

# Learning to Count: Tests for Evolutionary Innovation and Robust Sequence Distance Estimation

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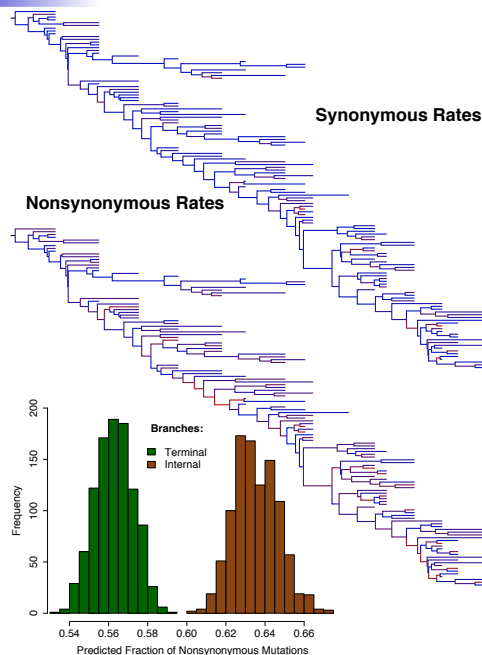
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## Classic Problem: Detecting Adaptation in Intra-host HIV Evolution



- **Data:** 129 HIV variants from one patient
- **Question:** Does adaptation occur along the backbone of evolution? (Suggests violations of neutrality)
- **Difficulty:** Branch/time-specific synonymous/non-synonymous rate models are too unwieldy
- **Solution?:** Count the expected # of labeled transitions

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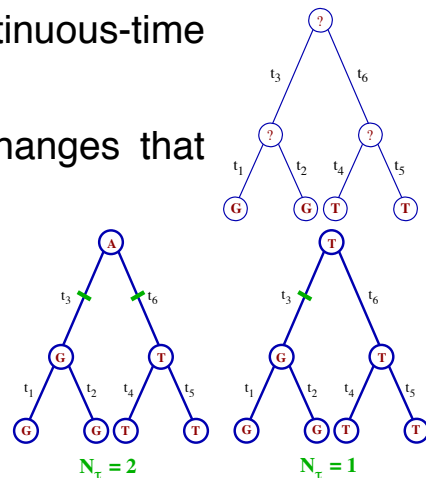
# Evolutionary Counting Processes: Current Approaches

**Model** trait evolution as a continuous-time Markov chain  $\Lambda$ , and

**Infer** the number  $N_\tau$  of state-changes that occur along the tree  $\tau$  via

**Stochastic Mapping** (Nielsen, 2002):

- Simulation-based
- Uses **rejection sampling**



**Can do one better?** Analytic solutions for  $\Pr(N_\tau = n | \mathbf{Y})$  and  $E(N_\tau^k | \mathbf{Y})$  enable (computationally) efficient statistical tests.

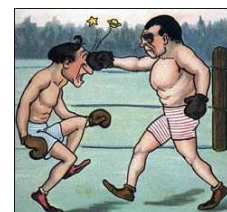
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## Punch-Line: Simulation Methods ... Could Be Better

**Computational efficiency / accuracy** comparison:

Simulants	Slow Evolving Site $N_\tau \approx 4$		Fast Evolving Site $N_\tau \approx 15$	
	Rejections/Simulant	Error	Rejections/Simulant	Error
100	100	0.0598	38845	0.4624
500	105	0.0255	39247	0.3319
1000	102	0.0259	42075	0.2905
10000	106	0.0205	40805	0.2809

- 61-state Markov chain (codon model) on 129-tip tree (HIV evolution)
- Counting labeled subsets of changes (synonymous/non-synonymous) on “internal” vs. “external” branches



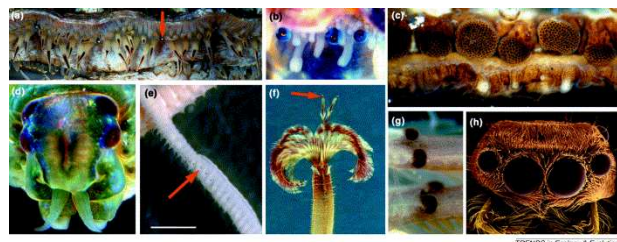
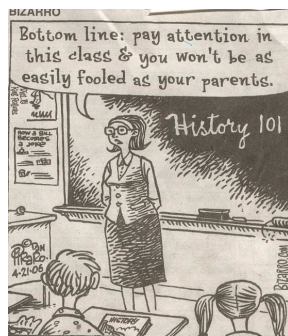
**1 min vs. 10 hrs**

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# Those Who Forget Their History ...

