

CHAPTER 25

TRACING PHYLOGENY

OUTLINE

I. The Fossil Record and Geologic Time

- A. Sedimentary rocks are the richest source of fossils
- B. Paleontologists use a variety of methods to date fossils
- C. The fossil record is a substantial, albeit incomplete, chronicle of evolutionary history
- D. Phylogeny has a biogeographical basis in continental drift
- E. The history of life is punctuated by mass extinctions followed by adaptive radiations of the survivors

II. Phylogeny and Systematics

- A. Taxonomy employs a hierarchical system of classification
- B. The branching pattern of a phylogenetic tree represents the taxonomic hierarchy
- C. Determining monophyletic taxa is a key to classifying organisms according to their evolutionary history
- D. Molecular biology provides powerful new tools for systematics
- E. The search for fossilized DNA continues despite recent setbacks: *science as a process*

III. The Science of Phylogenetic Systematics

- A. Phenetics increased the objectivity of systematic analysis
- B. Cladistic analysis uses novel homologies to define branchpoints on phylogenetic trees
- C. Phylogenetic systematics relies on both morphology and molecules

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Explain the importance of the fossil record to the study of evolution.
2. Describe how fossils form.
3. Distinguish between relative dating and absolute dating.
4. Explain how isotopes can be used in absolute dating.
5. Explain how continental drift may have played a role in history of life.
6. Describe how radiation into new adaptive zones could result in macroevolutionary change.
7. Explain how mass extinctions could occur and affect evolution of surviving forms.
8. List the major taxonomic categories from the most to least inclusive.

9. Explain why it is important when constructing a phylogeny to distinguish between homologous and analogous character traits.
10. Distinguish between homologous and analogous structures.
11. Describe three techniques used in molecular systematics and explain what information each provides.
12. Distinguish between a monophyletic and a polyphyletic group, and explain what is meant by a "natural taxon."
13. Describe the contributions of phenetics and cladistics to phylogenetic systematics.
14. Describe how cladistic analysis uses novel homologies to define branch points on phylogenetic trees.

KEY TERMS

phylogeny	binomial	monophyletic	DNA sequence analysis
systematics	genus (genera, pl.)	polyphyletic	phenetics
fossil record	specific epithet	paraphyletic	cladistic analysis
geological time scale	family	homology	clade
radiometric dating	order	convergent evolution	outgroup
half-life	class	analogy	synapomorphies
Pangaea	phylum (phyla, pl.)	DNA-DNA	parsimony
adaptive zone	kingdom	hybridization	phylogenetic biology
phylogenetic trees	taxon (taxa, pl.)	restriction maps	

LECTURE NOTES

Biologists reconstruct evolutionary history by studying the succession of organisms in the fossil record. Fossils are collected and interpreted by *paleontologists*.

Phylogeny = The evolutionary history of a species or group of related species

- Phylogeny is usually diagrammed with *phylogenetic trees* that trace inferred evolutionary relationships.

Systematics = The study of biological diversity in an evolutionary context; reconstructing phylogeny is part of the scope of systematics

- Biological diversity reflects past episodes of speciation and macroevolution.
- Encompasses the identification and classification of species (*taxonomy*) in its search for evolutionary relationships.

Fossil record = The ordered array in which fossils (any preserved remnant or impression left by an organism that lived in the past) appear within layers of rock that mark the passing of geological time

I. The Fossil Record and Geological Time

A. Sedimentary rocks are the richest sources of fossils

Sedimentary rocks are the richest sources of fossils.

- These rocks form from deposits of sand and silt that have weathered or eroded from the land and are carried by rivers to seas and swamps.
- Aquatic organisms and some terrestrial forms were swept into seas and swamps and became trapped in sediment when they died.
- New deposits pile on and compress older sediments below into rock. Sand is compressed into sandstone and mud into shale.
- A small proportion of the organisms left a fossil record.

Fossils usually form from mineral-rich hard parts of organisms (bones, teeth, shells of invertebrates) since most organic substances usually decay rapidly (see Campbell, Figure 25.1 a and b).

- Paleontologists usually find parts of skulls, bone fragments, or teeth; although nearly complete skeletons of dinosaurs and other forms have been found.
- Many of the parts found have been hardened by *petrification*, which occurs when minerals dissolved in groundwater seep into the tissues of dead organisms and replace organic matter (see Campbell, Figure 25.1c).

Some fossils, found as thin films pressed between layers of sandstone or shale, retain organic material.

- Paleontologists have found leaves millions of years old that are still green with chlorophyll and preserved well enough that their organic composition and ultrastructure could be analyzed (see Campbell, Figure 25.1d).
- One research team was even able to clone a very small sample of DNA from an ancient magnolia leaf.

