

Genomic data for evolutionary inference

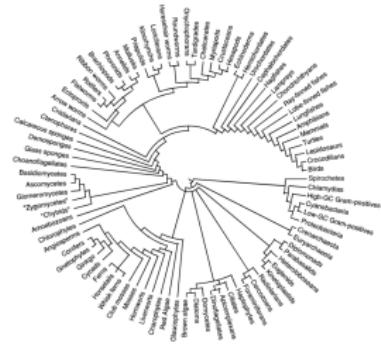
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How do you get from



to

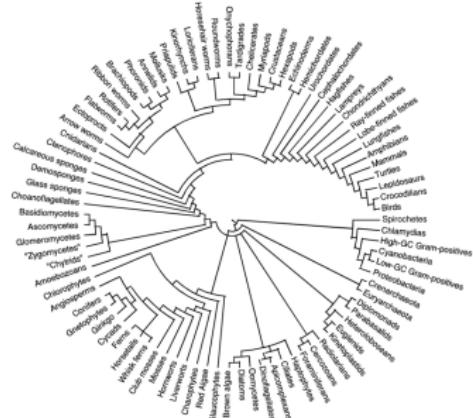


?

You've seen a lot about how to get from

```
A G C T T A C T T A A T T C C G G G C C G A A T T A G G T C  
A G T T T A T T A A T T C G A G C T G A A A C T A G G T C  
A G T C T A T T A A T T C G A G C A G A A C T T G G T C  
A G T T T A T T A A T T C G A G C T G A A C T T T G G C C  
A G T C T A C T A A T T C G A G C T G A A T T A G G T C  
A G A T T A T T A A T T C G A G C T G A A A C T T T G G T C  
A G A T T G C T A A T T C G A G C C G A A T T A G G T C  
A G A T T A T T A A T T C C G G G C T G A A T T A G G T C  
A G T C T A T T A A T T C G A G C T G A A T T A G G A C  
A G C T T A T T A A T T C G T G C T G A A A C T C G G A C  
A G C T T A T T A A T T C G A G C T G A A A C T C G G G G C  
A G C T T A T T A A T T C G A G C C G G A A C T C G G G G C
```

to



I'm going to talk about going from



to



to

A grid of DNA sequence data represented by colored letters (A, T, C, G) in a 10x10 grid. The colors correspond to the four bases: A (green), T (red), C (blue), and G (yellow).

A	G	C	T	T	A	C	T	C	C
A	G	T	T	T	A	T	T	G	C
A	G	T	C	T	A	T	T	G	C
A	G	T	T	T	A	T	T	G	C
A	G	T	C	T	A	T	T	G	C
A	G	A	T	T	A	T	T	G	C
A	G	A	T	T	A	T	T	G	C
A	G	A	T	T	A	T	T	G	C
A	G	A	T	T	A	T	T	G	C
A	G	A	T	T	A	T	T	G	C

I'm going to talk about going from



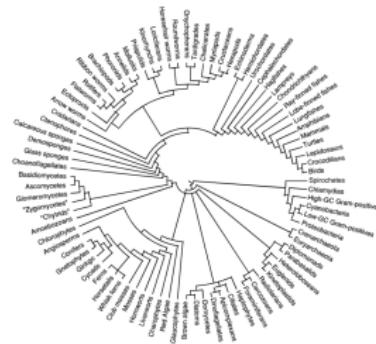
to



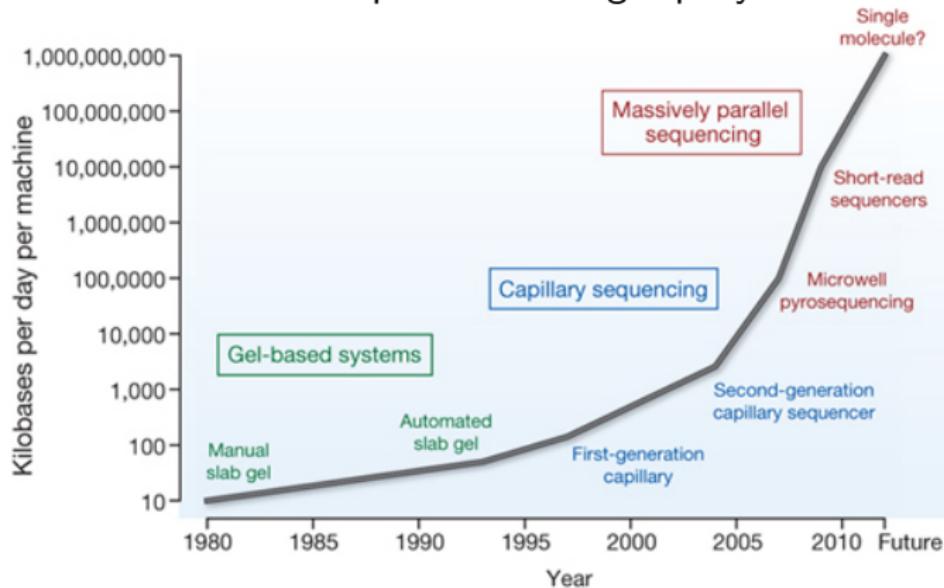
to

A grid of DNA sequence data showing a sequence of 10 base pairs across 10 different samples. The sequence is: A G C T T A C T A A T C C G G G C C G A A T T A G G T C. Each sample is represented by a different color: pink, purple, light blue, yellow, green, blue, orange, red, and black. The sequence is repeated for each sample.

and how those choices can affect



The quantity of available sequence data for inferring evolutionary relationships is increasing rapidly



<http://genome.wellcome.ac.uk/>

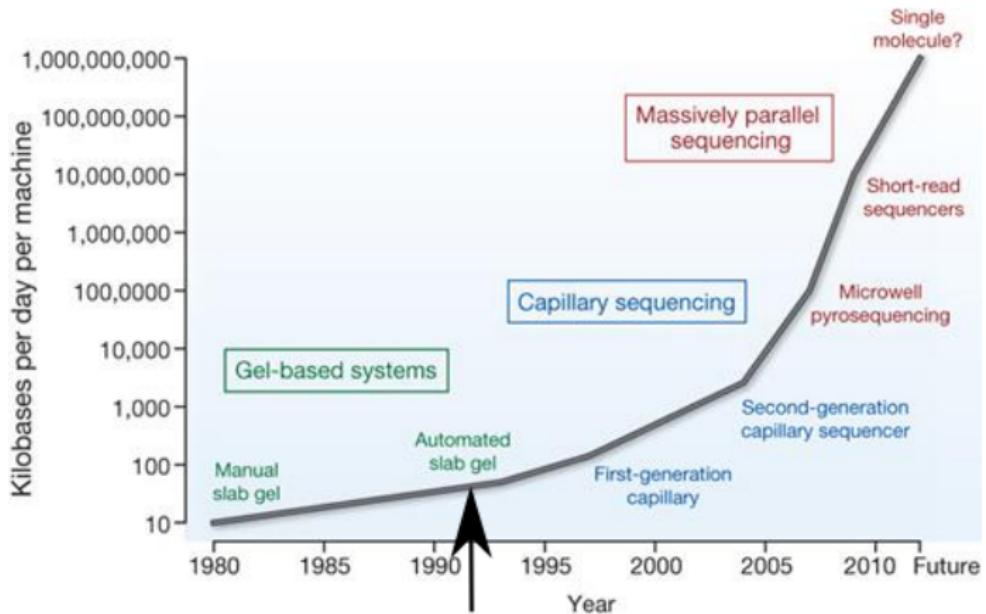
“With the advent of modern molecular biology, the ability to collect biological sequence data has out-paced the ability to adequately analyze these data”

– Jeff Thorne (Evolutionary biologist)

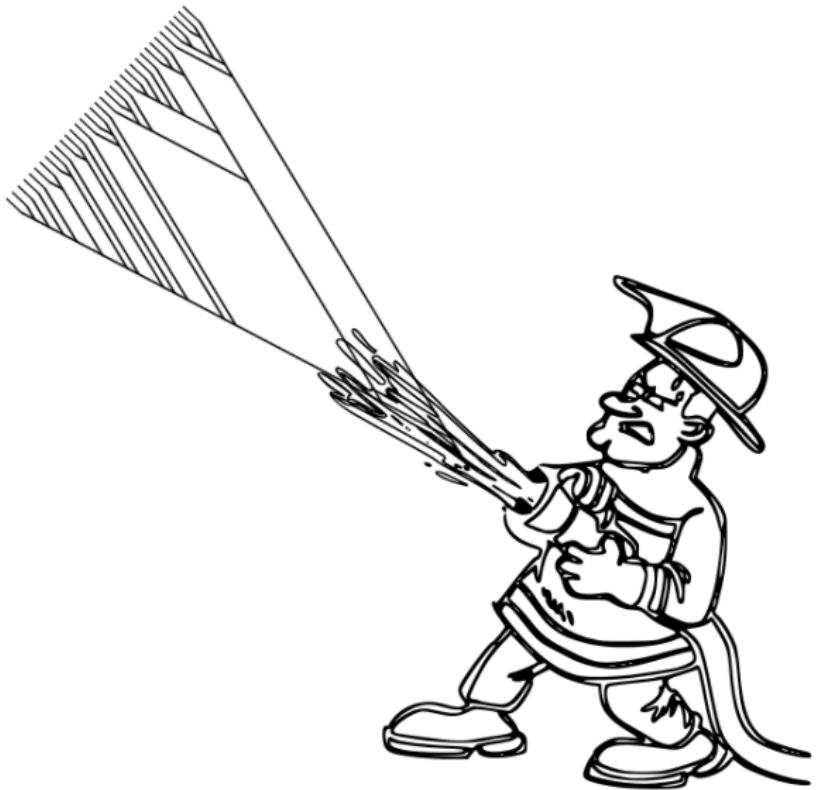
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Thorne et al., Journal of Molecular Evolution. 1991



<http://genome.wellcome.ac.uk/>



There are a lot of choices to make!

Biological questions

What do you want to know?

What do you already know?

Biological questions

What do you want to know?

What do you already know?

Technical questions

What data is right for our questions?

Is a closely related reference genome available?

How should we process and analyze our data?

What biases may be affecting our inferences?

General approach

- Decide what to sequence ( to )
- Consensus sequence, alignment, locus selection
( to )
- Evolutionary analyses ( to )
- Success!

What to sequence?



to



Different sequencing approaches enrich the samples for different components of the genome

Enrichment (smallest to largest proportion of genome)

Directed PCR

Targeted enrichment, Rad-tag etc

Transcriptome

Whole genome

Depending on your questions, any of these could be the best option!

Survey question! PollEv.com/emilyjanemctavish820

Directed PCR

Simple and cheap for a small number of genes

Doesn't scale so well to many genes

Doesn't sound fancy

Targeted enrichment (e.g. Ultra-conserved elements, probes for orthologous single copy genes, etc.)

Use hybridization to enrich particular regions

Works well even on degraded DNA

Need to synthesize probes specific to each region
- need data to get data!

Data sets can be combined across projects if same probe set applied

Non-targeted enrichment (RAD-tag, ddRAD etc.)

Select randomly distributed, but consistent, genome regions

Comparable across closely related taxa, but not more distant taxa

Each locus has very few variable sites (not good for generating gene trees)

Whole transcriptome

Enriched for expressed protein coding genes

Content will vary based on cell type,
environment, etc.

