kWIP: The k-mer Weighted Inner Product

And the rest of my PhD...

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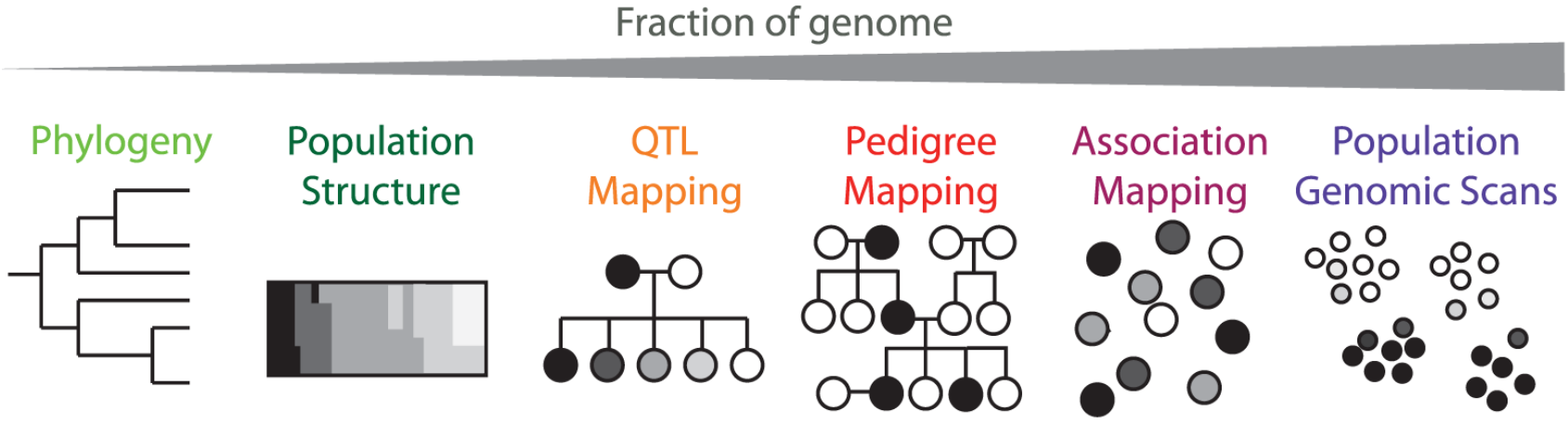
April 15, 2016

Large-scale population genomics

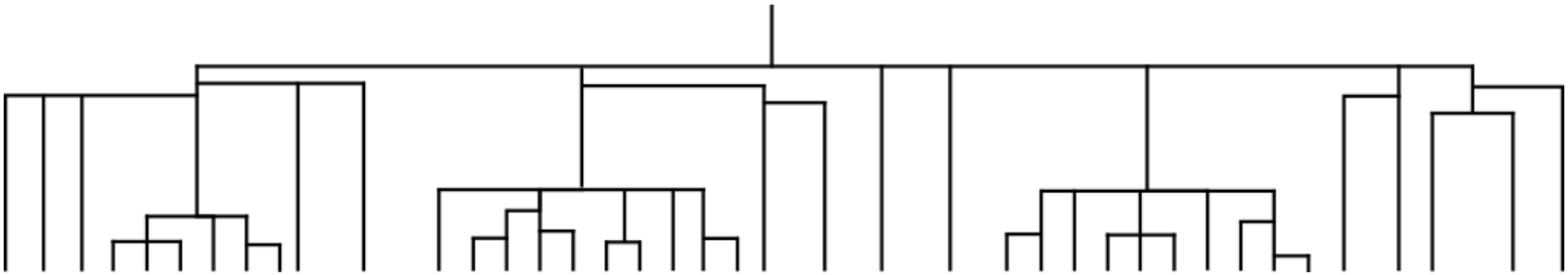
- Moving from 100s to 1,000s or 10,000s of samples *per study!*
- Efficient algorithms to analyse large-scale genomic data
  - Reference & alignment free: *less bias, de novo*
  - Platform/protocol agnostic: *future proof*
  - Computationally efficient: *not the bottleneck*



after Peterson *et al.* [1]

Genetic Similarity Estimation

- Rough approximation of sample relatedness required
  - For natural collections
  - As a technical control



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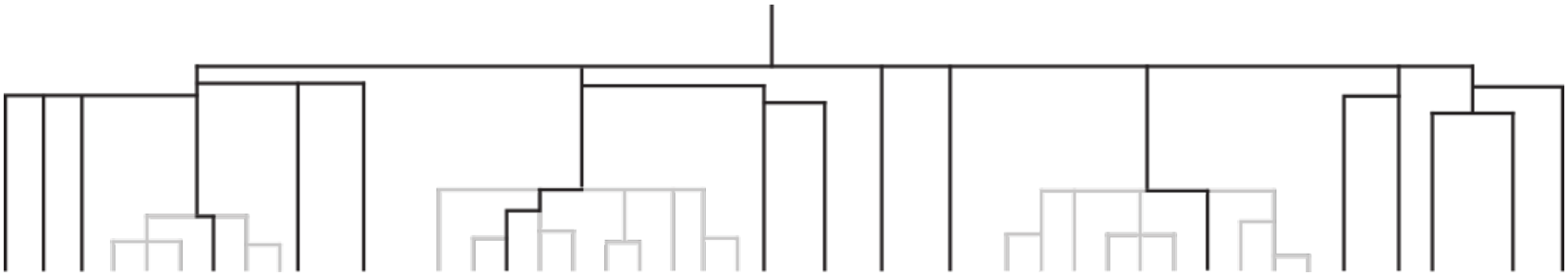

