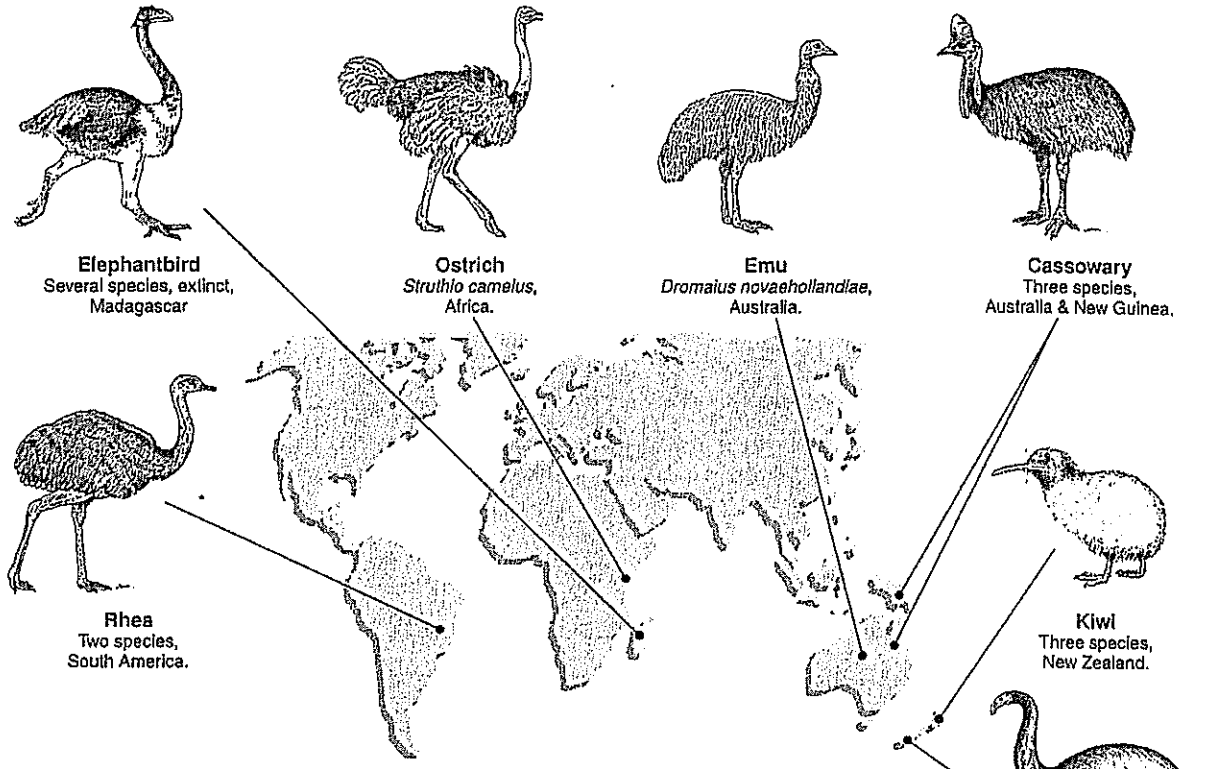
Marsupials and **placental** mammals were separated from each other very early in mammalian evolution (about 120 mya). Marsupials were initially widely distributed throughout the ancient supercontinent of Gondwana, and there are some modern species still living in the American continent. Gondwana split up about 100 million years ago. As the placentals developed, they displaced the marsupials in most habitats around the world. The island continent of Australia, because of its early isolation by the sea, escaped this competition and placentals did not reach the continent until the arrival of humans 35 000 to 50 000 years ago. The Australian marsupials evolved into a wide variety of forms (below left) that bear a remarkable resemblance to ecologically equivalent species of North American placentals (below right).

Placental mammals

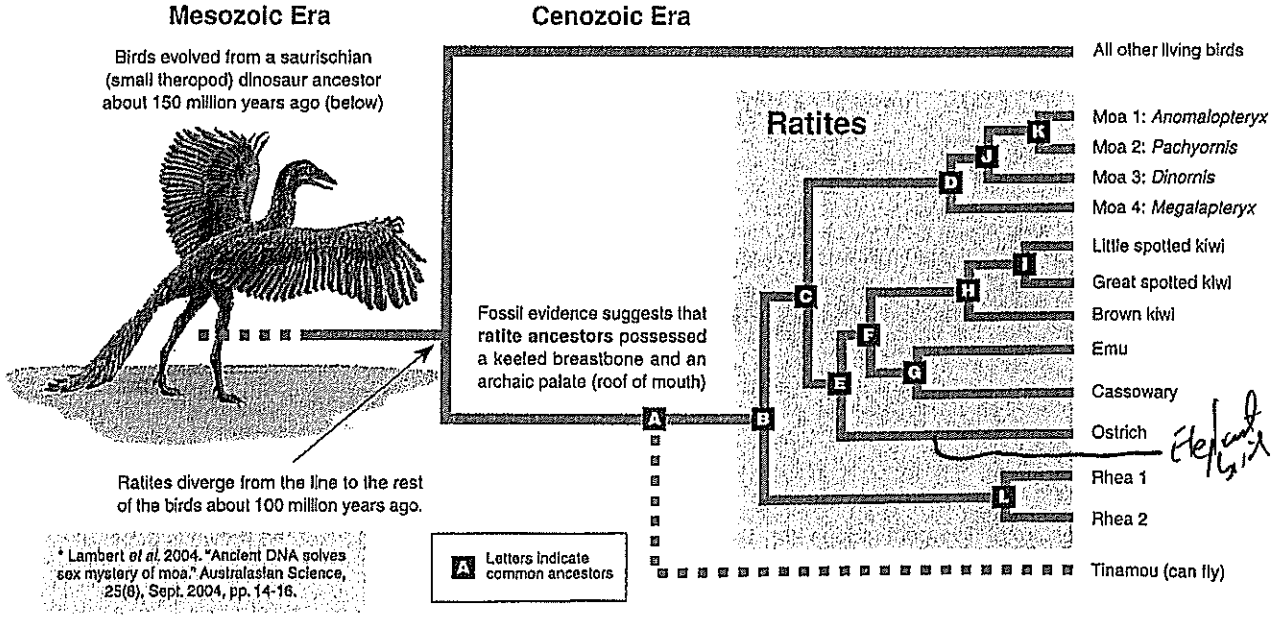
 Wombat	(a) Adaptations: Both have rodent-like teeth, eat roots and above ground plants, and excavate burrows. Selection pressures: Diet requires chisel-like teeth for gnawing. The need to seek safety from predators on open grassland.	 Wood chuck
 Flying phalanger	(b) Adaptations: SKIN FLAPS Selection pressures: AIR.	 Flying squirrel
 Marsupial mole	(c) Adaptations: CLAWS Selection pressures: DIGGING	 Mole
 Marsupial mouse	(d) Adaptations: SMALL Selection pressures: HOT ENVIRONMENT	 Mouse
 Tasmanian wolf (tiger)	(e) Adaptations: TEETH Selection pressures: CARNIVORE	 Wolf
 Long-eared bandicoot	(f) Adaptations: EARS Selection pressures: HEAR PREDATORS	 Jack rabbit

Adaptive Radiation in Ratites

The ratites evolved from a single common ancestor; they are a monophyletic group of birds that lost the power of flight very early on in their evolutionary development. Ratites possess two features distinguishing them from other birds: a flat breastbone (instead of the more usual keeled shape) and a primitive palate (roof to the mouth). Flightlessness in itself is not unique to this group. There are other examples of birds that have lost the power of flight, particularly on remote, predator-free islands. Fossil evidence indicates that the ancestors of ratites were flying birds living about 80 million years ago. These ancestors also had a primitive palate, but they possessed a keeled breastbone.



The geographical distribution of modern day and extinct ratite species can be partially explained in terms of continental drift. The ancestral ratite population existed at a time when the southern continents of South America, Africa and Australia (together with their major offshore islands) were joined as a single land mass called Gondwana. As the continents moved apart as a result of plate tectonics, the early ratite populations were carried with them. Subsequent speciation on each continent and some of the islands produced the variety of forms shown here. The 50 species of tinamou (see chart below) from South America, are considered a sister group to the ratites even though they can fly, because they possess the archaic palate. This relationship is confirmed by DNA sequence tests. The diagram below shows a possible phylogenetic tree based upon comparisons of mitochondrial DNA sequences. This view has been supported by the extensive comparison of skeletons from the different ratite species.



* Lambert *et al.* 2004. "Ancient DNA solves sex mystery of moa." Australasian Science, 25(8), Sept. 2004, pp. 14-16.

1. (a) Describe three physical features distinguishing all ratities from most other birds: LONG LEGS, NO FLIGHT, BEAK STRUCTURE

   (b) Identify the primitive feature shared by ratites and tinamou: BREASTBONE

2. Describe two anatomical changes, common to all ratites, which have evolved as a result of flightlessness. For each, describe the selection pressures for the anatomical change:

   (a) Anatomical change: BREASTBONE

   Selection pressure: FAST MOVEMENT

   (b) Anatomical change: BEAK

   Selection pressure: DIET

3. Name the ancient supercontinent that the ancestral ratite population inhabited: GONDWANA

4. (a) The extinct elephantbird from Madagascar is thought to be very closely related to another modern ratite. Based purely on the **geographical distribution** of ratites, identify the modern species that is the most likely relative: OSTRICH

   (b) Explain why you chose the modern ratite in your answer to (a) above: MADAGASCAR / AFRICA CLOSE

   (c) Draw lines on the diagram at the bottom of the previous page to represent the divergence of the elephantbird from the modern ratite you have selected above.

5. (a) Name two other flightless birds that are not ratites: PENGUINS

   (b) Explain why these other flightless species are not considered part of the ratite group: DIFFERENT SELECTION PRESSURES IN DIFFERENT AREAS AT DIFFERENT TIMES

6. Eleven species of moa is an unusually large number compared to the species diversity of the kiwis, the other ratite group found in New Zealand. The moas are classified into at least four genera, whereas kiwis have only one genus. The diets of the moas and the kiwis are thought to have had a major influence on each group's capacity to diverge into separate species and genera. The moas were herbivorous, whereas kiwis are nocturnal feeders, feeding on invertebrates in the leaf litter. Explain why, on the basis of their diet, moas diverged into many species, whereas kiwis diverged little: LARGER DIVERSITY OF FOOD SOURCES AVAILABLE

7. The DNA evidence suggests that New Zealand had two separate invasions of ratites, an early invasion from the moas (before the breakup of Gondwana) followed by a second invasion of the ancestors of the kiwis. Describe a possible sequence of events that could account for this: MOAS THERE BEFORE NZ SEPARATED

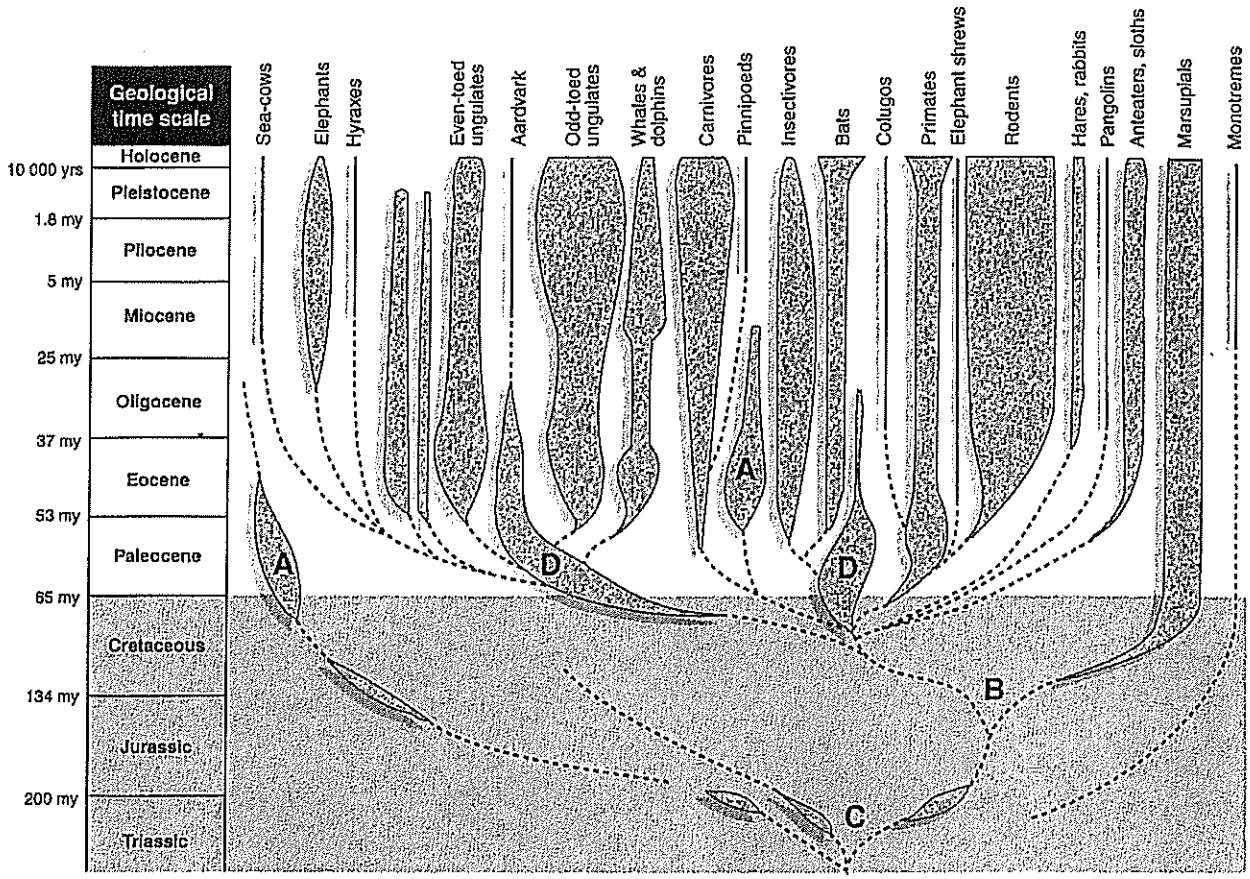
   KIWI ARRIVED LATER.

8. The common ancestors of divergent groups are labelled (A-L) on the diagram at the bottom of the previous page. State the letter identifying the **common ancestor** for:

   (a) The kiwis and the Australian ratites: F (b) The kiwis and the moas: C

Adaptive Radiation in Mammals

Adaptive radiation is diversification (both structural and ecological) among the descendants of a single ancestral group to occupy different niches. Immediately following the sudden extinction of the dinosaurs, the mammals underwent an adaptive radiation. Most of the modern mammal groups became established very early. The diagram below shows the divergence of the mammals into major orders; many occupying niches left vacant by the dinosaurs. The vertical extent of each gray shape shows the time span for which that particular mammal order has existed (note that the scale for the geological time scale in the diagram is not linear). Those that reach the top of the chart have survived to the present day. The width of a gray shape indicates how many species were in existence at any given time (narrow means there were few, wide means there were many). The dotted lines indicate possible links between the various mammal orders for which there is no direct fossil evidence.



1. In general terms, discuss the **adaptive radiation** that occurred in mammals:
   CA 'C' EVOLED INTO ALL MAMMALS ILLUSTRATED
2. Name the term that you would use to describe the animal groups at point **C** (above): COMMON ANCESTOR
3. Explain what occurred at point **B** (above):
   DIVERGENT EVOLUTION
4. Describe two things that the animal orders labeled **D** (above) have in common:
   (a) ANCESTORS TO OTHER MAMMALS
   (b) EXTINCT
5. Identify the two orders that appear to have been most successful in terms of the number of species produced:
   RODENTS, UNGULATES
6. Explain what has happened to the mammal orders labeled **A** in the diagram above:
   EXTINCT
7. Identify the **epoch** during which there was the most adaptive radiation: PALEOCENE

8. Describe two key features that distinguish mammals from other vertebrates:

(a) HAIR (b) MILK.

