Integrating hierarchical information in the analysis of microbiome data

C. Ambroise[1], A. Bichat[1,2,3], *M. Mariadassou*[2]

[1]LaMME, UEVE, Université Paris-Saclay, Evry, France
[2]MAIAGE, INRAE, Université Paris-Saclay, Jouy-en-Josas, France
[3]Enterome, Paris, France

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Outline

Microbiota

Ecological community of microorganisms that reside in an environmental niche.

Published associations with:

- Inflammatory bowel diseases
- Liver disease
- Vaccine efficiency
- Anxiety
- Muscular strength
- etc

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Strong interest in findings (groups of) microbes associated to a given condition.

Metabarcoding data (Ravel et al., 2011)

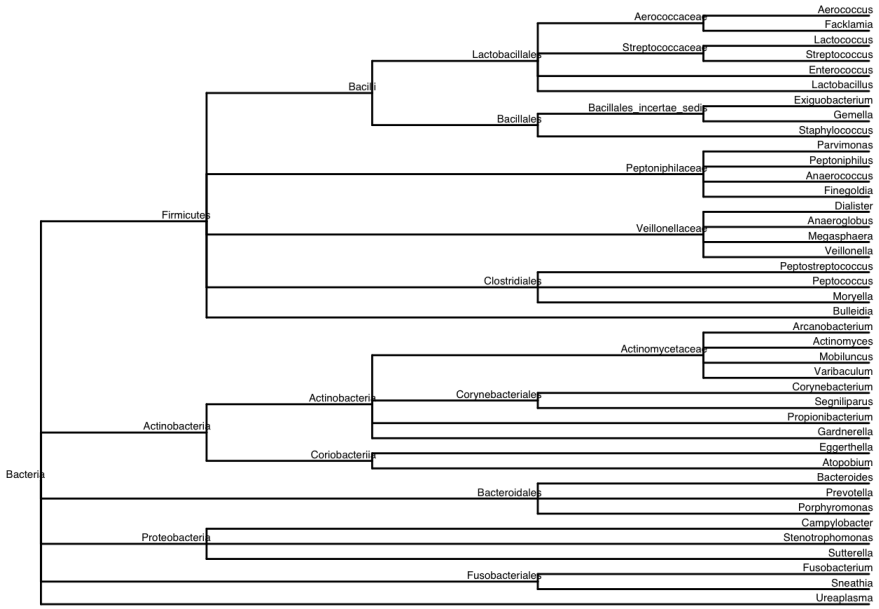
A species × sample count table

	Taxa	A1	A2	A3	B1	B2	B3
1	Lactobacillus	2318	1388	1361	2256	88	1770
2	Prevotella	0	1	1	0	525	7
3	Megasphaera	0	1	0	0	402	0
4	Sneathia	0	0	0	0	302	0
5	Atopobium	0	1	0	0	84	0
6	Streptococcus	0	0	3	0	0	0
7	Dialister	0	1	0	0	152	4
8	Anaerococcus	0	1	3	2	0	9
9	Peptoniphilus	0	1	0	0	7	2
10	Eggerthella	0	0	0	0	2	0

Metabarcoding data (Ravel et al., 2011)

Taxonomic / phylogenetic tree

	Phylum	Class	Order	Family	Genus
1	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinobaculum
2	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
3	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Arcanobacterium
4	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Mobiluncus
5	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Varibaculum
6	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium
7	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Gardnerella



Differential abundance analysis

- For each taxa i (in $\{1, \ldots, n\}$), test
  - H_{0i}: Abundances are equal in groups A and B
  - H_{1i}: Abundances are not equal in groups A and B
- Hundred of univariate tests and p-values
- Need for a multiple testing correction procedure

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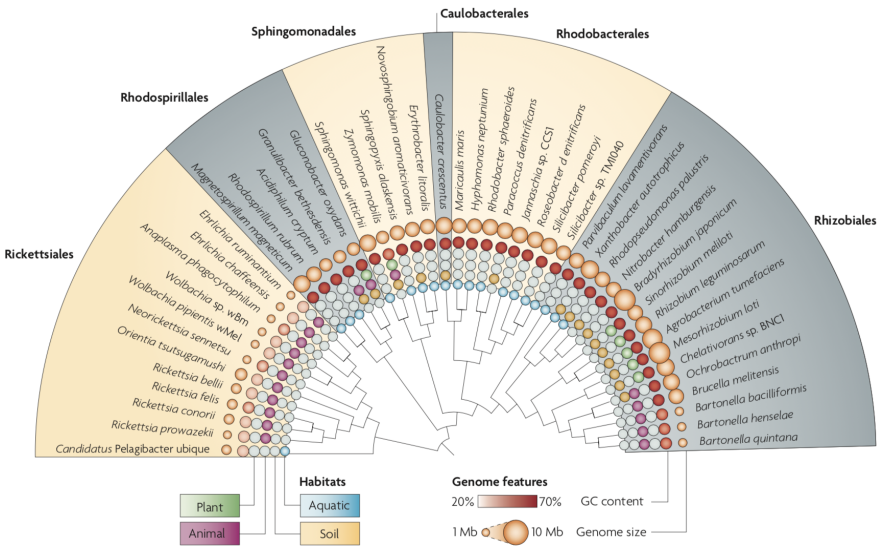


- Taxa / group associations may show a **phylogenetic signal**
- Similar taxa $\Rightarrow$ similar levels of association
- Can we leverage the tree when correcting the tests?

