Spring 2024

BIOS 477/877

Bioinformatics and Molecular Evolution

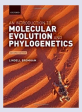
Lecture 3

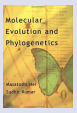
1

TODAY'S TOPICS

➢ Molecular Evolution

- Molecular and Genome Evolution, 1st ed. by Graur, 2016 Sinauer Associates, Inc. [ISBN: 1605354694]
- An Introduction to Molecular Evolution and Phylogenetics, 2nd ed. by Bromham, 2016. Oxford University Press [ISBN: 9780191070693]
- Molecular Evolution and Phylogenetics by Nei & Kumar, 2000 Oxford University Press. [ISBN: 0195135857]
  ➜ Molecular Evolutionary Genetics Analysis (MEGA): free software





MEGA
Molecular Evolutionary Genetics Analysis

https://www.megasoftware.net/
(for Windows, MacOS, Linux)

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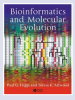
Molecular Evolution & Bioinformatics



"Bioinformatics and Molecular Evolution"
by Higgs and Attwood (2005) Blackwell Publishing [ISBN: 1405106832]
Ch 1: explains how population genetics and molecular evolution are important and related to bioinformatics
Ch 3: explains the basic mechanisms of molecular evolution
(PDF available on Canvas)

"Fundamental Concepts of Bioinformatics"
by Krane and Raymer (2003) Benjamin Cummings [ISBN: 0805346333]
Ch 3: presents the basics of molecular evolution
(PDF available on Canvas)

"Bioinformatics for Beginners"
by Choudhuri (2014) Elsevier [ISBN: 9780124104716]
Ch 2: presents the foundation of molecular evolution
(PDF available on Canvas)
(The book accessible on Elsevier website from UNL)

*PDF files available in "Molecular Evolution readings" page on Canvas.

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Molecular Evolution

DNA molecules:

➜ hereditary material for all living organisms

➜ "documents of evolutionary history"
(Emile Zuckerkandle, one of the pioneers of molecular evolution)

The purpose of molecular evolutionary study:

➜ to unravel historical records written in biological molecules,

➜ to fill in the missing gaps found in these records,

➜ to put the information in order, and

➜ to decipher its meaning (Graur and Li, 2000)

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Molecular Evolution

What we can do:

➜ reconstruct the evolutionary history of genes and genomes (evolution at the molecular level)

➜ reconstruct the evolutionary history of populations and species (evolution at the organismal level)
- can be tracked both in space and in time

➜ attempt to build a classification of the living world

➜ reconstruct the evolution of adaptation (function)

➜ identify the driving forces behind the evolutionary process

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Molecular Evolution

What's happening in this sequence?

```
>Seq1
ATGGCTCTCACCAACAAGAACATCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
ACCAGTCGCGAATTGGTCAAACGGGATCTCAAGGTTTGTTCAACTTTAATTATTTTGGTT
TTGTTTTCCAAAAAACTTACTTTGTTTTCCCGCTGGTTAGAACTTG
```

It is not easy to find any pattern from a single sequence

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Molecular Evolution

Comparison gives us a lot more power!

```
>Seq1
ATGGCTCTCACCAACAAGAACATCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
ACCAGTCGCGAATTGGTCAAACGGGATCTCAAGGTTTGTTCAACTTTAATTATTTTGGTT
TTGTTTTCCAAAAAACTTACTTTGTTTTCCCGCTGGTTAGAACTTG

>Seq2
ATGGCACTCACCAACAAAAACGTCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
ACCAGTCGCGAATTGCTTAAGCGGGATCTCAAGGTTTGTTCGGAGAAAAATCTTTGAAAGG
TTCCAAAGAATTACCTTTGTTTTTTTTGTTTTTTTTGTAGAACTTG
```


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Molecular Evolution

Sequence comparison = Alignment

```
Seq1  ATGGCTCTCACCAACAAGAACATCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
Seq2  ATGGCACTCACCAACAAAAACGTCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC

Seq1  ACCAGTCGCGAATTGGTCAAACGGGATCTCAAGGTTTGTTCAACTTTAATTATTTTGGTT
Seq2  ACCAGTCGCGAATTGCTTAAGCGGGATCTCAAGGTTTGTTCGGAGAAAAATCTTTGAAAGG

Seq1  TTGTTTTCCAAAAAACTTACTTTGTTTTCCCGCTGGTTAGAACTTG
Seq2  TTCCAAAGAATTACCTTTGTTTTTTTTGTTTTTTTTGTAGAACTTG
```


