Comparative Genomics and Visualisation BS32010

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Table of Contents

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What Is Comparative Genomics?

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The combination of genomic data, and comparative and evolutionary biology, to address questions of genome structure, evolution, and function.

Evolution is the central concept

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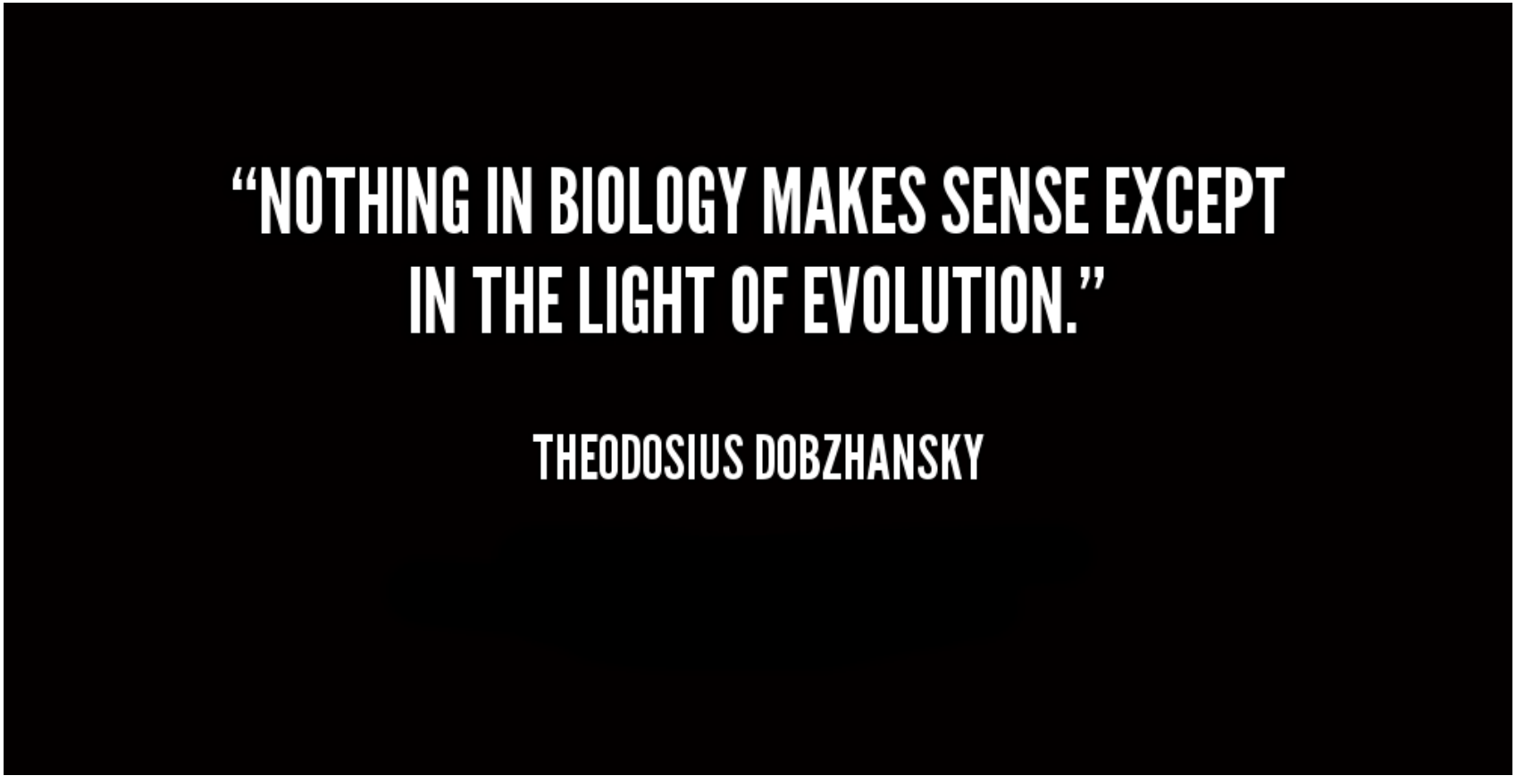

Comparison of physical features

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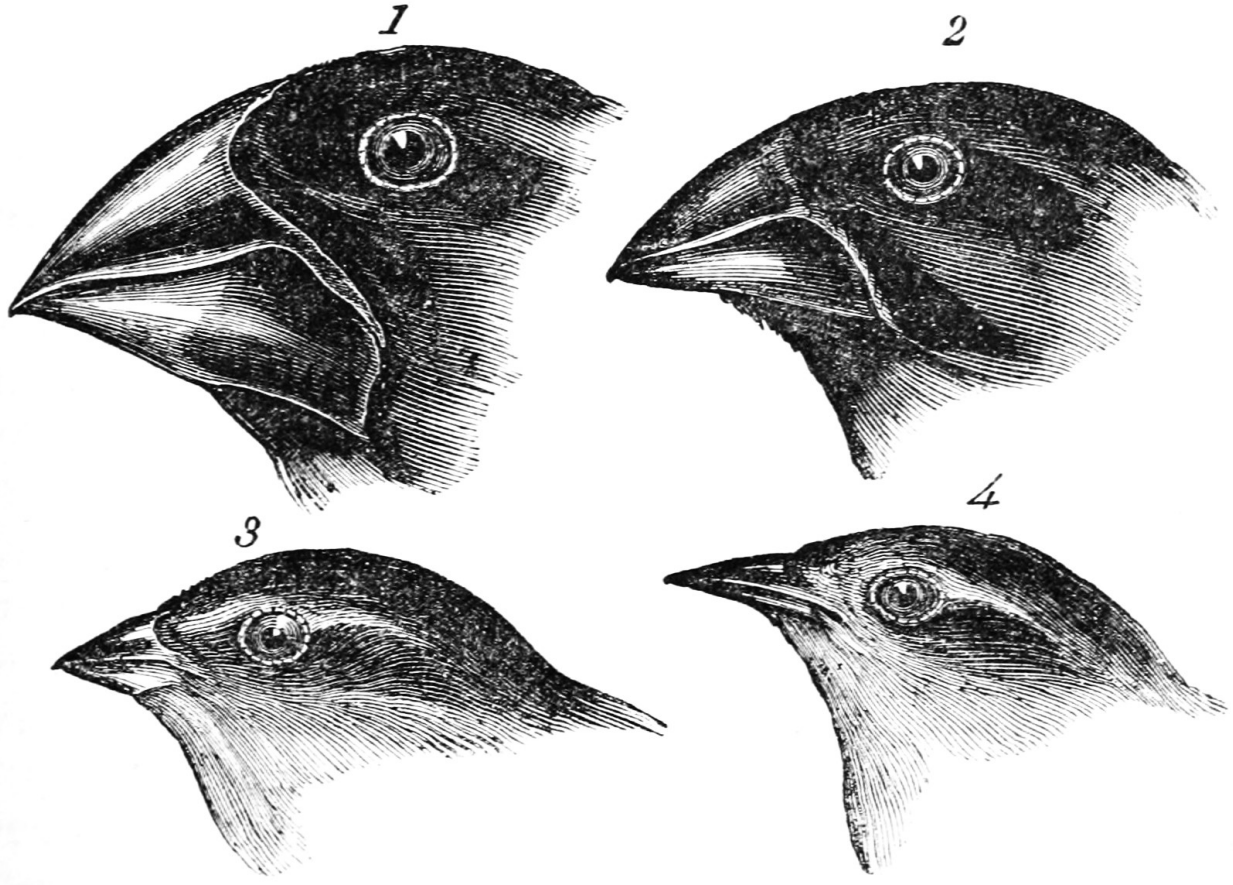
How do we determine that features are related, and evolved?



1. Geospiza magnirostris.
2. Geospiza fortis.
3. Geospiza parvula.
4. Certhidea olivasea.

Comparison of sequence features

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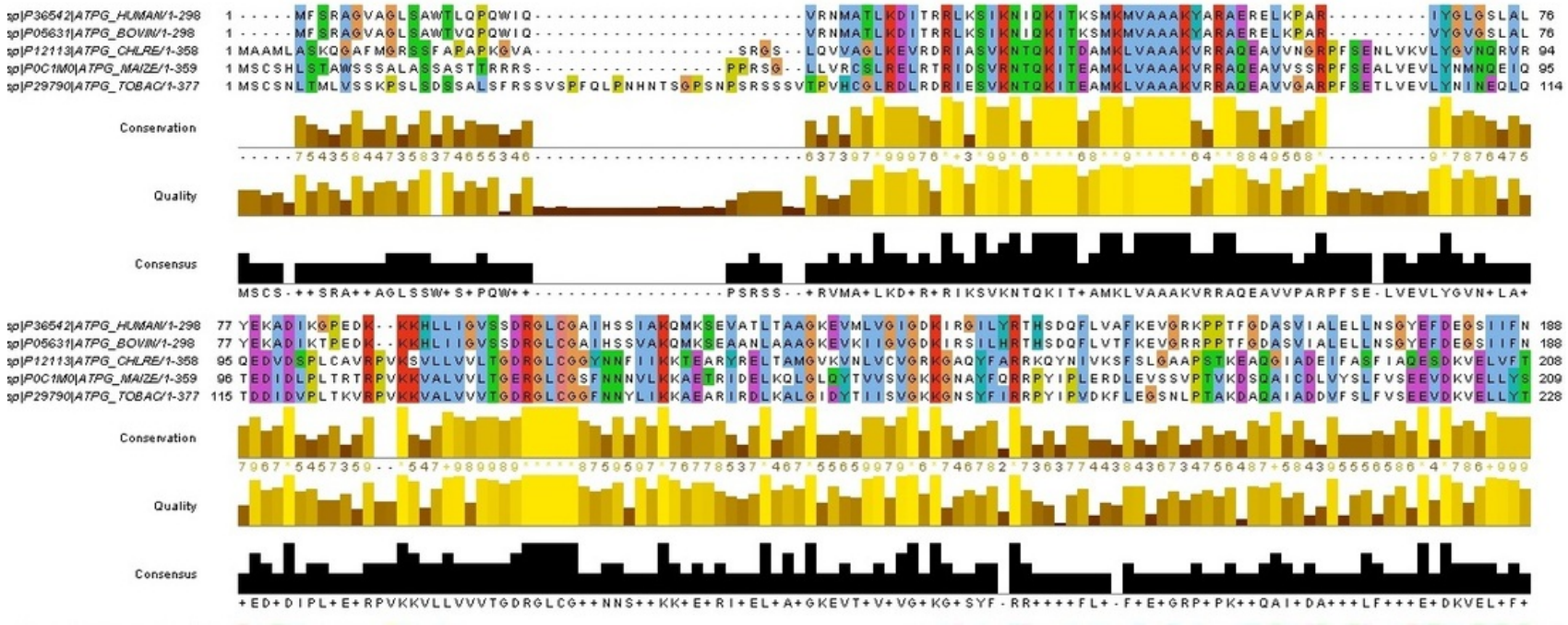
Multiple sequence alignment of ATP synthase



Comparison of genome features

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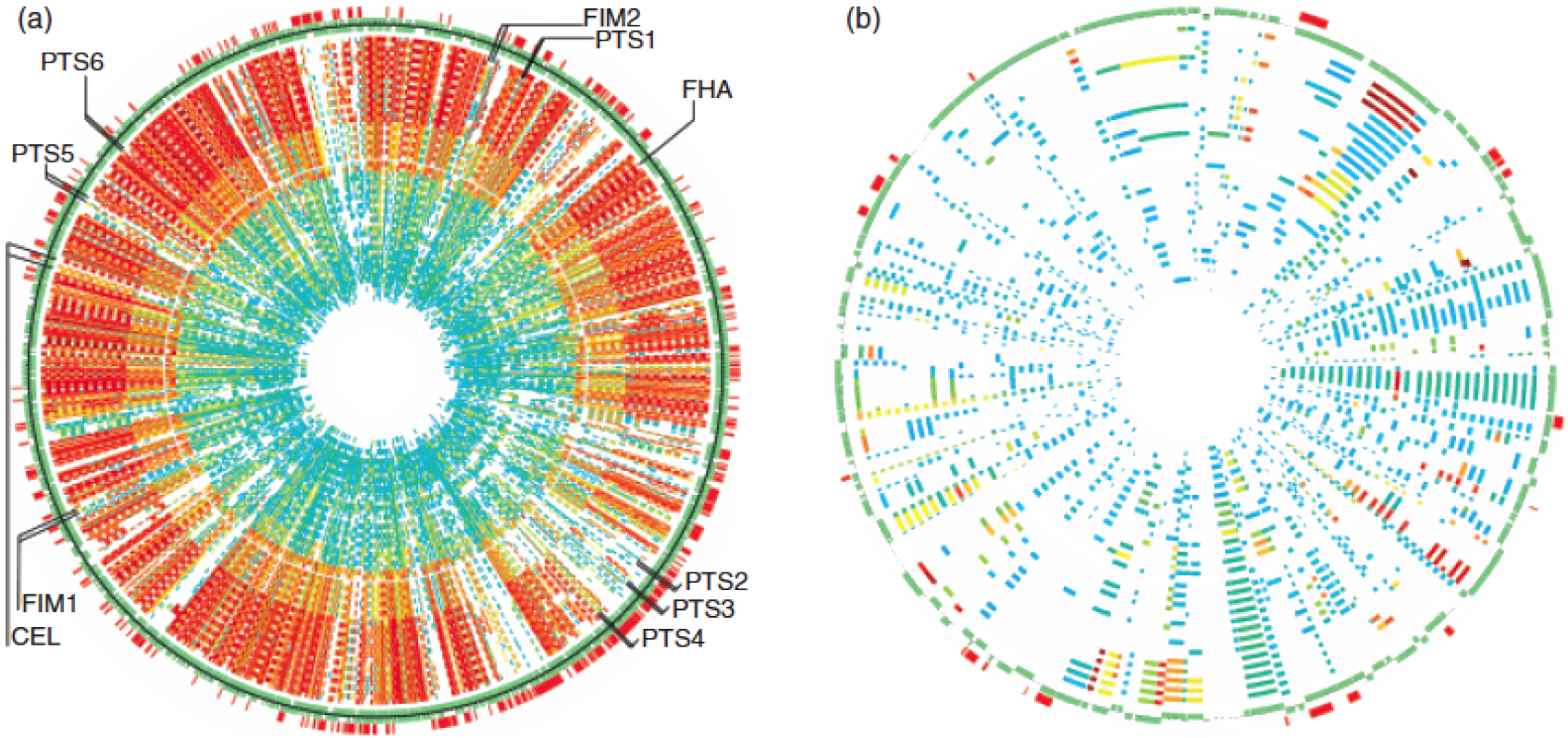
Similarity of individual features (feature sequence)



