

Phyloclustering: A Model-Based Approach for Identifying Microbial Populations

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Outline

Motivation

- Equine Infectious Anemia Virus (EIAV)

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- Mixture Multivariate Normal Distribution

- Model-based Clustering

- Clustering for Nucleotide Sequences

Phylocustering Approach

- Continuous Time Markov Chain (CTMC) Model

- Mixture Transition Probability

- EM Algorithm

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Data Analysis

- EIAV Result

Summary

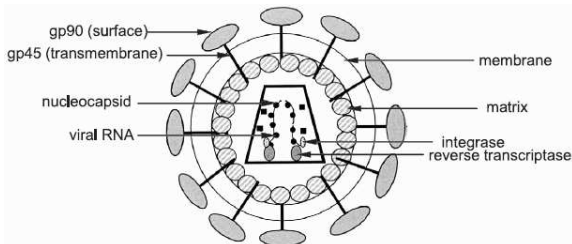
Motivation I

Equine Infectious Anemia Virus (EIAV)

- ▶ Leroux, Cadoré, and Montelaro (2004).
- ▶ "Country cousin" of HIV.
- ▶ Lentivirus in the Retrovirus family infect equines.
- ▶ A persistent infection characterized by recurring febrile episodes associating with viremia, thrombocytopenia, and wasting symptoms.



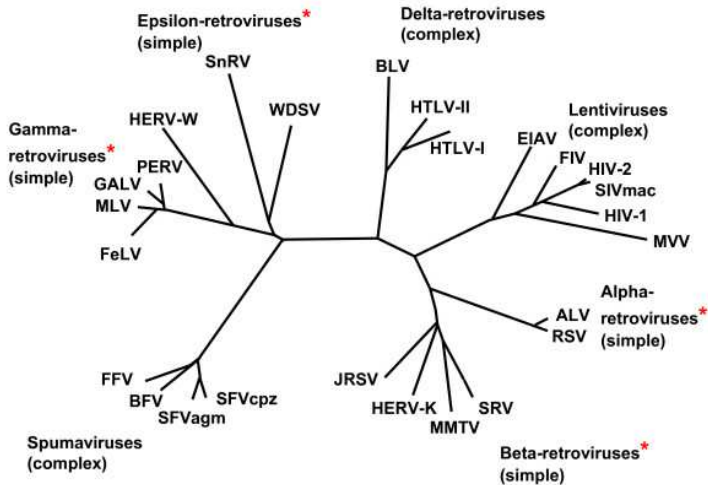
ISU Horse Barn (2006).



Leroux, Cadoré, and Montelaro (2004).

Motivation II

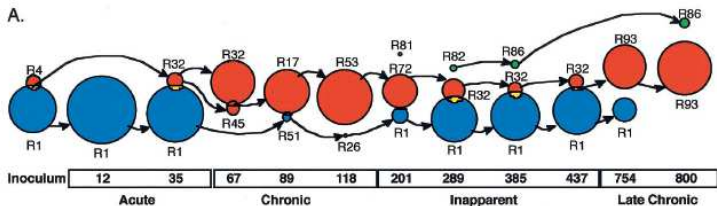
Phylogeny of Retroviruses



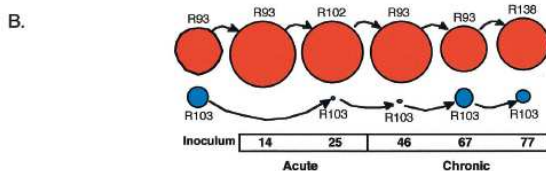
Weiss (2006).

Motivation III

PAQ: Partition Analysis of Quasispecies (Baccam et.al. (2001)).



Pony 524

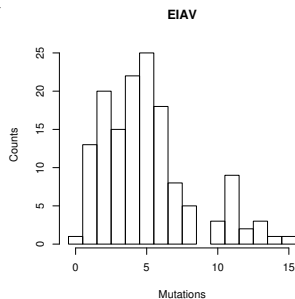
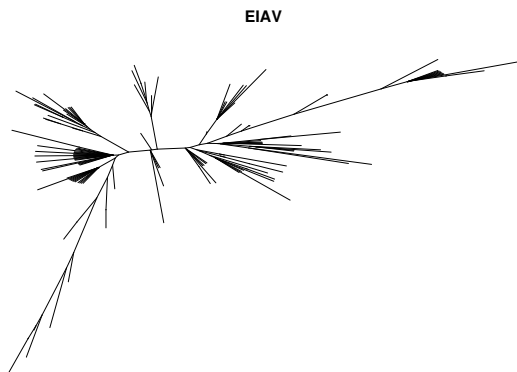


Pony 625

Baccam et al. (2003).

