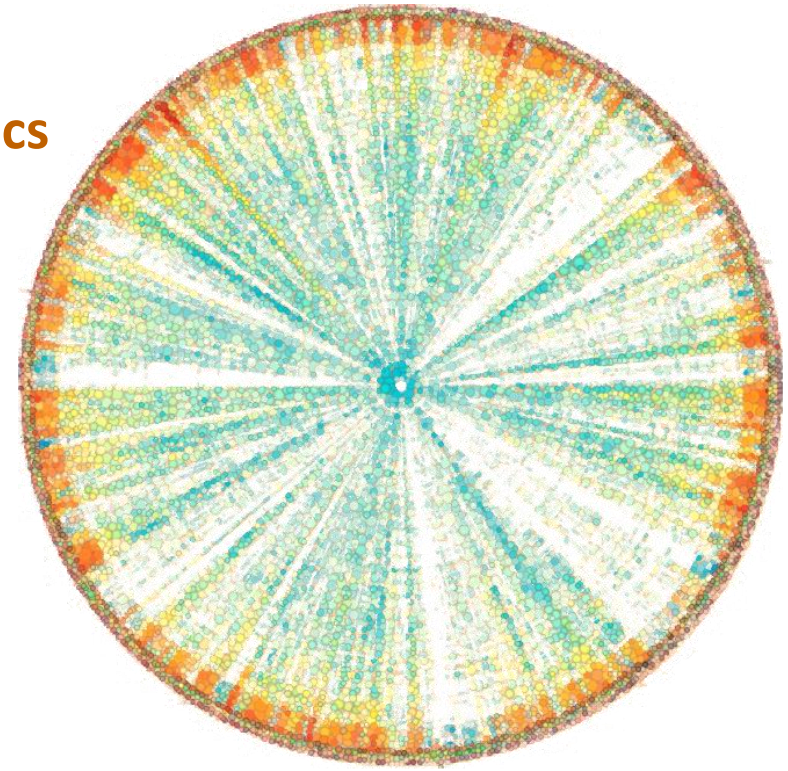
Comparative Genomics and Visualisation

- **What is comparative genomics?**
- **Levels of genome comparison**
  - bulk, whole sequence, features
- **Computational Comparative Genomics**
  - Bulk properties
  - Whole genome comparisons

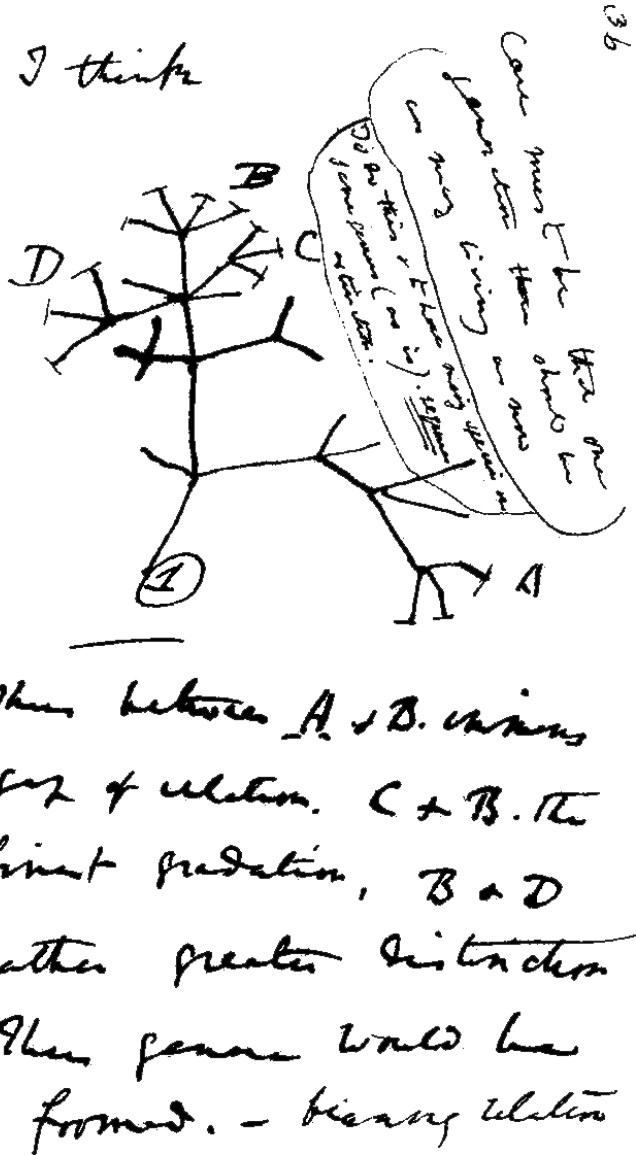


What is Comparative Genomics?

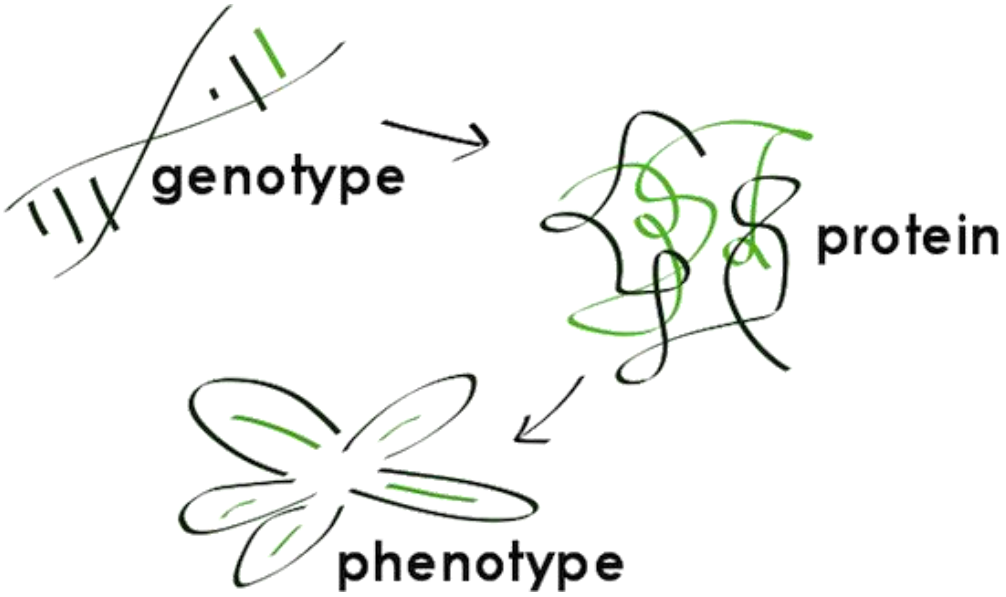
The combination of genomic data and comparative and evolutionary biology to address questions of genome structure, evolution and function.

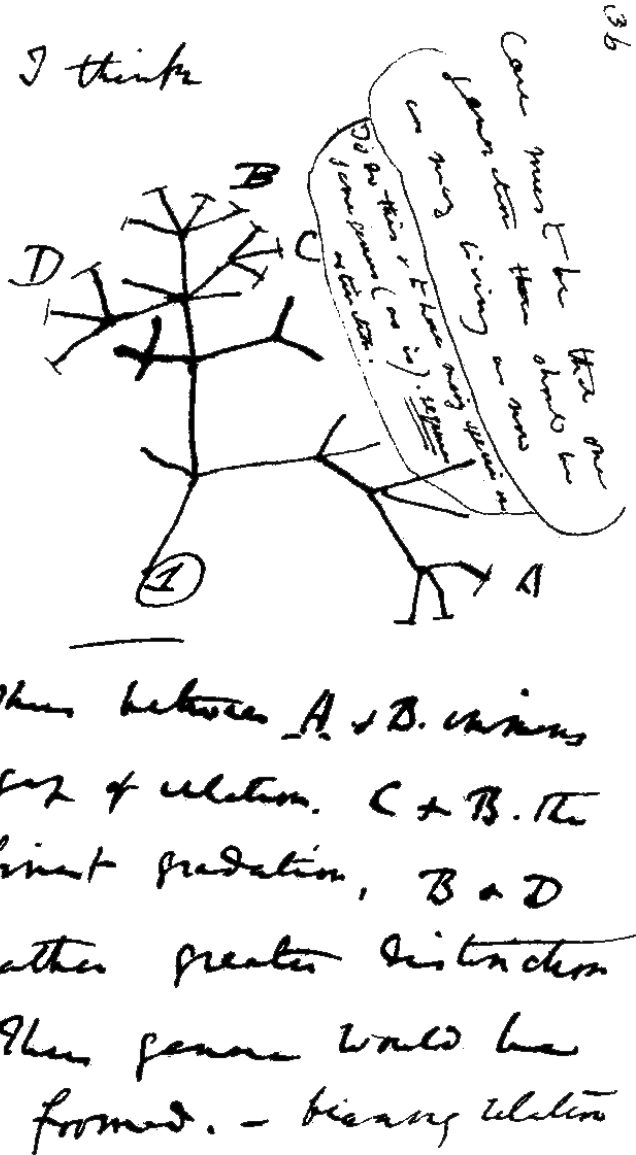
Why Comparative Genomics?

- Genomes describe heritable characteristics
- Related organisms share ancestral genomes
- Functional elements encoded in genomes are common to related organisms
- Functional understanding of model systems (*E. coli*, *A. thaliana*, *D. melanogaster*) can be transferred to non-model systems on the basis of genome comparisons
- Genome comparisons can be informative, even for distantly-related organisms



Why Comparative Genomics?

