

A Flexible Bayesian Framework to Study Viral Trait Evolution

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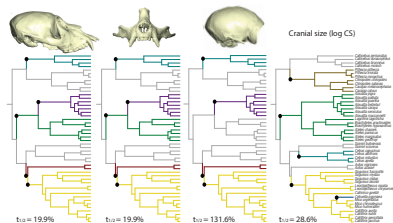
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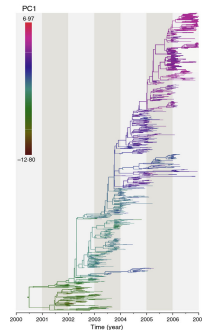
5 June 2019



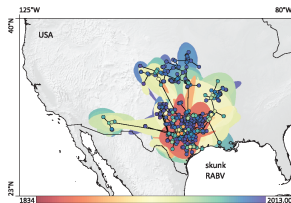
Phylogenetic Comparative Methods



Aristide et al. (2018)



Vrancken et al. (2015)



Dellicour et al. (2017)

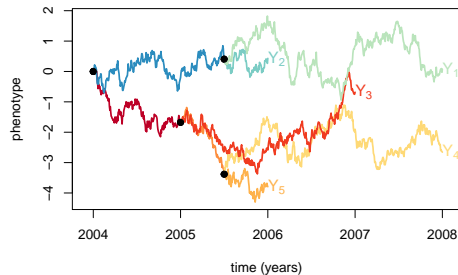
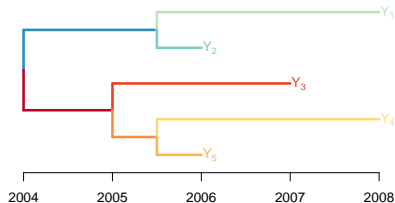
- Various time scales: Myr – decade.
- Various traits: morpho, geo, viral.

Question: Trait dynamics for an evolving organism ?

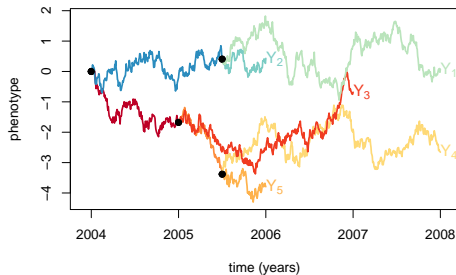
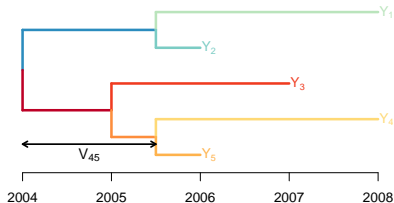
Outline

- ① Models of Trait Evolution
- ② Efficient Bayesian Inference
- ③ HIV Virulence Heritability Study

BM on a Tree



BM on a Tree

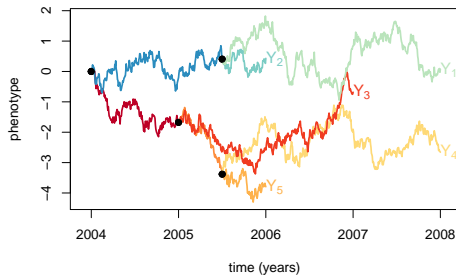
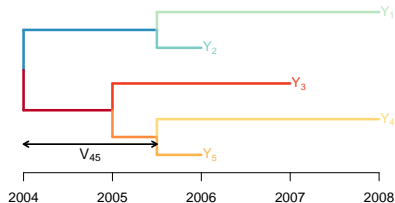


