codon substitution models and the analysis of natural selection pressure

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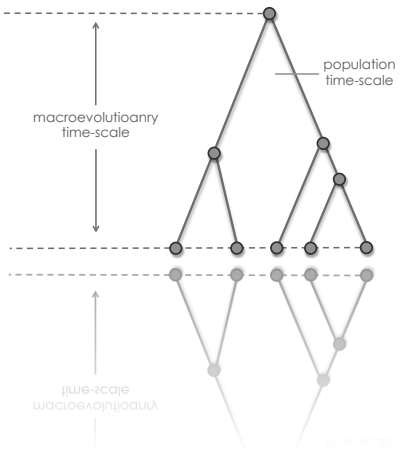
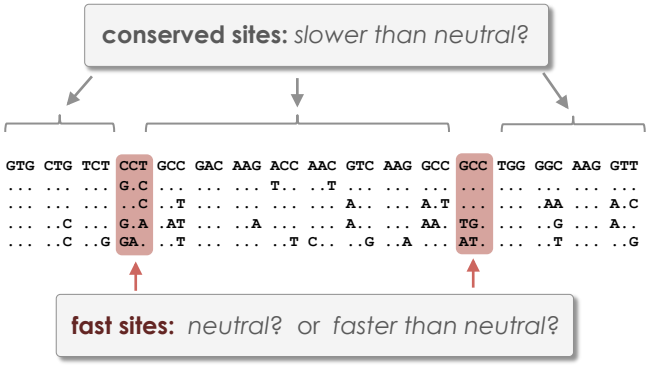
part 1: introduction



evolutionary rate depends on intensity of selection

selectively constrained = slower than neutral (drift alone)

adaptive divergence = faster than neutral (drift alone)



What is the neutral expectation?

neutral theory of molecular evolution (Kimura 1968)

the **number of new mutations** arising in a diploid population $2N\mu$

the **fixation probability** of a new mutant by drift $1/2N$

The **substitution (fixation) rate**, k $k = 2N\mu \times 1/2N$

the elegant simplicity of **neutral theory**: $k = \mu$

genetic code determines impact of a mutation

	U	C	A	G	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

http://www.langara.bc.ca/biology/mario/Assets/Geneticode.jpg

The genetic code determines how random changes to the gene brought about by the process of mutation will impact the function of the encoded protein.

Kimura (1983)

d_S: number of synonymous substitutions per synonymous site (K_S)

d_N: number of nonsynonymous substitutions per nonsynonymous site (K_A)

ω: the ratio d_N/d_S; it measures selection at the protein level

an index of selection pressure

rate ratio	mode	example
$dN/dS < 1$	purifying (negative) selection	histones
$dN/dS = 1$	neutral evolution	pseudogenes
$dN/dS > 1$	diversifying (positive) selection	MHC, Lysin

an index of selection pressure

Why use d_N and d_S?
(Why not use raw counts?)

example of counts:

300 codon gene from a pair of species
5 synonymous differences
5 nonsynonymous differences

5/5 = 1

why don't we conclude that rates are equal (i.e., **neutral evolution**)**?**

the genetic code & mutational opportunities

Relative proportion of different types of mutations in hypothetical protein coding sequence.

	Expected number of changes (proportion)			
Type	**All 3 Positions**	**1st positions**	**2nd positions**	**3rd positions**
Total mutations	549 (100)	183 (100)	183 (100)	183 (100)
Synonymous	134 (25)	8 (4)	0 (0)	126 (69)
Nonsyonymous	392 (71)	166 (91)	176 (96)	57 (27)
nonsense	23 (4)	9 (5)	7 (4)	7 (4)

Modified from Li and Graur (1991). Note that we assume a hypothetical model where all codons are used equally and that all types of point mutations are equally likely.

Why do we use d_N and d_S ?

same example, but using d_N and d_S:

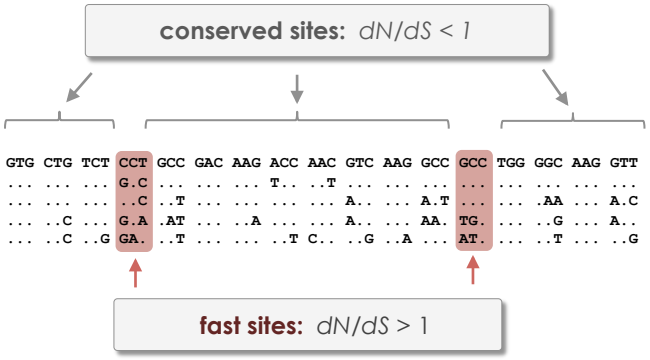
Synonymous sites = 25.5%
S = 300 × 3 × 25.5% = 229.5

Nonsynonymous sites = 74.5%
N = 300 × 3 × 74.5% = 670.5

So, d_S = 5/229.5 = 0.0218
d_N = 5/670.5 = 0.0075

d_N/d_S (ω) = 0.34, **purifying selection** !!!

an index of selection pressure acting on the protein



conclusion: *dN* differs from *dS* due to the effect of selection on the protein.

mutational opportunity vs. physical site

Relative proportion of different types of mutations in hypothetical protein coding sequence.

Type	Expected number of changes (proportion)			
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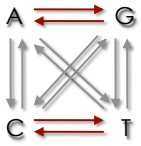
Note that by framing the counting of sites in this way we are using a "mutational opportunity" definition of the sites. Thus, a synonymous or non-synonymous site is not considered a physical entity!

Note that we assume a hypothetical model where all codons are used equally and that all types of point mutations are equally likely.

real data have biases (*Drosophila GstD1* gene)

transitions vs. **transversions**:



ts/tv = 2.71

preferred vs. **un-preferred** codons:

partial codon usage table for the *GstD* gene of *Drosophila*

Phe	F	TTT	0	Ser	S	TCT	0	Tyr	Y	TAT	1
		TTC	27			TCC	15			TAC	22
Leu	L	TTA	0			TCA	0	***	*	TAA	0
		TTG	1			TCG	1			TAG	0
Leu	L	CTT	2	Pro	P	CCT	1	His	H	CAT	0
		CTC	2			CCC	15			CAC	4
		CTA	0			CCA	3	Gln	Q	CAA	0
		CTG	29			CCG	1			CAG	14

Cys	C	TGT	0								
		TGC	6								
***	*	TGA	0								
Trp	W	TGG	8								
Arg	R	CGT	1								
		CGC	7								
		CGA	0								
		CGG	0								

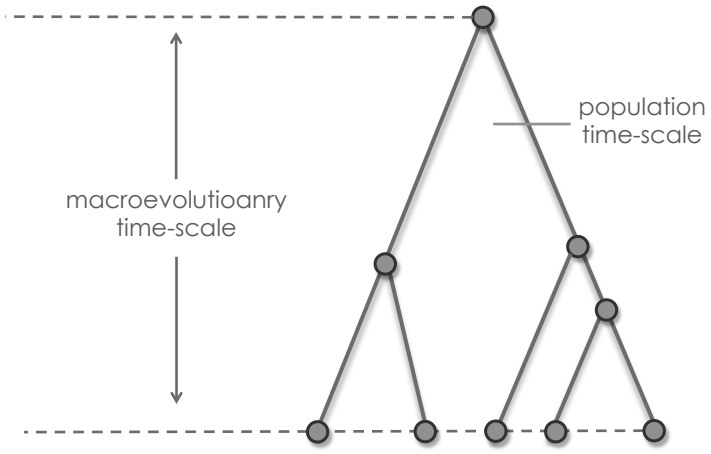
an index of selection pressure acting on the protein