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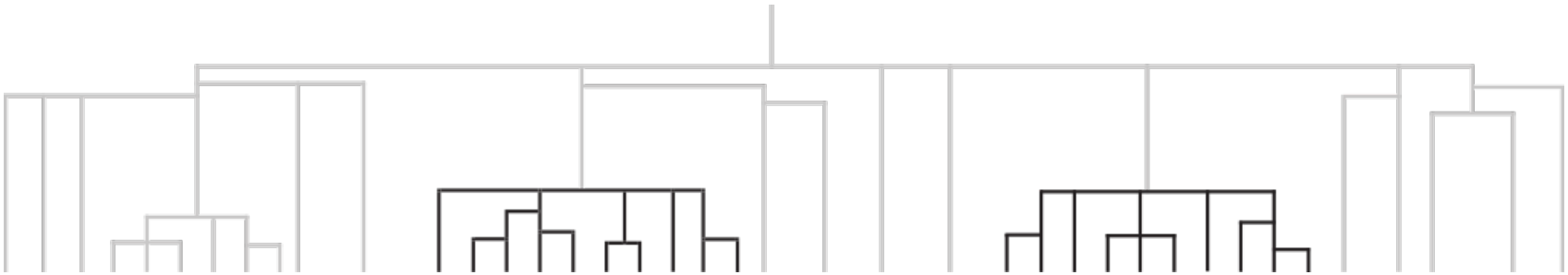

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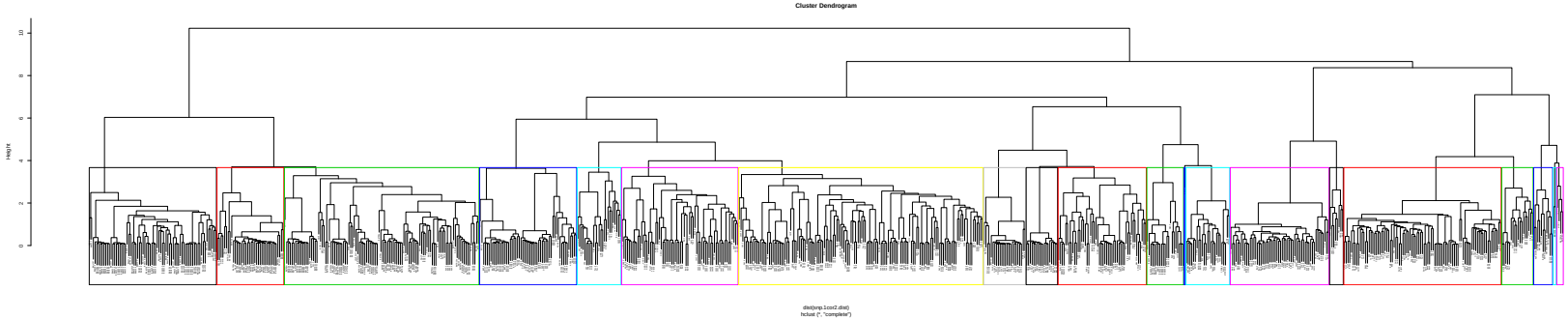

