

1 Phylogenetic History: The Evolution of Marine Mammals

Think for a moment about marine mammals: seals, walruses, dugongs and whales. Seals and walruses are primarily cold-water species that eat mostly fish and can spend part of their time on land (or ice). Dugongs and manatees are tropical herbivores and are entirely aquatic. Toothed whales and baleen whales are also entirely aquatic. All of these species share many similarities such as streamlined bodies and flippers. But they are all clearly mammals: they have fur, bear live young, lactate, and their skeletons match many features of terrestrial mammals.

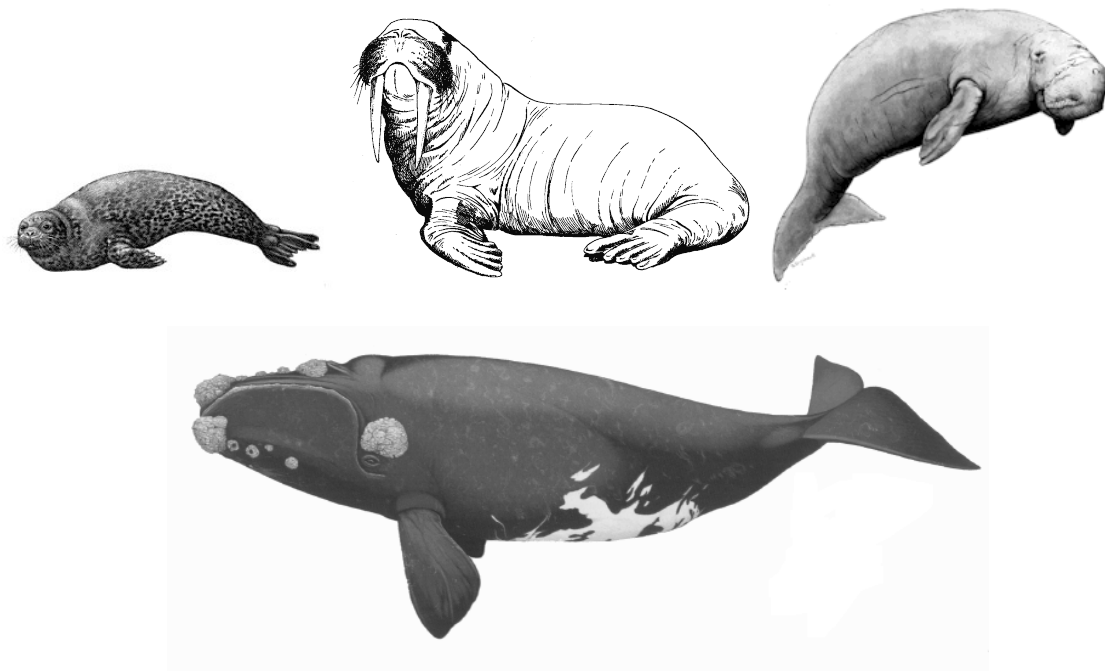


Figure 1. Harbor seal, walrus, dugong, and right whale)

The first mammals were certainly terrestrial. There is good evidence that mammals evolved from reptile-like ancestor. Therefore at some point there was a transition from terrestrial environment to an aquatic lifestyle in this group of species. How did that occur?

Charles Darwin spent a lot of time thinking about such evolutionary transitions. He argued that whales evolved from terrestrial ancestors and re-developed the streamlined bodies as an adaptation to their aquatic lifestyle. Here is a passage from the first edition of the *Origin of Species*, chapter 6:

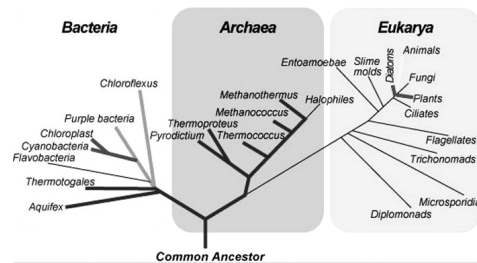
“I will now give two or three instances of diversified and of changed habits in the individuals of the same species. When either case occurs, it would be easy for natural selection to fit the animal, by some modification of its structure, for its changed habits, or exclusively for one of its several different habits. In North America the black bear was seen by Hearne swimming for hours with widely open mouth, thus catching, like a whale, insects in the water. *Even in so extreme a*

case as this, if the supply of insects were constant, and if better adapted competitors did not already exist in the country, I can see no difficulty in a race of bears being rendered, by natural selection, more and more aquatic in their structure and habits, with larger and larger mouths, till a creature was produced as monstrous as a whale."

It is interesting to note that the last sentence (in italics) was omitted in later editions of the book. Perhaps that was because of ridicule by some of his opponents. More likely it was because he knew that although bears and walruses share many anatomical features, whales are bears were not particularly similar, so the ancestor of a whale was probably not in the lineage that led to bears.