EDS: $dX_t = \sigma dB_t$

Variance: $\text{Cov}[Y_4; Y_5] = \sigma^2 \times V_{45}$ shared evolution time

Expectation: $\mathbb{E}[Y_i] = \mu$ ancestral root value

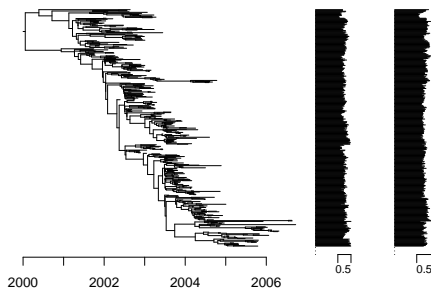
BM on a Tree



Distribution: Normal

$$\mathbf{Y} \sim \mathcal{N}(\mu \mathbf{1}_n, \sigma^2 \mathbf{V})$$

Multivariate BM

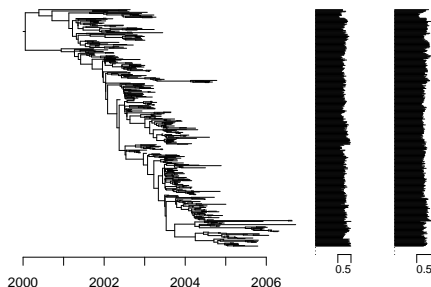


Data: Vectors of p traits

$$\mathbf{Y}_i^T = (Y_{i1}, \dots, Y_{ip})$$

Tree: Influenza H3N2 (Lemey et al., 2014)

Multivariate BM



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$$\mathbf{Y}_i^T = (Y_{i1}, \dots, Y_{ip})$$

EDS:

$$d\mathbf{X}_t = \mathbf{\Sigma} d\mathbf{B}_t$$

$$\mathbf{R} = \mathbf{\Sigma}^T \mathbf{\Sigma}$$

Variance:

$$\text{Cov}[Y_{ik}, Y_{jl}] = R_{kl} \times V_{ij}$$

shared evolution time

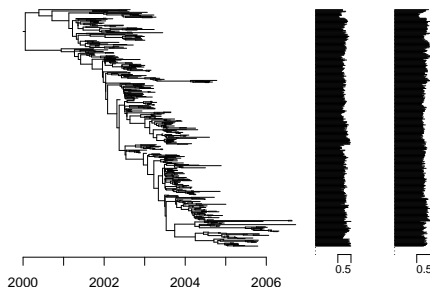
Expectation:

$$\mathbb{E}[\mathbf{Y}_{.k}] = \mu_k$$

ancestral root value

Tree: Influenza H3N2 (Lemey et al., 2014)

Multivariate BM



Data: Vectors of p traits

$$\mathbf{Y}_i^T = (Y_{i1}, \dots, Y_{ip})$$

Distribution: Matrix Normal

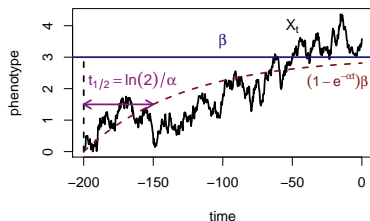
$$\mathbf{Y} \sim \mathcal{MN}(\mathbf{1}_n \boldsymbol{\mu}^T, \mathbf{V}, \mathbf{R})$$

$$\text{Var}[\text{vec}(\mathbf{Y})] = \mathbf{R} \otimes \mathbf{V}$$

Tree: Influenza H3N2 (Lemey et al., 2014)

Ornstein-Uhlenbeck Modeling

(Hansen, 1997)



$$dX_t = \alpha[\beta - X_t]dt + \sigma dB_t$$

Deterministic part:

- β : optimum value.
- α : selection strength (attenuation).

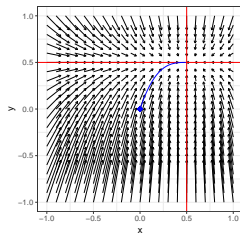
Stochastic part:

- X_t : trait value.
- $\sigma dB(t)$: Brownian fluctuations.

Multivariate OU Modeling

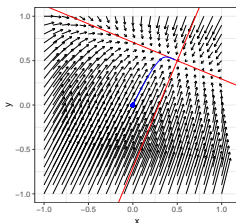
$$d\mathbf{X}_t = \mathbf{A}[\boldsymbol{\beta} - \mathbf{X}_t] dt + \boldsymbol{\Sigma} d\mathbf{B}_t$$

Diagonal



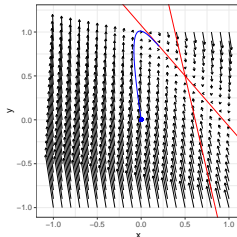
$$\mathbf{A} = \begin{pmatrix} 0.1 & 0 \\ 0 & 0.3 \end{pmatrix}$$

