

# **Cytoplasmic Genomes**

## **mtDNA and cpDNA**

- **Small relative to nuclear genome**
- **Often sex biased transmission**
- **More homogenous in sequence “type” than nuclear**
- **Homoplastic (i.e., haploid)**
- **High copy number**

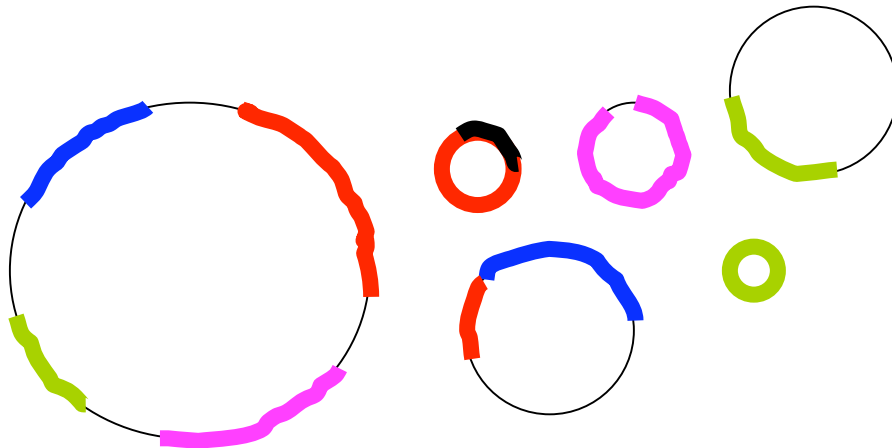
## Animal mitochondrial DNA

- Best known and most used
- Small number of genes
  - 2 ribosomal genes
  - 22 transfer RNA genes (dispersed)
  - 13 ATP synthesis genes
- Small overall size (14 – 30 kbp)
- Mostly coding
  - 1 region of non-coding used for replication called the control region of d-loop
- female transmitted
- homoplastic (mostly)
- rapid sequence evolution
  - may lack proof reading system
- generally conserved gene order within phyla but not between (and sometime not within)

**$N_e$  is then 1/4 of nuclear so population processes happen faster. Rapid evolution = higher  $\mu$  (mutation rate)**

## Plant mtDNA

- **Very, very different**
- **Almost never used (not in same way)**
- **Much less known about sequence**
- **10X larger or smaller than animal mtDNA**
- **Very unstable (homoplastic?)**
  - **Many sub-satellite genomes**



- **female and male transmitted (sometimes both)**
- **non-coding regions**

## **Plant cpDNA**

- **cpDNA to the rescue!**
- **Still larger than animal mtDNA**
  - **~10 times**
- **Some repeated DNA segments**
- **Coding and noncoding regions (introns)**
- **Slow rate of sequence evolution (and gene order)**  
**relative to animal mtDNA**
- **Female or male transmitted or both but usually female**
- **Homoplasmic**

**(two out of three isn't bad!)**

## Uses of mtDNA and cpDNA

- **Protein stuff?**
- **They are all DNA so...**
  - **DNA-DNA hybridization**
  - **Restriction analysis**
  - **Direct DNA sequencing**
  - **Can use PCR**
    - **RFLP**
    - **DNA sequencing**