What were the ancestors of whales and walruses? In particular, **did the transition to water occur once or more than once in the evolution of mammals?** To answer that we need to reconstruct the phylogenetic history of mammals.

1.1 The tree of life

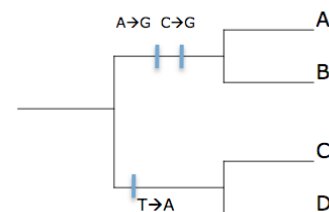


There is overwhelming evidence (from the universal genetic code and the fundamental similarity of all cells) that all life on earth has descended from a common ancestor. Therefore any set of species can eventually be traced back to a single common ancestor.

Evolutionary lineages are shown as branches on a tree diagram with the living taxa as leaves at the tips of the branches. The root of the tree is the common ancestor. Each node where two branches diverge shows a speciation event where one ancestral lineage diverged into two independent lineages.

In the simplest case we will imagine a set of completely asexual lineages which evolve through a simple branching process. An ancestral lineage undergoes a speciation event where it bifurcates into two branches which remain separate for all future generations. The various lineages will slowly diverge as they accumulate mutations.

Importantly, all of the future descendants will also inherit the new mutations. In this example species C and D will both inherit an A instead of a T as shown on the branching diagram. That "A" is a shared feature, a "**synapomorphy**", which unites C and D as part of the same evolutionary lineage.



1.2 How to read a phylogenetic tree

Trees can be drawn many different ways. Sometimes the branches are rectangular as in the examples above or they can be drawn with diagonal lines. Sometimes the taxa are arranged horizontally and other times vertically.

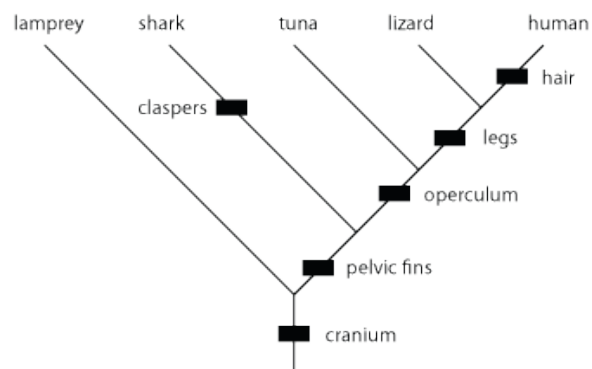
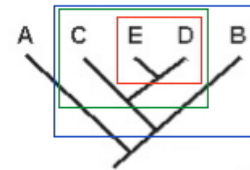
When you read a phylogenetic tree the order of taxa along the tips of the branches makes no difference. The information in a phylogenetic tree is shown *only* by the branching relationships. The tree in this example shows that species D and E are most closely related because they share the most recent common ancestor. Similarly, the common ancestor of the group C,D,E was more recent than the common ancestor of B and C.



These two trees depict exactly the same phylogenetic relationships. The only difference is that the branches have been rotated at the nodes.

It is also important to realize these trees do not imply that species B-E evolved *from* species A. All of the species are currently alive. What the tree does say is that species A shares a common ancestor with the other species.

Finally, when considering phylogenetic trees we will often be interested in the **monophyletic groups**. A monophyletic group, or **clade**, is defined as the *all* of the descendants of a single common ancestor. In this example D and E form a monophyletic group, as does the set of three species (C,D,E) and the set of four species (B,C,D,E). The set (B,C) is *not* a monophyletic group because their common ancestor is also the common ancestor of D and E.



From the information in this simple phylogeny of vertebrates, is a tuna more closely related to a shark or to a lizard? _____

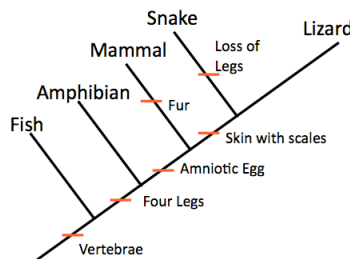
Do lizards and humans form a monophyletic group? _____

1.3 Reconstructing evolutionary relationships

What we can observe right now are the living species, the tips of the branches of the phylogenetic tree. The phylogenetic information comes from the similarities and differences among those species. Species that share many features in common are likely to be closely related. The key to reconstructing the phylogenetic history of a group is based on an insight from the German taxonomist Willi Hennig: evolutionary relationships are revealed only by **shared derived characters (“synapomorphies”)**. Shared ancestral traits don’t provide any evidence about the evolutionary relationships among taxa. That two species share an ancestral character is not surprising because they are of course descendants of an ancestor that also showed that trait.

Let’s consider a simple example: a lizard, and snake and a cat. The lizard and cat both have four legs, but that doesn’t provide evidence that they are most closely related. Their common vertebrate ancestor also had four legs. We need to focus instead on shared derived traits. In our example, derived characters (such as scaly skin or terrestrial eggs) that were not present in the common ancestor point instead to the close relationship between lizards and snakes. We infer that lizards and snakes shared a common ancestor that evolved scaly skin.