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Molecular Evolution

Sequence comparison = Alignment

```
Seq1  ATGGCTCTCACCAACAAGAACATCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
Seq2  ATGGCACTCACCAACAAAAACGTCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC

Seq1  ACCAGTCGCGAATTGGTCAAACGGGATCTCAAGGTTTGTTCAACTTTAATTATTTTGGTT
Seq2  ACCAGTCGCGAATTGCTTAAGCGGGATCTCAAGGTTTGTTCGGAGAAAAATCTTTGAAAGG

Seq1  TTGTTTTCCAAAAAACTTACTTTGTTTTCCCGCTGGTTAGAACTTG
Seq2  TTCCAAAGAATTACCTTTGTTTTTTTTGTTTTTTTTGTAGAACTTG
```


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Molecular Evolution

Can you see any pattern from the alignment below?

```
Seq1  ATGGCTCTCACCAACAAGAACATCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
Seq2  ATGGCACTCACCAACAAAAACGTCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC

Seq1  ACCAGTCGCGAATTGGTCAAACGGGATCTCAAGGTTTGTTCAACTTTAATTATTTTGGTT
Seq2  ACCAGTCGCGAATTGCTTAAGCGGGATCTCAAGGTTTGTTCGGAGAAAATCTTTGAAAGG

Seq1  TTGTTTTCCAAAAAACTTACTTTGTTTTCCCGCTGGTTAGAACTTG
Seq2  TTCCAAAGAATTACCTTTGTTTTTTTTGTTTTTTTTGTAGAACTTG
```

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Molecular Evolution

Which region is functionally more important?

Region A [more conserved]

```
Seq1  ATGGCTCTCACCAACAAGAACATCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
Seq2  ATGGCACTCACCAACAAAAACGTCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC

Seq1  ACCAGTCGCGAATTGGTCAAACGGGATCTCAAGGTTTGTTCAACTTTAATTATTTTGGTT
Seq2  ACCAGTCGCGAATTGCTTAAGCGGGATCTCAAGGTTTGTTCGGAGAAAAATCTTTGAAAGG

Seq1  TTGTTTTCCAAAAAACTTACTTTGTTTTCCCGCTGGTTAGAACTTG
Seq2  TTCCAAAGAATTACCTTTGTTTTTTTTGTTTTTTTTGTAGAACTTG
```

Region B [less conserved]

A: More conserved
B: Less conserved

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Molecular Evolution

By comparing sequences, we can speculate functional regions of the gene.

Region A [more conserved]

```
Seq1  ATGGCTCTCACCAACAAGAACATCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
Seq2  ATGGCACTCACCAACAAAAACGTCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC

Seq1  ACCAGTCGCGAATTGGTCAAACGGGATCTCAAGGTTTGTTCAACTTTAATTATTTTGGTT
Seq2  ACCAGTCGCGAATTGCTTAAGCGGGATCTCAAGGTTTGTTCGGAGAAAAATCTTTGAAAGG

Seq1  TTGTTTTCCAAAAAACTTACTTTGTTTTCCCGCTGGTTAGAACTTG
Seq2  TTCCAAAGAATTACCTTTGTTTTTTTTGTTTTTTTTGTAGAACTTG
```

Region B [less conserved]

[Eukaryotic gene: exons + introns]

Which is exon, A or B?

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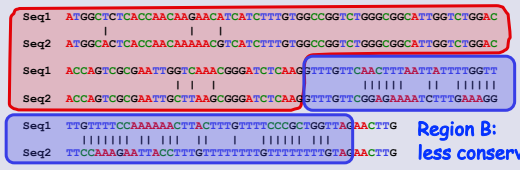
Molecular Evolution

Exons are functionally more important than introns.

Region A: more conserved



Region B: less conserved

usually

Exon (codes amino acids): functionally more important

Intron (does not code amino acids): less important

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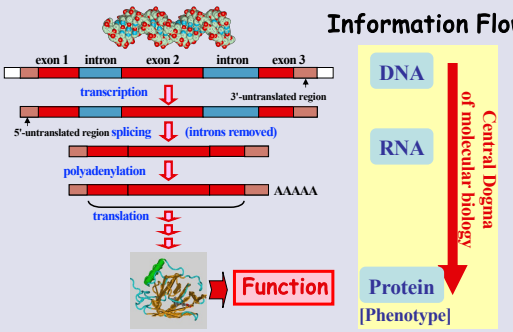
Information Flow