9. Describe the principal reproductive features distinguishing each of the major mammalian lines (sub-classes):

(a) Monotremes: EGGS

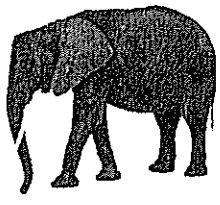
(b) Marsupials: POUCH

(c) Placentals: PLACENTA

10. There are 18 orders of placental mammals (or 17 in schemes that include the pinnipeds within the Carnivora). Their names and a brief description of the type of mammal belonging to each group is provided below. Identify and label each of the diagrams with the correct name of their Order:

Orders of Placental Mammals D.I.Y

Order	Description
Insectivora	Insect-eating mammals
Macroscelidae	Elephant shrews (formerly classified with Insectivores)
Chiroptera	Bats
Cetacea	Whales and dolphins
Pholidota	Pangolins
Rodentia	Rodents
Proboscidea	Elephants
Sirenia	Sea-cows (manatees)
Artiodactyla	Even-toed hoofed mammals
Dermoptera	Colugos
Primates	Primates
Xenarthra	Anteaters, sloths, and armadillos
Lagomorpha	Pikas, hares, and rabbits
Carnivora	Flesh-eating mammals (canids, raccoons, bears, cats)
Pinnipedia	Seals, sealions, walruses. (Often now included as a sub-order of Carnivora).
Tubulidentata	Aardvark
Hyracoidea	Hyraxes
Perissodactyla	Odd-toed hoofed mammals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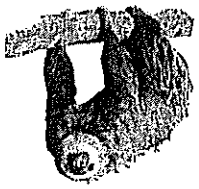
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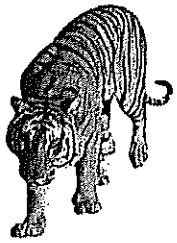
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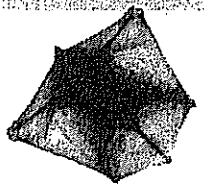
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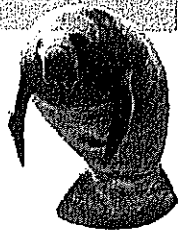
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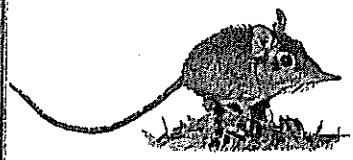
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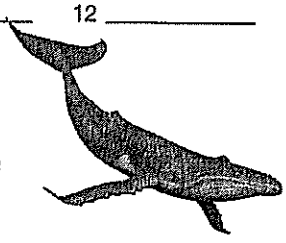
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11. For each of three named **orders** of placental mammal, describe one **adaptive feature** that allows it to exploit a different niche from other placentals, and describe a **biological advantage** conferred by the adaptation:

(a) Order: PROBOSCIDEA Adaptive feature: TRUNK.

Biological advantage: BROWSING.

(b) Order: _____ Adaptive feature: _____

Biological advantage: _____

(c) Order: _____ Adaptive feature: _____

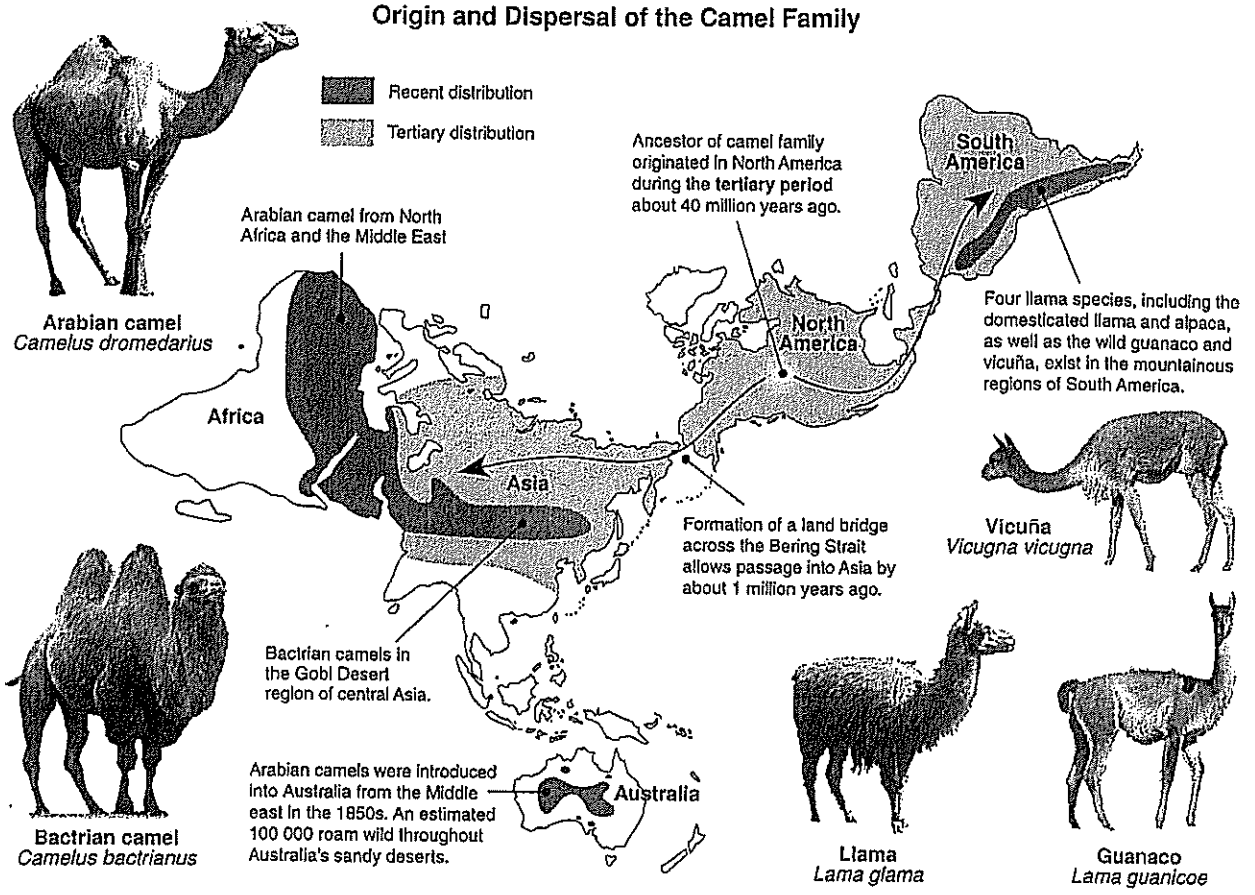
Biological advantage: _____

Geographical Distribution

The camel family, Camelidae, consists of six modern-day species that have survived on three continents: Asia, Africa and South America. They are characterized by having only two functional toes, supported by expanded pads for walking on sand or snow. The slender snout bears a cleft upper lip. The recent distribution of the camel family is fragmented. Geophysical forces such as plate tectonics and the ice age cycles have controlled the extent of their distribution. South America, for example, was separated from North America until the end of the Pliocene, about 2 million years ago. Three general principles about the dispersal and distribution of land animals are:

- When very closely related animals (as shown by their anatomy) were present at the same time in widely separated parts of the world, it is highly probable that there was no barrier to their movement in one or both directions between the localities in the past.
- The most effective barrier to the movement of land animals (particularly mammals) was a sea between continents (as was caused by changing sea levels during the ice ages).
- A scattered distribution of modern species may be explained by the movement out of the area they originally occupied, or by extinction in those regions between modern species.

Origin and Dispersal of the Camel Family



1. The early camel ancestors were able to move into the tropical regions of Central and South America. Explain why this did not happen in southern Asia and southern Africa:

   UNFAVORABLE ENVIRONMENT

2. Arabian camels are found wild in the Australian Outback. Explain how they got there and why they were absent during prehistoric times:

   IMPORTED

3. The camel family originated in North America. Explain why there are no camels in North America now:

   HUNTING

4. Suggest how early camels managed to get to Asia from North America:

   LAND BRIDGE

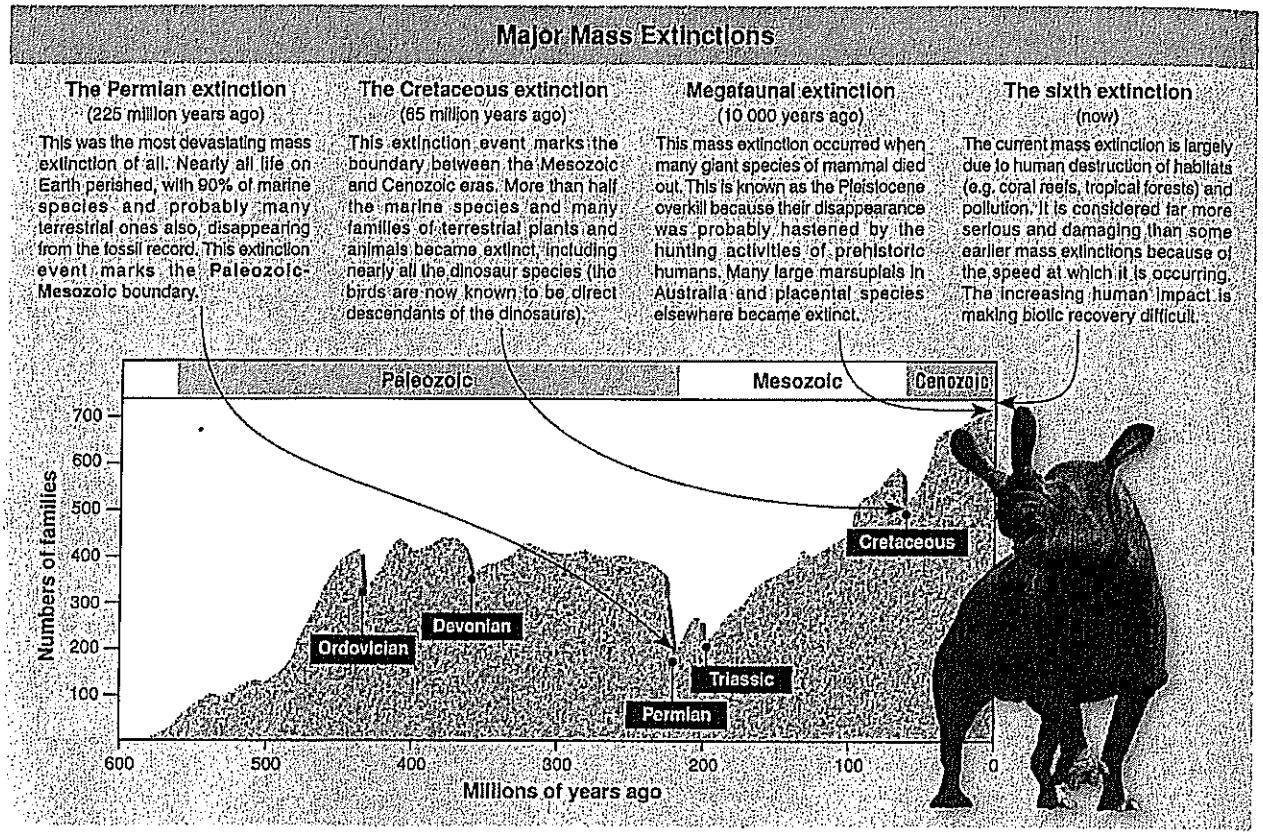
5. Describe the present distribution of the camel family and explain why it is scattered (discontinuous):

   ALL OVER BUT BETWEEN POPULATIONS

   UNFAVORABLE CONDITIONS

Extinction

Extinction is an important process in evolution as it provides opportunities, in the form of vacant niches, for the development of new species. Most species that have ever lived are now extinct. The species alive today make up only a fraction of the total list of species that have lived on Earth throughout its history. Extinction is a natural process in the life cycle of a species. Background extinction is the steady rate of species turnover in a taxonomic group (a group of related species). The duration of a species is thought to range from as little as 1 million years for complex larger organisms, to as long as 10-20 million years for simpler organisms. Superimposed on this constant background extinction are catastrophic events that wipe out vast numbers of species in relatively brief periods of time in geological terms. The diagram below shows how the number of species has varied over the history of life on Earth. The number of species is indicated on the graph by families; a taxonomic group comprising many genera and species. There have been five major extinction events and two of these have been intensively studied by palaeontologists.



1. Describe the main features (scale and type of organisms killed off) of each of the following major extinction events:

(a) Permian extinction: 220 MYA MARINE LIFE HALF FAMILIES

(b) Cretaceous extinction: 80 MYA DINOSAURS 70% FAMILIES

(c) Megafaunal extinction: 10,000 YA PLANTS/MAMALS SMALL NUMBER

2. Explain how human activity has contributed to the most recent mass extinction:

HUNTING, BUILDING

3. In general terms, describe the effect that past mass extinctions had on the way the surviving species **further evolved**:

OPPORTUNITY FOR MORE SUITABLE ORGANISMS TO EVOLVE

Related activities: Causes of Mass Extinctions
Web links: What Killed the Dinosaurs?
Periodicals: The sixth extinction

Protein Homologies

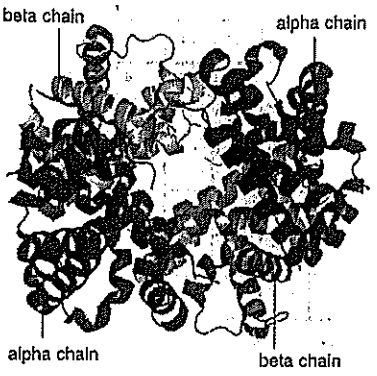
Traditionally, phylogenies were based largely on anatomical or behavioral traits and biologists attempted to determine the relationships between organisms based on overall degree of similarity or by tracing the appearance of key characteristics. With the advent of molecular techniques, homologies can now be studied at the molecular level as well and these can be compared to the phylogenies established using other methods. Protein sequencing provides an excellent tool for establishing **homologies** (similarities resulting from shared ancestry). Each protein has a specific number of amino acids arranged in a specific order. Any differences in the sequence reflect changes in the DNA sequence. Commonly studied proteins include blood proteins, such as **hemoglobin** (below), and the respiratory protein **cytochrome *c*** (overleaf). Many of these proteins are highly conserved, meaning they change very little over time, presumably because mutations would be detrimental to basic function. Conservation of protein sequences is indicated by the identical amino acid residues at corresponding parts of proteins.

Amino Acid Differences in Hemoglobin

Human beta chain	0
Chimpanzee	0
Gorilla	1
Gibbon	2
Rhesus monkey	8
Squirrel monkey	9
Dog	15
Horse, cow	25
Mouse	27
Gray kangaroo	38
Chicken	45
Frog	67

When the sequence of the beta hemoglobin chain (right), which is 146 amino acids long, is compared between humans, five other primates, and six other vertebrates, the results support the phylogenies established using other methods. The numbers in the table (left) represent the number of amino acid differences between the beta chain of humans and those of other species. In general, the number of amino acid differences between the hemoglobins of different vertebrates is inversely proportional to genetic relatedness.

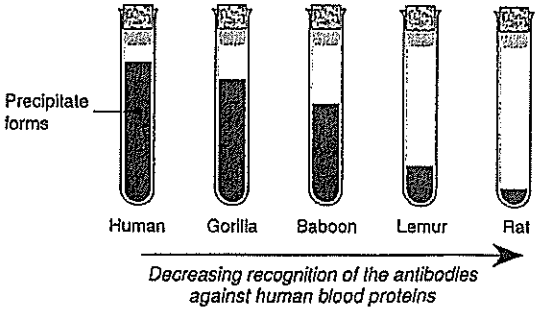
Shading indicates (from top) primates, non-primate placental mammals, marsupials, and non-mammals.

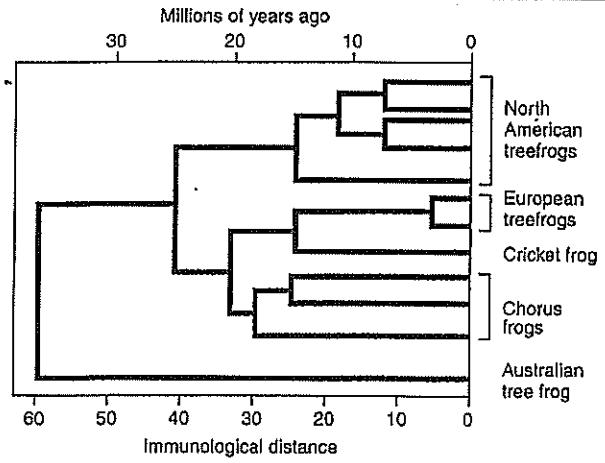


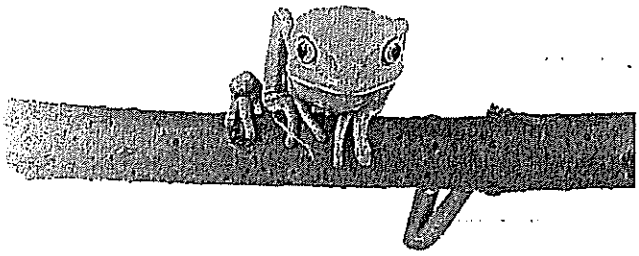
In most vertebrates, the oxygen-transporting blood protein hemoglobin is composed of four polypeptide chains, two alpha chains and two beta chains. Hemoglobin is derived from myoglobin, and ancestral species had just myoglobin for oxygen transport. When the amino acid sequences of myoglobin, the hemoglobin alpha chain, and the hemoglobin beta chain are compared, there are several amino acids that remain conserved between all three. These amino acid sequences must be essential for function because they have remained unchanged throughout evolution.