While it is possible that a derived character will evolve independently in two different lineages (a process called convergence or **homoplasy**), it is much more likely that the derived character evolved only once, in the common ancestor of the two species.



How can you tell which characteristics are ancestral and which are derived? Sometimes there is evidence from fossils. Another common method is to use an **outgroup**. The outgroup is a related taxon that is known (from other evidence) to be outside the group of interest. For our example of lizards, snakes and mammals the outgroup might be an amphibian such as a salamander or frog that we know are in a separate evolutionary lineage that diverged before reptiles and mammals. Characters that are present in both the outgroup and the ingroup are considered ancestral, whereas unique features present in some, but not all, of the ingroup are derived.

	Skin with Scales	Amniotic Eggs	Fur	4 Legs	Diapsid skull
Outgroup (e.g. frog)	No	No	No	Yes	No
Lizard	Yes	Yes	No	Yes	Yes
Snake	Yes	Yes	No	No	Yes
Cat	No	Yes	Yes	Yes	No

For the first character absence of scales is considered ancestral because it is present in both the ingroup and outgroup, whereas presence of scales is the derived character. For the second character we cannot determine whether aquatic or terrestrial eggs is ancestral using only this information. It is possible that the ancestral state was aquatic and that the evolution of amniotic eggs unites all three ingroup taxa. However it is equally likely that the ancestor terrestrial eggs and there was a mutation to aquatic eggs in the outgroup lineage.

For character 3, fur is a derived character. However that derived character is present in only one of these three organisms. Although there has been evolution at that site it tells us nothing about the relationship among lizards, snakes and cats.

Looking at character 5, is the diapsid skull (two openings behind the eye) an
ancestral or derived character? _____

To summarize, characters are phylogenetically informative if they are

- derived characters
- present in at least two, but not all, of the ingroup taxa.

1.4 Details of phylogenetic reconstruction

The basic procedure for inferring phylogenies is fairly straightforward. In principle, you identify a set of homologous characters and record whether each species has the ancestral or derived condition. Make a guess at the phylogenetic tree and calculate a score for that tree. Often the score is the number of evolutionary changes that are required. Finally you search all possible trees to find the tree with the best score (i.e. the one that requires the fewest evolutionary steps).

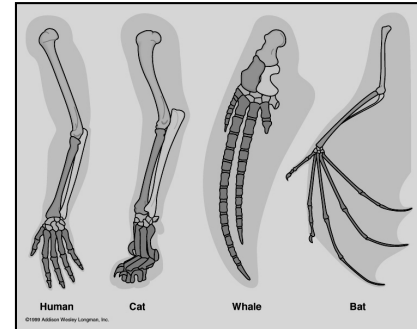
In practice, those simple steps are not always easy to implement. Some apparently similar characters may not really be homologous. This is a particular problem with DNA sequence data where there are only four possible character states. Second, the number of possible trees is enormous so it is often not possible to search the entire set of possible trees. Third, there may be two or more solutions that have the same score so it may not be possible to identify a single best tree. Finally the true tree may not in fact be the most parsimonious. We assume that the simplest tree is best but that is not necessarily the case. The parsimony principle is most likely to fail when some lineages have a higher rate of evolution than others. We'll examine each of those issues in turn.

Identify homologous characters

Characters two organisms are homologous if they are both derived from the same trait in a common ancestor.

The bones in the digits of a cat's foot or a bat's wing or whale's flipper are homologous because they are both modifications of the same bone in their common tetrapod ancestor. Morphological homologies are usually identified by comparing the details of the structure. We can identify the spines of a cactus as being homologous to the leaves of another plant because they both have same type of vascular architecture that is characteristic of the development of leaves.

Homology is also important when comparing molecular sequence data. For example when we say that the nucleotide at position 1 is a "C" in two different species, are those C's really homologous because they were inherited unchanged from a common ancestor? Or are they just the same character state, with two unrelated nucleotide positions both happening to have a C? Finding homology in molecular data is a problem of **alignment**.



For example, here are two short sequences that appear to have many differences in their base composition. However if we insert a gap in the second sequence we can bring the two into alignment so there is much more congruence among the bases at each position.

```

AGTCACGATA      AGTCACGATA
AGCACGACAG      →  AG-CACGACAG
  
```

In the aligned sequences show that there are really only two changes: the deletion of a T at position 3 and the change of T to C at position 9.

Alignment is easiest when the sequences are fairly similar so there are many constant sites on which to base the alignment. For highly divergent sequences where there have been substitutions at most site it may be very hard to find anchor points that show how the sequences line up.

When we align two sequences we are making the statement about homology: that nucleotide position 1 is a homologous character in both sequences.

