kWIP: The k-mer Weighted Inner Product

Estimating genetic similarity of sequencing runs

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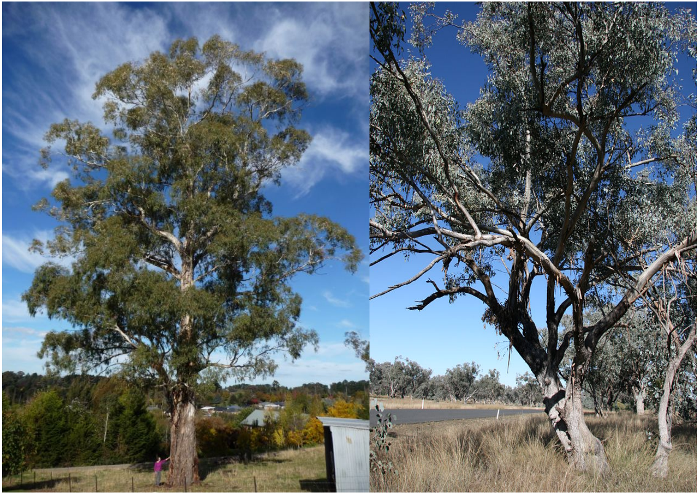
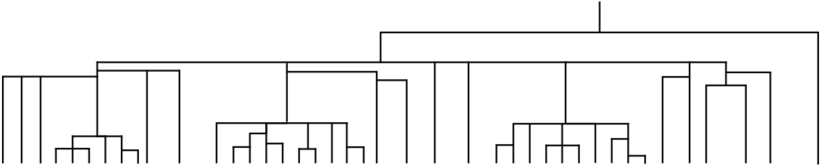
Natural Genetic Variation



Collection Re-structuring

- Collect 100s or 1,000s of natural samples
- “First look” at genetic relatedness
  - Assert replicates cluster, detect mixups
  - Carry best samples to detailed analysis



after Brachi *et al.* [1]

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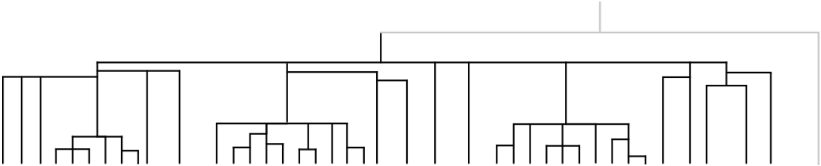

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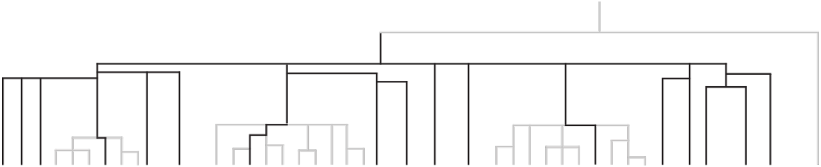
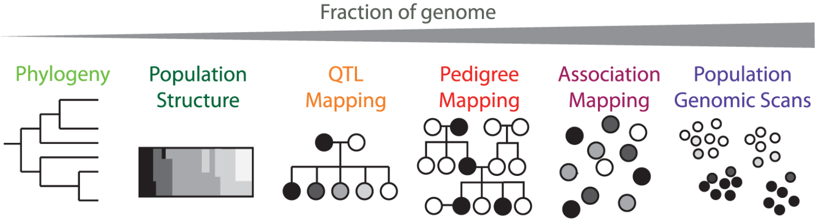


after Brachi *et al.* [1]

(Initial) Genetic Similarity Estimation

- Initial genetic analyses inspect
  - Outgroups (widest relationships)
  - Replicates
  - Mix-ups
  - Broad groupings
- Current genetic similarity, *not evolutionary history*



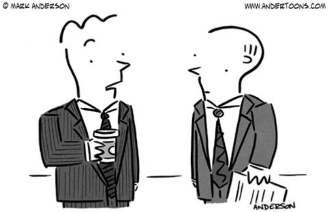
after Peterson *et al.* [2]

Genetic Similarity Estimation – Algorithms

- 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*

Alignment-free Sequence Comparison

- Many existing metrics, and tools
  - $D2$ and related statistics
  - `spaced` and other spaced-word approaches[3,4]
  - `Cnidaria` and other Jaccard index approaches[5]
  - `mash` and other MinHash approaches[6]
- Most require assembled gene/genome sequence
- **Most assume evolutionary history between samples**



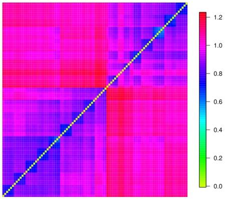
"You ever have one of those days where you just don't feel like aligning?"