Using Immunology to Determine Phylogeny

The immune system of one species will recognize the blood proteins of another species as foreign and form antibodies against them. This property can be used to determine the extent of homology between species. Blood proteins, such as albumins, are used to prepare **antiserum** in rabbits. The antiserum contains antibodies against the test blood proteins (e.g. human) and will react to those proteins in any blood sample they are mixed with. The extent of the reaction indicates how different the proteins are; the greater the reaction, the greater the homology. This principle is illustrated (right) for antiserum produced to human blood and its reaction with the blood of other primates and a rat.







The relationships among tree frogs have been established by immunological studies based on blood proteins such as immunoglobulins and albumins. The immunological distance is a measure of the number of amino acid substitutions between two groups. This, in turn, has been calibrated to provide a time scale showing when the various related groups diverged.

Related activities: Comparative Anatomy, DNA Homologies
Web links: Evidence for Evolution

Cytochrome *c* and the Molecular Clock Theory

Evolutionary change at the molecular level occurs primarily through fixation of neutral mutations by genetic drift. The rate at which one neutral mutation replaces another depends on the mutation rate, which is fairly constant for any particular gene.

If the rate at which a protein evolves is roughly constant over time, the amount of molecular change that a protein shows can be used as a molecular clock to date evolutionary events, such as the divergence of species.

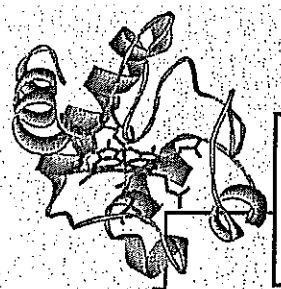
The molecular clock for each species, and each protein, may run at different rates, so scientists calibrate the molecular clock data with other evidence (morphological, molecular) to confirm phylogenetic relationships.

For example, 20 amino acid substitutions in a protein since two organisms diverged from a known common ancestor 400 mya indicates an average substitution rate of 5 substitutions per 100 my.

		1					6				10				14			17	18		20		
Human		Gly	Asp	Val	Glu	Lys	Gly	Lys	Lys	Ile	Phe	Ile	Met	Lys	Cys	Ser	Gln	Cys	His	Thr	Val	Glu	Lys
Pig												Val	Gln			Ala							
Chicken				Ile								Val	Gln										
Dogfish										Val		Val	Gln			Ala							Asn
Drosophila	<<									Leu		Val	Gln	Arg		Ala							Ala
Wheat	<<		Asn	Pro	Asp	Ala		Ala				Lys	Thr			Ala						Asp	Ala
Yeast	<<		Ser	Ala	Lys			Ala	Thr	Leu		Lys	Thr	Arg		Glu	Leu						

This table shows the N-terminal 22 amino acid residues of human cytochrome *c*, with corresponding sequences from other organisms aligned beneath. Sequences are aligned to give the most position matches. A shaded square indicates no change. In every case, the cytochrome's heme group is attached to the Cys-14 and Cys-17. In *Drosophila*, wheat, and yeast, arrows indicate that several amino acids precede the sequence shown.

The sequence homology of cytochrome c (right), a respiratory protein, has been used to construct a phylogenetic tree for species across a wide range of taxa. Overall, the phylogeny aligns well to other evolutionary data, although the tree indicates that primates branched off before the marsupials diverged from other placental mammals, which is incorrect based on a variety of other evidence. As indicated by the table above, cytochrome c is highly conserved, which means that its sequence changes very little despite speciation.



1. Explain why chimpanzees and gorillas are considered most closely related to humans, while monkeys are less so:

MORE CYTOCHROME AA IN COMMON

2. (a) Explain why a respiratory protein like cytochrome C would be highly conserved:

ALL ORGANISMS RESPIRE SO NEED PROTEIN

(b) Suggest why highly conserved proteins are good candidates for use in establishing protein homologies

ALL ORGANISMS HAVE THE PROTEIN

3. Discuss some of the limitations of using protein homology, specifically molecular clocks to establish phylogeny:

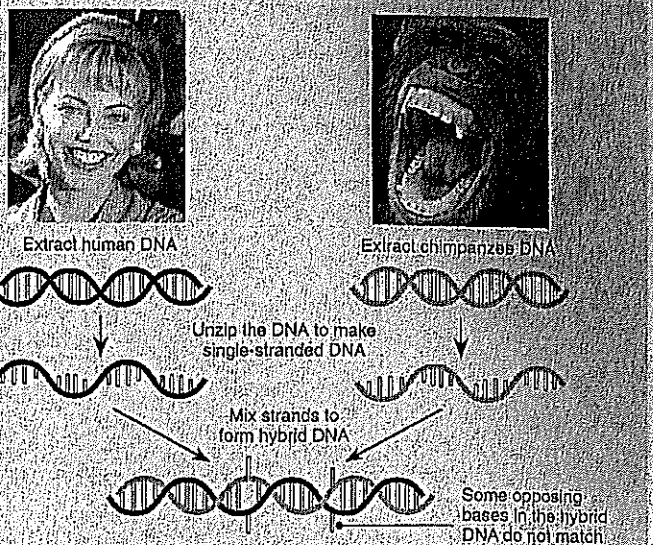
CLOCK MAY NOT ALWAYS RUN AT SAME TIME . . .

DNA Homologies

Establishing a phylogeny on the basis of homology in a protein, such as cytochrome c, is valuable, but it is also analogous to trying to see a complete picture through a small window. The technique of **DNA-DNA hybridization** provides a way to compare the total genomes of different species by measuring the degree of genetic similarity between pools of DNA sequences. It is usually used to determine the genetic distance between two species; the more closely two species are related, the fewer differences there will be between their genomes. This is because there has been less time for the point mutations that will bring about these differences to occur. This technique gives a measure of 'relatedness', and can be calibrated as a **molecular clock** against known fossil dates. It has been applied to primate DNA samples to help determine the approximate date of human divergence from the apes, which has been estimated to be between 10 and 5 million years ago.

DNA Hybridization

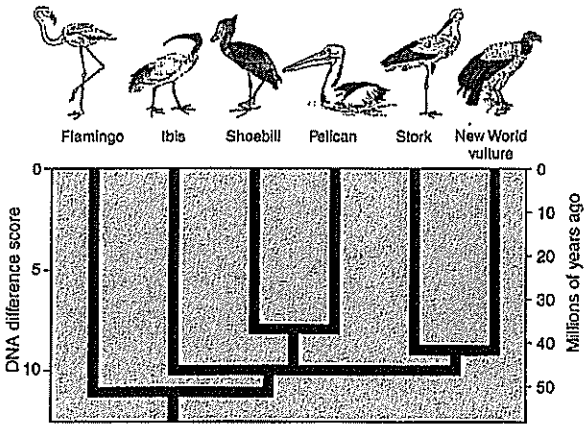
1. DNA from the two species to be compared is extracted, purified and cut into short fragments (e.g. 600-800 base pairs).
2. The DNA of one species is mixed with the DNAof another.
3. The mixture is incubated to allow DNA strands to dissociate and reanneal, forming hybrid double-stranded DNA.
4. The hybridized sequences that are highly similar will bind more firmly. A measure of the heat energy required to separate the hybrid strands provides a measure of DNA relatedness.



DNA Homologies Today

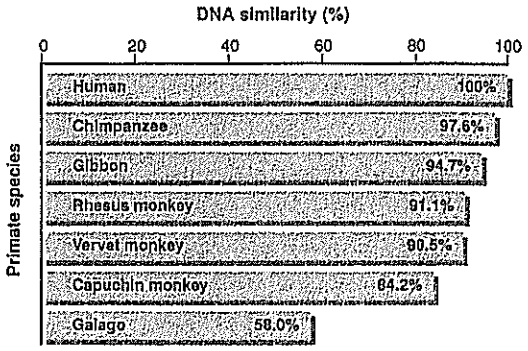
DNA-DNA hybridization has been criticized because duplicated sequences within a single genome make it unreliable for comparisons between closely related species.

Today, DNA sequencing and computed comparisons are more widely used to compare genomes, although DNA-DNA hybridization is still used to help identify bacteria.



The relationships among the New World vultures and storks have been determined using DNA-DNA hybridization. It has been possible to estimate how long ago various members of the group shared a common ancestor.

Similarity of human DNA to that of other primates



The genetic relationships among the primates has been investigated using DNA-DNA hybridization. Human DNA was compared with that of the other primates. It largely confirmed what was suspected from anatomical evidence.

1. Explain how **DNA hybridization** can give a measure of genetic relatedness between species:
   MORE NUCLEOTIDES IN COMMON THE MORE SIMILARLY RELATED.

2. Study the graph showing the results of a DNA hybridization between human DNA and that of other primates.
   (a) Identify which is the most closely related primate to humans: CHIMP
   (b) Identify which is the most distantly related primate to humans: GALAGO

3. State the DNA difference score for: (a) Shoebills and pelicans: 7.5 (b) Storks and flamingos: 11

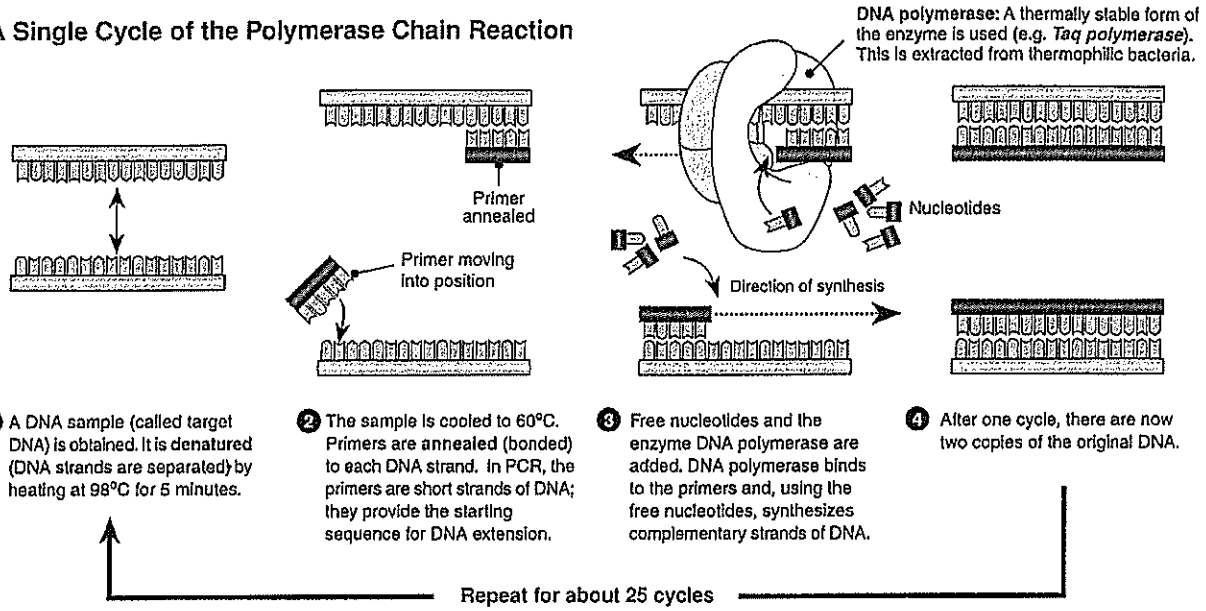
4. On the basis of DNA hybridization, state how long ago the ibises and New World vultures shared a common ancestor:
   65 MYA

Periodicals: Building a phylogenetic...
Related activities: Protein Homologies

Polymerase Chain Reaction

Many procedures in DNA technology (such as DNA sequencing and DNA profiling) require substantial amounts of DNA to work with. Some samples, such as those from a crime scene or fragments of DNA from a long extinct organism, may be difficult to get in any quantity. The diagram below describes the laboratory technique called **polymerase chain reaction (PCR)**. Using this technique, vast quantities of DNA identical to trace samples can be created. This process is often termed **DNA amplification**. Although only one cycle of replication is shown below, following cycles replicate DNA at an exponential rate. PCR can be used to make literally billions of copies in only a few hours. **Linear PCR** differs from regular PCR in that the same original DNA templates are used repeatedly. It is used to make many radio-labeled DNA fragments for DNA sequencing.

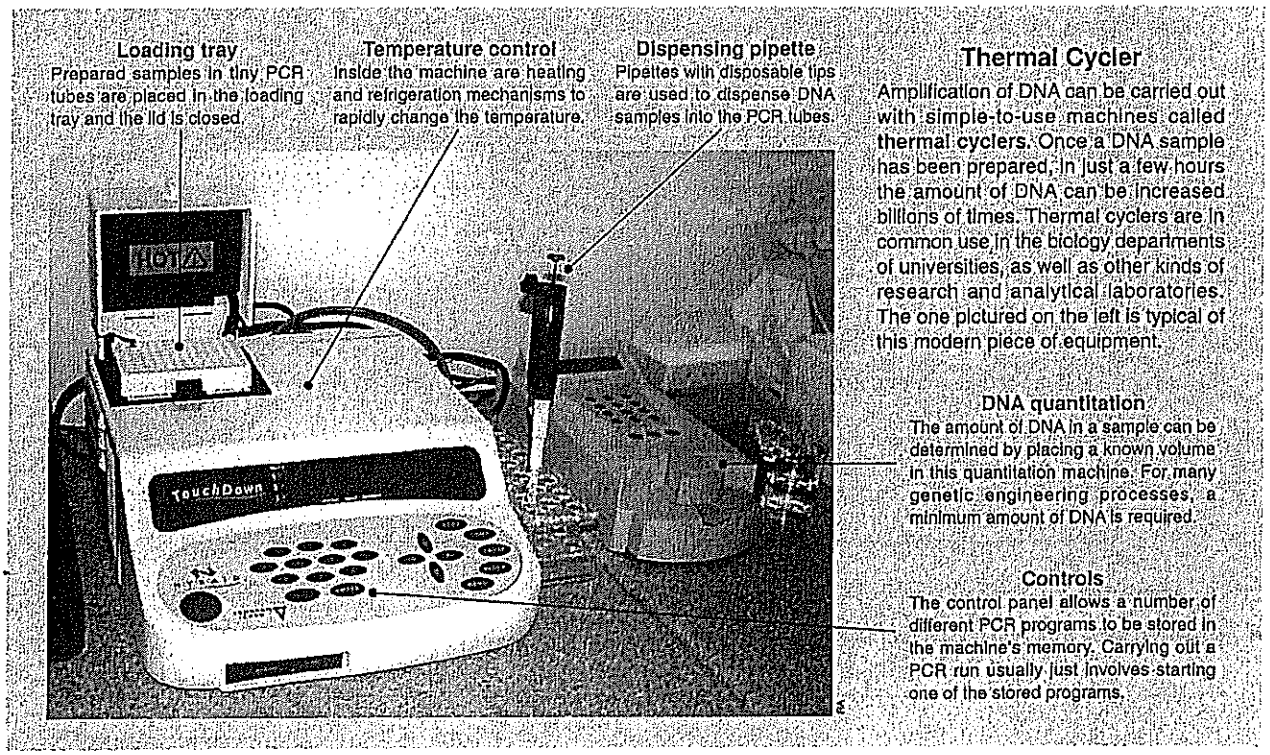
A Single Cycle of the Polymerase Chain Reaction



1. A DNA sample (called target DNA) is obtained. It is denatured (DNA strands are separated) by heating at 98°C for 5 minutes.
2. The sample is cooled to 60°C. Primers are annealed (bonded) to each DNA strand. In PCR, the primers are short strands of DNA; they provide the starting sequence for DNA extension.
3. Free nucleotides and the enzyme DNA polymerase are added. DNA polymerase binds to the primers and, using the free nucleotides, synthesizes complementary strands of DNA.
4. After one cycle, there are now two copies of the original DNA.