Determine whether the character is ancestral or derived

As discussed above, the polarity of a character is usually assessed by comparison with an outgroup. Traits that are shared by the outgroup and at least one ingroup taxon are most likely ancestral. For that reason it is important to choose the outgroup well. The outgroup should be fairly closely related to the species of interest but it must have diverged from the common ancestor prior to all of the rest. In other words it must be less closely related to the ingroup species than any of them are to each other.

Notice that we talk only about ancestral and derived characters, not organisms. Organisms will usually have a mix of some ancestral characters and some derived characters.

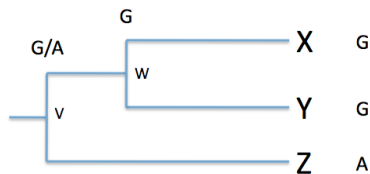
Choose a possible tree and calculate the score

There are lots of ways one might evaluate the fit of a particular tree to the data, but one of the most common methods is by the principle of parsimony. In that case the score is simply the number of evolutionary steps that are required to produce the observed set of characters.

Once again we will assume that character state changes are rare so we can use the principle of parsimony to infer ancestral states that require the fewest number of changes.

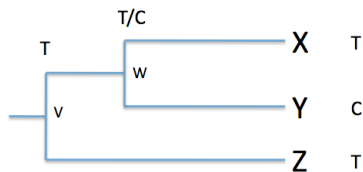
The general procedure is to start at the tips of the branches with a pair of sister taxa. If both species share a particular character state then it is most parsimonious to assume that the ancestor did too. If the two species differ then the character state of the ancestor could have been either. In that case we look at their next closest relative. If it shares one of those characters then it is likely that the ancestor also had that trait.

For example, imagine three species, X, Y, and Z. X and Y have an (unknown) common ancestor W, and all three share the common ancestor V. Let the character states for a given DNA base position be (G, G, and A).



To find the number of required changes we first need to determine the probable genotype of the two ancestors. Because both X and Y have a G we assume the W had a G also. Now compare W and Z. Because the two bases differ, V could be either G or A. The most parsimonious solution is either a single change from G to A on the branch leading to species Z, or a change from A to G on the branch leading to W. In either case it requires one evolutionary event so its parsimony score is 1.

Here is another example:



In this case X and Y differ so we cannot be sure which base ancestor W had. So we look at the next closest relative. Because Z has a T and that is one of the possibilities for W, we assume that W also had a T. In this case the most parsimonious solution is a single change from T to C on the branch leading to species Y.

Search all possible trees to find the one with the best score

The number of possible trees rises extremely quickly as the number of taxa increases. For three species there are only 3 possible phylogenetic histories. With four species the number of possibilities increases to 15.

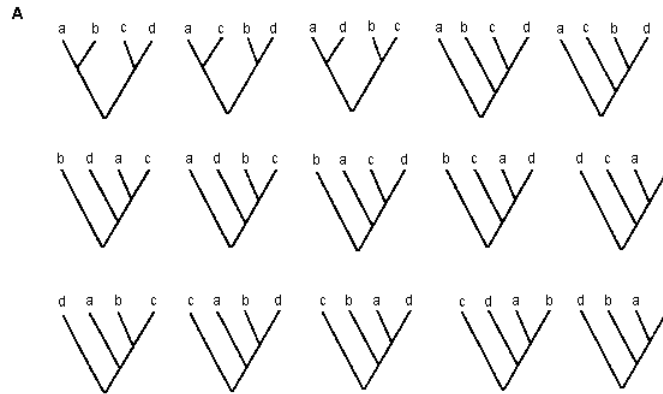


Figure ??. Fifteen possible trees for four taxa.
In general the number of possible rooted trees is $= (2N-3)!!$

For 10 taxa there are over 34 billion possible trees that must be evaluated. When more than about 10 species are considered it becomes impossible to actually evaluate all possible trees in a reasonable amount of time. Fortunately efficient computer algorithms have been developed to find solutions even without searching the entire set of trees. One common approach is to start with a tree that is close to correct by grouping the taxa based on their overall similarity. Then the computer algorithm randomly swaps branches to see if there is an improvement in the fit of the data. If the change improves the score it is accepted, otherwise a different pair of branches is swapped. Eventually it gets to the point where no changes will improve the fit.

The number of possible trees increases quickly as the number of species increases.

