## Whales on Trees!

# How Many Times Did Mammals Return to the Sea? 

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Cetaceans


Pinnipeds


Sirenians


## Evolution of the Whales



## Mesonychids: Wolf - Related or Wolf-Like?



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## Using DNA Evidence to Unravel the Story of the Evolution of the Whales

There are three groups of mammals that live in the oceans; the whales and dolphins (the cetaceans), the seals and sea lions (the pinnipeds) and the manatees and dugongs (the sirenians). We can use DNA evidence to determine if the mammals returned to the sea only once or sea-dwelling mammals evolved on several occasions. If the first case is true, then the cetaceans, pinnipeds and sirenians will be more closely related to each other than to any land-dwelling mammals. On the other hand, if each of these groups are more closely related to different land-dwelling mammals, this would indicate that the ability to live in the water evolved several times in the mammals.

The fossil evidence tells us that whales evolved from land dwelling mammals, known as mesonychids. These carnivorous animals have a wolf-like appearance. The fossil record shows a progression from mesonychids to the otter-like Pakicetus, to a walking whale, Amubulocetus, to progressively more whale-like creatures. All of these animals have certain unique bones in common, morphological evidence that they are related. So, does this tell us that whales are related to wolves, or were the mesonychids and Pakicetus, just wolf-like in appearance; an example of convergent evolution?

We will use DNA evidence to help us to answer these evolutionary questions. Even though we don't have DNA from these fossil animals, we can use DNA collected from contemporary animals to help us. We will compare the DNA from whales and various other animals using two computer programs. These programs are available on a website called Biology Workbench.

ClustalW will take a number of DNA sequences and align them or line them up so that we can compare them and see the differences between the sequences. The product of ClustalW is called a multiple alignment. Another program, DrawGram, will take the alignment, calculate the differences between each pair of sequences and use the data to construct a phylogenic tree. The phylogenetic tree shows us the evolutionary relatedness of these animals, based on the differences and similarities in their DNA. The phylogenetic trees will enable us to determine:
a. how related are the cetaceans, pinnipeds and sirenians, and
b. which living mammal is most closely related to the whales. (Is it a carnivore, like the wolf, or another group of mammals, that just happened to have meateating members in the past).

## Directions

## Placing DNA sequences in a text file:

1. Locate the nucleotide sequences in FASTA format.

Go the online site for this class
Select the appropriate sequence file:
Sea Mammal Nucleotide ( 12sRNA) ( section A of activity)
Or
Whale nucleotide (section B of activity)
(contains sequences from exon 7 of the casein gene)
2. Select all text and copy to a text editor on your computer.
3. Save as a text file on Notepad with the extension .txt

## Register an account at Biology Workbench:

4. Go to Biology Workbench at http://workbench.sdsc.edu
i. Follow the directions to set up a new account.

## Starting a new session:

5. Select Session Tools.
i. Name the session
ii. Select "Start a New Session" and hit the" Run" button
6. Select "Nucleic Tools"

## Add the DNA sequences to your session:

7. Add Your sequences:
i. Select "Add New Nucleic Sequences" and hit "Run"
ii. Select "Browse" to find your text file.
iii. Open the file
iv. Select Upload
v. Check that the file has been uploaded with each sequence in a separate window.
vi. Click on "Save"
vii. Your sequences are now permanently saved as part of this session.

## Creating a Multiple Alignment with ClustalW:

8. Select "select all sequences" from the menu and hit run.
9. Select ClustalW from the menu and hit run.
10. Hit the submit button.
11. When the program is done, scroll down to review your multiple alignment.
12. Hit the import key to allow other programs to use the alignment.
13. Click on the box next to the name of your alignment.

## Creating a Phyologenetic Tree with DrawGram:

14. Select DrawGram from the menu and hit run.
15. Click on "submit".
16. When the program is done, scroll down to view your phylogenetic tree.
17. Copy the tree into a Word document and print it.