Provides expression level data

Whole genome sequencing

Capture all the data

In a phylogenetic context, often only cost effective for small genomes

Annotation is hard! Often need transcriptome to get genes

Mapping or assembly can be slow

Need to put the pieces back together!



to

A	G	C	T	T	A	C	T	A	A	T	C	G	G	G	C	G	A	A	T	T	A	G	G	T	C				
A	G	T	T	T	A	T	T	A	A	T	T	C	G	A	G	C	T	G	A	A	C	T	A	G	G	T	C		
A	G	T	C	T	A	T	T	A	A	T	T	C	G	A	G	C	A	G	A	A	C	T	T	G	G	C			
A	G	T	T	T	A	T	T	A	A	T	T	C	G	A	G	C	T	G	A	A	C	T	T	G	G	C			
A	G	T	C	T	A	C	T	A	A	T	T	C	G	A	G	C	T	G	A	A	T	T	A	G	G	T	C		
A	G	A	T	T	T	A	T	T	A	A	T	T	C	G	A	G	C	T	G	A	A	C	T	T	G	G	C		
A	G	A	T	T	T	G	C	T	A	A	T	T	C	G	A	G	C	C	G	A	A	T	T	A	G	G	T	C	
A	G	A	T	T	T	G	C	T	A	A	T	T	C	G	A	G	C	G	G	C	A	A	T	T	A	G	G	T	C
A	G	C	T	T	A	T	T	A	A	T	T	C	G	T	G	C	T	G	A	A	C	T	C	G	G	A	C		
A	G	C	T	T	A	T	T	A	A	T	T	C	G	A	G	C	T	G	A	A	C	T	C	G	G	G	C		
A	G	T	C	T	T	T	T	A	A	T	T	C	G	A	G	C	T	G	A	A	T	T	A	G	G	A	C		

Genomic sequencing

You have all the data! 

You have to deal with all of the data. 

De novo assembly

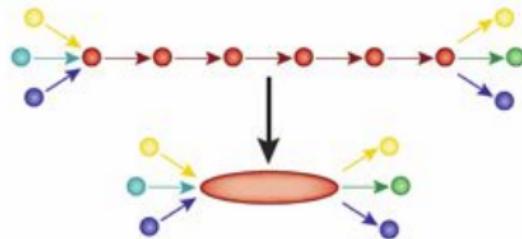
1. Fragment DNA and sequence



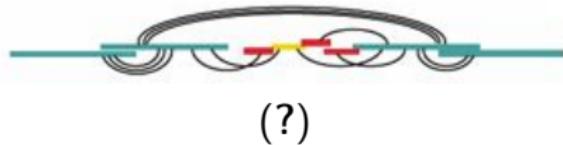
2. Find overlaps between reads

...AGCCTAGACCTACA**GGATGCGCGACACGT**
GGATGCGCGACACGTCGCATATCCGGT...

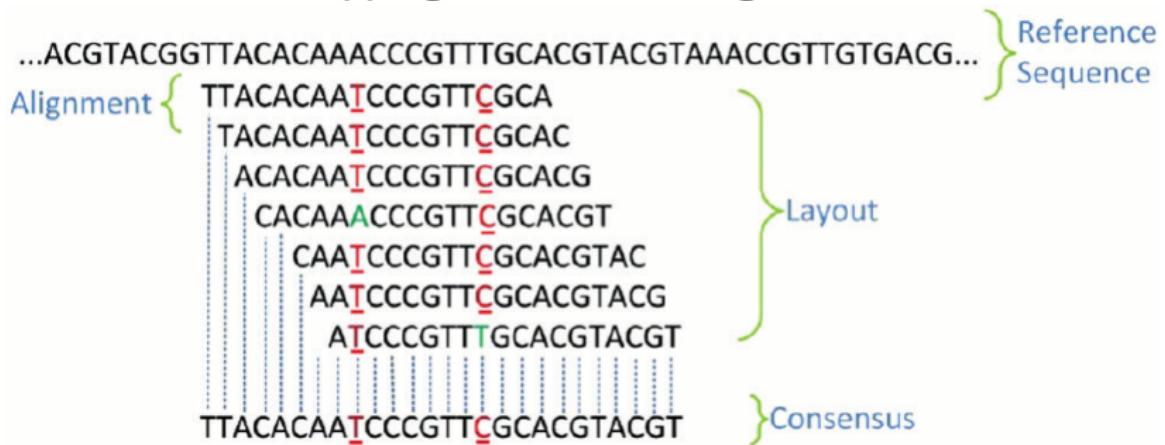
3. Assemble overlaps into contigs



4. Assemble contigs into scaffolds



Mapping to a reference genome

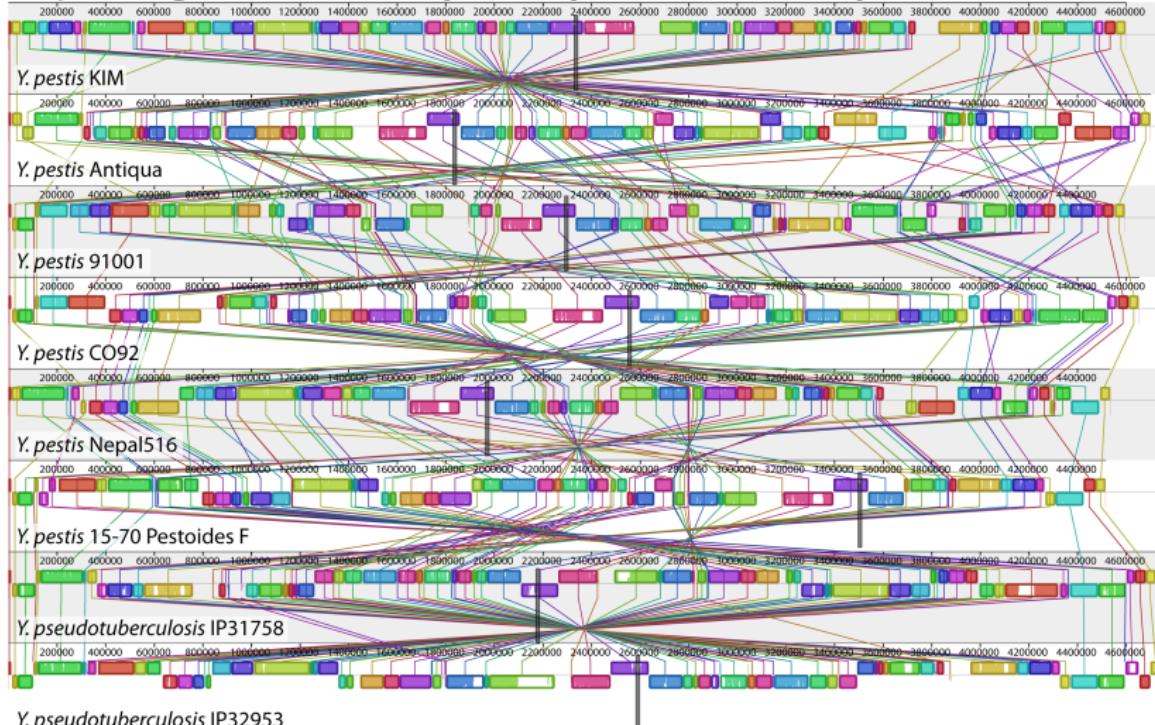


To make evolutionary statements, you need to align genomic regions across taxa.

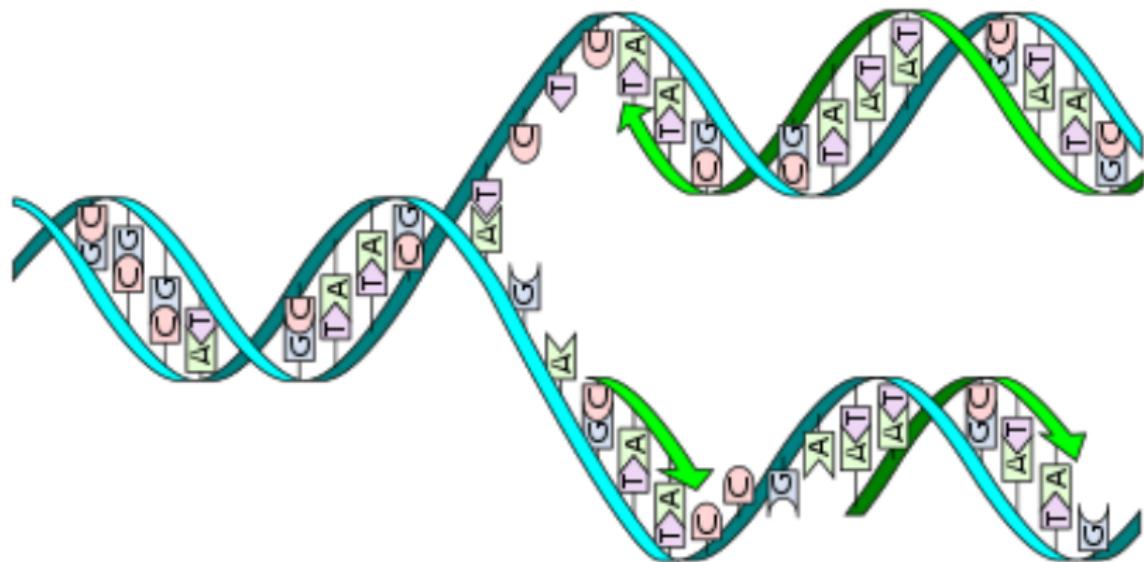
Depending on evolutionary history this can be easy or hard!

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Depending on evolutionary history this can be easy or hard!



An alignment is a statement of shared ancestry



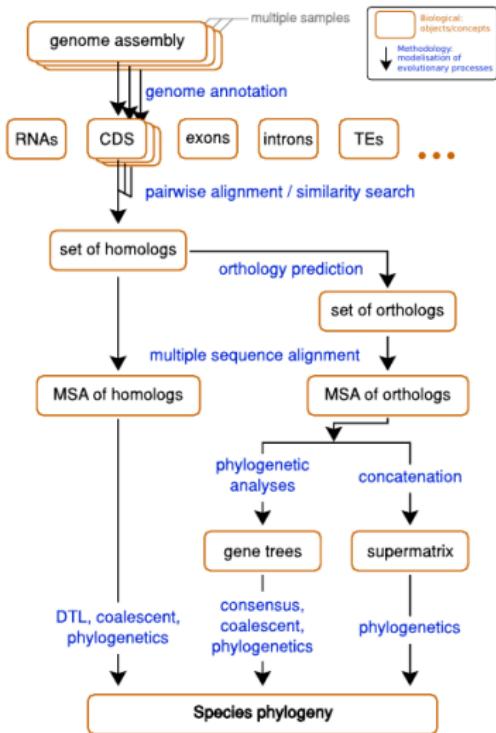
Gene tree (Locus tree)

The ancestry of a homologous region of the genome that has a single evolutionary history (no recombination)

Enrichment methods focus our sequencing efforts on these regions

Free textbook: Phylogenetics in the Genomic Era

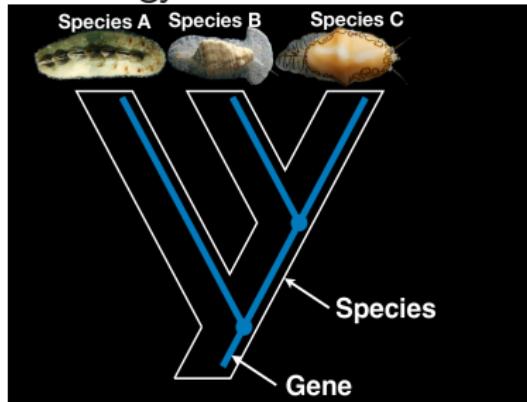
<https://inria.hal.science/PGE/page/table-of-contents>



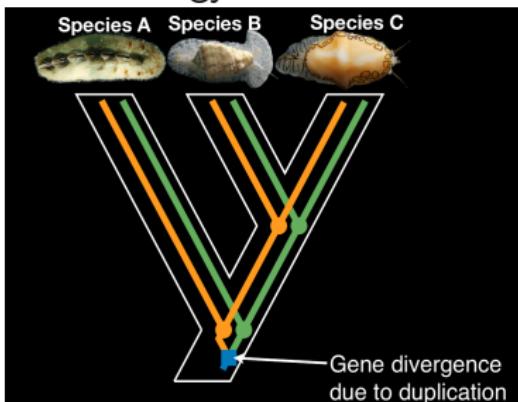
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Gene duplication and loss