Comparison of genome features

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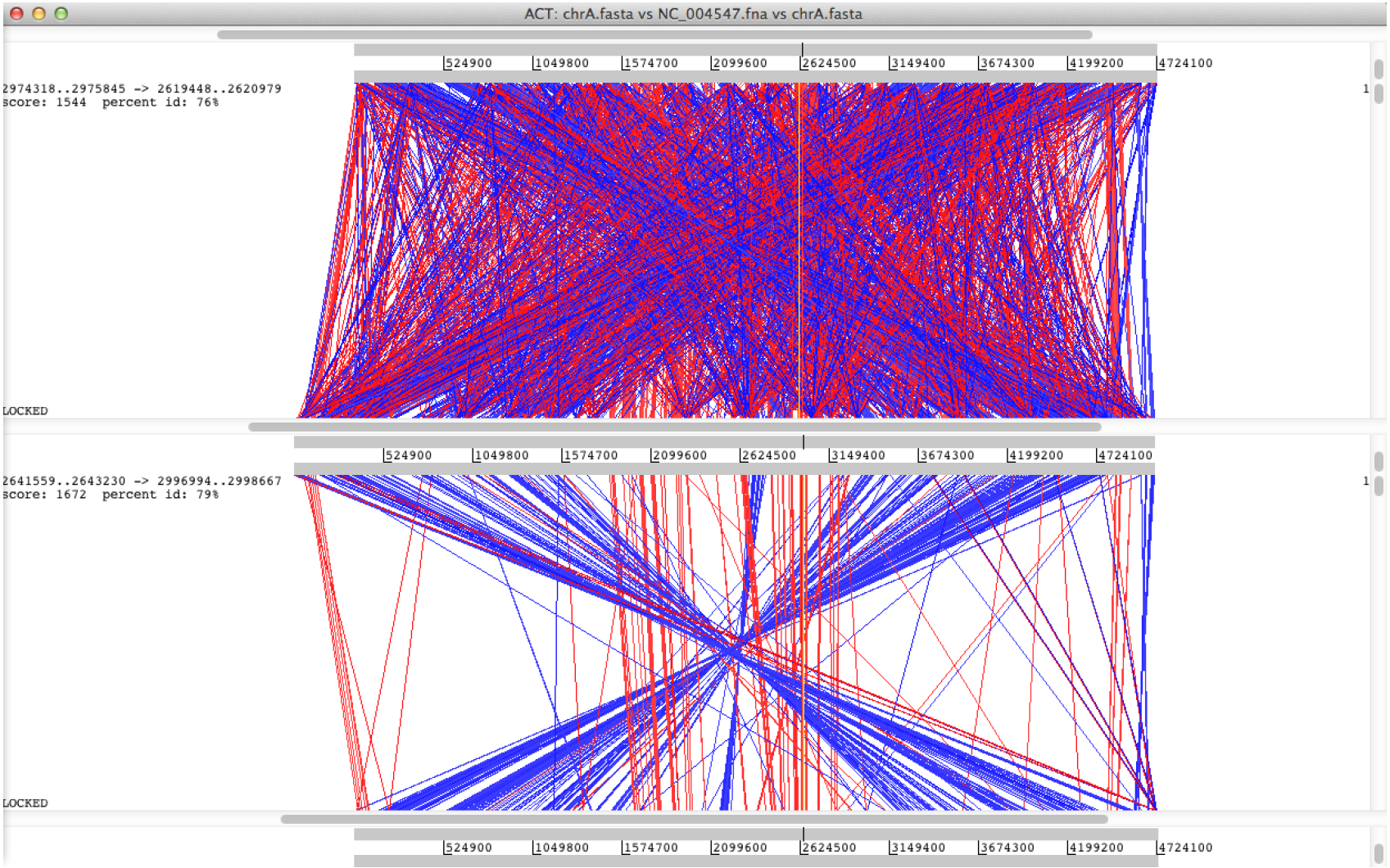
Similarity of individual features (ordering and arrangement)



Why comparative genomics?

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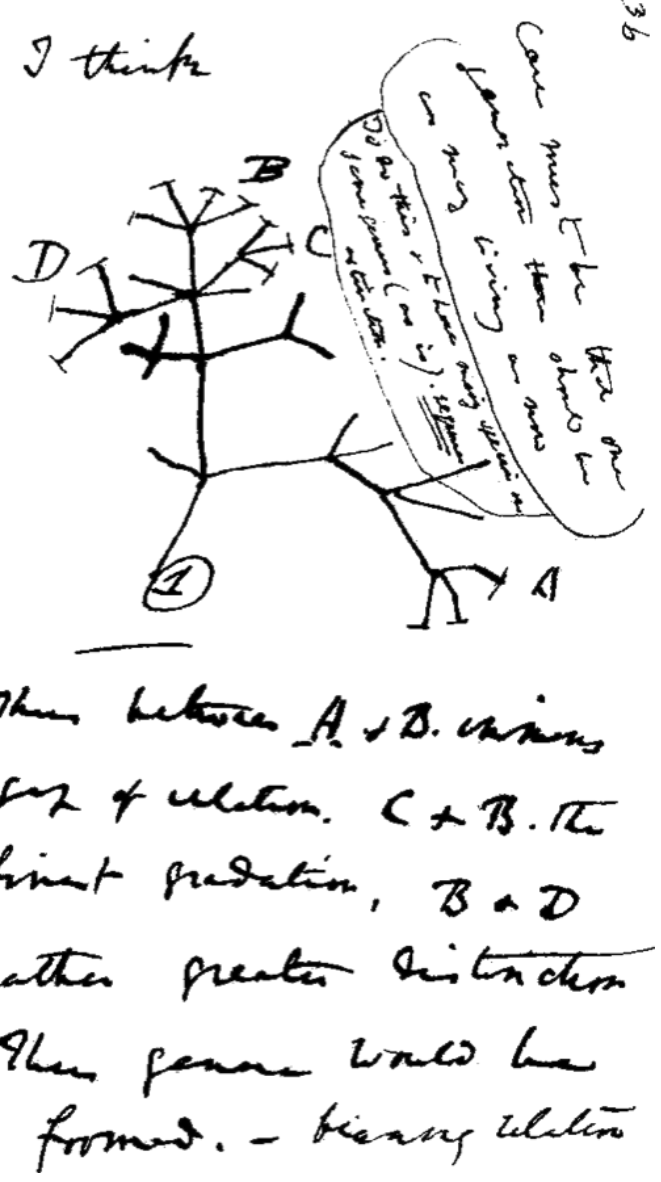
- Genome features are heritable characteristics
- Related organisms share ancestral genomes
- Related organisms inherit common genome features
- Genome similarity $\propto$ relatedness? (phylogenomics)



Why comparative genomics?

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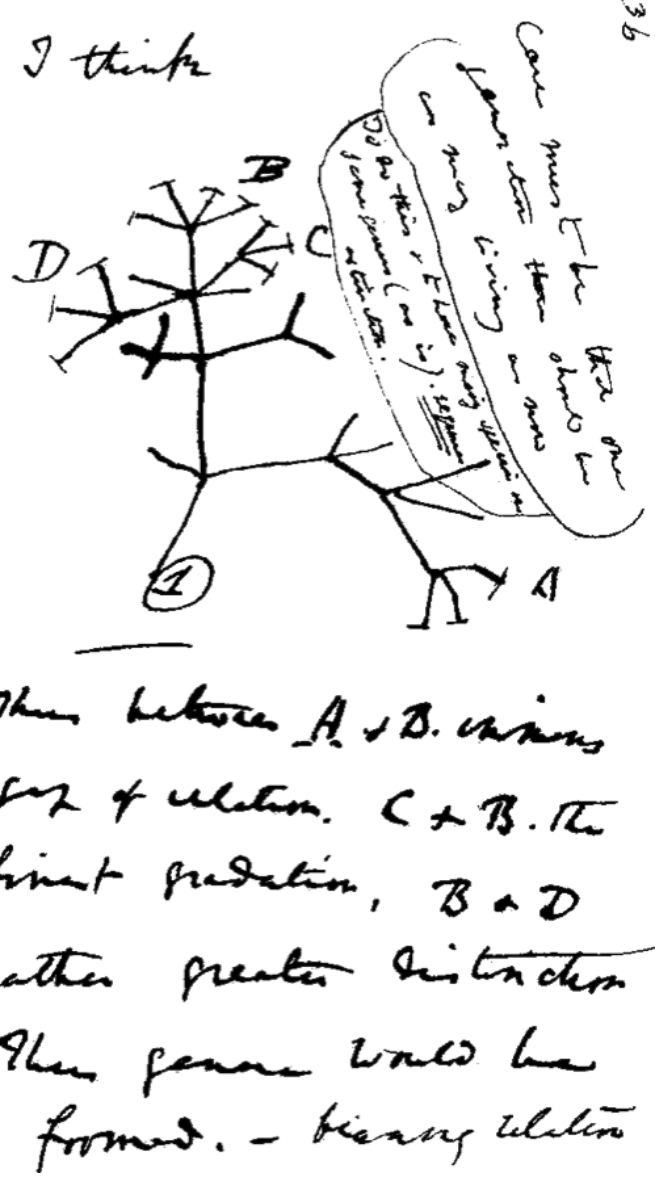
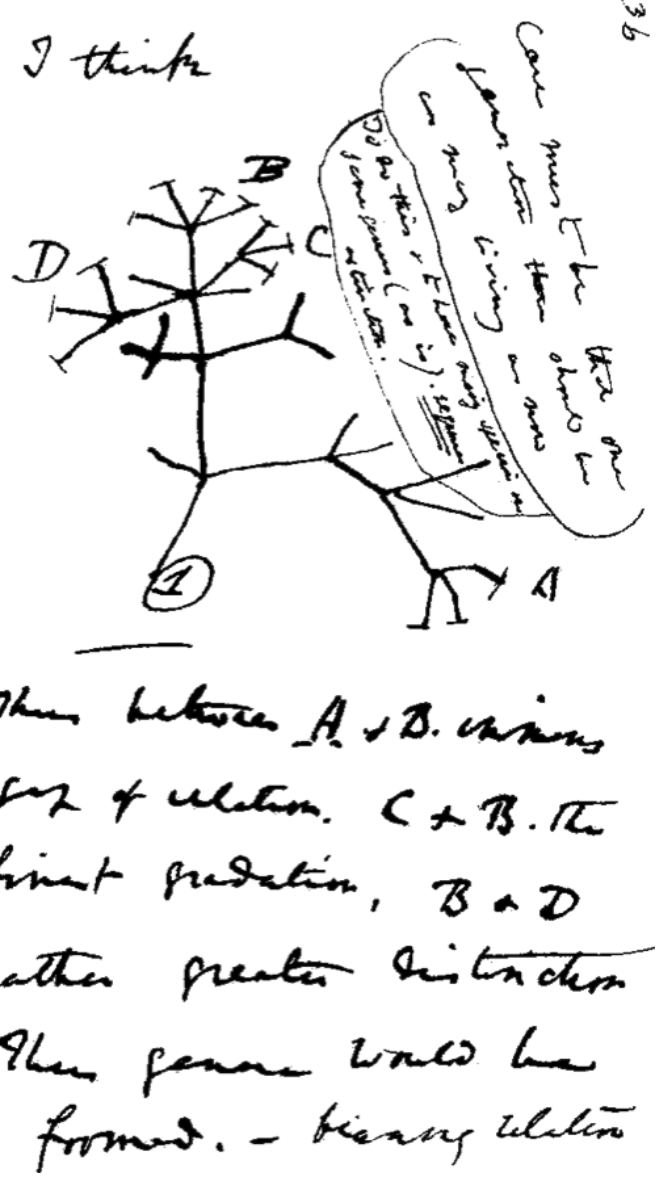
- Genomes carry functional elements under selection pressure
- Related organisms carry similar functional elements
- Deleterious functional elements are lost through selection
- Genome similarity $\propto$ phenotype? (functional genomics)



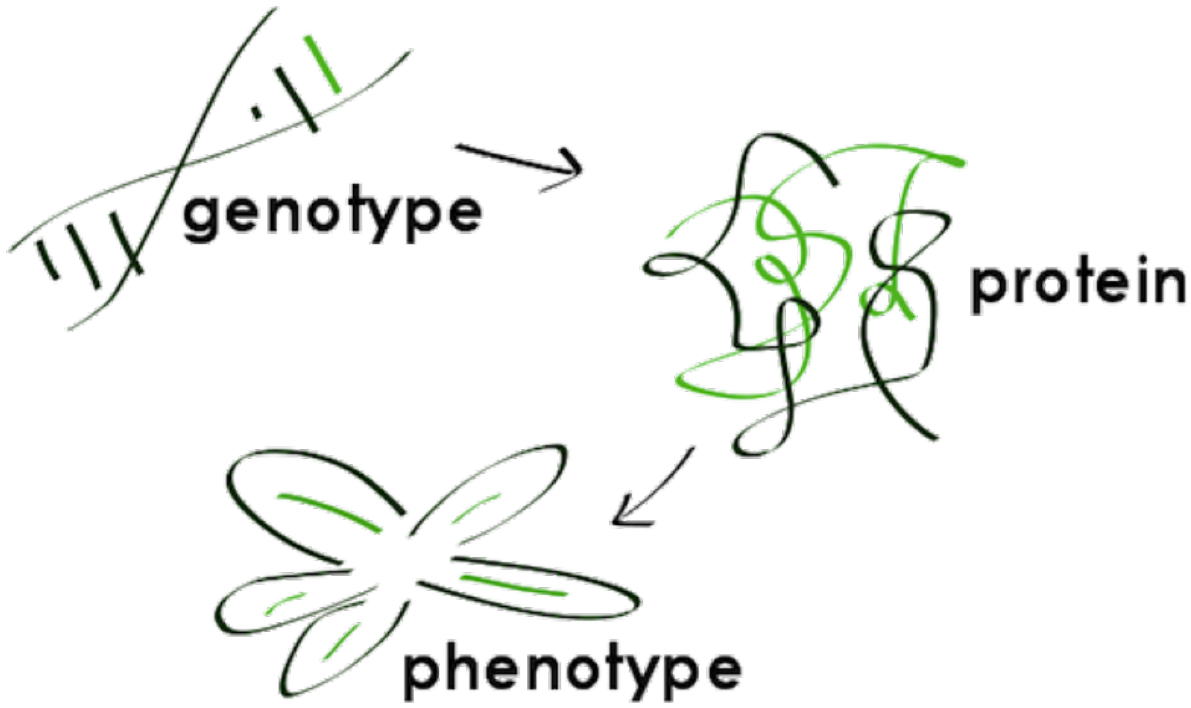
Why comparative genomics?

- Functional elements are selected over many generations
- Optimised functional elements may not change greatly
- (Functional elements can be transferred non-heritably)
- Genome feature similarity $\Longrightarrow$ common function? (genome annotation)
- Transfer functional information from model systems (*E. coli*, *A. thaliana*, *D. melanogaster*) to non-model systems



Genomes are informative, but...