Other fossils found by paleontologists are replicas formed in molds left when corpses were covered by mud or sand (see Campbell, Figure 25.1e).

- Minerals from the water which filled the mold eventually crystallized in the shape of the organism.

Trace fossils form in footprints, animal burrows, and other impressions left in sediments by animal activity. These can provide a great deal of information.

- Dinosaur tracks can provide information about the animals gait, stride length, and speed.

Rarely has an entire organism been fossilized. This only happens if the organism was buried in a medium that prevented bacteria and fungi from decomposing the body (see Campbell, Figure 25.1g).

B. Paleontologists use a variety of methods to date fossils

Several methods are used to determine the age of fossils, which makes them useful in studies of macroevolution.

1. Relative dating

Sedimentation may occur when the sea-level changes or lakes and swamps dry and refill.

- The rate of sedimentation and the types of particles that sediment vary with time when a region is submerged.
- The different periods of sedimentation resulted in formation of rock layers called *strata*.
- Younger strata are superimposed on top of older ones.
- The succession of fossil species chronicles phylogeny, since fossils in each layer represent organisms present at the time of sedimentation.

Strata from different locations can often be correlated by the presence of similar fossils, known as index fossils.

- The shells of widespread marine organisms are the best index fossils for correlating strata from different areas.
- Gaps in the sequence may appear in an area if it was above sea level (which prevents sedimentation) or if it was subjected to subsequent erosion.

Geologists have formulated a sequence of geological periods by comparing many different sites. This sequence is known as the *geological time scale* (see Campbell, Table 25.1).

- These periods are grouped into four eras with boundaries between the eras marking major transitions in the life forms fossilized in the rocks.
- Periods within each era are subdivided into shorter intervals called *epochs*.
- The divisions are not arbitrary, but are associated with boundaries that correspond to times of change.

This record of the rocks presents a chronicle of the relative ages of fossils, showing the order in which species groups evolved.

2. Absolute dating

Absolute dating is not errorless, but it does give the age in years rather than in relative terms (e.g., before, after).

The most common method for determining the age of rocks and fossils on an absolute time scale is *radiometric dating*.

- Fossils contain isotopes of elements that accumulated in the living organisms.
- Since each radioactive isotope has a fixed half-life, it can be used to date fossils by comparing the ratio of certain isotopes (e.g., ^{14}C and ^{12}C) in a living organism to the ratio of the same isotopes in the fossil.
- *Half-life* = The number of years it takes for 50% of the original sample to decay
- The half-life of an isotope is not affected by temperature, pressure or other environmental variables.

Carbon-14 has a half-life of 5600 years, meaning that one-half of the carbon-14 in a specimen will be gone in 5600 years; half of the remaining carbon-14 would disappear from the specimen in the next 5600 years; this would continue until all of the carbon-14 had disappeared (see Campbell, Figure 25.2).

- Thus a sample beginning with 8g of carbon-14 would have 4g left after 5600 years and 2g after 11,200 years.
- Carbon-14 is useful in dating fossils less than 50,000 years old due to its relatively short half-life.

Paleontologists use other radioactive isotopes with longer half-lives to date older fossils.

- Uranium-238 has a half-life of 4.5 billion years and is reliable for dating rocks (and fossils within those rocks) hundreds of millions of years old.
- This isotope was used to place the oldest fossil-containing rocks in the Cambrian period.
- An error of < 10% is present with radioactive dating.

Other methods may also be used to date some fossils.

- Amino acids can have either left-handed (L-form) or right-handed (D-form) symmetry.
- Living organisms only synthesize L-form amino acids to incorporate into proteins.
- After an organism dies, L-form amino acids are slowly converted to D-form.
- The ratio of L-form to D-form amino acids can be measured in fossils.
- Knowing the rate of chemical conversion (*racemization*) allows this ratio to be used in determining how long the organism has been dead.
- This method is most reliable in environments where the climate has not changed significantly, since the conversion is temperature sensitive.

The dating of rocks and fossils they contain has enabled researchers to determine the geological periods (see Campbell, Table 25.1)

C. The fossil record is a substantial, albeit incomplete, chronicle of evolutionary history

A fossil represents a sequence of improbable events:

- An organism had to die in the right place and at the proper time for burial conditions favoring fossilization.
- The rock layer containing the fossil had to escape geologic events (erosion, pressure, extreme heat) which would have distorted or destroyed the rock.
- The fossil had to be exposed and not destroyed.
- Someone who knew what they were doing had to find the fossil.

The fossil record is slanted in favor of species that existed for a long time, were abundant and widespread, and had shells or hard skeletons

Paleontologists thus work with an incomplete record for many reasons.

- A large fraction of species that have lived probably left no fossils.
- Most fossils that were formed have probably been destroyed.
- Only a small number of existing fossils have been discovered.