# Sequence Alignment

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Boulengero CAATTCATTAGAGG-----CTGGCTGTTTtagcacaagagtaggcagg----CTGAG-CCA
Serranochr CAATTCATTAGAGG-----CTGGCTGTTTtagcacaagagtaggcagg----CTGAG-CCA
Tylochromi CAATTCATTAGAGG-----CTGGCTGTATAGCACAAGAGTAGGCAGG----CTGAG-CCA
Tmoorii CAATTCATTAGAGG-----CTGGCTGTTTtagcacaagagtaggcagg----CTGAG-CCA
Astatoreoc CAATTCATTAGAGG-----CTGGCTGTTTtagcacaagagtaggcagg----CTGAG-CCA
Astatotila CAATTCATTAGAGG-----CTGGCTGTTTtagcacaagagtaggcagg----CTGAG-CCA
Labidochro CAATTCATTAGAGG-----CTGGCTGTTTtagcacaagagtaggcagg----CTGAG-CCA
Pelvicachr CAATTCATTAGAGG-----CTGGCTGTTTtagcacaagagtaggcagg----CTGAG-CCA
Hemichromi AAATTCATTAGAGG-----TTGGCTGTTTtagcacaagagtaggcagg----CTGAG-CCA
Crenicichl CAATTCATTAGAGG-----CTGGCTGTTTtagtgc--gagtaggcagt----CTGAG-ACA
Astronotus CAATTCATTAGAGG-----CCGGCTGTTTtagtgc--gagtaggcagg----CTGAG-GCA
Paretroplu CAATTCATTAGAGA-----CTGCCTGCTCAGCAGAAGAGTAGAGAGT----CTGAG-CCA
Etroplus CATTTCAATTAGAGA-----CTGGCTGCTTtagctgg-----TTGAG-CCA
Abudefduf CAGTT----AGACA-----CCGCCTGTTTtagcaggagagtaggcagg----CCGAG-CGG
D.trimacul CAATT----AGACA-----CTGTCTGTTTtagaggacgagtaggcagg----CCGAG-CTG
D.arcuanus CAATT----AGAAA-----CTGTCTGTTTtagaggccgagtagg-----CCGAG-CTG
Micrometru CAATTCATTAGACA-----CTGGCTGTTTtagcagaagagtaggtagt----TTGAG-CTG
Damalichth CACCTCATTAGCCA-----CTGGCTGTTTtagcagaagagtaggcagg----TTGAG-CTG
Perca CACTTCATTAGAGGCAACACTGGCTGTTTtagcagaagagtaggtaga----CTGAGGCGT

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Boulengero GCTG-----ACCAGTT-----AAAACAATATTTGG---TGTGTGTTTGTGCAC
Serranochr GCTG-----ACCAGTT-----AAAACAATATTTGG---TGTGTGTTTGTGCAC
Tylochromi GCTG-----ACCAGTT-----AAAACAATATTTGG---TGTGTGTTTGTGCAC
Tmoorii GCTG-----ACCAGTT-----AAAACAATATTTGG---TGTGTGTTTGTGCAC
Astatoreoc GCTG-----ACCAGTT-----AAAACAATATTTGG---TGTGTGTTTGTGCAC
Astatotila GCTG-----ACCAGTT-----AAAACAATATTTGG---TGTGTGTTTGTGCAC
Labidochro GCTG-----ACCAGTT-----AAAACAATATTTGG---TGTGTGTTTGTGCAC
Pelvicachr GCTG-----ACCAGTT-----AAAACAATATTTGG---TGTGTGTTTGTGCAC
Hemichromi GCCG-----ACCAGTT-----AAAACAATATTTGG---TGTGTGTTTGTGCAC
Crenicichl TCTG-----ACCAGTT-----AAAATAATATTTGG---TGTGTGTTTGTGCAC
Astronotus GCTG-----ACCACTT-----AAAATAATATTTGG---CGTGTGTTTGTGCAC
Paretroplu GCTG-----ACCAGTT-----AAAACAATATTCAC---TGTGTCTTTGTGCAC
Etroplus GCTG-----ACCAGTT-----AAAAAAAATATTCA--GTGTGCCTTTGTGCAC
Abudefduf GTTG-----TCCGAAT-----CAAAGAATATTTAA--GAGAGTGTGTTGTGTAC
D.trimacul GGTGTCCATT----TCAAATATTGTTGGAGACTATATTTAA--GGGTGTGTTTGTGTAC
D.arcuanus GGTGTCCATT----TCAAATAGCGTTG-AAACAATATTTAA--GGGTGTGTTTGTGTAC
Micrometru GTTG-----TCCAGTTGAAACGGCAACTATAACCAG-TGTGTGTGTTTGTGTGC
Damalichth GCCG-----TCCAGTTGAAACGGCAACTATAACCAG-TGTGTGTGTTTGTGTGC
Perca GTTGAGTTGGTTTGGTCTAATTGACAAGGCAACAATATTCAG-----TGTGTTGTGTGC

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## Types of Sequences

- Coding (e.g., cytochrome b)

3' – TGT CTG TGG CGT CCC TGC GAG TCA – 5'  
 5' – ACA GAC ACC GCA GGG ACG CTC AGT – 3'

↓  
 Transcription

– **ACA GAC ACC GCA GGG AGC CUC AGU** – mRNA

↓  
 Translation

– **Thr-Asp-Thr-Ala-Gly-Thr-Leu-Ser** –

<b>ACU GAU</b>	<b>GCU GGU</b>	<b>CUU AGU</b>
<b>ACC GAC</b>	<b>GCC GGC</b>	<b>CUC AGC</b>
<b>ACA</b>	<b>GCA GGA</b>	<b>CUA UCU</b>
<b>ACG</b>	<b>GCG GGG</b>	<b>CUG UCC</b>
		<b>UUA UCA</b>
		<b>UUG UCG</b>

### Genes with:

Little variation – use 3° sites only (or any!)