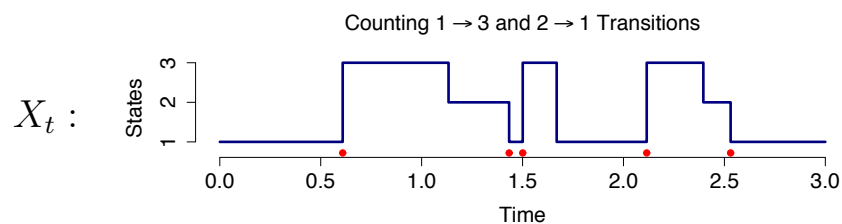
## Three Major Approaches:

- Examine process at stationarity/no conditioning on start/end points: **Ball**, **Neuts** (ion channel physics)
- Label only one specific transition: **Guttorp**, **Bruno**, **Hobolth**
- Via uniformization: **Siepel**



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# General Framework: Labeled Changes



- $R$  is a set of ordered index pairs that **label transitions**
- $\Lambda$  - generator,  $\Lambda_R = \{\lambda_{ij} \times 1_{\{(i,j) \in R\}}\}$ , and  $\Lambda_{\bar{R}} = \Lambda - \Lambda_R$



- Matrix  $Q(n, t)$  of probabilities ( $N_t = n, X_t = j \mid X_0 = i$ )
- Matrix  $M^{[k]}(t)$  of restricted, factorial moments ( $N_t^{[k]} 1_{\{X_t=j\}} \mid X_0 = i$ )

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## Derivation Sketch: A Moment's Reflection on One Branch

Start with **Kolmogorov's Forward** equation:

$$\frac{d}{dt} \mathbf{Q}(n, t) = \mathbf{Q}(n, t) \Lambda_{\bar{R}} + \mathbf{Q}(n-1, t) \Lambda_R$$

and the **matrix probability generating function**:

$$\mathbf{G}(r, t) = \sum_{n=0}^{\infty} r^n \mathbf{Q}(n, t)$$

Then  $\frac{\partial}{\partial t} \mathbf{G}(r, t) = \mathbf{G}(r, t) (\Lambda_{\bar{R}} + r \Lambda_R) \Rightarrow \mathbf{G}(r, t) = e^{(\Lambda_{\bar{R}} + r \Lambda_R)t}$  and  $\mathbf{M}^{[k]}(t) = \frac{\partial^k}{\partial r^k} \mathbf{G}(r, t)|_{r=1}$  - hard unless  $\Lambda$  and  $\Lambda_R$  commute!

Use **integration** instead  $\mathbf{M}^{[k]}(t) = k \int_0^t \mathbf{M}^{[k-1]}(t) \Lambda_R e^{\Lambda(t-\theta)} d\theta$

$$\mathbf{M}^{[1]}(t) = \sum_{i,j} \mathbf{B}_i \Lambda_R \mathbf{B}_j I_{ij}(t), \quad I_{ij}(t) = \begin{cases} t e^{d_i t} & \text{if } d_i = d_j, \\ \frac{e^{d_i t} - e^{d_j t}}{d_i - d_j} & \text{if } d_i \neq d_j. \end{cases}$$

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## Reaping the Rewards of Counting over Trees

Let  $\mathbf{H} = \sum_b h(X_t^{(b)})$  be an additive **summary**, where  $h(\cdot)$  **counts/rewards** (on possibly select branches), e.g.,

- Transitions
- Final states
- Dwell times
- And others ...



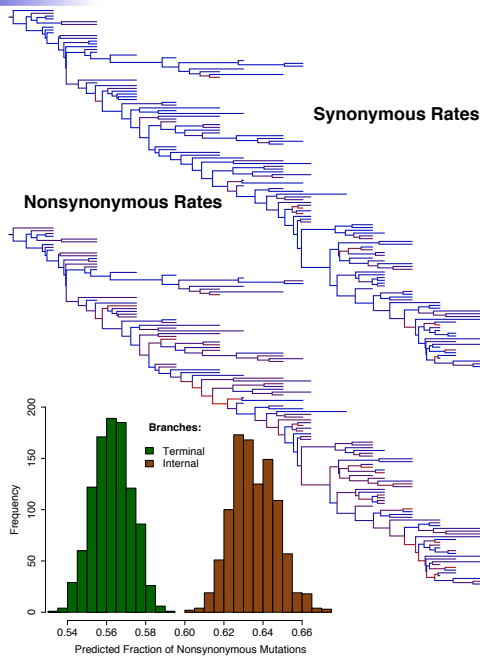
Then  $E(\mathbf{H})$  also has an analytic solution  $\Rightarrow$  no simulation of internal-node states or conditioning on ancestral reconstruction.