Presenting kWIP

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

```
@D9S4KXP1:261:C3L8WACXX:3:1101:2570:2264 1:N:0:
TGCTGAAGGCAGAAGATGACCAAGCAAGAGCACCAAGAAATCATGAGCC
+
DADHHBDFIIIIIIIEHIJIC9CGCCHECEGGIJIJEIIJGIIIGGGIG
@D9S4KXP1:261:C3L8WACXX:3:1101:2570:2264 2:N:0:
TGCAGAAAGTGCAGAATCAACCGACCCACCAAACTCACTAGGTTCATTC
+
FFFDHFFBFA<FHGGIIIGGGHHHHHGHHIIIIIIIGHGGIGHHGD=FH
@D9S4KXP1:261:C3L8WACXX:3:1101:3208:2295 1:N:0:
TGCAGGTGAAAGGAGAGATCAGACAGTGATTATAGAAACTGCTATGATT
+
FFFHHHFHIJJJJJJJJJJJIJJJJIJHHIJJJJIIJJJJJJJJJJJJIJ
@D9S4KXP1:261:C3L8WACXX:3:1101:3208:2295 2:N:0:
TGCAGATTTTATAAACATATTAAGTAATTCAGTCCGTGCAATGACCACA
+
FFFFHHHHHJJJIJJIIJJJJJJJIIJJJJJIIJJJJJJJJJJIJJIBGI
@D9S4KXP1:261:C3L8WACXX:3:1101:3632:2456 1:N:0:
TGCAGCGATTATCACTATTGTTTCAGTGGATGCATTTATTTCGTGCATT
+
FFFHHHHHJJJJJJJJJJJJJJJIIIHIGIJIJIJJIIJJGGEGHHGI
@D9S4KXP1:261:C3L8WACXX:3:1101:3632:2456 2:N:0:
TGCAGTATAAATTATCCTCTGTTTTATCAGACTTTTCTAGAAGAGTAGA
+
FFFFHHHHHJIJJJJJJJJJJJJJIJJJJJJJJJJJJJJJGEGIIJD?FH
```

→



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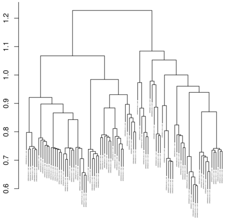
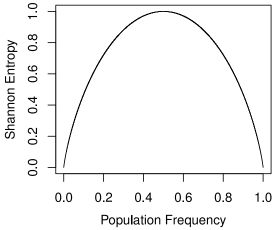


`kWIP` Algorithm

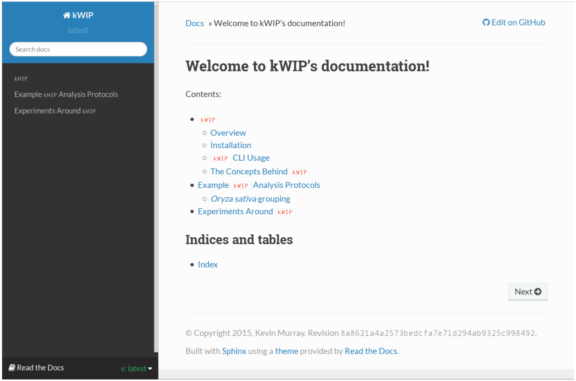
- Count each sample's *k*-mers probabilistically (**`khmer`**)
- Calculate information content of each *k*-mer
- Compute each pairwise distance using weighed inner product (WIP)

Sketch 1	4	1				1	6			1	1
Sketch 2	1					2	3			1	
Sketch 3		2		2		1		1			2
Sketch 4		2	1	7		1					2
Sketch 5	1	4				12	6			9	6
Frequency Sketch	0.6	0.8	0.2	0.4	0	1	0.6	0.2	0	0.6	0.8



kWIP – Software

- Uses khmer for k-mer counting
- Parallelised with OpenMP
- GNU GPL licensed, C++11 source code on GitHub
- Precompiled binaries provided
- Documentation & tutorials online



kWIP

latest

Search docs

kWIP

Example kWIP Analysis Protocols

Experiments Around kWIP

Read the Docs v: latest

Docs » Welcome to kWIP's documentation!