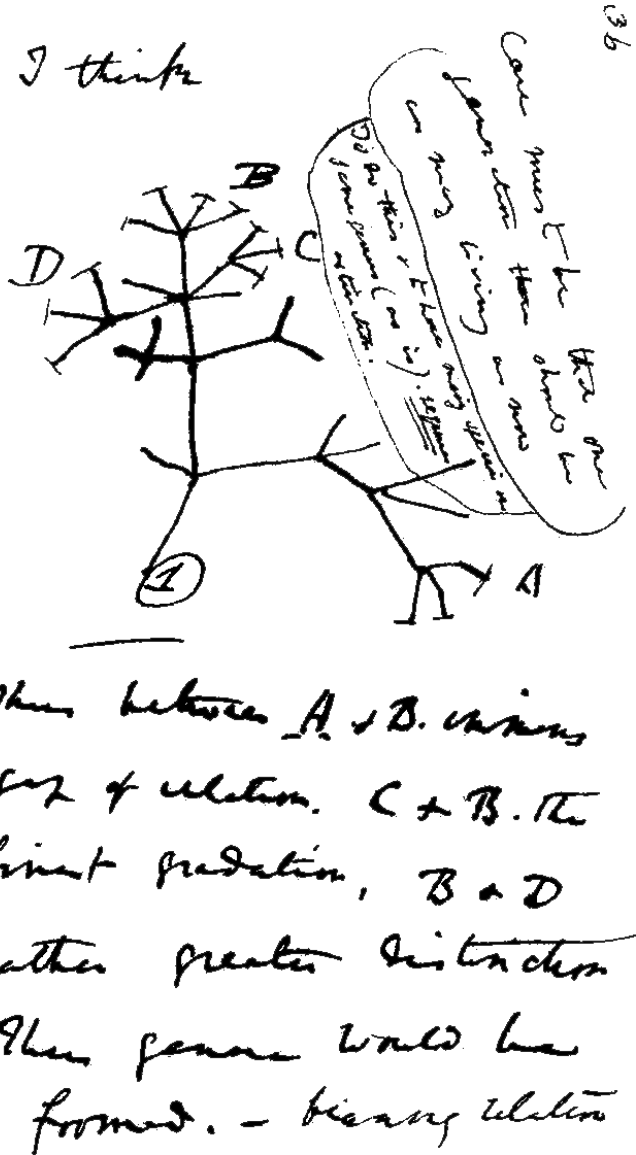




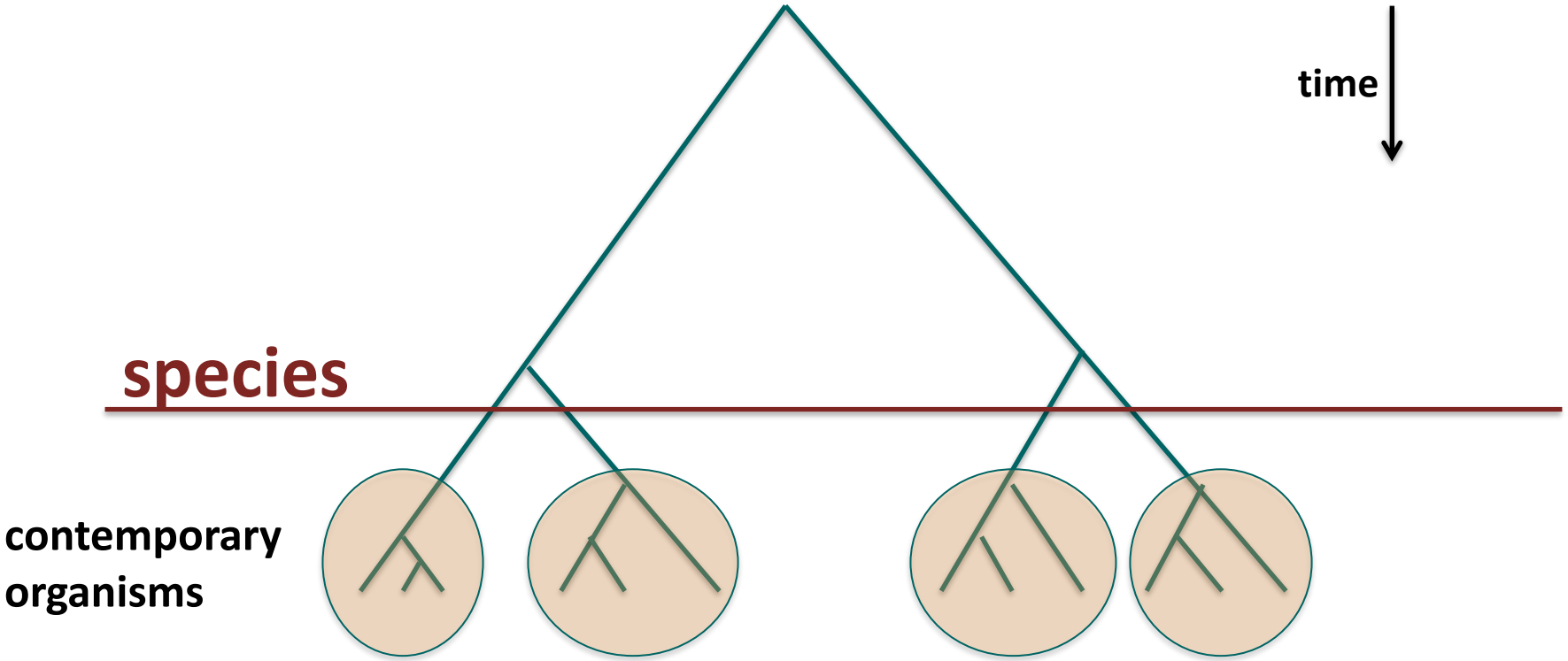
- **BUT:**
  - **Context:** epigenetics, tissue differentiation, mesoscale systems, etc.
  - **Phenotypic plasticity:** responses to temperature, stress, environment, etc.

Why Comparative Genomics?

- Genomic differences can underpin phenotypic (morphological or physiological) differences.
- Where phenotypes or other organism-level properties are known, comparison of genomes may give mechanistic or functional insight into differences (e.g. GWAS).
- Genome comparisons aid identification of functional elements on the genome.
- Studying genomic changes reveals evolutionary processes and constraints.



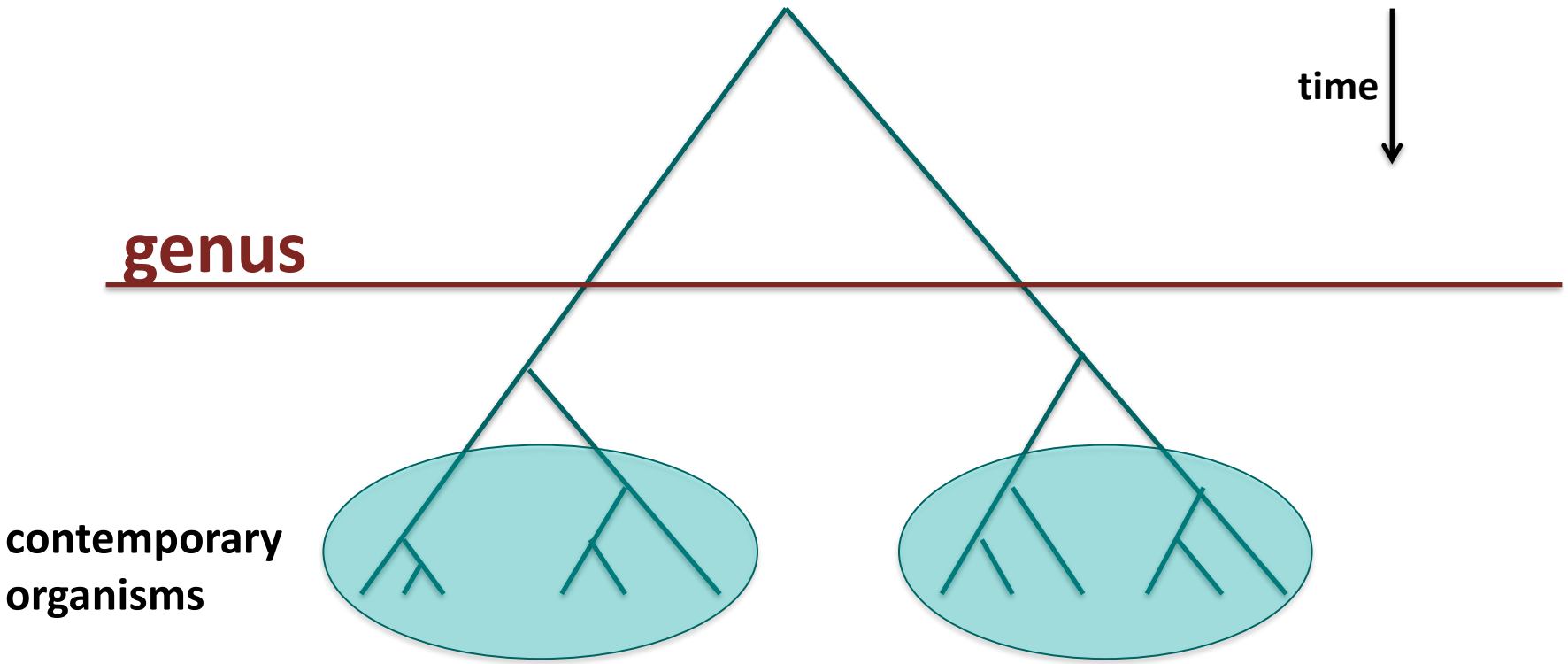
Why Comparative Genomics?



- Comparison within species (e.g. isolate-level – or even within individuals): which genome features may account for unique characteristics of organisms/tumours? Epigenetics in an individual.