Outline

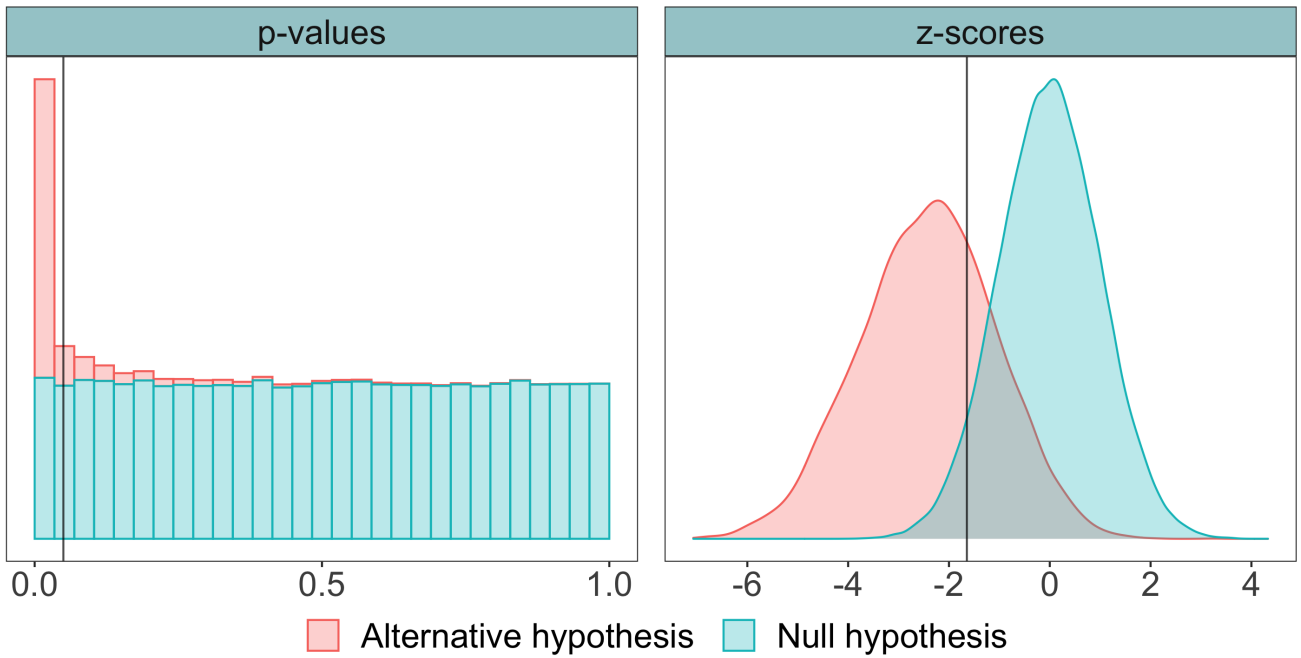
Mathematical Model

Standard assumptions on *p*-values

- Under $H_{0i}, p_i \sim \mathcal{U}(0,1)$
- Under $H_{1i}, p_i \preccurlyeq \mathcal{U}(0,1)$

Standard assumptions on *z*-scores

- Under H_{0i}, $z_i = \Phi^{-1}(p_i) \sim \mathcal{N}(0,1)$
- Under H_{1i}, $z_i = \Phi^{-1}(p_i) \sim \mathcal{N}(m_i, 1)$ with $m_i < 0$