Moderate variation – use 1° and 2° sites only

Lots of variation – use 2° sites only, AA sequence or transversions  
 (Purine to Purine or Pyrimidine to Pyrimidine).

## Transitions versus Transversions

Expect Ts:Tv ratio of 1:2

Observations are variable but generally see  $Tv > Ts$   
(sometimes 10-15X).

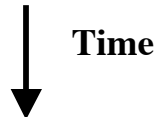
### Mutation Saturation

1 2 3 4 5 6 7 8 9 10 11 12 13 14  
... A C A G T T A G C T C T A G ...

$$P(\text{position 3 mutates}) = \frac{1}{14}$$

$$P(\text{position 3 does not mutate}) = \frac{13}{14}$$

$$P(\text{position has been previously mutated}) = 0$$



1 2 3 4 5 6 7 8 9 10 11 12 13 14  
... A **T** **G** G T **C** A G C **C** C **C** **G** G ...

$$P(\text{position has not been previously mutated}) = \frac{8}{14}$$

$$P(\text{position has been previously mutated}) = \frac{6}{14}$$

**P(position 3 mutates AND has been previously mutated)**

$$= \frac{1}{14} \times \frac{6}{14} = 3.06\%$$

**This results in multiple mutations to a site:**

1 2 3 4 5 6 7 8 9 10 11 12 13 14  
... A T A G T C A G C C C C G G ...

**A => G mutation**

... A T **G** G T C A G C C C C G G ...

**= 1 difference between**

**Reversions – a second mutation at the same site that changes the nucleotide back to the original**

**G => A (reversion)**

... A T **A** G T C A G C C C C G G ...

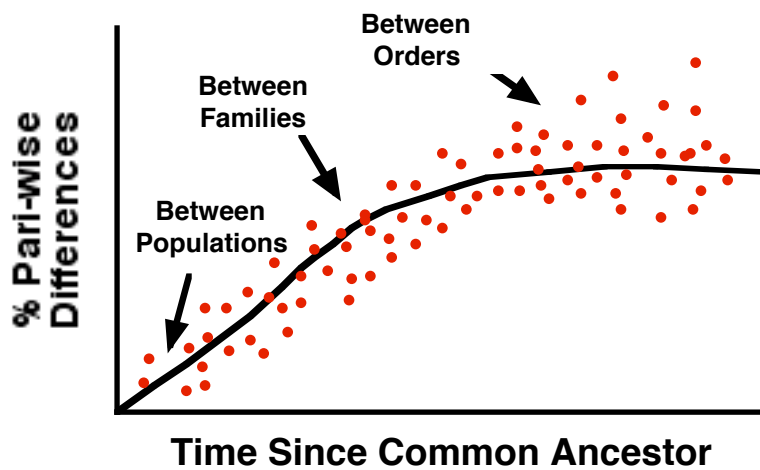
**= 0 differences between**

Any other mutation:  $G \Rightarrow C$   
 $G \Rightarrow T$

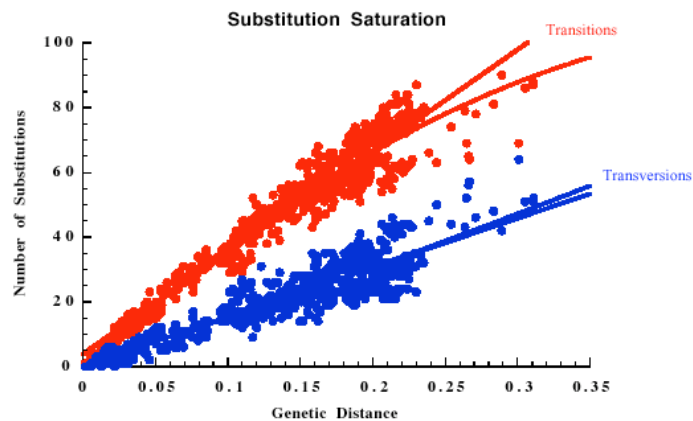
... A T **C** G T C A G C C C C G G ...