Repeat for about 25 cycles

Repeat cycle of heating and cooling until enough copies of the target DNA have been produced



Thermal Cycler

Amplification of DNA can be carried out with simple-to-use machines called thermal cyclers. Once a DNA sample has been prepared, in just a few hours the amount of DNA can be increased billions of times. Thermal cyclers are in common use in the biology departments of universities, as well as other kinds of research and analytical laboratories. The one pictured on the left is typical of this modern piece of equipment.

DNA quantitation
The amount of DNA in a sample can be determined by placing a known volume in this quantitation machine. For many genetic engineering processes, a minimum amount of DNA is required.

Controls
The control panel allows a number of different PCR programs to be stored in the machine's memory. Carrying out a PCR run usually just involves starting one of the stored programs.

1. Explain the purpose of PCR: AMPLIFY SPECIFIC SECTIONS OF DNA

2. Briefly describe how the **polymerase chain reaction** (PCR) works:

1. DENATURING
2. ANNEACING
3. REPLICATION

3. Describe three situations where only minute DNA samples may be available for sampling and PCR could be used:

(a) CRIME SCENE

(b) PRE NATAL DIAGNOSIS

(c) PATERNITY CASES

4. After only two cycles of replication, four copies of the double-stranded DNA exist. Calculate how much a DNA sample will have increased after:

(a) 10 cycles: 2^{10} (b) 25 cycles: 2^{25}

5. The risk of contamination in the preparation for PCR is considerable.

(a) Explain what the effect would be of having a single molecule of unwanted DNA in the sample prior to PCR:

TARGET SECTION IN CONTAMINANT MAY BE REPLICATED AS WELL

(b) Describe two possible sources of DNA contamination in preparing a PCR sample:

Source 1: MICROPIPETTE TIP CONTAMINATION

Source 2: HUMAN (BREATHING)

(c) Describe two precautions that could be taken to reduce the risk of DNA contamination:

Precaution 1: DISPOSABLE PIPETTE TIPS

Precaution 2: FACE MASK.

6. Describe two other genetic engineering/genetic manipulation procedures that require PCR amplification of DNA:

(a) GEL ELECTROPHORESIS (SCREENING)

(b) TRANSFORMATION

Restriction Enzymes

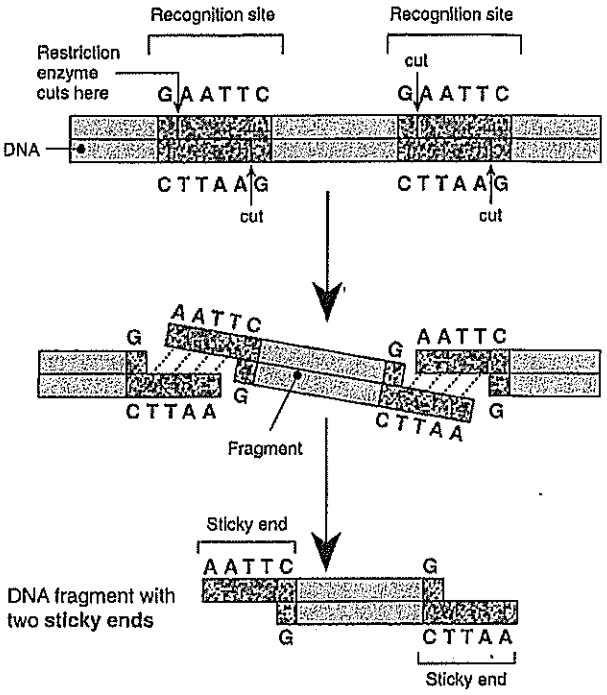
One of the essential tools of genetic engineering is a group of special **restriction enzymes** (also known as restriction endonucleases). These have the ability to cut DNA molecules at very precise sequences of 4 to 8 base pairs called **recognition sites**. These enzymes are the "molecular scalpels" that allow genetic engineers to cut up DNA in a controlled way. Although first isolated in 1970, these enzymes were discovered earlier in many bacteria (see panel on the next page). The purified forms of these bacterial restriction enzymes are used today as tools to cut DNA (see table on the next page for examples). Enzymes are named according to the bacterial species from which they were first isolated. By using a 'tool kit' of over 400 restriction enzymes recognizing about 100 recognition sites, genetic engineers can isolate, sequence, and manipulate individual genes derived from any type of organism. The sites at which the fragments of DNA are cut may result in overhanging "sticky ends" or non-overhanging "blunt ends". Pieces may later be joined together using an enzyme called **DNA ligase** in a process called **ligation**.

Sticky End Restriction Enzymes

1. A **restriction enzyme** cuts the double-stranded DNA molecule at its specific **recognition site** (see the table opposite for a representative list of restriction enzymes and their recognition sites).

2. The cuts produce a DNA fragment with two **sticky ends** (ends with exposed nucleotide bases at each end). The piece it is removed from is also left with sticky ends.

Restriction enzymes may cut DNA leaving an overhang or sticky end, without its complementary sequence opposite. DNA cut in such a way is able to be joined to other exposed end fragments of DNA with matching sticky ends. Such joins are specific to their recognition sites.

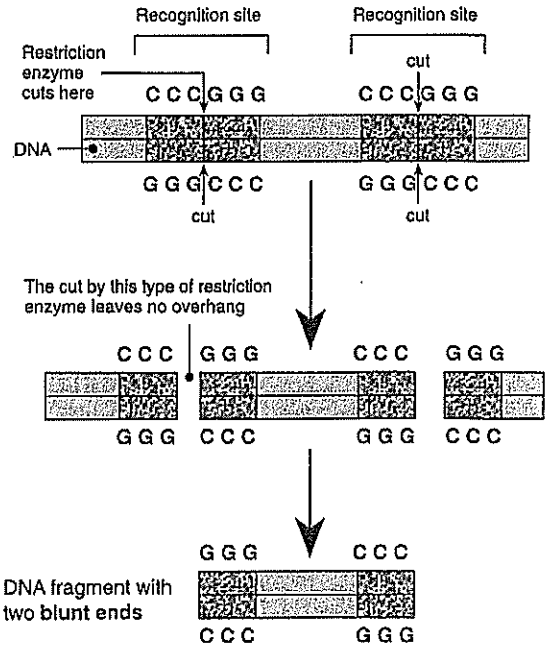


Blunt End Restriction Enzymes

1. A **restriction enzyme** cuts the double-stranded DNA molecule at its specific **recognition site** (see the table opposite for a representative list of restriction enzymes and their recognition sites).

2. The cuts produce a DNA fragment with two **blunt ends** (ends with no exposed nucleotide bases at each end). The piece it is removed from is also left with blunt ends.

It is possible to use restriction enzymes that cut leaving no overhang. DNA cut in such a way is able to be joined to any other blunt end fragment, but tends to be nonspecific because there are no sticky ends as recognition sites.



Related activities: Ligation
Web links: DNA Interactive: Cut and Paste

Origin of Restriction Enzymes

Restriction enzymes have been isolated from many bacteria. It was observed that certain *bacteriophages* (viruses that infect bacteria) could not infect bacteria other than their usual hosts. The reason was found to be that other potential hosts could destroy almost all of the phage DNA using *restriction enzymes* present naturally in their cells; a defense mechanism against the entry of foreign DNA. Restriction enzymes are named according to the species they were first isolated from, followed by a number to distinguish different enzymes isolated from the same organism.

Recognition sites for selected restriction enzymes

Enzyme	Source	Recognition Sites
*Eco*RI	*Escherichia coli* RY13	G A A T T C
*Bam*HI	*Bacillus amyloliquefaciens* H	G G A T C C
*Hae*III	*Haemophilus aegyptius*	G G C C
*Hin*dIII	*Haemophilus influenzae* Rd	A A G C T T
*Hpa*I	*Haemophilus parainfluenzae*	G T T A A C
*Hpa*II	*Haemophilus parainfluenzae*	C C G G
*Mbo*I	*Moraxella bovis*	G A T C
*Not*I	*Norcardia otitidis-caviarum*	G C G G C C G C
*Taq*I	*Thermus aquaticus*	T C G A

1. Explain the following terms, identifying their role in recombinant DNA technology:

   (a) Restriction enzyme: CUTTING DNA

   (b) Recognition site: WHERE THE RE CUTS

   (c) Sticky end: COVALENT BONDS CUT 2-6 BASES ALONG LEAVING EXPOSED ENDS.

   (d) Blunt end: COVALENT BONDS BROKEN STRAIGHT ACROSS

2. The action of a specific sticky end restriction enzyme is illustrated on the previous page (top). Use the table above to:

   (a) Name the **restriction enzyme** used: ECORI

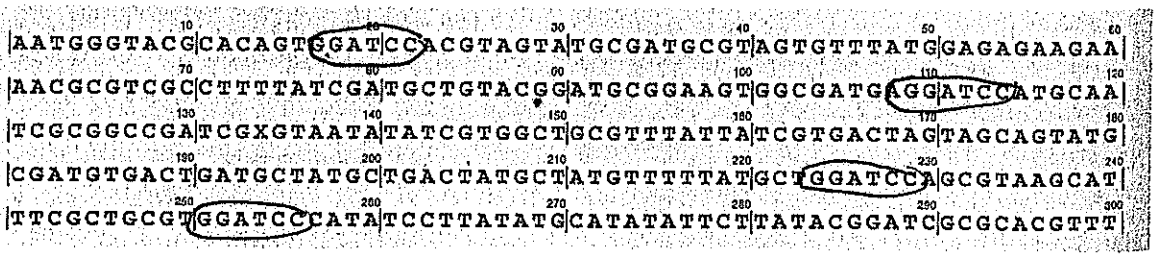
   (b) Name the organism from which it was first isolated: E. Coli

   (c) State the **base sequence** for this restriction enzyme's recognition site: GAATTC

3. A genetic engineer wants to use the restriction enzyme *Bam*HI to cut the DNA sequence below:

   (a) Consult the table above and state the recognition site for this enzyme: GGATCC

   (b) Circle every **recognition site** on the DNA sequence below that could be cut by the enzyme *Bam*HI:



   (c) State how many fragments of DNA were created by this action: 5

4. When restriction enzymes were first isolated in 1970 there were not many applications to which they could be put to use. They are now an important tool in genetic engineering. Describe the human needs and demands that have driven the development and use of restriction enzymes in genetic engineering:

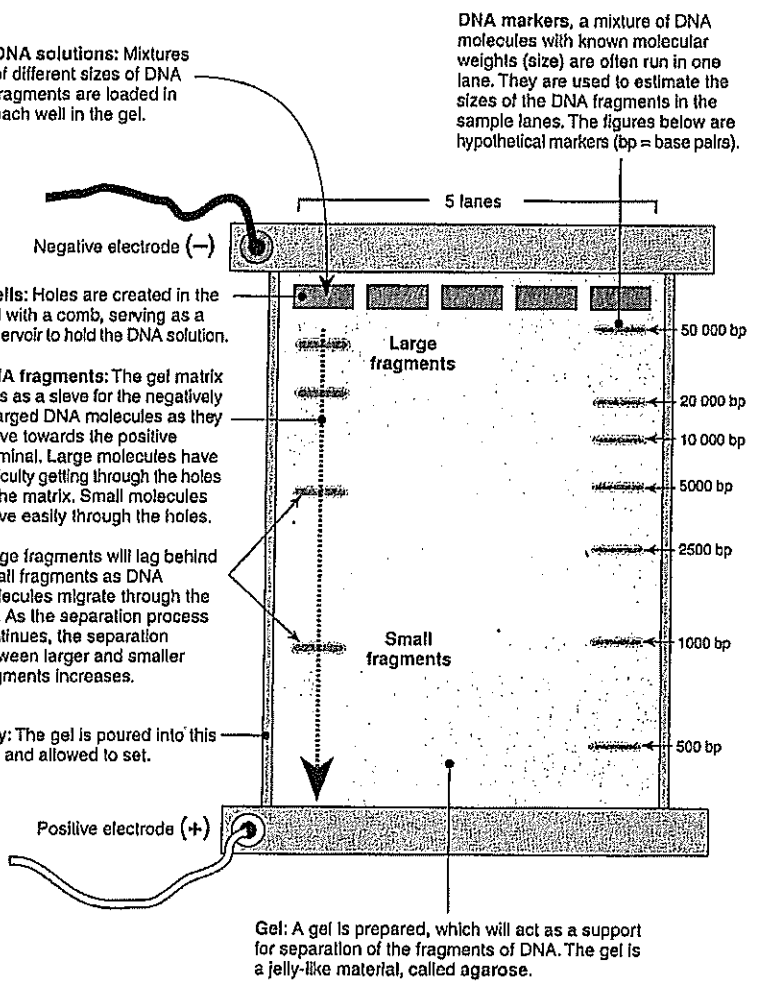
   TRANSGENIC ORGANISMS

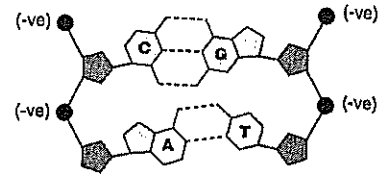
   GENETIC PROFILING

Gel Electrophoresis

Gel electrophoresis is a method that separates large molecules (including nucleic acids or proteins) on the basis of size, electric charge, and other physical properties. Such molecules possess a slight electric charge (see DNA below). To prepare DNA for gel electrophoresis the DNA is often cut up into smaller pieces. This is done by mixing DNA with restriction enzymes in controlled conditions for about an hour. Called **restriction digestion**, it produces a range of DNA fragments of different lengths. During electrophoresis, molecules are forced to move through the pores of a gel (a jelly-like material), when the electrical current is applied. Active electrodes at each end of the gel provide the driving force. The electrical current from one electrode repels the molecules while the other electrode simultaneously attracts the molecules. The frictional force of the gel material resists the flow of the molecules, separating them by size. Their rate of migration through the gel depends on the strength of the electric field, size and shape of the molecules, and on the ionic strength and temperature of the buffer in which the molecules are moving. After staining, the separated molecules in each lane can be seen as a series of bands spread from one end of the gel to the other.

Analyzing DNA using Gel Electrophoresis





DNA is negatively charged because the phosphates (black) that form part of the backbone of a DNA molecule have a negative charge.

Steps in gel electrophoresis of DNA

1. A tray is prepared to hold the gel matrix.
2. A gel comb is used to create holes in the gel. The gel comb is placed in the tray.
3. Agarose gel powder is mixed with a buffer solution (the liquid used to carry the DNA in a stable form). The solution is heated until dissolved and poured into the tray and allowed to cool.
4. The gel tray is placed in an electrophoresis chamber and the chamber is filled with buffer, covering the gel. This allows the electric current from electrodes at either end of the gel to flow through the gel.
5. DNA samples are mixed with a "loading dye" to make the DNA sample visible. The dye also contains glycerol or sucrose to make the DNA sample heavy so that it will sink to the bottom of the well.
6. A safety cover is placed over the gel, electrodes are attached to a power supply and turned on.
7. When the dye marker has moved through the gel, the current is turned off and the gel is removed from the tray.
8. DNA molecules are made visible by staining the gel with ethidium bromide which binds to DNA and will fluoresce in UV light.

