

Comparative Genomics and Visualisation

BS32010



**The James
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Institute**

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Levels of genome comparison

Types of genome comparison

Making Comparisons

In silico bulk genome comparisons

Whole genome comparisons

Genome feature comparisons



What Is Comparative Genomics?

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



Evolution is the central concept



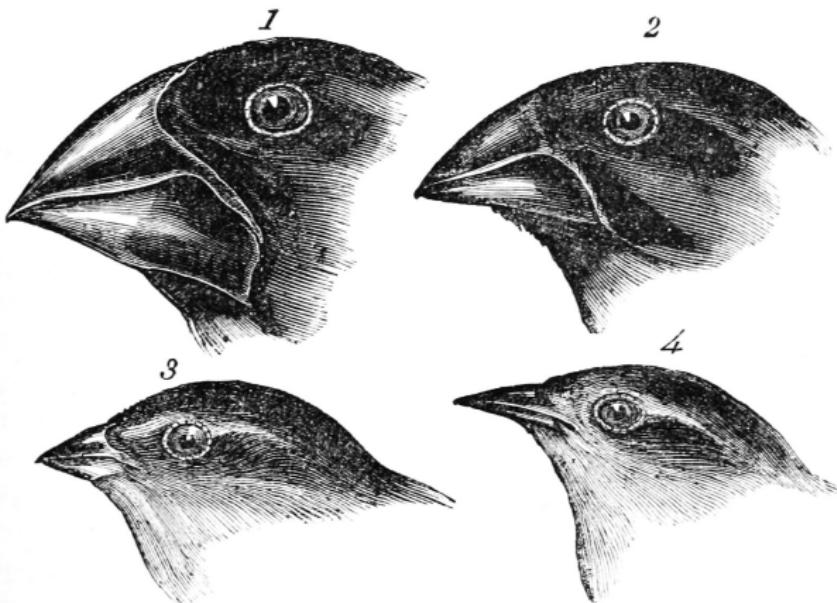
**“NOTHING IN BIOLOGY MAKES SENSE EXCEPT
IN THE LIGHT OF EVOLUTION.”**

THEODOSIUS DOBZHANSKY



Comparison of physical features

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



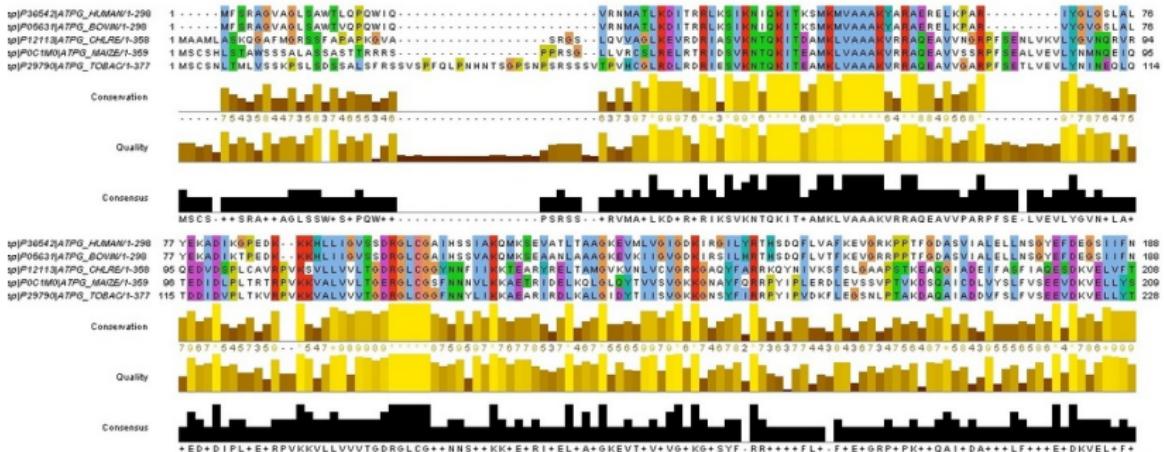
1. *Geospiza magnirostris*.
3. *Geospiza parvula*.

2. *Geospiza fortis*.
4. *Certhidea olivacea*.



Comparison of sequence features

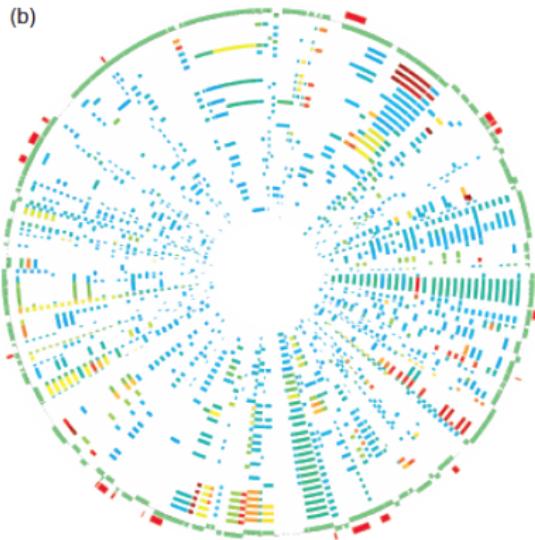
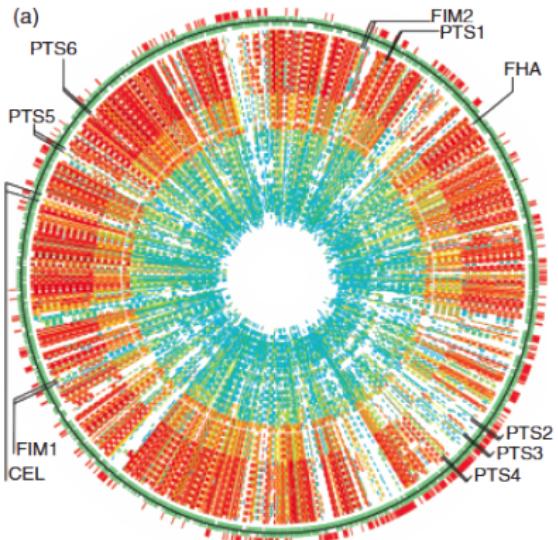
Multiple sequence alignment of ATP synthase





Comparison of genome features

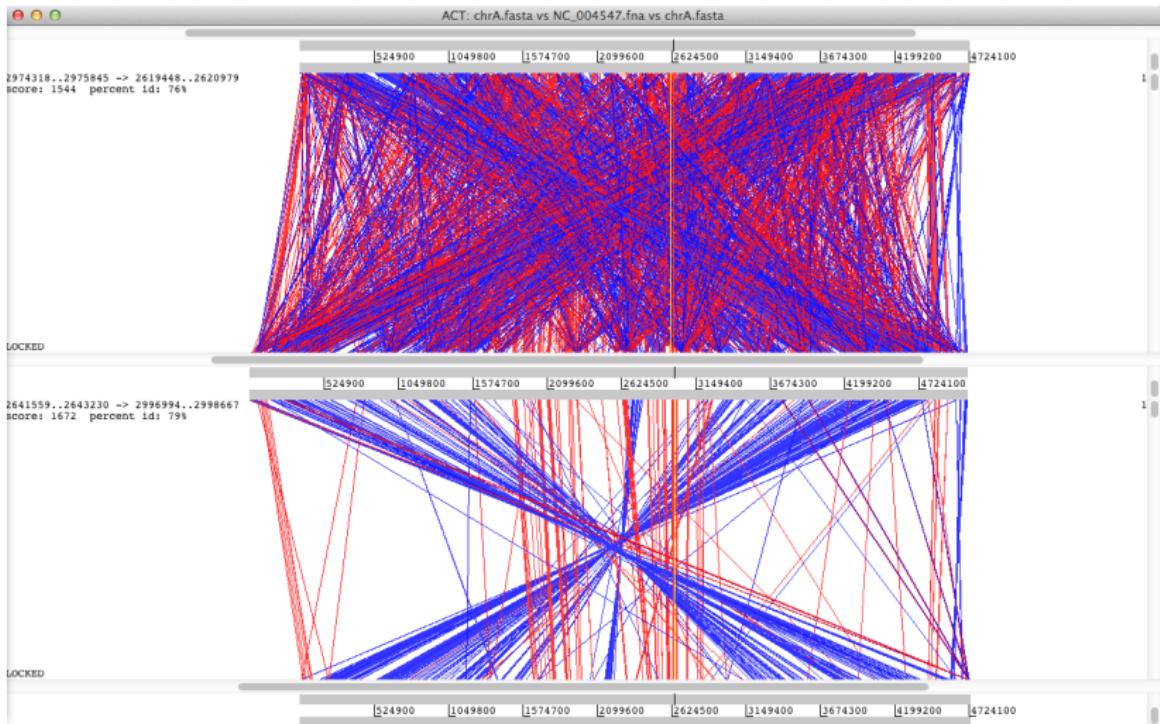
Similarity of individual features (feature sequence)





Comparison of genome features

Similarity of individual features (ordering and arrangement)



Why comparative genomics?