## Critical Thinking Exercises Are the Seals, Whales and Manatees Closely Related? (for Section A)

1. According to your phylogenetic tree, which mammal is most closely related to the whales? To what phylogenetic group do these mammals belong? Which mammal is most closely related to the pinnipeds? The sirenians?
2. According to your evaluation of the DNA evidence, how many times did the mammals return to the sea? Explain your answer.
3. Explain how comparing DNA extracted from different organisms can help us to look at the evolutionary past, even though we may not have DNA from fossils?

## Critical Thinking Exercises Are the Whales Descended from Wolves? (for part 2)

1. According to your phylogenetic tree, which mammal is most closely related to the whales? To what phylogenetic group do these mammals belong?
2. Does your evidence support the hypothesis that Pakicetus and the mesonychids were truly wolf-like or does it support the competing hypothesis that they just looked like wolves, but were related to something else (an example of convergent evolution)? Explain your answer.
3. What is meant by convergent evolution? Explain why the mesonychids may have looked like wolves.
4. How does the possibility of convergent evolution affect the way scientists analyze fossils?
5. Explain how comparing DNA extracted from different organisms can help us to look at evolution, even though we may not have DNA from fossils?

## Comparison of Mammalian 12S rRNA

| Scientific Name | Common Name |
| :--- | :--- |
| Vulpes vulpes | Fox |
| Canis familiaris | Dog |
| Giraffa camelopardalis | Giraffe |
| Physeter catodon | Sperm whale |
| Trichechus manatus | Caribbean manatee |
| Loxosonta Africana | African elephant |
| Leptonychotes weddellii | Weddell seal |

## Comparison of casein sequences

| Scientific Name | Common Name |
| :--- | :--- |
| Alces alces | Moose |
| Tragulus napu | Chevrotain |
| Giraffa camelopardalis | Giraffe |
| Pecari tajacu | Peccary |
| Delphinapterus leucas | Beluga whale |
| Baleaenoptera physalus | Finback whale |
| Uncia uncia | Snow leopard |
| Hippotamus amphibious | Hippopotamus |
| Camelus dromedarius | Arabian camel |
| Antilocapra Americana | Pronghorn antelope |
| Tapirus indicus | Malayan tapir |
| Equus grevyi | Zebra |