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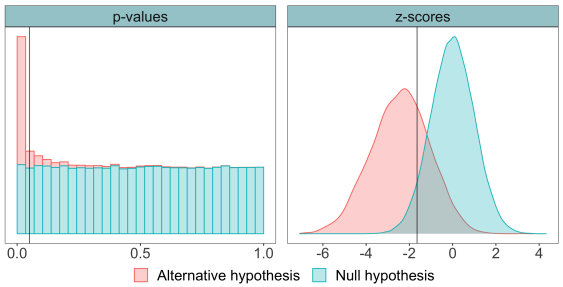
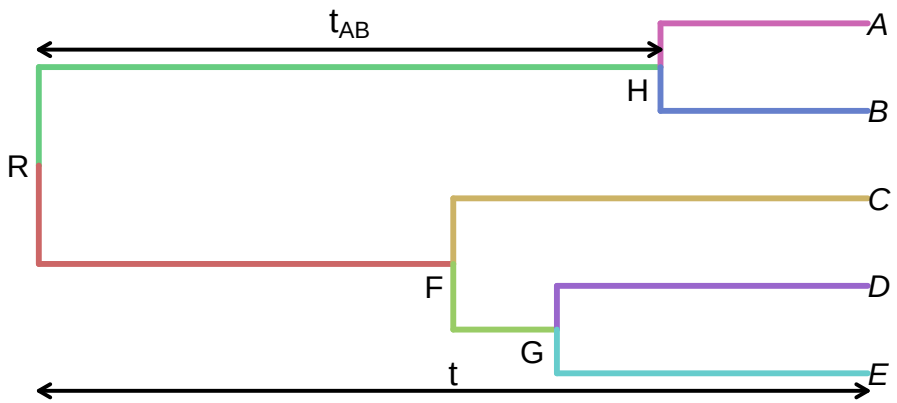
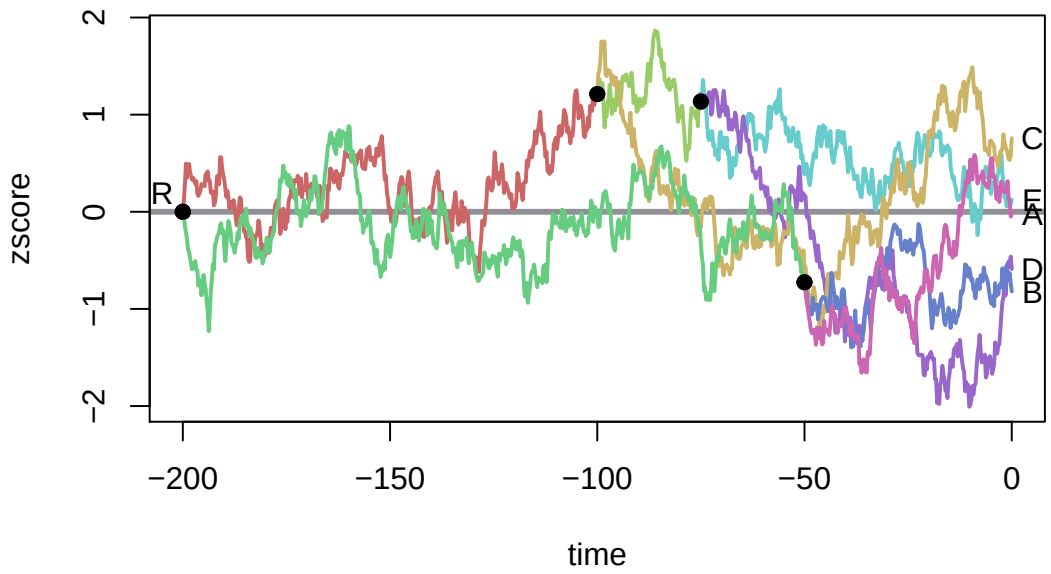


Hierarchical assumptions on z-scores

- The (z_i) are correlated according to a tree.

Stochastic Process on a Tree (Felsenstein, 1985)





The tree is known.

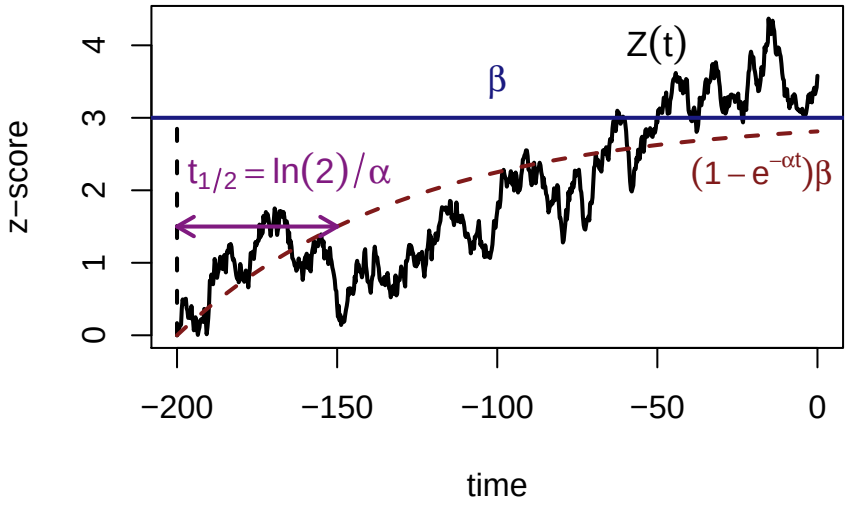
Only *tip* values are observed

Ornstein Uhlenbeck:

$$\mathbb{V}\mathrm{ar}\left[A \mid R\right] = \frac{\sigma^2}{2\alpha}(1-e^{-2\alpha t})$$

$$\mathbb{C}\mathrm{or}(A, B|R) = e^{-2\alpha(t-t_{AB})}$$

OU Modeling (Hansen, 1997)



$$dZ(t) = \alpha[\beta(t) - Z(t)]dt + \sigma dB(t)$$

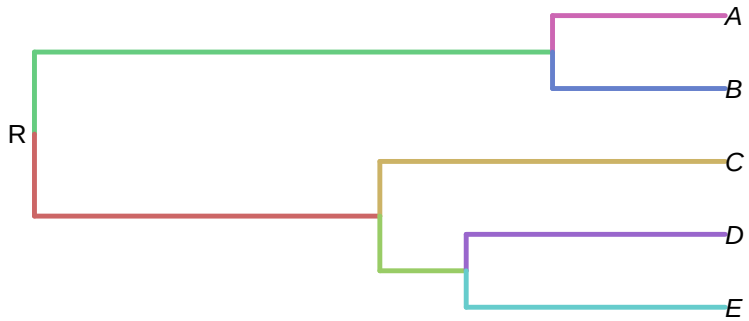
Deterministic part:

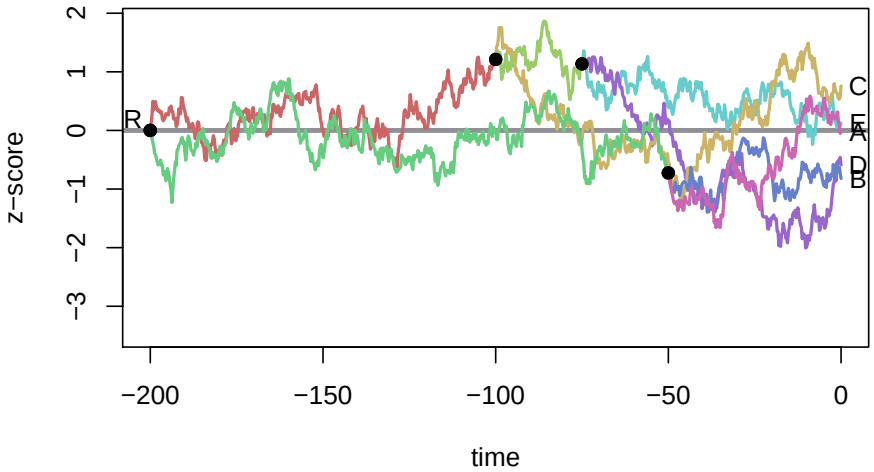
- $\beta(t)$: Effect size
- $\ln(2)/\alpha$: phylogenetic half live.

Stochastic part:

- $Z(t)$: z-scores $\simeq$ estimated effect size.
- $\sigma dB(t)$ Brownian fluctuations.

Shifts





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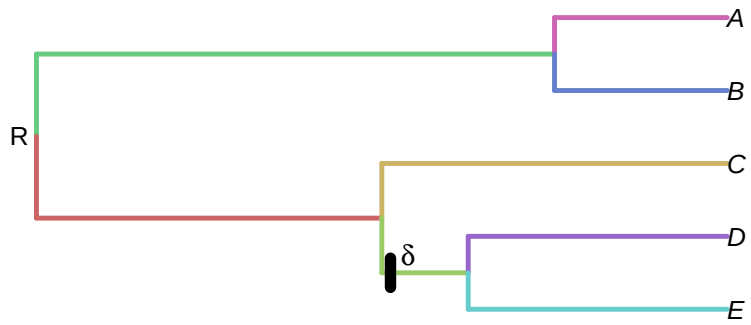

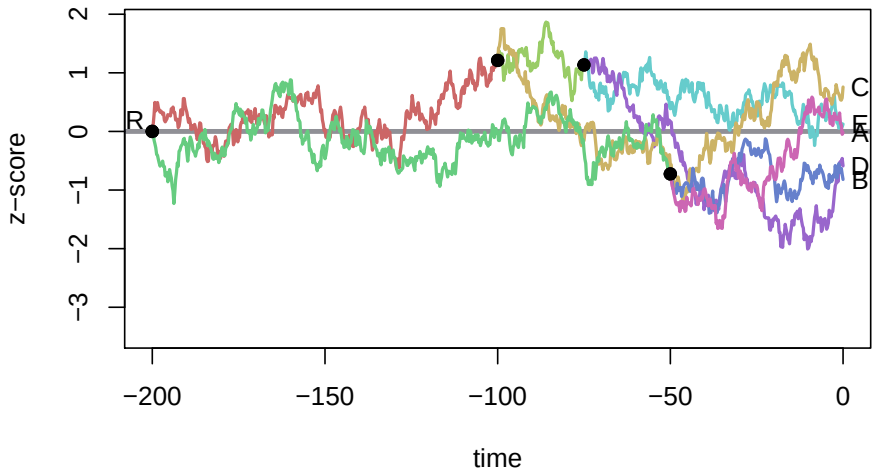

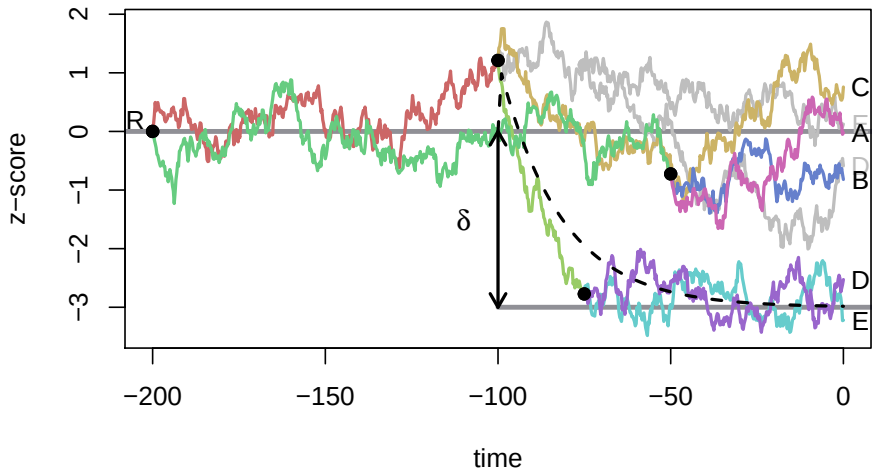

Negative shift $\Rightarrow E[Z] \leq 0 \Rightarrow$ Small p-values $\Rightarrow$ Differential abundance

Mathematical Model (Cont'd)

Statistical Model

Assume that z-scores evolve as an OU on the tree with a sign constraint on the mean.

- $Z = (z_1, \ldots, z_n) \sim \mathcal{N}(M, \Sigma_\alpha)$ where
  - $M = (m_1, \ldots, m_n) \in \mathbb{R}^n_-$
  - Σ_α is the variance matrix of an OU process on a tree.