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

I think



Then between A + B. *causes*
less relation. C + D. The
first generation, B + D
rather greater distinction
Then genome would be
formed. - binary relation



Why comparative genomics?

- 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)

I think



Then between A & B. *causes*
loss of relation. C & D. the
first generation, B & D
rather greater distinction
Then genome would be
formed. - binary relation



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 \Rightarrow common function? (genome annotation)
- Transfer functional information from model systems (*E. coli*, *A. thaliana*, *D. melanogaster*) to non-model systems

I think

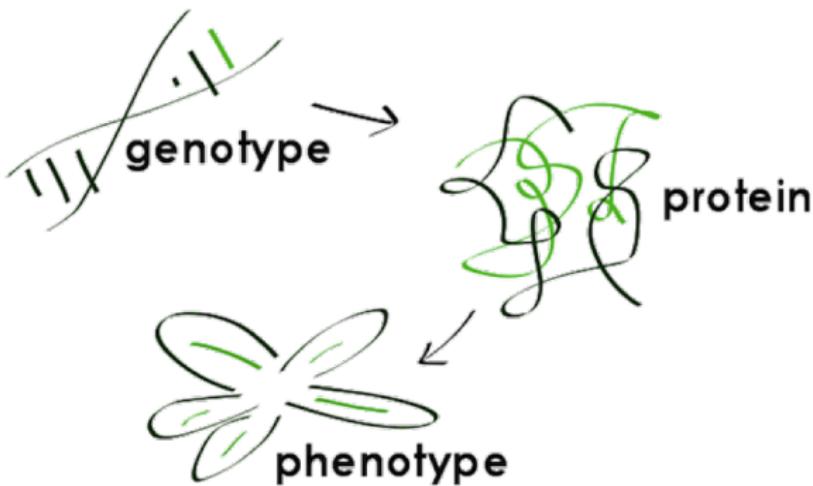


Then between *A* & *B*. *cis* or *trans* relation. *C* & *B*. *the* first generation, *B* & *D* rather greater distribution. Then genome would be formed. - binary relation



Genomes are informative, but...

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



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



Genomes to systems

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.

I think



Then between A & B. can
less of relation. C & B. the
first generation, B & D
rather greater distinction
Then genera would be
formed. - binary relation



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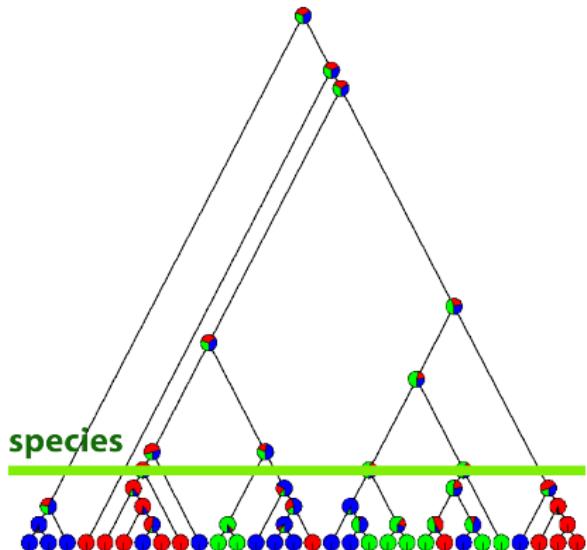
Genome feature comparisons



Types of comparison

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?

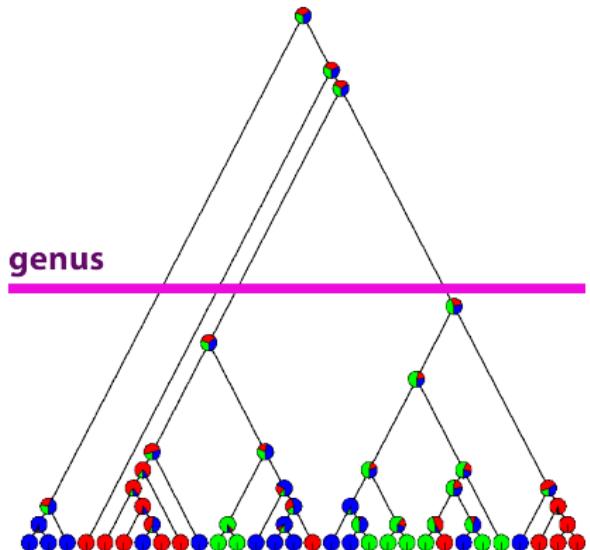




Types of comparison

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?

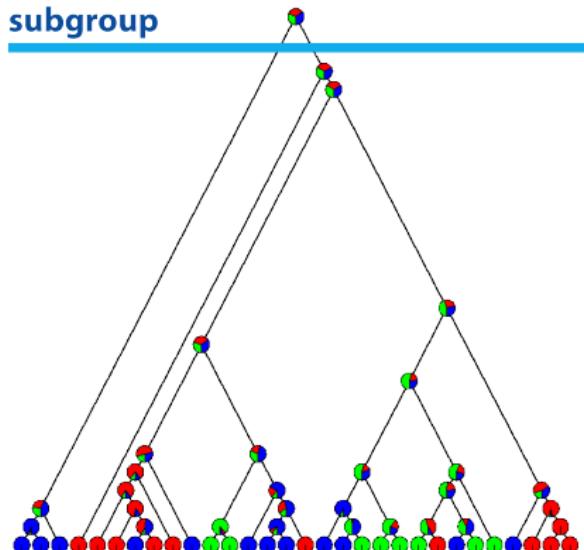




Types of comparison

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?





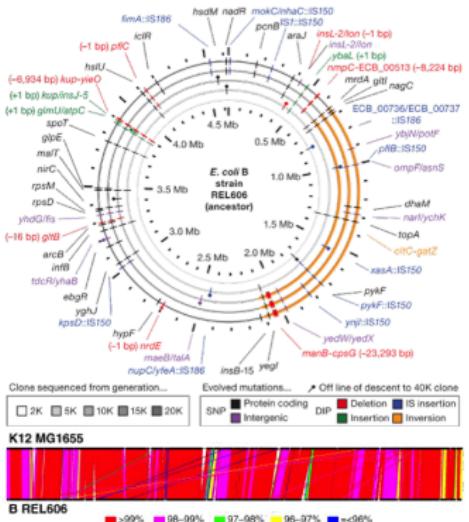
E. coli LTEE a b c

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

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

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

- 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



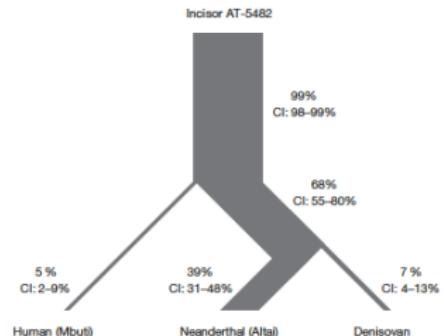
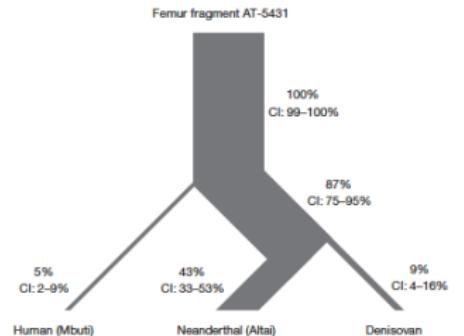


Comparative genomics in the news ^{a b}

^aBBC News 15/3/2016

^bMeyer 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:

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?