Edit on GitHub

Welcome to kWIP's documentation!

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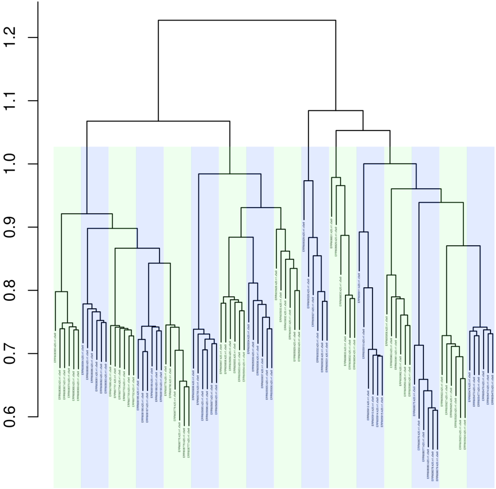
kWIP Case Studies

- Rice genomes project[7]
  - 3000 rice varieties (25k runs)
  - ≈ 2-fold sequencing per run
- Population genomics – Chlamydomonas[8]
  - High-coverage sequencing of 20 wild & lab strains
- Rice root-associated microbiome metagenomics
  - Shotgun sequencing of root-soil interface
- Simulation
  - Fake population genome sequencing studies

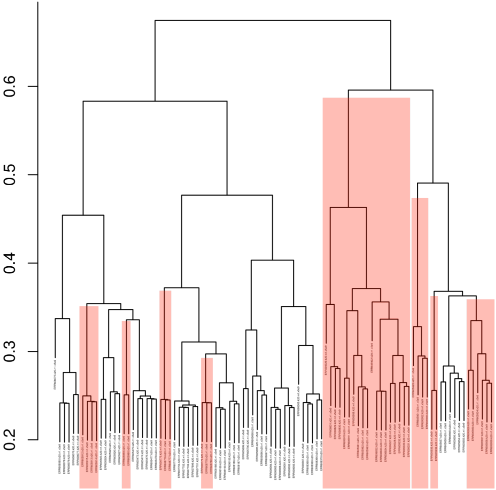
Replicate and Subspecies Clustering

- Set of 96 rice runs from 16 samples, 6 tech reps.
- ≈ 3-fold sequencing per run
- Expectations:
  - All runs cluster into samples of 6 reps
  - Big split between 2 major groups (Indica, Japonica)
- Recover known grouping w/ `kWIP`, not w/ unweighted IP
- Took 6 hours on 16 CPU, 64GB RAM supercomputer node
- Similar patterns observed over 100s of similar subsets

WIP

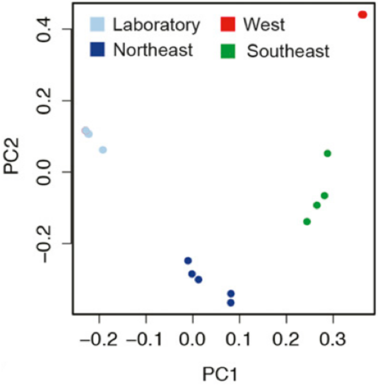


IP



Chlamydomonas

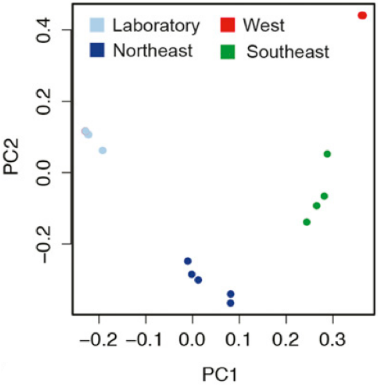
- Avoid reference bias with "leftover assembly"[8]
  - Sequence *very* deep (> 200x)
  - Map to reference
  - Assemble umapped reads
  - Map to reference + leftovers
  - Call variants
  - `SNPrelate` + PCA