Motivation IV

146 EIAV *rev* sequences of pony 524.



Mutation counts for 146 sequences.

Motivation V

Number of bifurcating unrooted trees N_U for $n \geq 3$ sequences is

$$N_U = \frac{(2n-5)!}{2^{n-3}(n-3)!}.$$

Number of sequences	Number of unrooted trees
2	1
3	1
4	3
5	15
6	105
7	945
\vdots	\vdots
17	6,190,283,353,629,375
18	191,898,783,962,510,625
19	6,332,659,870,762,850,625
20	221,643,095,476,699,771,875

Felsenstein (1978) or Graur and Li (2000).

Goals of Phyloclustering

- ▶ to identify population centers where sequences may diverge from,
- ▶ to establish a model based approach to cluster sequences with phylogenetic meaning,
- ▶ to distinguish population structure based on classifications, and
- ▶ to aggregate trustworthy sequence information.

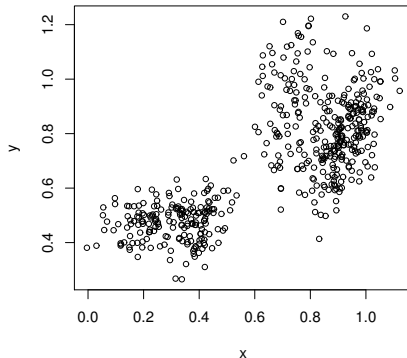
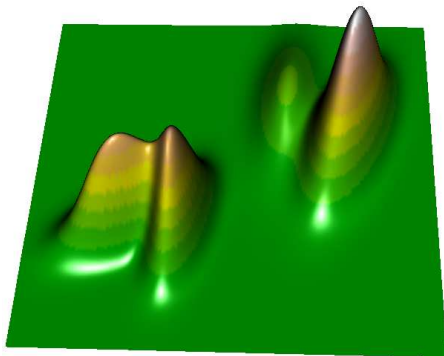
Mixture Multivariate Normal (MVN) Distribution

Mixture MVN with K components in p dimension:

$X_1, \dots, X_N \stackrel{iid}{\sim} \phi(\mathbf{x}|\boldsymbol{\mu}, \boldsymbol{\Sigma})$ and $\phi(\mathbf{x}|\boldsymbol{\mu}, \boldsymbol{\Sigma}) = \sum_{k=1}^K \eta_k \phi_k(\mathbf{x}|\boldsymbol{\mu}_k, \boldsymbol{\Sigma}_k)$
where

$$\phi_k(\mathbf{x}|\boldsymbol{\mu}_k, \boldsymbol{\Sigma}_k) = \frac{1}{(2\pi)^{p/2} |\boldsymbol{\Sigma}_k|^{1/2}} \exp \left\{ -\frac{1}{2} (\mathbf{x} - \boldsymbol{\mu}_k)' \boldsymbol{\Sigma}_k^{-1} (\mathbf{x} - \boldsymbol{\mu}_k) \right\}$$

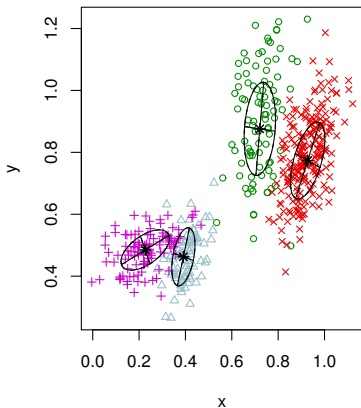
N=500



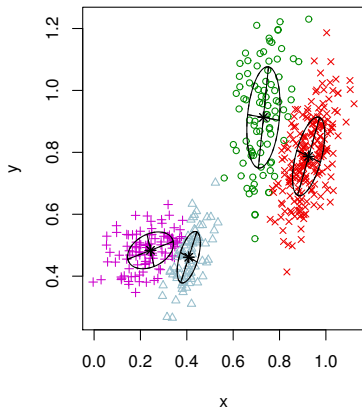
Question: Are there four clusters? Where are they?