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Levels of comparison

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



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



Genome comparisons predate NGS

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





Bulk genome comparisons

**Calculate values for individual genomes,
then compare them.**

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



Nucleotide frequency/genome size

Very easy to calculate from complete or draft genome sequence

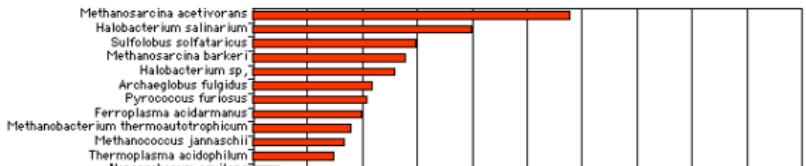
```
In [1]: from Bio import SeqIO
In [2]: s = SeqIO.read("data/NC_000912.fna", "fasta")
In [3]: a, c, g, t = s.seq.count("A"), s.seq.count("C"), s.seq.count("G"), s.seq.count("T")
In [4]: float(g + c)/len(s)
Out[4]: 0.40008010837904245
In [5]: float(g - c)/(g+c)
Out[5]: 0.002397259225467894
```

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

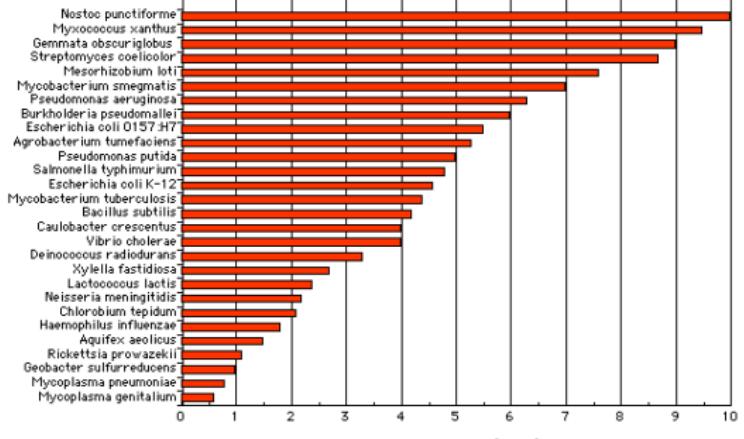


Genome Size

Archaea:

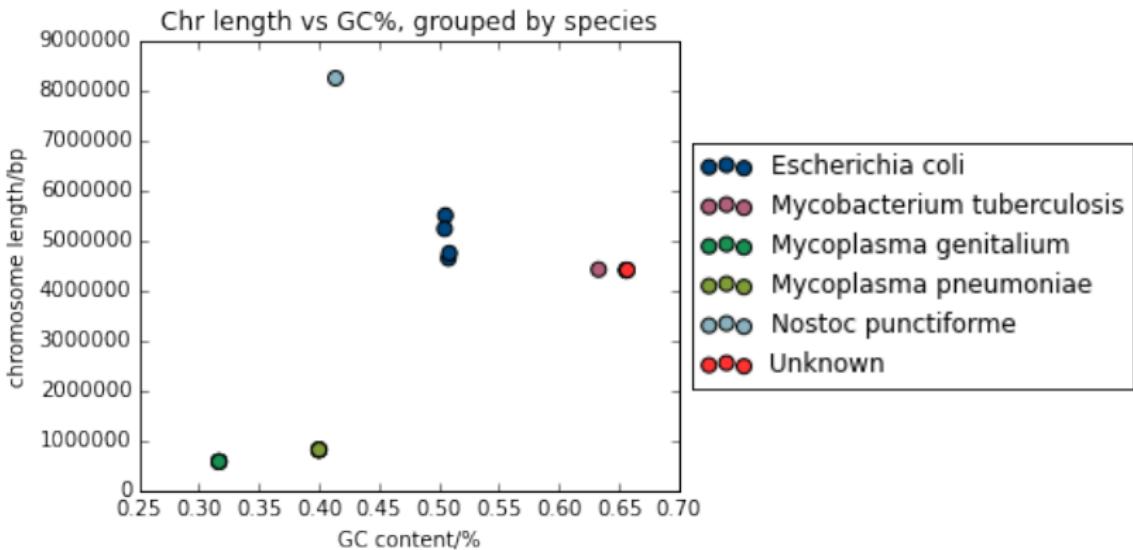


Bacteria:





Genome Size and GC%





Blobology ^{a b}

^aKumar & 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

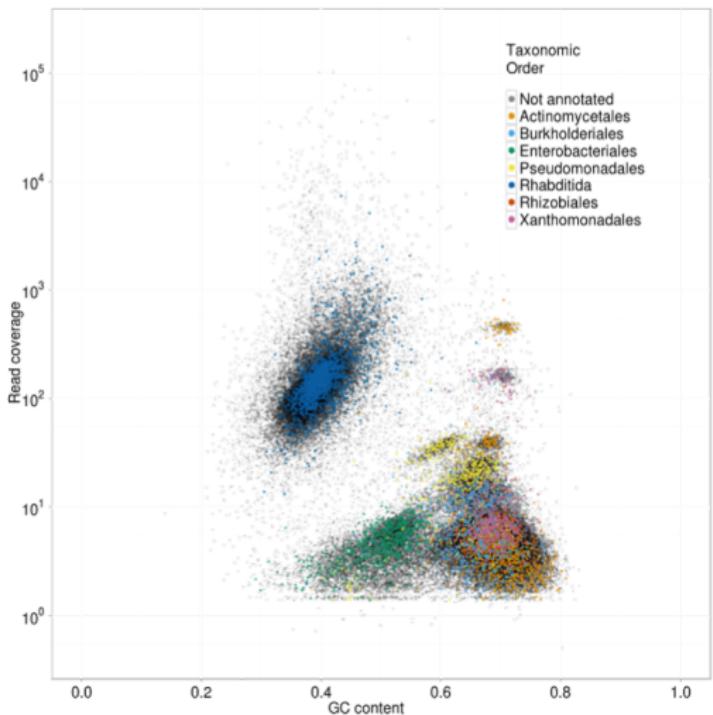
- Host and symbiont DNA have different %GC
- Host and symbiont DNA differ in coverage
- Assemble genome
- Map reads
- Plot coverage against %GC



Blobology ^{a b}

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

^b<http://nematodes.org/bioinformatics/blobology/>





Nucleotide k -mers

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

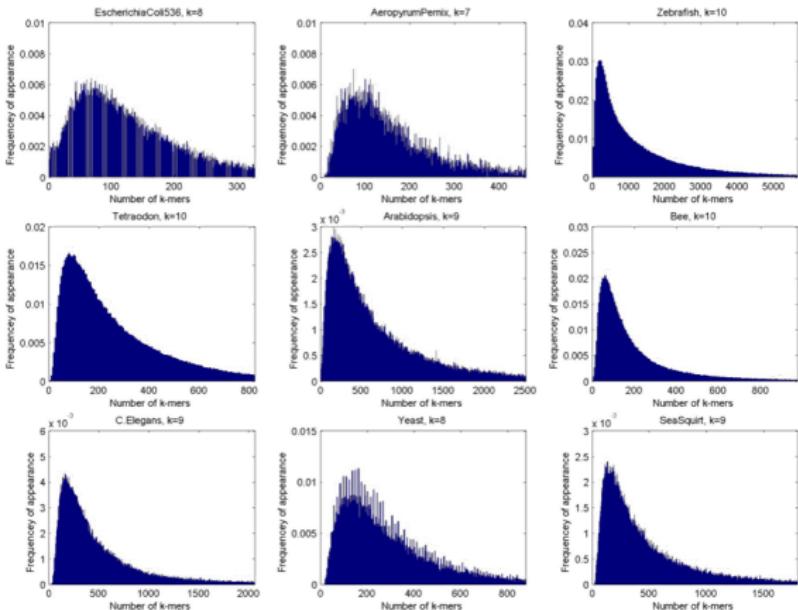
- Nucleotides, $k = 1$, 4 \times 1-mers
A, C, G, T
- Dinucleotides, $k = 2$, 16 \times 2-mers
AA, AC, AG, AT, CA, CC, CG, CT, GA, GC, GG, GT, TA, TC, TG, TT
- Triucleotides, $k = 3$, 64 \times 3-mers
- k -nucleotides, $4^k \times k$ -mers



