

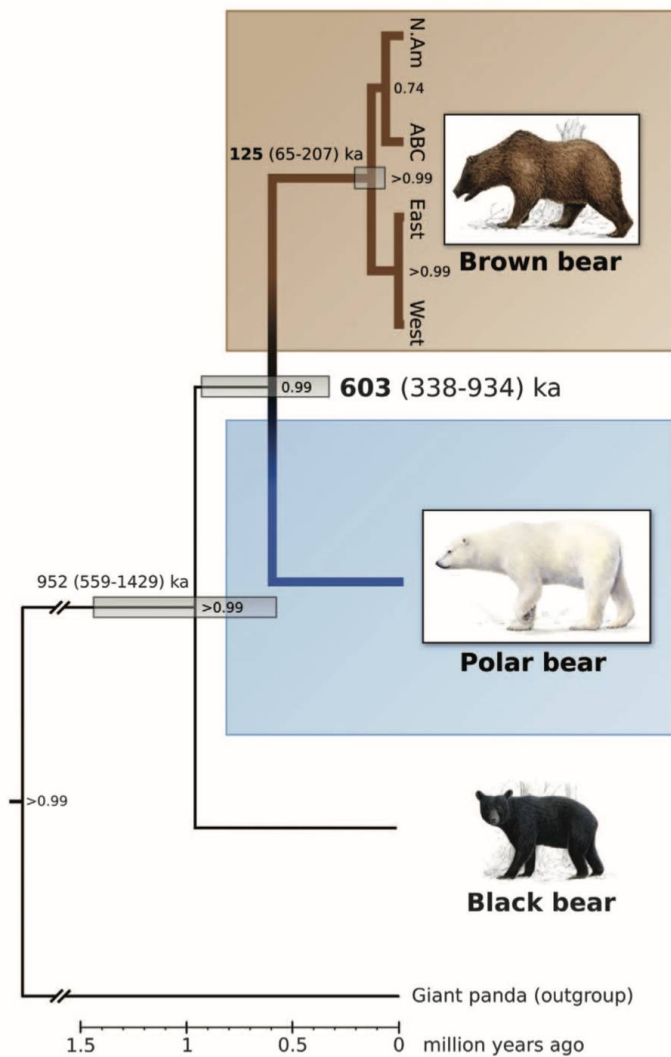
I. Polar bears

DNA was extracted from one giant panda and 45 individuals of polar, brown, and black bears. 14 independent loci (introns) were PCR-amplified and sequenced for each DNA sample. A Bayesian approach was used to reconstruct the phylogeny based on these 14x46 sequences (panel A). With high statistical support ($P > 0.99$), polar bears were recovered as a sister lineage to all brown bears.

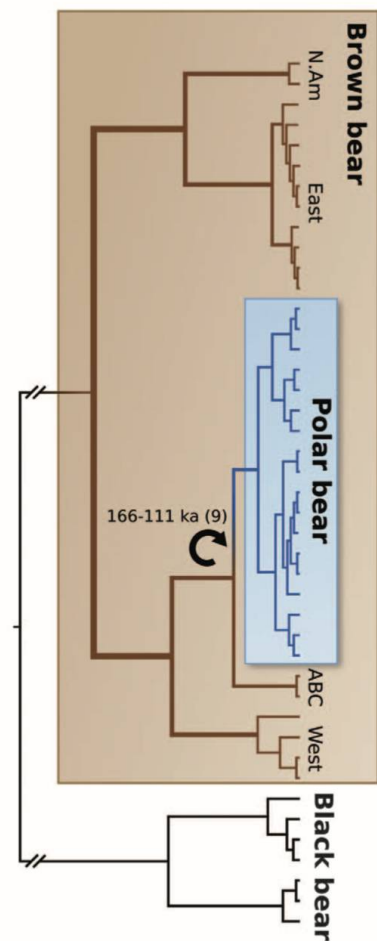
A few mitochondrial genes were also sequenced in several individuals polar, brown, and black bears and they produced another phylogenetic tree which is also highly robust statistically (panel B).

a. Interpret the two trees.