# species	# rooted trees
2	1
3	3
4	15
5	105
6	945
7	10,395
8	135,135
9	2,027,025
10	34,459,425
15	2.1×10^{14}
20	8.2×10^{21}

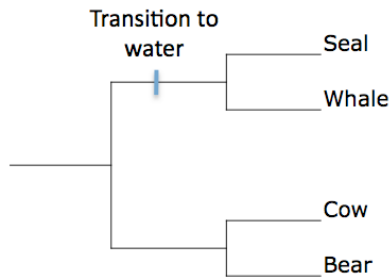
1.5 Back to seals and whales

We are now ready to answer the question posed at the beginning: did the evolution of aquatic mammals occur once, in which case all of the aquatic mammals would form a single monophyletic group? A second possibility is that various unrelated groups of

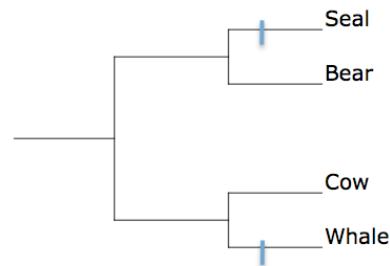
terrestrial mammals independently evolved the aquatic lifestyle. In that case different groups of aquatic mammals would be in different clades, each with a terrestrial ancestor. Again we will start with a very simple example using two aquatic mammals (seal and whale) and two terrestrial mammals (bear and cow).

Figure 2

A) Single transition to water



B) Multiple transitions



How can we distinguish those two possibilities?

Again, the basic steps to reconstruct the phylogeny will be to

- Identify synapomorphies
- Group species by their shared derived characters
- Evaluate possible phylogenies using parsimony, to find the tree that requires the fewest evolutionary steps.

In order to determine which characters are ancestral and which are derived we will use the kangaroo as an outgroup. Kangaroos are mammals but they are marsupials instead of placental mammals. Therefore we can be confident that they diverged from the common ancestor prior to the diversification of the ingroup species.

	terrestrial/aquatic	Placenta	Carnassial teeth	Elongate skull	Conical Canines
Kangaroo	Terrestrial	Absent	Absent	Absent	Absent
Bear	Terrestrial	Present	Present	Absent	Present
Seal	Aquatic	Present	Present	Absent	Present
Whale	Aquatic	Present	Absent	Present	Absent
Cow	Terrestrial	Present	Absent	Present	Absent

(Carnassial teeth are scissor-like pre-molars used for tearing flesh).

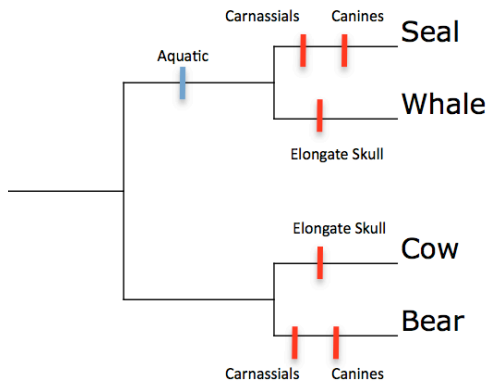
- Identify the derived character states in the table above.
 - From the pattern synapomorphies, which are the natural groupings of these species?

Notice that the derived condition of aquatic habit is not consistent with the pattern of synapomorphies for carnassial teeth, elongate skull and conical canines. That means that

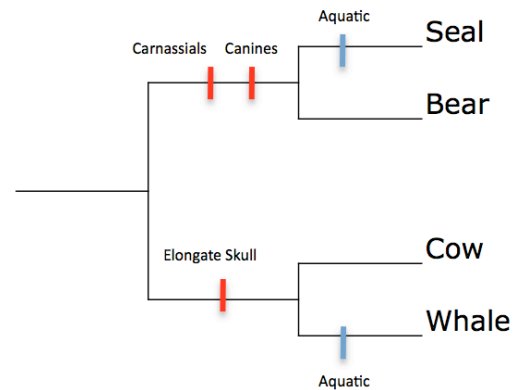
either the transition to water occurred more than once, or that the other characters evolved more than once.

Here is how those evolutionary events would map onto our two example trees:

Tree A: one transition



Tree B: multiple transitions

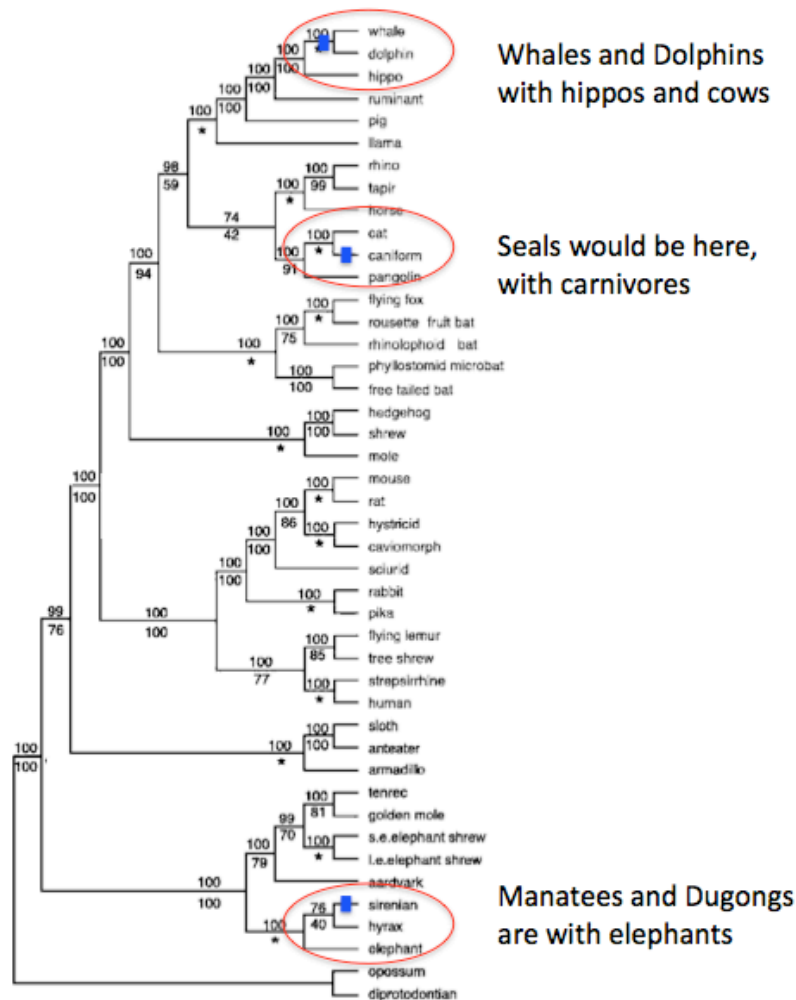


For our simple example, Tree A requires 7 total evolutionary steps whereas tree B requires only 5 steps. By the principle of parsimony these data suggest tree B is more likely. Therefore we conclude that the transition to water happened at least twice, once in the lineage leading to whales and again in the lineage leading to seals.

Darwin was a good anatomist and he saw that the skeletal details of the different marine species allied them with distinct groups of mammals. Dugongs and manatees were most similar to elephants; seals and walruses shared many similarities to carnivores, especially bears. Whales remained a mystery but by the late 1800s they were hypothesized to be related to ungulates (e.g., cows and hippos). Darwin regarded the general morphology of the species as convergent characters that independently evolved in response to the move from terrestrial to aquatic habitats. The true lines of descent are revealed by the many other anatomical similarities and differences. Here is another passage from the *Origin of Species*:

“The resemblance, in the shape of the body and in the fin-like anterior limbs, between the dugong, which is a pachydermatous animal, and the whale, and between both these mammals and fishes, is analogical. On my view of characters being of real importance for classification, only in so far as they reveal descent, we can clearly understand why analogical or adaptive character, although of the utmost importance to the welfare of the being, are almost valueless to the systematist. For animals, belonging to two most distinct lines of descent, may readily become adapted to similar conditions, and thus assume a close external resemblance; but such resemblances will not reveal — will rather tend to conceal their blood-relationship to their proper lines of descent. (1859 *Origin of Species*, chapter 13)

A rigorous test for the evolution of marine mammals would use many more species and more characters. But the general result holds: mammals made the transition to water at least three times: in pinnipeds (seals and walruses), in whales, and also in sirenians (dugongs and manatees). Here is a recent phylogenetic tree for placental mammals:



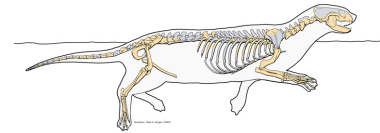
1.6 The phylogenetic tree represents a hypothesis about shared ancestry that can be tested.

Based on this tiny set of characters, Tree “B” (where seals are grouped with carnivores and whales with hoofed mammals) is more parsimonious. We’ll use that as a **phylogenetic hypothesis** about the evolution of marine mammals. Like any good hypothesis, there are various ways it could be tested.

Fossil evidence.

Fossils provide the only way to observe the morphology of species in this group over time. This hypothesis predicts that intermediate forms existed that, for example, contained the ancestral features of carnivores without all of the derived aquatic characteristics of seals. The main difficulty is that the fossil record is notoriously incomplete. The animal must be buried in such a way that the bones are preserved long enough to fossilize *and* that rare fossil must later be exposed in order to be found. Nevertheless, some intermediates have been found.

This fossil of an early member of the seal clade, *Puijila darwini*, was discovered in 2007. It was semi-aquatic, with webbed feet but without fully developed flippers and showing a number of skeletal features of carnivores. Rybczynski et al 2009



The existence of this fossil shows that there were ancient carnivores with some of the features of seals and walruses, but that does not mean this fossil was the actual *ancestor* of modern species. There were likely many ancient species that went extinct, leaving no descendants. We can, however, deduce that this ancient species shared a common ancestor with the lineage that led to modern pinnipeds.

Other characters.

Another way to test the hypothesis is to look at more characters. This hypothesis predicts that the examination of other details of the anatomy or genetics of these taxa ought to reveal more shared synapomorphies between seals and carnivores and between whales and artiodactyls.