k-mer spectra ^a

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

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





k-mer spectra ^a

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

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

Genomic regions also differ in this property

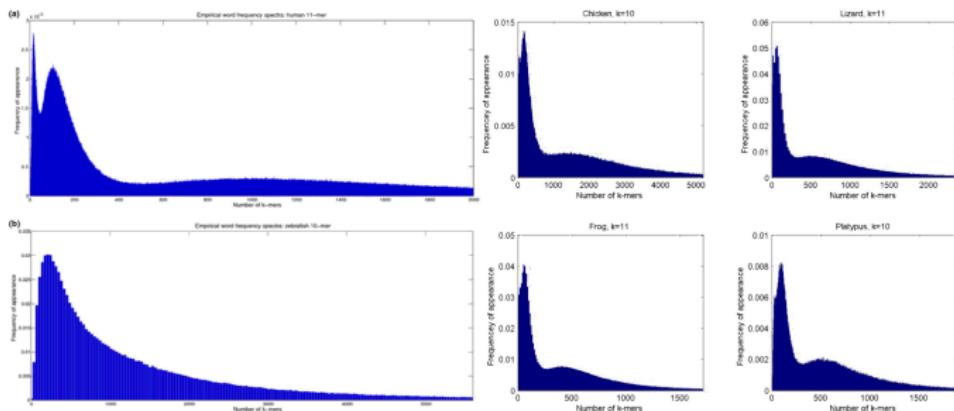




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Whole genome comparisons

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



Whole genome comparisons

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

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

Analogously, *in silico* methods mostly involve sequence alignment

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

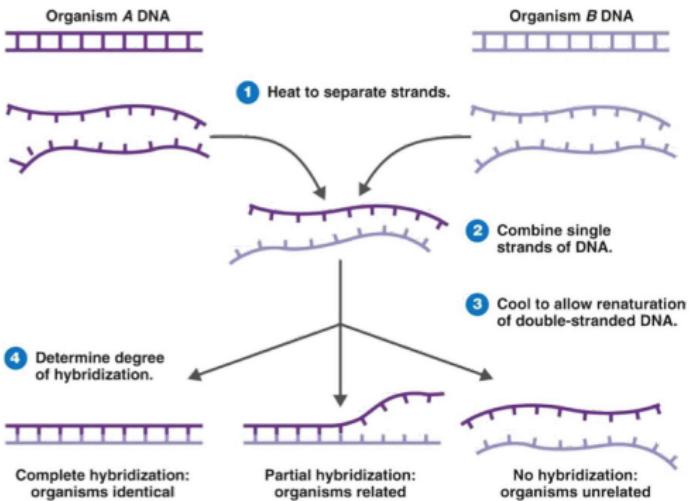


DNA-DNA hybridisation (DDH) ^a

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

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

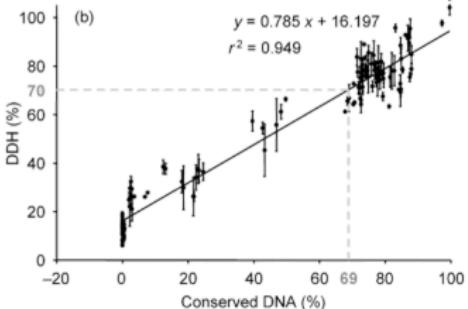
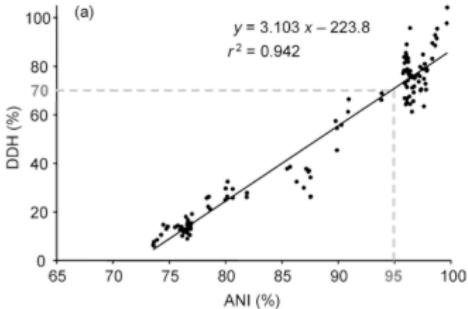


Average Nucleotide Identity (ANI) ^a

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

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

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



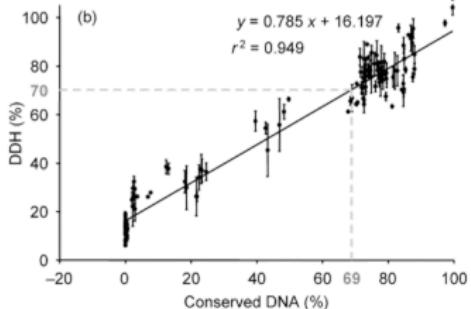
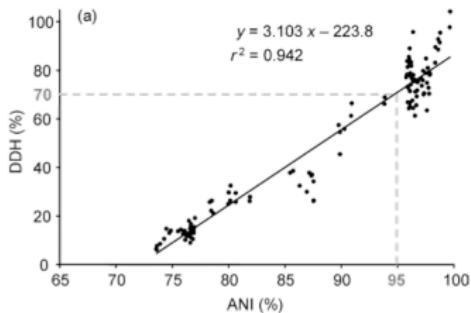


Average Nucleotide Identity (ANI) ^a

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

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





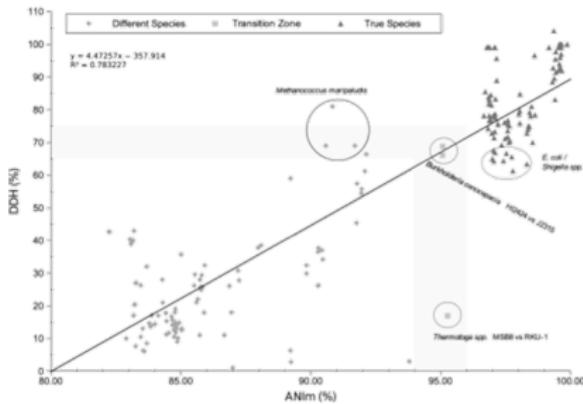
Average Nucleotide Identity (ANI) ^a

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

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

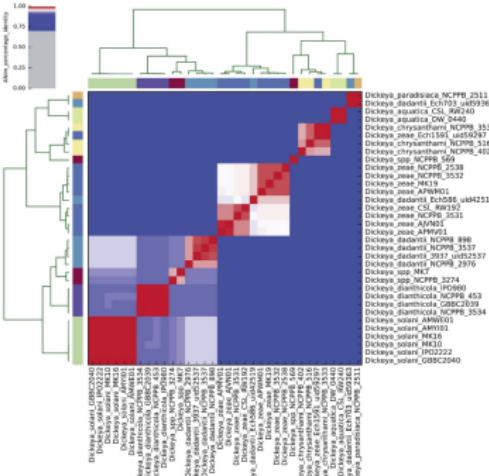


ANI in practice ^{a b}

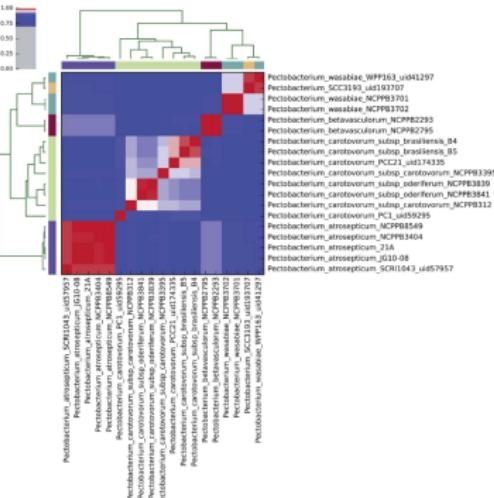
^a van der Wolf et al. (2014) *Int. J. Syst. Evol. Micr.* **64**:768-774 doi:10.1099/ijss.0.052944-0

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

Dickeya species structure



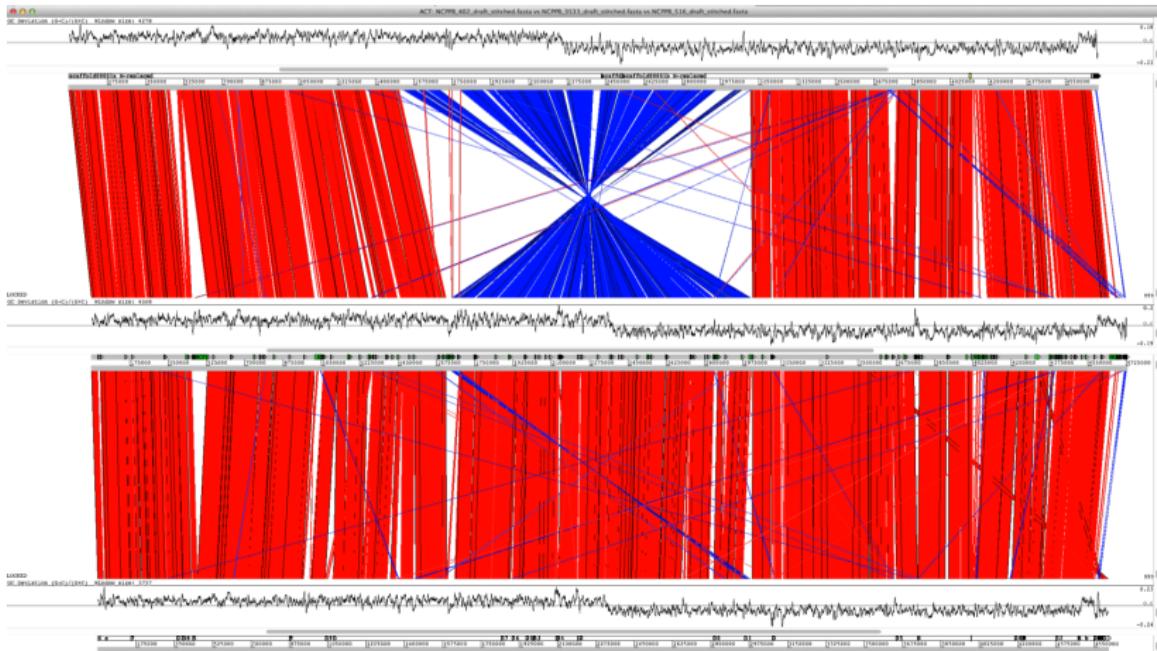
Pectobacterium species structure:





Pairwise genome alignments

Pairwise comparisons require alignment of similar regions.