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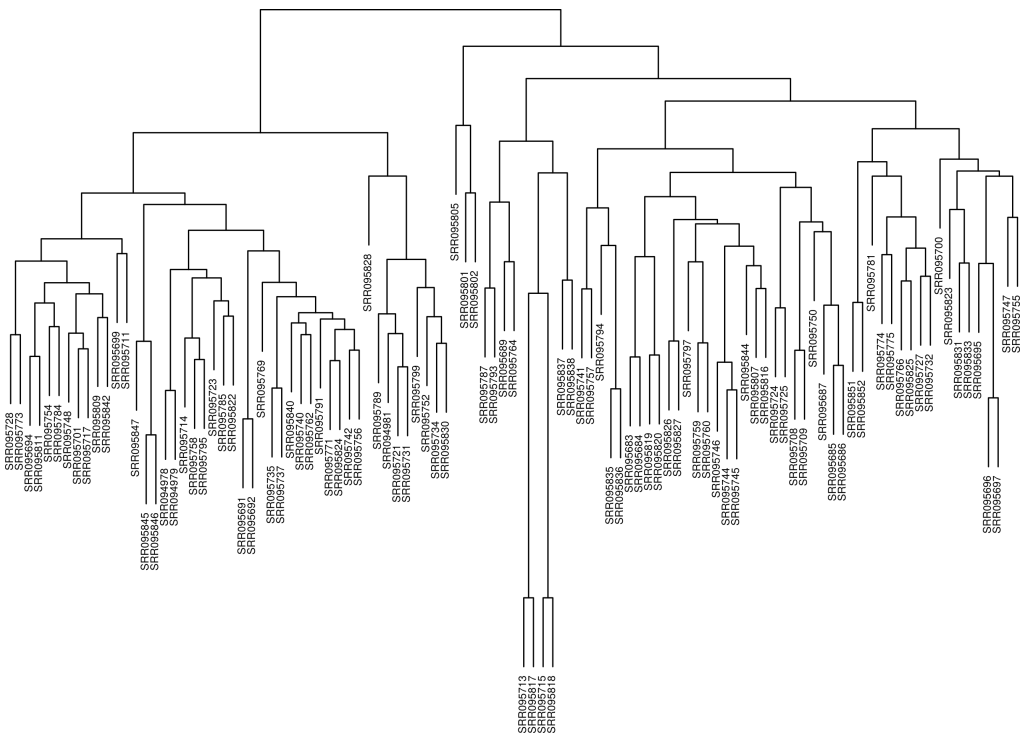
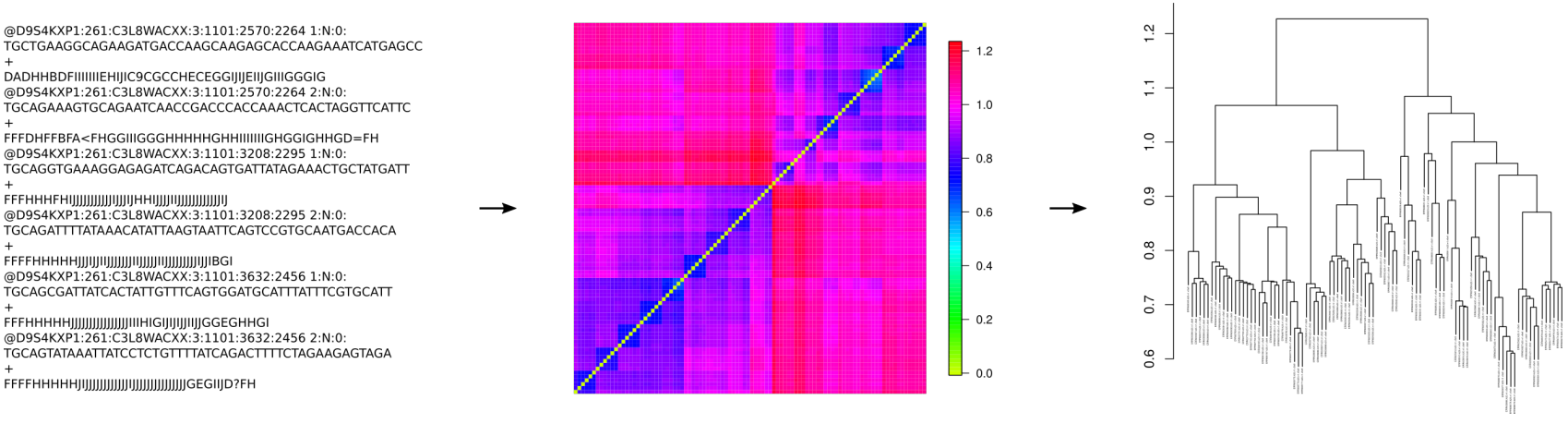


Genetic Similarity Estimation

Initially, we care mostly about the deepest and shallowest branches of the tree.

Presenting kWIP

- k-mer based *de novo* genetic relatedness estimator
- Produces a distance matrix from raw NGS reads
- Uses Weighted Inner Product between k-mer counts