Even though it is incomplete, the fossil record provides the outline of macroevolution, but the evolutionary relationships between modern organisms must be studied to provide the details.

D. Phylogeny has a biogeographical basis in continental drift

Evolution has dimension in space as well as time.

- Biogeography was a major influence on Darwin and Wallace in developing their views on evolution.
- Drifting of continents is the major geographical factor correlated with the spatial distribution of life (see Campbell, Figure 25.3a).

Continental drift results from the movement of great plates of crust and upper mantle that float on the Earth's molten core.

- The relative positions of two land masses to each other changes unless they are embedded on the same plate.
- North America and Europe are drifting apart at a rate of 2 cm per year.
- Where two plates meet (boundaries), many important geological phenomena occur: mountain building, volcanism, and earthquakes (see Campbell, Figure 25.3b).

⇒ Volcanism, in turn, forms volcanic islands (e.g., Galapagos), which opens new environments for founders and adaptive radiation.

Plate movements continually rearrange geography, however, two occurrences had important impacts on life: the formation of *Pangaea* and the subsequent breakup of *Pangaea* (see Campbell, Figure 25.4).

At the end of the Paleozoic era (250 million years ago), plate movements brought all land masses together into a super-continent called *Pangaea*.

- Species evolving in isolation were brought together and competition increased.
- Total shoreline was reduced and the ocean basins became deeper (draining much of the remaining shallow coastal seas).
- Marine species (which inhabit primarily the shallow coastal areas) were greatly affected by reduction of habitat.
- Terrestrial organisms were affected as continental interior habitats (and their harsher environments) increased in size.

- Changes in ocean currents would have affected both terrestrial and marine organisms.
- Overall diversity was thus impacted by extinctions and increased opportunities for surviving species.

During the early Mesozoic era (about 180 million years ago) Pangaea began to breakup due to continuing continental drift.

- This isolated the fauna and flora occupying different plates.
- The biogeographical realms were formed and divergence of organisms in the different realms continued.

Many biogeographical puzzles are explained by the pattern of continental separations. For example,

- Matching fossils recovered from widely separated areas.
 - ⇒ Although Ghana and Brazil are separated by 3000 km of ocean, matching fossils of Triassic reptiles have been recovered from both areas.
- Australia has unique fauna and flora.
 - ⇒ Australian marsupials are very diverse and occupy the same ecological roles as placental mammals on other continents.
 - ⇒ Marsupials probably evolved on the portion of Pangaea that is now North America and migrated into the area that would become Australia.
 - ⇒ The breakup of Pangaea isolated Australia (and its marsupial populations) 50 million years ago, while placental mammals evolved and diversified on the other continents.

E. The history of life is punctuated by mass extinctions followed by adaptive radiations of survivors

The evolution of modern life has included long, relatively quiescent periods punctuated by briefer intervals of more extensive turnover in species composition.

- These intervals of extensive turnover included explosive adaptive radiations of major taxa as well as mass extinctions.

1. Examples of major adaptive radiations

The evolution of some novel characteristics opened the way to new *adaptive zones* allowing many taxa to diversify greatly during their early history. For example,

- Evolution of wings allowed insects to enter an adaptive zone with abundant new food sources and adaptive radiation resulted in hundreds of thousands of variations on the basic insect body plan.
- A large increase in the diversity of sea animals occurred at the boundary between the Precambrian and Paleozoic eras. This was a result, in part, of the origin of shells and skeletons in a few key taxa.
 - ⇒ Precambrian rock contains the oldest animals (700 million years old) which were shell-less invertebrates that differed significantly from their successors found in Paleozoic rock.
 - ⇒ Nearly all the extant animal phyla and many extinct phyla evolved in less than 10 million years during the mid-Cambrian (early Paleozoic era).
 - ⇒ Shells and skeletons opened a new adaptive zone by making many new complex body designs possible and altering the basis of predator-prey relationships.
 - ⇒ It is possible that genes controlling development evolved during this time, resulting in a potential for increased morphological complexity and diversity.

An empty adaptive zone can be exploited only if the appropriate evolutionary novelties arise. For example,

- Flying insects existed for 100 million years before the appearance of flying reptiles and birds that fed on them.

Conversely, an evolutionary novelty cannot enable organisms to exploit adaptive zones that are occupied or that do not exist.

- Mass extinctions have often opened adaptive zones and allowed new adaptive radiations.
- For example, mammals existed 75 million years before their first large adaptive radiation in the early Cenozoic. This may have resulted from the ecological void created with the extinction of the dinosaurs.

2. Examples of mass extinctions

Extinction is inevitable in a changing world. The average rate of extinction has been between 2.0 and 4.6 families (each family may include many species) per million years.