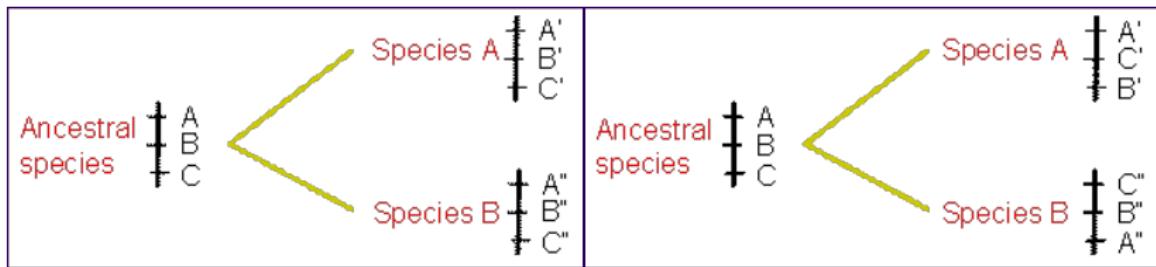




Synteny and Collinearity

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

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

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

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?



^aZhang *et al.* (2000) *J. Comp. Biol.* **7**(1-2): 203-214

^bKorf *et al.* (2003) *BLAST* O'Reilly & Associates, Sebastopol, CA

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>)

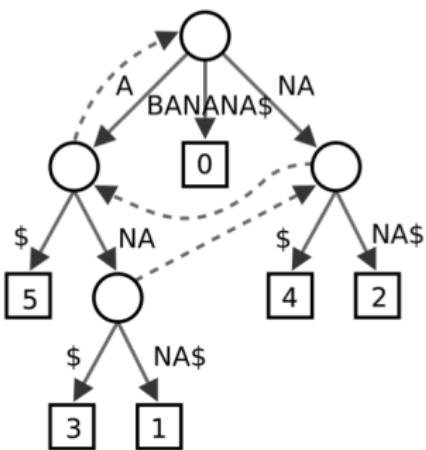


Conceptually completely different to BLAST/BLAT/megaBLAST
Uses *suffix trees* for pattern matching

- 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\$





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

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

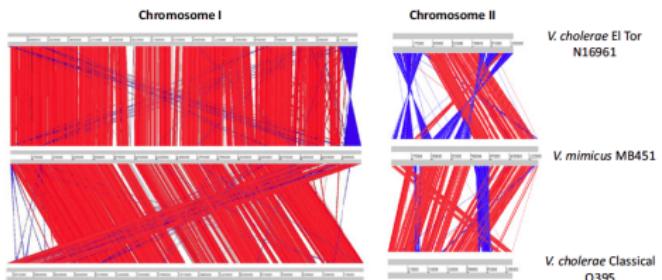


Fig. 2. Linear pairwise comparison of the *Vibrio mimicus* genome by Artemis Comparison Toll. Regions with similarity are highlighted by connecting red or blue lines between the genomes; red lines indicate homologous blocks of sequence, and blue lines indicate inversions. Gaps indicate unique DNA. The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

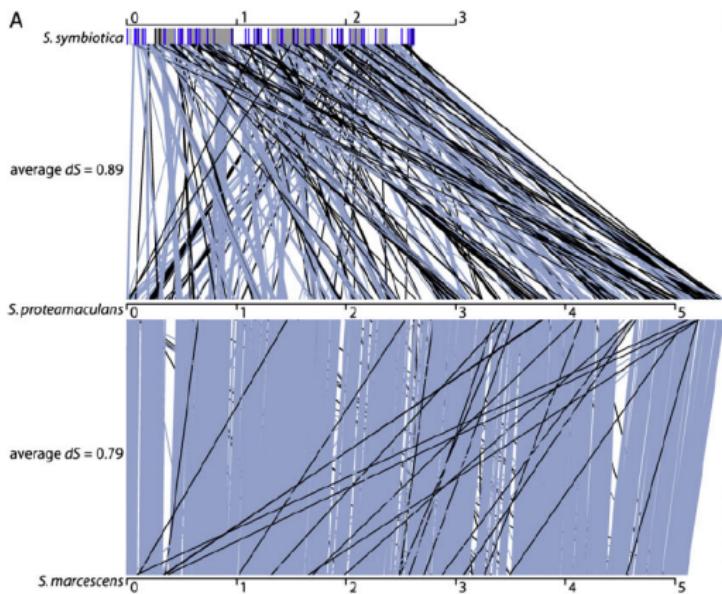
Suggests modularity of genome organisation, as a mechanism for adaptation (HGT, two-speed genome).



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

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

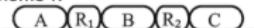
Many tools either positional homology or glocal alignment

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/>)

Given a set of genomes:

Genome 1:



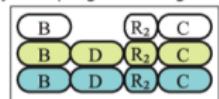
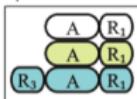
Genome 2:



Genome 3:



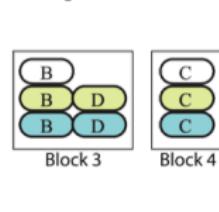
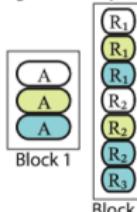
Ideal *positional homology* multiple genome alignment:



Block 1

Block 2

Ideal *glocal* multiple genome alignment:



Block 1

Block 2

Block 3

Block 4



Human:Mouse:Rat



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mouse rat





Human:Mouse:Rat ^a

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

Three-way progressive alignment

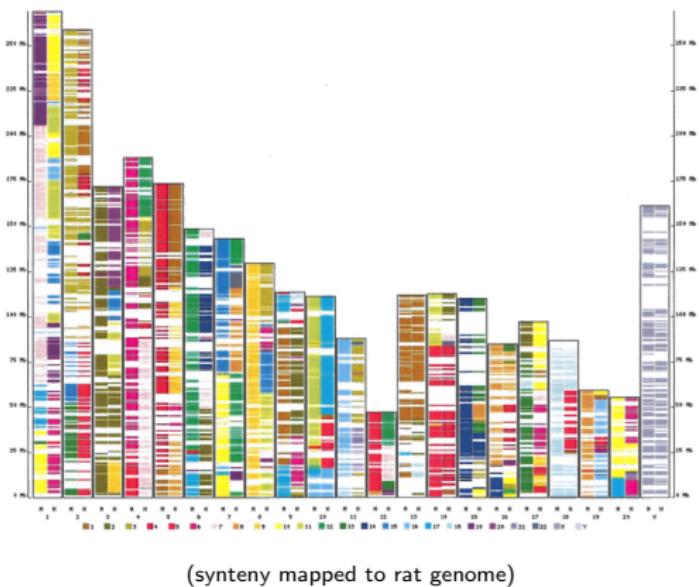
Initial alignments: BLAT

Synteny:

LAGAN/MLAGAN

Three-way synteny

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



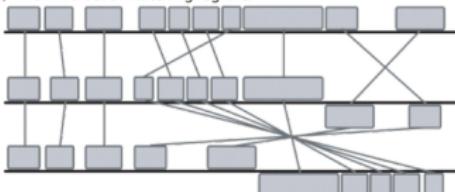


MAUVE/Progressive MAUVE: <http://gel.ahabs.wisc.edu/mauve/>

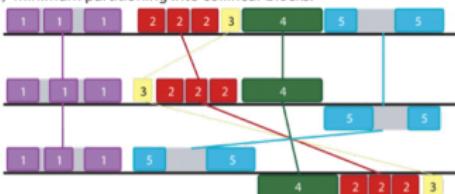
Algorithm:

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

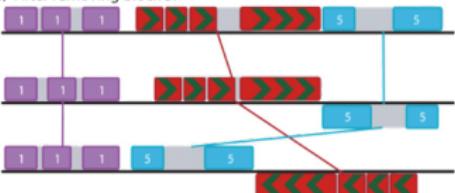
A) The initial set of matching regions:



B) Minimum partitioning into collinear blocks:



C) After removing block 3:





MAUVE alignment of LCBs in nine enterobacterial genomes Evidence for rearrangement of homologous backbone sequence