Model-based Clustering

N=500, K=4, Original



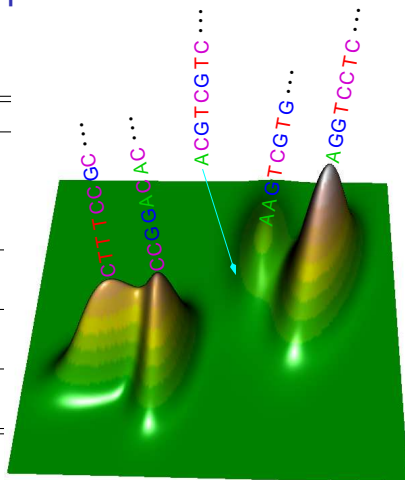
Estimated, AdjR=0.9



Model-based clustering based on the mixture MVN model.

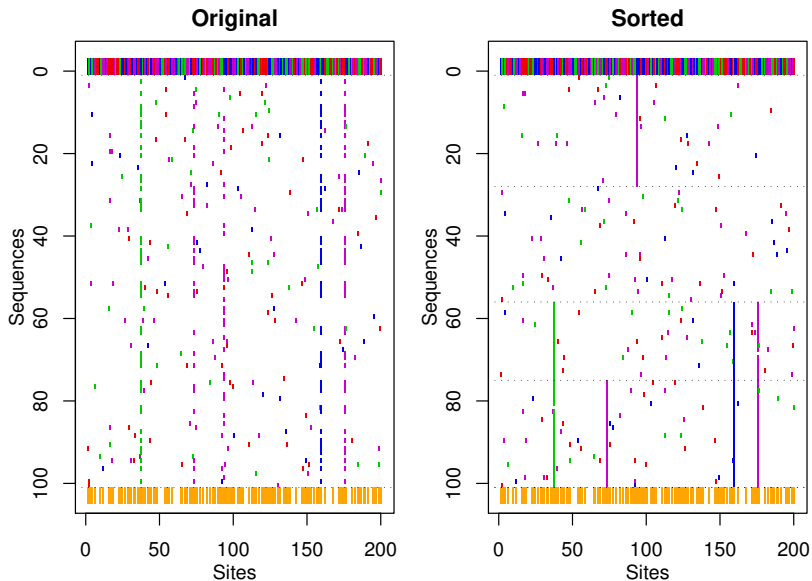
Clustering for Nucleotide Sequences

Id	Sequence	Center
1	ACGTCGTC...	AAGTCGTG...
2	AAGTCGTG...	
3	AAGTCGAG...	
4	AGGTCGCG...	
5	CCGGACAC...	CCGGACAC...
6	CCGGACAC...	
7	CTTGCCGC...	CTTTCCGC...
8	CTTTCCGC...	
9	AGGTCCTC...	AGGTCCTC...
10	AGGTCCTC...	



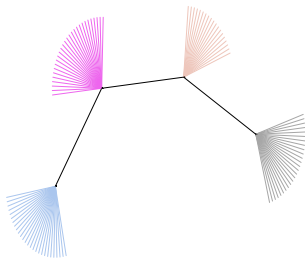
Question: How do we model/cluster this kind of data?

A Toy Dataset

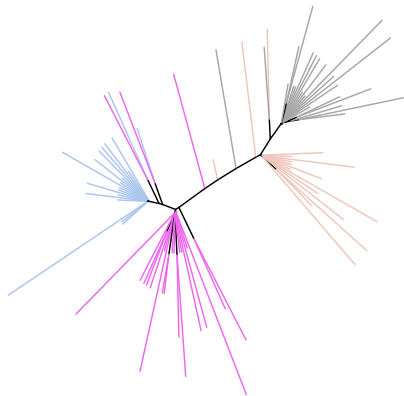


Phylogenetic Approach

True tree for the toy dataset



Neighbor joining tree (K80)



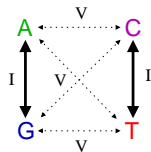
Question: What is the model for mutation process?

Continuous Time Markov Chain (CTMC) Model

Nucleotide substitution model: JC69 (Jukes & Cantor (1969)), K80 (Kimura (1980)), HKY85 (Hasegawa, Kishino & Yano (1985)).