1. Explain the purpose of gel electrophoresis: SEPARATE DNA FRAGMENTS ACCORDING TO SIZE

2. Describe the two forces that control the speed at which fragments pass through the gel:
   (a) ELECTRIC CURRENT
   (b) FRAGMENT SIZE

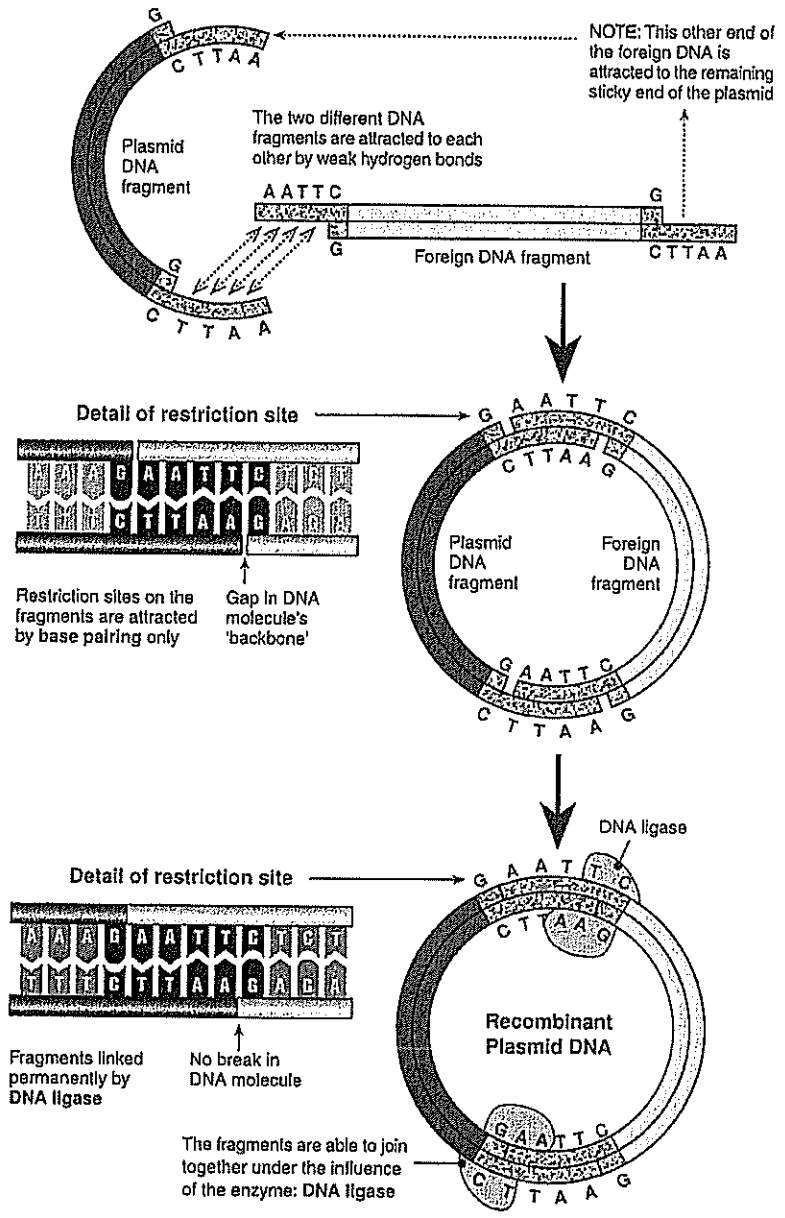
3. Explain why the smallest fragments travel through the gel the fastest: EASIER TO MOVE THROUGH THE GEL

Related activities: Nucleic Acids, DNA Profiling, Automated DNA Sequencing
Web links: DNA Extraction, Gel Electrophoresis

Ligation

DNA fragments produced using restriction enzymes may be reassembled by a process called **ligation**. Pieces are joined together using an enzyme called **DNA ligase**. DNA of different origins produced in this way is called **recombinant DNA** (because it is DNA that has been recombined from different sources). The combined techniques of using restriction enzymes and ligation are the basic tools of genetic engineering (also known as recombinant DNA technology).

Creating a Recombinant DNA Plasmid



1. If two pieces of DNA are cut by the same restriction enzyme, they will produce fragments with matching **sticky ends** (ends with exposed nucleotide bases at each end).

2. When two such matching sticky ends come together, they can join by base-pairing. This process is called **annealing**. This can allow DNA fragments from a different source, perhaps a **plasmid**, to be joined to the DNA fragment.

3. The joined fragments will usually form either a linear molecule or a circular one, as shown here for a **plasmid**. However, other combinations of fragments can occur.

4. The fragments of DNA are joined together by the enzyme **DNA ligase**, producing a molecule of **recombinant DNA**.

1. Explain in your own words the two main steps in the process of joining two DNA fragments together:

(a) Annealing: JOINING FRAGMENTS DUE TO COMPLIMENTARY SEQUENCES OF 'STICKY' ENDS

(b) DNA ligase: FRAGMENTS FORM COVALENT BONDS

2. Refer to the activity *DNA Replication* and briefly describe the usual role of DNA ligase in a cell:

NA: BUT PERFORMS SIMILAR ROLE

3. Explain why ligation can be considered the *reverse* of the restriction enzyme process:

LIGATION: FORM COVALENT BOND RESTRICT: BREAKS

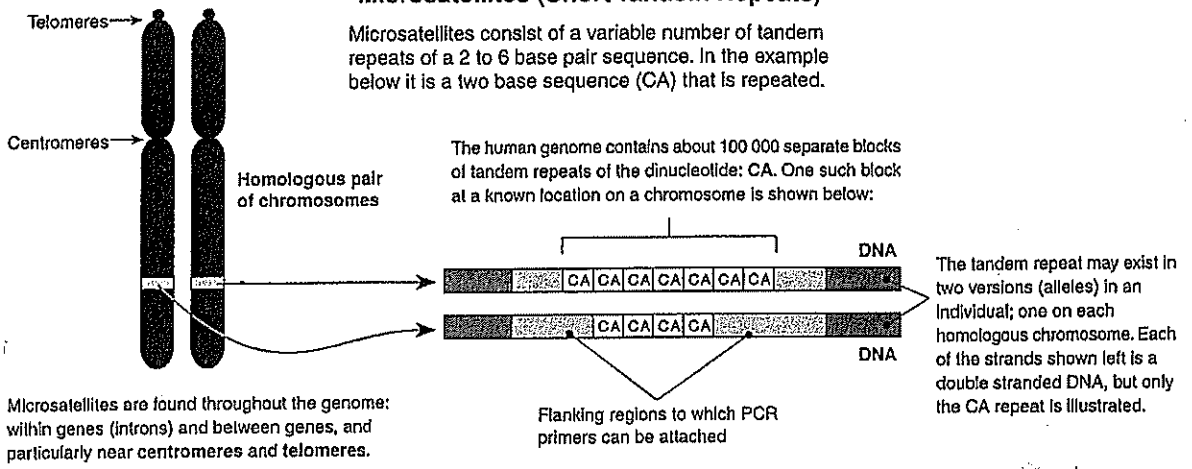
DNA Profiling Using PCR

In chromosomes, some of the DNA contains simple, repetitive sequences. These *noncoding* nucleotide sequences repeat themselves over and over again and are found scattered throughout the genome. Some repeating sequences are short (2-6 base pairs) called **microsatellites** or **short tandem repeats** (STRs) and can repeat up to 100 times. The human genome has numerous different microsatellites. Equivalent sequences in different people vary considerably in the numbers of the repeating unit. This phenomenon has been used to develop **DNA profiling**, which identifies the natural variations found in every person's DNA. Identifying such differences in the DNA of individuals is a useful tool for forensic investigations.

In 1998, the FBI's Combined Offender DNA Index System (CODIS) was established, providing a national database of DNA samples from convicted criminals, suspects, and crime scenes. In the USA, there are many laboratories approved for forensic DNA testing. Increasingly, these are targeting the 13 core STR loci recommended by the FBI; enough to guarantee that the odds of someone else sharing the same result are extremely unlikely (less than one in a thousand million). The CODIS may be used to solve previously unsolved crimes and to assist in current or future investigations. DNA profiling can also be used to establish genetic relatedness (e.g. in paternity or pedigree disputes), or when searching for a specific gene (e.g. screening for disease).

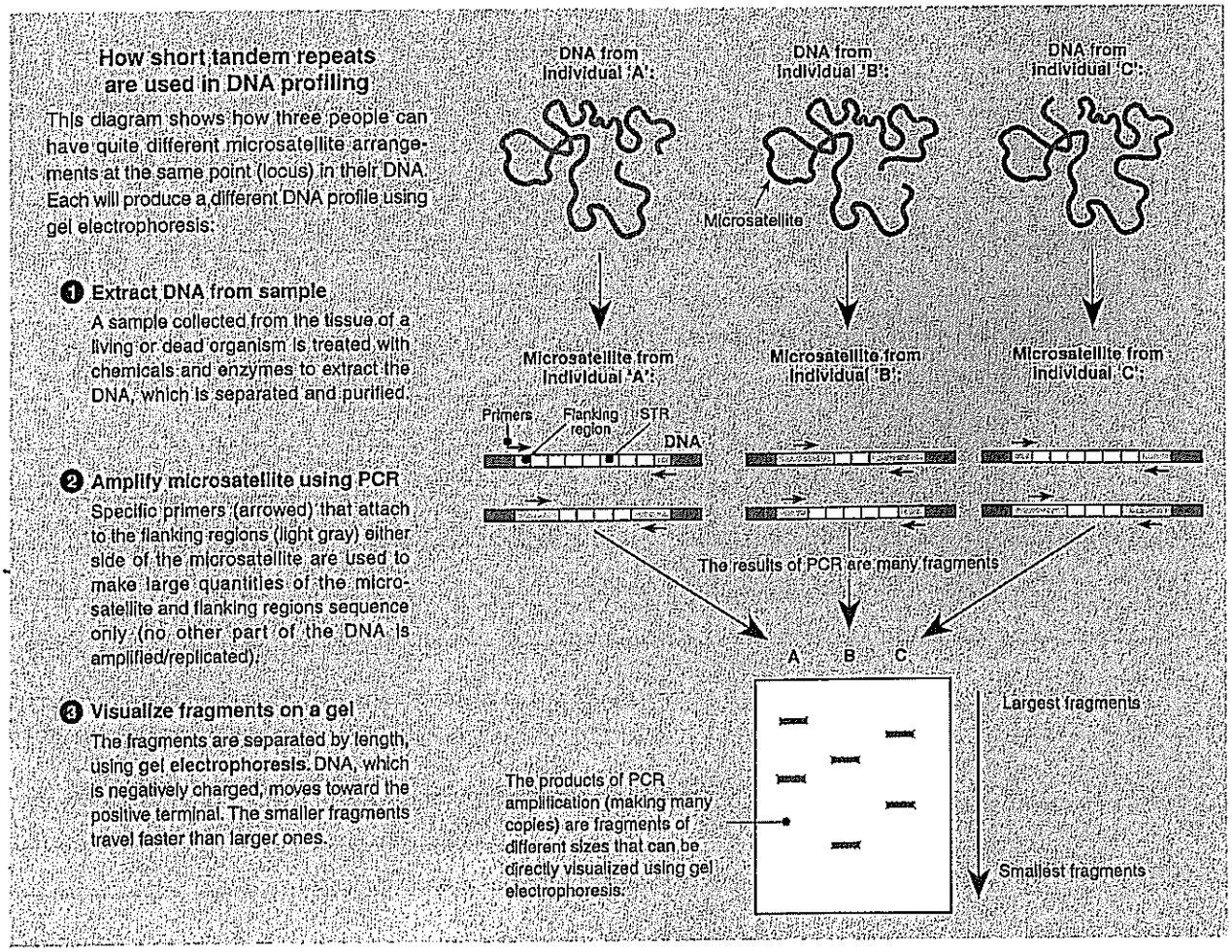
Microsatellites (Short Tandem Repeats)

Microsatellites consist of a variable number of tandem repeats of a 2 to 6 base pair sequence. In the example below it is a two base sequence (CA) that is repeated.



How short tandem repeats are used in DNA profiling

This diagram shows how three people can have quite different microsatellite arrangements at the same point (locus) in their DNA. Each will produce a different DNA profile using gel electrophoresis:



❶ Extract DNA from sample

A sample collected from the tissue of a living or dead organism is treated with chemicals and enzymes to extract the DNA, which is separated and purified.

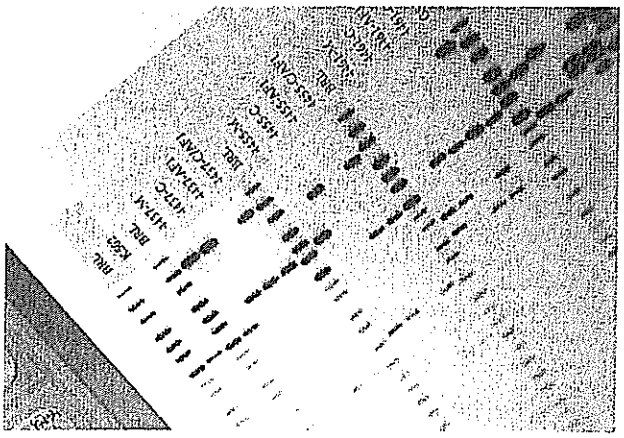
❷ Amplify microsatellite using PCR

Specific primers (arrowed) that attach to the flanking regions (light gray) either side of the microsatellite are used to make large quantities of the microsatellite and flanking regions sequence only (no other part of the DNA is amplified/replicated).

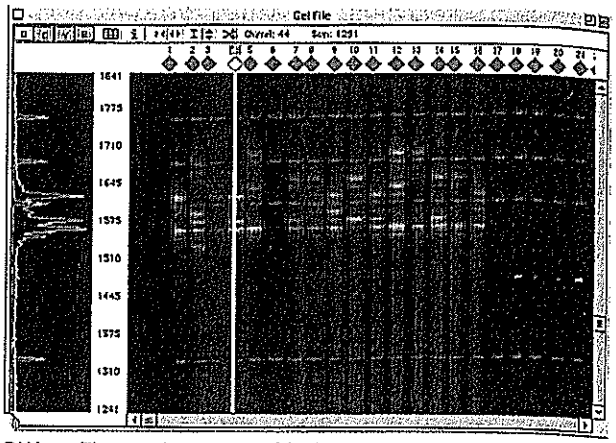
❸ Visualize fragments on a gel

The fragments are separated by length, using gel electrophoresis. DNA, which is negatively charged, moves toward the positive terminal. The smaller fragments travel faster than larger ones.

Related activities: Gel Electrophoresis, Forensic Applications of DNA Profiling, Polymerase Chain Reaction
Web links: DNA Profiling Using Probes



The photo above shows a film output from a DNA profiling procedure. Those lanes with many regular bands are used for calibration; they contain DNA fragment sizes of known length. These calibration lanes can be used to determine the length of fragments in the unknown samples.



DNA profiling can be automated in the same way as DNA sequencing. Computer software is able to display the results of many samples run at the same time. In the photo above, the sample in lane 4 has been selected. It displays fragments of different length on the left of the screen.

1. Describe the properties of **short tandem repeats** that are important to the application of **DNA profiling** technology:

   LOCI VARY IN SIZE

2. Explain the role of each of the following techniques in the process of DNA profiling:

   (a) Gel electrophoresis: SEPERATES FRAGMENTS ACCORDING TO SIZE

   (b) PCR: AMPLIFIES SPECIFIC TARGETED SECTIONS OF THE GENOME

3. Describe the three main steps in DNA profiling using PCR:

   (a) EXTRACT DNA FROM SUBJECT

   (b) AMPLIFY TARGET PCR

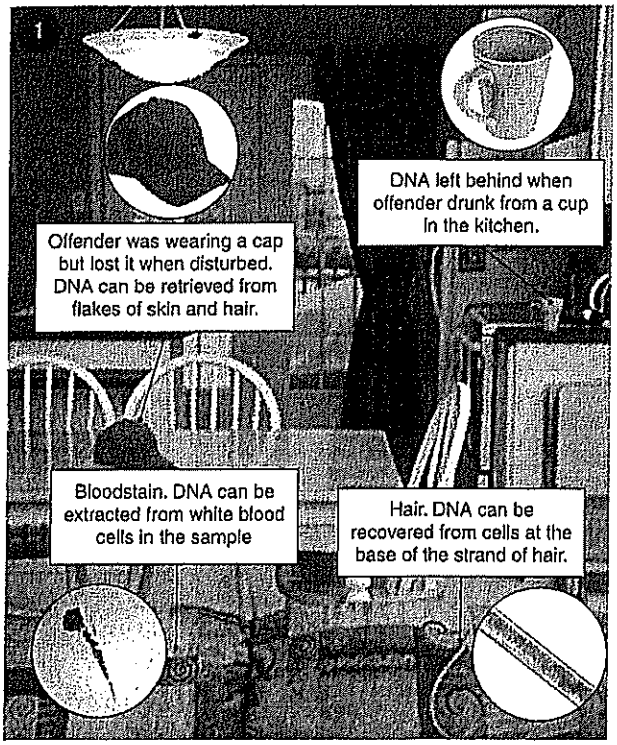
   (c) APPLY GEL ELECTROPHORESIS TO SEPERATE FRAGMENTS

4. Explain why as many as 10 STR sites are used to gain a DNA profile for forensic evidence:

   CLARITY.

Forensic Applications of DNA Profiling

The use of DNA as a tool for solving crimes such as homicide is well known, but it can also be used to as a solution to many other problems. DNA evidence has been used to identify body parts, solve cases of industrial sabotage and contamination, for paternity testing, and even in identifying animal products illegally made from endangered species.