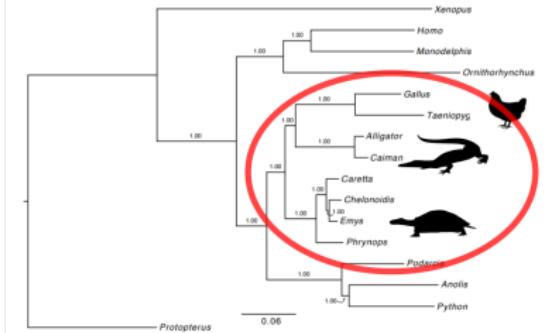
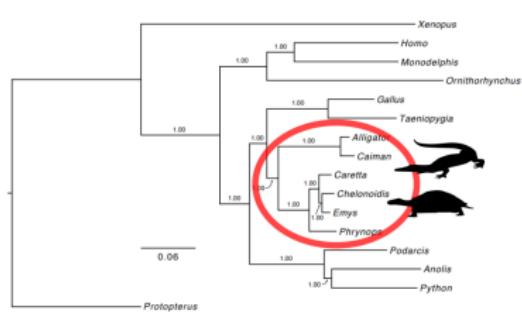
Orthology



Paralogy



Inference of homology is not incorrect! But our current models are limited. If you treat paralogs as orthologs, you can make incorrect inferences. figure from Casey Dunn



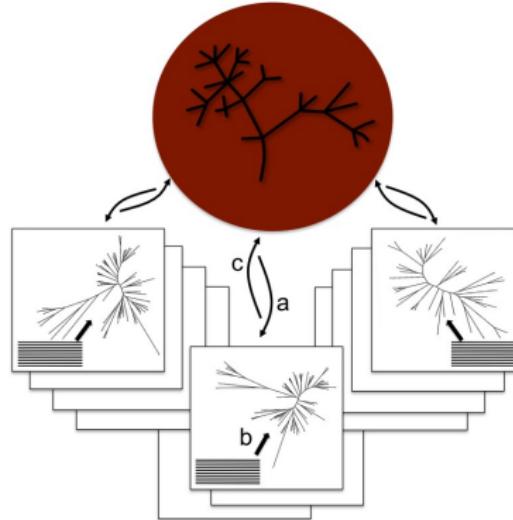
“investigation of genes with extreme support for turtle placement revealed unappreciated paralogy in a small proportion of alignments (<1%) that had an extraordinary influence on the inferred placement of turtles.”

(?) (?)

Challenge: The true (unknown) phylogenetic history is needed to assess orthology vs paralogy

Integrated approaches to Duplication, Transfer, and Loss (DTL)
can jointly estimate gene trees and species trees, but are very
computationally expensive.

$$L(T, S, N|A) = \prod_{G_i \in \mathcal{G}} L(G_i)$$



Phyldog; (?)

Using all Gene Families Vastly Expands Data Available for Phylogenomic Inference

Megan L. Smith *,^{1,2} Dan Vanderpool  and Matthew W. Hahn^{1,2}

“For most subsets of the data and inference methods, using all clusters (i.e. paralogs and orthologs) also results in consistent inferences of species tree topologies. Our results highlight the benefits of using data from all gene families by showing that the amount of data used can be increased by an order of magnitude”
(but there is sensitivity to inference method)

 CellPress

Trends in Genetics

Review

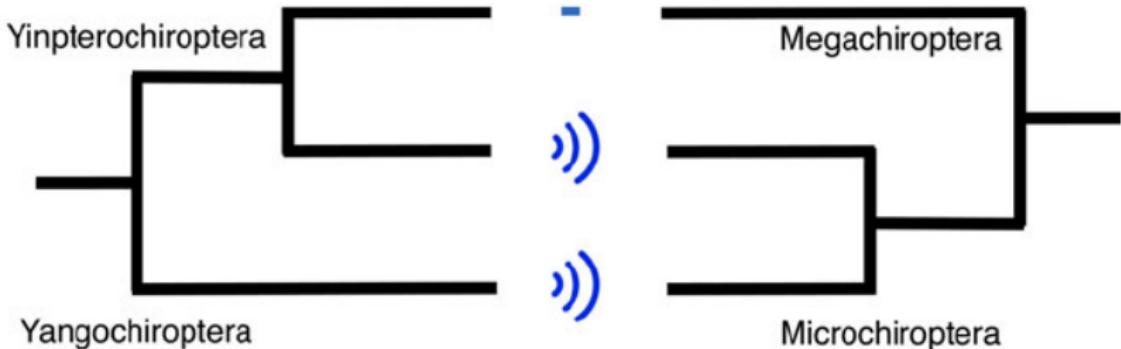
New Approaches for Inferring Phylogenies in
the Presence of Paralogs

Megan L. Smith^{1,*} and Matthew W. Hahn¹

??

Is the species tree even what you want?

Different gene trees can drive different conclusions

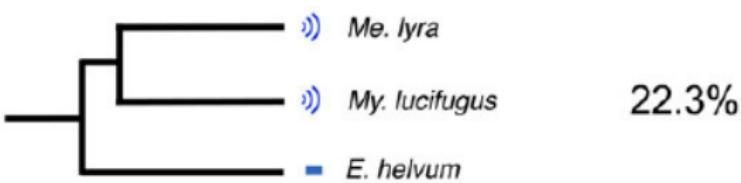
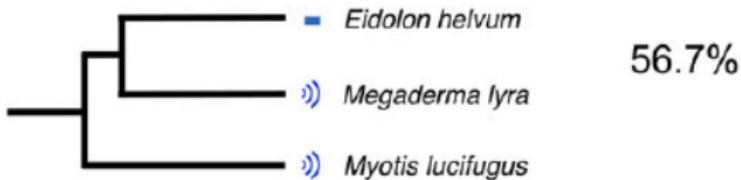


Species relationships between echolocating and nonecholocating bats (after Teeling 2009). Left: inferences from DNA sequence data.

Right: traditional species relationships inferred from morphological characters (and limited sequence data). (?)

If you are interested in a trait controlled by one or a few genes, the species tree may not describe the evolutionary history.

B



(?)

Do you need a whole genome to answer your questions?

Do you need a whole genome to answer your questions?

For phylogenetic and population genetic questions, not necessarily!

Most phylogenetic methods cannot directly handle whole genome data, but from whole genome sequencing you can get homologous loci, as well as a bunch of other stuff!

Data processing/ascertainment bias

How do the choices we make in



to



to

A sequence of DNA bases represented by colored boxes. The sequence is: A G C T T A C T A A T C C G G G C C G A A T T A G G T C
A G T T T A T T A A T T C G A G C T G A A C T A G G T C
A G T C T A T T A A T T C G A G C T G A A C T T G G T C
A G T T T A T T A A T T C G A G C T G A A C T T G G T C
A G T C T A T T A A T T C G A G C T G A A C T T G G T C
A G T T T A T T A A T T C G A G C T G A A C T T G G T C
A G T C T A T T A A T T C G A G C T G A A C T T G G T C
A G C T T A T T A A T T C G A G C T G A A C T C G G A C
A G C T T A T T A A T T C G A G C T G A A C T C G G A C
A G C T T A T T A A T T C G A G C T G A A C T C G G A C
A G C T T A T T A A T T C G A G C T G A A C T C G G A C
A G C T T A T T A A T T C G A G C T G A A C T C G G A C
A G C T T A T T A A T T C G A G C T G A A C T C G G A C
A G C T T A T T A A T T C G A G C T G A A C T C G G A C
A G C T T A T T A A T T C G A G C T G A A C T C G G A C

How do the choices we make in



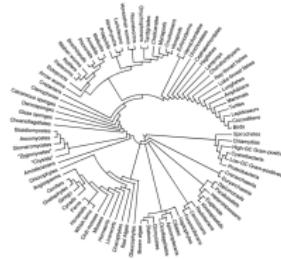
to



to

A G C T T A C T A A T C C G G G C C G A A T T A G G T C
A G T T T A T T A A T T C G A G C T G A A C T A G G T C
A G T C T A T T A A T T C G A G C A G C A G C T G A A C T T G G T C
A G T C T A C T A A T T C G A G C T G A A C T T A G G T C
A G A T T T A T T A A T T C G A G C T G A A C T T G G T C
A G A T T T A T T A A T T C G A G C C G A A T T A G G T C
A G A T T T A T T A A T T C G A G C T G A A A T T A G G T C
A G T C T A T T A A T T C G A G C T G A A C T T A G G T C
A G C T T A T T A A T T C G A G C T G A A C T C G G A C
A G C T T A T T A A T T C G A G C T G A A C T C G G G C
A G T C T A T T A A T T C G A G C T G A A C T C G G G C

affect



?

Ascertainment bias

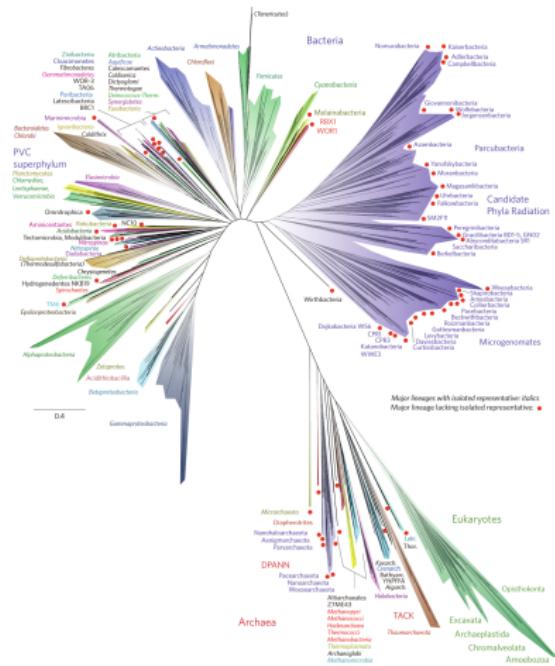
A bias in parameter estimation or testing caused by non-random sampling of the data.

(also sometimes overlapping with 'selection bias' or 'acquisition bias')

Ascertainment bias is ubiquitous!

- Surveying volunteers
- Studying undergraduates
- Sampling across 'species'
- Discarding rare outliers

Sampling across the tree of life

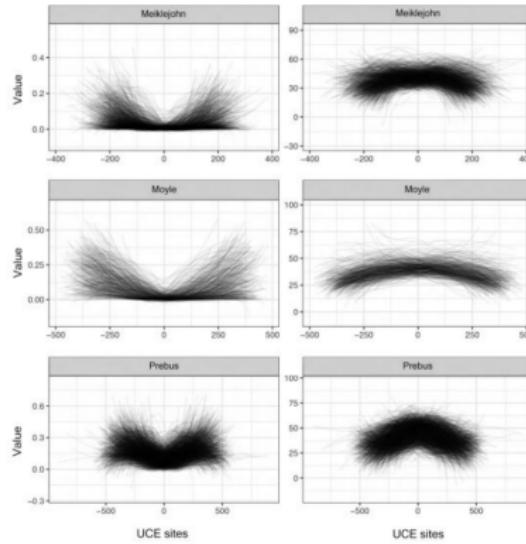


(?)

It is important to consider what models of evolution are appropriate for your data types

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Entropy (rate proxy), GC content



Extreme rate heterogeneity in Ultra Conserved Elements, can be handled with appropriate partitioning
 (?)

Analyzing only variable sites (e.g. Single Nucleotide Polymorphism (SNP) analyses)

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This affects our ability to estimate branch lengths using likelihood
Intuitively, will increase inferred branch lengths
can also affect tree topology

Short Tree

AAGTATACACATTATCGAATCAAAAGAAAAATTTCGAAAAACTATAGA
AAGTATACACATTATCGAATCAAAAGAAAAATTTCGAAAAACTATAGA
AAGTATACACATTATCGAATCAAAAGAAAAATTTCGAAAAACTATAGA
AAGTATACACATTATCGAATCAAAAGAAAAATTTCGAAAAACTATAGA
AAGTATACACATTATCGAATCAAAAGAAAAATTTCGAAAAACTATAGA

Short Tree

AAGTATACACATTATCGAAATCAAAAAGAAAAATTTCGAAAAACTATAGA
AAGTATACACATTATCGAAATCAAAAAGAAAAATTTCGAAAAACTATAGA
AAGTATACACATTATCGAAATCAAAAAGAAAATTTCGAAAAACTATAGA
AAGTATACACATTATCGAAATCAAAAAGAAAATTTCGAAAAACTATAGA
AAGTATACACATTATCGAAATCAAAAAGAAAATTTCGAAAAACTATAGA

Long Tree

CAGCAGGGTTTACCTTGCAAGAGGGAAAGCCCATTCCACCCACTTCCCTGGCAC
CACCAGATTTACCTTGCAAGGGCAAAACAGTCCACCCACTTCATGAACAC
CAGCAGGGTTTACCTTGCAAAAGGGAAAGCCATTTCCTTACCTTCAGGGAAC
CAGCAGGGTTTACCTTGCAAAAGAAAACAGTTTACCATTTCTTGGAAC
CAGCAGGGTTTACCTTGCAAGGGAAAAATCAATATAACACTTTTGCAATAAC

How surprised should we be to see no invariant sites?
Very surprising, unless branches are very long

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Very surprising, unless branches are very long
but only if we looked for them!