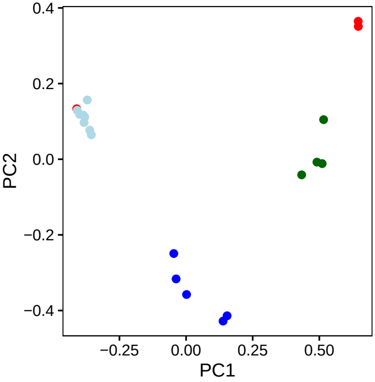


"Sample CC-4414 (red) is hidden behind the cluster of laboratory strains (light blue)"

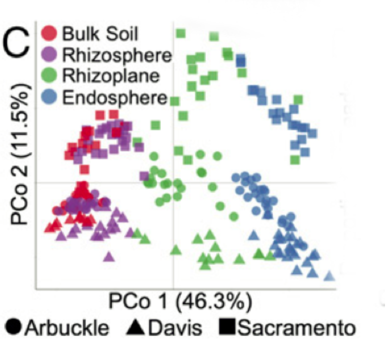
Chlamydomonas – with `kWIP`

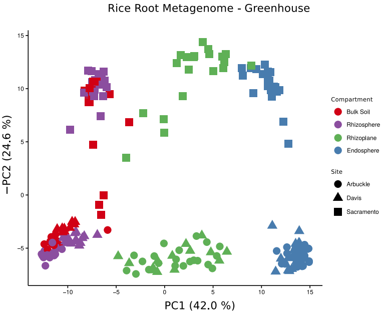
- Download SRA files
- Count k-mers
- Run `kWIP`





Metagenomes

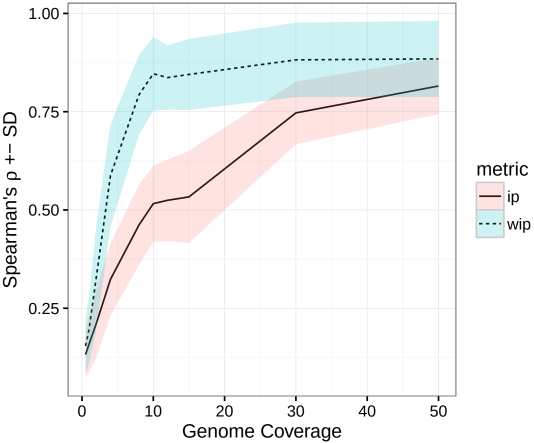




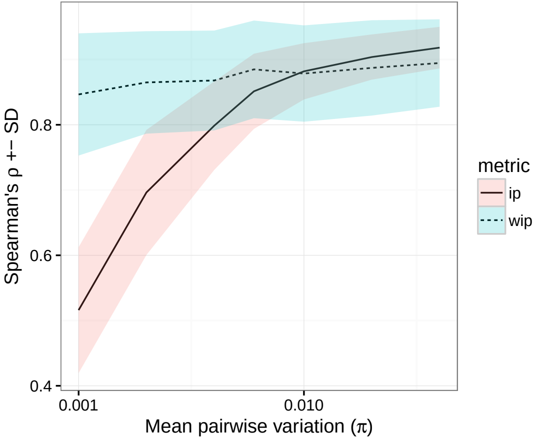
Simulation

- Perform simulated sequencing experiment (50 times):
  - Simulate natural population structure
  - Simulate sample genomes
  - Simulate sequencing runs (with random variation)
  - Sketch reads, `kWIP`
  - Compare `kWIP` results to known truth
  - Spearmans Rank Correlation (ρ), “Performance”
- kWIP quantitatively outperforms unweighted equivalent
  - Performs reasonably at low-moderate coverage
  - Performance stable across scale of variation

Performance vs Coverage



Performance vs Mean pairwise distance (π)



kWIP Summary

- `kWIP` is implemented, production ready
- Publicly available at `github.com/kdmurray91/kwip`
- Publication in review at PLoS Comp. Biol. (`bit.do/kwip`)
- Version 2 on the way
  - MPI parallel
  - More metrics
  - Even faster

Thanks

- Norman Warthmann, Justin Borevitz
- Christfried Webers, Cheng Soon Ong
- Sylvain Forêt
- AB^3ACBS Organisers and Yourselves

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