Symmetric



$$\mathbf{A} = \begin{pmatrix} 0.1 & 0.1 \\ 0.1 & 0.3 \end{pmatrix}$$

Diagonalizable in \mathbb{R}

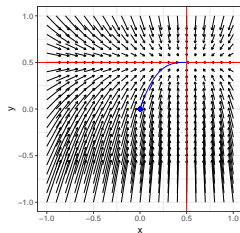


$$\mathbf{A} = \begin{pmatrix} -0.02 & -0.04 \\ 0.2 & 0.2 \end{pmatrix}$$

Multivariate OU Modeling

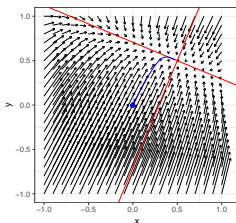
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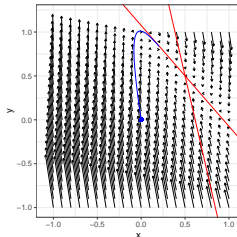
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Distribution on a Tree: Still Gaussian. No nice Kronecker product.

Bayesian Phylogenetics

Goal:

$$p(\theta, \mathcal{T}, \psi \mid \mathbf{Y}, \mathbf{S})$$

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$$p(\boldsymbol{\theta}, \mathcal{T}, \boldsymbol{\psi} \mid \mathbf{Y}, \mathbf{S}) \propto p(\mathbf{Y}, \mathbf{S} \mid \boldsymbol{\theta}, \mathcal{T}, \boldsymbol{\psi}) p(\boldsymbol{\theta}, \mathcal{T}, \boldsymbol{\psi})$$

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Assumption: \mathbf{Y} and \mathbf{S} independent conditionally on \mathcal{T} .

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This talk: \mathcal{T} fixed.

BM: Gibbs with Conjugate Priors

Likelihood:

$$\mathbf{Y}|\mathbf{R}, \boldsymbol{\mu} \sim \mathcal{MN}(\mathbf{1}_n \boldsymbol{\mu}^T, \mathbf{V}, \mathbf{R})$$

Conjugate Priors:

$$\mathbf{R} \sim \mathcal{IW}(\mathbf{R}_0, \nu)$$

$$\boldsymbol{\mu}|\mathbf{R} \sim \mathcal{N}(\boldsymbol{\mu}_0, \kappa_0^{-1} \mathbf{R})$$

Gibbs:

$$\mathbf{R}|\mathbf{Y}, \boldsymbol{\mu} \sim \mathcal{IW}(\mathbf{R}_n, \nu_n) \quad \text{with} \quad \mathbf{R}_n = f(\mathbf{Y}, \boldsymbol{\mu}, \mathbf{V})$$

↪ Automatic sampling in the space of variance matrices.

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OU: No Gibbs

Likelihood:

$$\mathbf{Y}|\mathbf{A}, \mathbf{R}, \mu \not\sim \mathcal{MN}$$

Conjugate Priors: ? → No Gibbs
 → Sample in **constrained** spaces (**A**, **R**)

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Conjugate Priors: ? \rightarrow No Gibbs

\rightarrow Sample in **constrained** spaces (\mathbf{A} , \mathbf{R})

Transformation:

$$f : \begin{cases} \mathcal{C}_q \rightarrow \mathbb{R}^q \\ \boldsymbol{\theta} \mapsto \boldsymbol{\nu} = f(\boldsymbol{\theta}) \end{cases} ; \quad \pi_{\boldsymbol{\theta}}(\boldsymbol{\theta}) = \pi_{\boldsymbol{\nu}}(f(\boldsymbol{\theta})) \times |J_f(\boldsymbol{\theta})|$$

Metropolis - Hasting Iterate:

- Draw $\boldsymbol{\nu}^*$ in $q(\boldsymbol{\nu} | \boldsymbol{\nu}^t)$.
- Set $\boldsymbol{\theta}^{(t+1)} = \boldsymbol{\theta}^* = f^{-1}(\boldsymbol{\nu}^*)$ with probability

$$r_t = \min \left\{ 1, \frac{p(\mathbf{Y} | \boldsymbol{\theta}^*)}{p(\mathbf{Y} | \boldsymbol{\theta}^t)} \frac{p(\boldsymbol{\theta}^*)}{p(\boldsymbol{\theta}^t)} \frac{q(\boldsymbol{\nu}^{(t)} | \boldsymbol{\nu}^*)}{q(\boldsymbol{\nu}^* | \boldsymbol{\nu}^{(t)})} \frac{|J_f(\boldsymbol{\theta}^{(t)})|}{|J_f(\boldsymbol{\theta}^*)|} \right\}.$$