**Noteworthy:** Integrating  $\mathbf{H}$  **generalizes** Felsenstein's Pruning Algorithm, the work-horse of modern phylogenetics.

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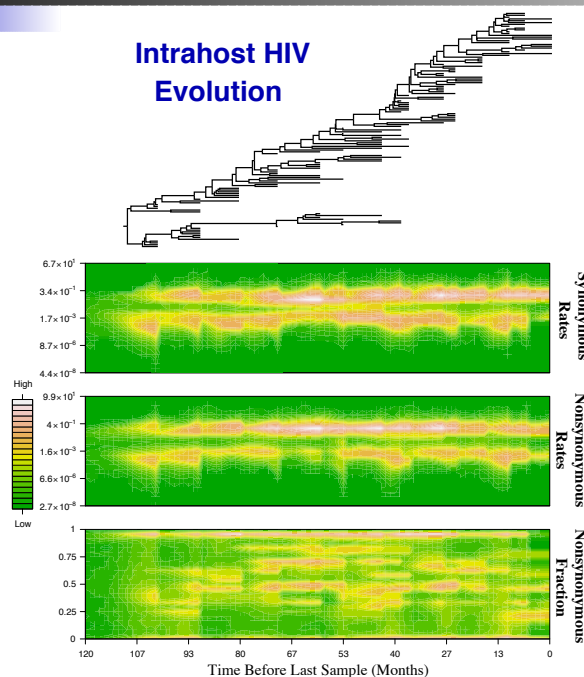
# Detecting Adaptation in Intra-host HIV Evolution



- **Data:** 129 HIV variants from one patient
- **Question:** Does adaptation occur along the backbone of evolution? (Suggests violations of neutrality)
- **Difficulty:** Branch-specific synonymous/non-synonymous rate change models are too unwieldy
- **Key:** Requires posterior simulation from only a **simple**, homogeneous rate model

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# Temporal Rate Variation in Intra-host HIV Evolution



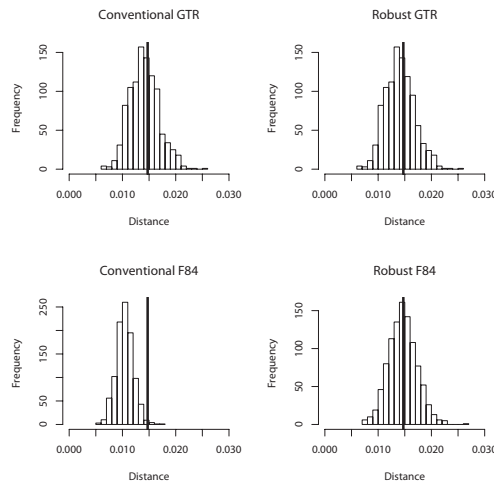
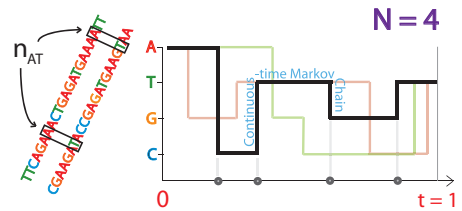
- Branch-specific counts enable rate projection onto **real time**
- **Bimodality** of both synonymous and non-synonymous distributions
- Early adaptation, followed by weakening selection

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# Pair-wise Robust Distance Estimation

Pairwise distance =  $E(N)$  w.r.t.

- **Stationary** ( $\pi_i \times p_{ij}$ ) distribution, vs.
- **Empirical** ( $f_{ij}$ ) distribution (**robust**) further straight-forward to **label**



Nucleotide simulation:

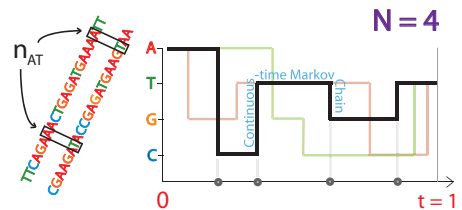
- True model = GTR
- Robust estimator using F84 (analytic calculations) performs as well as estimators under GTR

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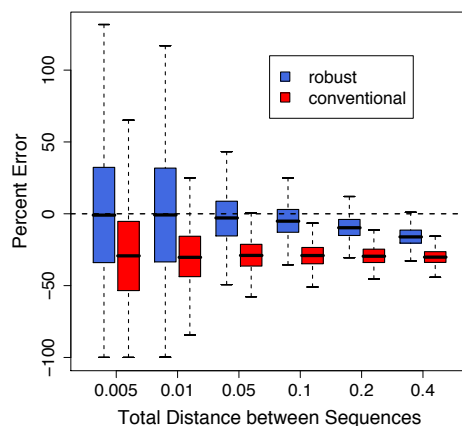
# Pair-wise Robust Distance Estimation - II

Pairwise distance =  $E(N)$  w.r.t.