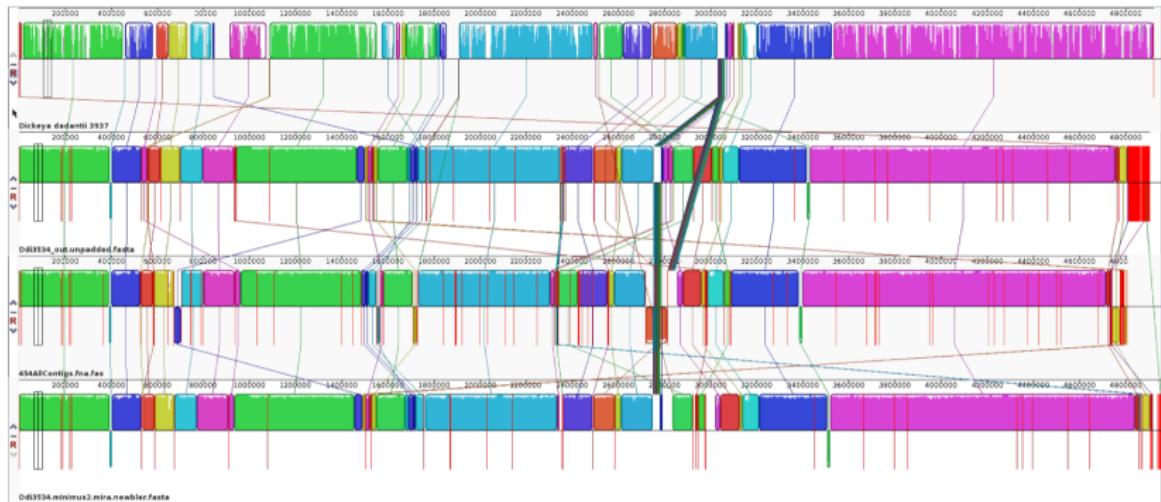


Draft genome alignment

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

^aYahara et al. (2013) *Mol. Biol. Evol.* 30:1454–1464 doi:10.1093/molbev/mst055

“Chromosome painting” infers recombination-derived ‘chunks’
Genome’s haplotype constructed in terms of recombination events
from a ‘donor’ to a ‘recipient’ genome



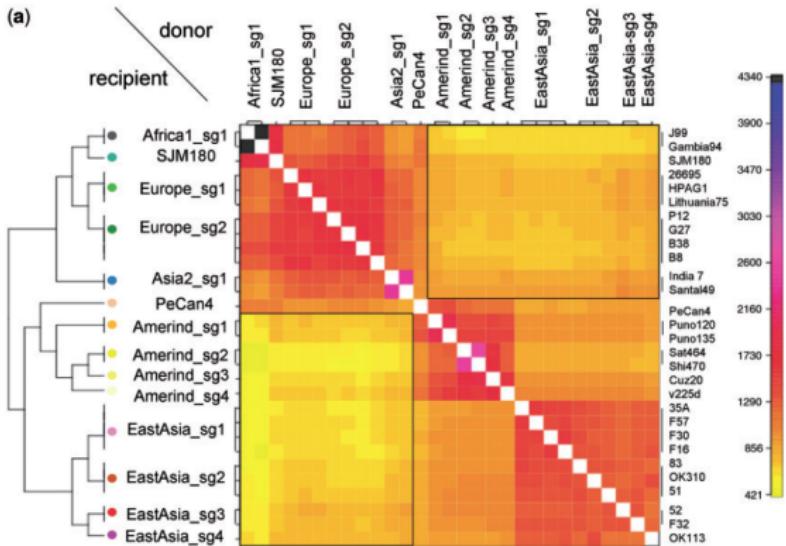
FIG. 1. Chromosome painting *in silico*. Each lane indicates the chromosome of a strain shown on the right. The strains are classified by fineSTRUCTURE into subgroups labeled by colors (table 1 and fig. 2) on the left. A color along the chromosome indicates the subgroup that donated a chunk of SNPs through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



Chromosome painting^a

^aYahara 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

Physical and computational genome comparisons

- Similar biological questions
- ∴ similar concepts

Modern biology: lots of sequence data

- Conservation ≈ evolutionary constraint
- Many choices of algorithms/software
- Many choices of visualisation tools/software



Table of Contents

Introduction

- What is comparative genomics?
- Levels of genome comparison
- Types of genome comparison

Making Comparisons

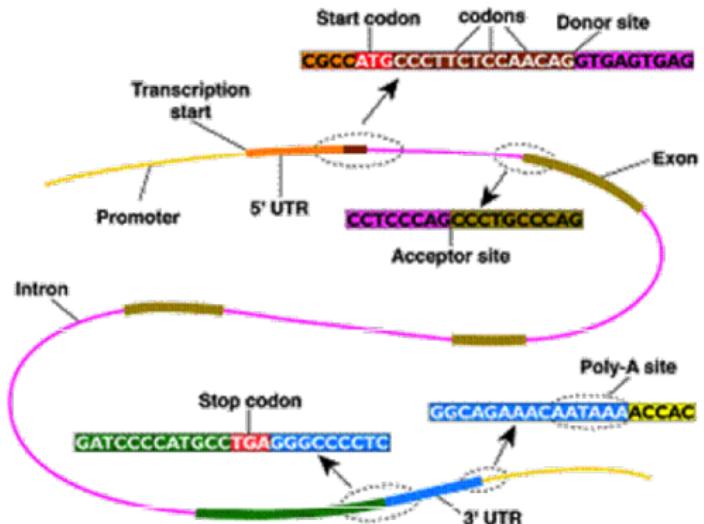
- In silico bulk genome comparisons
- Whole genome comparisons
- Genome feature comparisons



Gene features

Significant substructure, especially in eukaryotes

- translation start
- introns
- exons
- translation stop
- translation terminator

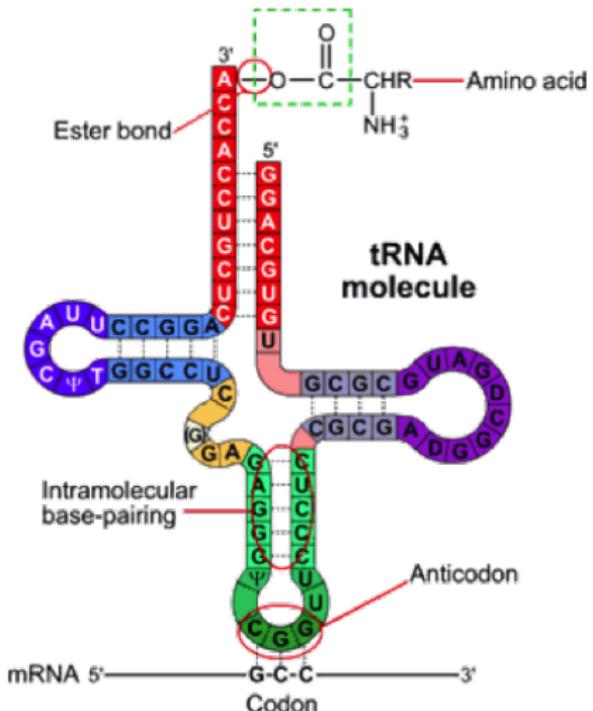




RNA features

RNA/ncRNA: characterised by complex secondary structure

- tRNA - transfer RNA
- rRNA - ribosomal RNA
- CRISPRs - prokaryotic defence, and genome editing
- many other functional classes, including enhancers

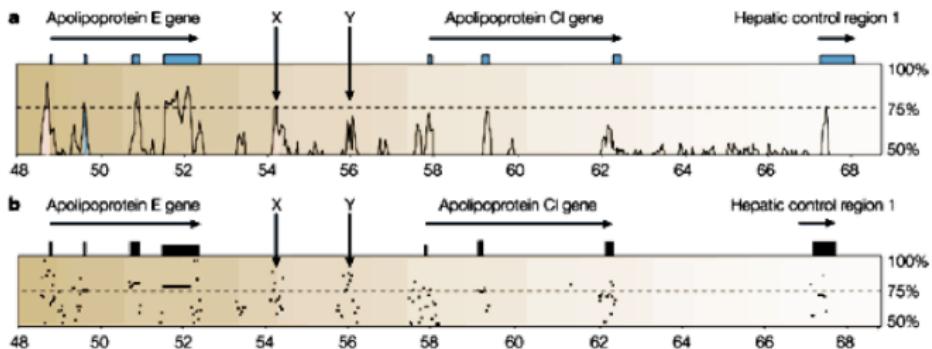




Regulatory features ^a

^a Pennacchio & Rubin (2001) *Nature Rev. Genet.* doi:10.1038/35052548

- transcription start sites (TSS)
- RNA polymerase (RNAP) binding sites
- transcription factor binding sites (TFBS)
- core, proximal and distal promoter regions





Gene finding ^{a b c}

^a Liang et al. (2009) *Genome Res.* doi:10.1101/gr.088997.108

^b Brent (2007) *Nat. Biotech.* doi:10.1038/nbt0807-883

^c Korf (2004) *BMC Bioinf.* doi:10.1186/1471-2105-5-59

At genome scales, we need to automate functional prediction

Empirical (evidence-based) methods:

- Inference from known protein/cDNA/mRNA/EST sequence
- Interference from mapped RNA reads (e.g. RNAseq)

Ab initio methods:

- Prediction on the basis of gene features (TSS, CpG islands, Shine-Dalgarno sequence, stop codons, nucleotide composition, etc.)