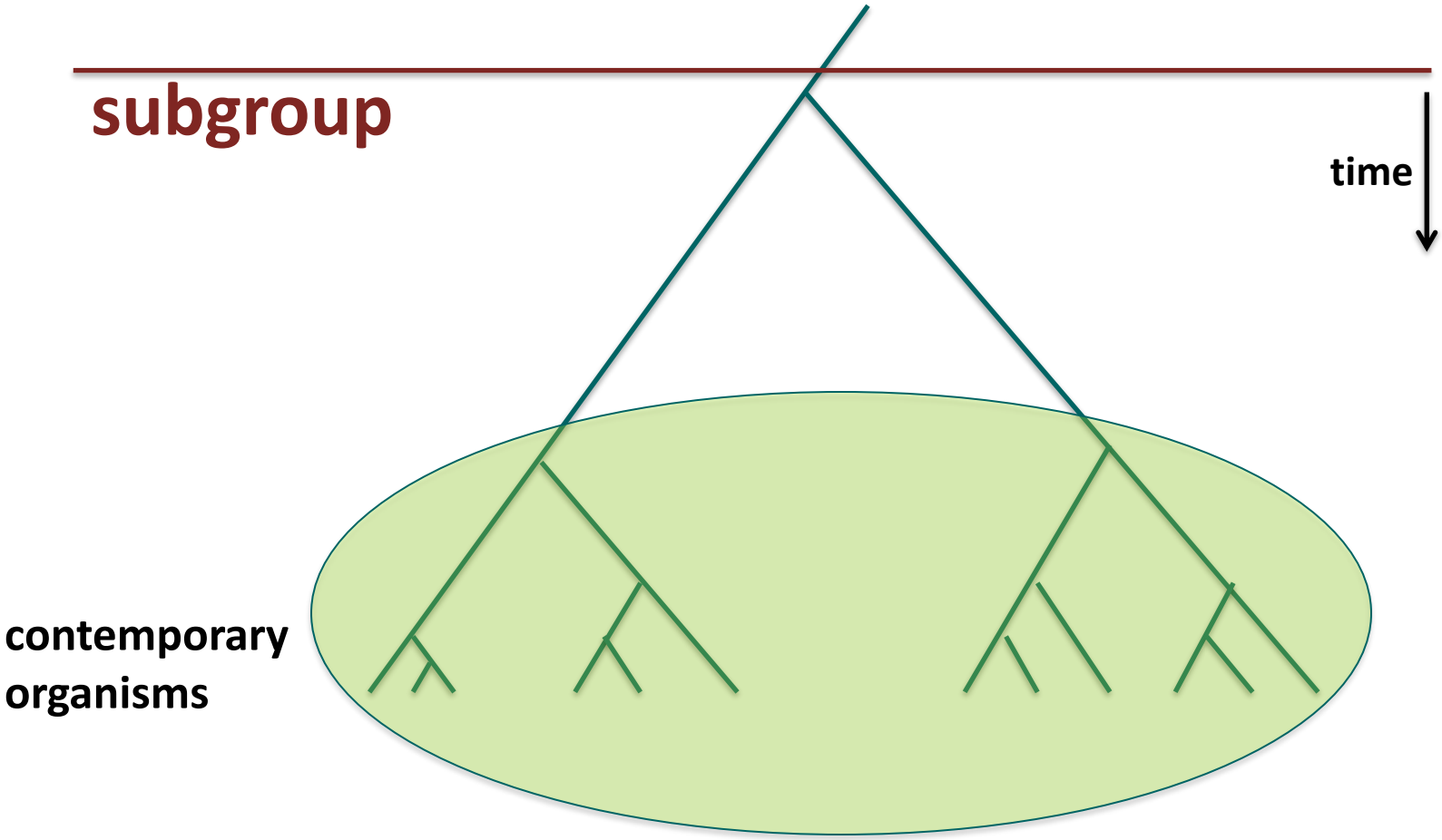
Adapted from Hardison (2003) *PLoS Biol.* doi:10.1371/journal.pbio.0000058

Why Comparative Genomics?



- Comparison within genus (e.g. species-level): what genome features show evidence of selective pressure, and in which species?

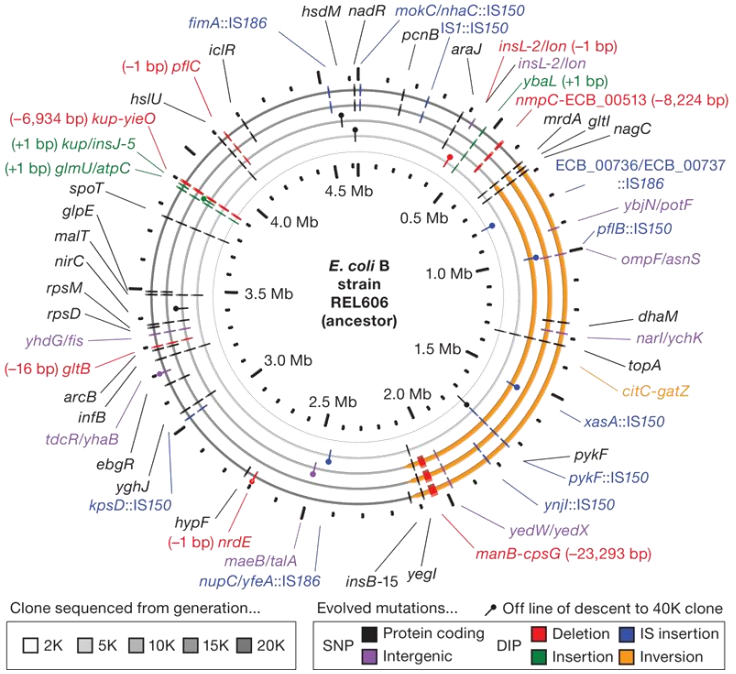
Why Comparative Genomics?

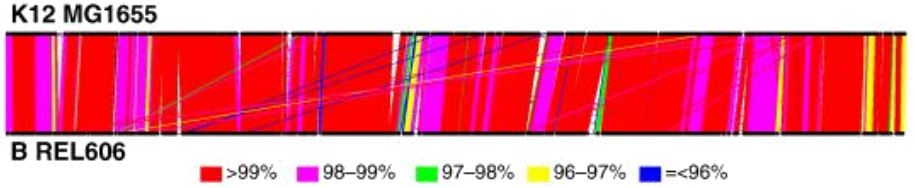


- Comparison within subgroup (e.g. genus-level): what are the core set of genome features that define a subgroup or genus?

The *E.coli* long-term evolution experiment

- 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 *Escherichia coli*!
  - Cultures propagated every day
  - Every 500 generations (75 days), mixed-population samples stored
  - Mean fitness estimated at 500 generation intervals





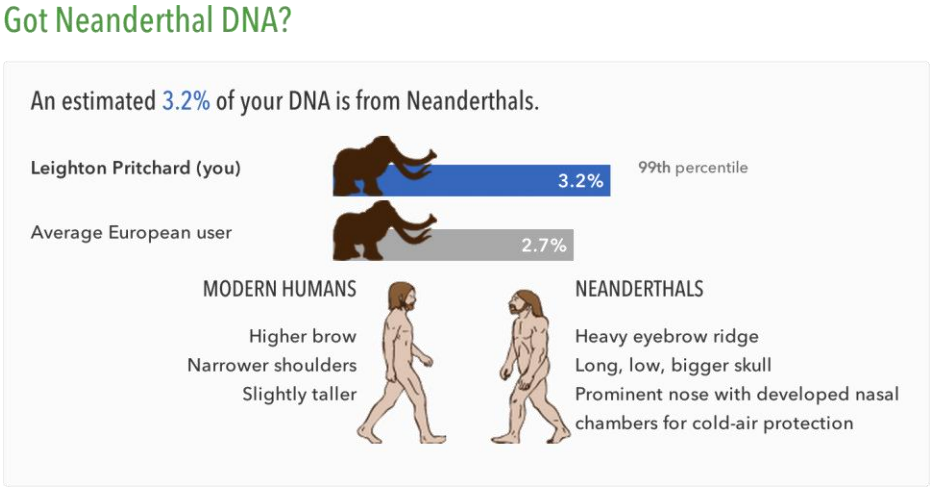
Jeong *et al.* (2009) *J. Mol. Biol.* doi:10.1016/j.jmb.2009.09.052

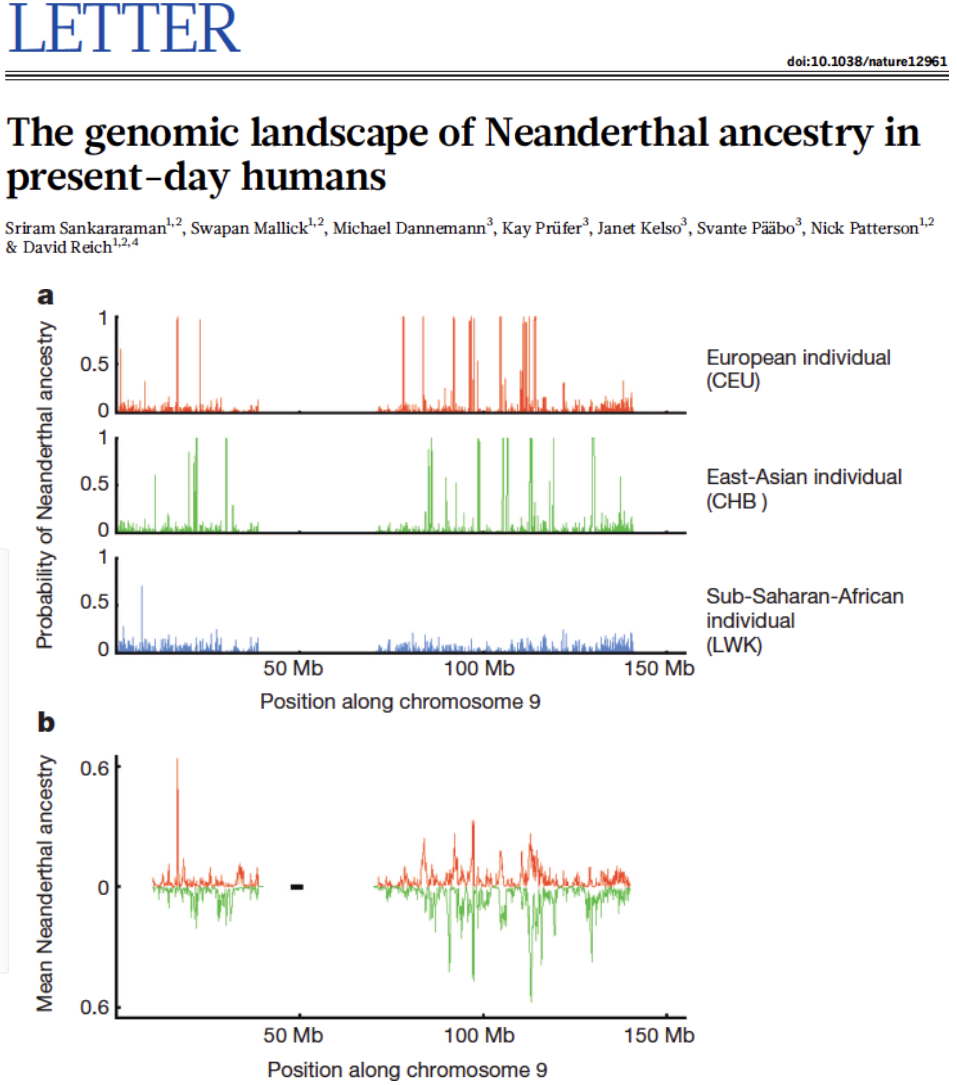
Barrick *et al.* (2009) Nature doi:10.1038/nature08480

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

Comparative Genomics in the News

- Neanderthal alleles:
  - Aid adaptation outwith Africa
  - Associated with disease risk
  - Reduce male fertility





Sankaraman *et al.* (2014) *Nature.* doi:10.1038/nature12961

Levels of Genome Comparison

Genomes are complex, and can be compared on a range of conceptual levels - both practically and *in silico*.