- **Stationary** ( $\pi_i \times p_{ij}$ ) distribution, vs.
- **Empirical** ( $f_{ij}$ ) distribution (**robust**) further straight-forward to **label**



Robust vs Conventional Distances



Nucleotide simulation:

- Vary “true” sequence distance from 0.005 to 0.4
- Robust inference **decreases bias** caused by model misspecification

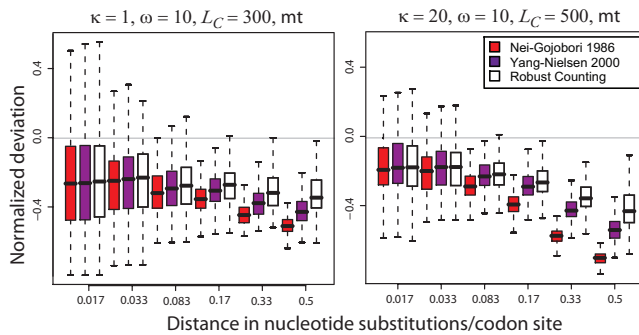
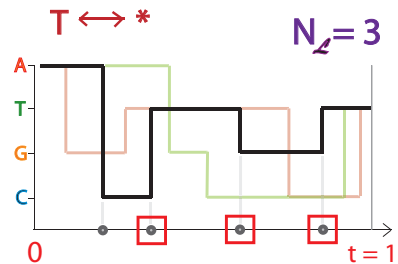
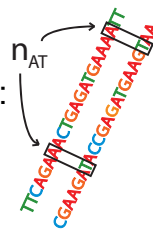
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# Robust Labeled Distance Estimation

Labeled distance =  $E(N_L)$

Go **robust** with a silly codon model:

- Composition  $3 \times F84s$
- **No numerical optimization**



**Synonymous** distance estimation outperforms:

- Nei and Gojobori (1986)
- Yang and Nielsen (2000) - specially tailored estimator

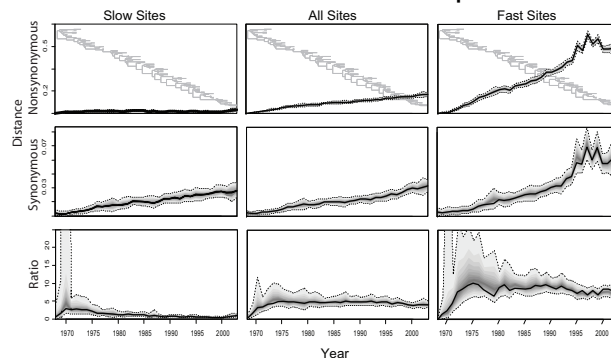
“Neutral” reconstruction possible?

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# Arbitrarily Sophisticated Distances: Codon Volatility Change in Influenza A H3N2

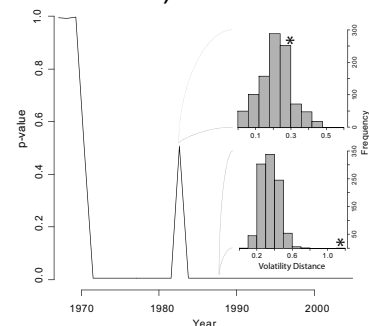
**Hypothesis:** Codon volatility correlates with selective pressures (Plotkin and Dushoff, 2003)

S/N distances for 96 HA sequences:



**Question:** Do volatility changes differ in the antibody interaction sites (consistent with the volatility hypothesis)?