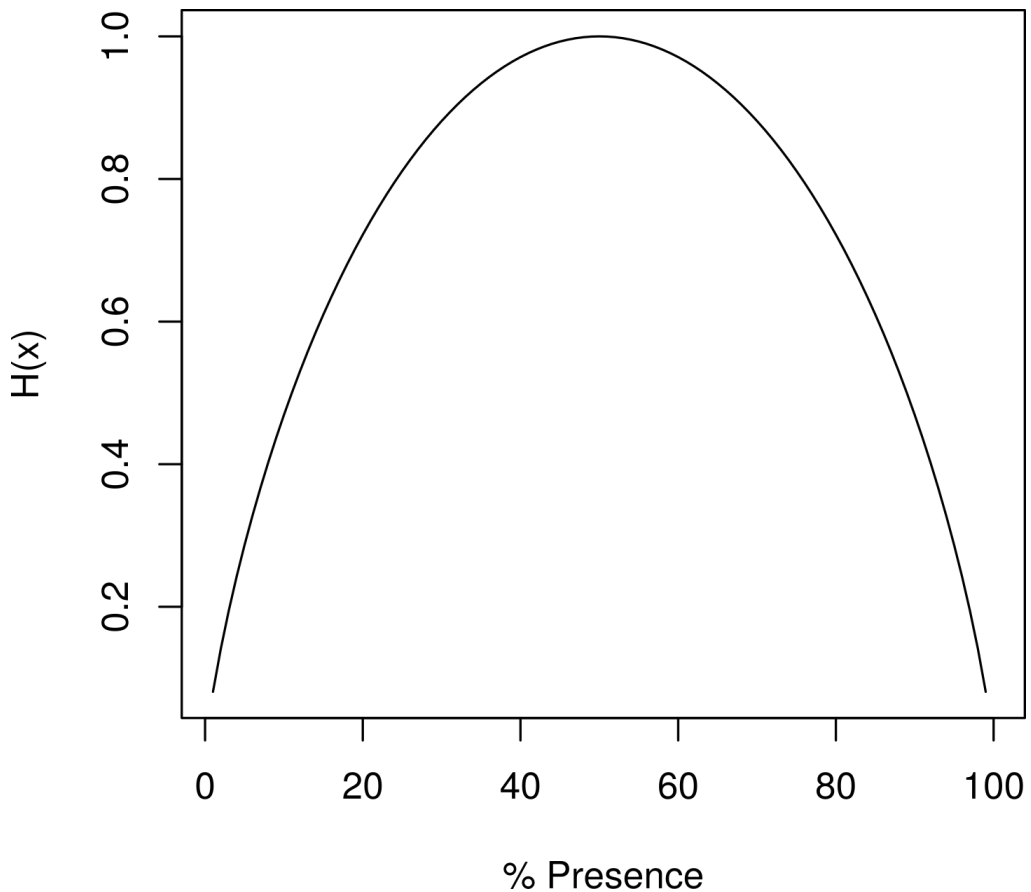


k-mer Sequence Comparison

- Many existing tools
  - $D2$ and related statistics
  - Early steps in many sequence aligners
  - `spaced` and other spaced-word approaches[3,4]
  - `Cnidaria` and other Jaccard distance approaches[5]
  - `mash` and other MinHash approaches[6]
- Most require assembled gene/genome sequence
- Most target deeper relationships
- Many use inner product between k-mer counts
- `kWIP` extends these tools:
  - No assembly required
  - Weights inner product to improve accuracy

Entropy Weighting

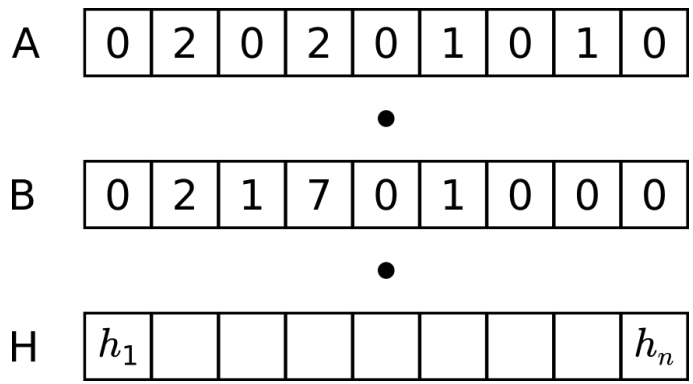
- `kWIP` weights by Shannon entropy: $H(frequency)$
- Shannon entropy: measure of information
- $-(P_i log_2(P_i) + (1 - P_i) log_2(1 - P_i))$



kWIP Algorithm

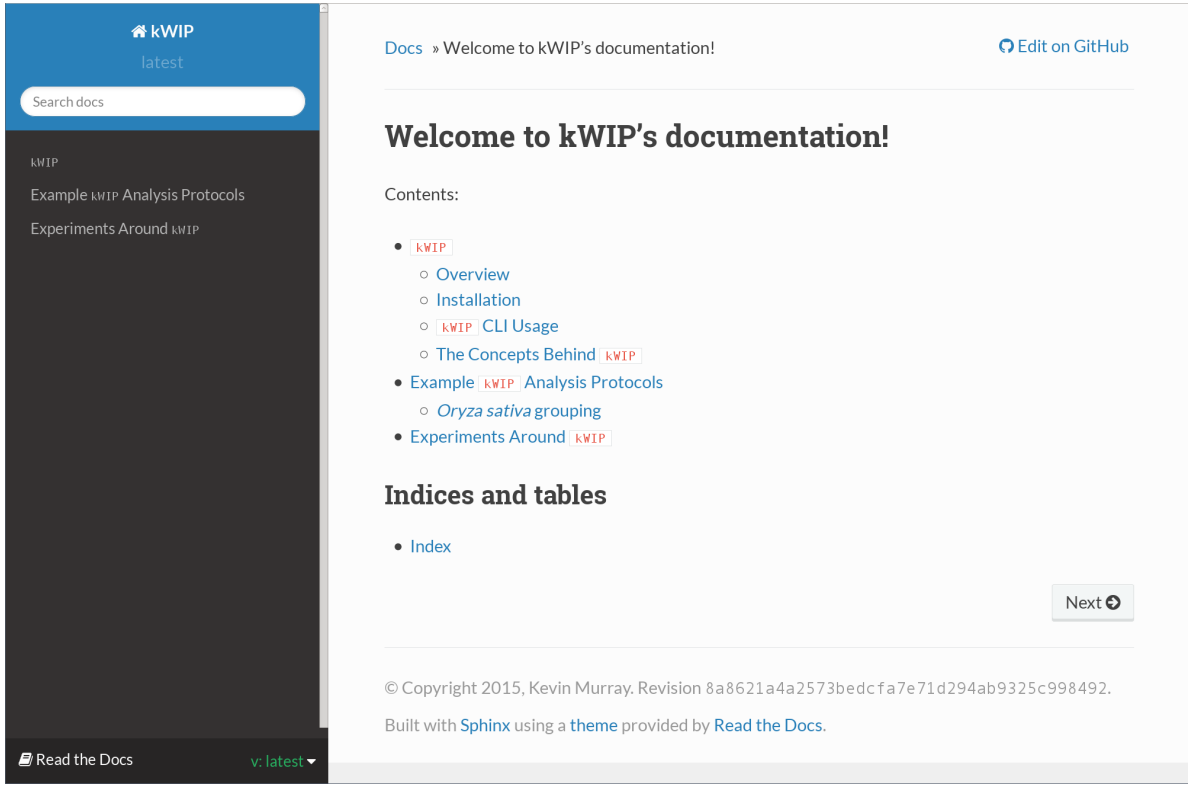
- For each run: count all k-mers probabilistically
- For each analysis set:
  - Calculate the entropy of k-mer frequency (H)
  - For each pair of runs with k-mer counts A and B, calculate

$$\sum_{i=1}^{n} A_i \cdot B_i \cdot H_i$$



kWIP

- The software:
  - C++11, ≈2000 lines of code
  - Uses **khmer** for k-mer counting
  - GNU GPL licensed, source code on GitHub
  - Precompiled binaries provided