Three broad levels of comparison

- 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.
- Bulk Properties
  - chromosome/plasmid counts and sizes,
  - nucleotide content, etc.
- Whole Genome Sequence
  - sequence similarity
  - organisation of genomic regions (synteny), etc.

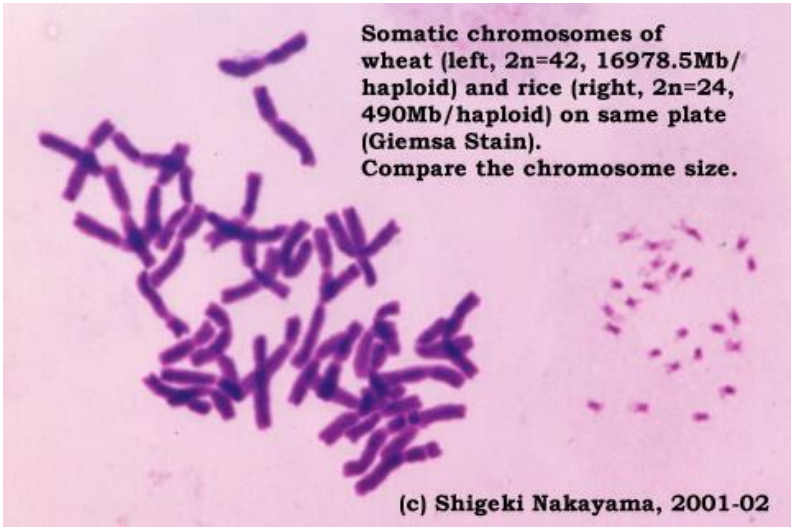
Bulk Genome Properties

- Large-scale summary measurements
- Measure genomes independently – compare values later
  - Number of chromosomes
  - Ploidy
  - Chromosome size
  - Nucleotide (A, C, G, T) frequency/percentage

Chromosome Counts/Size

- The chromosome counts/ploidy of organisms can vary widely
  - *Escherichia coli*: 1 (but plasmids…)
  - Rice (*Oryza sativa*): 24 (but mitochondria, plastids etc…)
  - Human (*Homo sapiens*): 46, diploid
  - Adders-tongue (*Ophioglossum reticulatum*): up to 1260
  - Domestic (but not wild) wheat somatic cells hexaploid, gametes haploid

- Physical genome size (related to sequence length) can also vary greatly
- Genome size and chromosome count do **not** indicate organism 'complexity'
- Still surprises to be found in physical study of chromosomes! (e.g. Hi-C)



Kamisugi *et al.* (1993) *Chromosome Res.* 1(3): 189-96
Wang *et al.* (2013) *Nature Rev Genet.* doi:10.1038/nrg3375

Nucleotide Content

- Experimental approaches for accurate measurement
  - e.g. use radiolabelled monophosphates, calculate proportions using chromatography

Nucleic Acids Research, Vol. 19, No. 19 5181–5185

Rapid determination of nucleotide content and its application to the study of genome structure

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Department of Genetics, Box 8232, Washington University School of Medicine, St Louis, MO 63110, USA

Received July 26, 1991; Revised and Accepted September 4, 1991

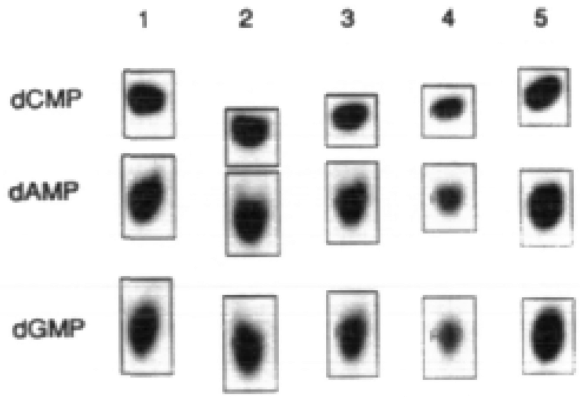


Table 4. Base composititon of yeast artificial chromosomes containing of human DNA inserts.

YAC	Replicate	Corrected G:A	Corrected C:A	%G+C from G:A	%G+C from C:A	Average GC content	Std dev.
yKM19-3	1	0 737	0.799	42.4	44.4	43.4	
(180 kb)	2	0.703	0.771	41.3	43.5	42.4	0 58
	3	0.707	0.768	41.4	43.4	42.4	
yCF-4	1	0.724	0.768	42.0	43.4	42 7	
(330 kb)	2	0.708	0.731	41.4	42.2	41 8	0.45
	3	0.701	0 759	41.2	43.2	42.2	
yW30-5	1	0.722	0 747	41.9	42.7	42.3	
(300 kb)	2	0 744	0.765	42.7	43.3	43.0	0.47
	3	0 713	0.739	41.6	42 5	42.1	
yJ311-2	1	0.725	0.747	42.0	42.8	42 4	
(230 kb)	2	0.728	0.757	42.1	42.4	42.6	0 10
	3	0.728	0.736	42.1	42.4	42.5	
yJ311-5	1	0.695	0 726	41.0	42.1	41.5	
(200 kb)	2	0 746	0.756	42.7	43.0	42.9	0.70
	3	0 731	0 733	42.2	42.3	42.3	
yHPRT	1	0 718	0.744	41.8	42 7	42.2	
(680 kb)	2	0 718	0.725	41.8	42.0	41 9	0.46
	3	0.694	0.713	41.0	41.6	41.3	

Karl (1980) *Microbiol. Rev.* 44(4) 739-796
Krane *et al.* (1991) *Nucl. Acids Res.* doi:10.1093/nar/19.19.5181

Chromosomal Rearrangements

- Genomes are dynamic, and undergo large-scale changes
- Hybridisation used to map genome rearrangement/duplication
  - Separate chromosomes electrophoretically
  - Apply single gene hybridising probes
  - Reciprocal hybridisations indicate translocations

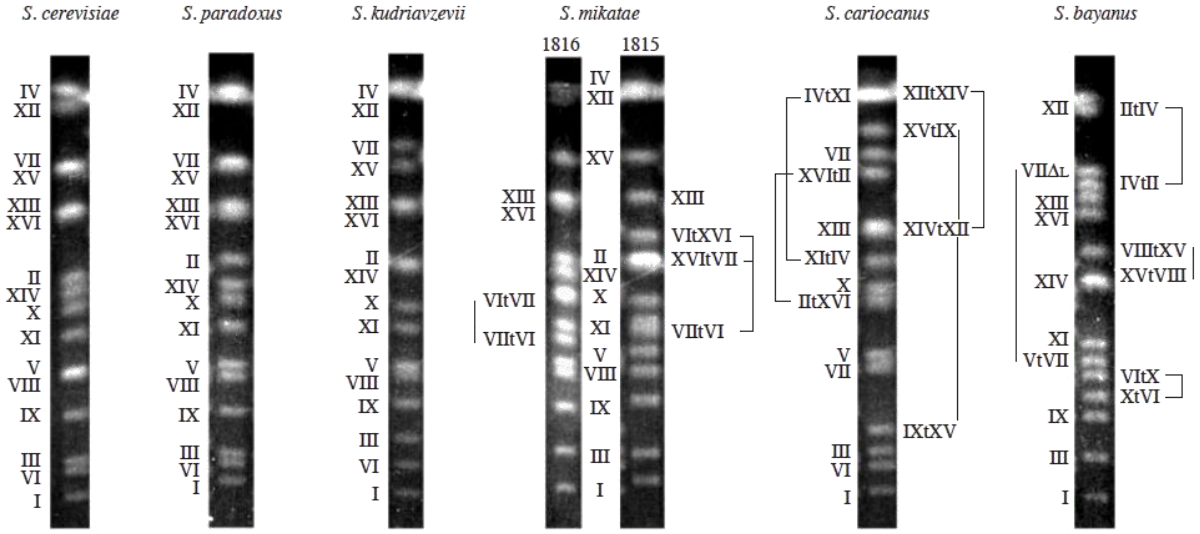


Figure 1 Electrophoretic karyotypes of the *Saccharomyces* 'sensu stricto' species. Strains presented here are *S. cerevisiae* Y55, *S. paradoxus* N17, *S. kudriavzevii* IFO 1802, *S. mikatae* IFO 1816 and IFO 1815, *S. cariocanus* IMUFRJ 50816 and S. *bayanus* CBS 7001. Chromosomes are labelled from I to XVI according to the *S. cerevisiae* nomenclature. Bands showing double intensities correspond to doublets where two non-homologous chromosomes run at the same position. A triplet involving chromosomes II, XIV and XVI t VII is present in the *S. mikatae* IFO 1815 karyotype. Pairs of chromosomes involved in a reciprocal translocation are connected. In *S. bayanus*, the non-reciprocal translocation event is depicted as V t VII connected to VII ΔL (deletion of the left arm of chromosome VII).

Fischer *et al.* (2000) *Nature.* doi:10.1038/35013058

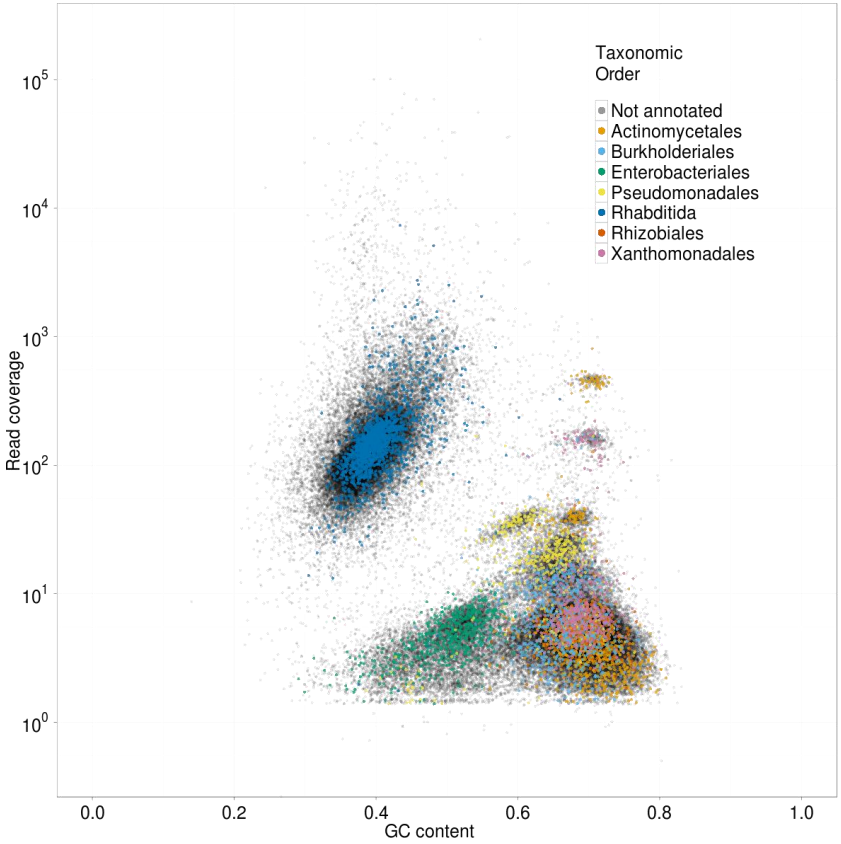
Nucleotide Frequencies/Genome Size

- Very easy to calculate from complete or draft genome sequence
  - (or in a region of genome sequence)