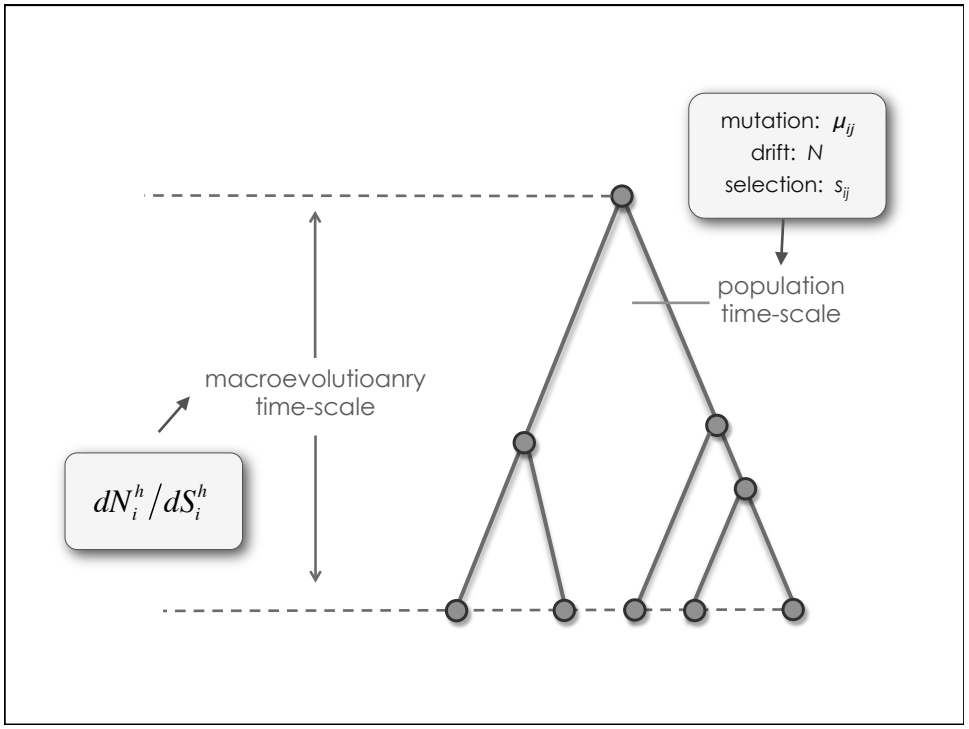
$$\omega = \frac{dN}{dS}$$

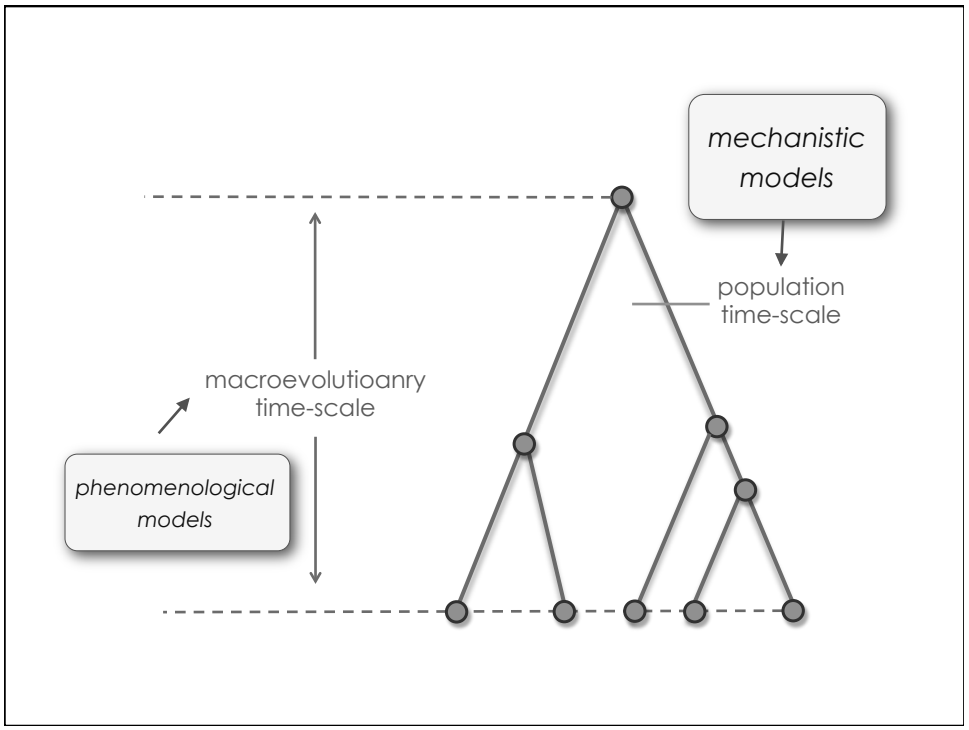
Don't worry: we will improve upon the counting method later in this lecture via likelihood!

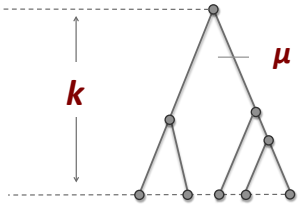
correcting *dS* and *dN* for underlying mutational process of the DNA makes them **sensitive to assumptions about the process of evolution**!

reconciling evolutionary time scales




mutation: μ_{ij}
drift: N
selection: s_{ij}
population
time-scale
macroevolutioanry
time-scale
dN_i^h / dS_i^h


mechanistic
models
population
time-scale
macroevolutioanry
time-scale
phenomenological
models



mechanistic models

- Wright-Fisher population
- drift: ***N***
- mutation: ***μ***
- selection: s_{ij}
- s_{ij} vary among sites AND amino acids
- expected dN^h/dS^h

"MUTSEL MODELS"

$$\Pr = \begin{cases} \mu_{ij} & \text{if neutral} \\ \mu_{ij} N \times \dfrac{2s_{ij}}{1-e^{-2Ns_{ij}}} & \text{if selected} \end{cases}$$

$$s_{ij} = \Delta f_{ij}$$

Halpern and Bruno (1998)

fixation probability with selection

population genetics at a single codon site (*h*)

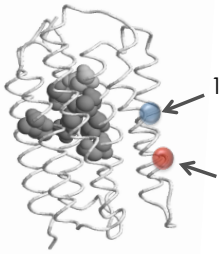
fitness coefficients $f^h = \langle f_1, \ldots, f_{61} \rangle$

selection coefficients $s_{ij}^h = f_j^h - f_i^h$

fixation probability (Kimura, 1962) $\Pr(s_{ij}^h) = \dfrac{2s_{ij}^h}{1-e^{-2Ns_{ij}^h}}$

fixation probability with selection

MutSel: selection favours amino acids with higher fitness (if *N* is large enough)