Inference from genome comparisons/sequence conservation



Regulatory element finding ^{a b c}

^aZhang *et al.* (2011) *BMC Bioinf.* doi:10.1186/1471-2105-12-238

^bKilic *et al.* (2013) *Nucl. Acids Re.* doi:10.1093/nar/gkt1123

^cVavouris & Elgar (2005) *Curr. Op. Genet. Deve.* doi:10.1016/j.gde.2005.05.002

Empirical (evidence-based) methods:

- Inference from protein-DNA binding experiments
- Interference from co-expression

Ab initio methods:

- Identification of regulatory motifs (profile/other methods; TATA, σ -factor binding sites, etc.)
- Statistical overrepresentation of motifs
- Identification from sequence properties

Inference from genome comparisons/sequence conservation



What makes genome features equivalent?



When we compare two features (e.g. genes) between two or more genomes, there must be some basis for making the comparison
That is, they have to be *equivalent* in some way, such as:

- common evolutionary origin
- functional similarity
- a family-based relationship

It's common to define equivalence of genome features in terms of evolutionary relationship.



Why look at equivalent features?



The real power of genomics is comparative genomics!

- Makes catalogues of genome components comparable between organisms
- Differences, e.g. presence/absence of equivalents may support hypotheses for functional or phenotypic difference
- Can identify characteristic signals for diagnosis/epidemiology
- Can build parts lists and wiring diagrams for systems and synthetic biology



Who let the -logues out?

Genome features can have complex evolutionary relationships

We need precise terms to describe these relationships





The -logues drop ^a

^aFitch et al. (1970) *Syst. Zool.* doi:10.2307/2412448



How do we understand the relationships between features in more than one genome?

- Functional similarity: **analogy**
- Evolutionary common origin: **homology, orthology, etc.**
- Evolutionary/functional/family relationship: **paralogy**

DISTINGUISHING HOMOLOGOUS FROM ANALOGOUS PROTEINS

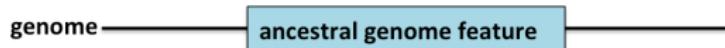
WALTER M. FITCH

Abstract

Fitch, W. M. (Dept. Physiological Chem., U. Wisconsin, Madison 53706) 1970. *Distinguishing homologous from analogous proteins.* *Syst. Zool.*, 19:99–113.—This work provides a means by which it is possible to determine whether two groups of related proteins have a common ancestor or are of independent origin. A set of 16 random



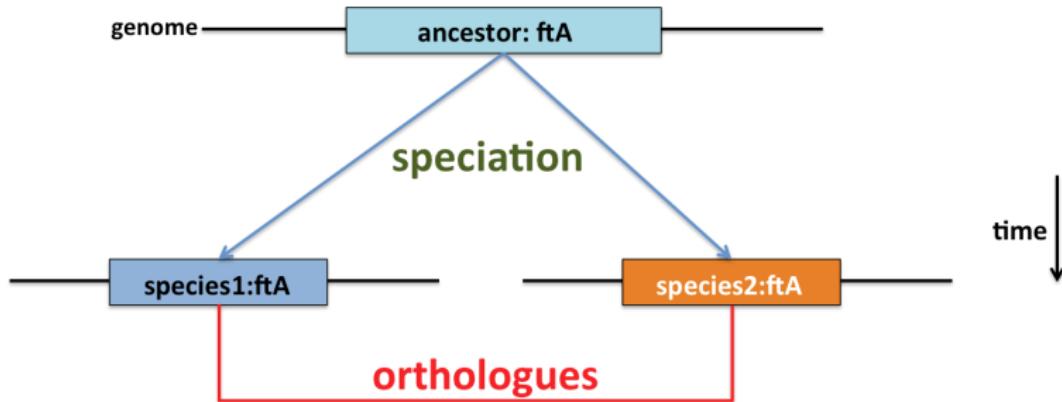
Who let the -logues out?



time
↓



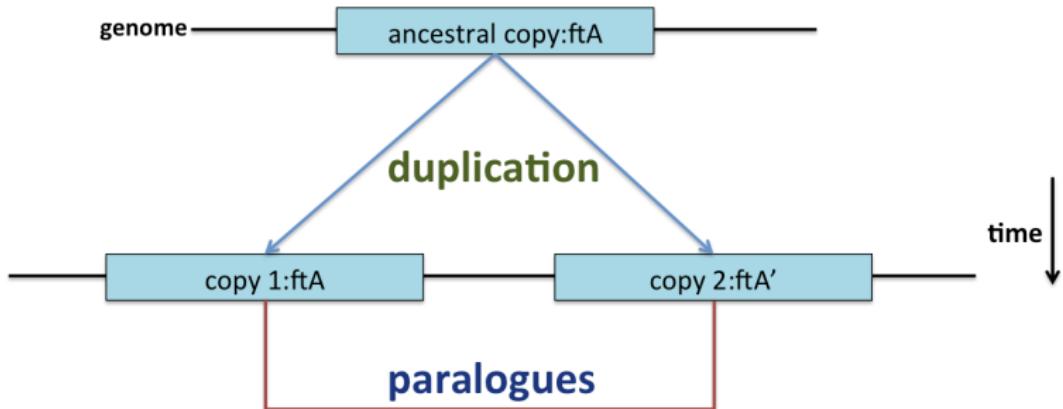
Who let the -logues out?



- **Orthologues:** homologues that diverged through speciation



Who let the -logues out?



Paralogues: homologues that diverged through duplication within the same genome



Orthology ^a

^aStorm & Sonnhammer (2002) *Bioinformatics* doi:10.1093/bioinformatics/18.1.92

- Frequently abused/misused as a term
- “Orthology” is an evolutionary relationship, bent into service as a functional descriptor
- Orthology is strictly defined *only for two species or clades!* (cf. OrthoMCL)
- Orthology is not transitive:
(A is an orthologue of C , and B is an orthologue of C , does **not** imply that A is an orthologue of B)

All classifications of orthology/paralogy are inferences!



The Ortholog Conjecture ^a ^b

^a Nehrt et al. (2011) *PLoS Comp. Biol.* doi:10.1371/journal.pcbi.1002073

^b Chen et al. (2012) *PLoS Comp. Biol.* doi:10.1371/journal.pcbi.1002784



Without duplication, a gene product is unlikely to change its basic function, because this would lead to loss of the original function, and this would be harmful.



Why focus on orthologues? ^{a b c}

^aChen and Zhang (2012) *PLoS Comp. Biol.* doi:10.1371/journal.pcbi.1002784

^bDessimoz (2011) *Brief. Bioinf.* doi:10.1093/bib/bbr057

^cAltenhoff and Dessimoz (2009) *PLoS Comp. Biol.* 5:e1000262 doi:10.1371/journal.pcbi.1000262

Formalisation of the idea of *corresponding genes* in different organisms.

Orthologues serve two purposes:

- **Evolutionary equivalence**
- **Functional equivalence** ("The Ortholog Conjecture")

Applications in comparative genomics, functional genomics and phylogenetics.

Over 30 databases attempt to describe orthologous relationships (http://questfororthologs.org/orthology_databases)



Finding orthologues ^{a b c}

^a Kristensen *et al.* (2011) *Brief. Bioinf.* **12**:379-391 doi:10.1093/bib/bbr030

^b Trachana *et al.* (2011) *Bioessays* **33**:769-780 doi:10.1002/bies.201100062

^c Salichos and Rokas (2011) *PLoS One* **6**:e18755 doi:10.1371/journal.pone.0018755.g006

Multiple methods and databases

- **Pairwise genome**

- RBBH (aka BBH, RBH),
RSD, InParanoid, RoundUp

- **Multi-genome**

- *Graph-based*: COG, eggNOG,
OrthoDB, OrthoMCL, OMA,
MultiParanoid
- *Tree-based*: TreeFam,
Ensembl Compara,
PhylomeDB, LOFT

List of orthology databases

If you know of any other database, please edit this page directly or contact us.