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but only if we looked for them!

Can correct by applying ? model for analysis of only variable sites
implemented inference software

Based on correction for problem of not counting un-observed
restriction sites in (?)



Pay attention to data '*clean-up*' steps.

e.g.

Minor allele frequency cutoffs

Removing non-biallelic sites (multiple hit)

Filtering out high rate regions

One method's “noise” is another method's data!

“it is possible in many cases to correct the ascertainment bias relatively easily, if reliable information is available regarding the details of the ascertainment scheme.” (?)

"it is possible in many cases to correct the ascertainment bias relatively easily, if reliable information is available regarding the details of the ascertainment scheme." (?)

This information is not always available. Bias can be driven by the true, evolutionary history you are attempting to estimate!

Despite the large volume of data in genomic studies, ascertainment bias is still an issue

Despite **because of** the large volume of data in genomic studies, ascertainment bias is still an issue

Case studies:

Phylogenetics of *Penstemon* using RADseq data

Tracing gonorrhea outbreaks

Phylogenetics of Penstemon using RADseq data

Question: How often have transitions between hummingbird and bee pollination occurred in Penstemon?



FIGURE 1. Flowers of selected *Penstemon* species. (A) *P. glaber*. (B) *P. barbatus*. (C) *P. barbatus*. (D)

Data:

Restriction site-associated DNA sequencing (RADSeq)

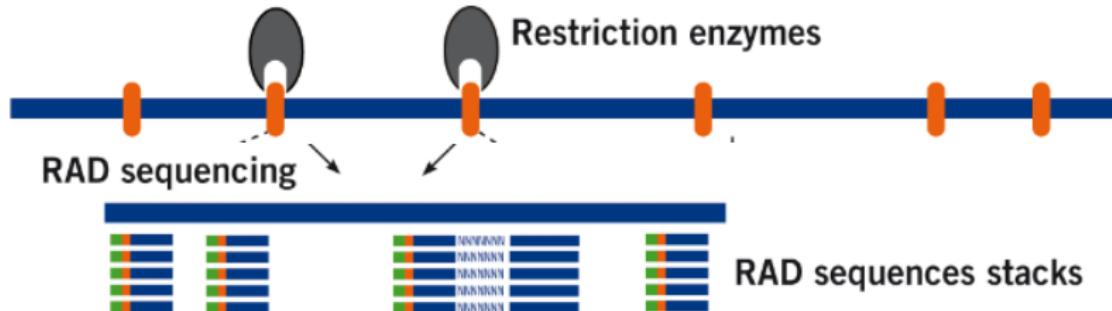
83 species, two samples per species

No closely related reference genome

RADseq

Uses restriction enzymes to fragment DNA

Targets sequencing to the same regions across taxa

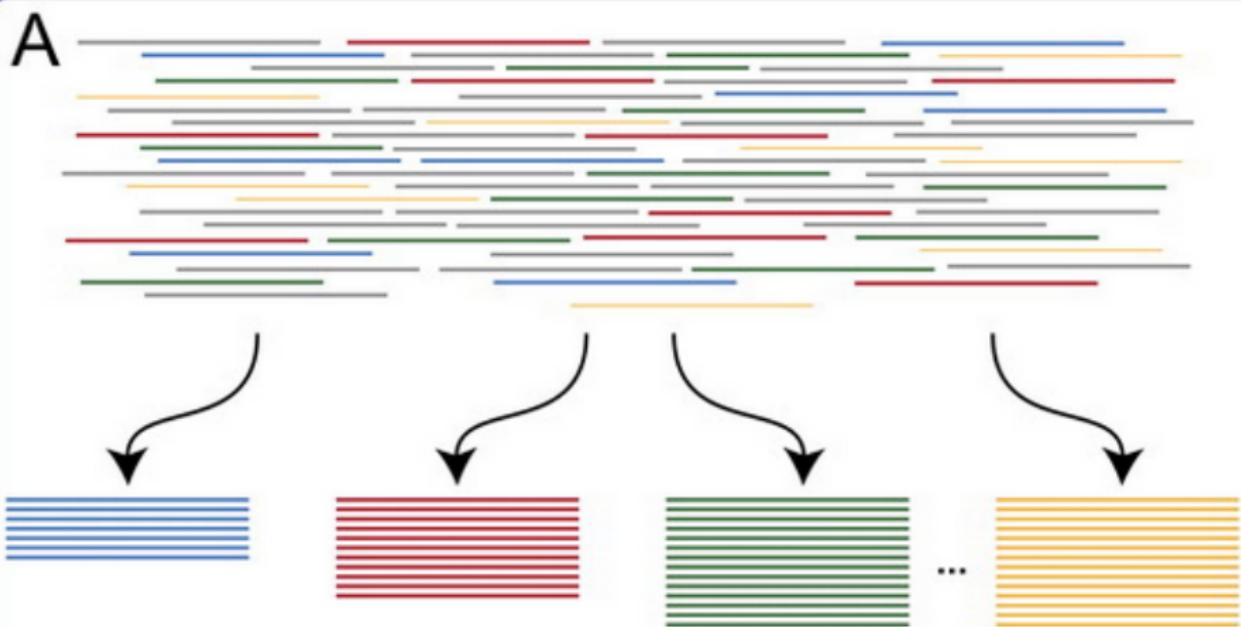


In comparison: Shotgun Sequencing



(figures from floragenex.com)

In the absence of a reference genome, you need to cluster reads
A 'cluster' is an inference of homology



Clustered using Stacks (?)

Several factors can cause drop-out of alleles in RAD-seq data (i.e. not observing homologous alleles)

- Mutations at restriction digest sites
- Clustering parameters exclude homologous regions
- Low coverage

There have been many conflicting studies on the importance of missing data in phylogenetic analyses,
broadly, as long as missing data is random, it shouldn't be very
problematic, but phylogenetically-biased missing data is likely to be.
(??)

Missing data in RADseq can mislead inference

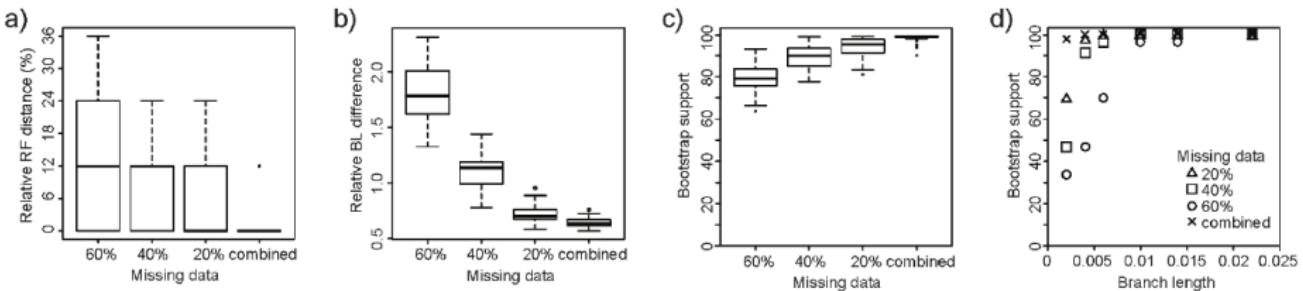


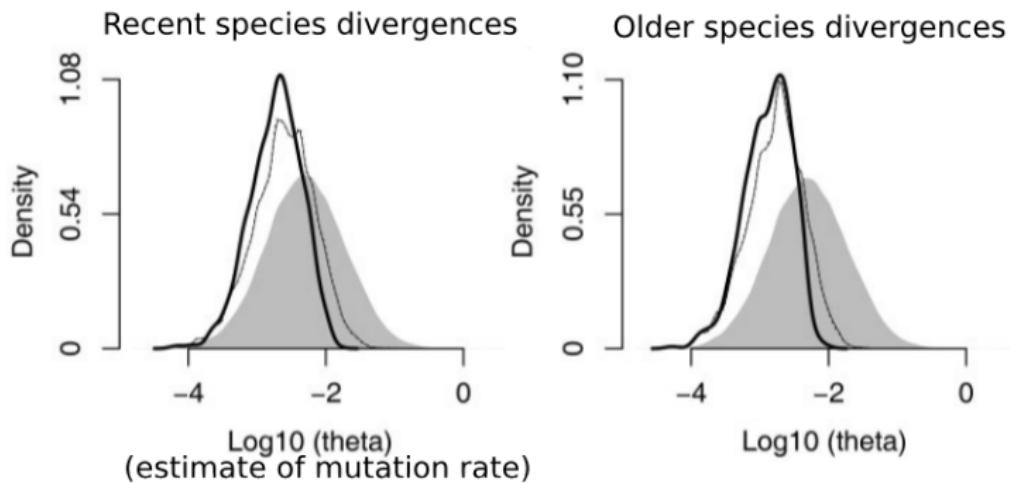
Figure 4: Properties of simulated RAD loci with different amounts of missing data. Loci that contain more missing data tend to result in discordant topologies (a), increased branch length errors (b), and lower bootstrap support (c). Loci that contain less missing data provide higher bootstrap support for shorter branches (d).

(?)

But excluding sites with high levels of missing data doesn't solve the problem.

But excluding sites with high levels of missing data doesn't solve the problem.

It biases rate estimation downwards by preferentially removing high rate loci



Gray shading is simulated rates, dashed line is shift due to loss of RAD sites, black line is shift due to loss of cut sites, black line shift due to loss of cut sites + post sequencing processing.

(?)

Advice?

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“Given that the data matrix reflects complex interactions between aspects of library construction and processing with the divergence history itself, our results also suggest that general rules-of-thumb are unlikely.”

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(?)