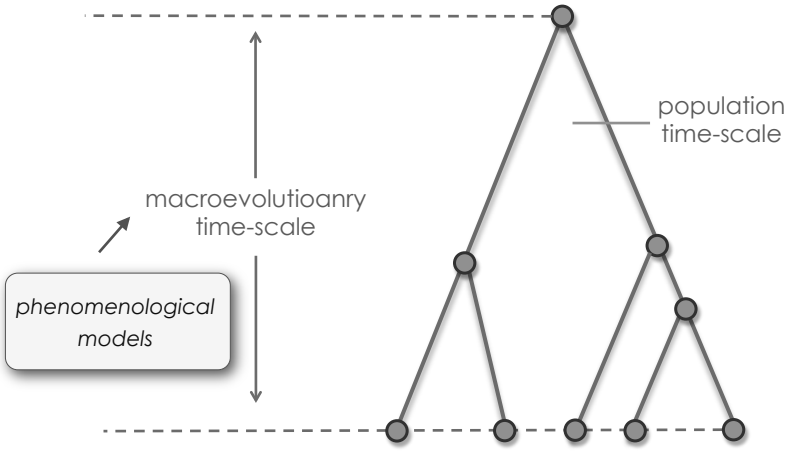


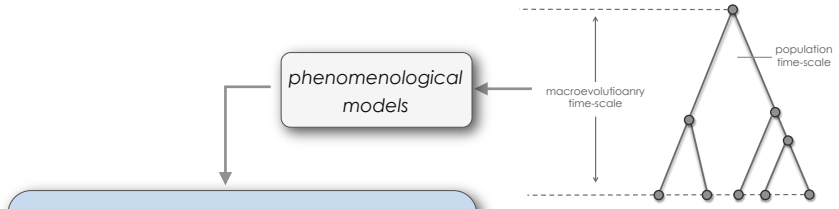
1. **A**TA (**Ile**) → **T**TA (**Leu**): $\Delta f^{h}_{Ile \to Leu}$ (**conservative**)

2. ATA (**Ile**) → A**A**A (**Lys**): $\Delta f^{h}_{Ile \to Lys}$ (**radical**)

realism: fitness expected to differ among sites and amino acids according to protein function

the cost of realism: too complex to fit such a model to real data (but simplified versions will allow new ways of data analysis)





"OMEGA MODELS"

$$q_{ij} = \begin{cases} 0 & \text{if } i \text{ and } j \text{ differ by} > 1 \\ \pi_j & \text{for synonymous tv.} \\ \kappa\pi_j & \text{for synonymous ts.} \\ \omega\pi_j & \text{for non-synonymous tv.} \\ \omega\kappa\pi_j & \text{for non-synonymous ts.} \end{cases}$$

Goldman and Yang (1994)
Muse and Gaut (1994)

- phenomenological parameters
- ts/tv ratio: κ
- codon frequencies: π_j
- $\omega = dN/dS$
- parameter estimation via ML
- *stationary process*

the instantaneous rate matrix, Q, is very big: 61 × 61

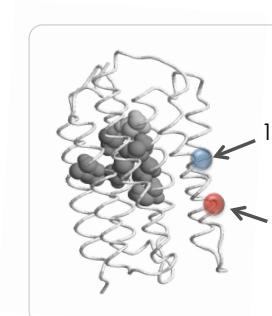
phenomenological codon models: just a few parameters are needed to cover the 3721 changes between codons!

	to codon below:							
From codon below:	TTT (Phe)	TTC (Phe)	TTA (Leu)	TTG (Leu)	CTT (Leu)	CTC (Leu)	⋯▸	GGG (Gly)
TTT (Phe)	—	$\kappa\pi_{TTC}$	$\omega\pi_{TTA}$	$\omega\pi_{TTG}$	$\omega\kappa\pi_{TTT}$	0	⋯▸	0
TTC (Phe)	$\kappa\pi_{TTT}$	—	$\omega\pi_{TTA}$	$\omega\pi_{TTG}$	0	$\omega\kappa\pi_{CTC}$	⋯▸	0
TTA (Leu)	$\omega\pi_{TTT}$	$\omega\pi_{TTC}$	—		0	0	⋯▸	0
TTG (Leu)	$\omega\pi_{TTT}$	$\omega\pi_{TTC}$	$\kappa\pi_{TTA}$	—	0	0	⋯▸	0
CTT (Leu)	$\omega\kappa\pi_{TTT}$	0	0	0	—	$\kappa\pi_{CTC}$	⋯▸	0
CTC (Leu)	0	$\omega\kappa\pi_{TTC}$	0	0	$\kappa\pi_{TTT}$	—	⋯▸	0
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋱	
GGG (Gly)	0	0	0	0	0	0	0	—

* This is equivalent to the codon model of Goldman and Yang (1994). Parameter ω is the ratio d_N/d_S, κ is the transition/transversion rate ratio, and π_i is the equilibrium frequency of the target codon (i).

substitution probability with selection

intentional simplification: all amino acid substitutions have the same ω!



1. **A**TA (**Ile**) → **T**TA (**Leu**): $\omega_{ile \to leu}$ (**conservative**)

2. ATA (**Ile**) → A**A**A (**Lys**): $\omega_{ile \to lys}$ (**radical**)

contradiction? selection should favour amino acids with higher fitness.

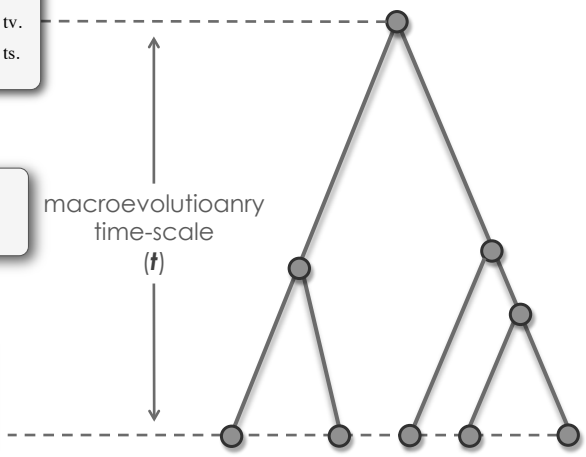
probability of substitution between codons over time, $P(t)$

$$Q_{ij} = \begin{cases} 0 & \text{if } i \text{ and } j \text{ differ by} > 1 \\ \pi_j & \text{for synonymous tv.} \\ \kappa\pi_j & \text{for synonymous ts.} \\ \omega\pi_j & \text{for non-synonymous tv.} \\ \omega\kappa\pi_j & \text{for non-synonymous ts.} \end{cases}$$

$P(t) = \{p_{ij}(t)\} = e^{Qt}$

recall that **Paul Lewis** introduced **Q matrices** and how to obtain **transition probabilities**

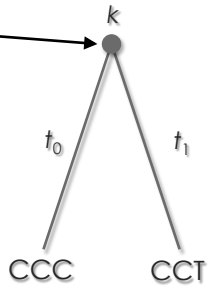
macroevolutioanry time-scale (t)



likelihood of the data at a site

$$L_h(CCC,CCT) = \sum_k \pi_k p_{kCCC}(t_0)\, p_{kCCT}(t_1)$$

the likelihood is a **sum over all possible ancestral codon states** that could have been observed at node k



recall that **Paul Lewis** described how to compute the likelihood of the data at a site for a DNA model. The only difference here is that the states are codons rather than nucleotides

note: analysis is typically done by using an unrooted tree

likelihood of the data at all sites

The likelihood of observing the entire sequence alignment is the product of the probabilities at each site.