DNA sequences represent one of the most common types of information used for phylogenetic analyses. We understand the rules of genetic inheritance so the genealogical relationship among parent and offspring base composition is clear. Mutations constantly generate new variants, allowing divergence among gene sequences in different lineages. Moreover, each of the thousands of bases in a DNA sequence provides an independent record of evolutionary change. Therefore gene sequences provide an almost limitless set of characters for phylogenetic analysis.

Here is a short aligned sequence from the end the 12s rRNA gene for several species of carnivores and pinnipeds, as well as an outgroup (kangaroo). There are numerous synapomorphies that unite pinnipeds with carnivores and whales with artiodactyls (in this case hippos).

Kangaroo	GCATTTAGCTTACACCTAAAAGATTTT	CAGCTAACCCCTGACCATTTGA-
Whale	GCATCTAGTTTACACCTAGAAGATTACACA	GCCCGTGCATATTTGA-
Dolphin	GCATCTAGTTTACACCTAGAAGATTCCACA	ACTCGTGCACATTTGA-
Hippopotamus	GCATCTAGTTTACACCCAGAAAGATTTT	CACATAAAGTGAATGCTTGA-
Bear	GCATCTAGCTTACACCCAGAGGATTTT	CACGCATG-TGACCGCTTGA-
Dog	GCCTCTGGCCTACACCCAGAAAGATTTT	CATTACTTA-TGGCCACTTGA-
Seal	GCCTCTGGCTTACACCCAGAAAGATTTT	CACACCCAA-TGACCACCTGAA
Walrus	GCATCTGGCTTACACCCAGAAAGATTTT	CACACCCAT-TGGCCACTTGA-

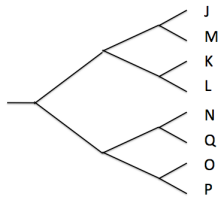
Can you find a synapomorphy that unites the placental mammals (i.e. separates them from the kangaroo)?

Can you find a synapomorphy that unites whales and dolphins?

1.7 Your turn:

In general when we are trying to reconstruct the phylogeny all we have are the currently living species at the tips of each branch. The interior nodes are never observed. So can we judge the accuracy of these phylogenetic methods? David Hillis and coworkers devised an ingenious test using laboratory populations of bacteriophage. A phage generation lasts only hours so they were able to let independent lineages evolve in the laboratory for hundreds of generations, mimicking evolutionary processes that may take thousands of years in other organisms.

They started with single phage isolate, the ancestral strain. That single strain was used to inoculate two independent cultures that remained completely isolated for the rest of the experiment. After many phage generations, each of those cultures was again split in two, creating four independent lineages. Again, after many generations, the cultures were divided to produce eight phage cultures. A ninth lineage, also derived from the ancestral stock, was maintained to serve as an outgroup. Throughout the experiment mutations occurred in the phage cultures so each separate lineage acquired a unique set of mutations.



From their experimental design they *knew* the true phylogenetic relationship among the eight lineages. They then sequenced some of the phage genes to see if they could decipher that true relationship among strains based on the DNA data alone, using the principle of maximum parsimony.

Here is a small subset of their data, showing only 30 sites (out of ?? bases) that had phylogenetically informative variation. Strain “R” is the outgroup and strains J -Q are the eight experimental lineages.

R	C	C	G	C	C	G	C	G	C	C	G	G	C	C	A	G	C	G	G	G	G	T	T	C	C	G	C	G	G	C
(outgroup)																														
J	C	C	G	C	C	G	T	A	C	C	G	G	T	C	A	A	C	G	G	G	G	T	T	C	T	G	C	A	G	T
K	T	C	G	C	C	G	C	A	C	C	G	A	T	C	A	A	T	G	G	G	G	G	C	T	G	C	A	G	T	
L	T	C	G	C	C	G	C	A	C	C	G	A	T	C	A	A	T	G	G	G	G	G	C	T	G	C	A	G	T	
M	C	T	G	C	C	G	T	A	C	C	G	G	T	C	A	A	C	G	G	G	G	T	T	C	T	G	T	A	G	T
N	C	C	G	T	T	A	C	G	T	T	A	G	C	T	G	G	C	A	A	A	A	T	T	T	C	A	C	G	A	C
O	C	T	A	C	C	G	C	G	C	T	G	G	C	C	G	G	C	A	G	A	A	T	T	C	C	A	T	G	A	C
P	C	C	A	C	C	A	C	G	C	T	G	G	C	C	G	G	C	A	G	A	A	T	T	C	C	A	C	G	A	C
Q	C	C	G	T	T	A	C	G	T	T	A	G	C	T	G	G	C	A	A	A	A	T	T	T	C	A	C	G	A	C

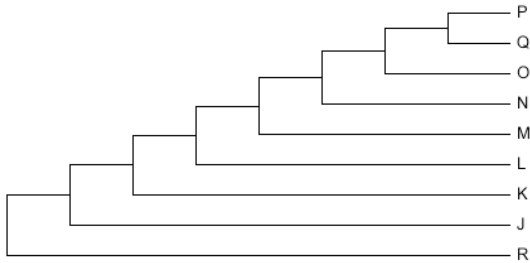
The synapomorphies at the first 10 sites have been highlighted.

