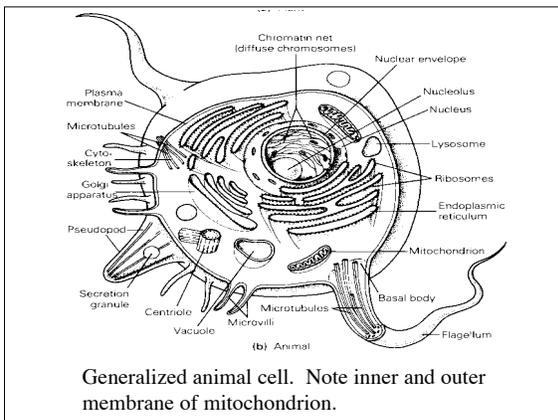
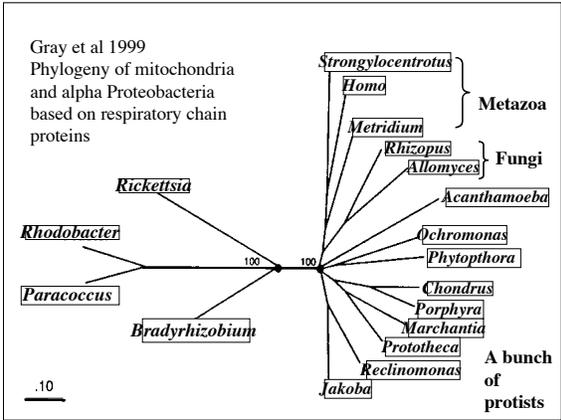


THE MOST COMMON DNA-BASED METHOD FOR ANIMAL SPECIES IDENTIFICATION: MITOCHONDRIAL DNA (mtDNA) SEQUENCING

Mitochondria are the cellular organelles responsible for oxidative phosphorylation. They are almost certainly descended from a free-living bacterium that became an endosymbiont* ~2 billion years ago.

*This sort of thing still happens.





MTDNA INCLUDES A SUBSET OF A TYPICAL BACTERIAL GENOME

- the number of genes observed varies from 97 to 5
- typically code for resp. proteins, and all tRNAs, some rRNAs needed to make them
- circular or linear

The genetic code is not universal, mammal mtDNA ≠ fly mtDNA ≠ nuclear DNA although they are quite similar

Although plants also have mitochondria, only animal mtDNA shows variation that is generally useful for forensic purposes.

Animal mtDNA is characterized by conserved gene order but variable sequence. Plant mtDNA is characterized by variable gene order but conserved sequence.

Individual animals of the same species usually vary (if at all) in their sequence, however there are examples of length variation. This may reflect a small indel or a relatively large insertion of tandem repeats.

*COMMON mtDNA
ARRANGEMENT IN ANIMALS*

16.5 KB CIRCULAR (HAPLOID) MOLECULE

37 GENES

22 tRNAs

13 PROTEINS

12s AND 16s rRNA

NO INTRONS, PSEUDOGENES, ETC.

Note about pseudogenes

- These probably were functioning genes that have stopped working and are free to mutate
- Although the mtDNA molecule has none, the nuclear genome has mtDNA pseudogenes that are sometimes accidentally amplified
- Best quality control method is to translate to the peptide (e.g. www.ebi.ac.uk/Tools/emboss/transeq/index.html). Also check if what should be overlapping sequences match.
- If it's a non-coding region, still look for an unusual result, such as a large number of variable sites that have never been observed before.

*TYPICAL ANIMAL mtDNA
CONTINUED...*

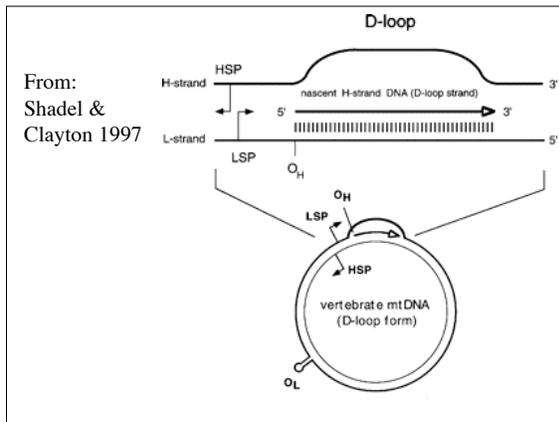
Single notable non-coding region:

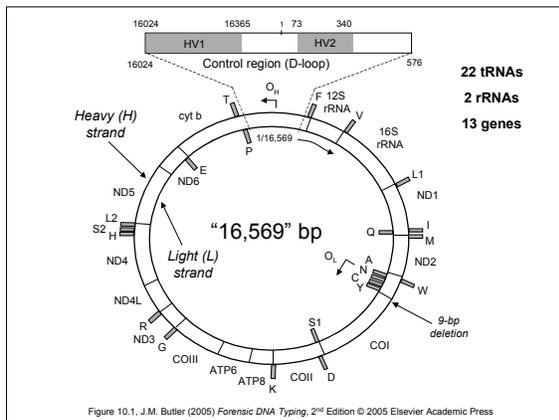
-Vertebrate "D-loop" or "Control Region"

-Invertebrate "A+T-rich Region"

-Each typically ~1 kb

“D-Loop” because in EM pictures the 2 strands are often displaced from each other by an RNA primer. “Control Region” because it is the origin of heavy strand replication.