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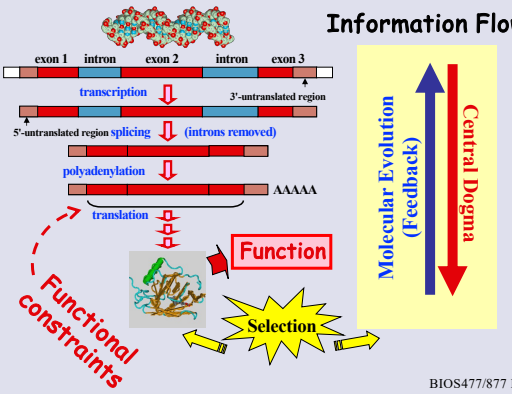
Information Flow



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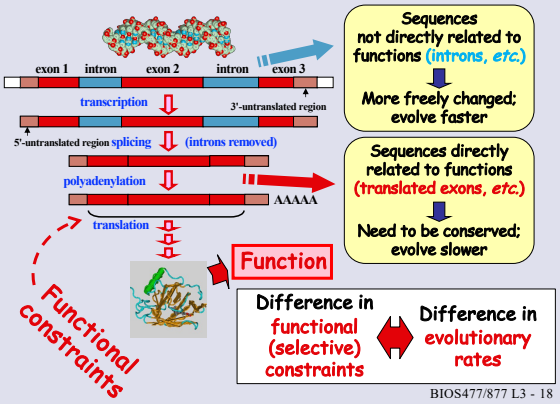

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Molecular Evolution

provides foundations and frameworks for bioinformatics methods

"Nothing in biology makes sense except in the light of evolution"

Dobzhansky (1973)

Molecular Evolution:
Mechanisms of sequence evolution

Molecular Evolutionary Methods:
Sequence distance calculation
Phylogenetic reconstruction
Positive selection detection
Population genetics analysis

Bioinformatics Methods:
Similarity search
Functional domain search
Protein family classification
Functional annotation
Gene prediction

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Molecular Evolution

provides foundations and frameworks for bioinformatics methods

"Nothing in bioinformatics makes sense except in the light of evolution"

Higgs and Attwood (Chapter 1)

Molecular Evolution:
Mechanisms of sequence evolution

Molecular Evolutionary Methods:
Sequence distance calculation
Phylogenetic reconstruction
Positive selection detection
Population genetics analysis

Bioinformatics Methods:
Similarity search
Functional domain search
Protein family classification
Functional annotation
Gene prediction

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Molecular Evolution

provides foundations and frameworks for bioinformatics methods

More conserved regions are predicted to be more functionally important

Molecular Evolution:
Mechanisms of sequence evolution

Molecular Evolutionary Methods:
Sequence distance calculation
Phylogenetic reconstruction
Positive selection detection
Population genetics analysis

Bioinformatics Methods:
Similarity search
Functional domain search
Protein family classification
Functional annotation
Gene prediction

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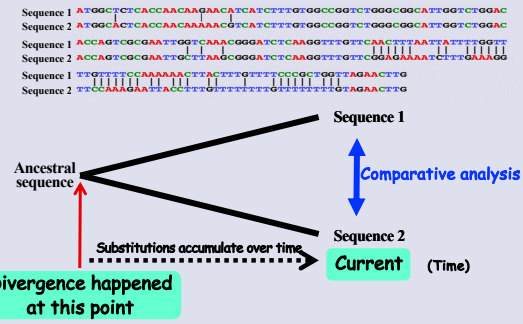
Molecular Evolution: Power of Comparison



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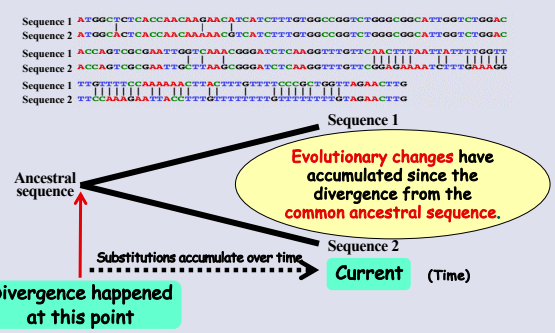
Molecular Evolution: Power of Comparison



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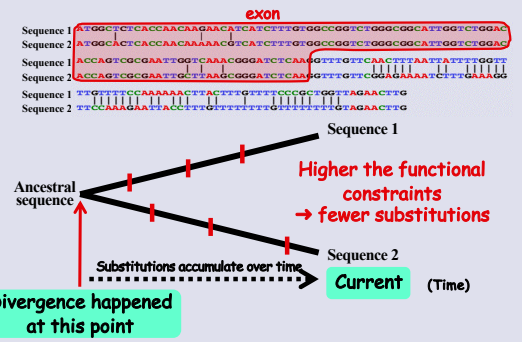
Molecular Evolution: Power of Comparison