>L.weddelli
CAAAGGTTTGGTCCTAGCCCTCCTATTGGTTTCTAATAAGATTATACATGCAAGCCTCCGCGCCCCGGTG AAAGTGCCCTCTAAATCTCATACAACTGATTAAAAGGAGCAGGTATCAAGCACACTAAACAAGTAGCTCA CAACGCCTTGCTCAACCACACCCCCACGGGATACAGCAGTAATAAAAATTAAGCTATGAACGAAAGTTCG ACTAAGCTATATTAAACATTCAGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAA ATCAATAGGTCCTCGGCGTAAAAAGTGTCAAAGATTTAATTAACACTAAAGTTAAAAACTAACCAAGCTG TAAAAAGCTACCGTTAATATAAAATAAACTACGAAAGTGACTTTACCAACTCCGACTACACGATAGCTAA GACCCAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAACACAAATAATTAATATAACAAAATTAT TCGCCAGAGAACTACTAGCAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTCACACCCCTCTAGAGGA GCCTGTTCTATAATCGATAAACCCCGATAAACCTCACCACTCCTTGCTAATTCAGTCTATATACCGCCAT CTTCAGCAAACCCTTAAAAGGAATAAAAGTAAGCATAATAATCACTACATAAAAAAGTTAGGTCAAGGTG TAACCTATGGAGCGGGAAGAAATGGGCTACATTTTCTAAACAAGAACAACCATACACGAAAGTTTTTATG AAACTAACAAACTAAAGGTGGATTTAGCAGTAAATTAAGAATAGAGAGCTTAATTGAATCGGGCCATGAA GCACGCACACACCGCCCGTCACCCTCCTCAAATAAACACTCTAAATTAAATAAAACATACACAAAACACA AGAGGAGACAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGGATAAAC
> Trichechus manatus
CAAAGGTTTGGTCCTGGCCTTCTTATTAGTTTTTAGTGAGCTTACACATGCAAGTATCCGCGCGCCAGTG AAAATGCCCTTCAAATCATTACTGACCATAAAGGAGCGGGTATCAAGCACACACCTATGTAGCTCACAAC ACCTTGCTTAGCCACACCCCCACGGGATACAGCAGTGATAAAAATTTAGCCATAAACGAAAGTTTGACTA AGCTACGCTAACACAGGGTTGGTAAATTTCGTGCCAGCCACCGCGGCCATACGATTAACCCAAATTAATA AGCATCGGCGTAGAGCGTGTTTAGGAGTCGACACACAAATAAAGTTAAATTAAATCTAAGCCGTAAAAAG CTAAGGATTAGACAAAAATAACCCACGAAAGTGACTTTACCAGCTCTGAATACACGATAGCTAGGACACA AACTGGGATTAGATACCCCACTATGCCTAGCCCTAAACCTCAATGGCTGCTAACAAAGCCATTCGCCAGA GAACTACTAGCAAAGGCTTAAAACTCAAAGGACTTGGCGGTGCTTTACATCCCCCTAGAGGAGCCTGTCC CATAATCGATAAACCCCGATAAACCTCACCACCACTAGCTAATTCAGCCTATATACCGCCATCTTCAGCC AACCCTTAGAAGGTCCAAAAGTGAGCTCGATCATAACCATAAAAAAGTTAGGTCAAGGTGTAGCCCATGT GGTGGTCCAAGATGGGCTACATTTTCTCTCAGAGAACAATCAAACTAACGGACGTTATTATGAAATAAAT GACTAAAGGAGGATTTAGTAGTAAATTAAGATCAGAGAGCTTAATTGAATTAGGCCATGAAGCACGCACA CACCGCCCGTCACCCTCCTCAAATACCCATAATTATCTTACCACAAACAATTTTAATAAGTATGAGAGGA GACAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGGATCACT
> Canis familiaris