Transformations

Variance Matrix: LKJ

(Lewandowski, Kurowicka, and Joe, 2009)

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Decomposition Use correlation matrix **C**.

$$\mathbf{R} = \begin{pmatrix} \sigma_1 & & 0 \\ & \ddots & \\ 0 & & \sigma_p \end{pmatrix} \begin{pmatrix} 1 & & C_{kl} \\ & \ddots & \\ C_{kl} & & 1 \end{pmatrix} \begin{pmatrix} \sigma_1 & & 0 \\ & \ddots & \\ 0 & & \sigma_p \end{pmatrix}$$

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Cholesky $\mathbf{C} = \mathbf{W}^T \mathbf{W}$ with:

- $W_{1k}^2 + \dots + W_{kk}^2 = 1$ (correlation)
- $W_{kk} > 0$ (identifiability)

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Attenuation Matrix:

Assumptions $\mathbf{A} = \mathbf{PAP}^{-1}$ $\lambda_k \in \mathbb{R}$ $\lambda_k > 0$

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Attenuation Matrix:

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Identifiability $\|\mathbf{P}_{\cdot k}\| = 1$ $\mathbf{P}_{pk} > 0$

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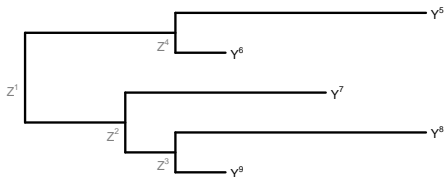
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General Model

BM, OU: Instance of a general Gaussian propagation model.

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$$\mathbf{X} = \begin{cases} \mathbf{Z} : \text{latent nodes} \\ \mathbf{Y} : \text{tips} \end{cases}$$

$$\mathbf{x}^1 \sim \mathcal{N}(\boldsymbol{\mu}, \boldsymbol{\Gamma})$$

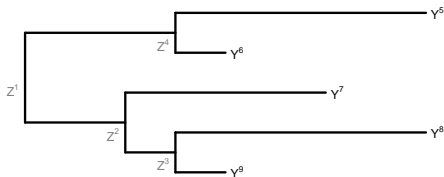
root

$$\mathbf{x}^j \mid \mathbf{x}^{\text{pa}(j)} \sim \mathcal{N}(\mathbf{q}_j \mathbf{x}^{\text{pa}(j)} + \mathbf{r}_j, \boldsymbol{\Sigma}_j)$$

tips and nodes

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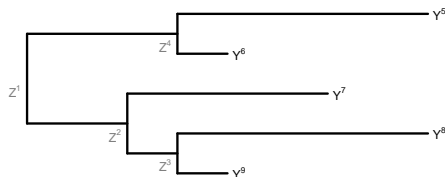
tips and nodes

Likelihood: $\log p(\mathbf{Y})$

one post-order traversal

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one post-order traversal

Gradient: $\frac{\partial}{\partial \phi_j} [\log p(\mathbf{Y})] = \mathbb{E} [\mathbf{F}(\mathbf{X}^j; \phi_j) \mid \mathbf{Y}]$

one pre-order traversal

Implementation

(Suchard et al., 2018)



BEAST
Bayesian Evolutionary Analysis Sampling Trees

- MCMC for tree estimation
- Comprehensive set of tools:
 - Factor model
 - Marginal Likelihood
 - ...
- Developed in Java since 2002.
- This is BEAST 1.10.

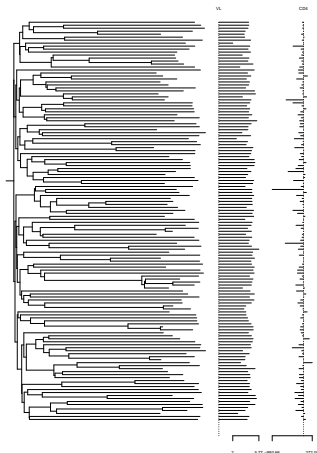
Don't ask about BEAST 2.