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Goal

Find the negative entries of M

Linear regression model

Tree-structure enforced by decomposition $M = TW(\alpha)\Delta$.

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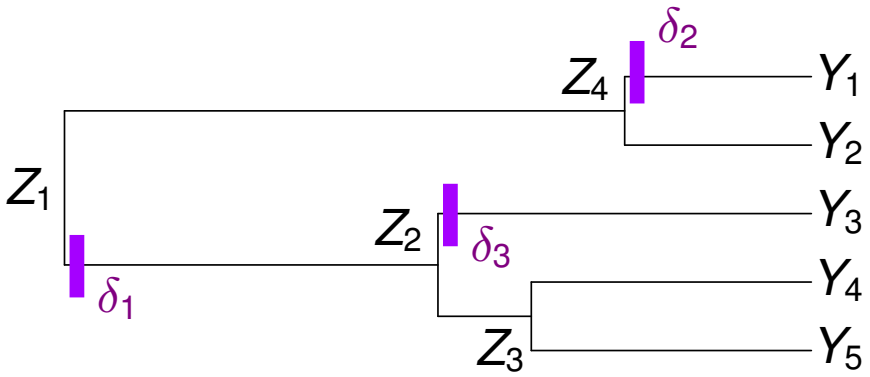

$$\Delta = \begin{pmatrix} 0 \\ \delta_1 \\ 0 \\ 0 \\ \delta_2 \\ 0 \\ \delta_3 \\ 0 \\ 0 \end{pmatrix} \qquad TW(\alpha)\Delta = \begin{pmatrix} w_5\delta_2 \\ 0 \\ w_2\delta_1 + w_7\delta_3 \\ w_2\delta_1 \\ w_2\delta_1 \end{pmatrix}$$

$$T = \begin{array}{c} \\ Y_1 \\ Y_2 \\ Y_3 \\ Y_4 \\ Y_5 \end{array} \begin{array}{c} \begin{array}{ccccccccc} Z_1 & Z_2 & Z_3 & Z_4 & Y_1 & Y_2 & Y_3 & Y_4 & Y_5 \end{array} \\ \begin{pmatrix} 1 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 1 & 1 & 0 & 0 & 0 & 0 & 1 & 0 \\ 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \end{array}$$

$$OU: \quad Z = TW(\alpha)\Delta + E$$

$$E \sim \mathcal{N}(0, V(\alpha))$$

$$W(\alpha) = \text{Diag}(1 - e^{-\alpha(h - t_{\text{pa}(i)})}, 1 \leq i \leq m + n)$$

Linear regression model

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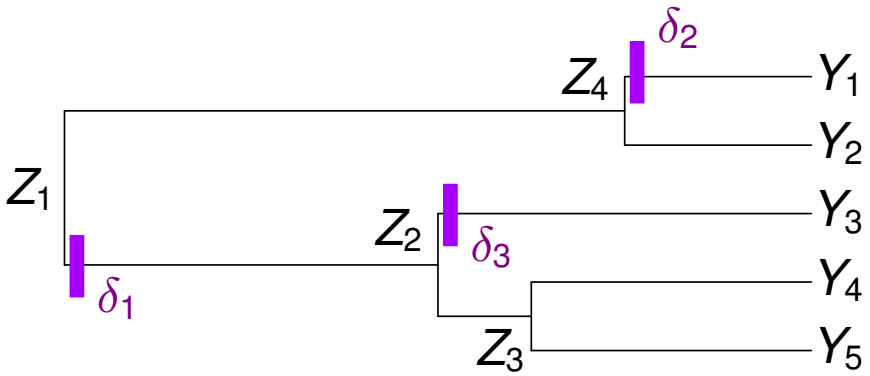

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Goal: Find $\{i : m_i < 0\}$ through $\{j : \Delta_j \neq 0\}$

Outline

Estimating M

The MLE of Δ (and in turn M) is solution to

$$\underset{\Delta \text{ s.t. } TW(\alpha)\Delta \leq 0}{\operatorname{argmax}} \|Z - TW(\alpha)\Delta\|^2_{2,\Sigma_\alpha^{-1}}$$

Equivalent to[1]:

$$\underset{\Delta \text{ s.t. } C\Delta \leq 0}{\operatorname{argmax}} \|Y - X\Delta\|^2_2$$

[1] with C, Y and X some simple transforms of Z and $TW(\alpha)$, Σ_α

[2] Using a variant of the LASSO shooting algorithm

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Add a ℓ_1-penalty to sparsify the solution and solve[2]