Extinctions may be caused by habitat destruction or by unfavorable environmental changes.

- Many very well adapted marine species would become extinct if the ocean's temperature fell only a few degrees.
- Changes in biological factors may cause extinctions even if physical factors remain stable.
- Since many species coexist in each community, an evolutionary change in one species will probably impact other species. For example,
⇒ The evolution of shells by some Cambrian animals may have contributed to the extinction of some shell-less forms.

There have been periods of global environmental change which greatly disrupted life and resulted in mass extinctions.

- During these periods, the rate of extinction escalated to as high 19.3 families per million years.
- These mass extinctions are recognized primarily from the decimation of hard-bodied animals in shallow seas which have the most complete fossil record.
- Two (of about a dozen) mass extinction episodes have been studied extensively by paleontologists.

The Permian extinctions (the boundary between the Paleozoic and Mesozoic eras) eliminated over 90% of the species of marine animals about 250 million years ago (see Campbell, Figure 25.5).

- Terrestrial life was probably also affected greatly. For example, eight of the 27 orders of Permian insects did not survive into the Triassic.
- This mass extinction took place in less than five million years and probably resulted from several factors.
⇒ Occurred about the time Pangaea was formed by the merging of continents which disturbed many habitats and altered the climate.
⇒ A period of extreme vulcanism and resulting volcanic debris (including carbon dioxide) in the atmosphere may have altered the global temperature.

The Cretaceous extinction (the boundary between the Mesozoic and Cenozoic eras) occurred about 65 million years ago.

- More than 50% of the marine species and many terrestrial plants and animals (including dinosaurs) were eliminated.

- During this time the climate was cooling and many shallow seas receded from continental lowlands.
- Increased volcanic activity during this time may have contributed to the cooling by releasing materials into the atmosphere and blocking the sunlight.

Evidence also indicates that an asteroid or comet struck the Earth (*impact hypothesis*) while the Cretaceous extinctions were in progress.

- Iridium, an element rare on earth but common in meteorites, is found in large quantities in the clay layer separating Mesozoic and Cenozoic sediments.
- Walter and Luis Alvarez (and colleagues), after studying this clay layer, proposed that it is fallout from a huge cloud of dust ejected into the atmosphere when an asteroid collided with the Earth.
- This cloud would have both blocked the sunlight and severely disturbed the climate for several months.
- Although the asteroid hit the earth during this time, some researchers feel it did not cause the mass extinction of this period.

The impact hypothesis consisted of two parts: a large asteroid or comet collided with the Earth and the collision caused the Cretaceous extinctions.

- Many forms of evidence support the idea that a large comet or small asteroid collided with Earth 65 million years ago.
 - ⇒ Many craters have been found and indicate that a large number of objects have fallen to the Earth's surface.
 - ⇒ A large crater (≈ 180 km in diameter) located beneath sediments on the Yucatan coast of Mexico has been located.
- Questions about the impact hypothesis are now concentrated on the second part: the collision caused the Cretaceous extinctions.
- Advocates of the impact hypothesis point to several items in their support (see Campbell, Figure 25.6):
 - ⇒ The large size of the impact would darken the Earth for several years and the reduction in photosynthesis output would be sufficient to cause food chains to collapse.
 - ⇒ Severe acid precipitation would result from the increased mineral content of the atmosphere.
 - ⇒ The content of sediments at the upper Cretaceous boundary indicated global fires were occurring and smoke from these fires would increase the atmospheric effects of the impact.
- Opponents of the impact hypothesis hold that the impact occurred within the period of mass extinction, but that the two occurrences are not a cause and effect event.
 - ⇒ Many paleontologists and geologists believe that the climatic changes which occurred were due to continental drift, increased vulcanism, and other processes.
 - ⇒ They also feel these events were sufficient to cause the mass extinction.

Many paleontologists are now trying to determine how sudden and uniform the Cretaceous extinctions were (on a geological time scale).

- Disappearance of diverse groups (from microscopic marine plankton to dinosaurs) during a short time span would support the impact hypothesis.
- A gradual decline with different groups disappearing at different rates, would support hypotheses emphasizing terrestrial causes.

- It is possible that the impact was a final, sudden event in environmental changes that were affecting the biota of the late Cretaceous.

Mass extinctions, whatever the cause, profoundly affect biological diversity.

- Not only are many species eliminated, but those that survive are able to undergo new adaptive radiations into the vacated adaptive zones and produce new diversity.

II. Phylogeny and Systematics

One of the main goals of systematics is to make biological classification reflect phylogeny.

A. Taxonomy employs a hierarchical system of classification

The taxonomic system used today was developed by Linnaeus in the eighteenth century. This system has two main features: the assignment of a binomial to each species and a filing system for grouping species (see Campbell, Figure 25.7).