- GC content/chromosome size can be characteristic of an organism
- **[ACTIVITY]**
  - **`bacteria_size_gc`** iPython notebook
  - **`ipython notebook --pylab inline`** in **`bacteria_size`** directory

Blobology

- Metazoan sequence data can be contaminated by microbial symbionts.
  - Host and symbiont DNA have different %GC (and are present in different amounts/coverage)
  - Preliminary genome assembly, followed by read mapping
  - Plot contig coverage against %GC = **Blobology**



- http://nematodes.org/bioinformatics/blobology/

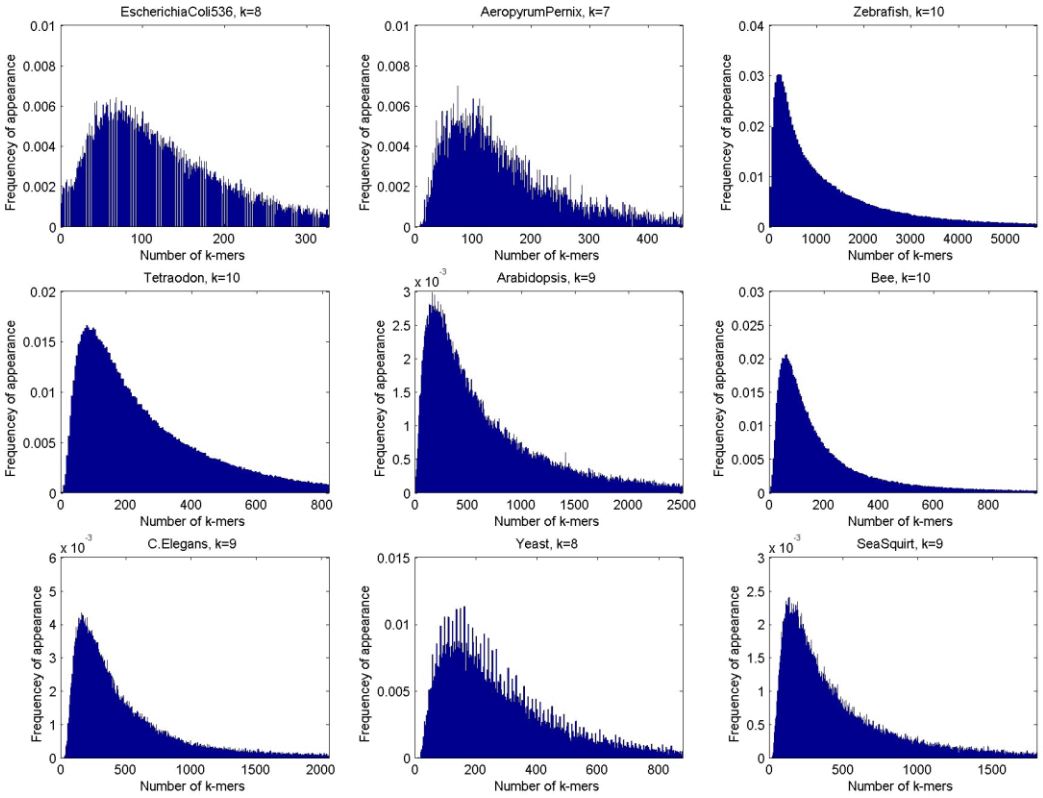
Kumar & Blaxter (2011) *Symbiosis* doi:10.1007/s13199-012-0154-6

Nucleotide *k*-mers

- **Sequence data is required to determine *k*-mers**
- Nucleotide frequencies:
  - A, C, G, T
- Dinucleotide frequencies:
  - AA, AC, AG, AT, CA, CC, CG, CT, GA, GC, GG, GT, TA, TC, TG, TT
- Trinucleotide frequencies:
  - 64 trinucleotides
- *k*-nucleotide frequencies:
  - 4^k *k*-mers
- **[ACTIVITY]**
  - `runApp("shiny/nucleotide_frequencies")` **in RStudio**

k-mer Spectra

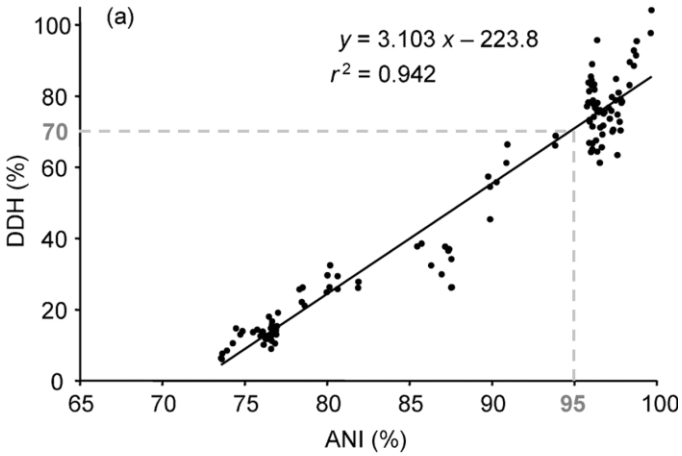
- ***k*-mer spectrum:**
  - Frequency distribution of observed *k*-mer counts
  - Most species have a unimodal *k*-mer spectrum

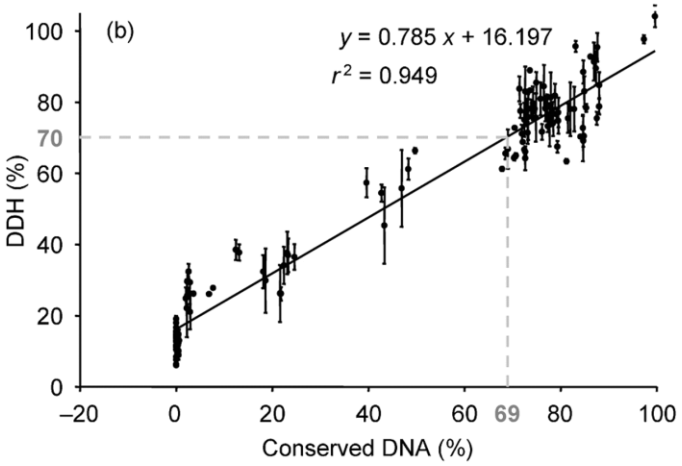


Chor *et al.* (2009) *Genome Biol.* doi:10.1186/gb-2009-10-10-r108

Average Nucleotide Identity (ANI)

- ANI introduced as a substitute for DDH in 2007:
  - 70% identity (DDH) = **"gold standard"** prokaryotic species boundary
  - **70%** identity (DDH) ≈ **95%** identity (ANI)
- Original method emulates physical experiment:
  1. break genome into 1020nt fragments
  2. align fragments using BLASTN
  3. ANI = mean identity of all BLASTN matches with >30% identity over 70% alignable length





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

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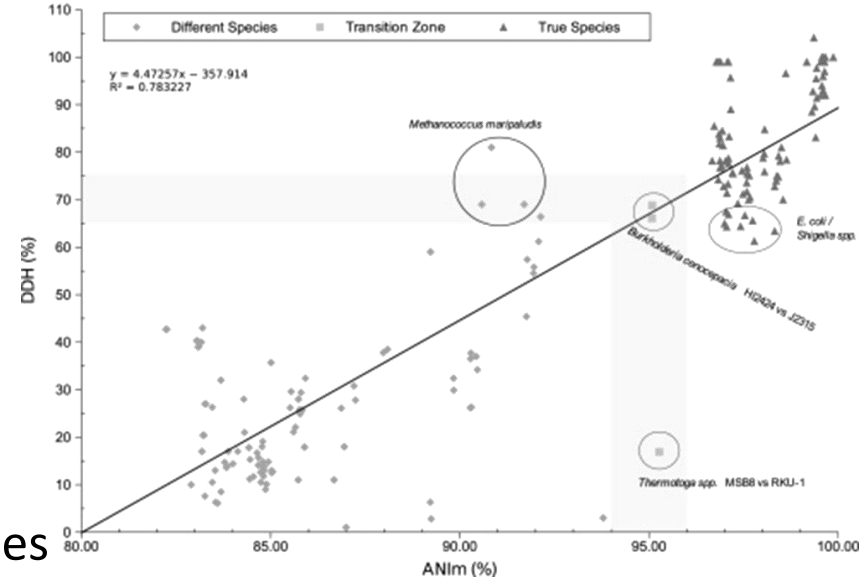
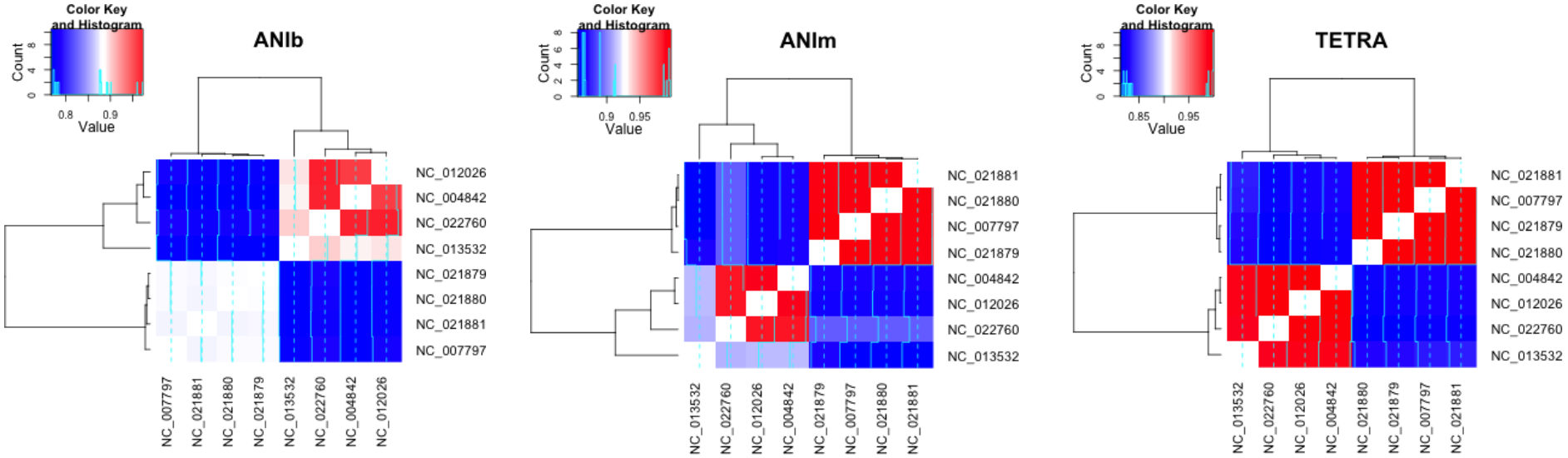