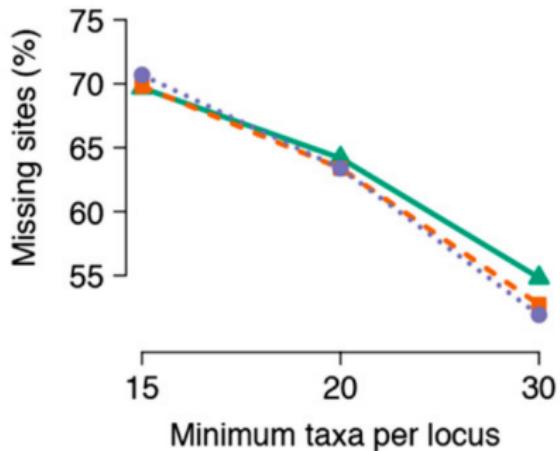
Tradeoffs:

Decreasing similarity cutoff captures more loci shared across the tree, at risk of incorrect homology

Decreasing taxon representation threshold allows you to capture more loci, but representing fewer individuals

Approach

Investigate a range of parameters

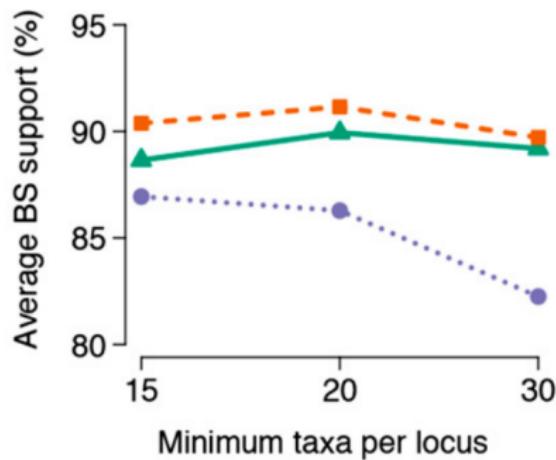


▲ $W_{\text{clust}} = 0.80$

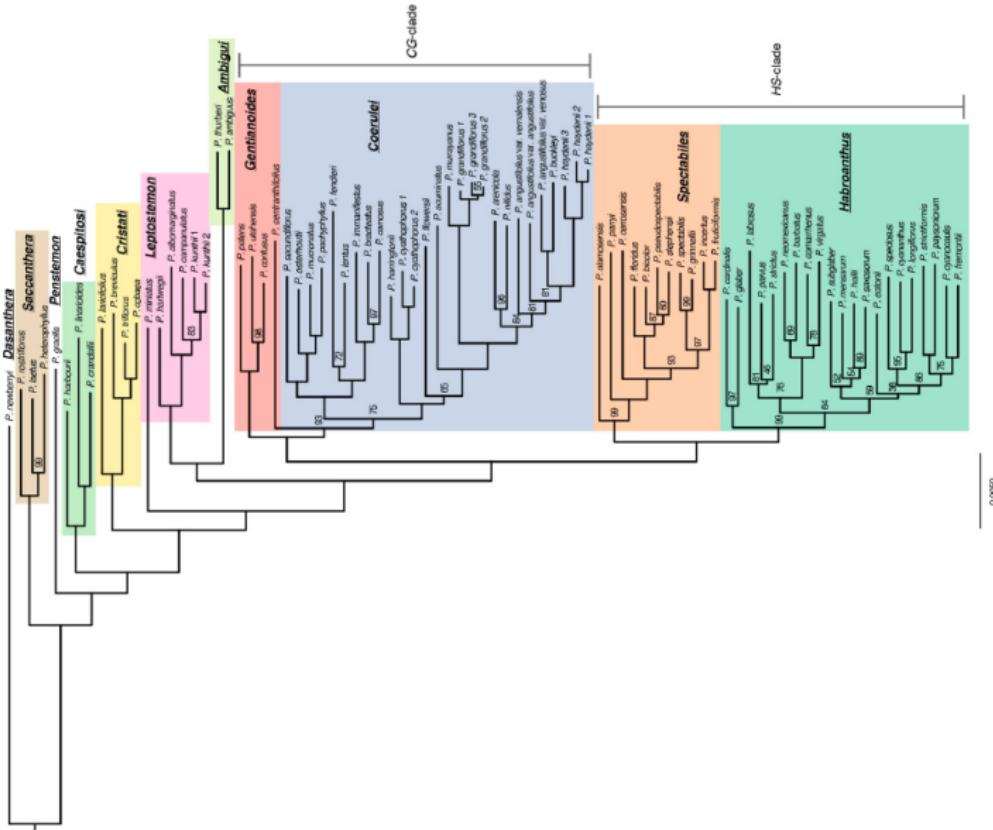
■ $W_{\text{clust}} = 0.90$

● $W_{\text{clust}} = 0.95$

(?)



Missing data is phylogenetically biased



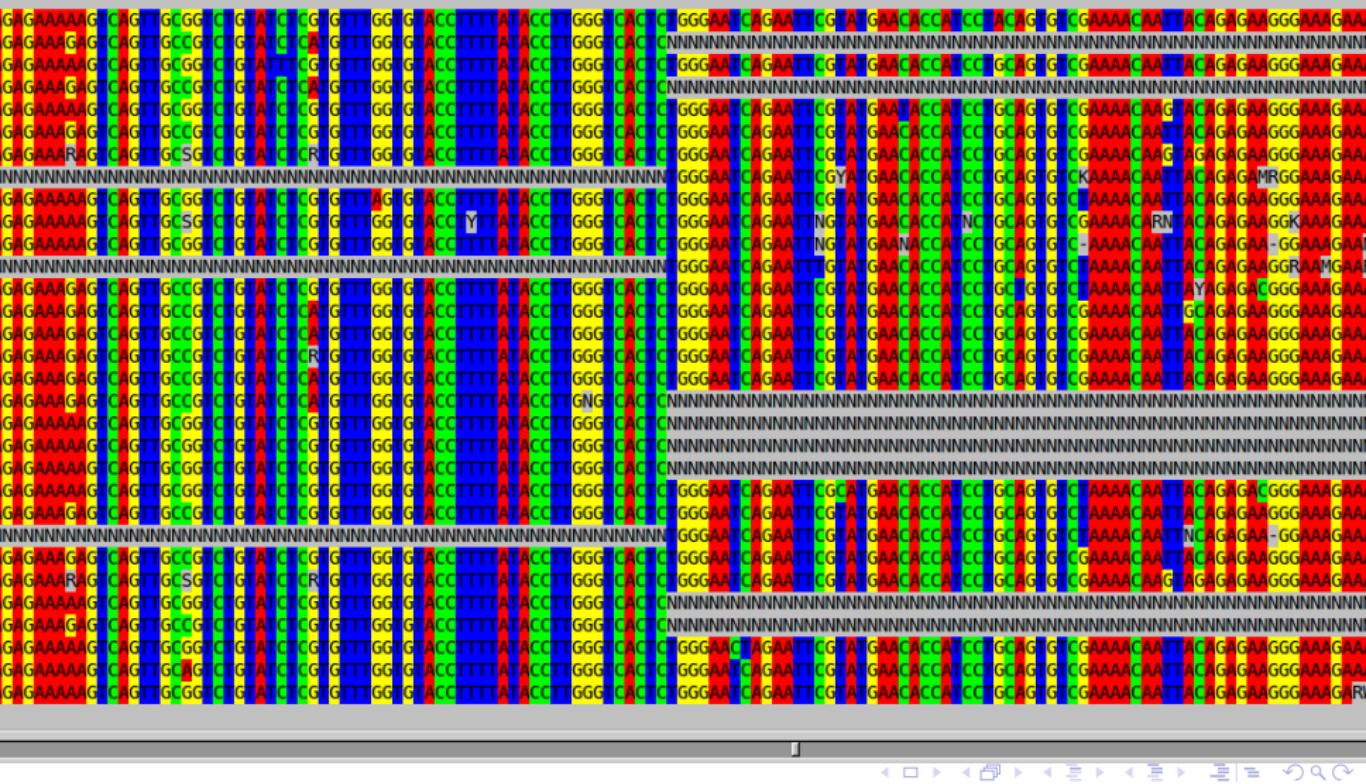
Across full dataset, many loci are only found in one of the major clades

ees Search: Goto: Help

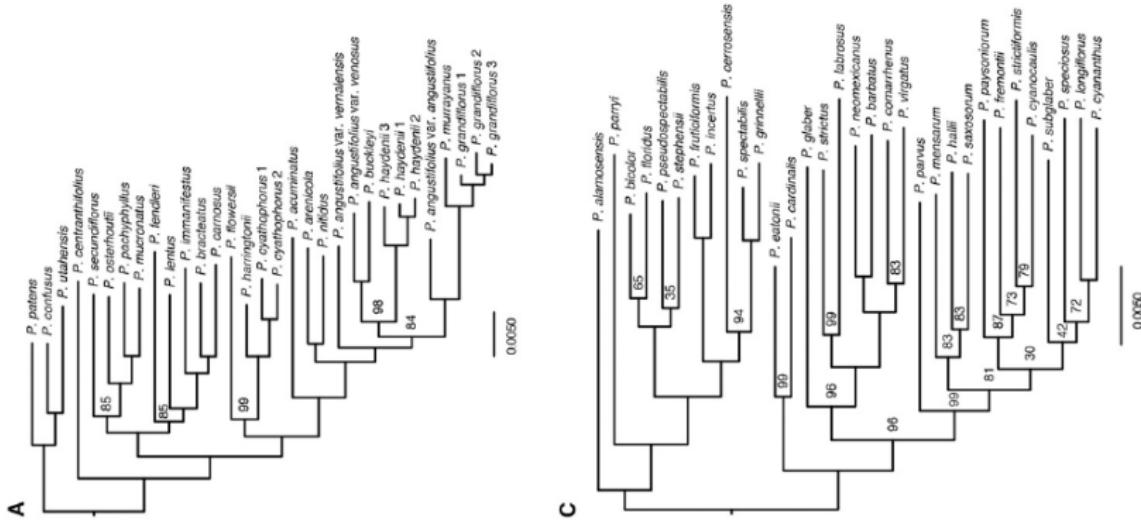
The visualization displays a large dataset of DNA sequences. The sequences are color-coded according to the four nucleotides: Adenine (green), Thymine (red), Cytosine (blue), and Guanine (yellow). The alignment shows that many loci (sites) are conserved across all samples, appearing as a single color (e.g., green for Adenine). Other loci show significant variation, with multiple colors appearing at the same position. This pattern is characteristic of a phylogenetic tree where different clades have diverged at specific sites. The sequences are presented in a grid format, with each row representing a different sequence and each column representing a position in the sequence.

Variation within clades is better captured by dividing the data set and clustering separately

rees | Search: | Goto: | Help |



Build (and report!) multiple trees using different filtering parameters



Trees from separate clade analyses (?)

Summary:

Bias:

Clustering parameters drive non-random missing data

Potential effect on inference:

No topological resolution

Tip branch lengths are shortened

Non-homologous regions align

Mitigation:

Estimate relationships under a range of filtering parameters

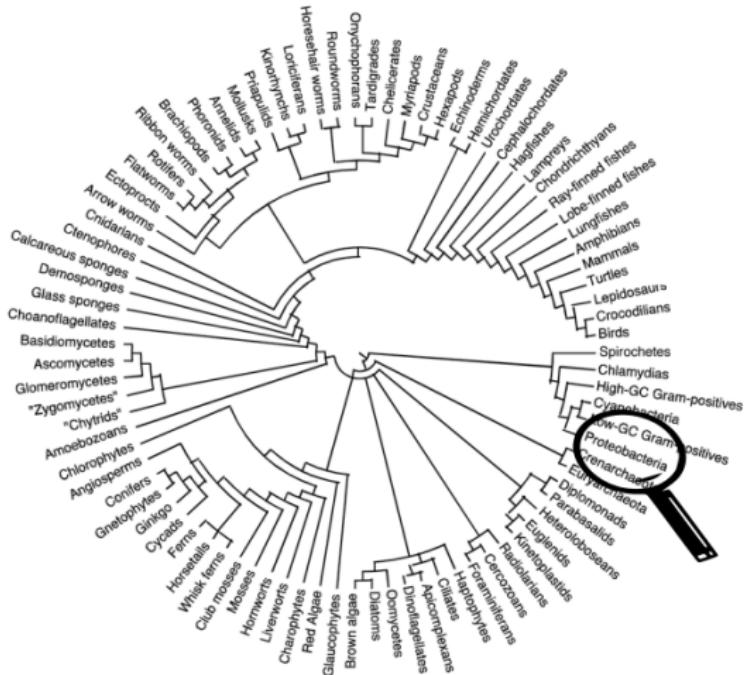
Conclusions:

Branch lengths and bootstrap support differ across filtering parameters

Different data sets may be appropriate at different phylogenetic scales

Evolutionary inferences about pollinator shifts need to be robust to this uncertainty