TAAAGGTTTGGTCCTAGCCTTCCTATTAGTTTTTTAGTAGACTTACACATGCAAGCCTCCACGCCCCAGTG AGAATGCCCTTAAAATCACCAGTGATCTAAAGGAGCAGGTATCAAGCACACTCTTAAGTAGCTCATAACA CCTTGCTAAGCCACACCCCCACGGGATACAGCAGTGATAAAAATTAAGCCATAAACGAAAGTTTGACTAA GCCATACTAAATAGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAACTAATAGG CCTACGGCGTAAAGCGTGTTCAAGATATTTTTACACTAAAGTTAAAACTTAACTAAGCCGTAAAAAGCTA CAGTTATCATAAAATAAACCACGAAAGTGACTTTATAATAATCTGACTACACGATAGCTAAGACCCAAAC TGGGATTAGATACCCCACTATGCTTAGCCCTAAACATAGATAATTTTACAACAAAATAATTCGCCAGAGG ACTACTAGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTATATCCCTCTAGAGGAGCCTGTTCTA TAATCGATAAACCCCGATAAACCTCACCACCTTTCGCTAATTCAGTCTATATACCGCCATCTTCAGCAAA CCCTCAAAAGGTAGAACAGTAAGCACAATCATTTTTACATAAAAAAGTTAGGTCAAGGTGTAACTTATGAG GTGGGAAGAAATGGGCTACATTTTCTACCCAAGAACATTTCACGAATGTTTTTATGAAATTAAAAACTGA AGGAGGATTTAGTAGTAAATTAAGAATAGAGAGCTTAATTGAATAGGGCCATGAAGCACGCACACACCGC CCGTCACCCTCCTCAAGTAATAAGACACAACCATAACCATATTAACTTAACTAAAACACAAGAGGAGACA AGTCGTAACAAGGTAAGCATACCGGAAGGTGTGCTTGGATTAAT
> V.vulpes
CAAAGGTTTGGTCCTGGCCTTCCTATTAGTCCTTAGTAAACTTACACATGCAAGCCTCCACGCCCCCAGTG AGAATGCCCTTAAAATCGCTAACGATCTAAAGGAGCAGGTATCAAGCACACTCCTAAGTAGCTCATAACA CCTTGCTAAGCCACGCCCCCACGGGATACAGCAGTGATAAAAATTAAGCCATGAACGAAAGTTCGACTAA GTTATACTAAAGAGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCGAACTAATAGG CCCACGGCGTAAAGCGTGTTTAAGATAACATATTACTAAAGTTAAAACTTAACTAAGCCGTAAAAAGCTA CAGTTACCATAAAATATACTACGAAAGTGACTTTAAAATTTTCTGATTACACGATAGCTAAGACCCAAAC TGGGATTAGATACCCCACTATGCTTAGCCCTAAACATAAATAGTTCTATAACAAAACAATTCGCCAGAGA ACTACTAGCAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTATATCCCTCTAGAGGAGCCTGTTCTA TAATCGATAAACCCCGATAAACCTCACCATCCCTTGCTAATACAGTCTATATACCGCCATCTTCAGCAAA CCCTTAAAAGGTAGAGCAGTAAGCAGGATCATCACGCATAAAAAAGTTAGGTCAAGGTGTAACTTATGGG ATGGGAAGAAATGGGCTACATTTTCTATTTTAAGAACACTTTACGAAAGTTTTTATGAAACTAGAAACTG AAGGAGGATTTAGTAGTAAATTAAGAATAGAGAGCTTAATTGAATAGGGCCATGAAGCACGCACACACCG CCCGTCACCCTCCTCAAGTAATAAGACTGAGGCCATAAACATATTAACTCACATCAAAACACGAGAGGAG ATAAGTCGTAACAAGGTAAGCATACCGGAAGGTGTGCTTGGATTAAC
> Physeter catodon
CAAAGGTTTGGTCCCAGCCTTCCTATTAGCTCCCAACAGATTTACACATGCAAGCATCTACATCCCAGTG AAAATGCCCTCCAAGTCACAAAGATCAAAAGGAGCTGGTATCAAGCACGCTTCACTAGCAGCTCACAACA CCTTGCTTAGCCACACCCCCACGGGACACAGCAGTGATAAAAATTAAGCCATGAACGAAAGTTTGACTAA GTCATGGTTGATTAGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTGACCCAAGCTAATAG GCATACGGCGTAAGGAGTGTTAAAGAGTTATACAAAATAAAGTCAAGCCTTGACTAAACCGTAAAAAGCC ATAGTTAAAATTAAGCTAAACCACGAAAGTGACTTTGGTACAATCTACTGCACGACAACTAAGACCCAAA CTGGGATTAGATACCCCACTATGCTTAGCCGTAAACCCAGGTAGTCATAAAACAAGACTATTCGCCAGAG TACTACTAGCAACAGCCTAAAACTCAAAGGACTTGGCGGTGCTTCATACCCCCCTAGAGGAGCCTGTTCT ATAACCGATAAACCCCGATCAACCTCACCAACCCTTGCTACTTCAGTCTATATACCGCCATCTTCAGCAA