During the initial investigation, samples of material that may contain DNA are taken for analysis. At a crime scene, this may include blood and body fluids as well as samples of clothing or objects that the offender might have touched. Samples from the victim are also taken to eliminate them as a possible source of contamination.

1. In the above case two sets of DNA profiles are shown. Describe the purpose of lane A in each set of profiles:

   MARKER LANE

2. Explain why DNA profiles are obtained for both the victim and investigator:

   CONTAMINATION ISSUES

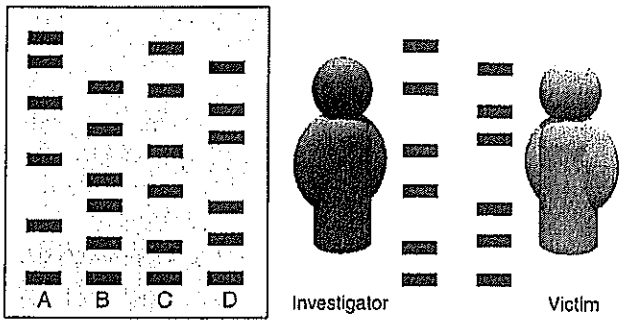
3. Use the evidence to decide if the alleged offender is innocent or guilty and explain your decision:

   NOT GUILTY
   PROFILE NOT IN SAMPLE

4. Explain how DNA profiling could be used to refute official claims of the number of whales being captured and sold in fish markets:

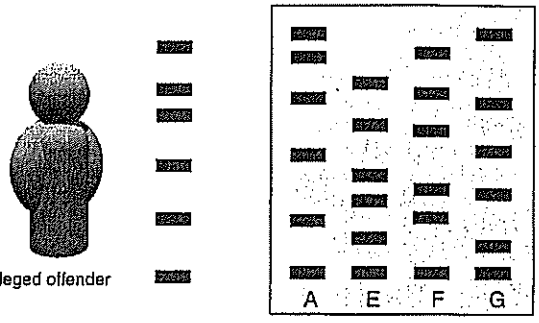
   EACH WHALE UNIQUE
   SAMPLING WHALE MEAT
   GIVE IDEA OF NUMBERS.

2 DNA is isolated and profiles are made from all samples and compared to known DNA profiles such as that of the victim.



Profiles from collected DNA

3 Unknown DNA samples are compared to DNA databases of convicted offenders and to the DNA of the alleged offender.



Alleged offender

Profiles from DNA database

4 Although it does not make a complete case, DNA profiling, in conjunction with other evidence, is one of the most powerful tools in identifying offenders or unknown tissues.

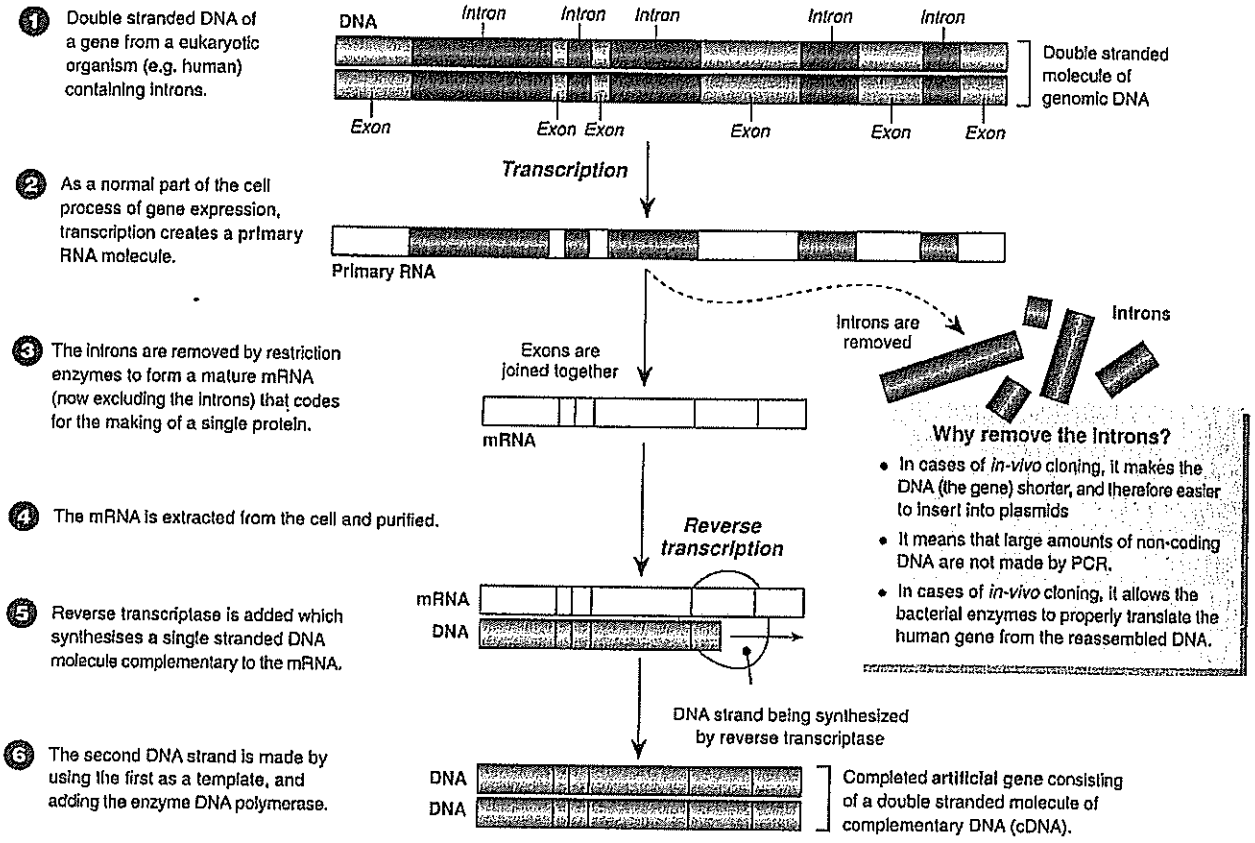
Whale DNA: Tracking Illegal Slaughter

Under International Whaling Commission regulations, some species of whales can be captured for scientific research and their meat sold legally. Most, including humpback and blue whales, are fully protected and to capture or kill them for any purpose is illegal. Between 1999 and 2003 Scott Baker and associates from Oregon State University's Marine Mammal Institute investigated whale meat sold in markets in Japan and South Korea. Using DNA profiling techniques, they found around 10% of the samples tested were from fully protected whales including western gray whales and humpbacks. They also found that many more whales were being killed than were being officially reported.

Preparing a Gene for Cloning

Gene cloning is the process of making large quantities of a piece of DNA once it has been isolated. Its purpose is to yield large quantities of an individual gene or its protein product when the gene is expressed. Genes can be cloned *in vitro* in an automated process called PCR, which amplifies the DNA. Genes can also be cloned when they are part of an organism, in a technique called *in vivo* cloning. A gene of interest (e.g. a human gene) is inserted into the DNA of a vector, resulting in a **recombinant DNA molecule** called a **molecular clone**. This technique uses the self-replicating properties of the vector to make copies of the gene. The genes of interest are rarely ready for cloning in their native form because they include pieces of non-protein coding DNA, called **introns**, which must be removed. Molecular biologists have a handy tool in the form of an enzyme, called **reverse transcriptase**, which makes this possible. Reverse transcriptase is a common name for an enzyme that functions as a RNA-dependent DNA polymerase and it is used to copy RNA into DNA. This task is integral to both *in vitro* and *in vivo* gene cloning because it produces a reconstructed gene that is ready for amplification.

Preparing a Gene For Cloning



1. Explain the role of restriction enzymes in preparing a clone:

   SO GENE CAN BE ISOLATED AND WITH STICKY ENDS (FOR TRANSFER)

2. (a) Explain why introns are removed before cloning a gene:

   SO ONLY THE DNA CODING FOR PROTEIN REMAINS

   (b) Describe the role of reverse transcriptase in this process:

   CONVERTS RNA → DNA

3. Describe the normal role of reverse transcriptase:

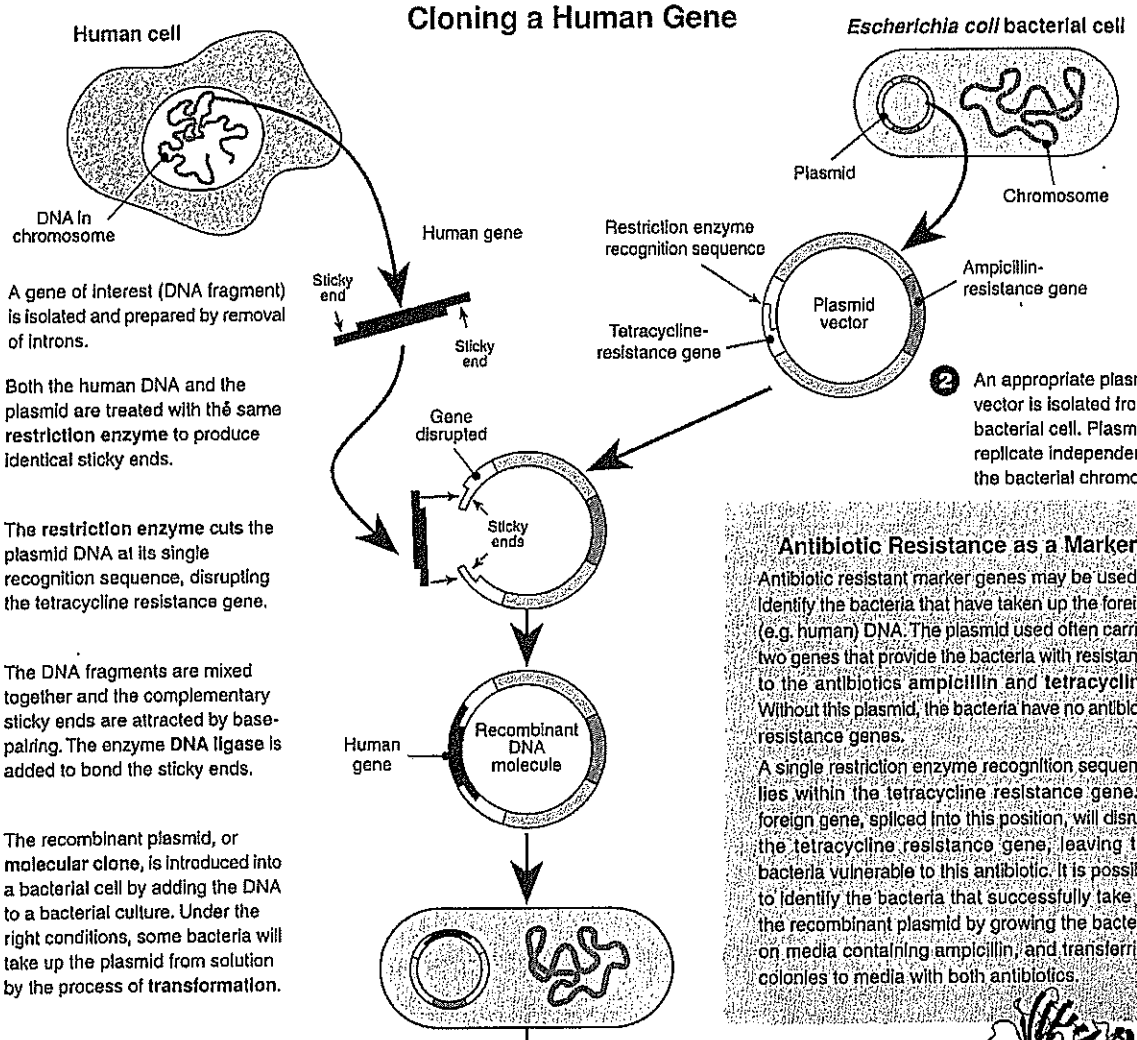
   VIRAL CONVERSION OF RNA → DNA

Related activities: In Vivo Gene Cloning, Polymerase Chain Reaction

In Vivo Gene Cloning

It is possible to use the internal replication machinery of a cell to clone a gene, or even many genes, at once. By using cells to copy desired genes, it is also possible to produce any protein product the genes may code for. Recombinant DNA techniques (restriction digestion and ligation) are used to insert a gene of interest into the DNA of a vector (e.g. plasmid or viral DNA). This produces a **recombinant DNA molecule** that can used to transmit the gene of interest to another organism. To be useful, all vectors must be able to replicate inside their host organism, they must have one or more sites at which a restriction enzyme can cut, and they must have some kind of **genetic marker** that allows them to be easily identified. Viruses, and organisms such as bacteria and yeasts have DNA that behaves in this way. Bacterial plasmids are commonly used because they are easy to manipulate, their restriction sites are well known, and they are readily taken up by cells in culture. Once the recombinant plasmid vector (containing the desired gene) has been taken up by bacterial cells, and those cells are identified, the gene can be replicated many times as the bacteria grow and divide.

Cloning a Human Gene



1. A gene of interest (DNA fragment) is isolated and prepared by removal of introns.
2. An appropriate plasmid vector is isolated from a bacterial cell. Plasmids replicate independently of the bacterial chromosome.
3. Both the human DNA and the plasmid are treated with the same **restriction enzyme** to produce identical sticky ends.
4. The **restriction enzyme** cuts the plasmid DNA at its single recognition sequence, disrupting the tetracycline resistance gene.
5. The DNA fragments are mixed together and the complementary sticky ends are attracted by base-pairing. The enzyme **DNA ligase** is added to bond the sticky ends.
6. The recombinant plasmid, or **molecular clone**, is introduced into a bacterial cell by adding the DNA to a bacterial culture. Under the right conditions, some bacteria will take up the plasmid from solution by the process of **transformation**.

Antibiotic Resistance as a Marker

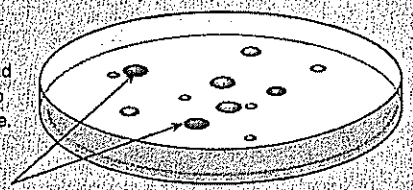
Antibiotic resistant marker genes may be used to identify the bacteria that have taken up the foreign (e.g. human) DNA. The plasmid used often carries two genes that provide the bacteria with resistance to the antibiotics **ampicillin** and **tetracycline**. Without this plasmid, the bacteria have no antibiotic resistance genes.

A single restriction enzyme recognition sequence lies within the tetracycline resistance gene. A foreign gene, spliced into this position, will disrupt the tetracycline resistance gene, leaving the bacteria vulnerable to this antibiotic. It is possible to identify the bacteria that successfully take up the recombinant plasmid by growing the bacteria on media containing ampicillin, and transferring colonies to media with both antibiotics.

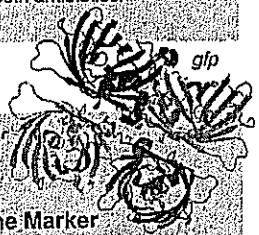
Preparation of the clone up to this point

Cloning the gene starts here

7. The actual gene cloning process (making multiple copies of the human gene) occurs when the bacterium with the recombinant plasmid is allowed to reproduce.
8. Colonies of bacteria that carry the recombinant plasmid can be identified by the fact that they are resistant to ampicillin but sensitive to tetracycline.



Agar plate with bacterial colonies. Only some have the plasmid with the human gene.



gfp as a Gene Marker

Most often today, another gene plays the role of the tetracycline resistance gene. The gene for Green Fluorescent Protein (*gfp*, above) isolated from the jellyfish *Aequorea victoria*, has become well established a a marker for gene expression. The *gfp* gene is recombined with the gene of interest and transformed cells can then be detected by the presence of the fluorescent product.