Paul Lewis covered this with the **"AND" rule** in his likelihood lecture

$$L = L_1 \times L_2 \times L_3 \times \ldots \times L_N = \prod_{h=1}^{N} L_h$$

see **Paul Lewis's** lecture slides for more about likelihoods vs. log-likelihoods

The log likelihood is a sum over all sites.

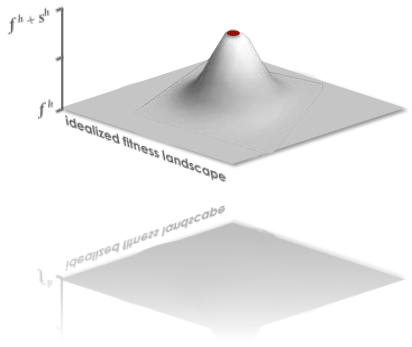
$$\ell = \ln\{L\} = \ln\{L_1\} + \ln\{L_2\} + \ln\{L_3\} + \ldots + \ln\{L_N\} = \sum_{h=1}^{N} \ln\{L_h\}$$

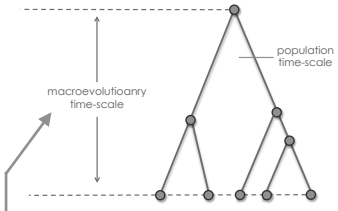
we made some progress…

1. we are now being explicit about **phenomenological and mechanistic models**
2. we are more **cautious about mechanistic interpretation** of phenomenological parameters
3. we have learned how to **connect evolutionary mechanisms to the substitution process**
4. we introduced the ***idea*** that we can **compute expectations** from mechanistic parameters

Lets look at some mechanism of evolution and "see" what we should expect!

part 2: mechanistic processes of codon evolution





mechanistic models

"MUTSEL FRAMEWORK"

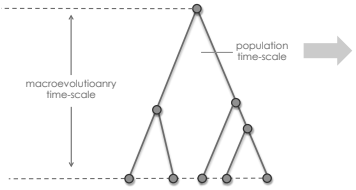
$$A_{ij}^{h} = \begin{cases} \mu_{ij} & \text{if } s_{ij}^{h} = 0 \\ \mu_{ij} N \times \dfrac{2 s_{ij}^{h}}{1 - \mathrm{e}^{-2 N s_{ij}^{h}}} & \text{otherwise} \end{cases}$$

$$s_{ij} = \Delta f_{ij}$$

Halpern and Bruno (1998)
Jones et al. (2016)

phenomenological models

site-specific MutSel rate matrix



MUTSEL RATE MATRIX

$$A_{ij}^{h} = \begin{cases} \mu_{ij} & \text{if } s_{ij}^{h} = 0 \\ \mu_{ij} N \times \dfrac{2 s_{ij}^{h}}{1 - \mathrm{e}^{-2 N s_{ij}^{h}}} & \text{otherwise} \end{cases}$$

- MutSel time-scale is infinitesimal compared to substitution scale
- MutSel probabilities approximate the instantaneous site-specific rate matrix, A
- μ_{ij} = nucleotide GTR process (before the effect of selection)

site-specific MutSel rate matrix

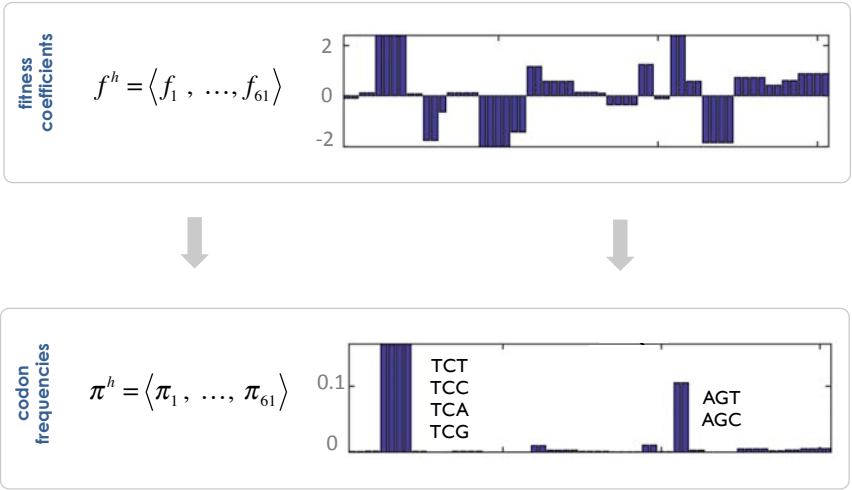
two explicit ways to reconcile **population genetics** and **macroevolution**:

1. map fitness to equilibrium frequencies

2. macroevolution index of selection intensity

(1) Sella and Hirsh 2005; (2) Jones et al. 2016

1. fitness coefficients map to stationary codon frequencies



2. from fitness coefficients to *dN*/*dS*

MUTSEL RATE MATRIX

$$dN^h / dS^h = \frac{E[\text{evolution w/ selection}]}{E[\text{evolution by drift alone}]}$$

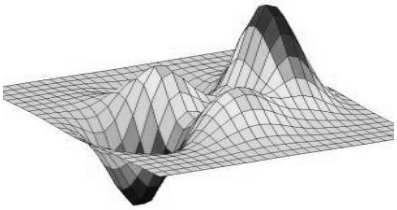
$$dN^h / dS^h = \frac{\sum_{i \neq j} \pi_i^h A_{ij}^h I_N}{\sum_{i \neq j} \pi_i^h \mu_{ij} I_N}$$

- *dN*/*dS* = ω when matrix A^h is replaced by matrix Q of model M0
- *dN*/*dS* is an analog of ω under MutSel

1932: adaptive landscapes and "shifting balance"