Case study - tracing gonorrhea outbreaks



Rapid phylogenetic updating to trace gonorrhea outbreaks



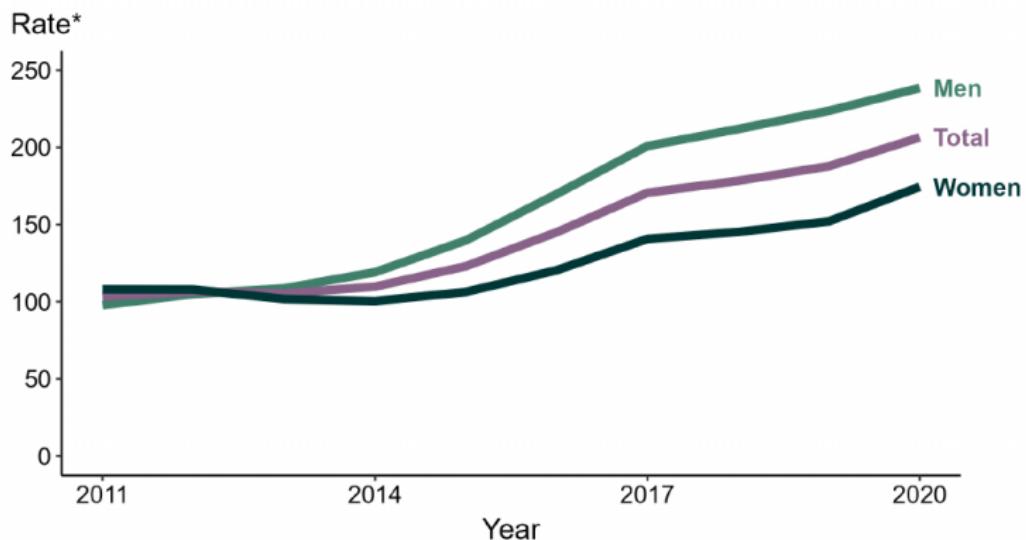
Collaboration with
Jack Cartee [CDC](#), Jeanine Abrams-McLean [CDC](#), and Jasper Toscani Field
(PhD student, UC Merced)

Neisseria gonorrhoeae

- Gram-negative, diplococci bacteria
- Responsible for the sexually transmitted infection known as gonorrhea
- One of two pathogenic *Neisseria* species known to infect humans
- WHO estimated 82 million new cases among adults worldwide in 2020



Gonorrhea rates over time by sex

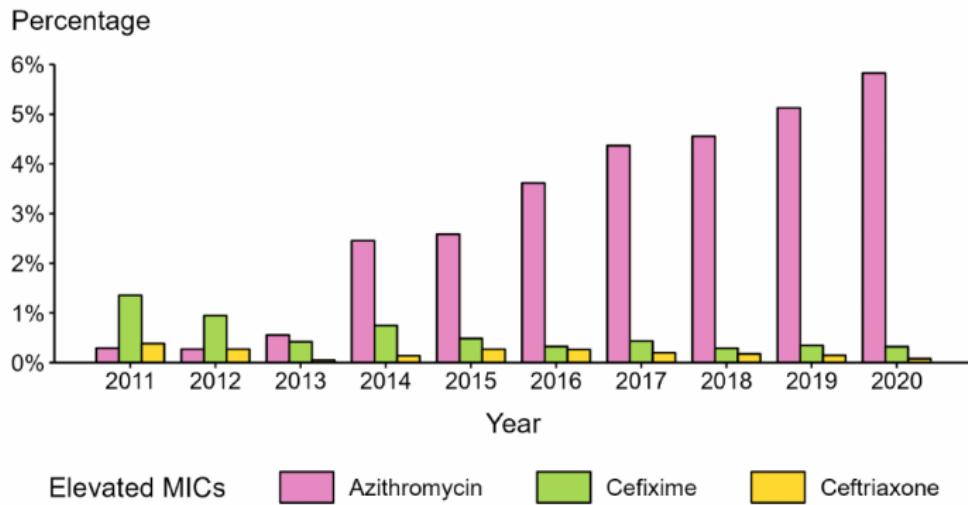


<https://www.cdc.gov/std/statistics/2020/figures/GC-2.htm>

Recent increase in rates of gonorrhea infections

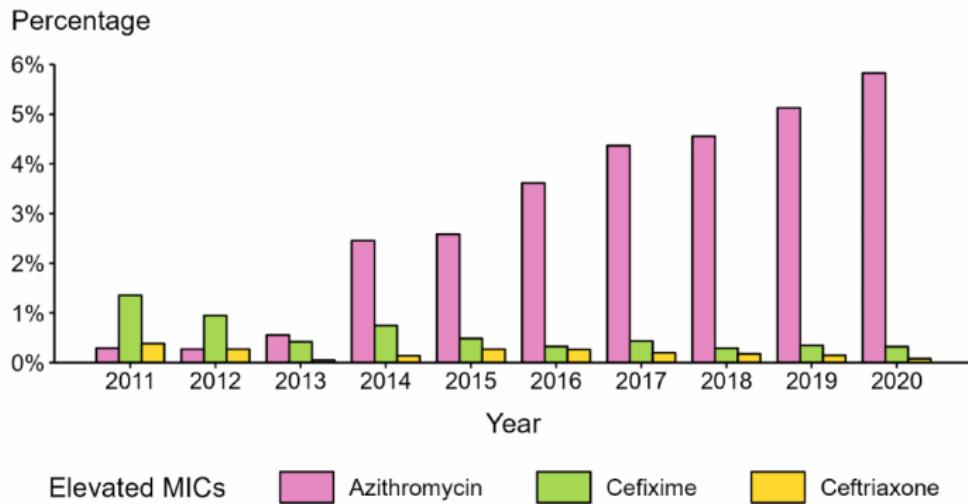
Neisseria gonorrhoeae has progressively developed resistance to each single dose antibiotic.

Percentage of isolates with antibiotic resistance



Neisseria gonorrhoeae has progressively developed resistance to each single dose antibiotic.

Percentage of isolates with antibiotic resistance

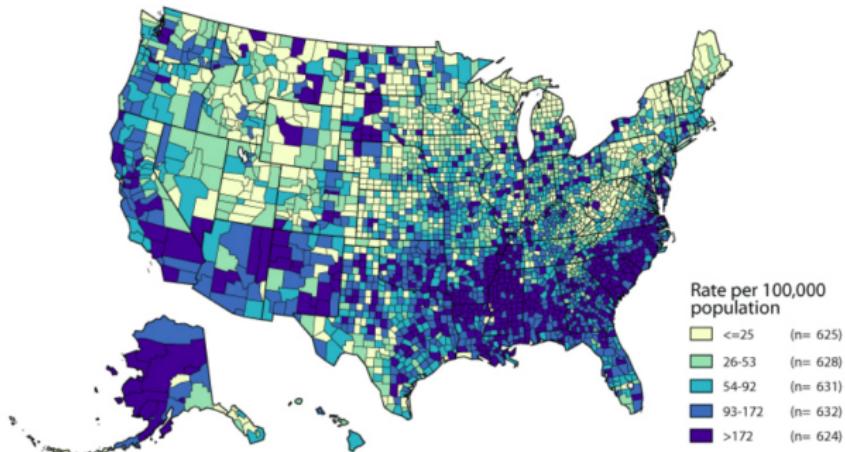


Only remaining recommended treatment option is dual therapy with a ceftriaxone plus azithromycin

"It is widely recognised that few antimicrobials remain effective in the treatment of *Neisseria gonorrhoeae* infection and that gonorrhoea could become untreatable in the future."
(Chisholm et al. Sex Transm Infect 2015)

To track and control outbreaks, the CDC is tracing evolutionary history of gonorrhea, across the US and globally.

Gonorrhea – Rates of Reported Cases by County, United States, 2017



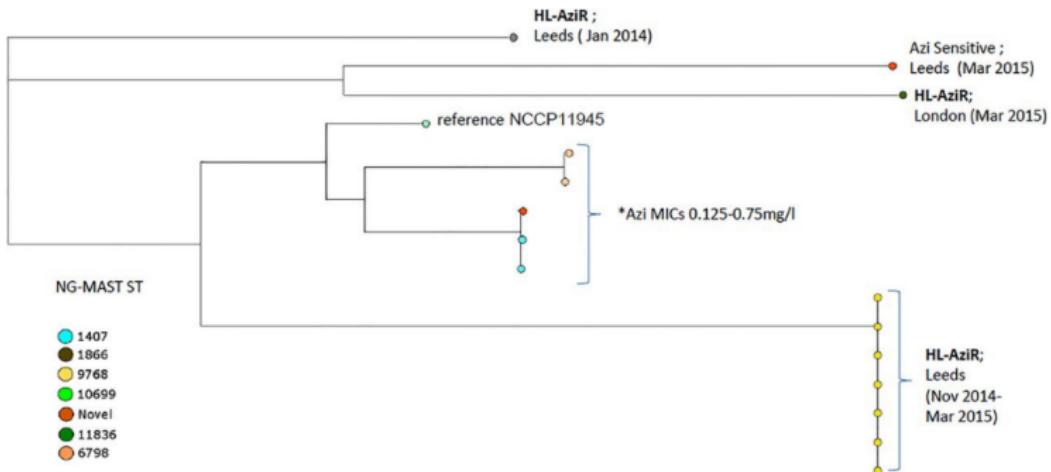
<https://www.cdc.gov/std/stats17/fignatpro.htm#gon>

Approach:

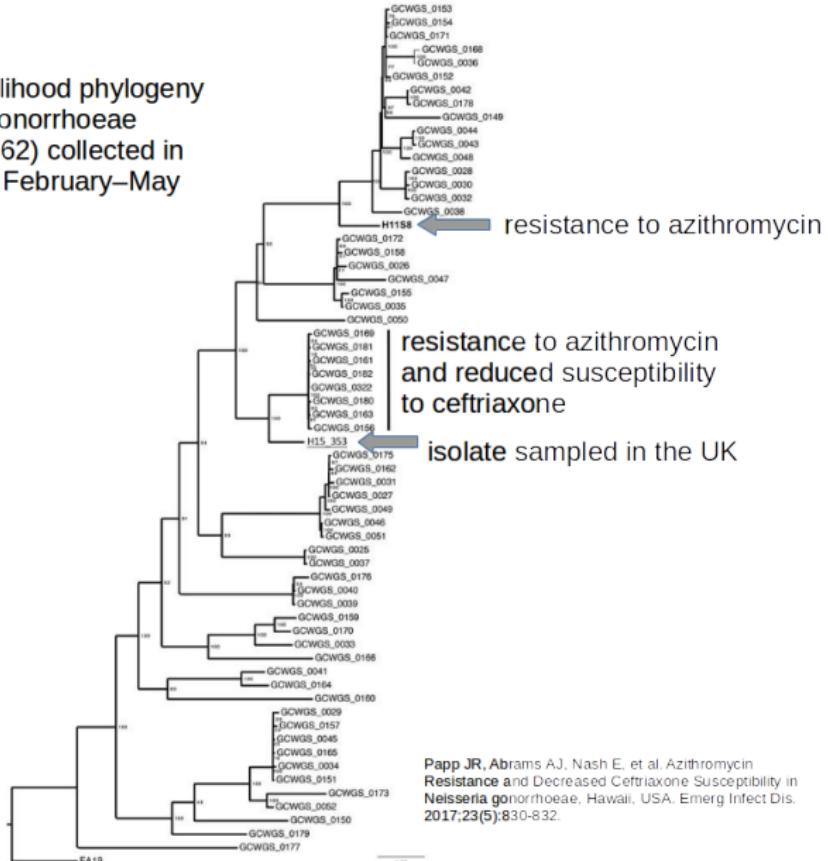
Whole genomic sequencing of *Neisseria gonorrhoea* isolates - up to thousands of lineages

Phylogenetic inference to track geographic spread and horizontal gene transfer of resistance genes

**Combining geographic and evolutionary information can trace transmission,
and transfer of resistance alleles across lineages**



Maximum-likelihood phylogeny
of *Neisseria gonorrhoeae*
samples (N = 62) collected in
Hawaii during February–May
2016



Challenges:

Thousands of samples; new isolates sequenced every day

Speed from sampling → phylogeny important

Need to rely on phylogenies for public health action (requires high confidence)

Often very little nucleotide variability, but horizontal gene transfer is common.