A Nuclear DNA



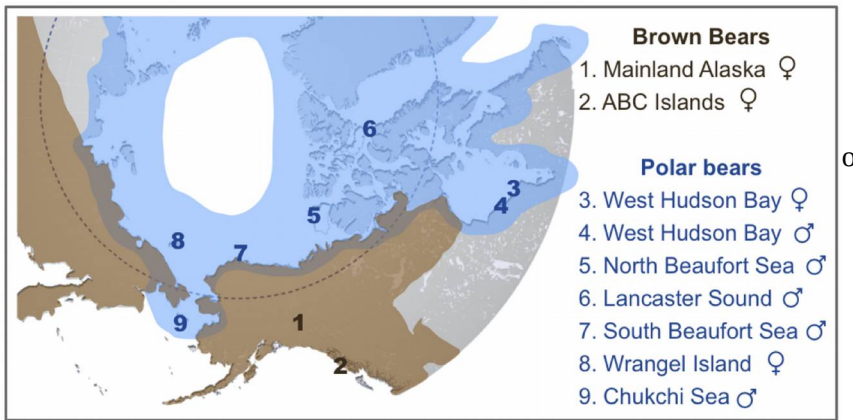
B Mitochondrial DNA



ABC = brown bears from Admiralty, Baranof, and Chichagof (ABC) Islands off the coast of Alaska .

Note that the probability of joining “ABC” bears to “N. Am” bears as sister groups based on nuclear DNA (panel A) has a probability of 0.74, suggesting that the mitochondria-based scenario may be too simplistic.

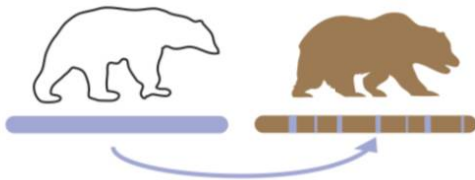
To try to resolve better the evolutionary history of the polar bears and the ABC brown bears, random genomic shotgun libraries were sequenced from seven polar bears, two brown bears (see map below for origin of each bear) and one black bear from Pennsylvania.



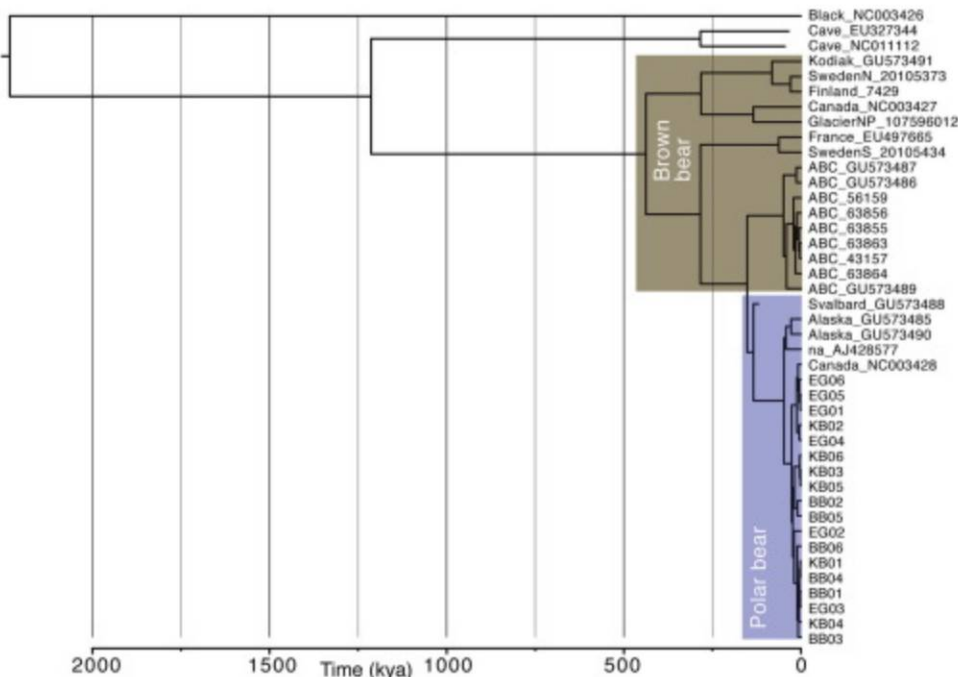
Genetic diversity was found to be lower for polar bears than for brown bears: polar bear alleles differ at about 4 sites in 10,000 whereas brown bears differ at about 17 sites in 10,000.

b. What can you infer from this observation?