At the next site, which base (G or A) is the derived condition? _____

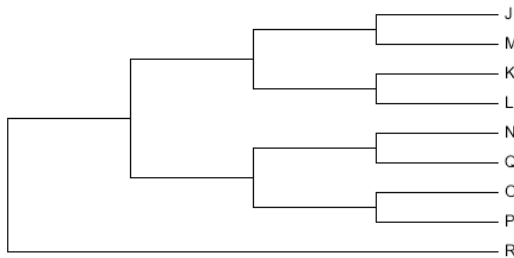
Here are just 2 of the 135,000 possible trees for 8 taxa.

- Using the first 10 highlighted synapomorphies, indicate the location of those changes on both trees (use a short hash mark on the appropriate branch).
- How many evolutionary changes are required by each tree? _____
- Which is most parsimonious? _____

Tree 1)



Tree 2)



It gets tedious to place all of the characters by hand, and this would have to be done for all 135,000 possible trees. Fortunately there are good computer algorithms to search among possible trees and calculate the number of evolutionary steps required for each.

Tree 2 is in fact the most parsimonious tree found using the complete sequence information. It requires 66 evolutionary changes (vs. 84 for tree 1)

How does that tree compare to the true relationship among lines based on the experimental procedure? _____

At least for this simple example the principle of parsimony holds. The most parsimonious tree is in fact identical to the true evolutionary history of the cultures.

Answers:

p. 3. A tuna is more closely related to a lizard than it is to a shark (because the tuna and the lizard share a more recent common ancestor than either does with a shark).

Yes, lizards and humans form a monophyletic group.

p. 5. The diapsid skull is derived, because it is not in the outgroup and it is in some, but not all, of the ingroup.

p. 9. Derived characters states are in bold:

	terrestrial/ aquatic	Placenta	Carnassial teeth	Elongate skull	Conical Canines
Kangaroo <i>outgroup</i>	Terrestrial	Absent	Absent	Absent	Absent
Bear	Terrestrial	Present	Present	Absent	Present
Seal	Aquatic	Present	Present	Absent	Present
Whale	Aquatic	Present	Absent	Present	Absent
Cow	Terrestrial	Present	Absent	Present	Absent

From these data alone we cannot tell if the placenta is ancestral or derived. (But yes, it is a derived feature based on other outgroup comparisons).

There are two synapomorphies uniting bears and seals so that is fairly well supported. There is also one uniting whales and cows. However the aquatic habit is not consistent with the other three characters.

p. 12. The C at position 5 and the G at position 19 are present in all of the placental mammals and absent in the kangaroo. The C at position 39 is unique to whales and dolphins.

```

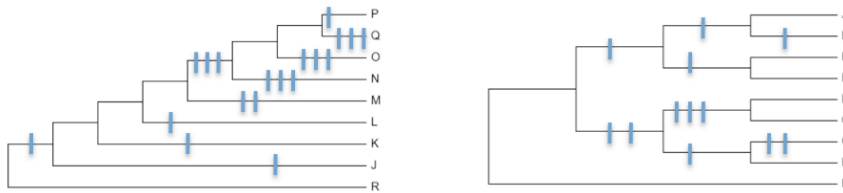
Kangaroo  GCATTTAGCTTACACCTAAAGATTTCAGCTAACCTGACCATTTGA-
Whale     GCATCTAGTTTACACCTAGAAAGATTACACA- GCCCGTGCATATTTGA-
Dolphin   GCATCTAGTTTACACCTAGAAAGATTCCACA- ACTCGTGCACATTTGA-
Hippopotamus GCATCTAGTTTACACCCAGAAAGATTTCACA- ATAAGTGAATGCTTGA-
Bear      GCATCTAGCTTACACCCAGAGGATTTCACGCATG- - TGACCGCTTGA-
Dog       GCGTCTGGCCTACACCCAGAAAGATTTCACTACTTA- TGGCCACTTGA-
Seal      GCGTCTGGCTTACACCCAGAAAGATTTCACACCCAA- TGACCACTTGAA
Walrus    GCATCTGGCTTACACCCAGAAAGATTTCACACCCAT- TGGCCACTTGA-

```

p. 13. At position 11, A is derived and G is ancestral

p. 14. Several solutions are possible. Here is one solution for the first 10 characters. Tree A requires 19 steps. Tree B requires only 12.

Therefore Tree B is more parsimonious.



Tree B is in fact identical to the experimental design.