Sewall Wright

- introduces "ADAPTIVE LANDSCAPE" as a metaphor



- introduces "SHIFTING BALANCE" as a model

(SBT **more complex** than I will present)

positive selection: 3 evolutionary scenarios

1. frequency dependent selection
2. episodic adaptation

dynamic fitness landscape

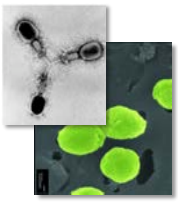
3. non-adaptive shifting balance

static fitness landscape

scenario 1: frequency dependent selection

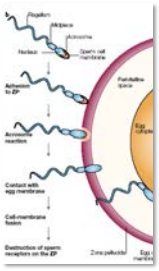
1. antagonistic evolutionary interaction

host-pathogen

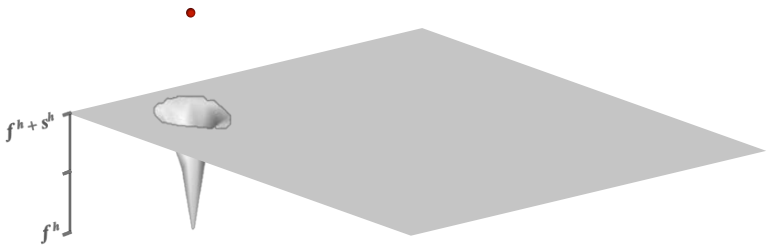


molecular-interactions

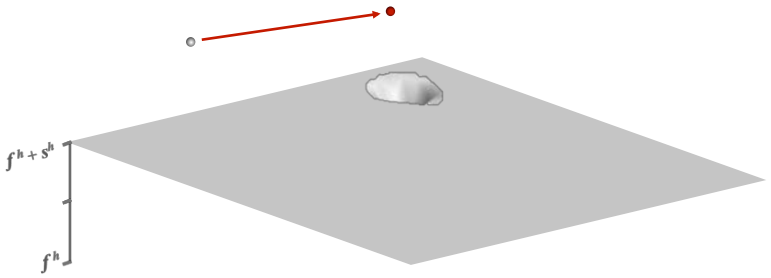
sexual-conflict



frequency-dependent adaptive landscape (weird)

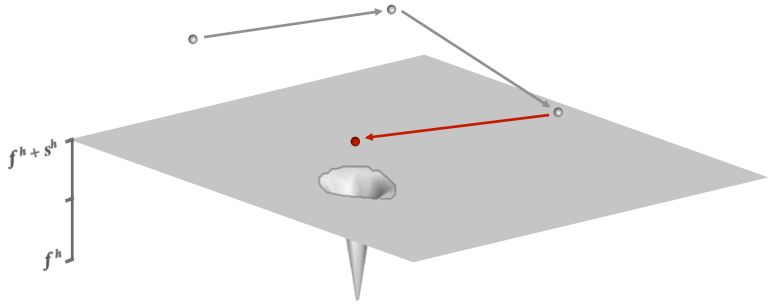


frequency-dependent adaptive landscape



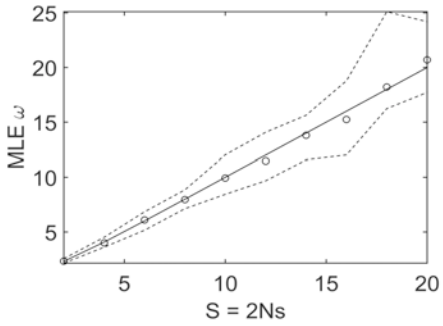
frequency-dependent selection: MutSelM0

1. amino acid at a site has f^h; all others have $f^h + s$
2. fitness values swap when a substitution occurs



MutSelM0: (1) and (2) above imply Markov chain properties with the same rate matrix Q as **codon model M0**

frequency-dependent selection: MutSelM0



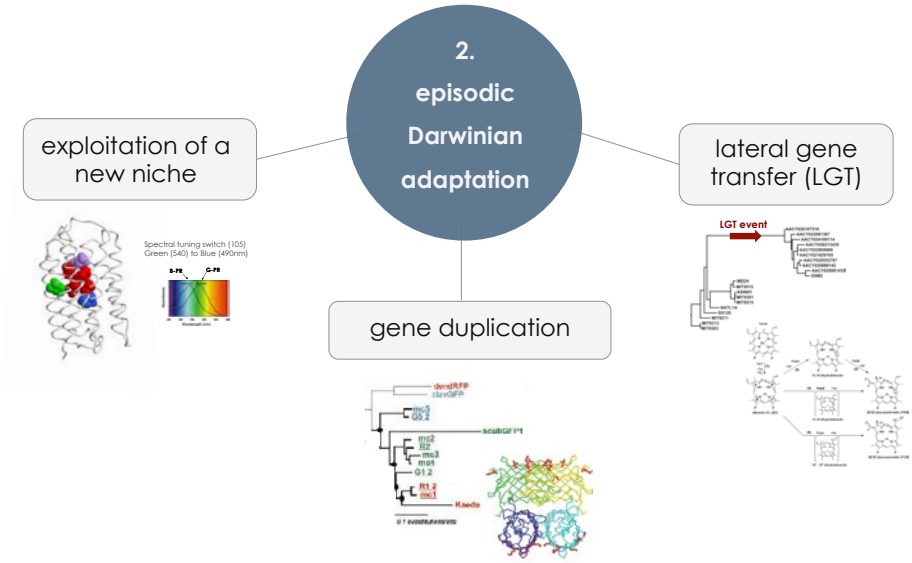
generating process:
MutSelM0
expectation = dN^h/dS^h
symbol = ——

fitted model:
model M0
inference = MLE ω
symbol = O

conclusion: phenomemologcial codon models assume frequency-dependent selection

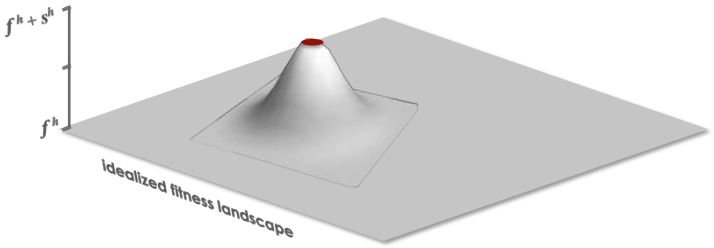
[dos Reis (2015); Jones et al. (2016)]

scenario 2: adaptive peak shift



adaptive peak shift: evolution of novel function

optimal function in a stable environment



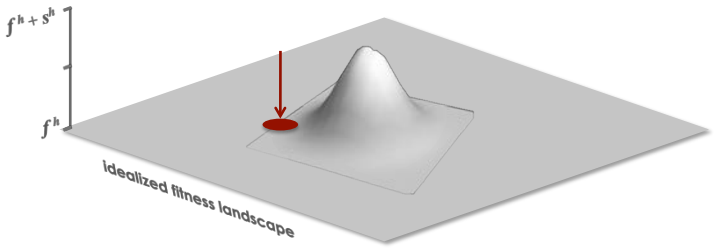
population: at fitness peak

fitness peak: stationary

FFTNS: keeps population at peak

adaptive peak shift: evolution of novel function

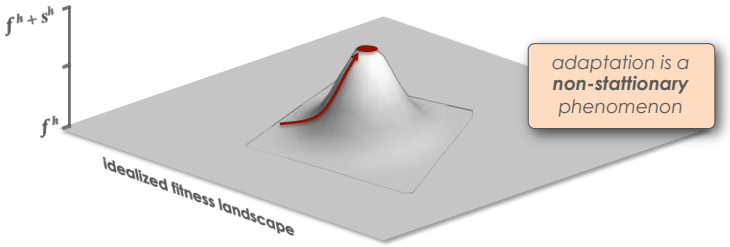
sub-optimal function in a novel environment



population: lower fitness
fitness peak: moving
FFTNS: increase population mean fitness
(non-stationary process)

adaptive peak shift: evolution of novel function

episodic adaptive evolution of a novel function



population: returns to peak
fitness peak: stabilized
FFTNS: increases population mean
fitness until at peak

adaptive peak shift: MutSelES model

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Research

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Molecular evolution

How to calculate the non-synonymous to synonymous rate ratio of protein-coding genes under the Fisher–Wright mutation–selection framework