Distribution (epitope vs. elsewhere):

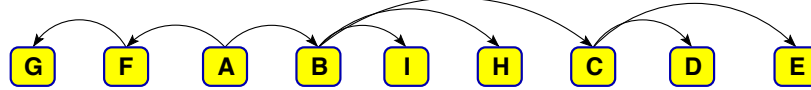


Antigenic shift in 1982

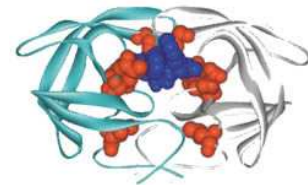
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# Handling Convergent Evolution in HIV

## Transmission Network



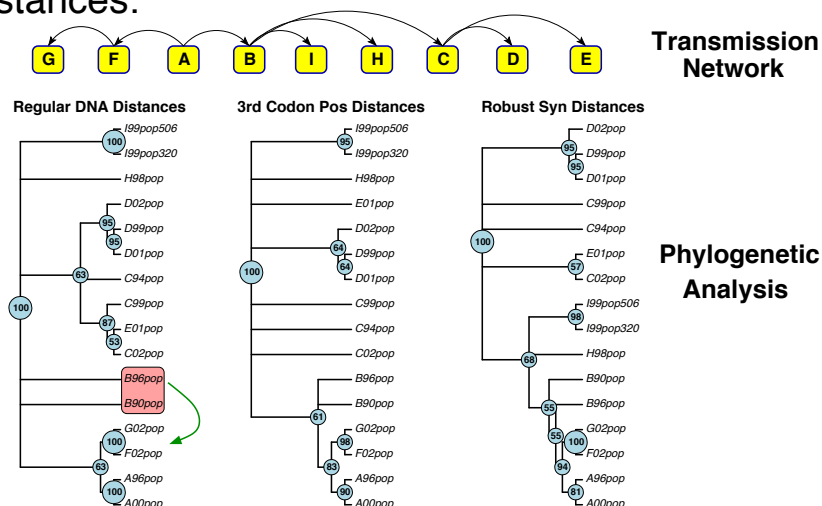
- Lemey et al. (2005) examine the genetic signature of a known HIV transmission network in the face of **convergent evolution**
- HIV *pol* and *env* sequences from 9 subjects
- Distance-based reconstructions using NG86 measures
- **Trouble:** *pol* phylogenies **conflict** with network



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# HIV Convergent Evolution-II

- Compare **“synonymous tree”** estimates using 3rd codon positions (**throw away data**) vs our robust synonymous distances:

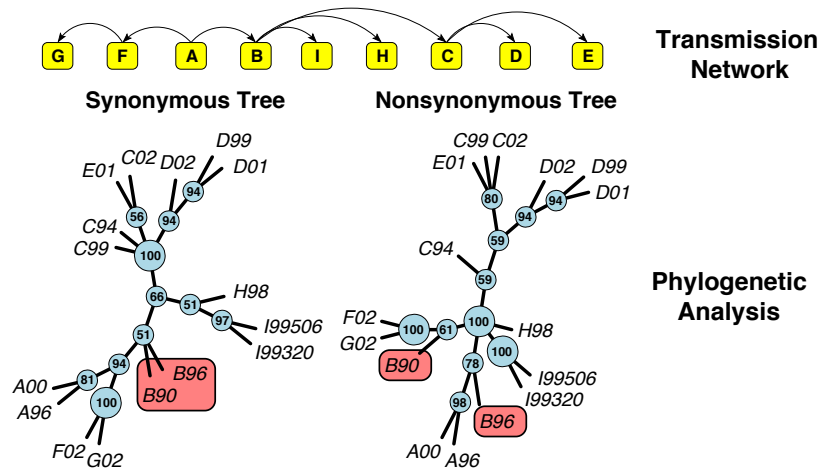


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# HIV Convergent Evolution-III

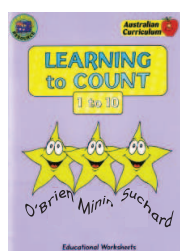
- Compare “**synonymous**” and “**nonsynonymous**” trees:



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## A Few Summary Comments

- Analytic expressions for evolutionary counting processes are derivable and flexible to use:
  - For moderate  $|\Lambda|$ : **substantial increase** in computational efficiency over simulation-based methods
  - For large  $|\Lambda|$ : now **tractable**
- Complex posterior  $p$ -value tests require simulation only under the null (simple) model for which **standard software exists**

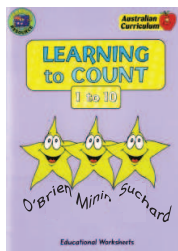


- Minin and Suchard, *Journal of Mathematical Biology*, 2008
- Minin and Suchard, *Proceedings of the Royal Society B*, 2008

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# A Few (More) Summary Comments

- We introduce a general framework for computing arbitrary **labeled distances**; appears **robust to model misspecification**
- How robust???  $\Leftrightarrow$  an open question
- O'Brien, Minin and Suchard, *Molecular Biology and Evolution*, 2009



- R package **markovjumps**
- Recently integrated into **BEAST** and ready for release; anyone want to try it?