For example, HKY85 defines $\mathbf{Q}_{x,y} = (q_{xy})_{4 \times 4}$ as

$$q_{xy} = \begin{cases} \pi_y & \text{if } x \text{ and } y \text{ differ by a transversion (V),} \\ \kappa \pi_y & \text{if } x \text{ and } y \text{ differ by a transition (I),} \end{cases}$$

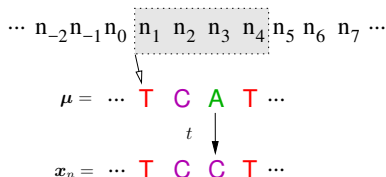


for $y \neq x$, $q_{xx} = -\sum_{y \neq x} q_{xy}$ where $x, y \in \{\text{A, G, C, T}\}$.

$$\begin{matrix} & \text{A} & \text{G} & \text{C} & \text{T} \\ \begin{matrix} \text{A} \\ \text{G} \\ \text{C} \\ \text{T} \end{matrix} & \begin{pmatrix} 1 - \kappa\pi_{\text{G}} - \pi_{\text{C}} - \pi_{\text{T}} & \kappa\pi_{\text{G}} & \pi_{\text{C}} & \pi_{\text{T}} \\ \kappa\pi_{\text{A}} & 1 - \kappa\pi_{\text{A}} - \pi_{\text{C}} - \pi_{\text{T}} & \pi_{\text{C}} & \pi_{\text{T}} \\ \pi_{\text{A}} & \pi_{\text{G}} & 1 - \pi_{\text{A}} - \pi_{\text{G}} - \kappa\pi_{\text{T}} & \kappa\pi_{\text{T}} \\ \pi_{\text{A}} & \pi_{\text{G}} & \kappa\pi_{\text{C}} & 1 - \pi_{\text{A}} - \pi_{\text{G}} - \kappa\pi_{\text{C}} \end{pmatrix} \end{matrix}$$

CTMC: if $\mathbf{Q}_{x,y} = \mathbf{U}\mathbf{D}\mathbf{U}^{-1} \Rightarrow \mathbf{P}_{x,y}(t) = e^{\mathbf{Q}_{x,y}t} = \mathbf{U}e^{\mathbf{D}t}\mathbf{U}^{-1}$

Transition Probability



- ▶ $\mathbf{x}_n = (x_{n1}, \dots, x_{nL}) \in \mathcal{S}^L$ where $x_{nl} \in \mathcal{S} = \{\text{A}, \text{G}, \text{C}, \text{T}\}$.
 - ▶ Assume mutations among sites are independent.
 - ▶ Assume \mathbf{x}_n evolves from a population center $\mu = (\mu_1, \dots, \mu_L) \in \mathcal{S}^L$.
 - ▶ Assume an substitution model, $\mathbf{Q}_{x,y}$.
 - ▶ Assume evolving time t between μ and \mathbf{x}_n .

Transition probability: $p_{\mu, \mathbf{x}_n}(t) = \prod_{l=1}^L P_{\mu_{kl}, x_{nl}}(t)$.

- ▶ Distribution of mutation process:

$$\phi(\mathbf{x}_n | \mu, \mathbf{Q}, t) = p_{\mu, \mathbf{x}_n}(t).$$

Mixture Transition Probability

Mixture Transition Probability:

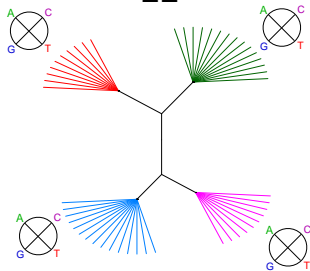
- ▶ Mixture proportion: $\boldsymbol{\eta} = (\eta_1, \dots, \eta_K)$, $\eta_k > 0$, and $\sum_{k=1}^K \eta_k = 1$.
- ▶ Dominant sequence (Center): $\boldsymbol{\mu}_k = (\mu_{k1}, \dots, \mu_{kL}) \in \mathcal{S}^L$ where $\mu_{kl} \in \mathcal{S}$.
- ▶ CTMC model (Dispersion): \mathbf{Q}_k and t_k .