Mario dos Reis

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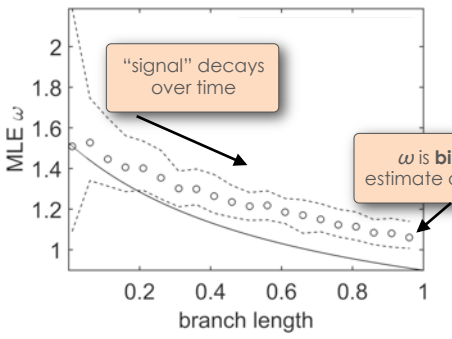
First principles of population genetics are used to obtain formulae relating the non-synonymous to synonymous substitution rate ratio to the selection coefficients acting at codon sites in protein-coding genes. Two theoretical cases are discussed and two examples from real data (a chloroplast gene and a virus polymerase) are given. The formulae give much insight into the dynamics of non-synonymous substitutions and may inform the development of methods to detect adaptive evolution.

4. The non-synonymous rate during adaptive evolution

adaptive peak shift: MutSelES



generating process:
MutSelES
expectation = dN^h/dS^h
symbol = ——

fitted model:
model M0
inference = MLE ω
symbol = o

conclusion : episodic models "work" because w>1 is a consequence of a system moving towards a new fitness peak.

conclusion : episodic models "work" because they are sensitive to non-stationary behavior

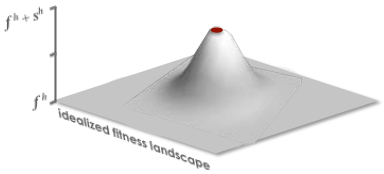
[dos Reis (2015); Jones et al. (2016)]

Scenario 3: non-adaptive evolution

3. fitness coefficients are constant (fixed-peak)

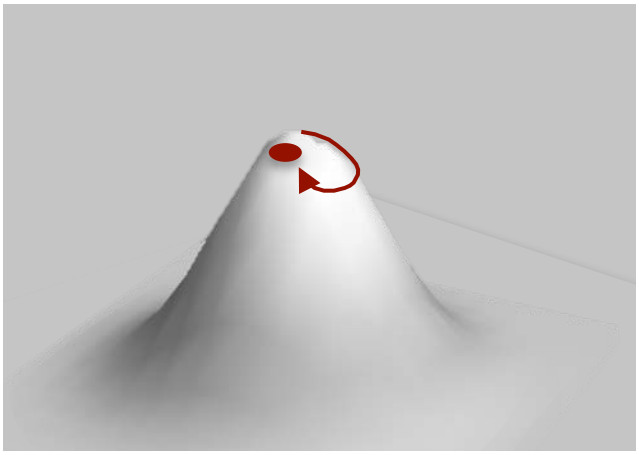
Spielman and Wilke (2015)

- *dN*/*dS* must be ≤1 when fitness coefficients are fixed.
- positive selection is not possible on a stationary fitness peak



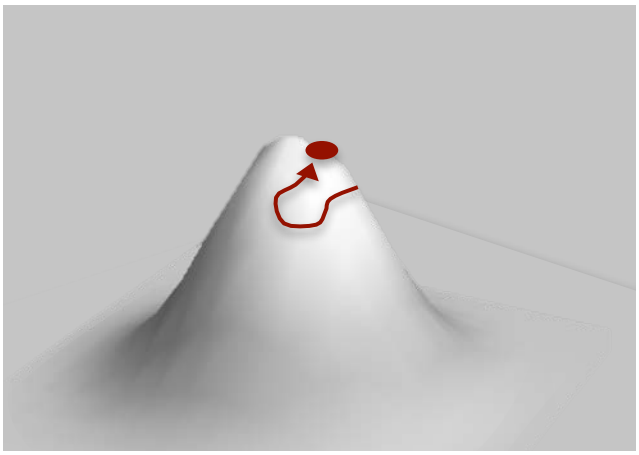
[Spielman and Wilke, (2015); Jones at al., (2016)]

shifting balance: movement around peak



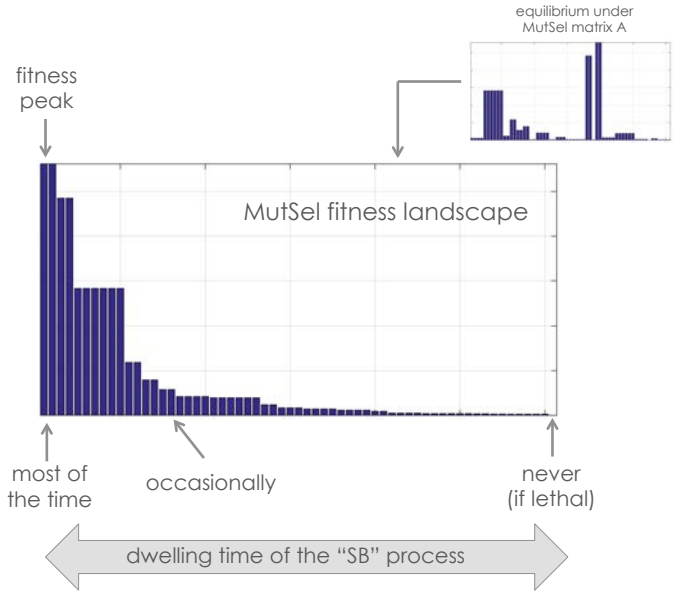
mutation and **drift** can move a pop. off a fitness peak

shifting balance: movement around peak

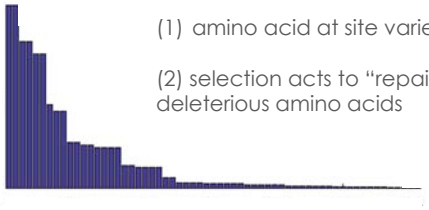


mutation and **drift** can move a pop. off a fitness peak

shifting balance: the MutSel landscape (Jones at el. 2016)



shifting balance: positive selection on a MutSel landscape



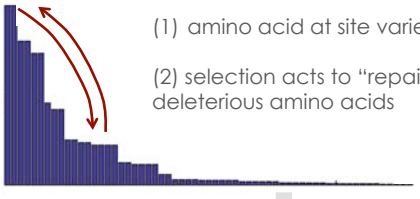
(1) amino acid at site varies over time

(2) selection acts to "repair" shifts to deleterious amino acids

EXPECTED PROPORTION OF MUTATIONS FIXED BY SELECTION

$$p_+^h = \frac{\sum_{(i,j)} \pi_i^h \left(A_{ij}^h - \mu_i \right) I_+}{\sum_{i \neq j} \pi_i^h A_{ij}^h}$$