The *binomial* (two part Latin name) assigned to each species is unique to that species.

- The first word of the binomial is the *genus* (pl. genera); the second word is the *specific epithet* of the species.
- The scientific name of a species combines the genus and specific epithet.
- Each genus can include many species of related organisms. For example, *Felis silvestris* is the domestic cat; *Felis lynx* is the lynx.
- Use of the scientific name defines the organism referred to and removes ambiguity.

The filing system for grouping species into a hierarchy of increasingly general categories formalizes the grouping of organisms.

- Binomial nomenclature is the first step in grouping: similar species are grouped in the same genus.
- The system then progresses into broader categories:
 - ⇒ Similar genera are grouped into the same family.
 - ⇒ Families are grouped into orders.
 - ⇒ Orders are grouped into classes.
 - ⇒ Classes are grouped into phyla.
 - ⇒ Phyla are grouped into kingdoms.
- Each taxonomic level is more inclusive than the one below. The more closely related two species are, the more levels they share:

Category	Domestic			
	Cat	Bobcat	Lion	Dog
specific epithet	silvestris	rufus	leo	familiaris
genus	Felis	Felis	Panthera	Canis
family	Felidae	Felidae	Felidae	Canidae
order	Carnivora	Carnivora	Carnivora	Carnivora
class	Mammalia	Mammalia	Mammalia	Mammalia
phylum	Chordata	Chordata	Chordata	Chordata
kingdom	Animalia	Animalia	Animalia	Animalia

The two main objectives of taxonomy are to sort out and identify closely related species and to order species into the broader taxonomic categories.

- In sorting, closely related organisms are assigned to separate species (with the proper binomial) and described using the diagnostic characteristics which distinguish the species from one another.
- In categorizing, the species are grouped into broader categories from genera to kingdoms.
- In some cases, intermediate categories (e.g., subclasses; between orders and classes) are also used.
- The named taxonomic unit at any level is called a *taxon* (pl. taxa).
- Rules of nomenclature have been established: the genus name and specific epithet are italicized, all taxa from the genus level and higher are capitalized.

B. The branching pattern of a phylogenetic tree represents the taxonomic hierarchy

The goal of systematics is to have classification reflect the evolutionary affinities of species. The taxonomic hierarchy is set up to fit evolutionary history (see Campbell, Table 25.2).

- In general, groups subordinate to other groups in the taxonomic hierarchy should represent finer and finer branching of phylogenetic trees (see Campbell, Figure 25.8)

Classification schemes and phylogenetic trees are hypotheses of history based on current data. Like all hypotheses, they may be refined with further study.

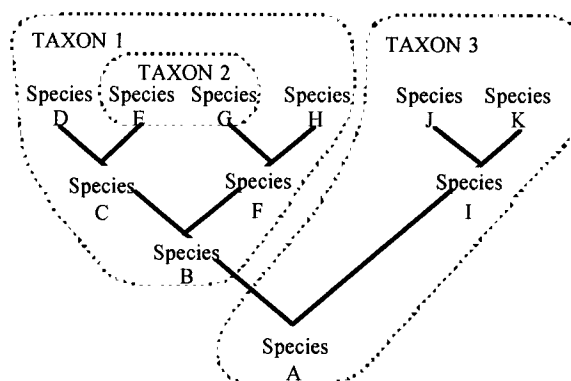
C. Determining monophyletic taxa is key to classifying organisms according to their evolutionary history

In order for a classification scheme to reflect the evolutionary history of an organism, the species must be grouped into taxa that are monophyletic.

- A *monophyletic taxon* is one where a single ancestor gave rise to all species in that taxon and to no species placed in any other taxon. For example, Family Ursidae evolved from a common ancestor (Taxon 1) (see also Campbell, Figure 25.9).

By contrast, other kinds of taxa do not accurately reflect evolutionary history

- A *polyphyletic taxon* is one whose members are derived from two or more ancestral forms not common to all members. For example, Kingdom Plantae includes both vascular plants and mosses which evolved from different algal ancestors (Taxon 2).
- A *paraphyletic taxon* is one that excludes species that share a common ancestor that gave rise to the species included in the taxon. For example, Class Reptilia excludes the Class Aves although a reptilian ancestor common to all reptiles is shared (Taxon 3).



1. Sorting homology from analogy

Systematists classify species into higher, preferably monophyletic, taxa based on the extent of similarities in morphology and other characteristics.

Homology = Likeness attributed to shared ancestry

- The forelimbs of mammals are homologous, they share a similarity in the skeletal support that has a genealogical basis.
- Homology must be distinguished from analogy in evolutionary trees.