Possible CTMC models:

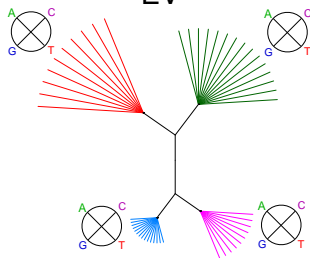
- ▶ EE: $\mathbf{Q}_1 = \mathbf{Q}_2 = \dots = \mathbf{Q}_K$ and $t_1 = t_2 = \dots = t_K$.
- ▶ EV: $\mathbf{Q}_1 = \mathbf{Q}_2 = \dots = \mathbf{Q}_K$ and $t_1 \neq t_2 \neq \dots \neq t_K$.
- ▶ VE: $\mathbf{Q}_1 \neq \mathbf{Q}_2 \neq \dots \neq \mathbf{Q}_K$ and $t_1 = t_2 = \dots = t_K$.
- ▶ VV: $\mathbf{Q}_1 \neq \mathbf{Q}_2 \neq \dots \neq \mathbf{Q}_K$ and $t_1 \neq t_2 \neq \dots \neq t_K$.

Examples of CTMC models

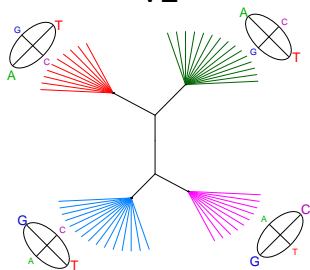
EE



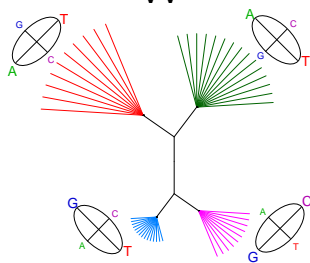
EV



VE



VV



EM Algorithm for Mixture Model

- ▶ Log likelihood: let $\Theta = \{\eta, \mu, \mathbf{Q}, t\}$,

$$\log L(\Theta|\mathbf{x}) = \sum_{n=1}^N \log \left[\sum_{k=1}^K \eta_k \phi_k(\mathbf{x}_n | \mu_k, \mathbf{Q}_k, t_k) \right].$$

- ▶ Augment data for missing information:

$$Z_{nk} = I(n \in \mathcal{G}_k) \text{ for } n = 1, \dots, N \text{ and } k = 1, \dots, K.$$

- ▶ Log complete-data likelihood:

$$\log L_c(\Theta, \mathbf{Z}|\mathbf{x}) = \sum_{n=1}^N \sum_{k=1}^K Z_{nk} [\log \eta_k + \log \phi_k(\mathbf{x}_n | \mu_k, \mathbf{Q}_k, t_k)].$$

- ▶ EM algorithm: (Dempster et.al. 1977)

1. E-step: $Q(\Theta|\mathbf{x}) = \mathbb{E}_{\mathbf{Z}}[\log L_c(\Theta, \mathbf{Z}|\mathbf{x})]$.
2. M-step: $\max_{\Theta} Q(\Theta|\mathbf{x})$.
3. Iterate E- and M-steps until convergence which yields

$$\hat{\Theta} = \underset{\Theta}{\operatorname{argmax}} \log L(\Theta|\mathbf{x}).$$

EM Algorithm for Phyloclustering with EE Model

- ▶ E-step:

$$z_{nk}^{(s)} = \mathbb{E}_{\mathbf{Z}}[Z_{nk} | \mathbf{x}, \boldsymbol{\Theta}^{(s-1)}] = \frac{\eta_k^{(s-1)} \phi_k(\mathbf{x}_n | \boldsymbol{\mu}_k^{(s-1)}, \mathbf{Q}^{(s-1)}, t^{(s-1)})}{\phi(\mathbf{x}_n | \boldsymbol{\mu}^{(s-1)}, \mathbf{Q}^{(s-1)}, t^{(s-1)})}$$

where $n = 1, \dots, N$ and $k = 1, \dots, K$.

- ▶ M-step:

- ▶ $\eta_k^{(s)} = \sum_{n=1}^N z_{nk}^{(s)} / N$.

- ▶ $\boldsymbol{\mu}_k^{(s)}(\mathbf{Q}, t)$ obtained by comparing transition probabilities,