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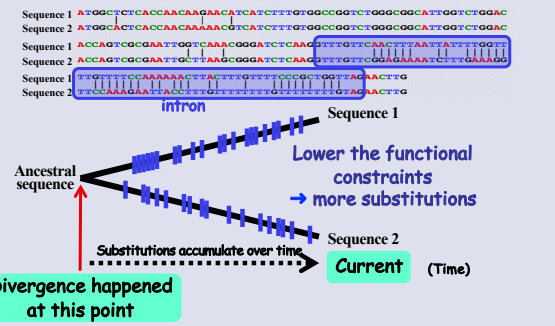
Molecular Evolution: Power of Comparison



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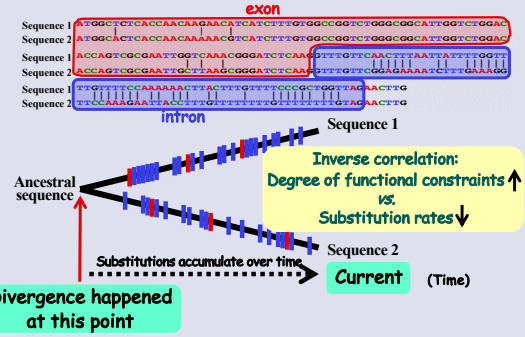
Molecular Evolution: Power of Comparison



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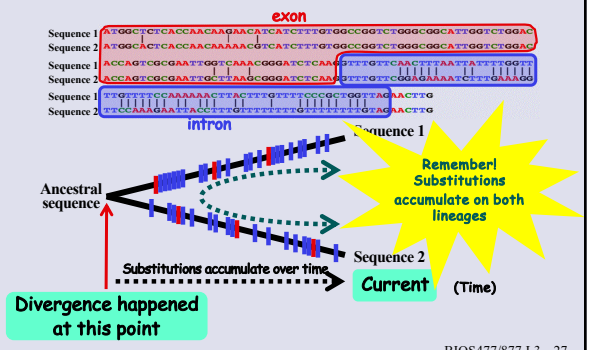
Molecular Evolution: Power of Comparison



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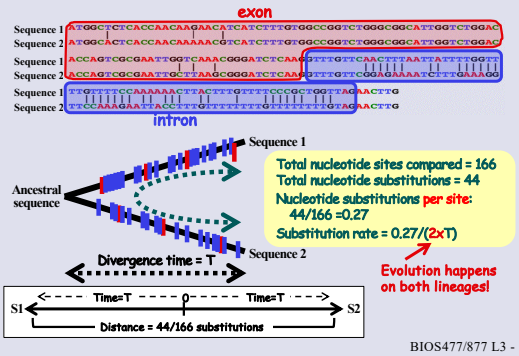
Molecular Evolution: Power of Comparison



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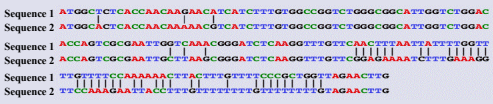
Causes of Molecular Evolution



How do nucleotide or amino acid substitutions happen?
What are substitutions?
What is molecular evolution?

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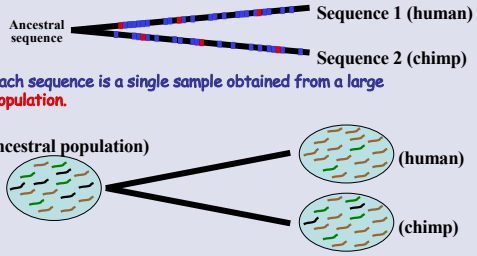
Causes of Molecular Evolution



Evolution happens at the population level.

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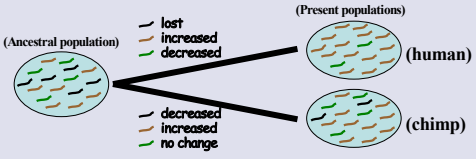
Causes of Molecular Evolution



Evolution happens at the population level.