Analogy = Similarities due to *convergent evolution*, not common ancestry

Convergent evolution = Acquisition of similar characteristics in species from different evolutionary branches due to sharing similar ecological roles with natural selection shaping analogous adaptations (see Campbell, Figure 25.10)

The distinction between homology and analogy is sometimes relative.

Example: The wings of birds and bats are modifications of the vertebrate forelimb.

- ⇒ The appendages are thus homologous.
- ⇒ As wings they are analogous since they evolved independently from the forelimbs of different flightless ancestors.
- Insect wings and bird wings are analogous.
- ⇒ They evolved independently and are constructed from entirely different structures.
- Convergent evolution has produced analogous similarities between Australian marsupials and placental mammals on other continents.

Homology must be sorted from analogy to reconstruct phylogenetic trees on the basis of homologous similarities.

- Generally, the greater the amount of homology, the more closely related the species and this should be reflected in their classification.
- Adaptation and convergence often obscure homologies, although studies of embryonic development can expose homology that is not apparent in mature structures.
- Additionally, the more complex two similar structures are, the less likely it is that they have evolved independently.

Example: The skulls of humans and chimpanzees are composed of many bones which are fused together and match almost perfectly. It is unlikely such a complex structure would have evolved independently in separate groups.

D. Molecular biology provides powerful new tools for systematics

Molecular comparisons of proteins and DNA have added another useful method for studying the evolutionary relationships between species.

- Inherited nucleotide sequences in DNA program the corresponding sequences of amino acids in proteins.
- Examination of these macromolecules provide much information about evolutionary relationships.

Molecular comparisons are:

- Objective and quantitative
- Used to assess relationships between species so distantly related that no morphological similarities exist

1. Protein comparisons

The primary structure of proteins is genetically programmed and a similarity in the amino acid sequence of two proteins from different species indicates that the genes for those proteins evolved from a common gene present in a shared ancestor.

Studies of cytochrome *c* (an ancient protein common to all aerobic organisms) have been used to compare many diverse species.

- The amino acid sequence has been determined for species ranging from bacteria to complex plants and animals.
- The sequence in cytochrome *c* is identical in chimpanzees and humans.
- The sequence in humans and chimpanzees differs at only one of the 104 amino acid positions in the rhesus monkeys.
- Chimpanzees, humans, and rhesus monkeys belong to the Order Primates.
⇒ Comparing these sequences with nonprimate species shows greater differences (13 with the dog and 20 with the rattlesnake).
- Phylogenetic trees based on cytochrome *c* are consistent with evidence from comparative anatomy and the fossil record.

One disadvantage to the use of amino acid data for phylogenetic studies is that such sequences provide information only about those genes that code for protein; this can be a small fraction of the genome (e.g., 2% in humans)

2. DNA and RNA comparisons

The most direct measure of common inheritance from shared ancestors is a comparison of the genes or genomes of two species. Comparison can be made by three methods: DNA-DNA hybridization, restriction maps, and DNA sequencing.

DNA-DNA hybridization can compare whole genomes by measuring the degree of hydrogen bonding between single-stranded DNA obtained from two sources.

- DNA is extracted from different species and the complementary strands separated by heating.
- The single-stranded DNA from the two species is mixed and cooled to allow double-stranded DNA reformation which results from hydrogen bonding.
- The hybrid DNA is then reheated to separate the double strands.
- The temperature necessary to separate the hybrid DNA is indicative of the similarity in the DNA from the two species.
⇒ The temperature correlation is based on the degree of bonding between the strands of the two species with more bonding occurring with greater similarity.
⇒ The more extensive the pairing, the more heat is needed to separate the hybrid strand.
- Evolutionary trees constructed through DNA-DNA hybridization usually agree with those based on other methods, however, this technique is very beneficial in settling taxonomic debates that have not been finalized by other methods.

Restriction maps provide precise information about the match-up of specific DNA nucleotide sequences.

- Restriction enzymes are used to cut DNA into fragments which can be separated by electrophoresis and compared to restriction fragments of other species.
- Two samples of DNA with similar maps for the locations of restriction sites will produce similar collections of fragments.
- The greater the divergence of two species from the common ancestor, the greater the differences in restriction sites and less similarity of the restriction fragments.
- This method works best when comparing small fragments of DNA.

- Mitochondrial DNA (mtDNA) is best suited for this type of comparison since it is smaller than nuclear DNA (produces smaller fragments) and mutates about ten times faster than nuclear DNA.
- The faster mutation rate of mtDNA allows it to be used to determine phylogenetic relationships between not only closely related species, but also populations of the same species.
 - ⇒ mtDNA was used to establish the close relationship among the Pima, Mayan, and Yanomami groups of Native Americans.
 - ⇒ The results supported linguistic evidence that these groups descended from the first wave of immigrants to cross the Bering land bridge from Asia during the late Pleistocene.