Related activities: Restriction Enzymes, Preparing a Gene for Cloning
Web links: Gene Cloning

1. Explain why it might be desirable to use *in vivo* methods to clone genes rather than PCR:
LIMITLESS PRODUCTION OF GENE + PROTEIN

2. Explain when it may not be desirable to use bacteria to clone genes:
NOT NEED PROTEIN OR ONLY USED IN PROFILING

3. Explain how a human gene is removed from a chromosome and placed into a plasmid.
DNA EXTRACTION
PCR
RESTRICTION ENZYMES
MIX WITH PLASMID (ALSO RESTRICTED)
LIGASE

4. A bacterial plasmid replicates at the same rate as the bacteria. If a bacteria containing a recombinant plasmid replicates and divides once every thirty minutes, calculate the number of plasmid copies there will be after twenty four hours:
2^{48}

5. When cloning a gene using **plasmid vectors**, the bacterial colonies containing the recombinant plasmids are mixed up with colonies that have none. All the colonies look identical, but some have taken up the plasmids with the human gene, and some have not. Explain how the colonies with the recombinant plasmids are identified:
ANTIBIOTIC RESISTANCE GENES ON PLASMID
GROW BACTERIA IN ANTIBIOTIC

6. Explain why the *gfp* marker is a more desirable gene marker than genes for antibiotic resistance:
GFP NOT HARMFUL TO ENVIRONMENT
COMPARED TO USING ~~BACTERIA~~ PLASMIDS
THAT CONFER A BACTERIA RESISTANT TO ANTIBIOTICS

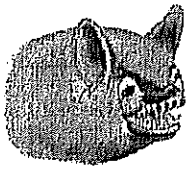
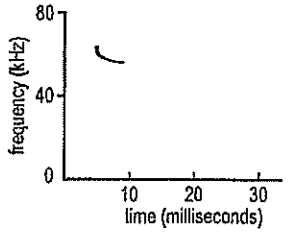
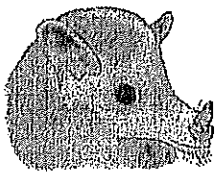
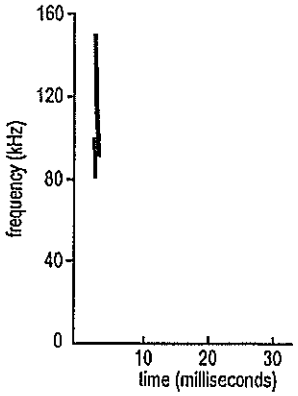
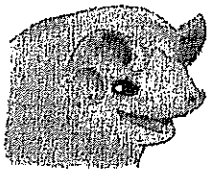
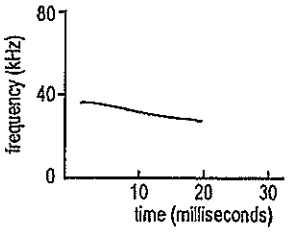
7. Viruses are also used in *in vivo* gene cloning even though they have no replication machinery themselves. Explain how viruses can be used to clone genes:
INFECT CELL LINES AND REPLICATE
WITHIN THEM

Question 5

Barro Colorado Island is a small island covered by tropical forest in Central America. Seventy-four different species of bats live in the forest.

Bats are nocturnal, flying mammals. To find their way around in darkness, many bat species emit high-frequency sound pulses that bounce off obstacles and prey. These pulses enable them to judge the distance to an object. This behaviour is called echolocation.

Three of the Barro Colorado Island species are described in the table below.

Species name	Facial appearance of bat	Diet	Feeding location	Echolocation signal
Black myotis bat (*Myotis nigricaris*)		insects	around trees at forest's edge and in clearings	
Mexican long-tongued bat (*Choeronycteris mexicana*)		nectar and pollen flowers that open at night, for example cactus, agave	narrow gaps and small spaces	
Velvety free-tailed bat (*Molossus molossus*)		insects	above trees, in open spaces	

a. From the information provided, state one selection pressure operating on the bats of Barro Colorado Island.

AVAILABILITY OF FOOD

1 mark

b. i. In terms of time, which of the three species emits the longest echolocation signal?

VELVETY BAT

ii. Explain how this could be a selective advantage for this bat species.

BETTER CHANCE FINDING INSECTS

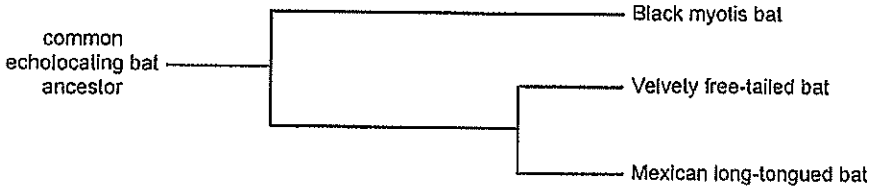
1 + 1 = 2 marks

c. A biologist suggested that the three species shown evolved from a recent common ancestor. What is this type of evolution called?

DIVERGENT

1 mark

The biologist wanted to establish the order in which each species had evolved from the common ancestor. DNA hybridisation between the various species was carried out. After analysing the results, the scientist drew the following phylogenetic tree.



d. What results would have been obtained from the DNA hybridisation that led the biologist to construct this phylogeny?

VELVET & MEXICAN MORE DNA NUCLEOTIDES IN COMMON (HIGHER T_m) BOTH HAVE LESS ~~DNA~~ NUCLEOTIDES IN COMMON WITH BLACK (LOWER T_m)

2 marks

e. A student suggested that the evolution of the three species was an example of allopatric speciation. Explain why you agree or disagree with the student.

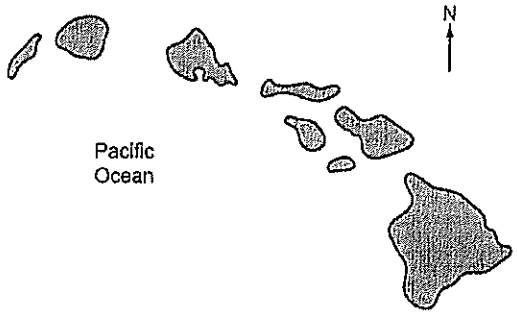
DISAGREE

NO GEOGRAPHIC BARRIER

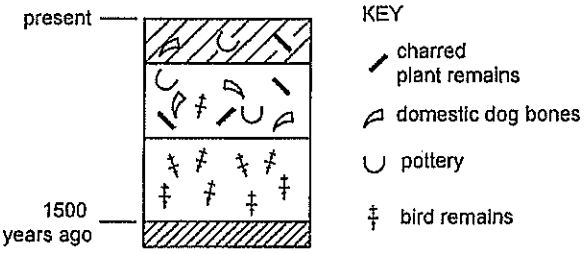
2 marks

Question 7

The islands of Hawaii in the Pacific Ocean were formed as a result of volcanic action in which small land masses were thrown up by submarine volcanoes. The youngest of the islands lies to the east of the oldest.



A similar pattern of deposition has been found across all islands, shown by the profile below.



a. What assumption is made about the formation of strata when interpreting profiles such as this?

TOP STRATA YOUNGER

1 mark

b. i. State a hypothesis to account for the disappearance of many of the bird species from the groups of islands.

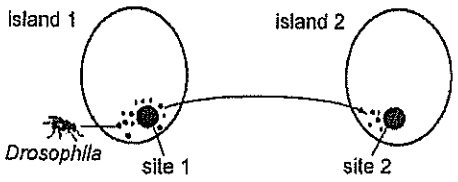
PREDATION

ii. Provide evidence to support your hypothesis.

PRESENCE OF DOG BONES

1 + 1 = 2 marks

Biologists studied many species of the fruit fly, *Drosophila*, living on the Hawaiian islands. The species vary widely in appearance, behaviour and habitat. The diversity of *Drosophila* can be explained by the successive colonisation of newly formed islands by a small number of individuals 'island-hopping' from the neighbouring westerly island. This is represented in the diagram below.



c. i. What name is given to this small group of colonising individuals?

FOUNDERS

ii. Explain how the new and old colonies became separate species.

POPULATIONS SEPERATED

SELECTION OCCURS

REUNITING REVEALS INABILITY TO SUCCESSFULLY BREED

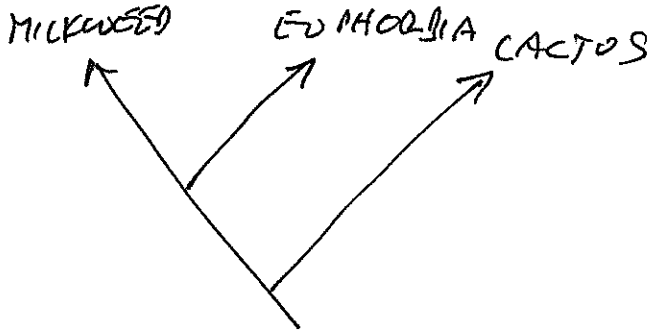
1 + 3 = 4 marks

Total 7 marks

Question 5

Three different kinds of plants, a cactus, euphorbia and milkweed, have similar adaptations for growing in desert environments. They all have long, fleshy stems for water storage, protective spines and reduced leaves. Both the euphorbia and milkweed plants are believed to have evolved from leafy plants adapted to more temperate climates. They share a more recent ancestor than either one does to a cactus.

a. Draw an evolutionary tree (also called phylogenetic tree or cladogram) to demonstrate this relationship.



1 mark

b. Name the process by which the ancestral leafy euphorbia plant could have given rise to the desert-adapted species described above.

NATURAL SELECTION

1 mark

c. What is a possible explanation for there being so few cactus fossils?

LACK HARD PARTS

1 mark

Total 3 marks

Question 5

Eastern tiger snakes (*Notechi scutatus*) living on desolate islands off mainland Australia have longer jaws than the mainland populations of snakes. The diet of island snakes includes large prey, such as seagull chicks, while the diet of the mainland snakes consists of small prey, such as frogs and mice.

Researchers set up experiments using baby snakes from both locations. Snakes were fed either large or small mice over several months, until they reached maturity. The method and results are indicated in the table below.

	experiment 1		experiment 2	
	group A island snakes	**group B island snakes**	**group C mainland snakes**	**group D mainland snakes**
Length of eastern tiger snakes' jaws at birth	long	long	normal	normal
Type of prey given over several months	small mice	large mice	small mice	large mice
Length of eastern tiger snakes' jaws at maturity	normal	long	normal	normal

a. What were the researchers investigating in these experiments?

EFFECT OF DIET ON JAW SIZE

1 mark

b. What was the independent variable in experiment 1?

SIZE OF PREY

1 mark

c. What evidence from the results suggests that the size of eastern tiger snakes' jaws is

i. a genetically inherited trait

GROUP C & D HAD NORMAL JAWS AT MATURITY

ii. affected by environmental factors?

GROUP A & B CHANGED JAW SIZE

1 + 1 = 2 marks

At present the island and mainland populations are both classified as the same species.

It has been proposed that the two populations of snakes may eventually evolve into two separate species.

d. Outline the steps involved in the process of speciation, with particular reference to the snakes in the two populations. You may use a labelled diagram or flow chart to illustrate your answer.

VARIATION

ISOLATION GEOGRAPHICALLY

DIFFERENT SELECTION PRESSURES

INABILITY TO REPRODUCE SUCCESSFULLY

IF REINTRODUCED

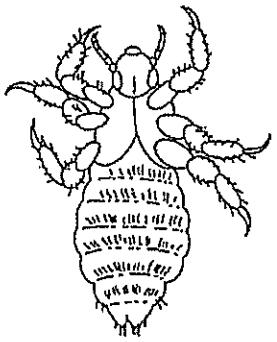
3 marks

Total 7 marks

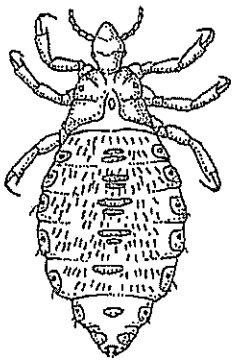
Question 9

There are two varieties of lice which live on humans: body lice which only live in clothing but feed on the body, and head lice which only live in hair and feed on the scalp.

It is not known when humans began wearing clothes. It is difficult to find evidence of cultural evolution in early humans as changes in behaviour are rarely reflected in physical changes visible in fossils. However, indirect evidence can be found.



head louse *Pediculus humanus capitis*



body louse *Pediculus humanus corporis*

A scientist used DNA hybridisation to measure differences between the DNA of head lice and body lice. He estimated that the two groups diverged about 72 000 years ago.

a. Explain how DNA hybridisation can be used to determine evolutionary relationships.

COMBINING SINGLE STRANDS OF DNA FROM DIFFERENT SPECIES THEN MEASURING HOW MUCH TEMP NEEDED TO SEPARATE THE MORE SIMILARITY THE MORE CLOSELY RELATED.

2 marks

b. A scientist claimed to have found other evidence showing the time at which humans began wearing clothes. What might this evidence have been?

ARTIFACTS

1 mark

c. Explain a possible advantage for lice of living in clothing.

PROTECTION

1 mark

Total 4 marks

END OF QUESTION AND ANSWER BOOK

Question 7

Embryonic studies of zebra fish and humans have shown common features exist. DNA studies have shown there are common genes for particular traits, such as body pigments. The nucleotide sequence of the zebra fish and human gene coding for body pigment are about 70% identical.

a. Explain why zebra fish and humans have a gene coding for the same trait but have variation within the gene.

COMMON ANCESTOR CARRIED SAME GENE
DIVERGENT EVOLUTION
MUTATIONS IN SEPERATE GENES ACCUMULATE
OVER TIME

2 marks

In Victoria, regulations require fishermen who catch golden perch fish (*Macquaria ambigua*) to return small fish to the water. Only medium-sized and large fish can be kept. In a Biology class, some students stated that returning small fish to the water was an example of selective breeding. Other students thought it was an example of natural selection.

b. Explain the difference between selective breeding and natural selection.

SELECTIVE – CHOOSING PHENOTYPES TO
BREED
NATURAL – VARIANTS MORE SUITED TO
CHANGING ENVIRONMENT

2 marks

Salmon is a species of fish. A biotechnology company has engineered a faster-growing salmon by splicing genes from another species of fish into the salmon DNA.

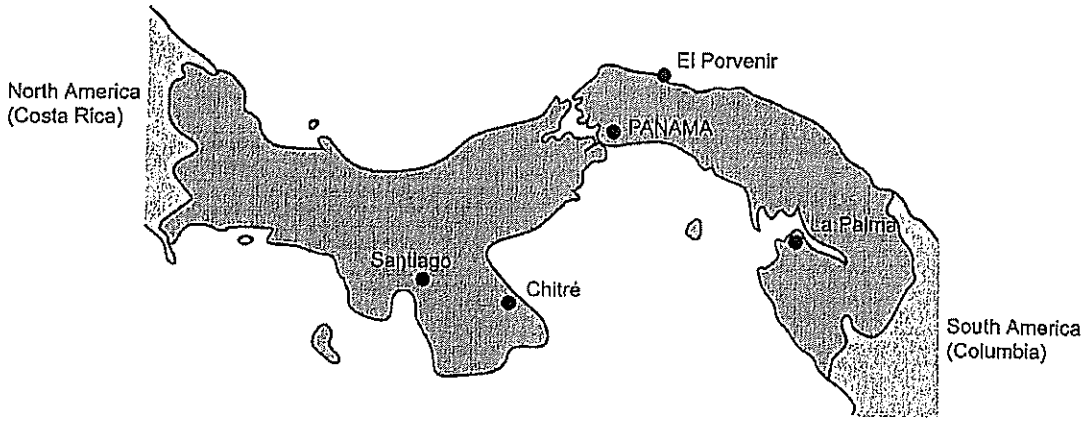
c. What general name is given to an organism that contains genes from other species?

TRANSGENIC

1 mark

Question 8

The Isthmus of Panama is a narrow strip of land that joins North and South America.
The land bridge formed approximately 3 million years ago.



Snapping shrimps, genus *Alpheus*, can be found on either side of the land bridge. The two groups are phenotypically similar. However when the males and females from either side of the land bridge were brought together they snapped aggressively at each other and would not mate. They are now considered to be two different species.

a. Why is the inability to mate sufficient evidence to call the two groups different species?

REDUCED GENE FLOW

1 mark

b. What type of speciation has occurred in the snapping shrimp?

ALLOPATRIC

1 mark

c. Explain how the differences between the shrimp on either side of the land bridge could have arisen.

VARIATION
SEPERATION
SELECTION
SPECIES FORMATION

2 marks

Thylacinus cyanocephalus (Tasmanian tiger) was the largest living marsupial carnivore in Australia at the time of European settlement. The thylacine is believed to have become extinct on 7 September 1936 when the last captive thylacine died in the Hobart Zoo.

There are thylacine fossils found in Tasmania and mainland Australia, but when Europeans arrived in Australia living thylacines were only found in Tasmania.

d. Suggest why thylacines were not found in mainland Australia at the time of European settlement.

HUNTED BY HUMANS

1 mark

Since 1936 there have been many reported sightings of thylacines in Tasmania and along the southern coast of Victoria.

e. Explain why scientists still believe thylacines are extinct.

NO SCIENTIFIC EVIDENCE

1 mark

The dingo is a eutherian mammal and the thylacine is a marsupial mammal. Scientists regard these two carnivores as an example of convergent evolution.

f. Explain why scientists would regard the thylacine and the dingo as an example of convergent evolution.