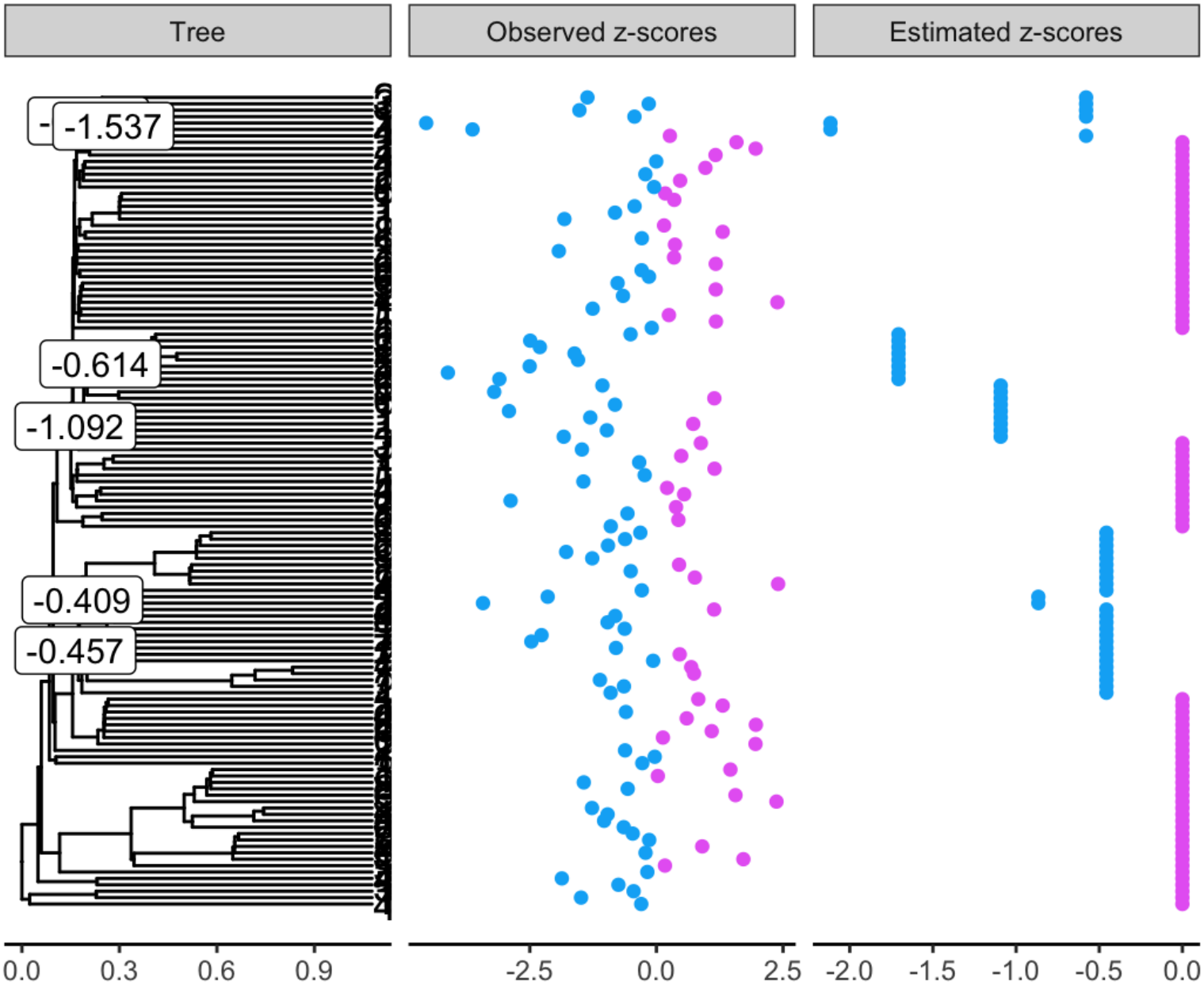
$$\hat{\Delta} = \underset{\Delta \text{ s.t. } C\Delta \leq 0}{\operatorname{argmax}} \|Y - X\Delta\|_2^2 + \lambda\|\Delta\|_1$$

using penalized likelihood for selection of α and λ

[1] with C, Y and X some simple transforms of Z and $TW(\alpha)$, Σ_α

[2] Using a variant of the LASSO shooting algorithm

Illustration



Confidence intervals for m_i

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Debiasing (Zhang and Zhang, 2014)

- One-step correction of the LASSO estimator

$$\hat{\Delta}_j^{debias} = \hat{\Delta}_j + \frac{S_j^\top (Y - X\hat{\Delta})}{S_j^\top X_j}$$