[Definition]
Evolution: any change in the frequency of alleles within a gene pool from one generation to the next.
(population)

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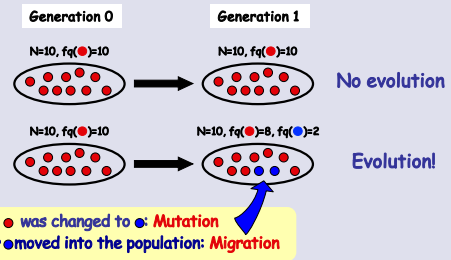
Causes of Molecular Evolution

➢ What causes changes in allele frequencies?



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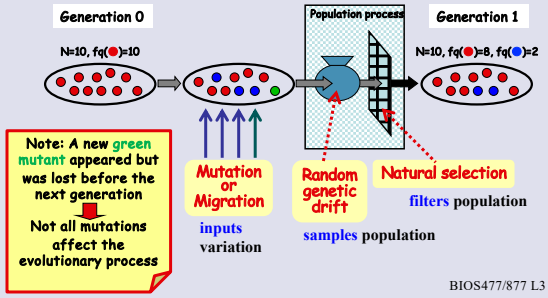
Causes of Molecular Evolution

➢ What causes changes in allele frequencies?



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Causes of Molecular Evolution

➢ Three forces that change allele frequencies

1. Mutation
   - ➜ Ultimate source of variation
   - ➜ Point mutations (*e.g.*, A -> T)
   - ➜ Recombinations 
   - ➜ Insertions and deletions (indels) 

(Migration can also introduce new variation)

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Causes of Molecular Evolution

➢ Three forces that change allele frequencies

1. Mutation
   - ➜ Mutation rates are usually very low (10^{-8}~10^{-10}/base/year)
   - ➜ Higher mutation rates are found in some genomes

     [due to weak (or no) repair systems, *etc.*]

     e.g., animal mitochondrial DNA

     some RNA viruses (*e.g.*, retroviruses) (10^{-3} ~ 10^{-2} /base/year!)

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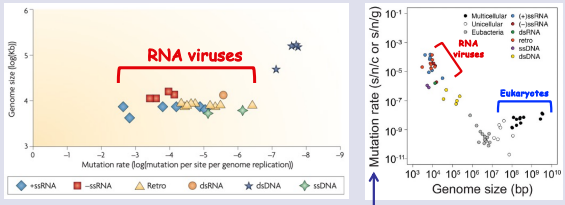
Causes of Molecular Evolution

➢ Three forces that change allele frequencies

1. Mutation



+ssRNA (SARS-CoV-2, West Nile, dengue, Zika, *etc.*)
-ssRNA (Ebola, influenza, *etc.*)
Retroviruses (HIV, *etc.*)

Duffy *et al.* (2008)

Peck & Lauring (2018)

s/n/c: substitutions per nucleotide site per cell infection
s/n/g: substitutions per nucleotide site per generation

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Causes of Molecular Evolution

➢ Three forces that change allele frequencies

2. Natural Selection

[Definition]
Differential reproduction of genetically distinct individuals or genotypes within a population

- ➜ Negative (purifying) selection
  Deleterious mutations are selected against and removed from the population quickly
- ➜ Positive (advantageous) selection
  Advantageous mutations will be fixed in the population
- ➜ Neutral mutations: free from natural selection

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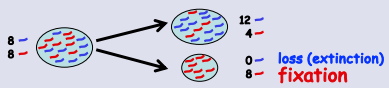
Causes of Molecular Evolution

➢ Three forces that change allele frequencies

3. Random Genetic Drift
   - ➜ Random fluctuations in allele frequencies produced by random sampling of gametes in the process of reproduction
   - ➜ Direction of the change is random
   - ➜ Its effect is larger in small populations



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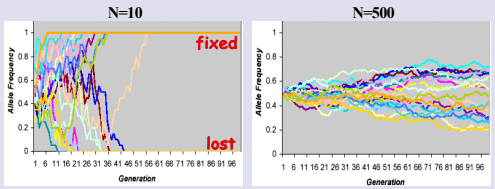
Causes of Molecular Evolution

- **Random Genetic Drift** and population size



N: population size
2 alleles: A or a (both neutral)
Fq(A) = Fq(a) = 0.5 at Generation 0

Population size affects how long neutral mutations persist

Simulation program:
POPULUS: https://cbs.umn.edu/populus

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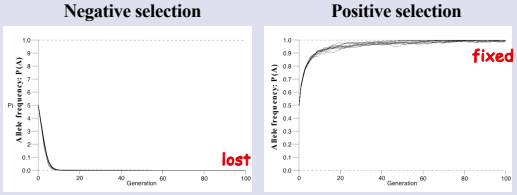
Causes of Molecular Evolution

- **Natural Selection**



Simulation program:
POPULUS: https://cbs.umn.edu/populus

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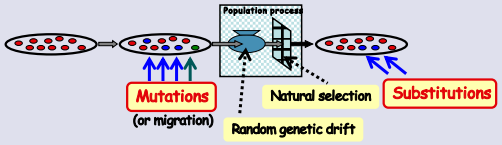
Mutation *vs.* Substitution

- **Mutations** are changes in nucleotide sequences that occur due to **errors in replication or repair.**
- **Substitutions** are mutations that have **passed through population process (drift and selection).**



Mutations ≠ Substitutions !!