DIFFERENT ANCESTRY
SIMILAR ENVIRONMENTAL PRESSURES

1 mark

Total 7 marks

END OF QUESTION AND ANSWER BOOK

Question 8

DNA includes sections that are called short tandem repeats (STR). Mutations in STRs occur, on average, every 500 generations.

Different numbers of these repeats have no obvious effect on the individual.

a. What is the likely reason for this?

INTRONS

1 mark

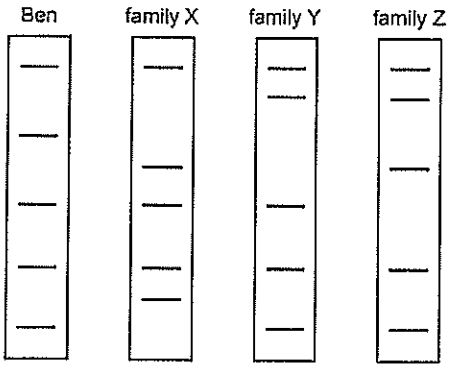
A young man, Ben, wants to find out more about his genetic ancestry. He sends a sample of cells, obtained from a swab of his mouth, to a laboratory. On receipt of the sample, the laboratory treats the cells to release the DNA to enable identification of STR markers.

b. Name the process used to produce many copies of the STR markers.

PCR. (USE WORDS)

1 mark

Each of the STR markers produced is labelled with a dye and subjected to gel electrophoresis. Five of Ben's STR markers were compared with three family groups who have the same surname as him. The following gels resulted.



c. Explain which family is Ben's most recent common ancestor.

Y

4 STRS IN COMMON WITH BEN

OTHERS HAVE LESS (X: 3, 2: 3)

2 marks

Total 4 marks

Question 5

Some people prefer to eat Wagyu cattle because of the high level of marbling (fat) in the meat. Four separate DNA markers are used to test for marbling in an animal. Tested cattle are scored on a scale of zero to eight, eight indicating the highest degree of marbling.

a. What does the use of four markers suggest about the inheritance of this characteristic?

4 GENES / POLYGENIC : PROB NOT ON COURSE

1 mark

A Wagyu breeder discovered a small number of individuals in her elite herd that were suffering from Chediak-Higashi Syndrome (CHS). CHS is an autosomal recessive condition that can affect species other than cattle. The breeder required further information.

Gene probing was used to target *CHS1*, the allele responsible for the condition. The genetic probes for the Wagyu CHS locus were derived from human alleles.

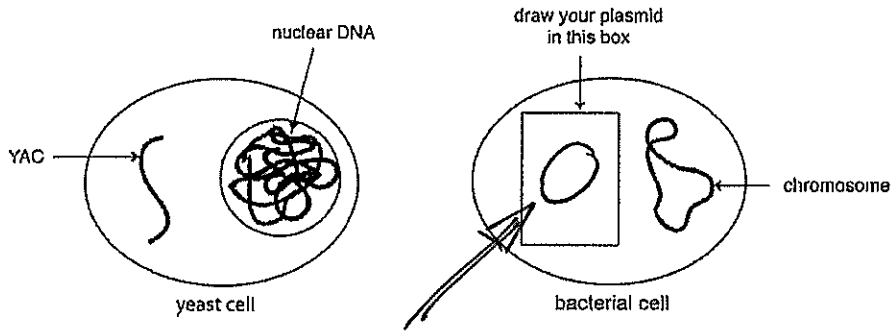
b. Given that the gene probe for a human works for the Wagyu, what can you infer about the chemical code for this allele?

UNIVERSAL

1 mark

The Wagyu *CHS1* allele was isolated and given a fluorescent tag. It was introduced into a yeast cell as a large, independent, cytoplasmic chromosomal segment called a Yeast Artificial Chromosome (YAC). In addition to the allelic DNA, a YAC includes a centromere and a replication sequence. The yeast cells are then incubated in the presence of growth stimulants and given time to replicate.

This procedure is similar to genetic engineering of bacterial plasmids, however the YAC is able to contain much larger pieces of DNA than a plasmid.



c. **i.** In the bacterial cell **above**, **draw** a plasmid in the **blank box**.

ii. Bacterial plasmids lack a centromere. Why are YACs made with a centromere?

SO CAN BE INVOLVED IN CELL DIVISION

NOTE: NOT ON COURSE

iii. What term describes the process of copying a gene?

CLONING

1 + 1 + 1 = 3 marks

A test was developed to identify each of the normal and mutant alleles. Two cows were chosen for testing.

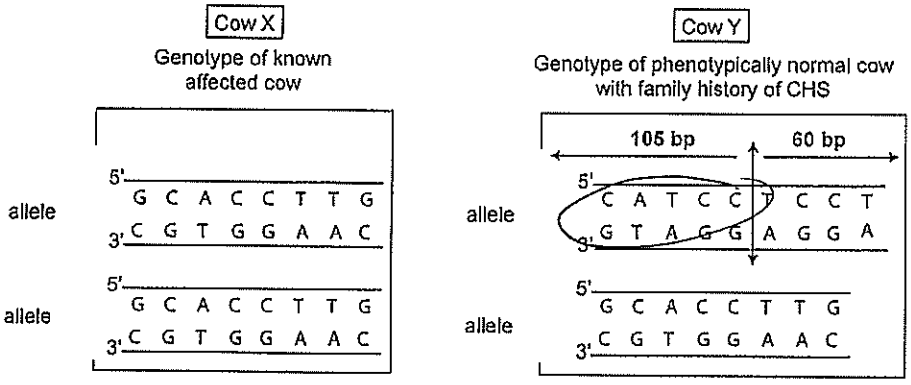
Cow X – a cow known to have the autosomal recessive disease CHS

Cow Y – a phenotypically normal cow with a family history of CHS

The CHS locus was isolated from each, amplified and then treated with *FokI* restriction enzyme which recognises the nucleotide sequences

5'–GGATG–3' and 5'–CATCC–3'

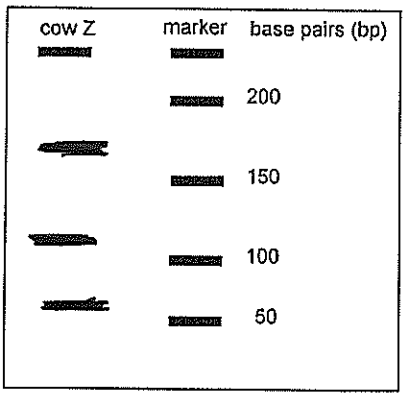
The genotypes at the CHS locus for the two cows are shown in the following figure.



d. i. Explain whether Cow Y is heterozygous or homozygous at the CHS locus.

HETEROZYGOUS : NOT ON COURSE

ii. On the electrophoretic gel diagram below, draw in the band(s) that would accurately show a profile for an unaffected cow Z with no history of CHS in the family.



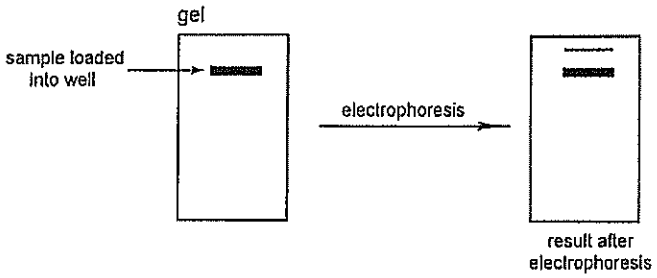
165

105

60

1 + 2 = 3 marks

A farmer suspected that one of his cows was a CHS carrier. He sent a sample of the cow's hair follicles for testing. A technician ran a gel of DNA sequences from the hair follicles and obtained the following result.



e. What mistake must the technician have made in his procedures to obtain this result?

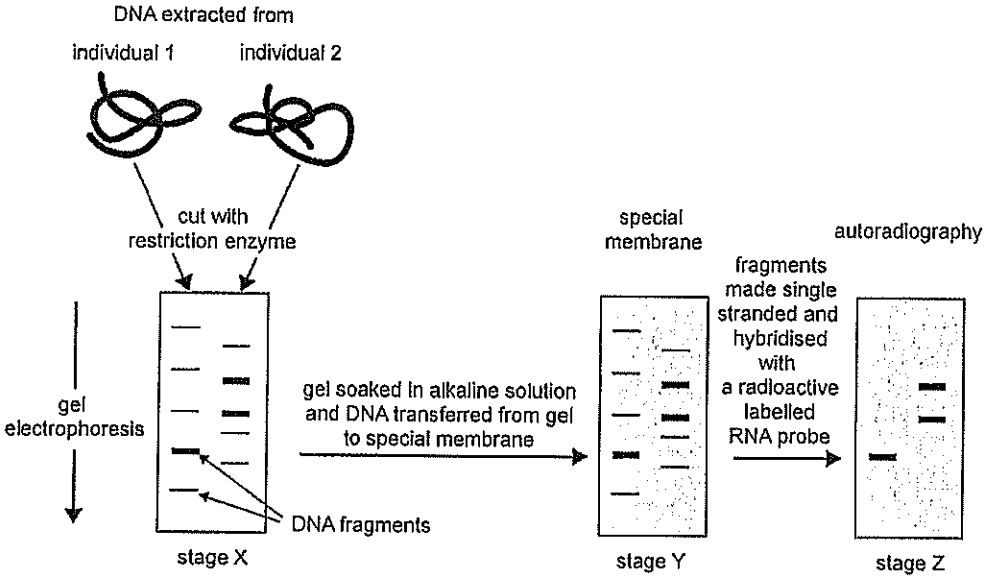
CONNECTED THE ELECTRODES INCORRECTLY

1 mark

Total 9 marks

Question 6

RFLP (Restriction Fragment Length Polymorphism) analysis is commonly used to determine genetic variation between individuals. The procedure is summarised below.



In this procedure, scientists select a particular restriction enzyme from an available range.

a. Explain the reason for their choice.

KNOWN RE SITES WITHIN RFLP.

1 mark

Electrophoresis uses electrical current to sort DNA fragments.

b. i. Describe one characteristic of this sorting process.

SIZE DEPENDANT

ii. Explain why the DNA of each individual produces a different pattern of fragments after gel electrophoresis, even when the same restriction enzyme is used.

DIFFERENT SEQUENCES IN RFLPs SO CUTS IN A VARIETY OF SITES

1 + 1 = 2 marks

Examine stages Y and Z.

c. Describe, at the molecular level, what is meant by the term 'hybridised'. Why is it necessary to carry out hybridisation?

COMBINING SINGLE STRANDED FRAGMENTS OF DNA.

2 marks

Total 5 marks

Question 2

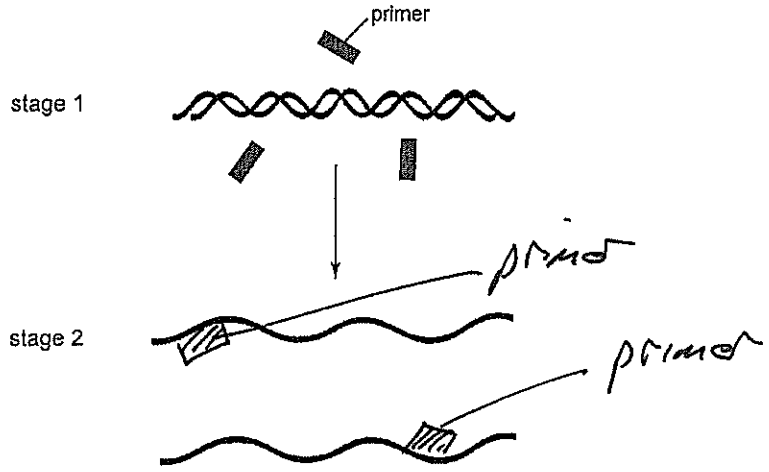
Victoria Police forensic scientists conduct DNA profiling using samples taken from crime scenes. Traces of DNA of less than 1 nanogram can be amplified and then profiled.

a. Name the process which is used to amplify the DNA.

PCR.

1 mark

Below is a diagram showing part of this process.



b. What must be done between stages 1 and 2 to separate the strands of the DNA molecule?

HEAT TO ABOVE 90°C

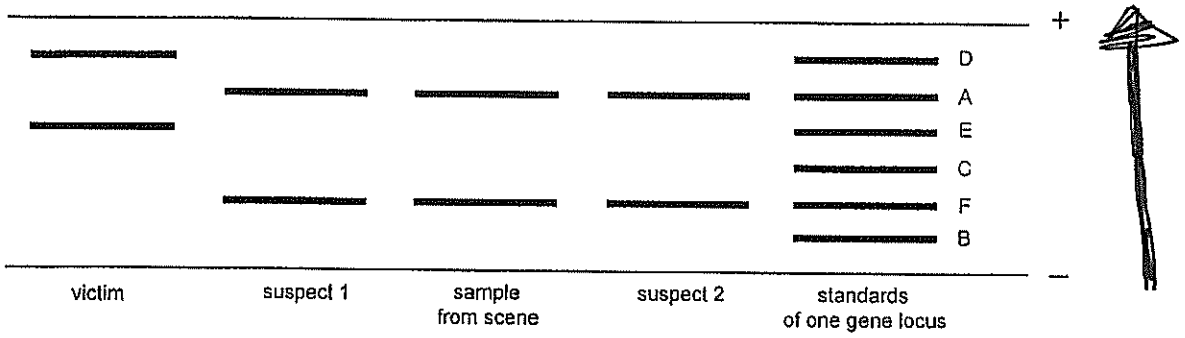
1 mark

c. Complete and label the diagram at stage 2.

2 marks

Small pieces of DNA of differing length can be compared to determine whether or not a sample could have come from a particular person. In a case, samples of DNA from the victim and the crime scene were compared with samples from two suspects.

The DNA samples were treated with restriction enzymes, amplified and run through gel electrophoresis. The results for one gene locus are shown in the diagram below.



d. Draw an arrow on the right-hand side of the diagram to indicate the direction of movement of the DNA fragments.

1 mark

e. What do the standards consist of, and what is their purpose?

KNOWN LENGTHS OF DNA USED TO DETERMINE SIZE OF UNKNOWN FRAGMENTS BEING TESTED

2 marks

f. From these results, give a conclusion which could be drawn about the sample taken from the crime scene.

SAME PROFICE FOR S1 and S2

1 mark

g. What further action would you recommend to the forensic scientists investigating this case?

TEST MORE GENE LOCI

1 mark

Total 9 marks

SECTION B – continued
TURN OVER

Question 4

a. Describe the appearance of a bacterial plasmid.

CIRCULAR.

1 mark

A bacterial plasmid was modified in the laboratory so that it contained a gene for an enzyme which provided resistance to the antibiotic tetracycline.

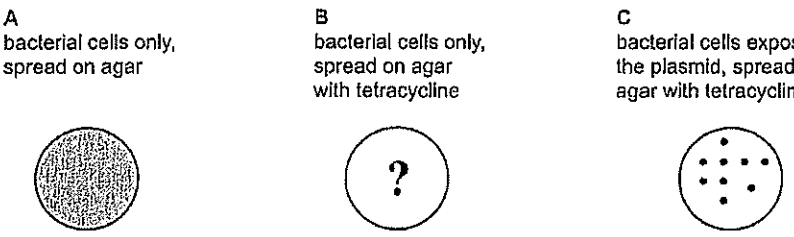
Bacterial cells, which in their natural environment were sensitive to the antibiotic tetracycline, were mixed with the modified plasmid. The bacterial cells were treated so that they could take up the plasmid.

b. What is the name of the process in which a bacterial cell takes up a plasmid and expresses the genes of the plasmid?

TRANSFORMATION

1 mark

The outcome of an experiment is shown below.



With respect to the growth of bacteria the results of plates A and C are shown. On plate A there is a continuous growth of bacteria over the surface of the agar. On plate C the colonies are distinguishable from each other.

c. i. What result would you expect on plate B with respect to the growth of the bacteria?

NO GROWTH

ii. Explain your answer to **c.i.**

NO TRANSFORMED BACTERIA SO NO ANTIBIOTIC RESISTANCE

1 + 1 = 2 marks

d. Explain why there is a difference in the way the bacteria have grown on plates A and C.

ONLY SOME BACTERIA ARE TRANSFORMED

GROW INTO COLONIES (ABOUT 10) COMPARED TO 1000s OF SMALLER COLONIES

2 marks

Total 6 marks