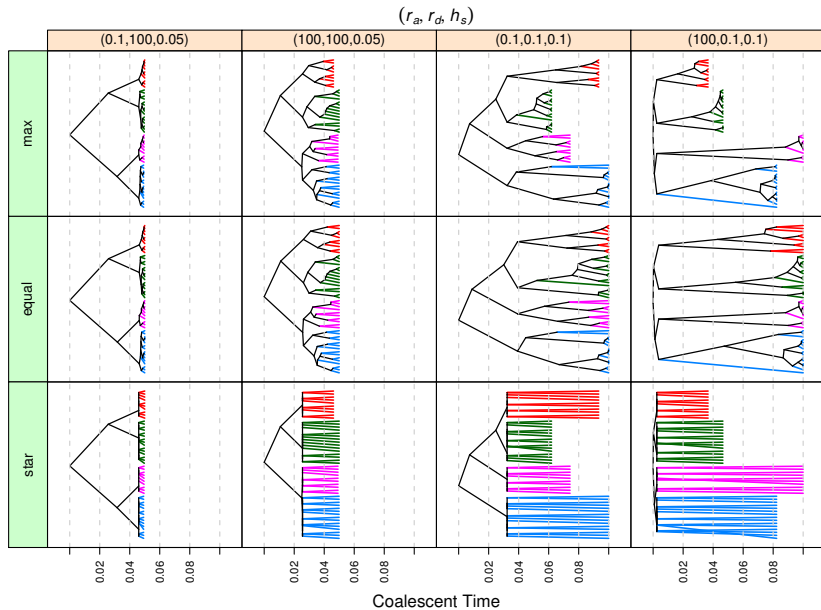
$$\begin{aligned} \mu_{kl}^{(s)}(\mathbf{Q}, t) &= \operatorname{argmax}_{\mu \in \mathcal{S}} \sum_{n=1}^N z_{nk}^{(s)} \log \phi_k(x_{nl} | \mu(\mathbf{Q}, t), \mathbf{Q}, t) \\ &= \operatorname{argmax}_{\mu \in \mathcal{S}} \sum_{a \in \mathcal{S}} \left[\left(\sum_{n \ni x_{nl}=a} z_{nk}^{(s)} \right) N_{\{x_l=a\}} \log p_{\mu, s}(t) \right]. \end{aligned}$$

- ▶ $\mathbf{Q}^{(s)}$ and $t^{(s)}$ obtained numerically to maximize profile likelihood.

Challenges of EM Algorithm

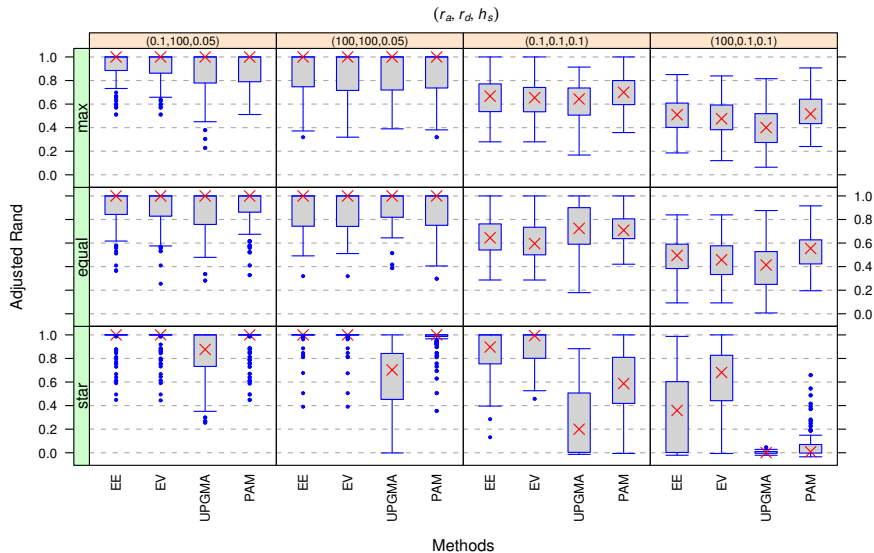
1. Improve slow convergence of EM algorithm:
 - ▶ ECM (Meng & Rubin (1993)).
 - ▶ AECM (Meng & van Dyk (1997)).
 - ▶ APECM (Chen & Maitra (2011)).
2. Initialization schemes to improve convergent results:
 - Method:
 - ▶ Neighbor joining tree (Saitou & Nei (1987))
 - ▶ Partition Around Medoids (PAM) (Kaufman & Rousseeuw (1990))
 - ▶ K-Medoids (Theodoridis & Koutroumbas (2006))
 - ▶ Manually
 - Procedure:
 - ▶ em-EM (Biernacki, Celeux, & Govaert (2003))
 - ▶ Rand-EM (Maitra (2007))
 - ▶ Exhausted EM