To obtain more data, 79 Greenlandic polar bears and ten brown bears from Fennoscandia; mainland US; and the Admiralty, Baranof, and Chichagof (ABC) Islands off the coast of Alaska were sequenced. ABC brown bears were found to carry pieces of polar bear genomes, but not the other brown bears:



A phylogeny based on all mitochondrial data was redone and gave the same result as the first study, with polar bears being the sister group of ABC brown bears:



c. Propose a scenario for the origin of polar bears and ABC bears that matches all available data.

II. Evolution of lice

Humans can be infected by three types of lice: *Pediculus humanus capitis* (human head louse), *Pediculus humanus corporis* (human body louse) and *Pthirus pubis* (crab louse). Our ape relatives are infected by only one type of louse species.

Pediculus humanus capitis and *Pediculus humanus corporis* are very similar morphologically and can still hybridize in the laboratory although they live on different places on the human body and possess slightly different DNA sequences. DNA comparison suggest that the most recent common ancestor of *Pediculus humanus capitis* and *Pediculus humanus corporis* lived less than 70,000 years ago.

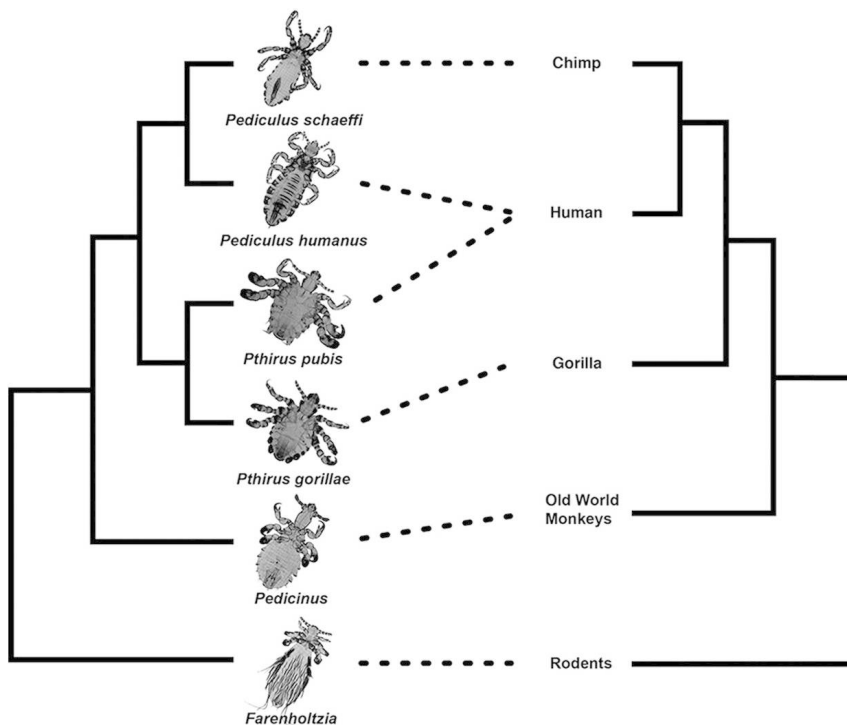
a. What can you infer about the evolution of clothes in humans?

Greater genetic diversity is found in lice from Africa compared to non-African lice.

b. Can you explain this result?

Below is a phylogeny based on both nuclear and mitochondrial genes for mammals (right) and for lice (left).

c. What can you infer? (at least two scenarios are possible)



d. Use Timetree (<http://www.timetree.org/>) to add time estimates on relevant nodes and find which scenario is most probable.