Potential issues:

Sequencing error

Effect of choice of reference genome

Sequencing error

Potentially problematic when real variable sites are rare

Sequencing errors are likely to be singletons

Will overestimate tip branch lengths

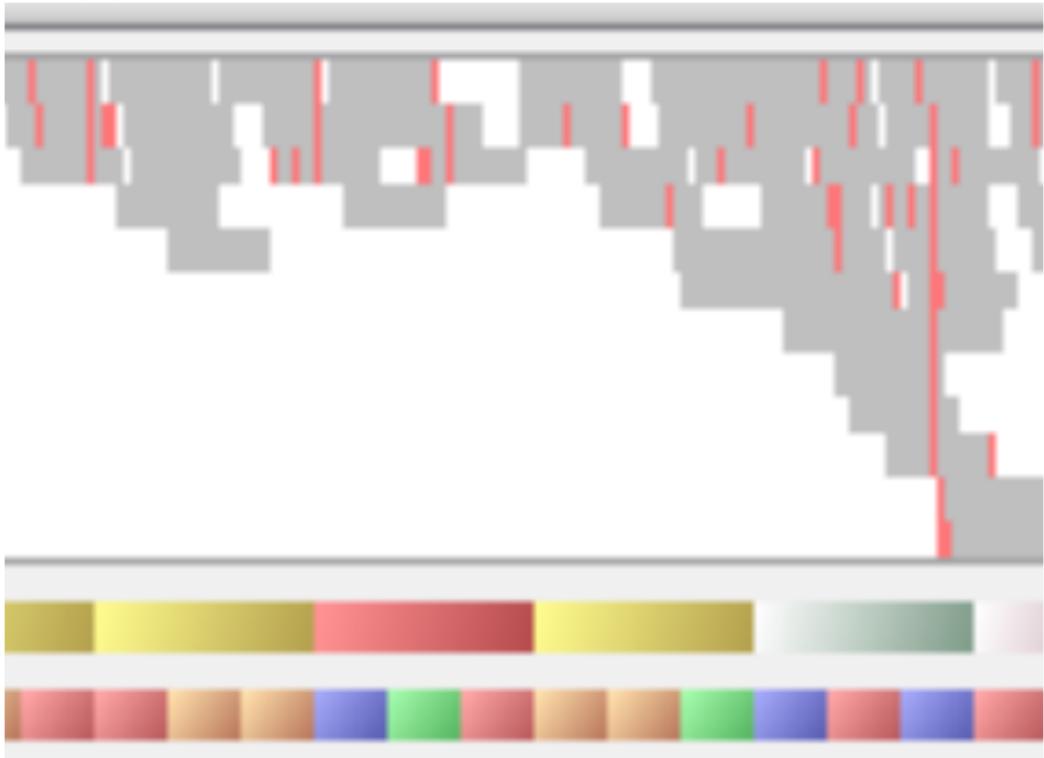
Currently, coverage and error information from sequence reads are discarded following  to 

We have information on confidence in individual base calls, but don't use it



? developed a correction for sequencing error in maximum likelihood phylogenetic inference.
Uses a constant expected error per site

Could use a “genotype likelihood”, capturing coverage and read quality (?)

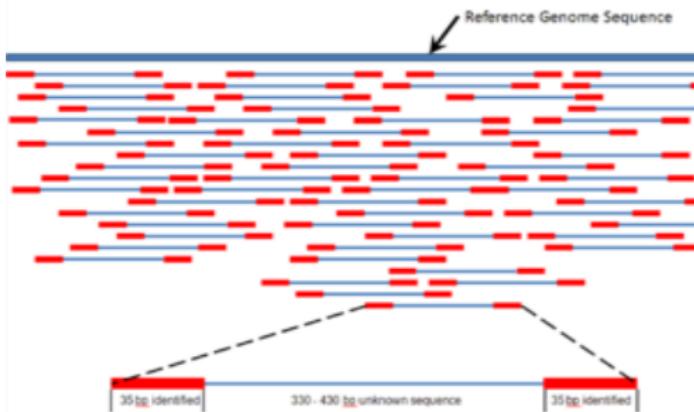


Not currently implemented in phylogenetic likelihood models

At high coverage, effect of sequencing error is likely low!

Effect of reference choice

Reference based mapping of short reads can speed up generating a consensus sequence.



BUT: Reference choice can affect evolutionary inference

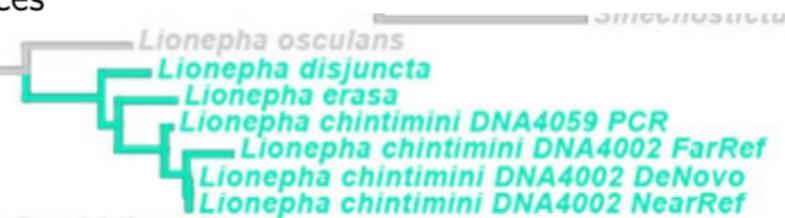
BUT: Reference choice can affect evolutionary inference

In humans, in highly polymorphic regions variant calling is biased toward the reference base (?)

BUT: Reference choice can affect evolutionary inference

In humans, in highly polymorphic regions variant calling is biased toward the the reference base (?)

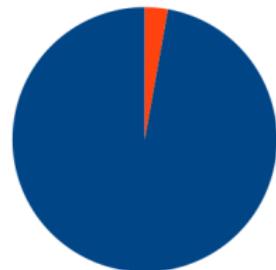
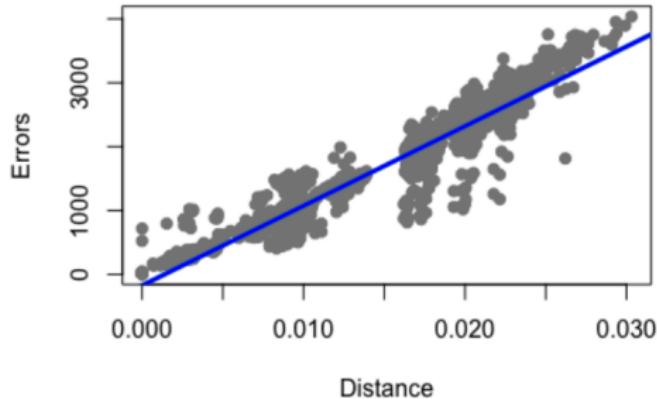
In fragmented DNA samples from beetles, branch lengths change based on reference choices



(?)

Error rate is correlated with distance to reference genome, and errors are strongly biased to the reference base

Unambiguous errors vs. Distance to reference

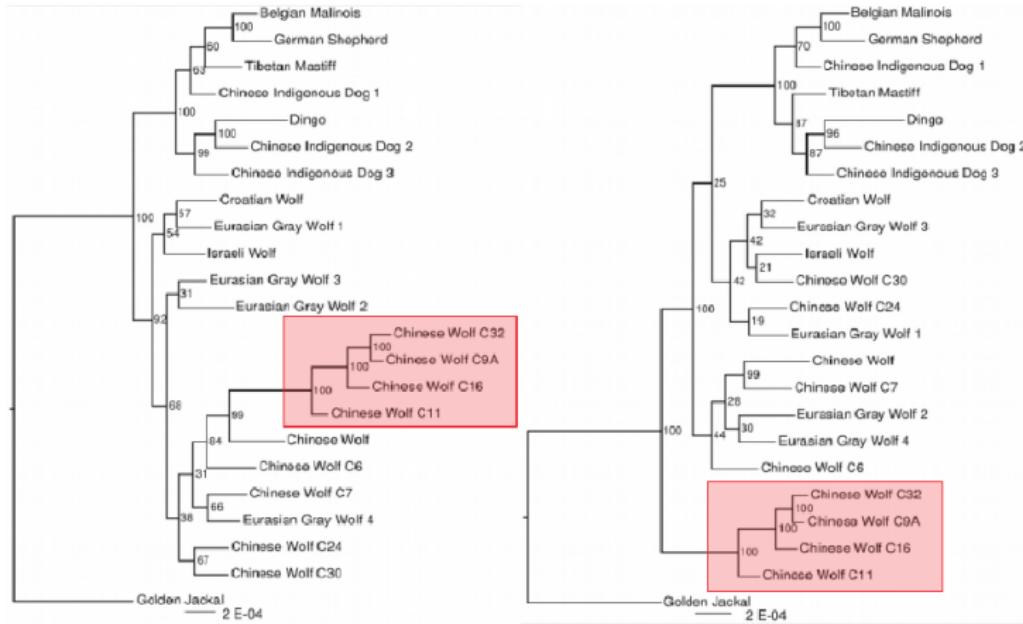


Base call errors match the reference base 97% of the time

(Toscani-Field and McTavish, work in progress)

A reference mapping based approach will discard information about structural variants not found in the reference

Reference choice can affect topology



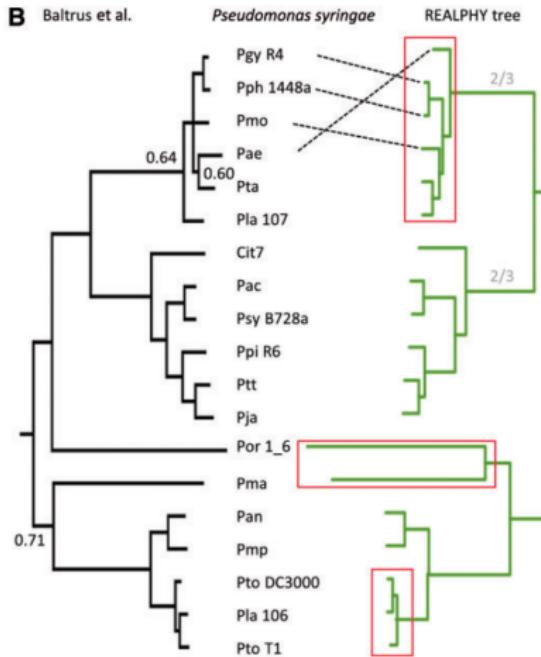
Dog Reference

?

Wolf Reference

Reference choice can affect topology inference

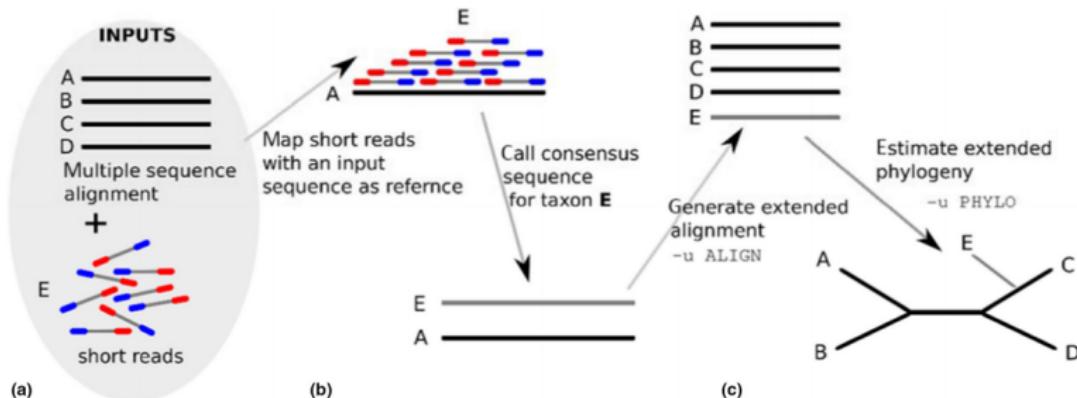
B Baltrus et al.



Mapping sequencing reads to reference genomes requires similarity cutoffs that generate biased missing data (?)

Problem: The true (unknown) phylogenetic history will affect how reads map across the genome.

Phylogenetically informed phylogenomic updating approach:



Assembles only homologous regions of interest

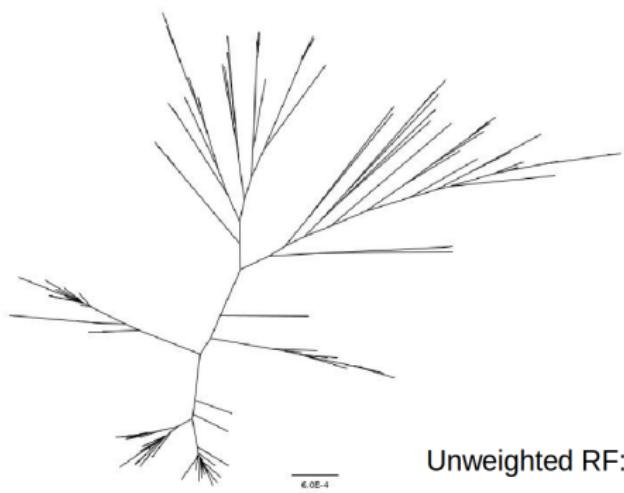
Can use multiple references to generate consensus sequence

Tree search speed up due to starting tree

github.com/mctavishlab/extensiphypipeline

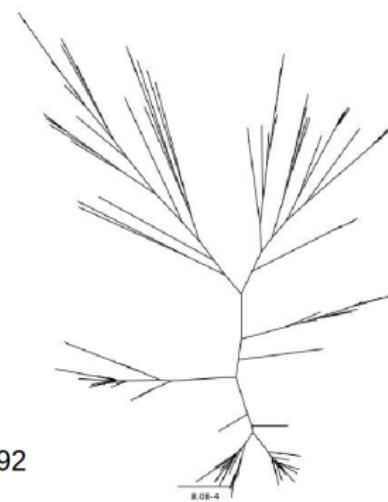
?