- ANIm and TETRA introduced (2009)
  1. Align sequences using NUCmer
  2. ANI = mean %identity of matches
- TETRA:
  1. Calculate tetranucleotide frequencies
  2. Determine each tetramer deviation from expectation (Z-score)
  3. TETRA = Pearson correlation coefficient of tetramer Z-scores

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

Average Nucleotide Identity (ANI)

- ANIb discards useful information that ANIm retains
- TETRA reflects bulk genome properties rather than selection on sequence



- Data for *Anaplasma marginale* (3), *A.phagocytophilum* (4), *A.centrale* (1)

- TETRA scores are prone to false positives; ANIb scores are prone to false negatives

Average Nucleotide Identity (ANI)

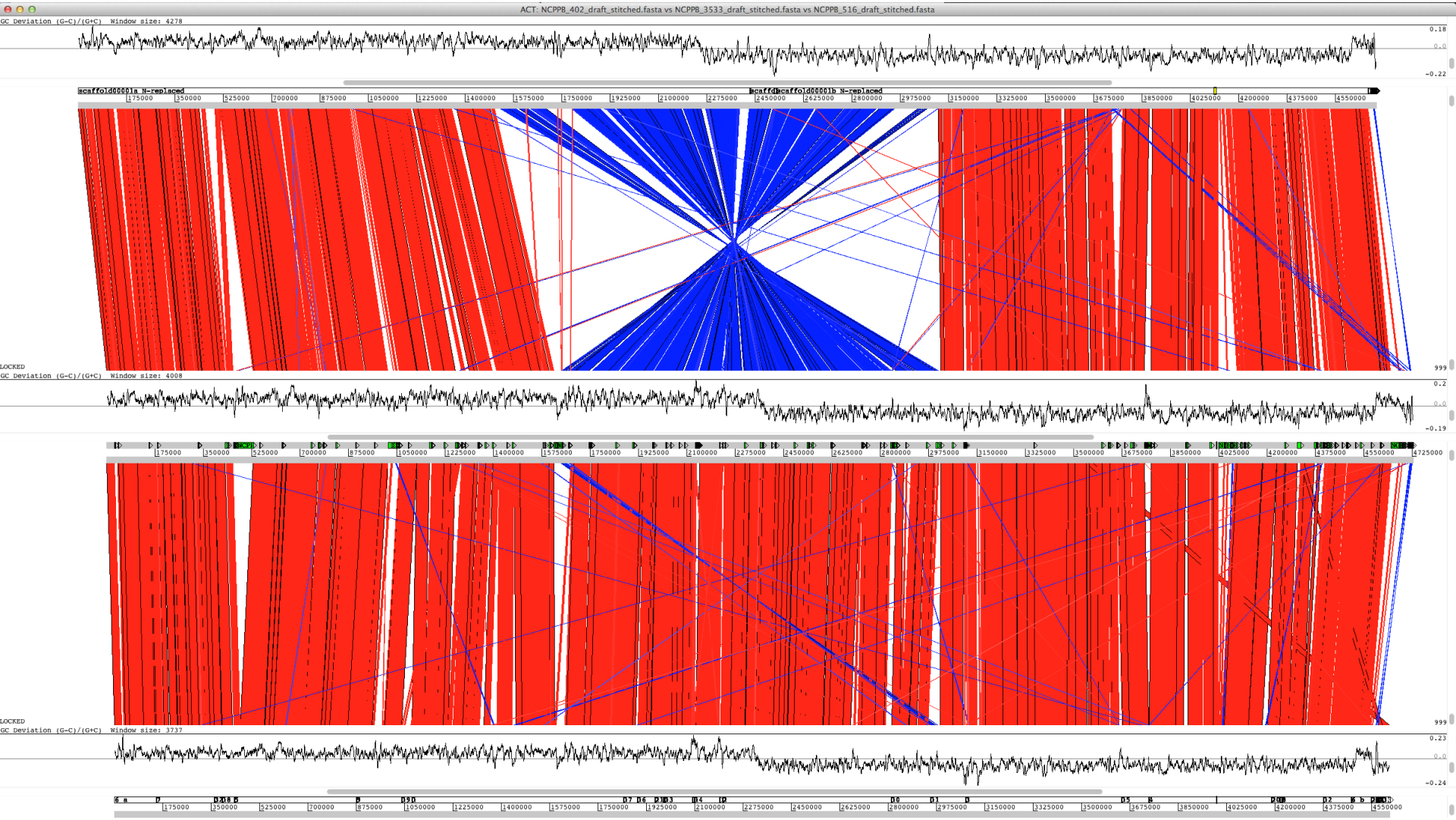
- Jspecies (http://www.imedea.uib.es/jspecies/)
  - WebStart
  - `java -jar -Xms1024m -Xmx1024m jspecies1.2.1.jar`
- Python script
  - `scripts/calculate_ani.py`
- **[ACTIVITY]**
  - `average_nucleotide_identity/README.md` Markdown

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

Whole Genome Sequence Comparisons

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

Whole Genome Alignment



Whole Genome Alignment

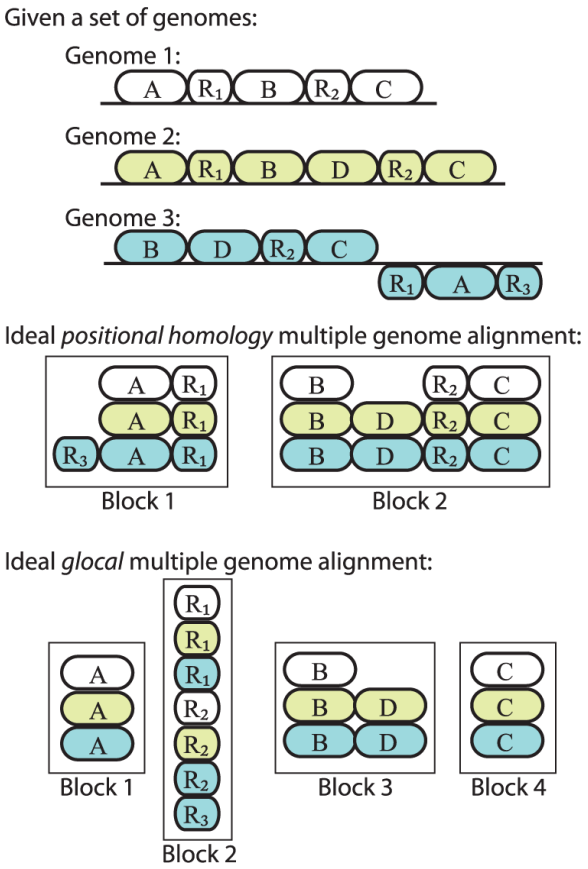
- Which genomes should you align? (or not bother aligning)
- For reasonable analysis, genomes should:
  - derive from a sufficiently **recent** common ancestor: so that homologous regions can be identified.
  - derive from a sufficiently **distant** common ancestor: so that sufficiently "interesting" changes are likely to have occurred
  - help answer your biological question:
    - is your question organism or phenotype specific?
    - are you investigating a process?
- This may be more involved for metazoans (vertebrates, arthropods, nematodes, etc.) than prokaryotes…

Whole Genome Alignment

- Naïve alignment algorithms (e.g. Needleman-Wunsch/Smith-Waterman) are not appropriate:
  - Do not handle rearrangements
  - Computationally expensive on large sequences
- Many whole-genome alignment algorithms proposed, including:
  - LASTZ (http://www.bx.psu.edu/~rsharris/lastz/)
  - **BLAT** (http://genome.ucsc.edu/goldenPath/help/blatSpec.html)
  - Mugsy (http://mugsy.sourceforge.net/)
  - **megaBLAST** (http://www.ncbi.nlm.nih.gov/blast/html/megablast.html)
  - **MUMmer** (http://mummer.sourceforge.net/)
  - LAGAN (http://lagan.stanford.edu/lagan_web/index.shtml)
  - WABA, etc…

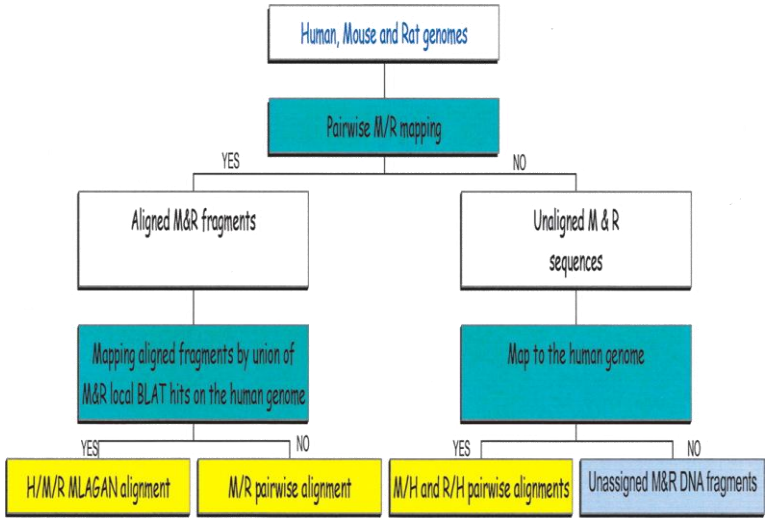
Multiple Genome Alignment

- 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/)
- Positional homology vs. *glocal*