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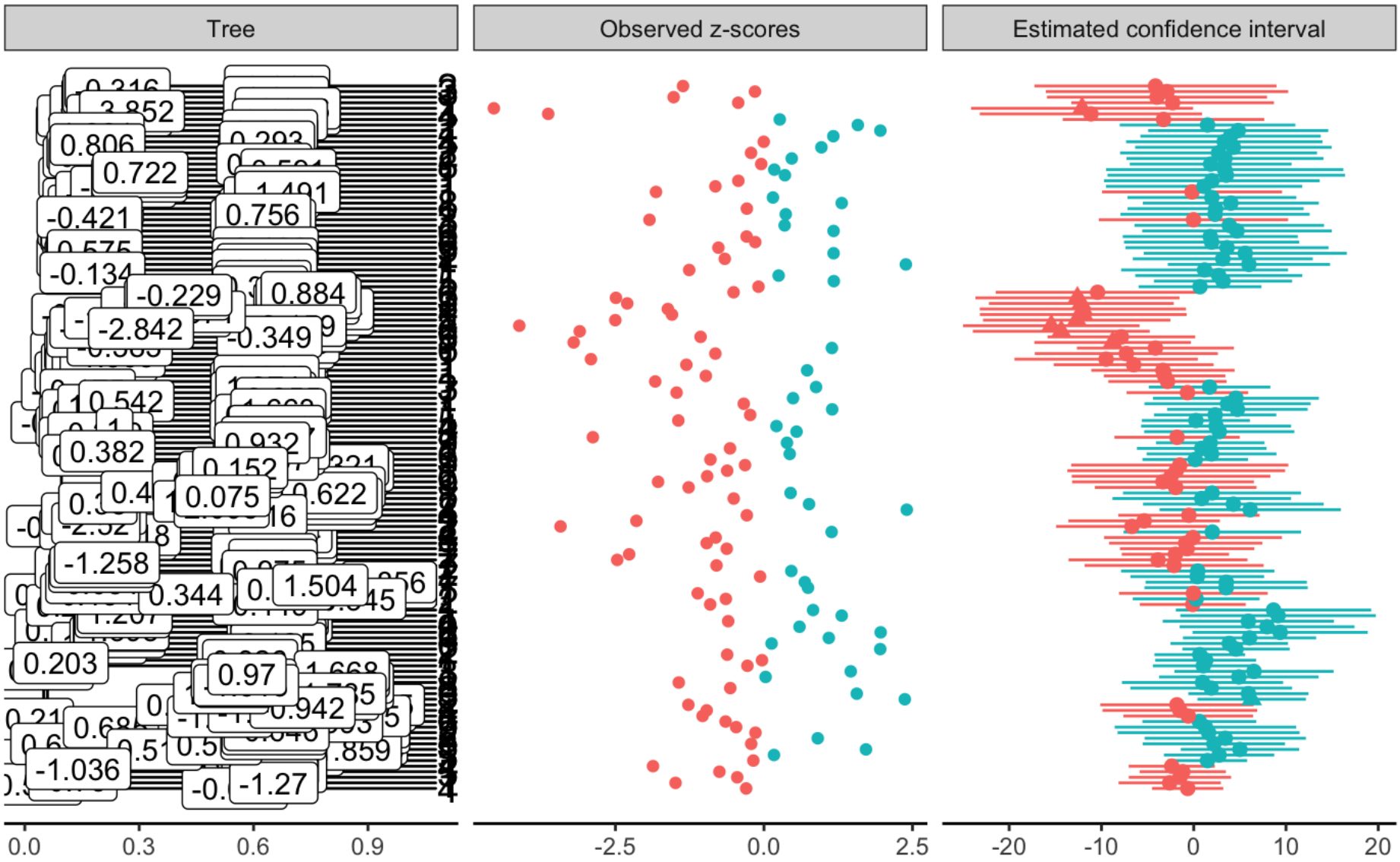
Confidence intervals (Javanmard et al., 2019)

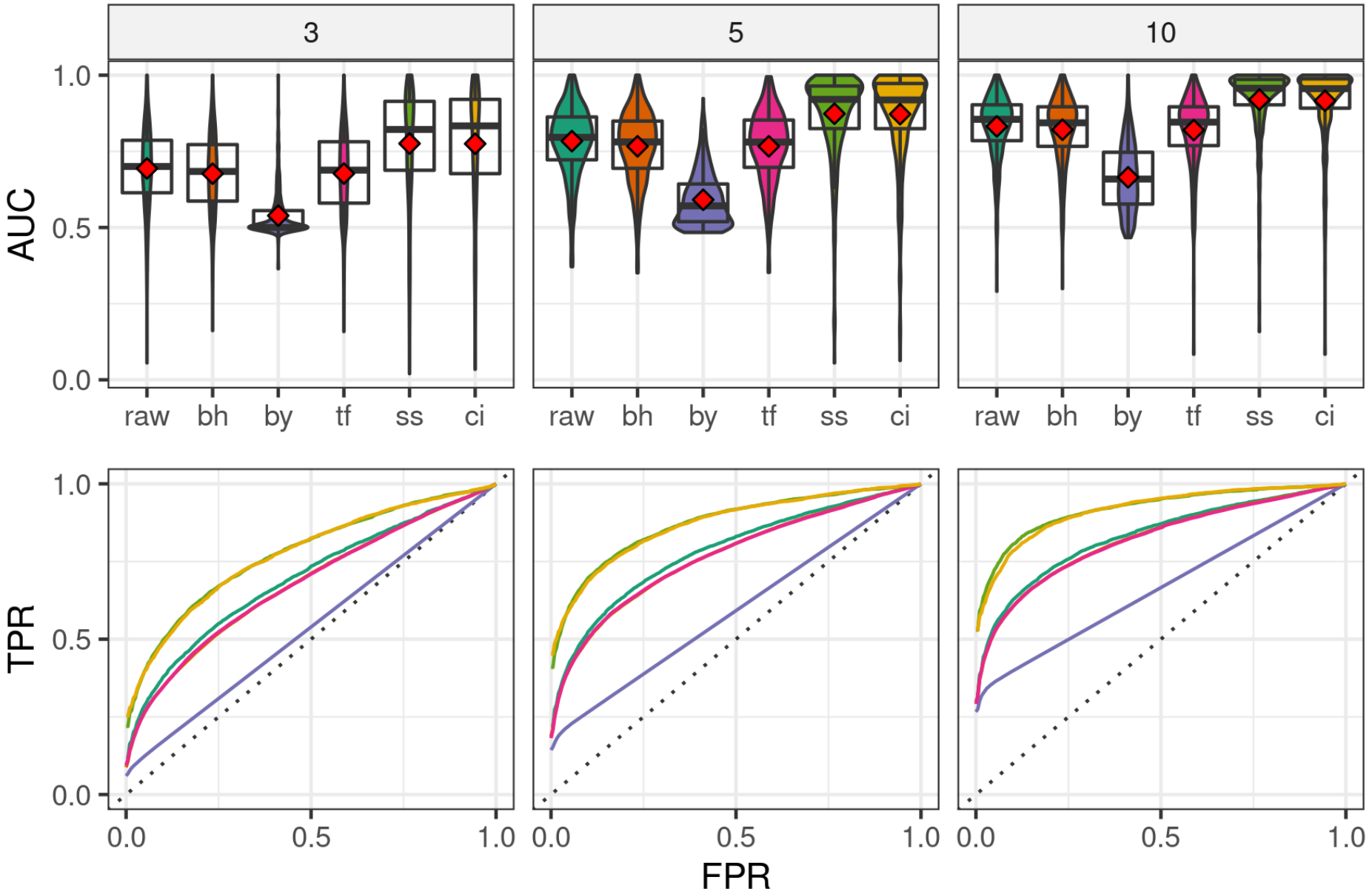
- BH-like procedure based on asymptotic normality of the $\hat{\Delta}_j^{debias}$