Simulation Study I



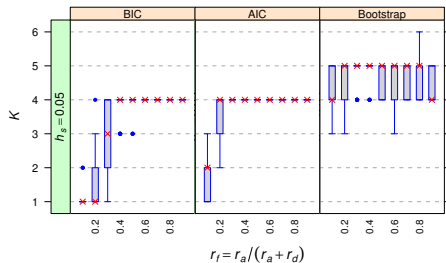
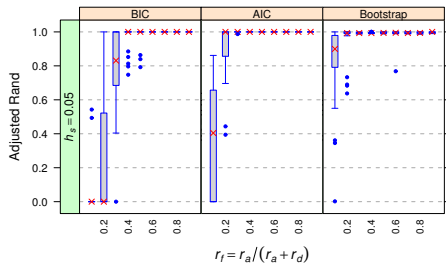
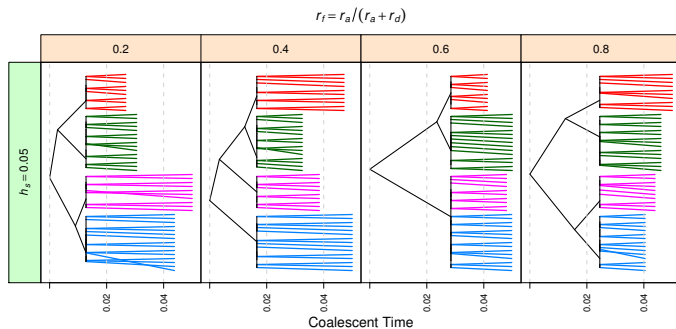
r_a : growth rate of ancestor tree, r_d : growth rate of descendent tree, h_s : total height.

Results of Simulation Study I



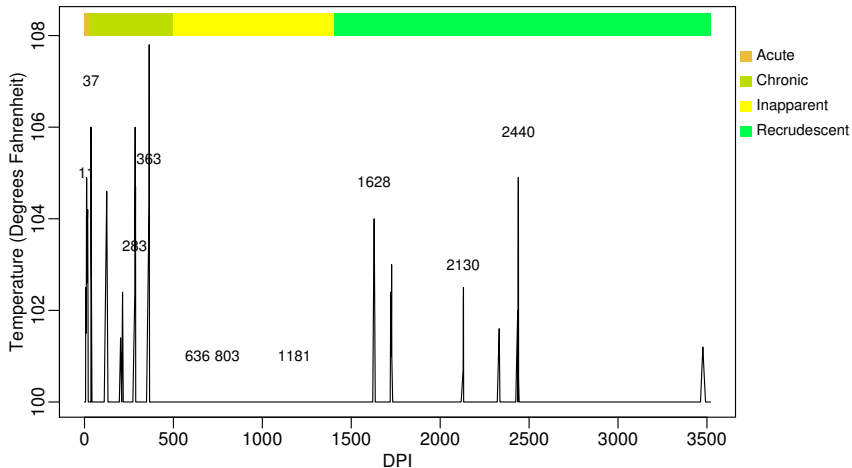
Results of EE (phyclust), EV (phyclust), UPGMA, and PAM.