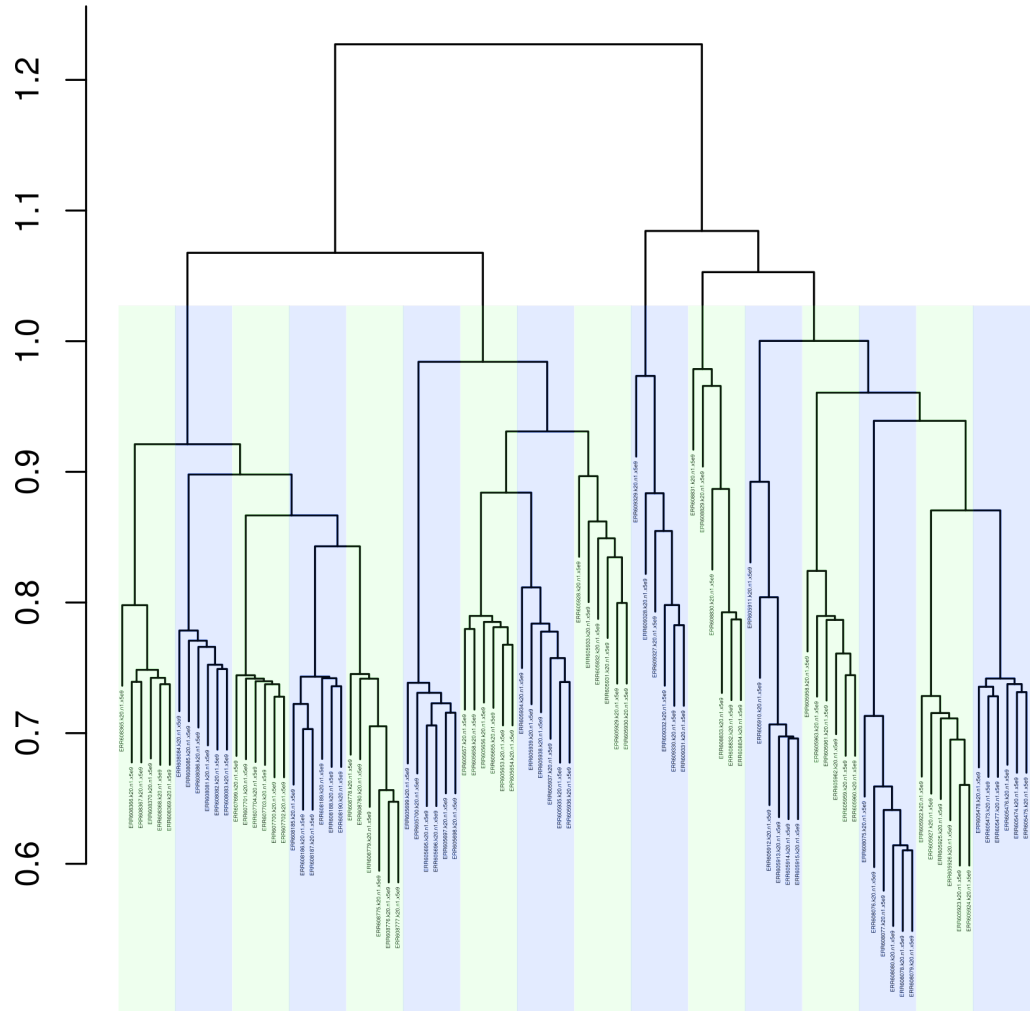
kWIP Case Studies

- 3000 rice genomes
  - 3000 rice samples (25k runs)
  - The 3,000 rice genomes project [7]
- Chlamydomonas
  - $\approx$ 20 lines from USA
  - Flowers *et al.* [8]
- Simulation
  - Fake population genome sequencing studies