BIOLOGICAL CONTEXT: epigenetics, tissue differentiation, mesoscale systems, etc.



PHENOTYPIC PLASTICITY: responses to temperature, stress, environment, etc.

Genomes to systems

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Functional Genomics

- Genome differences underpin phenotype (morphological, physiological) differences.
- If phenotypes known, genome comparisons can indicate genomic origin of differences (e.g. GWAS).
- Genome comparisons can reveal evolutionary processes and constraints, and suggest phenotypic outcomes.

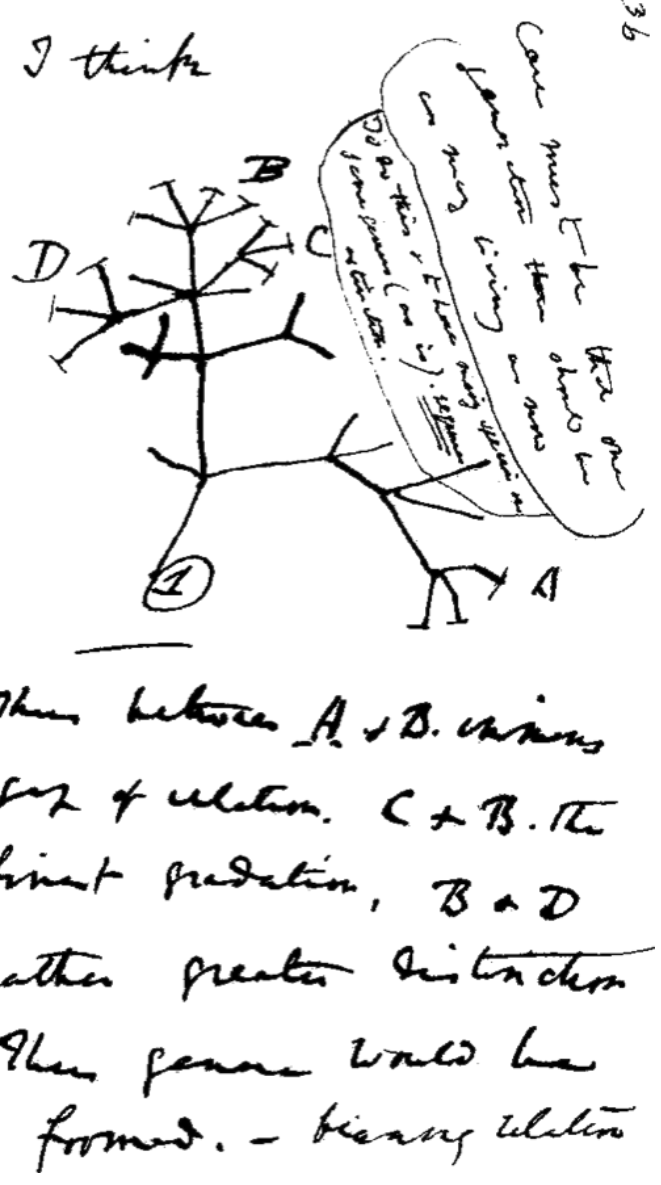

Table of Contents

Types of comparison

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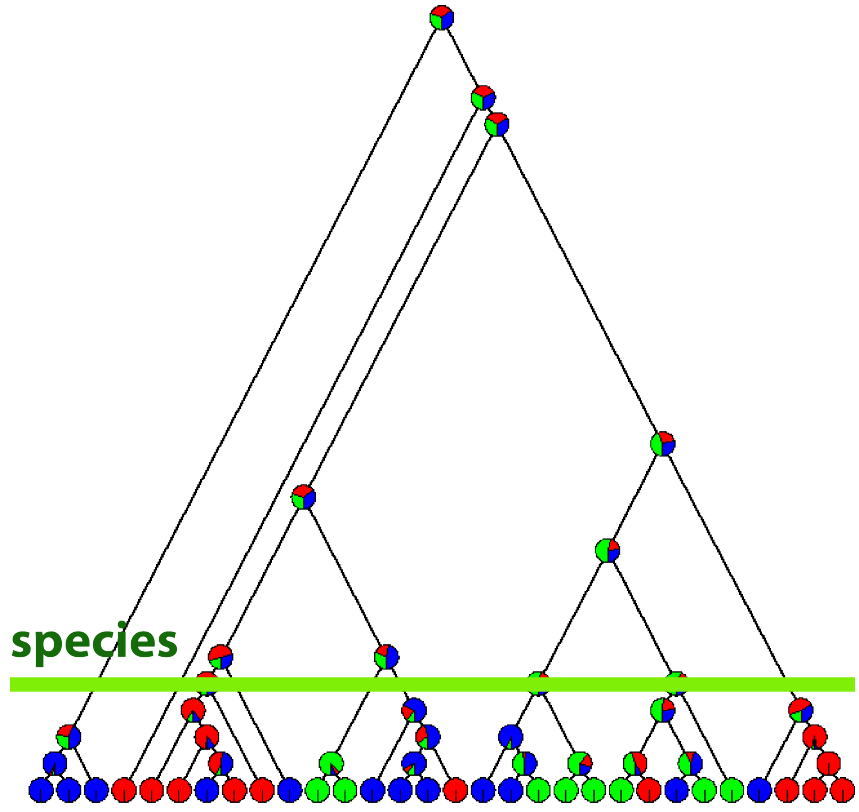
Within species

- e.g. between isolates/individuals (or between tissues...)
- Which genome features may account for unique characteristics of organisms or cell-types (e.g. tumours)?
- what epigenetic changes occur in an individual?



Types of comparison

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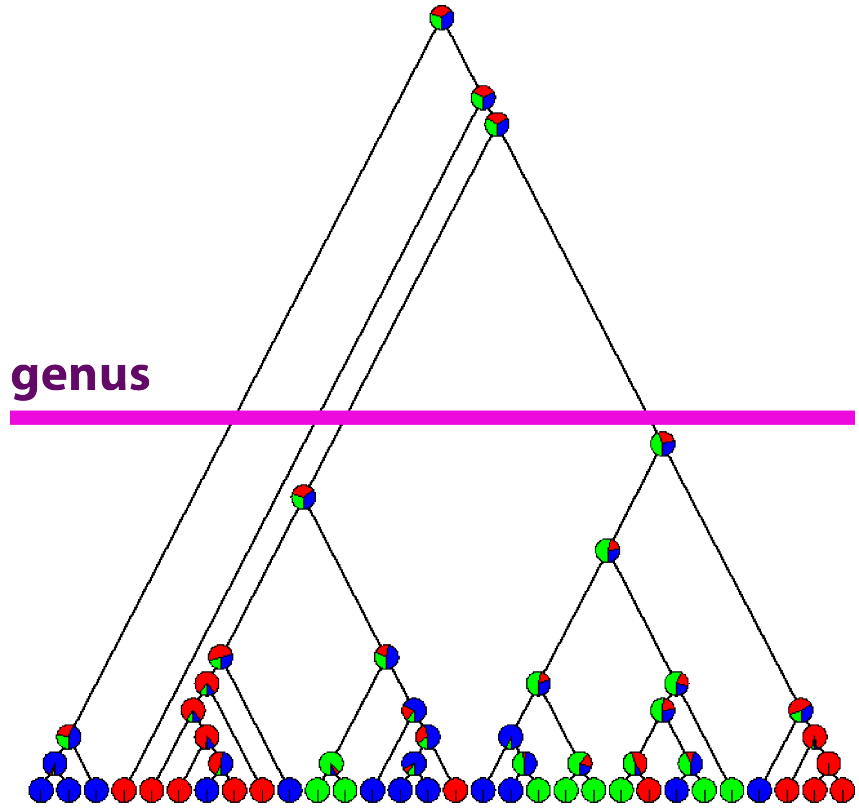
Within genera/between species

- comparison between groups of individuals
- what genome features show evidence of selective pressure?
- which features/changes are associated with species phenotype?



Types of comparison

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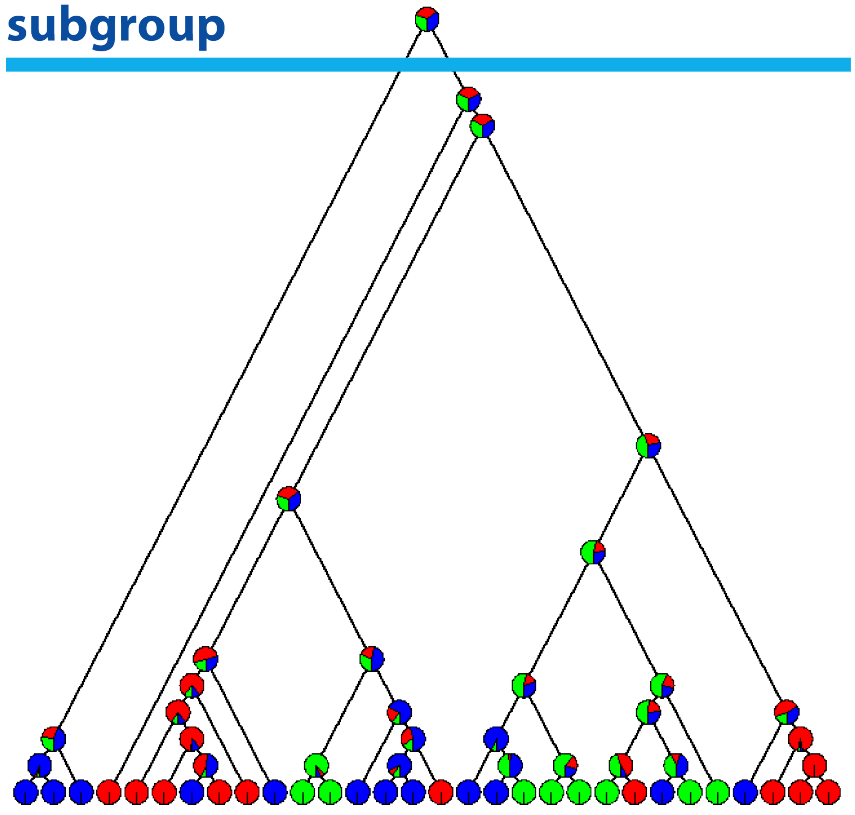
Between subgroups

- e.g. comparisons across many diverse individuals
- what are the *core set* of genome features that define a subgroup or genus?
- what functions are present/absent between groups?



E. coli LTEE [a] [b] [c]

[a] Jeong *et al.* (2009) *J. Mol. Biol.* doi:10.1016/j.jmb.2009.09.052

[b] Barrick *et al.* (2009) *Nature* doi:10.1038/nature08480

[c] Wiser *et al.* (2013) *Science* doi:10.1126/science.1243357

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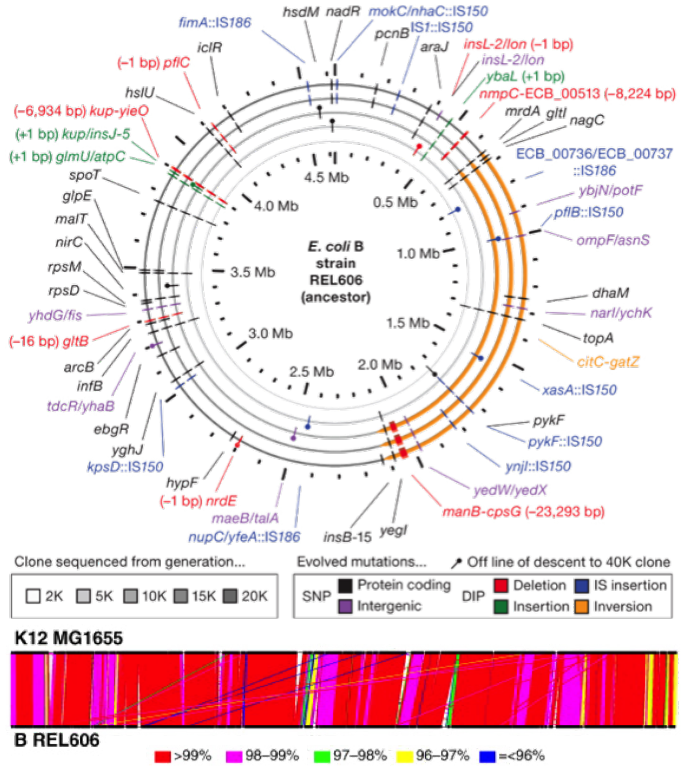
- Run by the Lenski lab, Michigan State University since 1988 (http://myxo.css.msu.edu/ecoli/)
- 12 flasks, citrate usage selection
- >50,000 generations of *E coli*!
  - Cultures propagated every day
  - Every 500 generations (75 days), mixed-population samples stored
  - Mean fitness estimated at 500 generation intervals

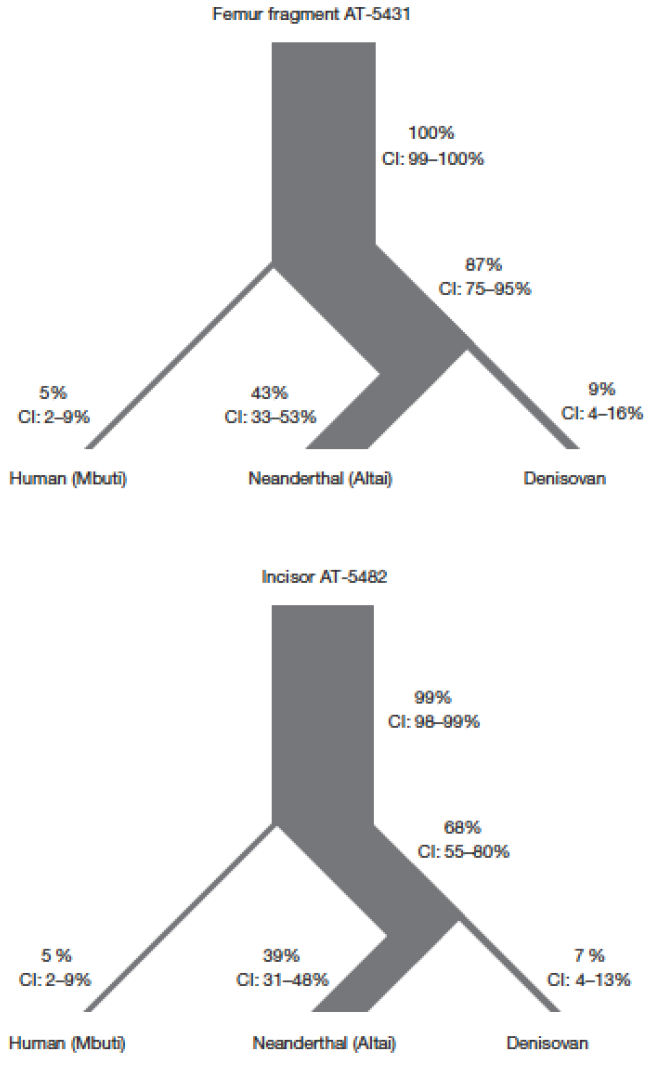


Comparative genomics in the news [a] [b]

[a] BBC News 15/3/2016

[b] Meyer *et al.* (2016) *Nature* doi:10.1038/nature17405

- Oldest DNA ever recovered from a human (430kya) - 0.1% of genome
- 28 individuals, Sima de los Huesos, N. Spain
- mitoDNA more similar to Siberian Denisovans than to modern humans
- Modern humans derived from wave out of Africa 250kya, with mitochondrial turnover?