III. Cytochrome b and fungicide resistance

Quinone outside inhibiting (QoI) fungicides represent one of the most widely used groups of fungicides used to control agriculturally important fungal pathogens. QoIs inhibit mitochondrial respiration in fungi by binding to the Qo site of the cytochrome bc1 complex (which includes the protein cytochrome b), blocking electron transfer and halting ATP synthesis. QoI fungicides usually have low toxicity towards birds, mammals (including humans), and bees at limited doses. We will study the evolution of resistance to QoI.

One gene has been involved in such resistance in the grapevine downy mildew.

a. Find the case in www.Gephebase.org by searching for “grapevine downy mildew”.

b. Why are there two entries?

c. Complete the table (to compare with the lactase case that we studied in class):

Gene	<i>lactase</i>	<i>cytochrome b</i>
Type of gene	nuclear	
Trait	lactose tolerance	
Taxonomic status	intraspecific	
Presumptive Null	no	
Molecular Type	cis-regulatory	
Aberration Type	SNP	
Molecular Details of the Mutation	T14009G (rs869051967) (ss 820486563)	

Click on “cytochrome b” to see if there are other entries in Gephebase for this gene.

d. Is it correct to call QoI molecules fungicides?

To see all the mutations in cytochrome c, click on “Split Mutations”, “Submit” and then the tab “Other” (or go to <https://www.gephebase.org/search-criteria?and+Gene%20Gephebase=%5Ecytochrome%20b%5E/and+splitMutations=true>)

e. Can you find any amino acid change that evolved multiple times in cytochrome b besides Gly143Ala?

The G143A mutation confers high (complete) resistance, which is always associated with the failure of QoIs to control disease.

We will check whether the mutation G143A is also found in other species.

f. Let’s first retrieve the cytochrome b protein sequence of *Plasmopara viticola*:

Click on one of the *Plasmopara viticola* Gephebase entry (for example <https://www.gephebase.org/view-gephe/2041>), click on the Main Reference. You should then see the paper in NCBI PubMed. In the bottom right, click on “>Related Information >Protein”. Select one of the *Plasmopara viticola* protein sequence for “apocytochrome b”.

g. Check position 143 in the protein. Can you see the G143A mutation?

h. Let’s search for other cytochrome b proteins with the same G143A mutation:

Copy one of cytochrome b protein sequences of *Plasmopara viticola* harboring the G143A mutation (for example <https://www.ncbi.nlm.nih.gov/protein/ABF29522.1>). Perform a BLAST to search for similar sequences in the nr database (choose the right BLAST search type among blastn, blastp, tblastn and blastx in

<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Click on “Alignments” and “Flat query-anchored with dots for identities” to see all the protein sequences aligned to your query. Find position 143.

i. Can you find other sequences with the G143A mutation? Which species do they correspond to? (use right-click to open the pages in other tabs)

There may be other species with the mutation but by default BLAST searches only show the first 100 target sequences. To retrieve more sequences, go back to the BLAST initial page, click on “Algorithm parameters” and select “5000” for Max target sequences. This run will take a bit longer.

j. Can you find more sequences with the G143A mutation? Which species do they correspond to?

Experimental data from *Saccharomyces cerevisiae* and *Strobilurus tenacellus* suggest that the N261D mutation in *cytochrome b* also confers resistance.

k. Use the same strategy as above to check whether the mutation N261D is also found in other species:

Let’s first retrieve the cytochrome b protein sequence in *Strobilurus tenacellus*:

Click on the *Strobilurus tenacellus* Gephabase entry (<https://www.gephabase.org/view-gephe/2045>), click on the Main Reference. You should then see the paper in NCBI PubMed. In the bottom right, click on “>Related Information >Protein”. Select the *Strobilurus tenacellus* protein sequence.

Check position 261 in the protein. Perform a BLAST to search for similar sequences in the nr database. Click on “Alignments” and “Flat query-anchored with dots for identities” to see all the protein sequences aligned to your query. Find position 261.

Can you find other sequences with the N261D mutation? Which species do they correspond to?

l. optional: search for the G143A mutation in humans.