Simulation Study II and Results



EIA Disease Progress

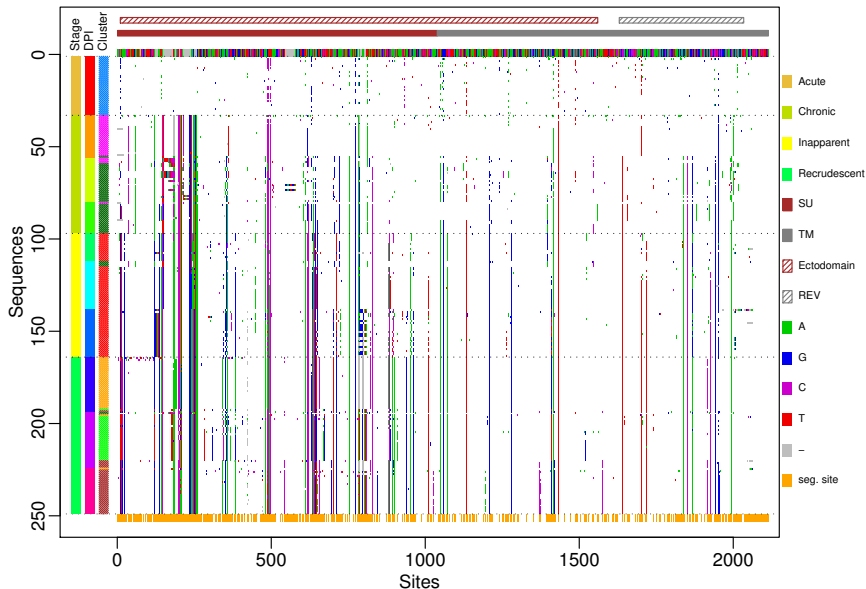
Pony 618 Fever Chart



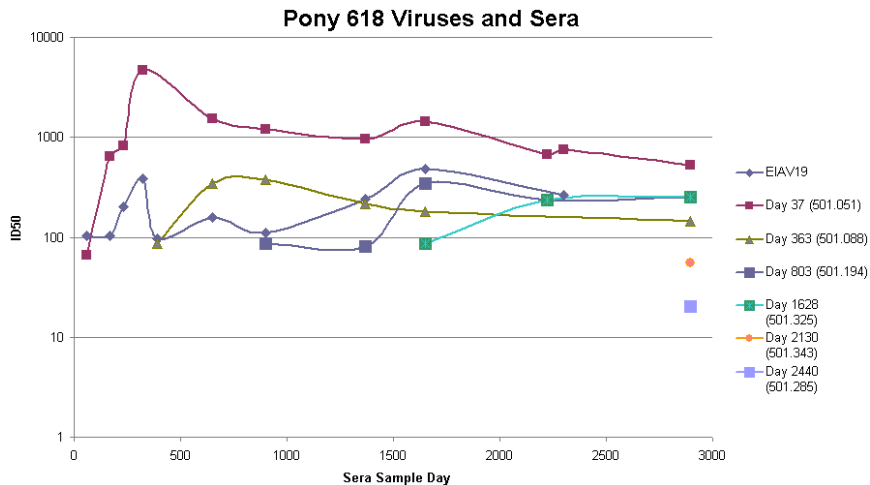
Cierra Pairett (2011), "Longitudinal analysis of genetic and antigenic variation in EIAV env", Iowa State University.

EIAV Phyloclustering Results

Pony 618, SGA (all), K=7



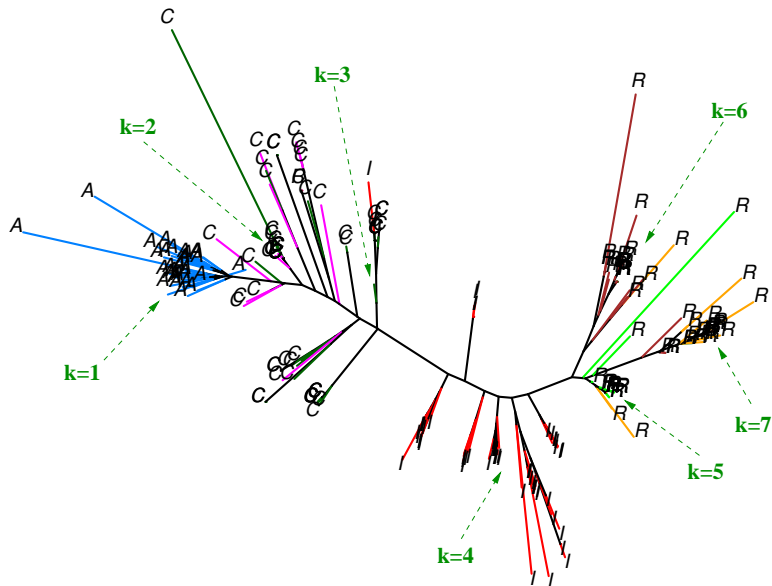
EIAV ID50 Result



Cierra Pairett (2011), "Longitudinal analysis of genetic and antigenic variation in EIAV env", Iowa State University.

EIAV Tree

Pony618, SGA (all), K=7



A: Acute, C: Chronic, I: Inapparent, R: Recrudescent.

Summary

- ▶ **phyclust**: an R package for Phylogenetic Clustering (<https://cran.r-project.org/package=phyclust>).
- ▶ Identify number of clusters.
- ▶ Initialization problem for EM algorithm.
- ▶ Potential extensions:
 - ▶ Reduce number of parameters (Hierarchical model for center sequences.)
 - ▶ Dependent structure along sites (Hidden Markov model.)

Acknowledgement

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- ▶ Dr. Susan Carpenter
- ▶ Cierra Pairett

Thank you!