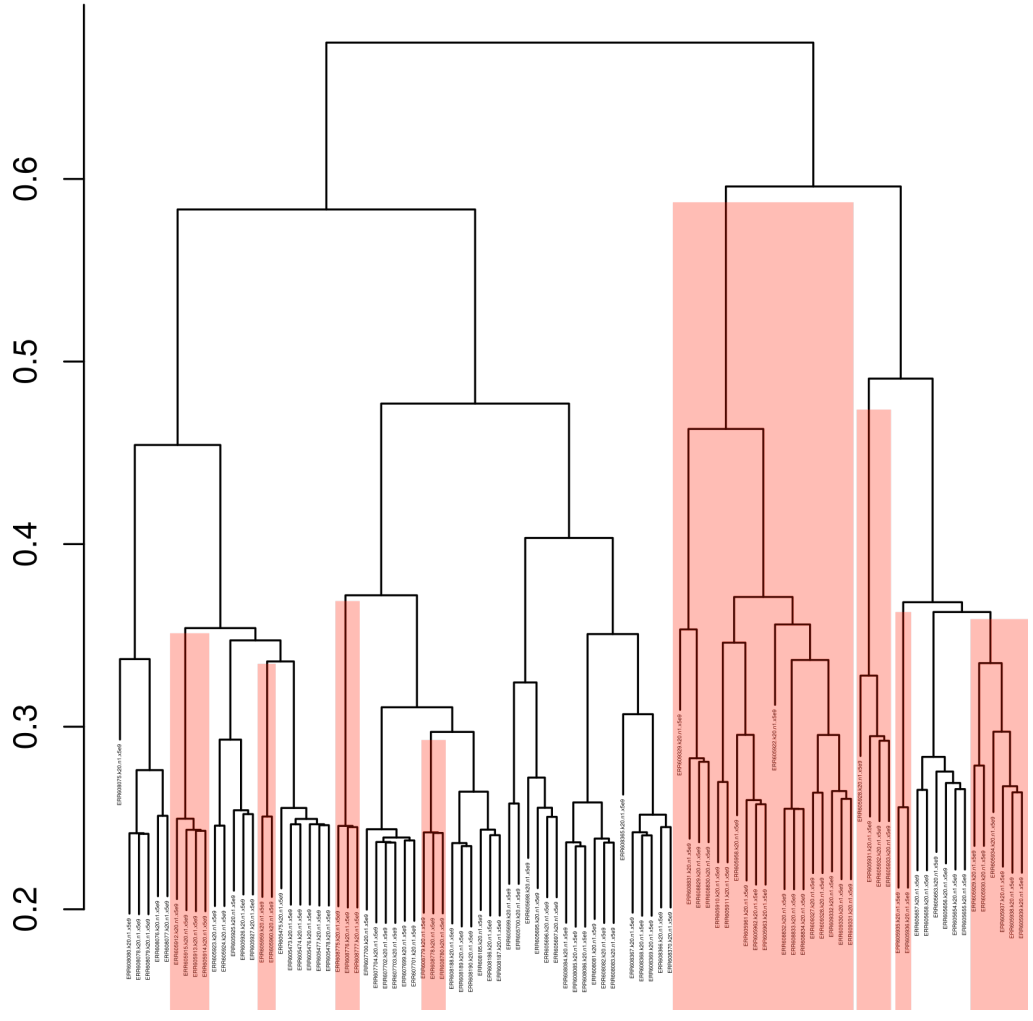
96 Rice Runs

- Set of 96 rice runs from 16 samples (6 tech reps ea)
- About half/half from 2 major groups (Indica, Japonica)
- Expectations:
  - All runs cluster into groups of 6 reps (16 samples)
  - Big split between two groups: (7 and 9 respectively here)
- Recover known grouping w/ `kWIP`, not w/ unweighted IP
- Sensitive to read depth
- Took 6 hours on 16 CPU, 64GB RAM supercomputer node

WIP



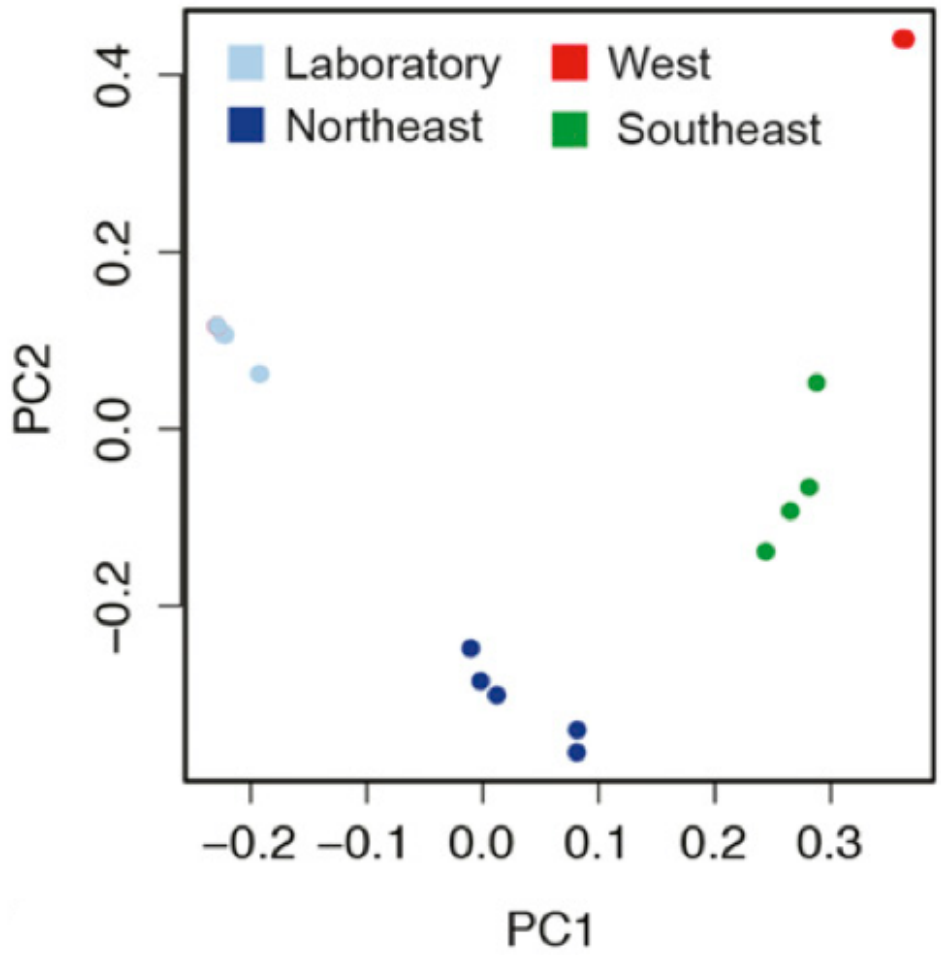
IP



Chlamydomonas

- High coverage re-sequencing with leftover assembly
  - Map to reference
  - Assemble missing sample genome from leftover reads
  - Map again to reference + leftovers
  - Call variants
  - Calculate distance
- Compare kWIP to SNP-based distance calculation
  - Compare PCA visualisation of each