Question:

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Which of these questions can be answered by comparative genomics?

- A: Are genes in a metabolic pathway always collocated, in species X?
- B: What genes does a transcription factor regulate?
- C: Which genes in a genome are under positive selective pressure?
- D: What genome features can be used to define a species?

Table of Contents

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Levels of comparison

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Bulk Properties

- chromosome/plasmid counts and sizes, nucleotide content, etc.

Whole Genome Sequence

- sequence similarity
- organisation of genomic regions (synteny), etc.

Genome Features/Functional Components

- numbers and types of features (genes, ncRNA, regulatory elements, etc.)
- organisation of features (synteny, operons, regulons, etc.)
- complements of features
- selection pressure, etc.

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Bulk genome comparisons

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You don't have to sequence genomes to compare them
(but it helps)

Genome comparisons predate NGS

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- Sequence data wasn't always cheap and abundant
- Practical, experimental genome comparisons were needed

Bulk genome comparisons

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Calculate values for individual genomes, then compare them.

- Number of chromosomes
- Ploidy
- Chromosome size
- Nucleotide (A,C,G,T) frequency

Nucleotide frequency/genome size

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Very easy to calculate from complete or draft genome sequence

```
In [1]: from Bio import SeqIO
In [2]: s = SeqIO.read("data/NC_000912.fna", "fasta")
In [3]: a, c, g, t = s.seq.count("A"), s.seq.count("C"), s.seq.count("G"), s.seq.count("T")
In [4]: float(g + c)/len(s)
Out[4]: 0.40008010837904245
In [5]: float(g - c)/(g+c)
Out[5]: 0.002397259225467894
```

GC content, chromosome size can be characteristic of an organism.

Genome Size

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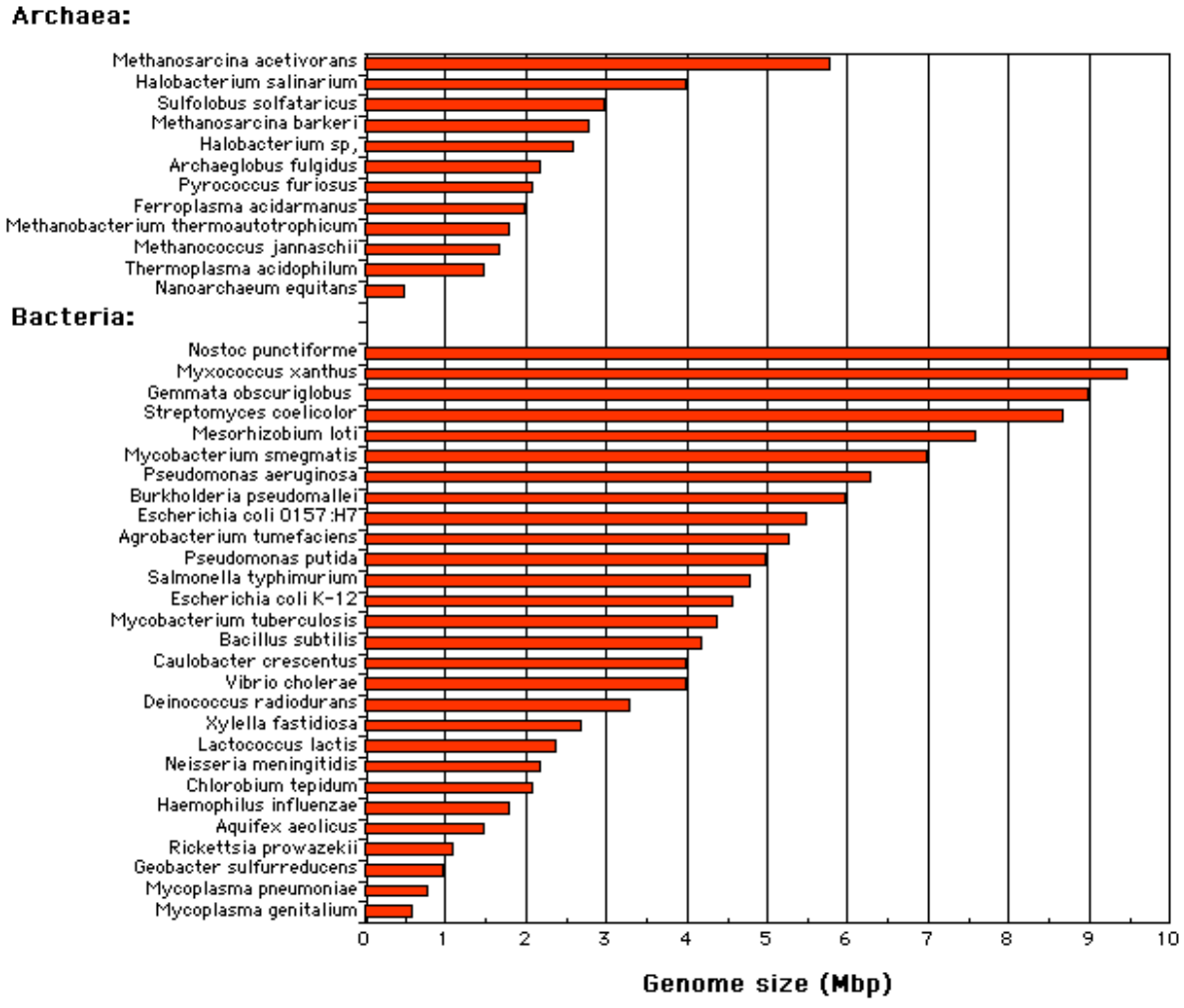
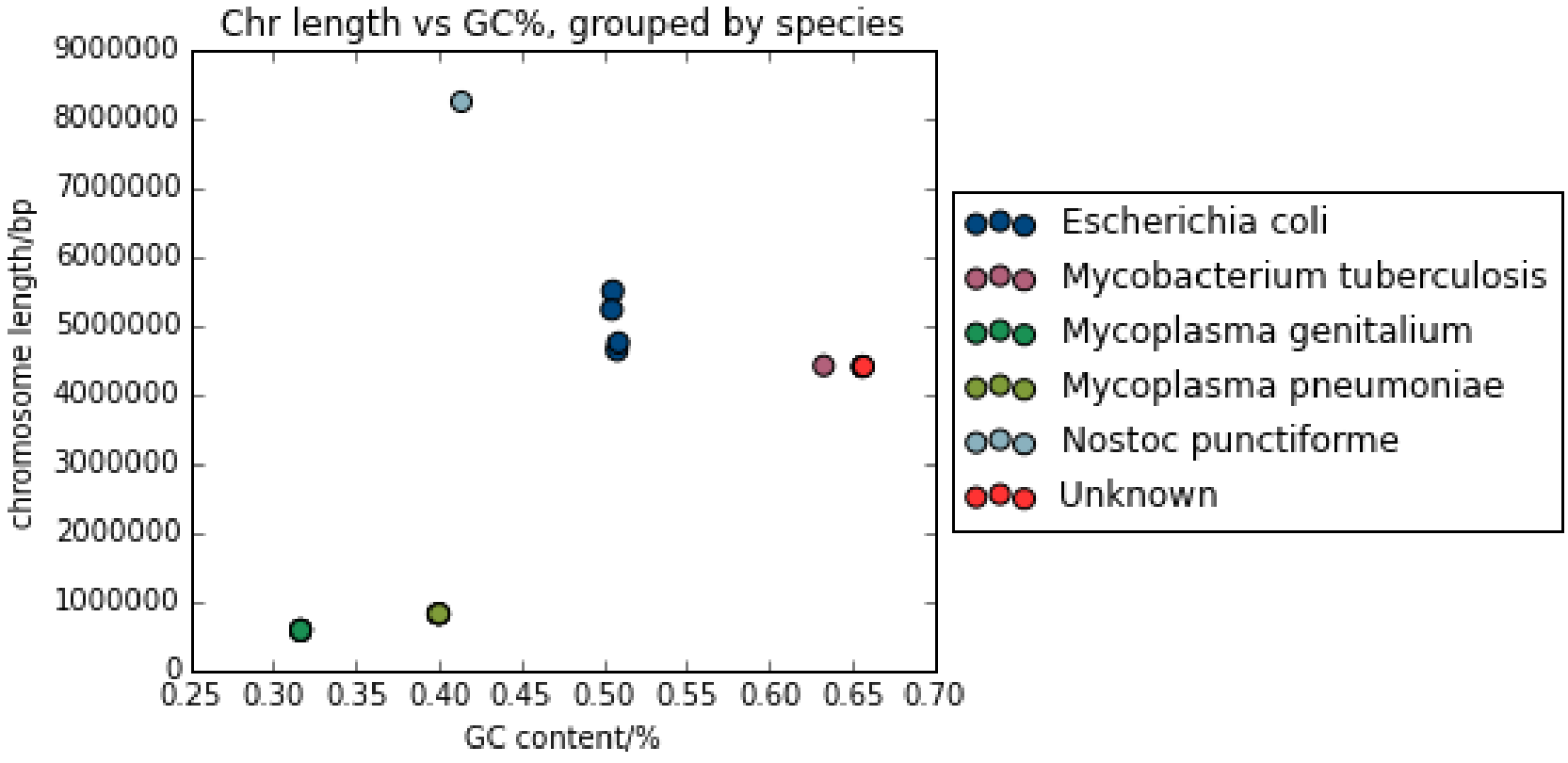


Genome Size and GC%



Blobology [a] [b]

[a] Kumar & Blaxter (2011) *Symbiosis* doi:10.1007/s13199-012-0154-6
[b] http://nematodes.org/bioinformatics/blobology/

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Sequence data can be contaminated by other organisms

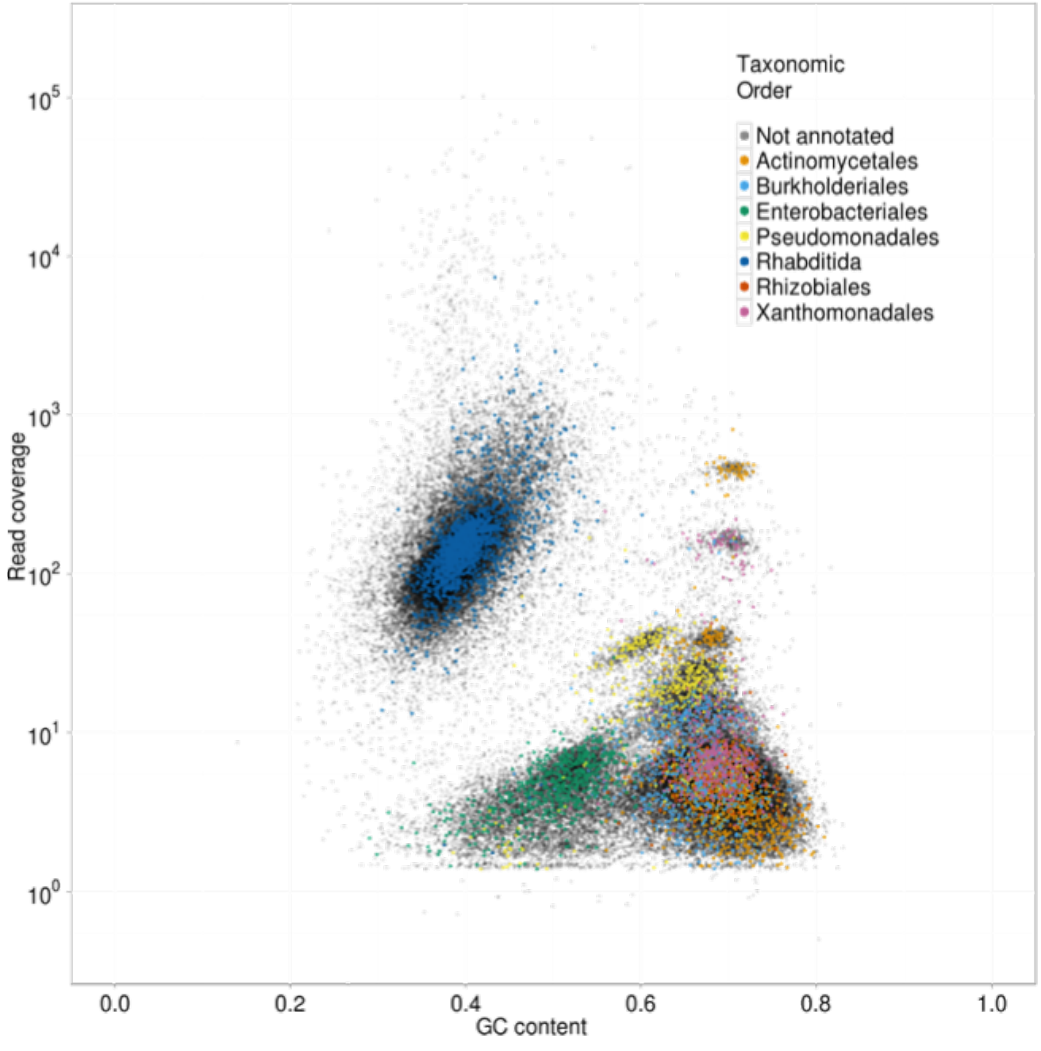
- Host and symbiont DNA have different %GC
- Host and symbiont DNA differ in coverage

- Assemble genome
- Map reads
- Plot coverage against %GC

Blobology [a] [b]

[a] Kumar & Blaxter (2011) *Symbiosis* doi:10.1007/s13199-012-0154-6

[b] http://nematodes.org/bioinformatics/blobology/



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Nucleotide k-mers

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Sequence data is necessary to determine k-mers/frequencies
Not possible by experiment

- Nucleotides, $k = 1$, 4x1-mers
  `A,C,G,T`
- Dinucleotides, $k = 2$, 16x2-mers
  `AA,AC,AG,AT,CA,CC,CG,CT,GA,GC,GG,GT,TA,TC,TG,TT`
- Triucleotides, $k = 1$, 64x3-mers
- k-nucleotides, 4^kxk-mers

k-mer spectra [a]