Tree from traditional method



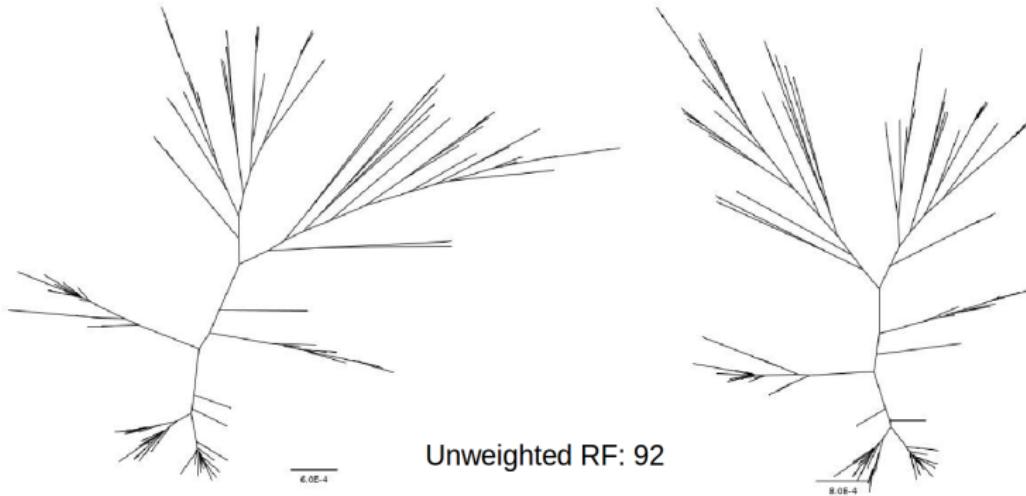
Unweighted RF: 92

Updated tree



Results:

Ok... 🤔 the tree is different! but is it better or worse?



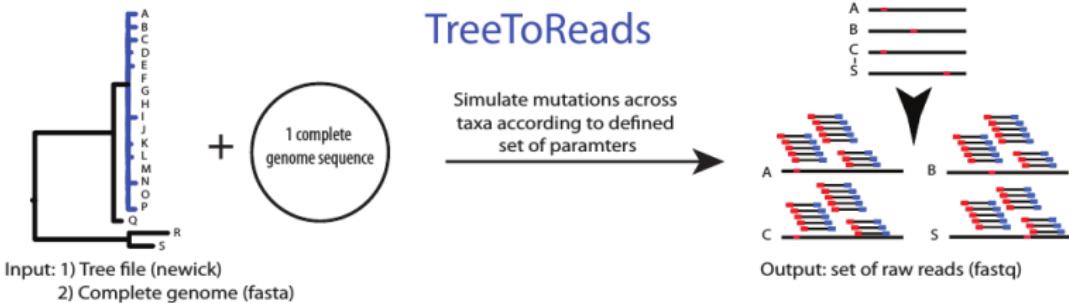
Testing the approach using simulations:

TreeToReads

Takes into account:

- Phylogeny and model of evolution
- Insertions and deletions
- Distribution of mutations across the genome
- Read coverage
- Sequencing error profiles (observed or estimated)

Generates short read data with which to test assembly, alignment and inference pipelines.

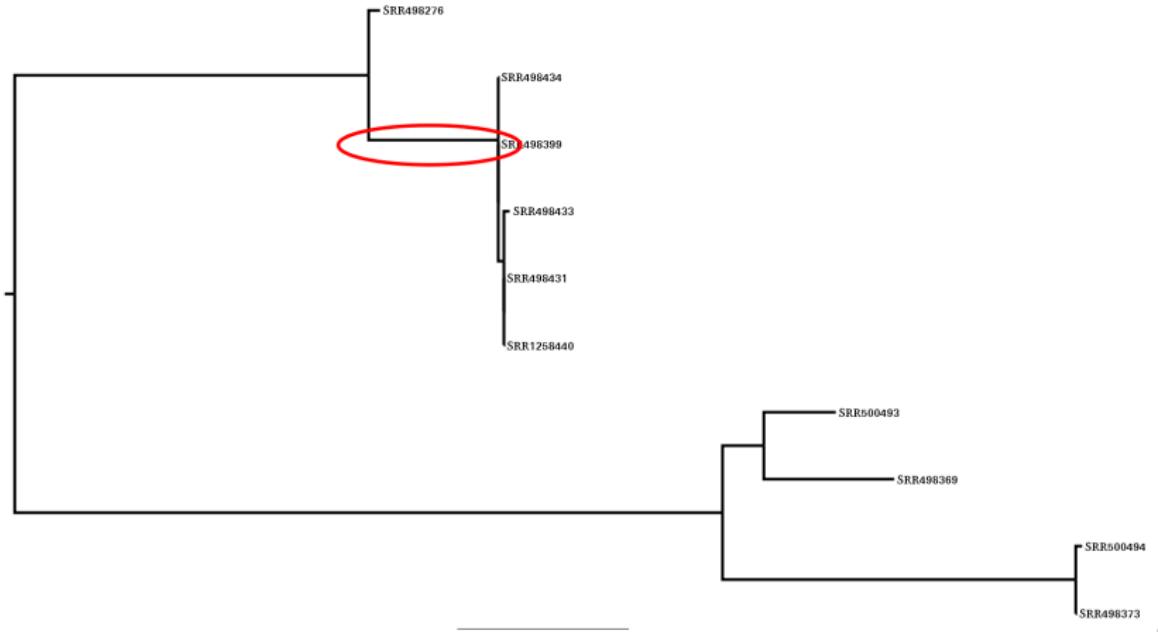


Input genome for simulation is a tip on simulated tree
 Can test alignment to other empirically observed genomes
 (?)

github.com/snacktavish/treetoreads

Other new approaches for generating reads from phylogenies:
NGSphy (?), *Jackalope* (R package) (?)

Take observed outbreak tree

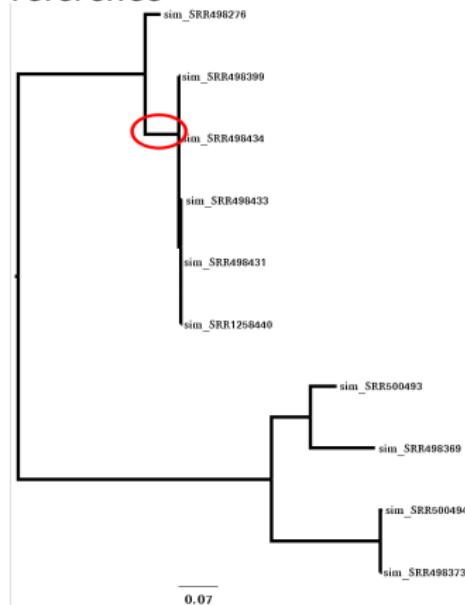


Simulate reads using empirical parameters

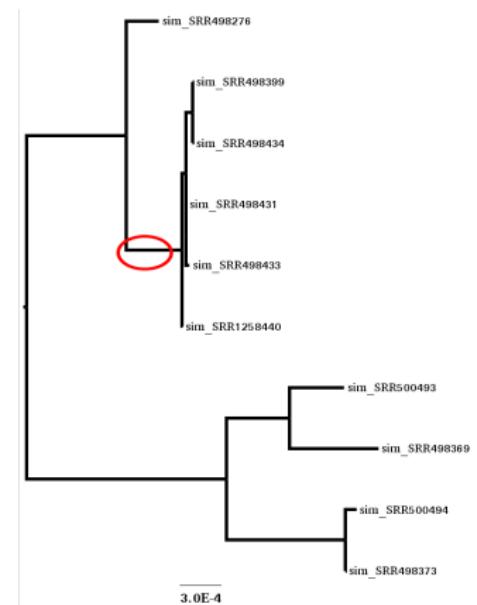
Infer trees from reads using two different reference genomes.

Reference within outbreak

reference



Distant (1% sequence divergence)



Simulation summary

- In this example, even distant reference genome did not affect parameter of interest (monophyly of outbreak), although it did affect branch lengths
- Effects of read mapping parameters and reference genome choice are likely to be idiosyncratic
- By using empirical estimates for evolutionary model, can investigate effects on parameters of interest
- Currently applying this approach to test gonorrhea phylogenetic updating procedure

Summary

Bias: Sequencing error, reference choice

Effect on inference:

- Sequencing error can increase terminal branch lengths relative to internal branches

- Not mapping reads on lineages more distant from reference genome will decrease those branch lengths

Mitigation: Use multiple reference genomes, simulation based tests to assess accuracy

Conclusions:

- When a closely related reference is available, alternatives worsen inference

- At high (around 40x) coverage all mutations are confidently recovered

- Even at lower coverage (around 5x) high confidence in monophyly of outbreak clade

Big picture

All data sets are biased, genome scale data is no exception

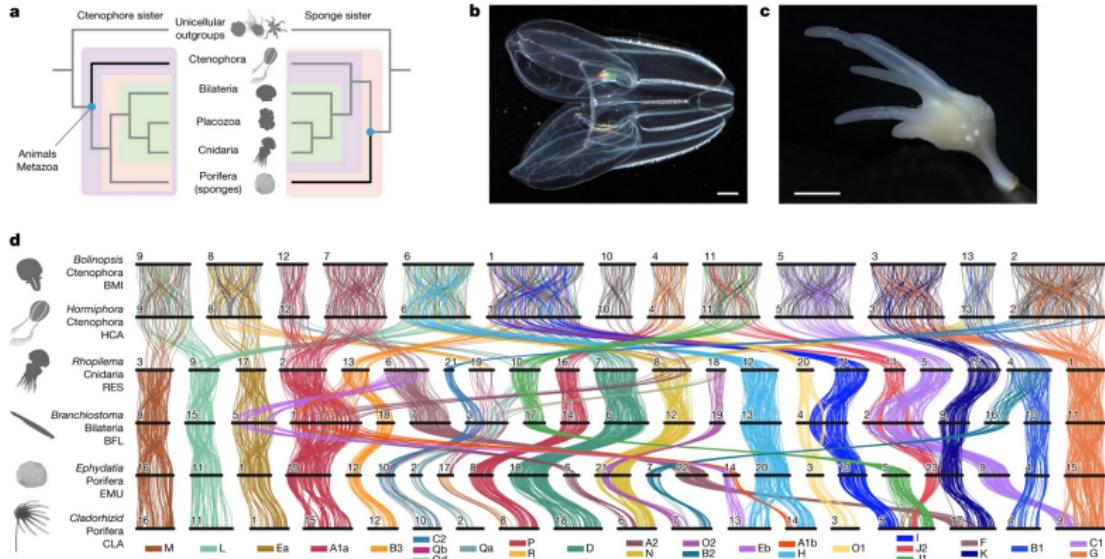
Careful project planning helps

Interrogate potential biases in data sets

What to do?

- What data will answer **your** questions?
- Are there existing data you want to be able integrate with?
- Consider in which direction biases are likely to sway results
- Use the most appropriate available model for your data
- Re-sample your data to test if your key conclusions are robust to choices
- Simulation approaches to test if parameters of interest are affected by sampling and ascertainment schemes

Treating genomes holistically, rather than as a collection of nucleotides, codons, or proteins, helps answer hard evolutionary questions.



Ancient gene linkages support ctenophores as sister to other animals ?

“The phylogenomic approach is, despite its flaws, surprisingly robust, as most pipelines will lead to the recovery of a similar species tree topology.

This can be explained by the sheer quantity of phylogenetic signal accumulated when thousands of molecular markers are combined.”

?

Questions?

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