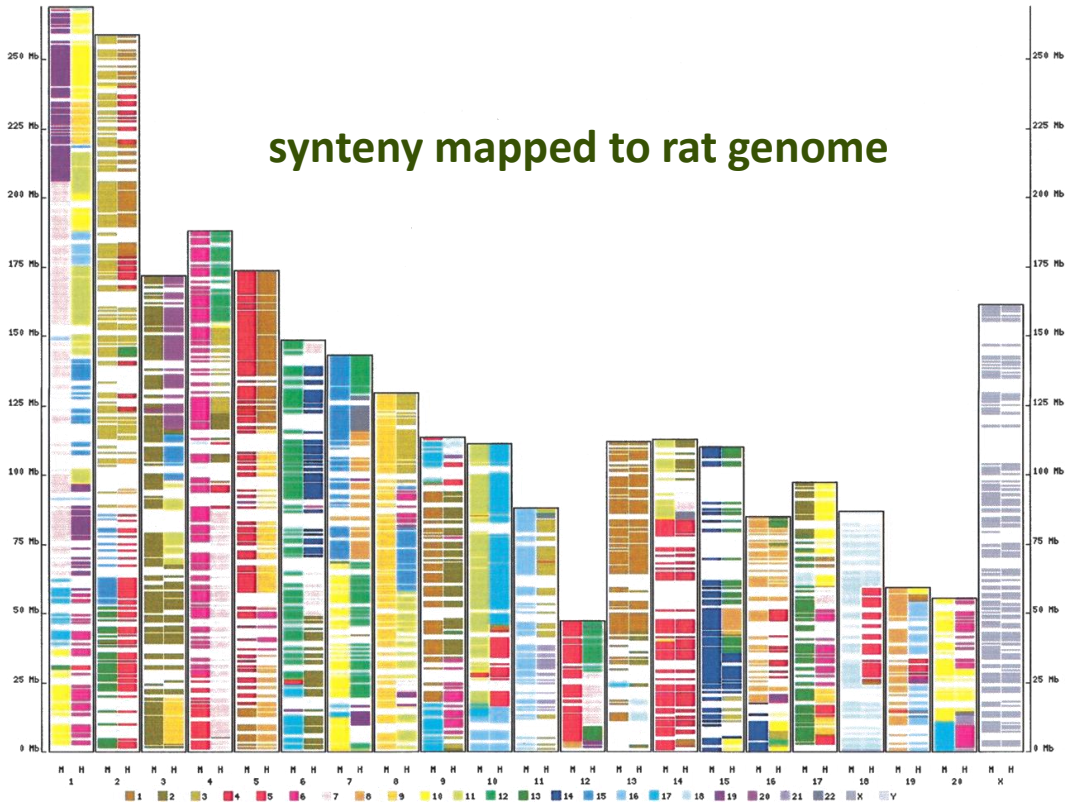


Human-Mouse-Rat Alignment

- Three-way progressive alignment, identifying:
  - Homologous (H/M/R), rodent-only (M/R) and human-mouse or human-rat (H/M, H/R) homologous regions
- Three-way synteny

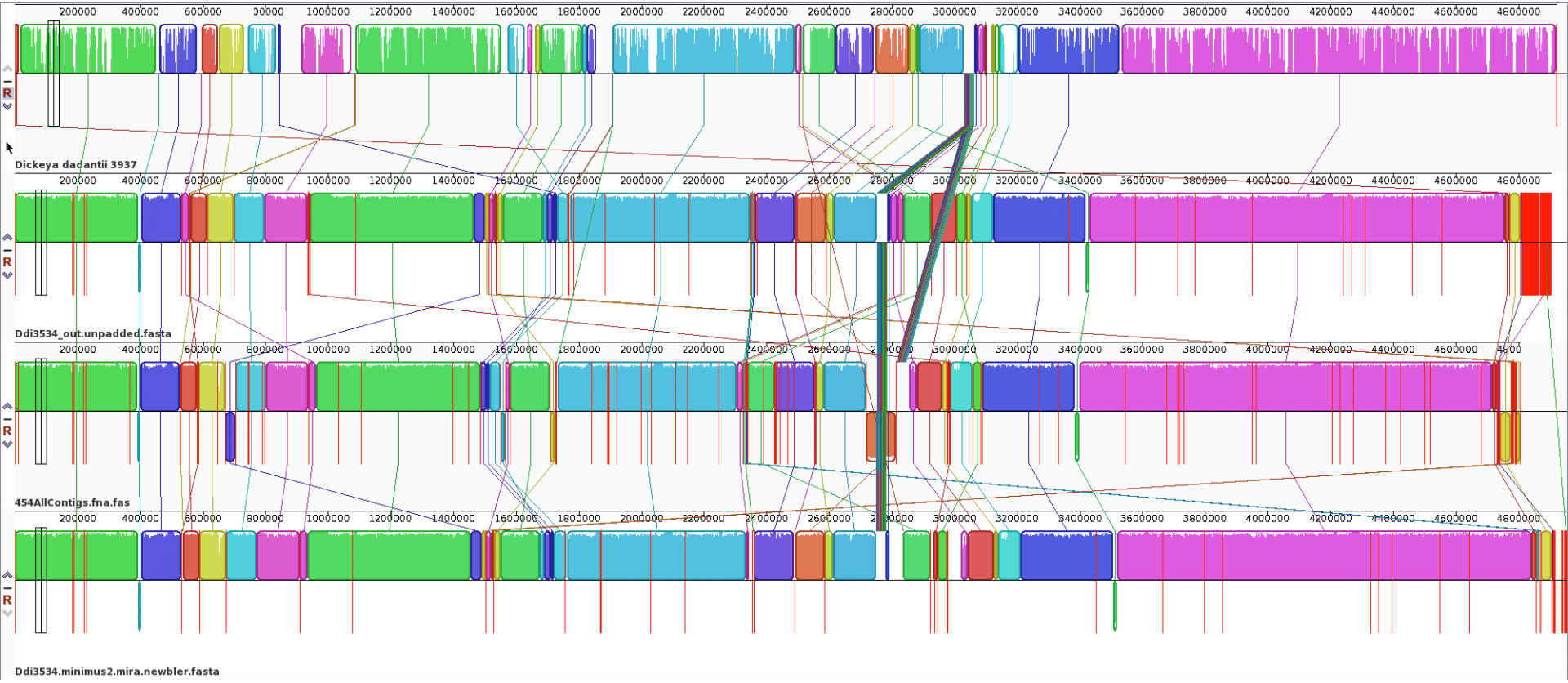


Initial alignments by BLAT
Syntenous regions aligned with LAGAN



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

Draft Genome Alignment



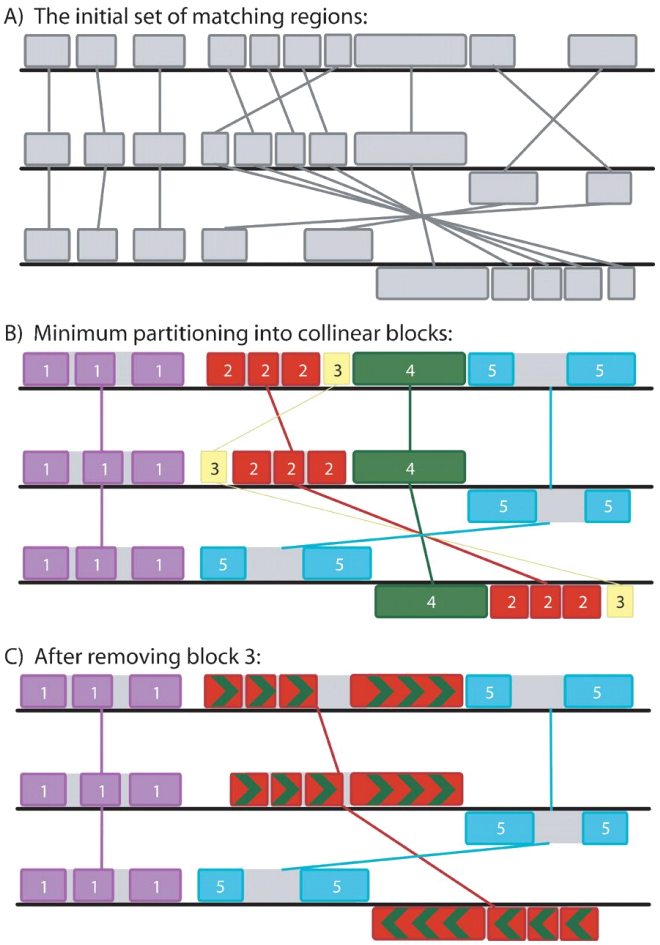
Draft Genome Alignment

- Whole genome alignments useful for scaffolding assemblies
  - High-throughput sequence assemblies come in fragments (contigs)
  - Contigs can sometimes be ordered if paired reads or long read technologies are used
  - Can also align to a known reference genome
- MUMmer
  - Can use NUCmer or, for more distant relations, PROmer
- Mauve/Progressive Mauve
  - http://gel.ahabs.wisc.edu/mauve/

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

Mauve

- **Mauve**'s alignment algorithm
  1. Find local alignments (multi-MUMs – seed & extend)
  2. Construct phylogenetic guide tree from multi-MUMs
  3. Select subset of multi-MUMs as anchors.
     - Partition anchors into Local Collinear Blocks (LCBs) – *consistently-ordered subsets*
  4. Perform recursive anchoring to identify further anchors
  5. Perform progressive alignment (similar to CLUSTAL), against guide tree