[a] Chor *et al.* (2009) *Genome Biol.* doi:10.1186/gb-2009-10-10-r108

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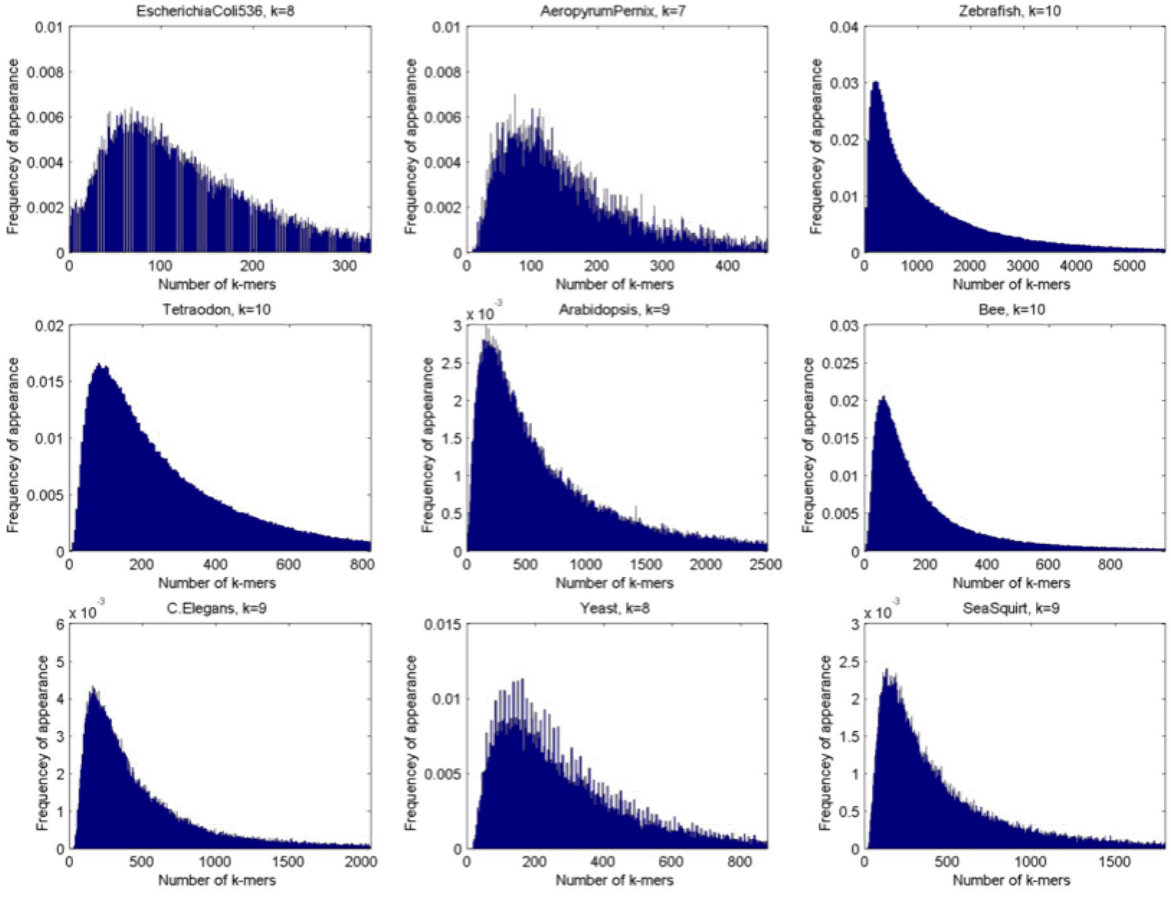
k-mer spectrum: frequency distribution of observed *k*-mer counts.

Most species have a unimodal *k*-mer spectrum



k-mer spectra [a]

[a] Chor *et al.* (2009) *Genome Biol.* doi:10.1186/gb-2009-10-10-r108

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All mammals tested (and some other) species have *multimodal k-mer spectra*

Genomic regions also differ in this property

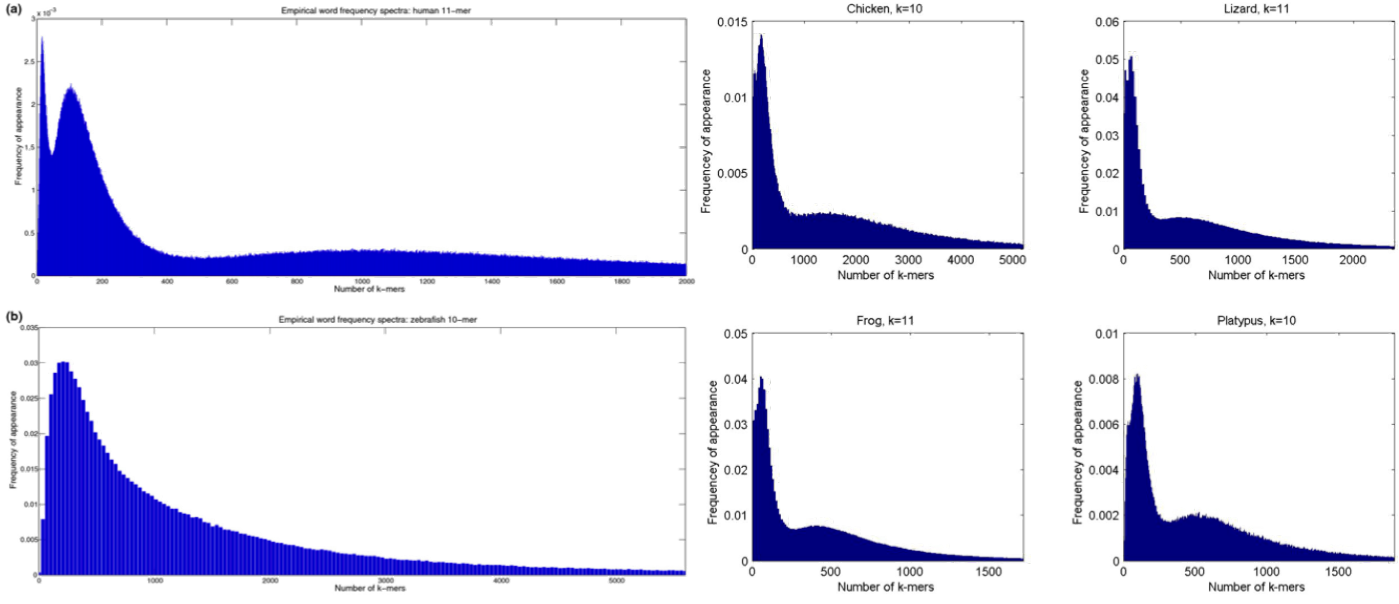

Table of Contents

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Whole genome comparisons

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Comparisons of one whole or draft genome with another
(. . .or many others)

Whole genome comparisons

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Minimum requirement: **two genomes**

- Reference Genome
- Comparator Genome

The experiment produces a comparative result *that is dependent on the choice of genomes*.

Whole genome comparisons

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Experimental methods mostly involve direct or indirect DNA hybridisation

- DNA-DNA hybridisation (DDH)
- Comparative Genomic Hybridisation (CGH)
- Array Comparative Genomic Hybridisation (aCGH)

Whole genome comparisons

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Analogously, *in silico* methods mostly involve sequence alignment

- Average Nucleotide Identity (ANI)
- Pairwise genome alignment
- Multiple genome alignment

DNA-DNA hybridisation (DDH) [a]

[a] Morelló-Mora & Amann (2011) *FEMS Microbiol. Rev.* doi:10.1016/S0168-6445(00)00040-1

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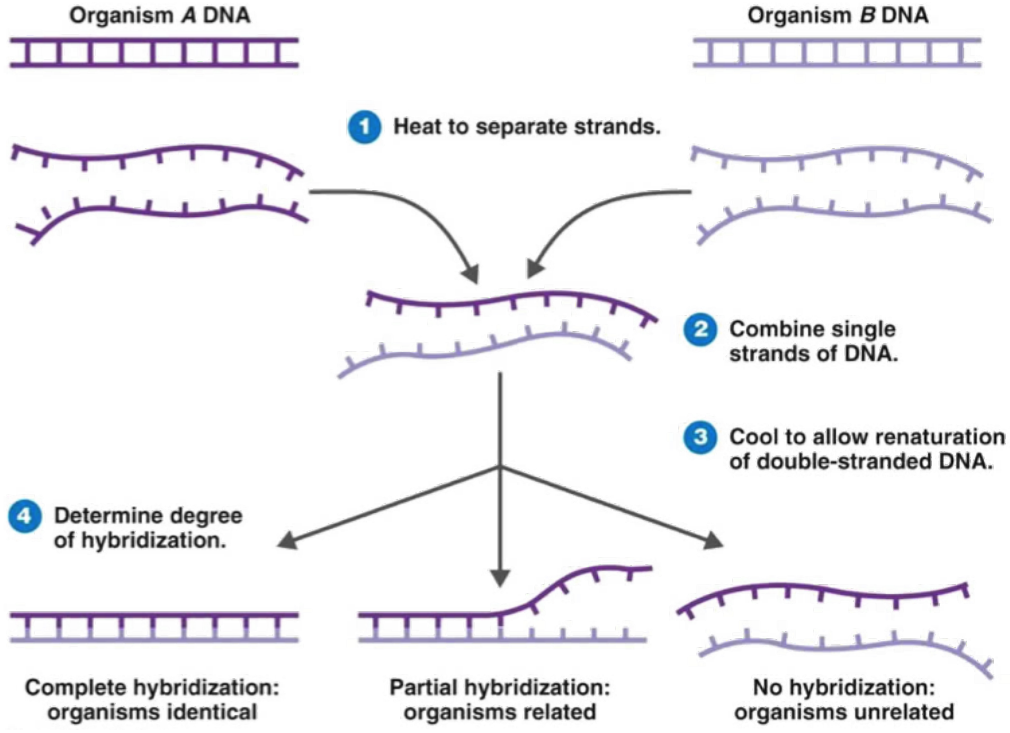
Several similar methods based on the same principle

- Denature gDNA mixture for organisms *A*, *B*
- Allow gDNA to anneal; hybrids result



Reassociation of gDNA $\approx$ sequence similarity

Average Nucleotide Identity (ANI) [a]

[a] Goris *et al.* (2007) *Int. J. System. Evol. Biol.* doi:10.1099/ijs.0.64483-0

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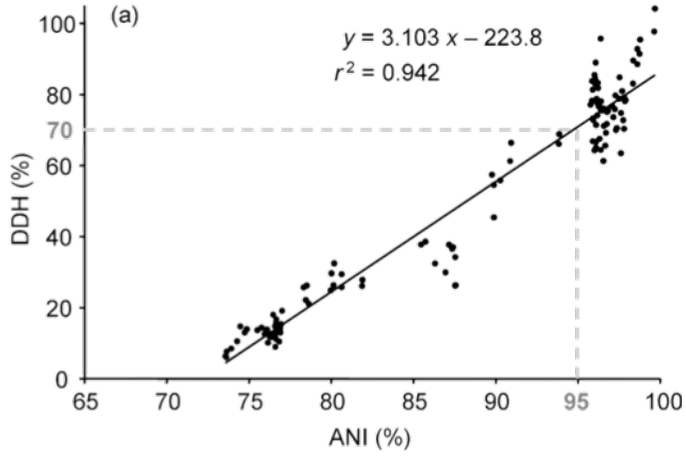
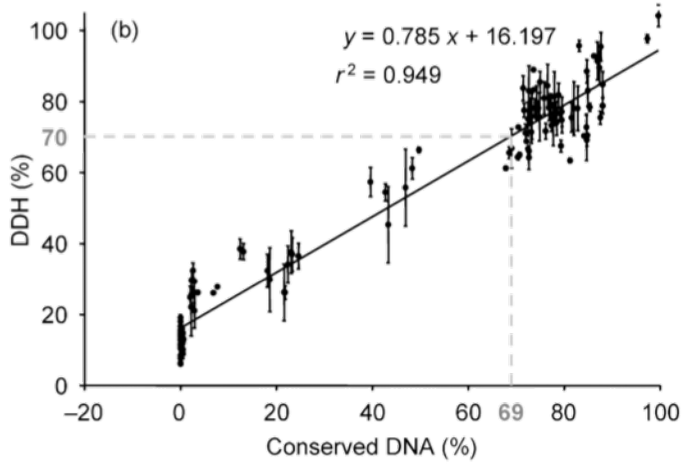
Introduced as an *in silico* substitute for DDH in 2007:

- 70% identity (DDH) = "gold standard" prokaryotic species boundary
- 70% identity (DDH) ≈ 95% identity (ANI)





Average Nucleotide Identity (ANI) [a]

[a] Goris *et al.* (2007) *Int. J. System. Evol. Biol.* doi:10.1099/ijs.0.64483-0

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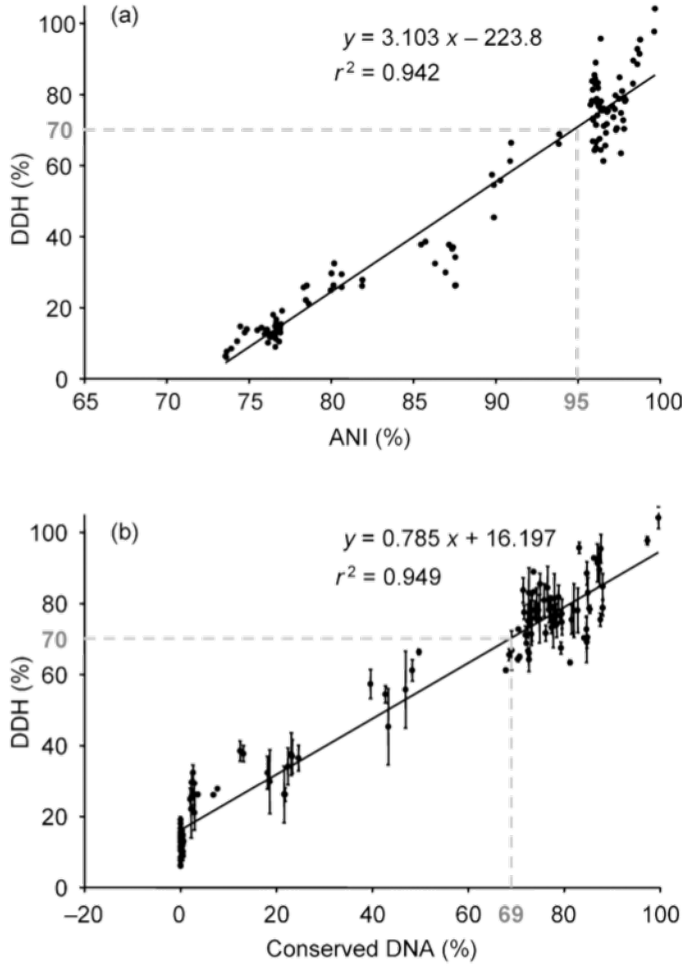
Original method emulated physical experiment:

1. break genome into 1020nt fragments
2. align all fragments with BLASTN
3. ANI = mean identity of all matches with $>$ 30% identity, $>$ 70% coverage



Average Nucleotide Identity (ANI) [a]

[a] Richter & Rosselló-Móra *et al.* (2009) *Proc. Natl. Acad. Sci. USA* doi:10.1073/pnas.0906412106

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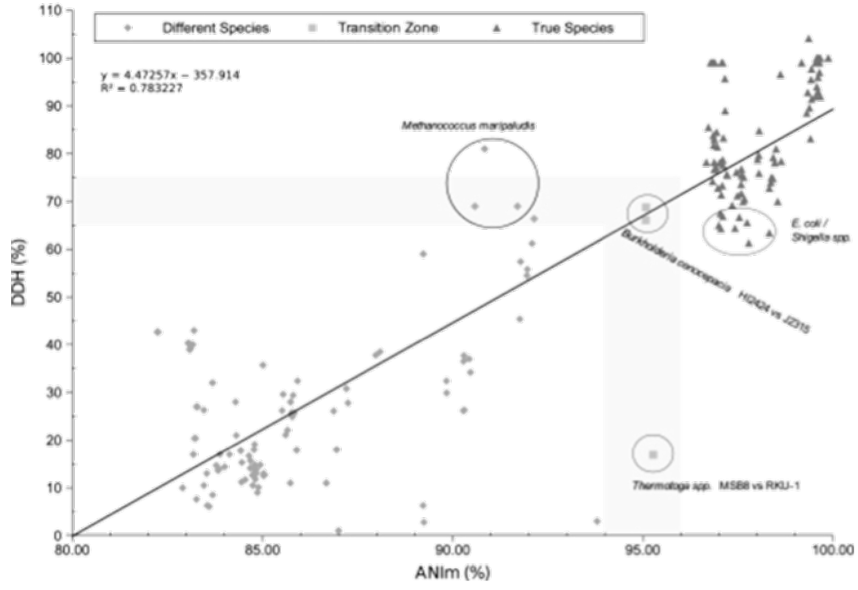
ANIm and TETRA variants introduced in 2009:

ANIm

1. Align sequences with NUCmer (no fragmentation)
2. ANI = mean identity of matches



TETRA (a bulk measure!)