What's new:

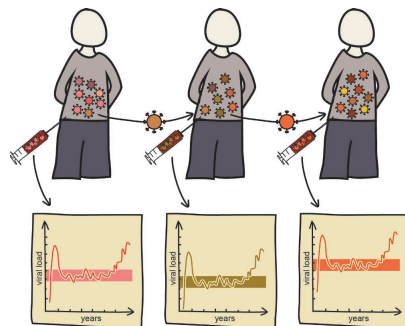
- Flexible OU models
- Efficient sampling of variance
- Efficient HMC (in progress)

HIV virulence heritability

(Alizon et al., 2010; Vrancken et al., 2015)



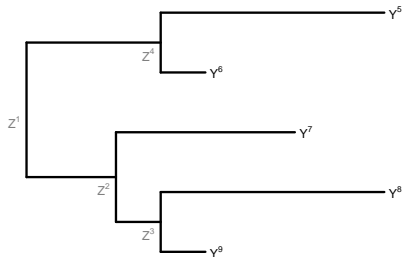
CD4: CD4+ T cells decline rate
VL: Set point viral load



Fraser et al. (2014)

Questions: Is virulence “heritable”? What model of trait evolution?

Heritability



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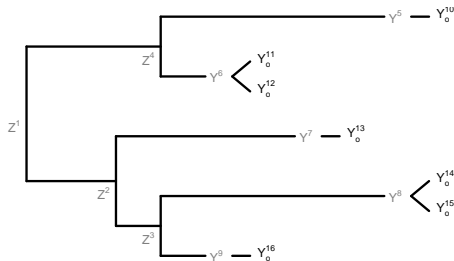
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tips and nodes

Heritability



$$\mathbf{X} = \begin{cases} \mathbf{Z} : \text{latent nodes} \\ \mathbf{Y} : \text{latent tips} \\ \mathbf{Y}_o : \text{observed traits} \end{cases}$$

$$\mathbf{X}^1 \sim \mathcal{N}(\boldsymbol{\mu}, \boldsymbol{\Gamma})$$

root

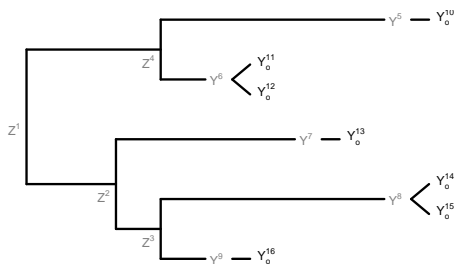
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latent tips and nodes

$$\mathbf{Y}_o^i \mid \mathbf{Y}^{\text{pa}(i)} \sim \mathcal{N}(\mathbf{Y}^{\text{pa}(i)}, \mathbf{S})$$

observations

Heritability



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latent tips and nodes

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observations

“Heritability”:

$$h_k^2 = \frac{V(\mathbf{Y}_{\cdot k})}{V(\mathbf{Y}_{o \cdot k})} \approx \frac{\sigma_k^2 \tilde{t}}{\sigma_k^2 \tilde{t} + s_k}$$

Results

Model:

VL OU (stabilizing selection)

CD4 BM (no selection)

The two traits can be correlated.

Heritability:

VL $h^2 = 17\% [0.007, 82.5]\% (95\% \text{ CI})$

CD4 $h^2 = 0.02\% [0.0024, 0.16]\%$

↪ “Consistent” with previous estimates.

Conclusion and Perspectives

A general framework for trait evolution with dated tips.

Main Features:

- Flexible models and implementation
- Efficient algorithms
- Applicable to virology

Perspectives:

- Develop HMC
- Identifiability
- Other questions: geographical spread, comparative studies, ...

Bibliography

- Alizon, von Wyl, Stadler, et al. 2010. *PLoS Pathog.* 6.
- Aristide, Bastide, dos Reis, et al. 2018. *Evolution.* 72:2697–2711.
- Dellicour, Rose, Faria, et al. 2017. *Mol. Biol. Evol.* 34:2563–2571.
- Fraser, Lythgoe, Leventhal, et al. 2014. *Science (80-.).* 343:1243727–1243727.
- Hansen. 1997. *Evolution.* 51:1341.
- Lemey, Rambaut, Bedford, et al. 2014. *PLoS Pathog.* 10:e1003932.
- Lewandowski, Kurowicka, Joe. 2009. *J. Multivar. Anal.* 100:1989–2001.
- Suchard, Lemey, Baele, et al. 2018. *Virus Evol.* 4:1–5.
- Vrancken, Lemey, Rambaut, et al. 2015. *Methods Ecol. Evol.* 6:67–82.

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Appendices