*TYPICAL ANIMAL mtDNA
CONTINUED...*

~200-2000 copies per cell (~5/mitochondrion)
Maternal inheritance (rare examples of paternal leakage) with genetic bottleneck at oogenesis
No recombination (some debate)
Mutation rate in coding regions ~10X nuclear DNA

The high mutation rate of animal mtDNA apparently reflects the lack of a repair mechanism for mutations that arise during replication, as well as a chemically reactive environment full of free radicals.

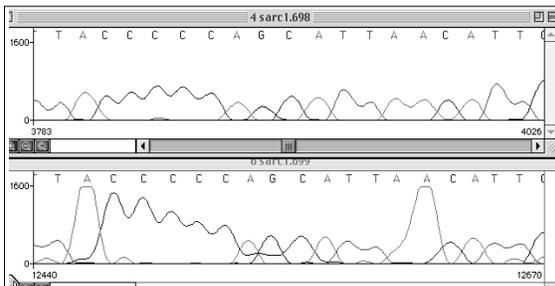
In contrast, mtDNA appears to have greater postmortem stability compared to nucDNA.

In Summary, mtDNA is
-a single haploid locus
-with very high copy number and mutation rate,
- inherited as a maternal, clonal, lineage.
-relatively resistant to postmortem degradation

WHAT'S IT USUALLY GOOD FOR?

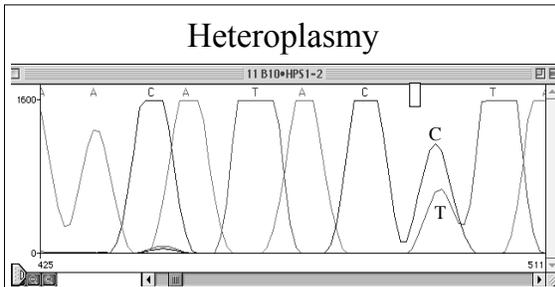
- Abundance and haploid nature make mtDNA easy to extract and analyze, sometimes from very degraded specimens (e.g., Pleistocene age)
- Genetic variation suitable for questions at the taxonomic Family to the individual level

MOST STUDIED GENETIC LOCUS



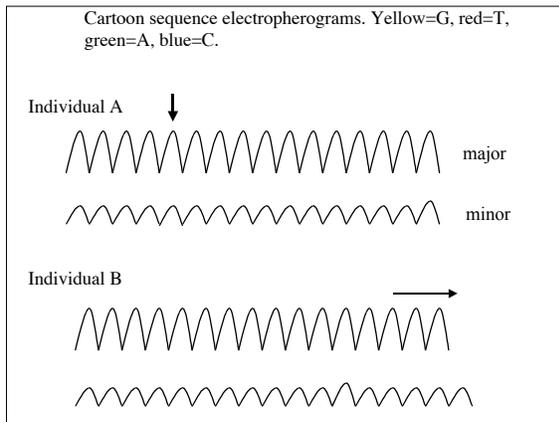
Complementary strands of same PCR product. Note background signal from C-stretch that we interpret as “noise”

Heteroplasmy

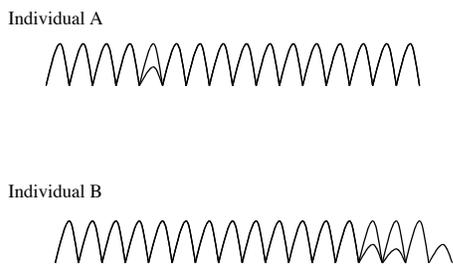


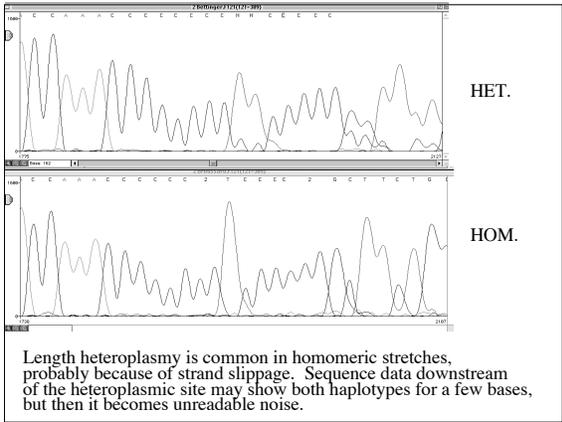
The detection of >1 haplotype in a single organism

The two haplotypes may be the same length but differ in sequence (a point substitution) or because of an indel (length).

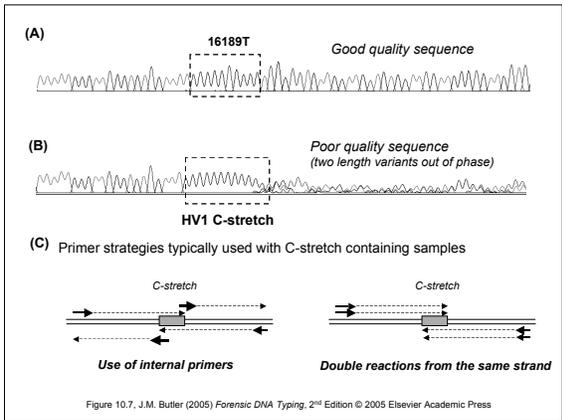


With electropherograms superimposed such as you would see if both haplotypes were present in the same DNA extraction.





The mix of haplotypes associated with a homomeric stretch can usually be reconstructed if both PCR strands are sequenced.



Although heteroplasmy is of great concern for human identity testing, point mutation heteroplasmy is not much of a problem for species determination. It is just a manifestation of intraspecific variation.