1. Calculate 4-mer frequencies
2. Determine Z-score for 4-mer deviation from expected value, given %GC content
3. TETRA = Pearson correlation coefficient of Z-scores

ANI in practice [a] [b]

[a] van der Wolf *et al.* (2014) *Int. J. Syst. Evol. Micr.* **64**:768-774 doi:10.1099/ijs.0.052944-0

[b] Pritchard *et al.* (2016) *Anal. Methods* **8**:12-24 doi:10.1039/C5AY02550H

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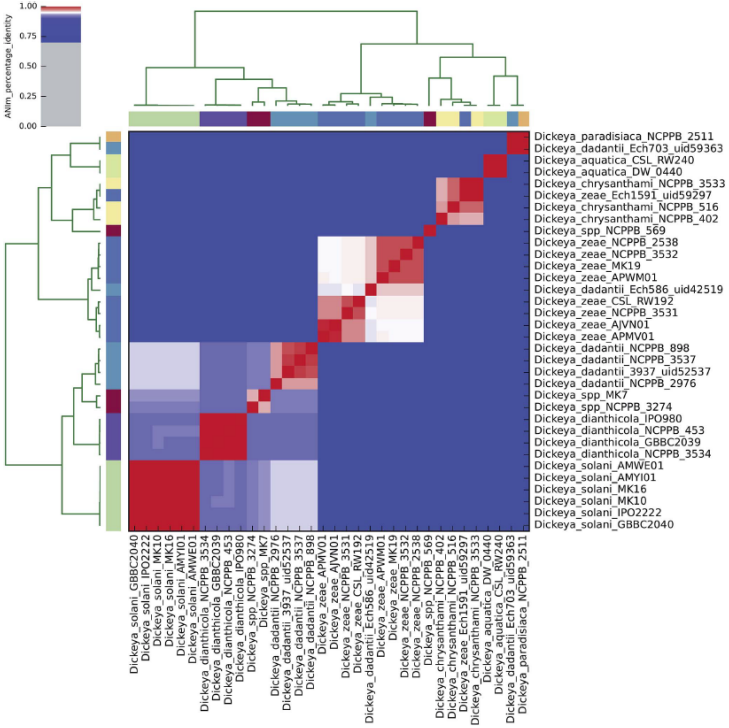
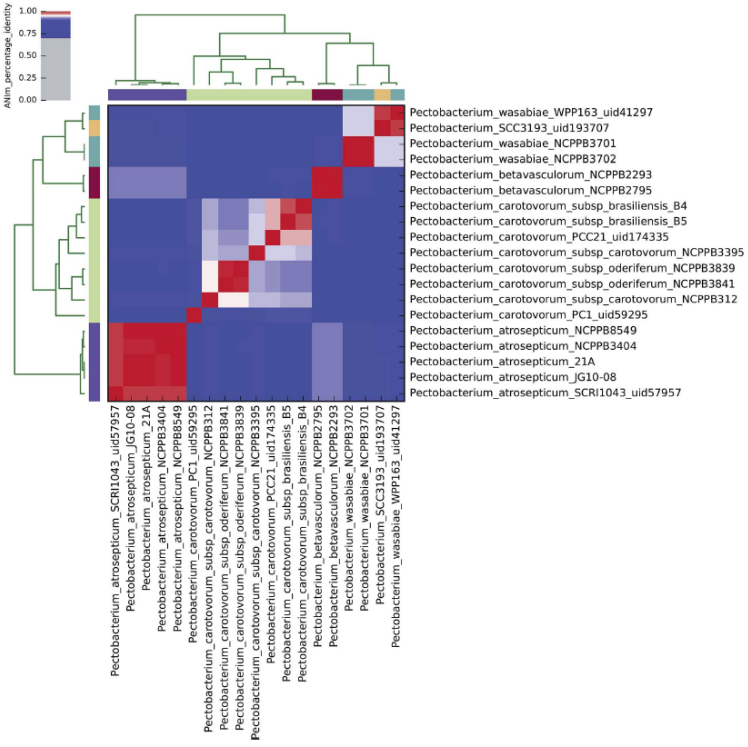
Dickeya species structure



Pectobacterium species structure:



Pairwise genome alignments

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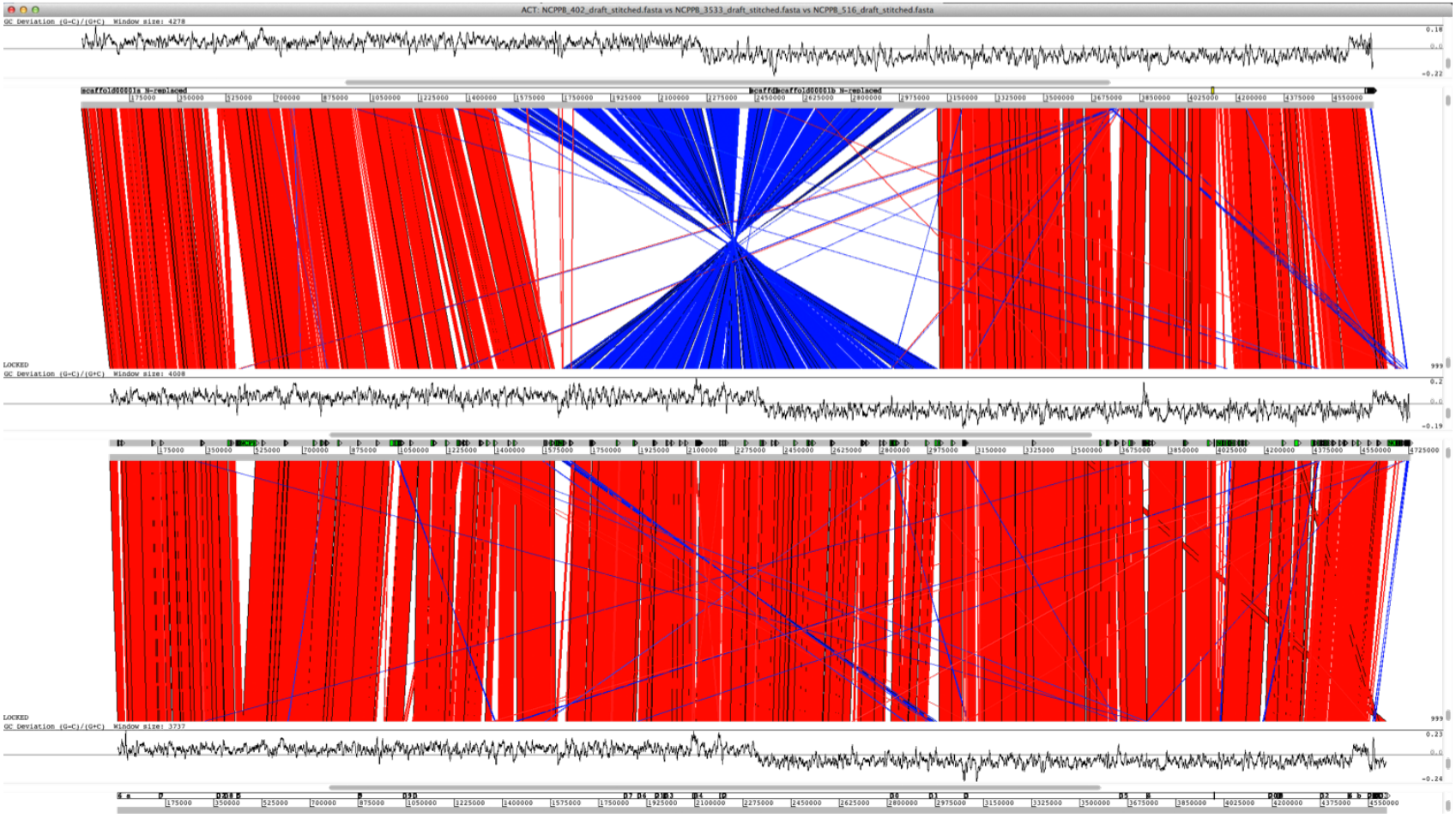
Pairwise comparisons require alignment of similar regions.



Synteny and Collinearity

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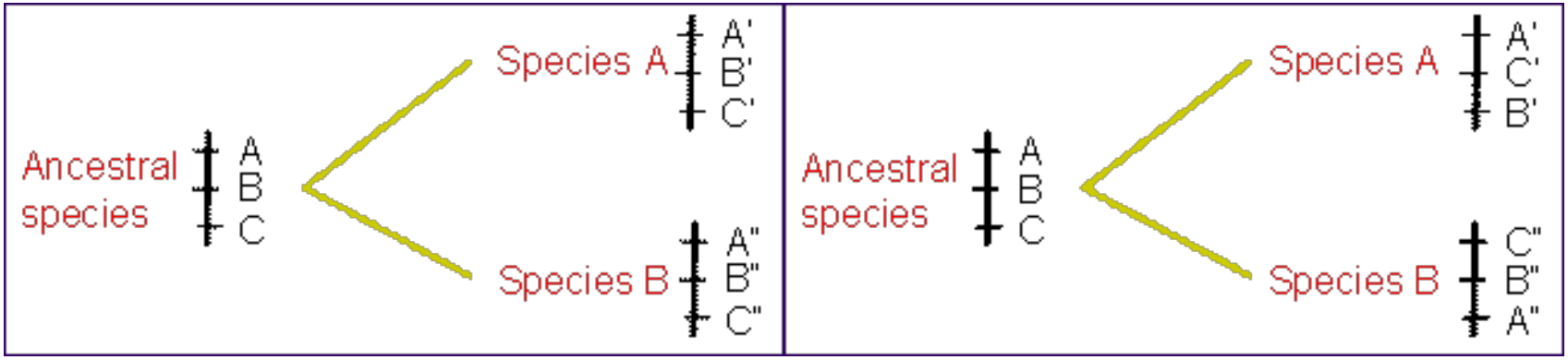
Genome rearrangements may occur post-speciation

Sequence similarity, and order of similar regions, may be conserved



- *collinear* conserved elements lie in the same linear sequence
- *syntenous* (or *syntenic*) elements:
  - (*orig.*) lie on the same chromosome
  - (*mod.*) are collinear

Evolutionary constraint (e.g. indicated by synteny) may indicate a functional constraint

Pairwise genome alignments

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Which genomes should you align (or not bother with)?
For reasonable analysis, genomes should:

- derive from a sufficiently recent common ancestor, so that homologous regions can be identified
- derive from a sufficiently distant common ancestor, so that biologically meaningful changes are likely to be found

Alignment algorithms/programs

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I assume you're familiar with BLAST

Naïve alignment algorithms are not appropriate:

- Needleman-Wunsch: optimal global alignment
- Smith-Waterman: optimal local alignment

Cannot handle rearrangement

Computationally expensive

Alignment algorithms/programs

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Many whole-genome alignment algorithms proposed

Handle genome-scale evolutionary processes, scalable

- LASTZ (http://www.bx.psu.edu/~rsharris/lastz/)
- **BLAT** (http://genome.ucsc.edu/goldenPath)
- Mugsy (http://mugsy.sourceforge.net/)
- **megaBLAST** (http://www.ncbi.nlm.nih.gov/blast/)
- **MUMmer** (http://mummer.sourceforge.net/)
- LAGAN (http://lagan.stanford.edu/lagan_web/index.shtml)
- WABA, etc?

megaBLAST [a] [b]

[a] Zhang *et al.* (2000) *J. Comp. Biol.* **7**(1-2): 203-214
[b] Korf *et al.* (2003) *BLAST* O'Reilly & Associates, Sebastopol, CA

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Optimised for:

- speed and genome-level searching
- queries on large sequence sets: "query-packing"
- long alignments of very similar sequences (`dc-megablast` for divergent sequences)

Uses Zhang et al. greedy algorithm, **not BLAST algorithm**

BLASTN+ defaults to megaBLAST algorithm
(see http://www.ncbi.nlm.nih.gov/blast/Why.shtml)

MUMmer [a]

[a] Kurtz *et al.* (2004) *Genome Biol.* doi:10.1186/gb-2004-5-2-r12

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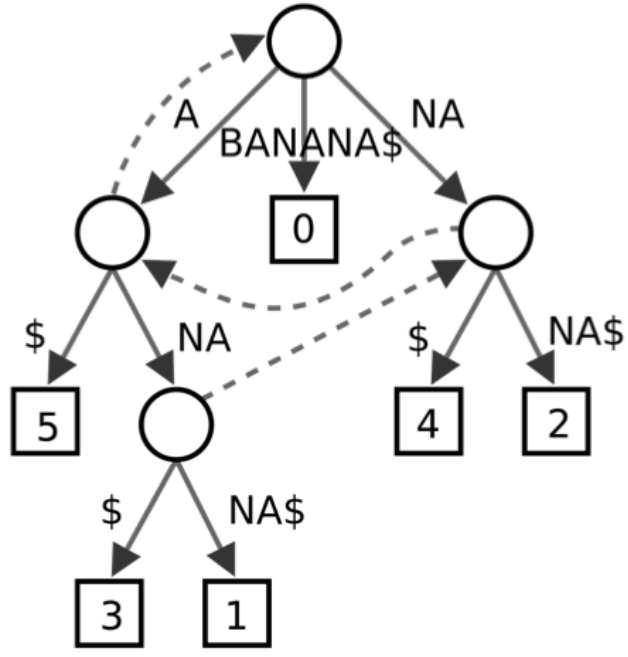
Conceptually completely different to BLAST/BLAT/megaBLAST

Uses *suffix trees* for pattern matching

- Finds maximal exact matches
- Memory use depends only on reference sequence size

Suffix Tree:

- Constructed and searched in $O(n)$ time
- Useful algorithms are nontrivial
- `BANANA$`



MUMmer [a]

[a] Kurtz *et al.* (2004) *Genome Biol.* doi:10.1186/gb-2004-5-2-r12

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Process:

1. Identify non-overlapping set of maximal exact matches (MUMs: *maximal unique matches*)
2. Cluster MUMs into *alignment anchors*
3. Extend between anchors to produce final (gapped) alignment

The approach is very flexible

1. Used in a suite of programs (`mummer`, `nucmer`, `promer`, ...)
2. Nucleotide and "conceptual protein" (sensitive!) alignments
3. Used for comparisons, scaffolding, repeat detection
4. Basis of other aligners and assemblers, (`Mugsy`, `AMOS`, ...)