conclusion: $p_+ > 0$ as long as number of viable amino acids > 1 at a site



(1) amino acid at site varies over time

(2) selection acts to "repair" shifts to deleterious amino acids

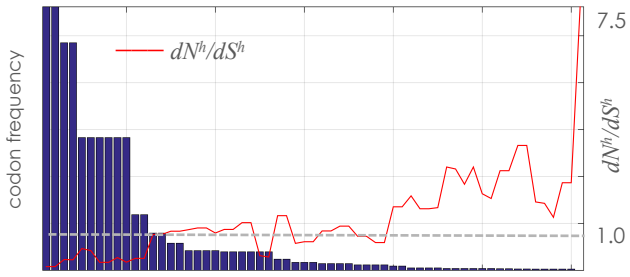
key result:

purifying selection: $p_+ = p_-$

(static landscape)

shifting balance: the MutSel landscape

dN^h/dS^h depends on the current amino acid

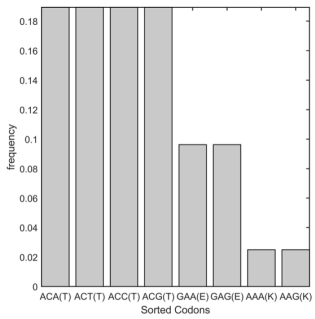


temporal average dN^h/dS^h = 0.61

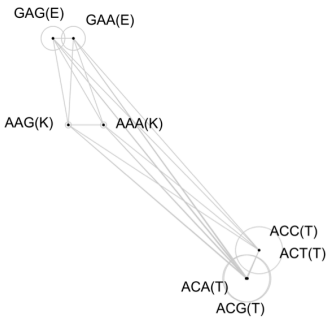
conclusion: positive selection operates on a stationary fitness peak in the same way as when there is an adaptive peak shift

landscapes have unique structures

MutSel landscape



McCandlish landscape

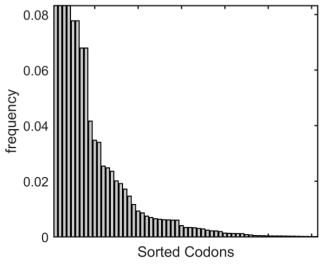


conclusion: A population can get to a sub-optimal codon (E) by drift and reside there for some time (b/c moving between T and E requires changes ≥ 2 codons).

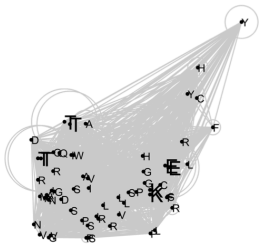
landscape structure depends on N

same site... 10x decrease in *N* (f^h have not changed!)

MutSel landscape



McCandlish landscape

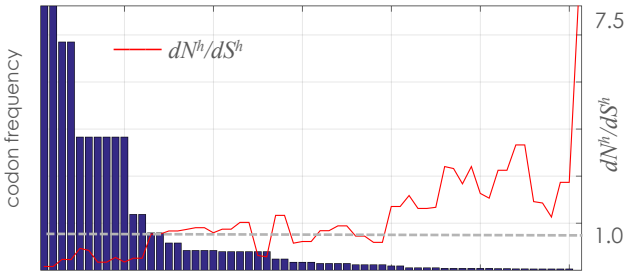


conclusion: decreasing N changes:

i. the "space" for shifting balance
ii. mean dN/dS
iii. equilibrium frequencies

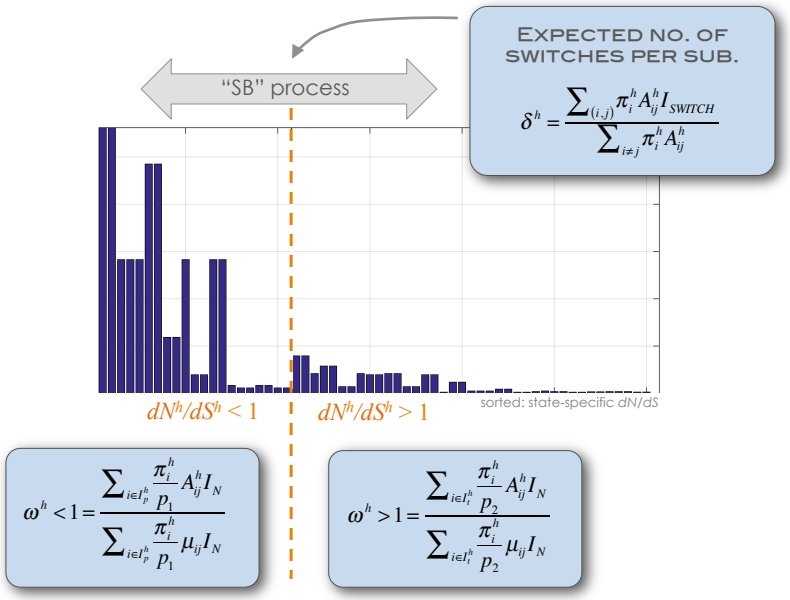
shifting balance: the MutSel landscape

dN^h/dS^h depends on the current amino acid



temporal average dN^h/dS^h = 0.61

shifting balance: a mechanistic model



shifting balance: a mechanistic model

	shifting balance over landscape: high	moderate	low
median switching rate (δ)	0.45	0.25	<0.01
expected probability of a site being in the "tail" of the landscape ($p_{w>1}$)	high (>20%)	moderate (1%-25%)	very low (<0.1%)
Expected ***dN/dS*** in the "tail" of the landscape	~ 1.1	1-3	>>1
Expected ***dN/dS*** near the "peak" of the landscape	~ 0.95	<0.4	<0.01
rate of evolution (i.e., "type of site")	**"fast"**	**"informative"**	**"conserved"**

landscapes:
250 f^h
σ: {0.0001, 0.001, 0.01}
N = 1000

Let's look at a **site pattern distribution** for real data

```
human
cow
rabbit
rat
opossum

GTG CTG TCT CCT GCC GAC AAG ACC AAC GTC AAG GCC GCC TGG GGC AAG GTT GGC GCG CAC
... ... ... G.C ... ... ... T.. ..T ... ... ... ... ... ... ... ... ... .GC A..
... ... ... ..C ..T ... ... ... ... A.. ... A.T ... ... ... .AA ... A.C ... AGC ...
... ..C ... G.A .AT ... ..A ... ... A.. ... AA. TG. ... ..G ... A.. ..T .GC ..T
... ..C ..G GA. ..T ... ... ..T C.. ..G ..A ... AT. ... ..T ... ..G ..A .GC ...

GCT GGC GAG TAT GGT GCG GAG GCC CTG GAG AGG ATG TTC CTG TCC TTC CCC ACC ACC AAG
... ..A .CT ... ..C ..A ... ..T ... ... ... ... ... ... AG. ... ... ... ... ...
.G. ... ... ... ..C ..C ... ... G.. ... ... ... ... T.. GG. ... ... ... ... ...
.G. ..T ..A ... ..C .A. ... ... ..A C.. ... ... ... GCT G.. ... ... ... ... ...
..C ..T .CC ..C .CA ..T ..A ..T ..T .CC ..A .CC ... ..C ... ... ... ..T ... ..A

ACC TAC TTC CCG CAC TTC GAC CTG AGC CAC GGC TCT GCC CAG GTT AAG GGC CAC GGC AAG
... ... ... ..C ... ... ... ... ... ... ..G ... ... ..C ... ... ... ... G..
... ... ... ..C ... ... ... T.C .C. ... ... ... .AG ... A.C ..A .C. ... ... ...
... ... ... T.T ... A.T ..T G.A ... .C. ... ... ... ... ..C ... .CT ... ... ...
..T ... ... ..C ... ... ... ... TC. .C. ... ..C ... ... A.C C.. ..T ..T ..T ...
```