DNA sequence analysis is the most precise method of comparing DNA as it determines the actual nucleotide sequence of a DNA segment.

- Uses polymerase chain reaction (PCR) technology to clone traces of DNA.
- PCR is coupled with automated sequencing to provide a simpler and faster method of collecting sequence data.
- DNA sequencing and comparisons show exactly how much divergence there has been in the evolution of two genes derived from the same ancestral gene.
- Ribosomal RNA (rRNA) sequencing is a similar technique which can provide information about some of the earliest branching in phylogenetic relationships since DNA coding for rRNA changes very slowly.
- rRNA sequencing has been very useful in examining the relationships among bacteria.

3. Identifying and comparing homologous DNA sequences

Comparing nucleotide sequences between corresponding DNA segments from different species has the potential of telling us how much divergence there has been in the evolution of two genes derived from the same gene.

To measure differences between two species, it is necessary to identify homologous nucleotide sequences. Following sequencing, the sequences are aligned and evaluated.

- Common ancestry is clear when two sequences of the same gene from two species that have diverged very recently are the same or differ by only a few bases.
- Mutations tend to accumulate as species diverge; the number of differences is a measure of evolutionary distance.
- Once a match is achieved, usually with the aid of computer programs, the investigator can develop a phylogenetic hypothesis (see Campbell, Figure 25.12).

4. Molecular clocks

Different proteins and nucleic acids evolve at different rates, although each type of molecule evolves at a relatively constant rate over time.

When comparing homologous proteins and nucleotide sequences from taxa that are known to have diverged from common ancestors, the number of amino acid substitutions is proportional to the elapsed time since divergence.

Example: Homologous proteins of bats and dolphins are more similar than those of sharks and tuna.

- This is consistent with fossil evidence showing that tuna and sharks have been separated much longer than bats and dolphins.

DNA comparisons may be even more reliable than protein comparisons.

- Phylogenetic branching based on nucleotide substitutions in DNA generally approximates dates determined from the fossil record.
- The difference in DNA between two taxa is more closely correlated with the time since divergence than is morphological difference.

Molecular clocks (DNA and protein) are calibrated by graphing the number of nucleotide or amino acid differences against the times for a series of evolutionary branch points known from the fossil record.

- The graph can then be used to determine the time of divergence between taxa for which no substantial fossil record is available.

The assumption that mutation rates for genes (and their protein products) are relatively constant is the basis for using molecular clocks in evolutionary biology.

- This assumption is relatively solid when comparing groups of closely related species.
- Molecular clocks are less reliable when comparing more distantly related groups since differences in generation times and metabolic rates affect mutation rates.

Among closely related species, the constant mutation rate for specific genes implies that the accumulation of selectively neutral mutations changes the genome more than adaptive mutations.

- Many evolutionary biologists doubt the prevalence of neutral variation, so they also question the use of molecular clocks to accurately date the time of divergence.
- There is less skepticism about the value of molecular clocks for determining the relative sequence of branch points in phylogeny.
- Modern systematists use available molecular data along with all other evidence to reconstruct phylogeny.

E. The search for fossilized DNA continues despite recent setbacks: *science as a process*

Science is a dynamic process that continuously tests hypotheses; sometime the hypotheses are supported and sometimes they are rejected

The nucleotide sequences in DNA traces recovered from fossils that retain organic material can be analyzed by using PCR.

- Fossilized DNA from 17 million-year-old magnolia leaves was first reported in 1990 .
- Since 1990, DNA fragments have been sequenced from a frozen mammoth (40,000 years old), an insect fossilized in amber (40 million years old), a 65-million-year-old *Tyrannosaurus rex* from Montana, a 30,000-year old fossil arm bone from an extinct member of the human family, and a frozen Stone Age man (5000 years old).

It now appears that most of the DNA first reported to be ancient is actually DNA from contaminating fungus or other organisms.

III. The Science of Phylogenetic Systematics

The two significant features of a phylogenetic tree are the location of branch points along the tree and the degree of divergence between branches.

- The locations of branch points along the tree symbolize the relative times of origin for different taxa.
- The degree of divergence between branches represents how different two taxa have become since branching from a common ancestor.

Initially, phylogenies were devised largely on morphology and were considered by many to be too subjective. In the 1960s, new computational technology helped usher in two new, more objective analytical approaches: phenetics and cladistics.

A. Phenetics increased the objectivity of systematic analysis

Phenetics makes no evolutionary assumptions and decides taxonomic affinities entirely on the basis of measurable similarities and differences.