Vibrio mimicus [a]

[a] Hasan *et al.* (2010) *Proc. Natl. Acad. Sci. USA* **107**:21134-21139 doi:10.1073/pnas.1013825107

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Chromosome C-II carries genes associated with environmental adaptation; C-I carries virulence genes.
C-II has undergone extensive rearrangement; C-I has not.

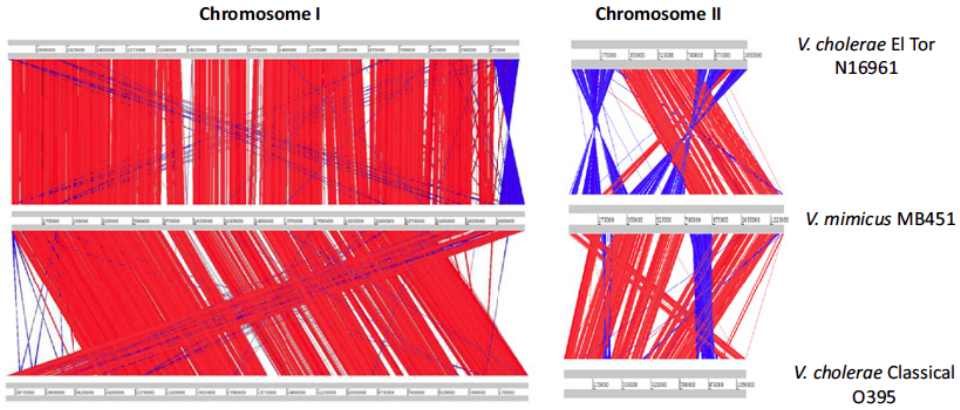


Fig. 2. Linear pairwise comparison of the *Vibrio mimicus* genome by Artemis Comparison Toll. Regions with similarity are highlighted by connecting red or blue lines between the genomes; red lines indicate homologous blocks of sequence, and blue lines indicate inversions. Gaps indicate unique DNA. The gray bars represent forward and reverse strands.

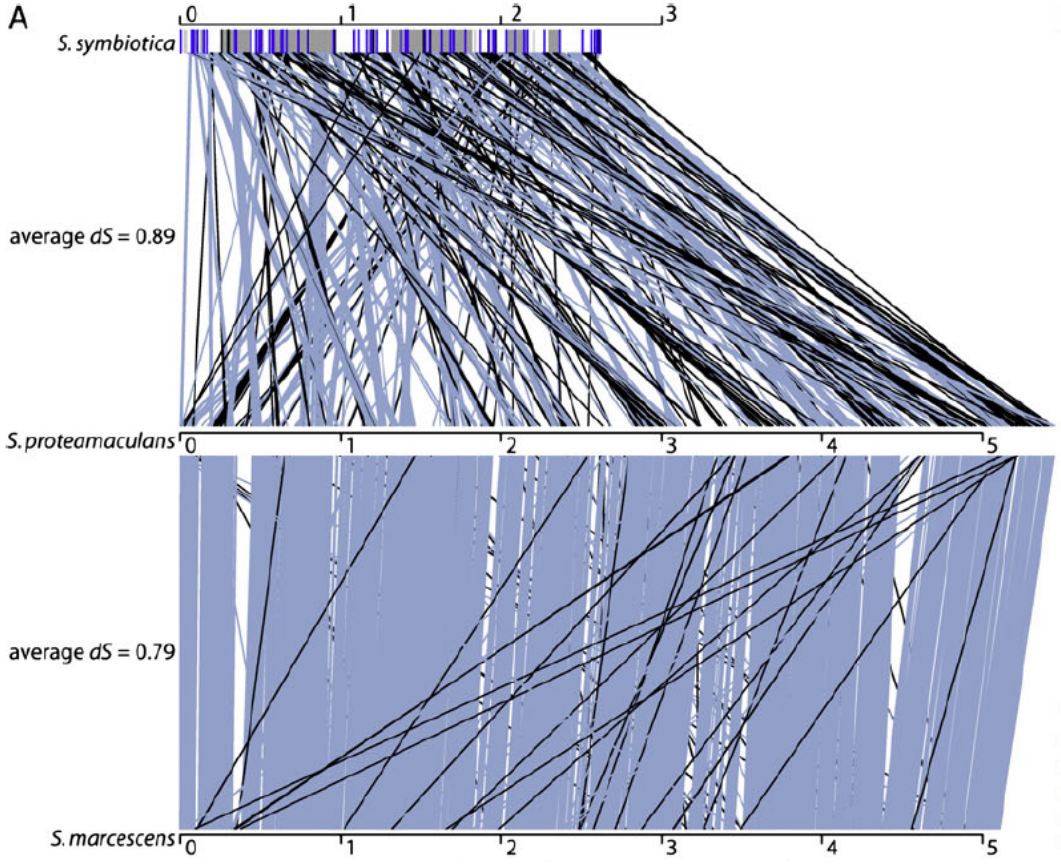
Suggests modularity of genome organisation, as a mechanism for adaptation (HGT, two-speed genome).

Serratia symbiotica [a]

[a] Burke and Moran (2011) *Genome Biol. Evol.* **3**:195-208 doi:10.1093/gbe/evr002

S. symbiotica is a recently evolved symbiont of aphids

Massive genomic decay is an adaptation to the new environment.



Multiple genome alignments

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Multiple genome alignments are "harder" than pairwise

- Computationally difficult to produce
- Lead to NP-complete optimisation problems!

Solutions: **heuristics**

- Progressive (build a tree, combine pairwise alignments)
- Iterative (realign initial sequences as new genomes added)
- Positional homology
- *Glocal* alignments

Multiple genome alignment

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Many tools either positional homology or glocal alignment

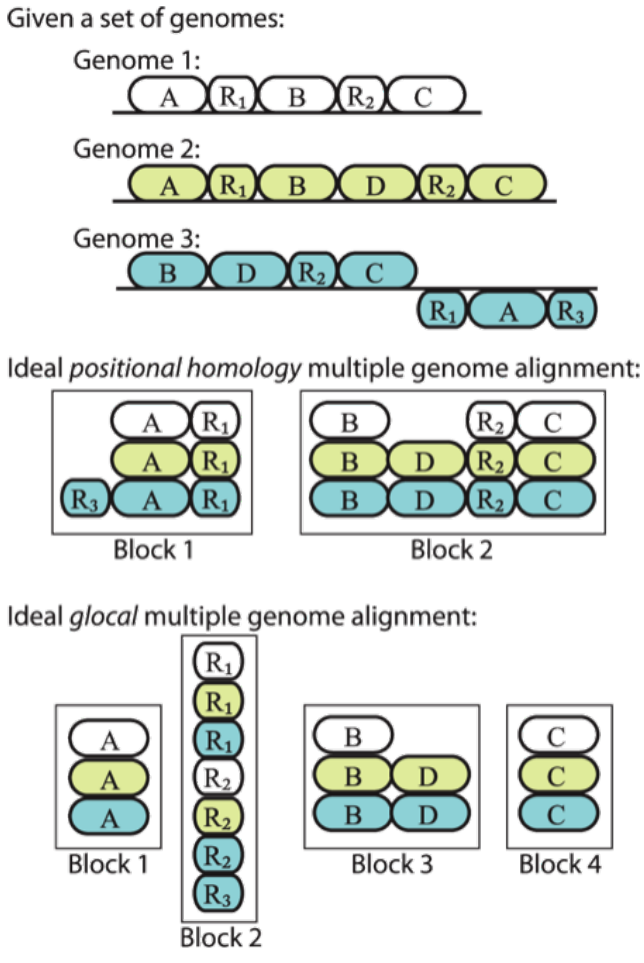
Several tools:

- Mugsy: (http://mugsy.sourceforge.net/)
- MLAGAN:

  (http://lagan.stanford.edu/lagan_web/index.shtml)
- TBA/MultiZ:

  (http://www.bx.psu.edu/miller_lab/)
- **Mauve**:

  (http://gel.ahabs.wisc.edu/mauve/)



Human:Mouse:Rat

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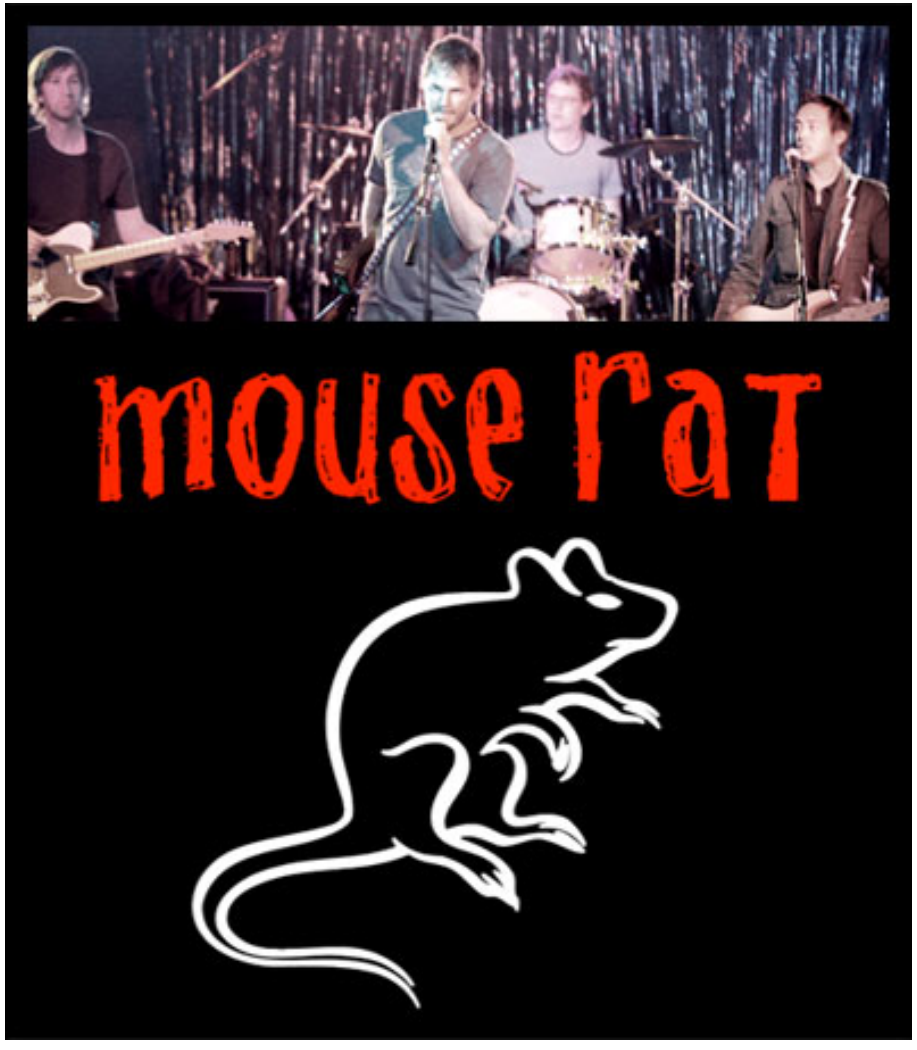

Human:Mouse:Rat [a]

[a] Brudno *et al.* (2004) *Genome Res.* doi:10.1101/gr.2067704

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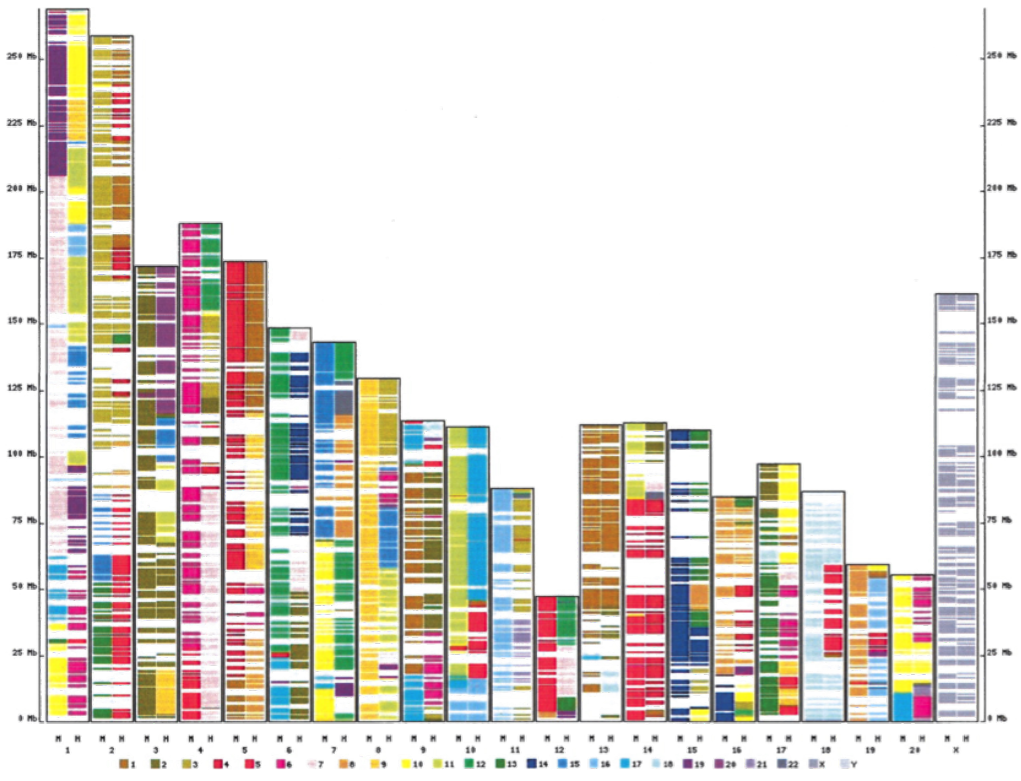
Three-way progressive alignment