covarion-like model of evolution

$$Q = \left(\begin{array}{c|c} \textbf{evolutionary regime 1}: \omega_1 = \text{low} \quad (\text{“near the peak”}) & \textbf{switching process:} \quad \omega_1 \rightarrow \omega_2 \\ \hline \textbf{switching process:} \quad \omega_1 \leftarrow \omega_2 & \textbf{evolutionary regime 2}: \omega_2 = \text{high} \quad (\text{“in the tail”}) \end{array} \right)$$

[Guindon et al., (2004); Jones et al. (2016); Jones et al. (2018); Jones et al. (*in review*)]

covarion-like model of evolution
(phenomenological)

2 selective regimes (low & high): sites CAN switch regime

LOW (ω_1) HIGH (ω_2)

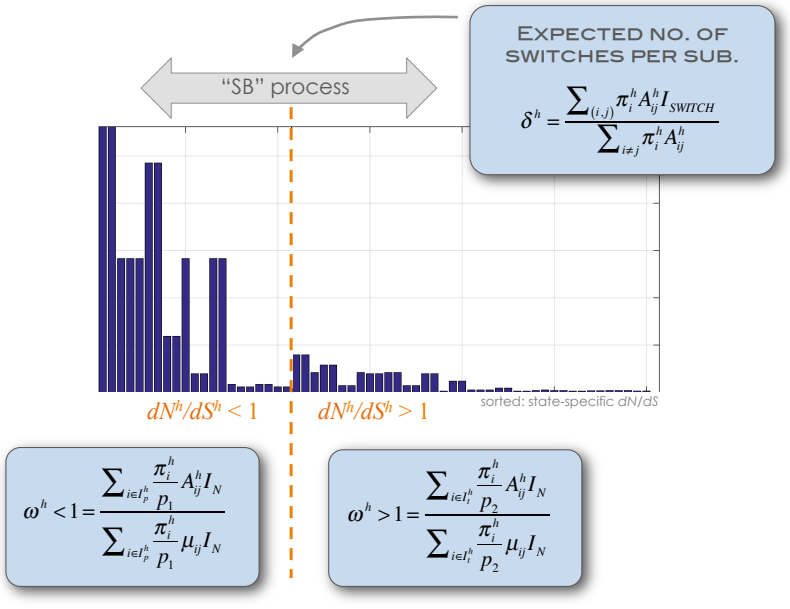
p_1: proportion of time sites are in ω_1

p_2: proportion of time sites are in ω_2

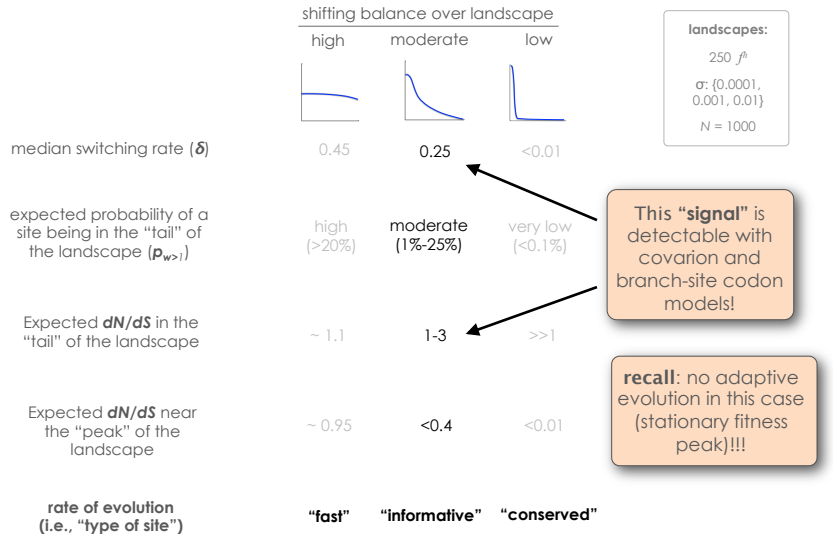
switching: δ

the covarion-like codon model can be **fit to real data**

shifting balance: a mechanistic model



shifting balance: a mechanistic model



hopefully you now have **more intuition about process** that generates your sample of real data

```
human
cow
rabbit
rat
opossum

GTG CTG TCT CCT GCC GAC AAG ACC AAC GTC AAG GCC GCC TGG GGC AAG GTT GGC GCG CAC
... ... ... G.C ... ... ... T.. ..T ... ... ... ... ... ... ... ... ... .GC A..
... ... ... ..C ..T ... ... ... ... A.. ... A.T ... ... .AA ... A.C ... AGC ...
... ..C ... G.A .AT ... ..A ... ... A.. ... AA. TG. ... ..G ... A.. ..T .GC ..T
... ..C ..G GA. ..T ... ... ..T C.. ..G ..A ... AT. ... ..T ... ..G ..A .GC ...

GCT GGC GAG TAT GGT GCG GAG GCC CTG GAG AGG ATG TTC CTG TCC TTC CCC ACC ACC AAG
... ..A .CT ... ..C ..A ... ..T ... ... ... ... ... ... AG. ... ... ... ... ...
.G. ... ... ... ..C ..C ... ... G.. ... ... ... ... T.. GG. ... ... ... ... ...
.G. ..T ..A ... ..C .A. ... ... ..A C.. ... ... ... GCT G.. ... ... ... ... ...
..C ..T .CC ..C .CA ..T ..A ..T ..T .CC ..A .CC ... ..C ... ... ... ..T ... ..A

ACC TAC TTC CCG CAC TTC GAC CTG AGC CAC GGC TCT GCC CAG GTT AAG GGC CAC GGC AAG
... ... ... ..C ... ... ... ... ... ... ..G ... ... ..C ... ... ... ... G..
... ... ... ..C ... ... ... T.C .C. ... ... ... .AG ... A.C ..A .C. ... ... ...
... ... ... T.T ... A.T ..T G.A ... .C. ... ... ... ... ..C ... .CT ... ... ...
..T ... ... ..C ... ... ... ... TC. .C. ... ..C ... ... A.C C.. ..T ..T ..T ...
```