Chlamydomonas

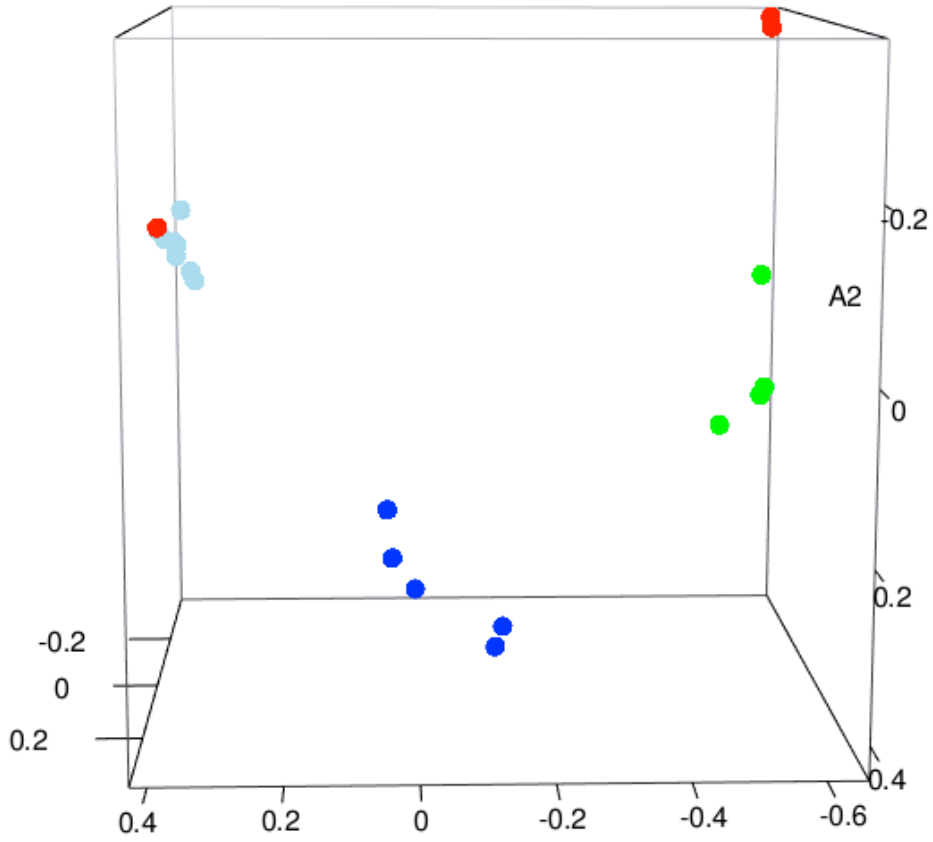


"Sample CC-4414 (red) is hidden behind the cluster of laboratory strains (light blue)"

Population structure of *Chlamydomonas* in USA

Data from Flowers *et al.* [8]

Chlamydomonas



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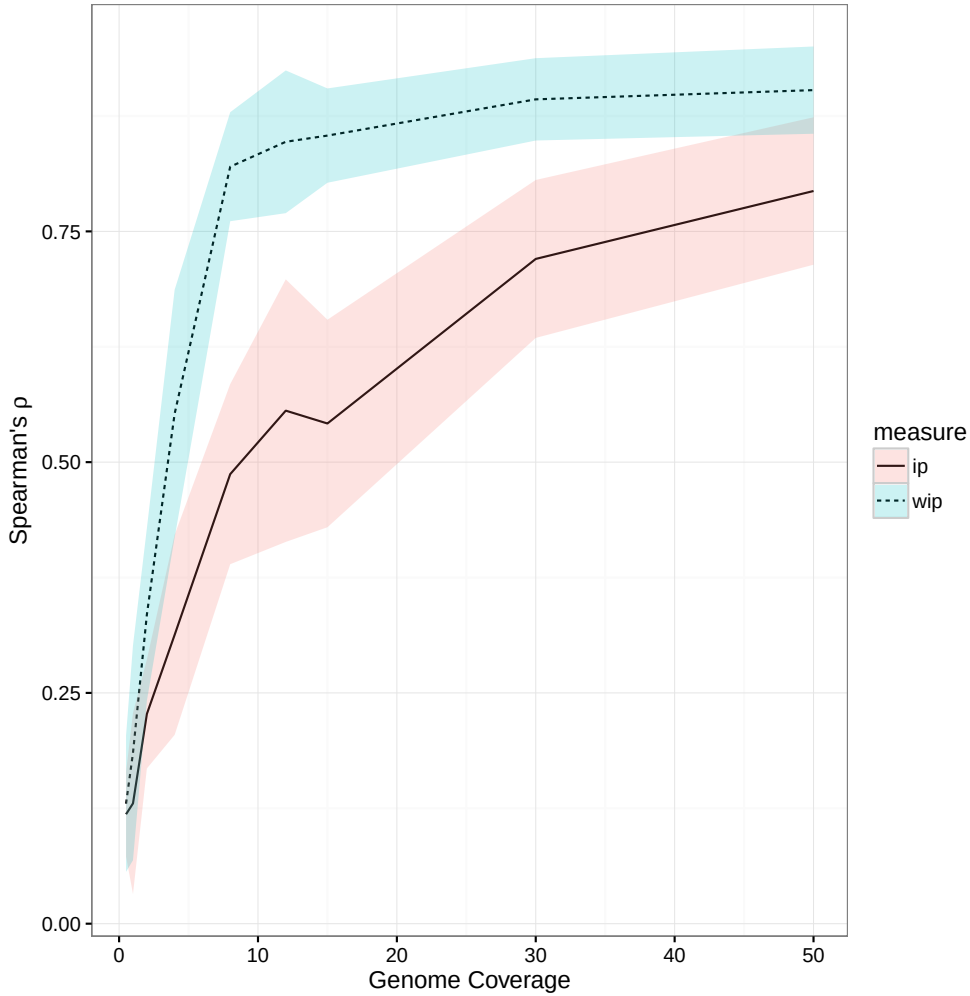
Simulation