Initial alignments: BLAT

Synteny: LAGAN/MLAGAN

Three-way synteny

- Homologous (HMR)
- Rodent-only (MR)
- Human-mouse (HM)
- Human-rat (HR)



(synteny mapped to rat genome)

MAUVE [a]

[a] Darling *et al.* (2003) *Genome Res.* doi:10.1101/gr.2289704

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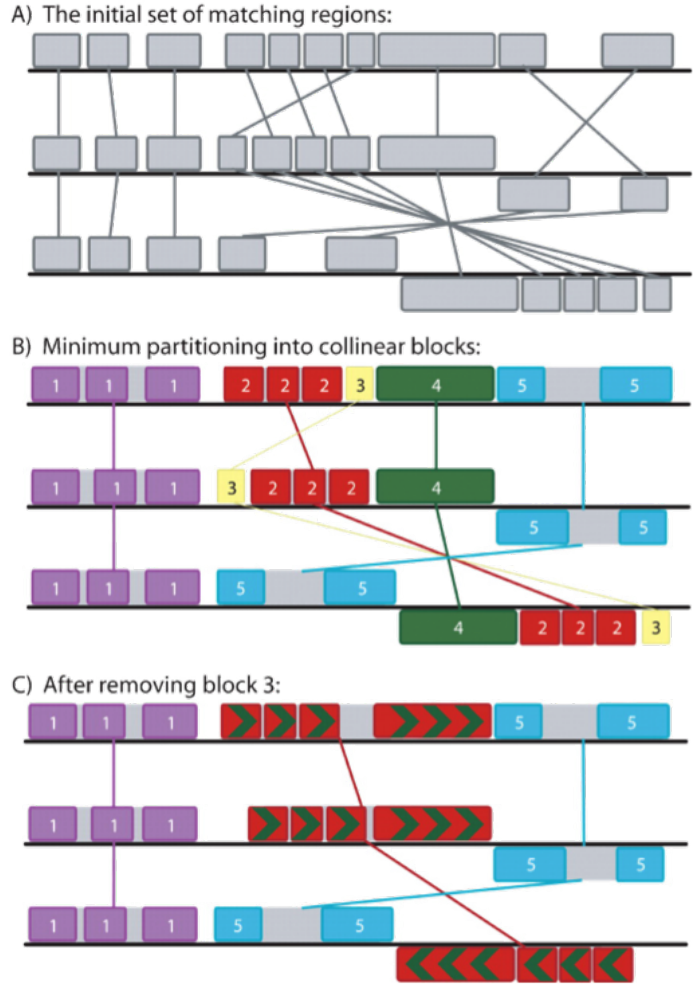
MAUVE/Progressive MAUVE: http://gel.ahabs.wisc.edu/mauve/

Algorithm:

1. Find local alignments (*multi-MUMs*, A)
2. Build guide tree from multi-MUMs
3. Select subset of multi-MUMs (*anchors*, B)
   - Partition into *local collinear blocks* (LCBs)
4. Recursive anchoring to refine anchors (C)
5. Progressive alignment against guide tree



MAUVE [a]

[a] Darling *et al.* (2003) *Genome Res.* doi:10.1101/gr.2289704

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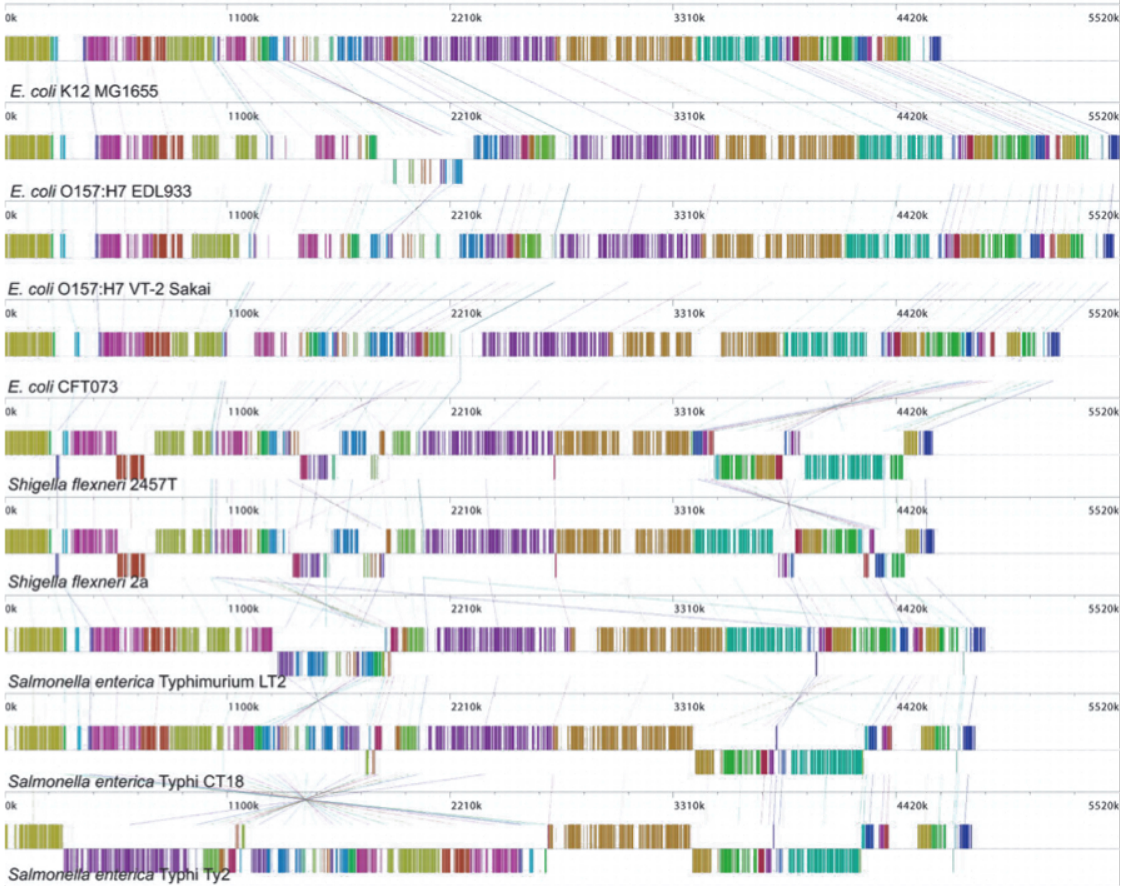
MAUVE alignment of LCBs in nine enterobacterial genomes

Evidence for rearrangement of homologous backbone sequence



Draft genome alignment

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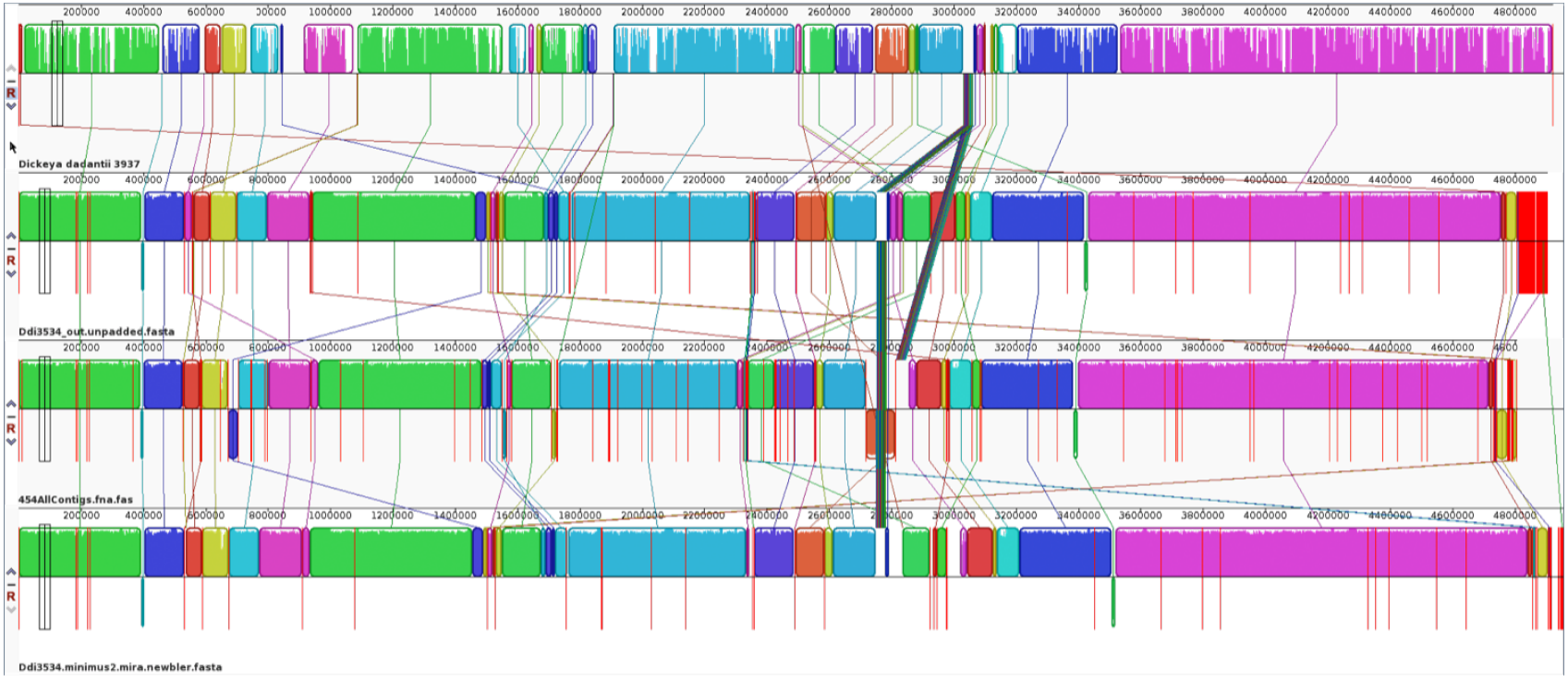
High-throughput genome assemblies may be fragments (contigs)

Contigs can be ordered (*scaffolded*):

- without alignment, by long or paired-end reads
- by alignment, to complete *reference* genomes
- by alignment, to other draft incomplete genomes



Chromosome painting[a]

[a]Yahara *et al.* (2013) *Mol. Biol. Evol.* **30**:1454-1464 doi:10.1093/molbev/mst055

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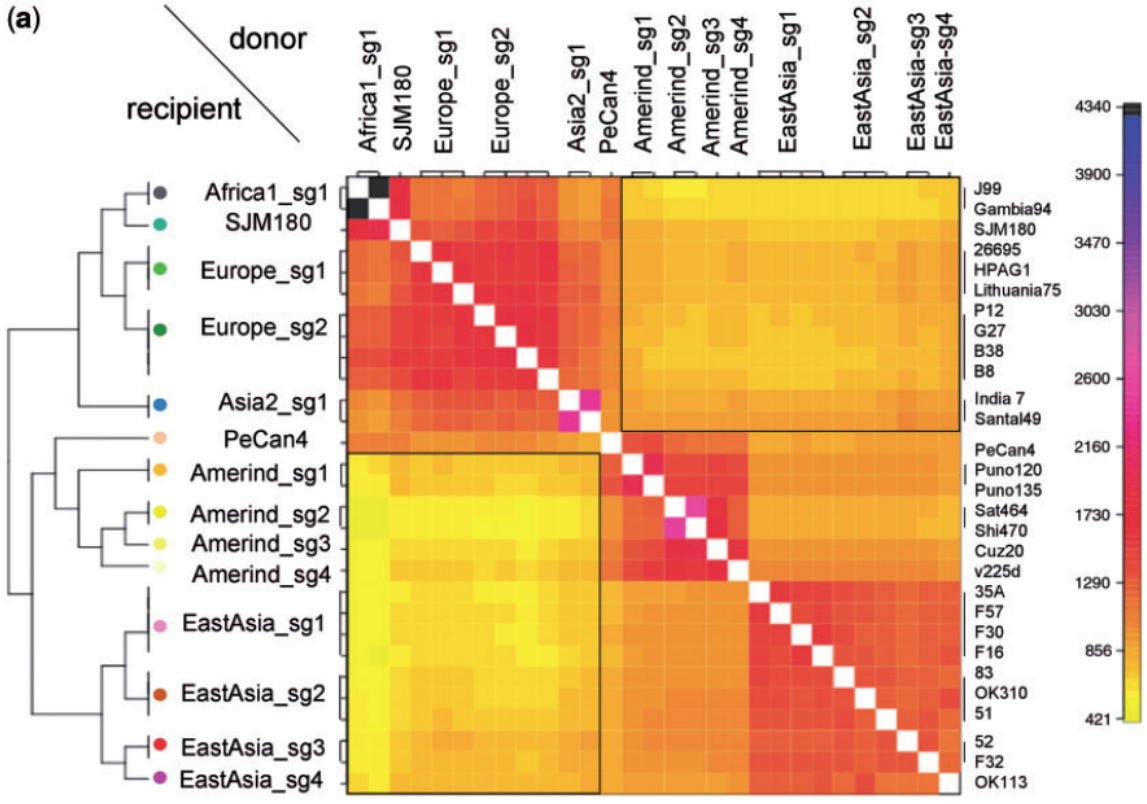
"Chromosome painting" infers recombination-derived 'chunks'
Genome's haplotype constructed in terms of recombination events from a 'donor' to a 'recipient' genome

FIG. 1. Chromosome painting in silico. Each lane indicates the chromosome of a strain shown on the right. The strains are classified by fineSTRUCTURE into subgroups labeled by colors (table 1 and fig. 2) on the left. A color along the chromosome indicates the subgroup that donated a chunk of SNPs through homologous recombination. All genomic positions are transformed to those of a reference strain (26695).

Chromosome painting[a]

[a] Yahara *et al.* (2013) *Mol. Biol. Evol.* **30**:1454-1464 doi:10.1093/molbev/mst055

Recombination events summarised in a *coancestry matrix*.
H. pylori: most within geographical bounds, but asymmetrical donation from Amerind/East Asian to European isolates.



Conclusions

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Physical and computational genome comparisons

- Similar biological questions
- $\therefore$ similar concepts

Modern biology: lots of sequence data

- Conservation $\approx$ evolutionary constraint
- Many choices of algorithms/software
- Many choices of visualisation tools/software

Table of Contents

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