- Mauve Contig Mover (MCM) for ordering contigs

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

Draft Genome Alignment

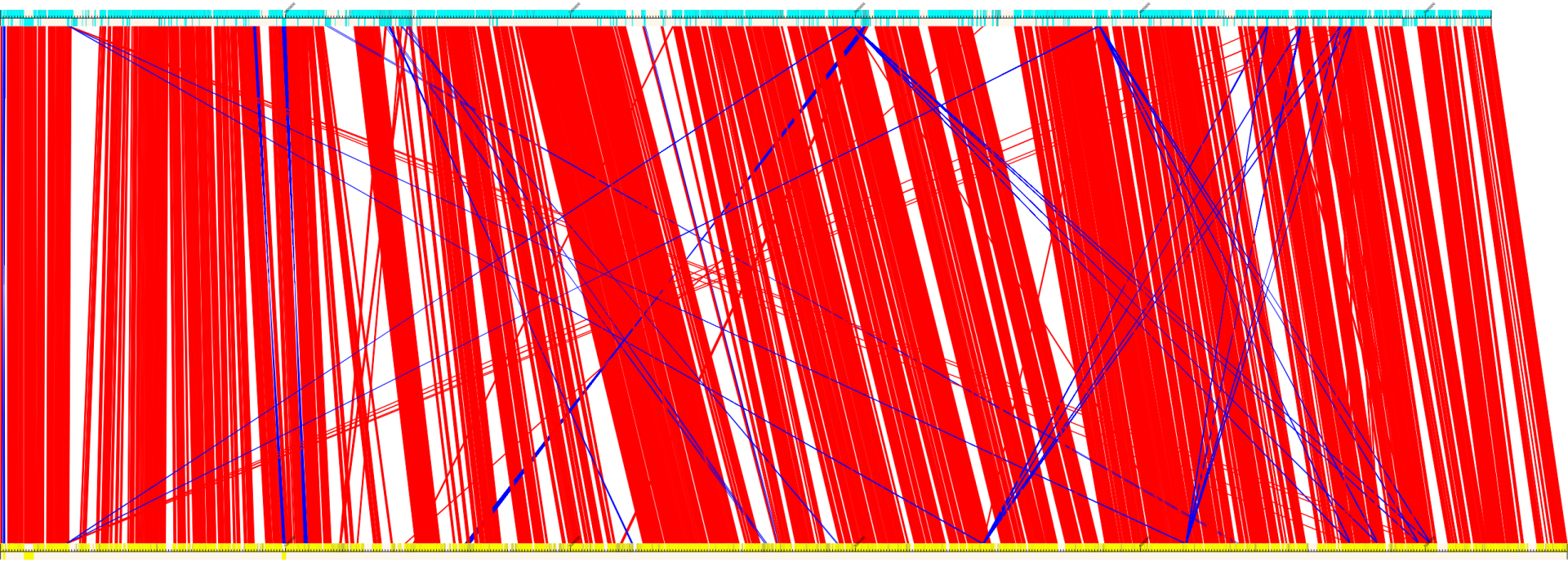
- **[OPTIONAL ACTIVITY] (useful for exercise)**
  - Alignment and reordering of draft genome contigs
  - **`whole_genome_alignments_B.md`** Markdown
  - https://github.com/widdowquinn/Teaching/blob/master/Comparative_Genomics_and_Visualisation/Part_1/whole_genome_alignment/whole_genome_alignments_B.md
- **[ACTIVITY]**
  - Visualisation of whole genome alignment with Biopython
  - **`biopython_visualisation`** iPython notebook

Collinearity and Synteny

- Rearrangements may occur post-speciation
- Different species still exhibit conservation of sequence similarity and order
  - Two elements are *collinear* if they lie in the same linear sequence
  - Two elements are *syntenous* (*syntenic*) if:
    - (*orig.*) they lie on the same chromosome
    - (*mod.*) conservation of blocks of order within the same chromosome
- Signs of evolutionary constraints, including synteny, may indicate functional genome regions
- More about this in **Part 2**, related to genome features

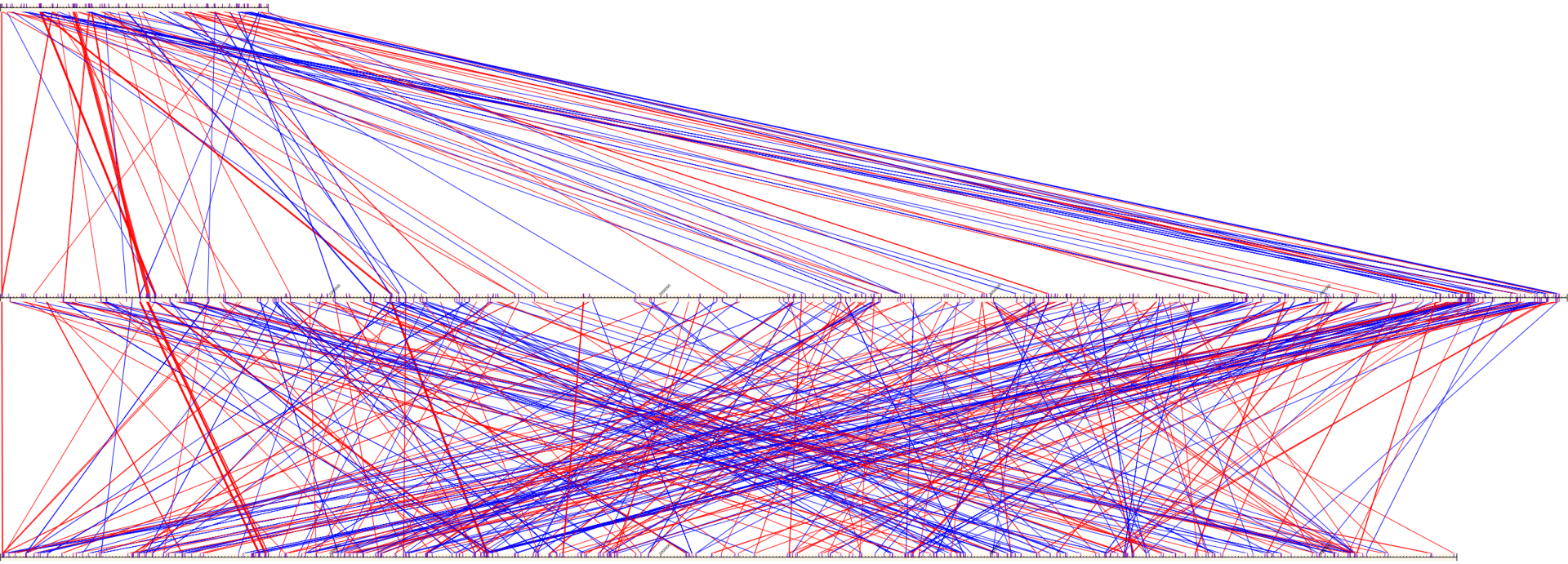
Syntenous

- **`example1.png`** from **`biopython_visualisation`** activity



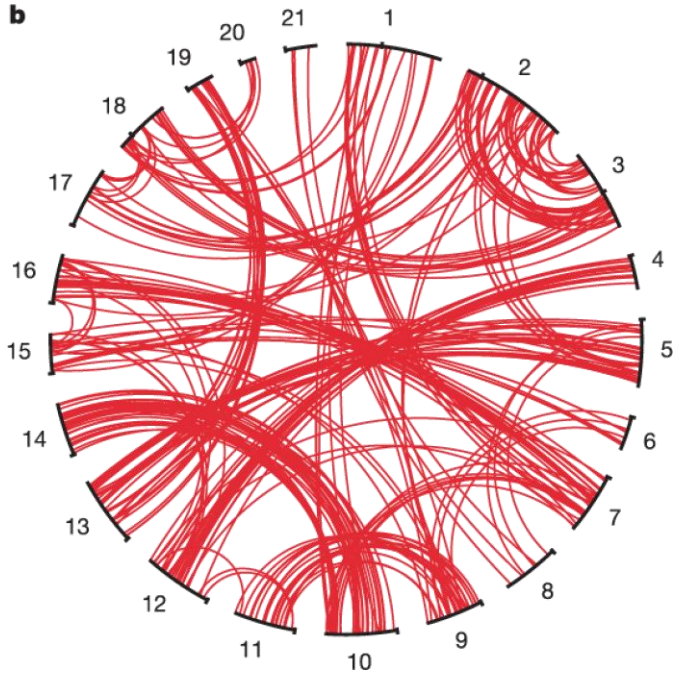
Nonsyntenous

- **`example2.png`** from **`biopython_visualisation`** activity

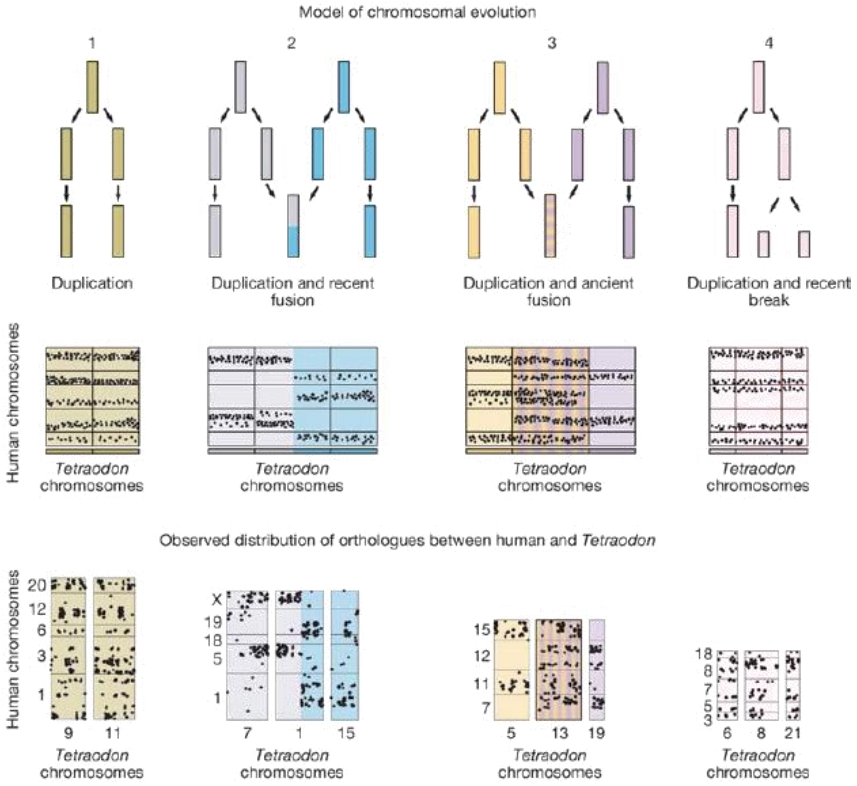


Whole Genome Duplication

- Puffer fish *Tetraodon nigroviridis* (smallest known vertebrate genome)
  - Whole-genome duplication, subsequent to divergence from mammals.
  - Ancestral vertebrate genome inferred to have 12 chromosomes.



Duplicated genes (ExoFish) on 21 chromosomes



Jaillon *et al.* (2004) *Nature* doi:10.1038/nature03025

Conclusion

- Physical and computational genome comparisons:
  - Similar biological questions -> similar concepts
- Lots of sequence data in modern biology
- Conservation ≈ evolutionary constraint
- Many choices of algorithms/analysis software
- Many choices of visualisation software/tools