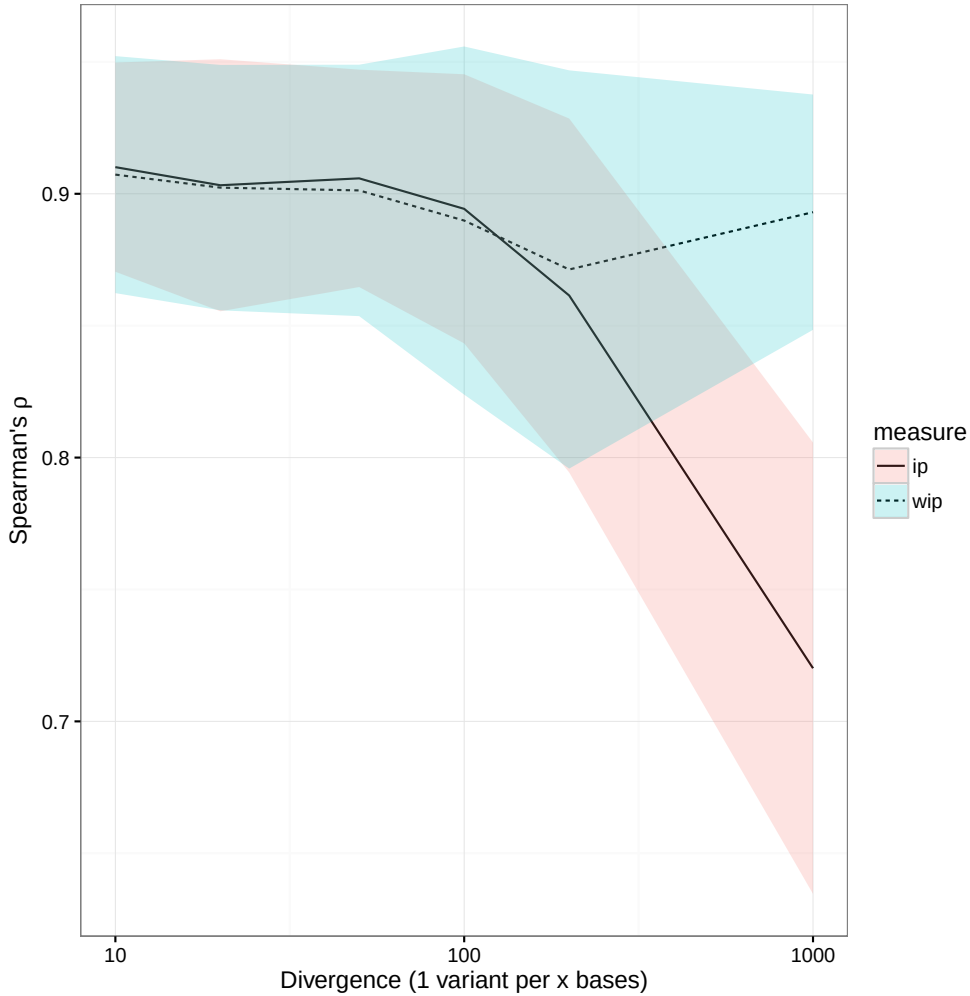
- Perform simulated sequencing experiment:
  - Simulate natural population structure
  - Simulate sample genomes, sequencing runs
  - Hash reads, kWIP
  - Compare known truth to kWIP results
    (with Spearmans Rank Correlation, ρ)
- kWIP quantitatively outperforms unweighted equivalent
  - Effect of coverage on accuracy
  - Accuracy across scale of variation

Simulation Results



Coverage vs Accuracy

Simulation Results



Average variation vs Accuracy

kWIP Summary

- kWIP is implemented, no known bugs
- Publicly available at github.com/kdmurray91/kwip
- We show the utility of kWIP
- Publication coming soon

What's next?

- Finish `kWIP` paper
- Complete & Publish GBS analysis tools
  - GBS is a quick & cheap sequencing method
  - Have written improved analysis tools
  - These need publication

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- Further k-mer analysis methods
  - Marrying assembly and k-mer comparison concepts
  - Pan-genome representations
- *Eucalyptus* population genomics
  - Collaboration with Rose Andrew @ UNE
  - Extending a pilot study in a hybrid population of *E. sideroxylon* and *E. albens*.

Thanks

- My `kWIP` collaborators
  - Norman Warthmann, Christfried Webers, Cheng Soon Ong, Sylvain Forêt
- Supervisors:
  - Justin Borevitz, Gavin Huttley, Barry Pogson, Sylvain Forêt
- `khmer` folks (DIB-lab, UC Davis)
- Yourselves

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