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Mutation *vs.* Substitution

- **Mutations** are changes in nucleotide sequences that occur due to **errors in replication or repair.**
- **Substitutions** are mutations that have **passed through population process (drift and selection).**

Mutations ≠ Substitutions !!

- It is very difficult to directly estimate mutation rates.
- Changes observed by comparing sequences are **substitutions** (after drift and selection).
  e.g. nucleotide substitutions, amino acid substitutions

Only if no drift nor selection, substitution rate = mutation rate

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Worksheet for calculating the number of nucleotide substitutions

exon

Sequence 1 ATGGCTCTCACCAACAAGAACATCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
Sequence 2 ATGGCACTCACCAACAAAAACGTCATCTTTGTGGCCGGTCTGGGCGGCATTGGTCTGGAC
Sequence 1 ACCAGTCGCGAATTGGTCAAACGGGATCTCAAGGTTTGTTCAACTTTAATTATTTGGTT
Sequence 2 ACCAGTCGCGAATTGCTTAAGCGGGATCTCAAGGTTTGTTCGGAGAAAATCTTTGAAAGG
Sequence 1 TTGTTTTCCAAAAAACTTACTTTGTTTCCCGCTGGTTAGAACTTG
Sequence 2 TTCCAAAGAATTACCTTTGTTTTTTTTGTTTTTTTTGTAGAACTTG

intron

(This is the same alignment used in this lecture)

[exon]

Number of nucleotide sites compared within the exon = _____
Number of nucleotide substitutions within the exon = _____
Number of nucleotide substitutions per site within the exon = _____

[intron]

Number of nucleotide sites compared within the intron = _____
Number of nucleotide substitutions within the intron = _____
Number of nucleotide substitutions per site within the intron = _____

(Downloadable from Canvas)

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>Adh (partial CDS)
D. melanogaster atg tcg ttt act ttg acc aac aag aac gtg att ttc gtt gcc ggt ctg gga ggc att ggt
D. pseudoobscura --- *** *** t-- c-c --- --- --- --c g-- --- --g --- --- --- --c --- --- --c

D. melanogaster ctg gac acc agc aag gag ctg ctc aag cgc gat ctg aag aac ctg gtg atc ctc gac cgc
D. pseudoobscura --- --- --- --- cg- --- t-- g-- --- --t a-- --- --- --- --- --c --- --g --t ---

D. melanogaster att gag aac ccg gct gcc att gcc gag ctg aag gca atc aat cca aag gtg acc gtc acc
D. pseudoobscura --- --c --t --- --- --- --- --- --a --- --- --- --- --- --c --- --- --- a-- ---

D. melanogaster ttc tac ccc tat gat gtg acc gtg ccc att gcc gag acc acc aag ctg ctg aag acc atc
D. pseudoobscura --- --t --- --c --- --- --t --- --- g-c --t --- --- --- --- --c --- --- --- ---

D. melanogaster ttc gcc cag ctg aag acc gtc gat gtc ctg atc aac gga gct ggt atc ctg gac gat cac
D. pseudoobscura --t --- --- g-- --- --a a-- --- --- --- --- --- --t --- --c --- --- --- --- --t

(*: gap; exclude the gap sites from the calculation)

[1st codon position]
Number of nucleotide sites compared in the 1st codon position = _____
Number of nucleotide substitutions in the 1st codon position = _____
Number of nucleotide substitutions per site in the 1st codon position = _____

[2nd codon position]
Number of nucleotide sites in the 2nd codon position = _____
Number of nucleotide substitutions in the 2nd codon position = _____
Number of nucleotide substitutions per site in the 2nd codon position = _____

[3rd codon position]
Number of nucleotide sites in the 3rd codon position = _____
Number of nucleotide substitutions in the 3rd codon position = _____
Number of nucleotide substitutions per site in the 3rd codon position = _____

(Downloadable from Canvas)

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