Illustration

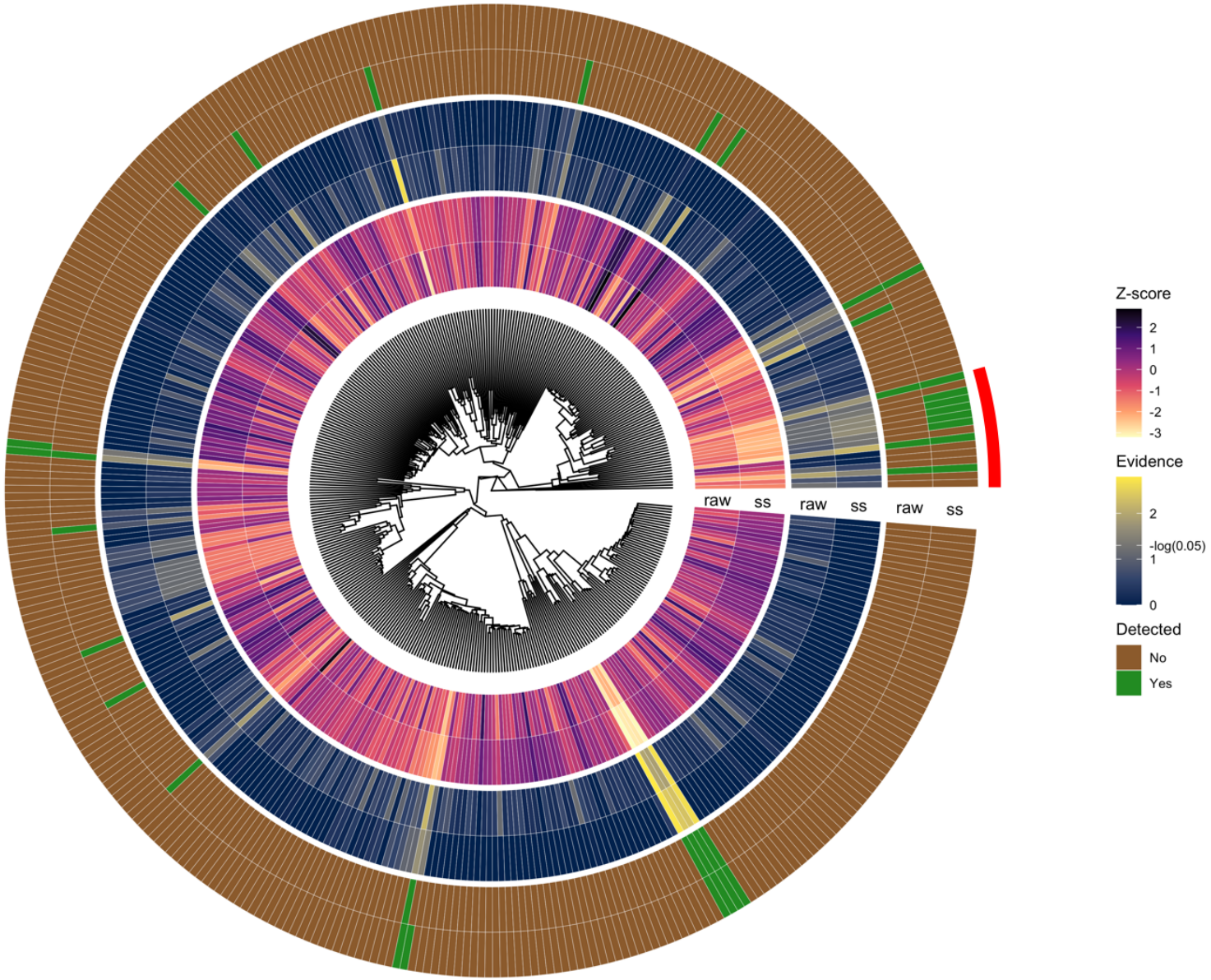


Outline

Simulation results: zazou (ss/ci) is competitive



Fiji adult and children gut microbiome (Brito et al., 2016)



Conclusion

- A framework for differential analyses that integrates taxonomic information
- Based on combining LASSO, trees and stochastic processes
- That performs well on simulations
- Implemented as a github R package (`abichat/zazou`)
- Technical details available in Bichat et al. (`arXiv:2009.13335`)

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