= 1 differences between although there has actually been two mutations to the same site

### Substitution Saturation:

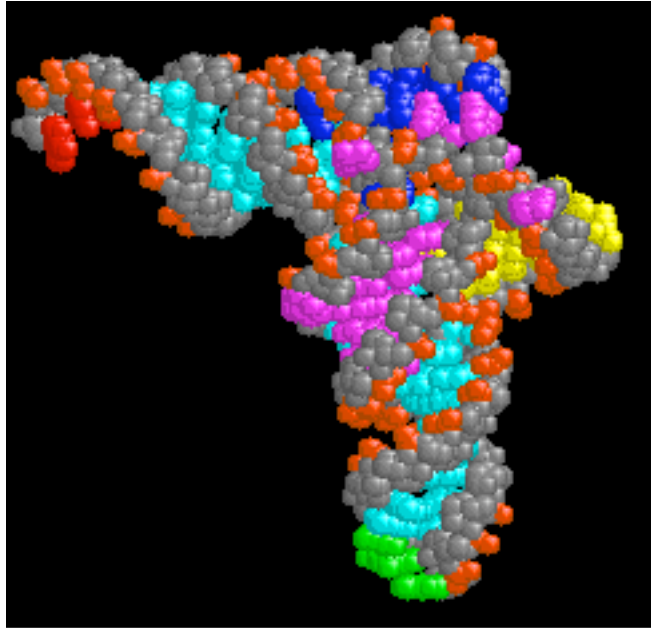


### Saturation of Transitions versus Transversions



## **Types of Sequences – noncoding**

**Transfer RNAs, ribosomal RNAs, d-loop.**



### **Aspects:**

- 1. Is the secondary structure known?**
- 2. Is the molecular as a whole generally considered conserved or variable?**
- 3. Are there known active sites and hyper-variable sites?**
- 4. Will alignment to other sequences be difficult due to insertions/deletions?**
- 5. Is there a known transition:transversion bias?**

## **MtDNA considerations**

- 1) fast and slow evolving genes**
- 2) coding and non-coding regions**
- 3) primers available**
- 4) what other information is available**
- 5) structural/functional information**
- 6) probability of homoplasy/divergence**

## Applications of Cytoplasmic DNA

**Description and distribution of 13 distinct mtDNA genotypes (A-M) in sea turtles.**

<b>Code</b>	<b>MtDNA Genotype</b>	<b>Rookery Location</b>	<b>Sample Size</b>
<b>A</b>	<b>ACCCCCCCCCCCCCDCC</b>	<b>Florida, USA,</b>	<b>21</b>
		<b>Tortuguero, Costa Rica</b>	<b>154</b>
		<b>Aves Island, Venezuela</b>	<b>1</b>
<b>B</b>	<b>ACCCBCCCCCCCCDCC</b>	<b>Florida, USA</b>	<b>3</b>
<b>C</b>	<b>ACCCCCCBCCCCCCC</b>	<b>Galibri, Surinam</b>	<b>15</b>
		<b>Aves Island, Venezuela</b>	<b>7</b>
<b>D</b>	<b>ACCCCCCCCCCCCCCCC</b>	<b>Ascension Island, UK</b>	<b>34</b>
		<b>Atol das Rocas, Brazil</b>	<b>15</b>
<b>E</b>	<b>ACCCCCCCCCCCCCCCD</b>	<b>Ascension Island, UK</b>	<b>1</b>
<b>F</b>	<b>BCCCCBCCCCCCCCCCC</b>	<b>Atol das Rocas, Brazil</b>	<b>1</b>
<b>G</b>	<b>ACCCCCCCCCDCCCCC</b>	<b>Lara, Cyprus</b>	<b>10</b>
<b>H</b>	<b>CCCCCBDCCCCDBBCD</b>	<b>Ras Al Hadd, Oman</b>	<b>15</b>
		<b>Galapagos, Ecuador</b>	<b>8</b>
		<b>Michoacan, Mexico</b>	<b>7</b>
		<b>Hawaii, USA</b>	<b>6</b>
<b>I</b>	<b>CCCCBECCCCDBBCD</b>	<b>Hawaii, USA</b>	<b>16</b>
<b>J</b>	<b>BCCCCBECCCCACBBCC</b>	<b>French Polynesia</b>	<b>2</b>