- A comparison is made of as many characters (anatomical characteristics) as possible without attempting to sort homology from analogy.
- Pheneticists feel that the contribution of analogy to overall similarity will be overridden by the degree of homology if enough characters are compared.
- Critics of phenetics argue that overall phenotypic similarity is not a reliable index of phylogenetic proximity.
- While supported by few systematists, the emphasis of phenetics on multiple quantitative comparisons has made important contributions to systematics.
⇒ Especially useful for analyzing DNA sequence data and other molecular comparisons between species.

B. Cladistic analysis uses novel homologies to define branchpoints on phylogenetic trees

Cladistic analysis, which has become synonymous with phylogenetic systematics, classifies organisms according to the order in time that branches arise along a phylogenetic tree, without considering the degree of divergence.

- This produces a cladogram, a dichotomous tree that branches repeatedly.
⇒ Each branch point is defined by novel homologies unique to the various species on that branch or *clade*.

1. Outgroup comparison

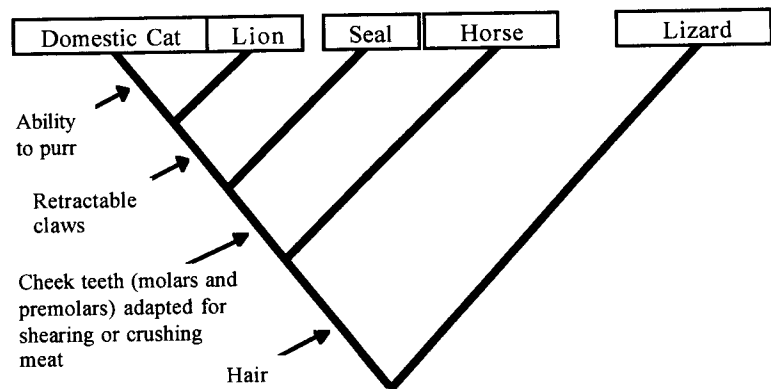
Each taxon has a mixture of primitive characters that existed in the common ancestor and characters that evolved more recently.

- The sharing of primitive characters indicates nothing about the pattern of evolutionary branching from a common ancestor.

Cladistic analysis uses a concept called outgroup comparison as an objective means of defining the roots of a phylogenetic tree and thereby distinguishing among the shared characters that are more primitive (see Campbell, Figures 25.13 and 25.14).

Outgroup = A species or group of species that is relatively closely related to a group of species being studied, but clearly not as closely related as any study-group member is to any other study-group member

- All members of the study group are compared as a whole to the outgroup



- Characters common to the outgroup and group being studied are likely to have been present in a common ancestor and are considered shared primitive characters

2. Use of synapomorphies (shared derived characters) and parsimony

A major difficulty in cladistic analysis is finding characters that are appropriate for identifying each branch point.

- Branch points of a phylogenetic tree can be identified by finding shared derived characters, or *synapomorphies*.
- These are homologies that evolved in an ancestor common to all species on one branch of a fork of the tree, but not common to the other branch.

A guiding principle of cladistic analysis is parsimony, the quest for the simplest, and probably most likely, explanation for observed phenomena.

- In systematics, parsimony means that a phylogenetic tree using the minimum number of changes to illustrate evolutionary relationships has the greatest likelihood of being correct.

3. Acceptance of only monophyletic taxa

By focusing on phylogenetic branching, cladistic analysis accepts only monophyletic taxa. As a result, some taxonomic surprises are produced by cladistic systematics:

- The branch point between crocodiles and birds is more recent than between crocodiles and other reptiles (a fact also supported by the fossil record).
⇒ Crocodiles and birds have synapomorphies not present in lizards and snakes.
- In a strict cladistic analysis, the Class Aves and the Class Reptilia, as we know them now, would be eliminated.
⇒ The birds would be included in a cladogram of the animals we know as reptiles.
- Birds are deemed superficially different because of the morphological changes associated with flight which have developed since their divergence from reptilian ancestors.
- Continued debate over how life evolved is indicative that evolutionary biology is an active science.

C. Phylogenetic systematics relies on both morphology and molecules

Modern systematics depends on cladistic analysis to formulate hypotheses about the history of life.

Cladistic analysis now relies upon both morphology and on molecular data; the strongest support for any phylogenetic hypothesis is agreement between data derived from both of these sources.

- Scientists have been collecting phenotypic information about living and extinct organisms for centuries. The amount of this morphological information exceeds by far the current molecular data.
- Molecular databases are expanding rapidly. While no means complete, the genome contains more information about an organism's evolutionary history than its anatomical features.

Phylogenetic biology, or the application of cladistic analysis in the study of evolutionary history and its relations to all aspects of life, pervades virtually every field of biology.

- Physiology - evolution of body temperature regulation
- Plant development - evolution of genes regulating flower development

- Behavior - evolutionary history of animal social systems
- Conservation biology - evaluates genetic differences among populations of endangered species

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