ACCCTAAAAAGGAATGAAAGTAAGCATAACTATCCTACGTAAAAACGTTAGGTCAAGGTGTAACCCATGG GATGGGAAGAAATGGGCTACATTTTCTACACCATAGAACACCCTATACCCCACGAAAGTTTTTATGAAAC CTAAAAACCAAAGGAGGATTTAGCAGTAAATTAAGAACAGAGTGCTTAATTGAATAAGGCCATGAAGCAC GCACACACCGCCCGTCACCCTCCTCAAGTATCACAGCGAACGCCCAATCCACTAATTTATGCCGAGCGCC CCCACAAGAGGAGACAAGTCGTAACAAGGTAAGCATACTGGAAGGTGTGCTTGGATAAAA > Giraffa camelopardalis TATAGGTTTGGTCCCGGCCTTCCCATTAGCCCCCAATAGACTTACACATGCAAGCATCTGCGCCCCGGTG AAAATGCCCTTCAAGTTAACCAAACTAAAAGGAGCTGGTATCAAGCACACATCTGTAGCTAACGACACCT TGCTTAACCACACCCCCACGGGAGACAGCAGTGATAAAAATTAAGCTATGAACGTAAGTTTGACTAAGTT ATATTGATTAGGGTCGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAGTTAATAGGAAT ACGGCGTAAAGCGTGTCAAAGCATTTCACCAAATACAGTTAAATTCTAATTAAGCCGTAAAAAGCCACAA TTATAATGAAAATAGATAACGAAGGTAGCTCTACAACCGCTGACACACGATAGCTAAGATTCAAACTGGG attagataccccactatgcttagccttaiacacaaitaittatacaiacaaaiattattcgccagaginct ACTAGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTATATCCCTCTAGAGGAGCCTGTTCTATAA TCGATAAACCCCGATAAACCTCACCAGTCCTTGCCAATACAGTCTATATACCGCCATCTTCAGCAAACCC TAAAAAGGAACAAAAGTAAGCGAAACCATACTACATAAAAACGTTAGGTCAAGGTGTAACCTATGGAATG GGAAGAAATGGGCTACATTTTTCTACTCTAAGAAAATCCAAATACGAAAGTTATTATGAAACTAATGACTA AAGGAGGATTTAGTAGTAAACTAAGAATAGAGTGCTTAGTTGAATTAGGCCATGAAGCACGCACACACCG CCCGTCACCCTCCTCAAATAGACACAATACACTCAAACTTATTAATACGTATTAACCATGTGAGAGGAGA TAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGGACAAAT
>Loxodonta africana
AAAGGTTTGGTCCCGGCCTTCTTATTGGTTACTAGGAAACTTATACATGCAAGTATCCGCCCGCCAGTGA ATACGCCTTCTAAATCATCACCGATCAAAGAGAGCTGGCATCAAGCACACACTCTAAGTGTAGCTCATGA CGTCTCGCCTAGCCACACCCCCACGGGAAACAGCAGTAGTAAATATTTAGCAATTAACAAAAGTTAGACT AAGGTATCCTAATAAAGGACTGGTCAATTTCGTGCCAGCAACCGCGGCCATACGATTAGTCCAAATTAAT AAGCATACGGCGTAAAGCGTATCAGAAGAATTAAGAAAATAAAGTTAAATCTTATACTAGCTGTTTAAAG CTCAAGATAAGACATAAATAACCTACGAAAGTGACTTTAATAATCCTAAACATACGATAGCTAGGGTACA AACTGAGATTAGATACCTCACTATGCCTAGCCCTAAACTTTGATAGCTACCTTTACAAAGCTATCCGCCA GAGAACTACTAGCCAGAGCTTAAAACTTAAAGGACTTGGCGGTGCTTTATATCCACCTAGGGGAGCCTGT CTCGTAACCGATGAACCCCGATATACCTTACCGTCACTTGCTAATTCAGTCCATATACCACCATCTTCAG CAAACCCCCATAGGGCACAAAAGTGAGCTTAATCATAACCCATGAAAAAGTTAGGCCGAGGTGTCGCCTA CGTGACGGTCAAAGATGGGCTACATTTTCTATTATAGAACAGACAAACGGATATCACTCTGAAATGGGTG GTTGAAGGCGGATTTAGTAGTAAACTAAGAATAGAGAGCTTAATTGAACAAGGCTATGAAGCGCGTACAC ACCGCCCGTCACTCTCCTCAAGTATCCCCACATCAAACAATCATATTACAGATTTAAACAAATACAAGAG GAGACAAGTCGTAACAAGGTAAGCGTACTGGAAAGTGCGCTTGGATAACT