## Geographical Distribution of mtDNA haplotypes

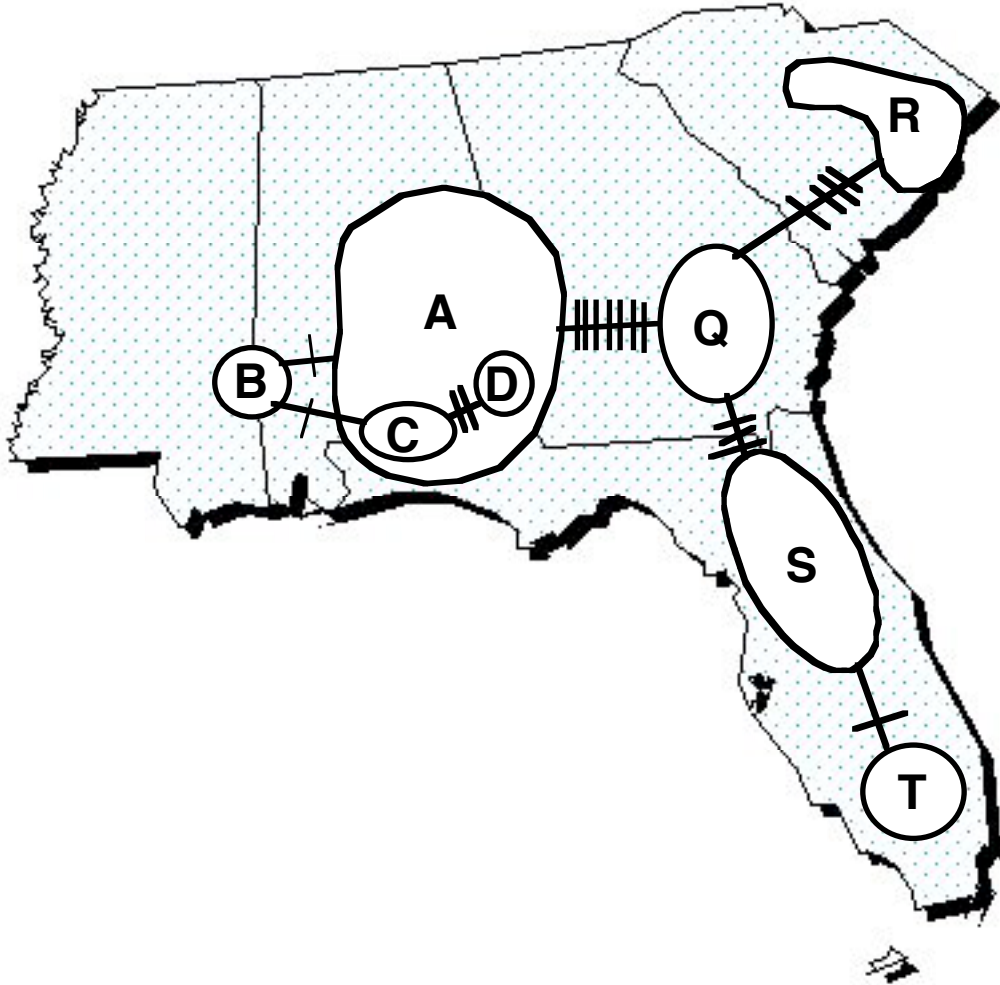
### Haplotype

	A	B	C	D	E	F	G	H	I	J	K	L	M
<b>Costa Rica</b>	15	—	—	—	—	—	—	—	—	—	—	—	—
<b>Florida</b>	21	3	—	—	—	—	—	—	—	—	—	—	—
<b>Venezuela</b>	1	—	7	—	—	—	—	—	—	—	—	—	—
<b>Suriname</b>	—	—	15	—	—	—	—	—	—	—	—	—	—
<b>Ascension</b>	—	—	—	34	1	—	—	—	—	—	—	—	—
<b>Brazil</b>	—	—	—	15	—	1	—	—	—	—	—	—	—
<b>Cyprus</b>	—	—	—	—	—	—	10	—	—	—	—	—	—
<b>Mexico</b>	—	—	—	—	—	—	—	7	—	—	—	—	—
<b>Galapagos</b>	—	—	—	—	—	—	—	8	—	—	—	—	—
<b>Hawaii</b>	—	—	—	—	—	—	—	6	16	—	—	—	—
<b>Polynesia</b>	—	—	—	—	—	—	—	—	—	2	1	—	—
<b>Australia</b>	—	—	—	—	—	—	—	—	—	—	15	—	—
<b>Japan</b>	—	—	—	—	—	—	—	—	—	—	1	5	—
<b>Oman</b>	—	—	—	—	—	—	—	15	—	—	—	14	—



## So? What do you get?

– ORDERED, highly polymorphic, rapidly evolving, discrete characters.



# Conservation Genetics