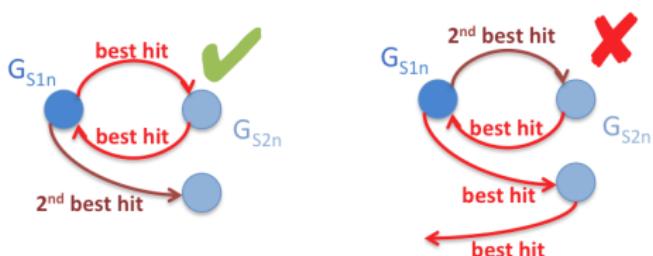
1. COG/WEGeKOGs
2. COGe-COCO-CL
3. COGe-LOFT
4. eggNOG
5. EGO
6. Ensembl Compara
7. Gene-Oriented Ortholog Database
8. GreenPhyDB
9. HCDP
10. Hemisite
11. HOGENOM
12. HOVERGEN
13. HOMOLENS
14. HOPS
15. INVHOGEN
16. JnParanoid
17. KEGG Orthology
18. MetaphOrn
19. MiGD
20. MiGD
21. OMA
22. OrthoDB (OrthoDB on Wikipedia)
23. OrthoID
24. ORTHOLUGE
25. OrthoInspector
26. OrthoMCL
27. Panther
28. PhOG
29. PHOG
30. PhylomeDB
31. PLAZA
32. P-POD
33. ProMMap
34. Proteinortho
35. RoundUp
36. TreeFam
37. YOBY



Reciprocal Best BLAST Hits ^a

^aOn Reciprocal Best BLAST Hits 19/7/2012

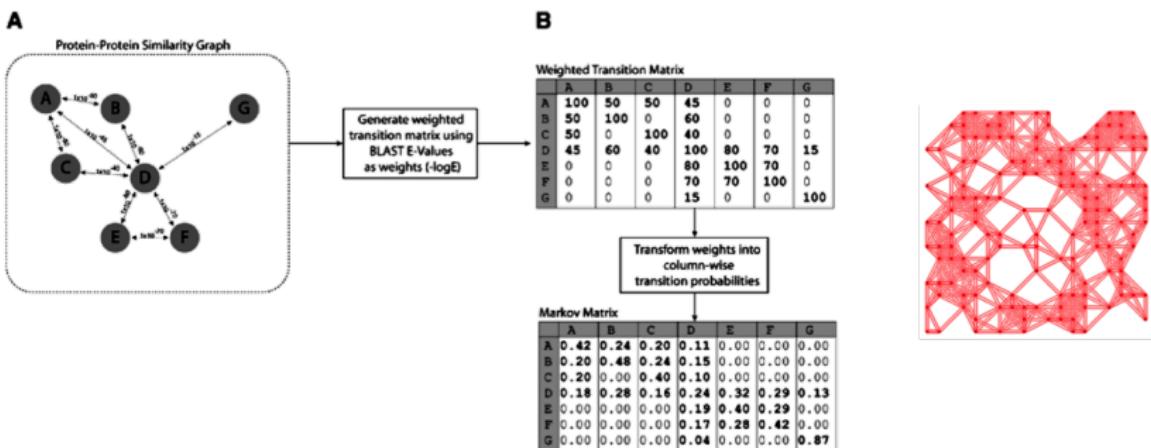
- S_1, S_2 are the gene sequence sets from two organisms
- Use sequence search tool (BLAST/FASTA):
 - Query= S_1 , Subject= S_2
 - Query= S_2 , Subject= S_1

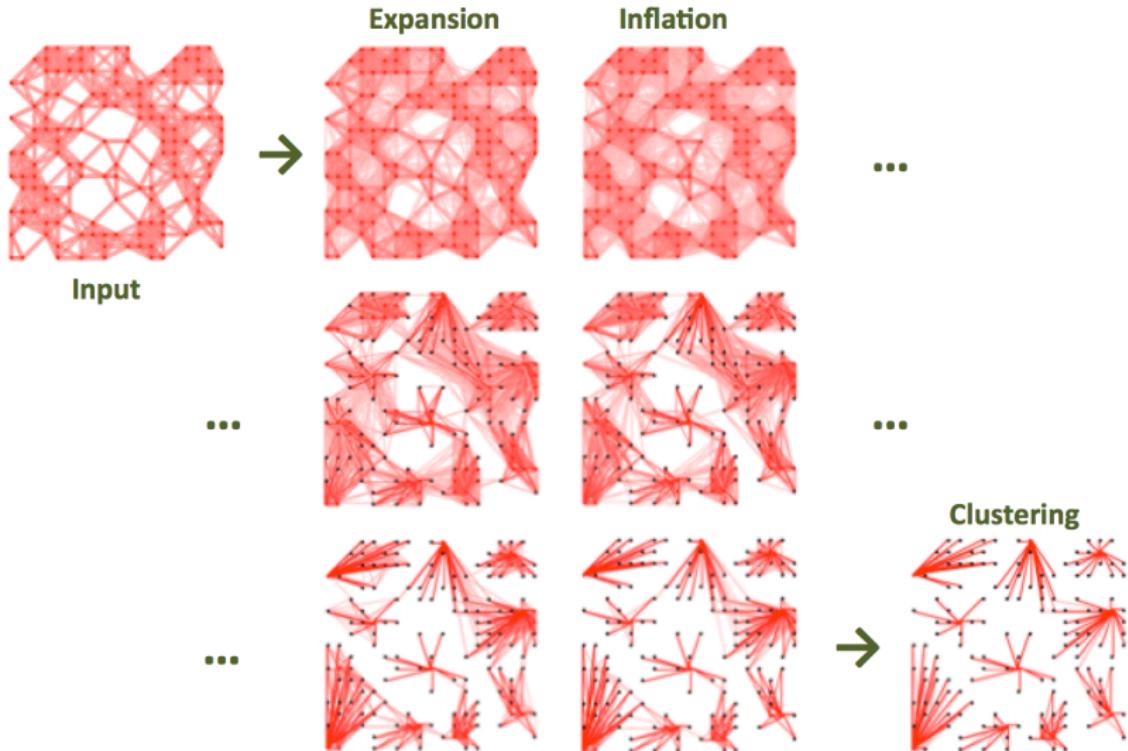


- Optionally filter hits (e.g. on %identity and %coverage)
- Find all pairs of sequences $\{G_{S1n}, G_{S2n}\}$ in S_1, S_2 where G_{S1n} is the best BLAST match to G_{S2n} and G_{S2n} is the best BLAST match to G_{S1n} .



- MCL constructs a network (*graph*) from all-against-all BLAST results
- Matrix operations (*expansion, inflation*) are applied
- Expansion, inflation iterated until the network converges







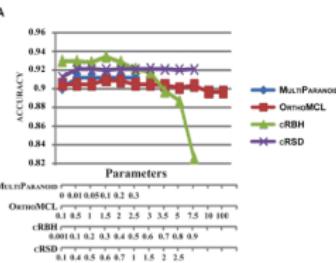
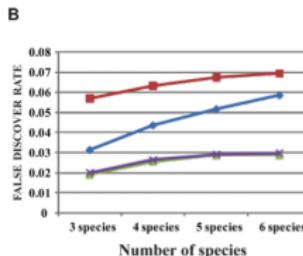
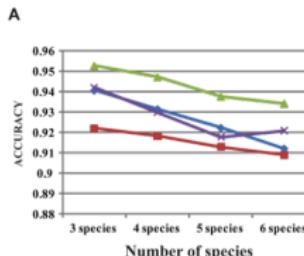
Which prediction methods work best? ^a

^aSalichos and Rokas (2011) *PLoS One* 6:e18755 doi:10.1371/journal.pone.0018755.g006

Four methods tested against 2,723 curated orthologues from six *Saccharomycetes*

- RBBH (and cRBH); RSD (and cRSD); MultiParanoid; OrthoMCL
- Rated by statistical performance metrics: sensitivity, specificity, accuracy, FDR

cRBH most accurate and specific, with lowest FDR.





Which prediction methods work best?

- Performance varies by choice of method, and interpretation of “orthology”
- Biggest influence is genome annotation quality
- Relative performance varies with choice of benchmark
- **(clustering) RBH outperforms more complex algorithms under many circumstances**



Selection Pressure

Comparative genomics helps identify selection pressures at a structural and sequence level



How orthologues help

Defining core groups of genes as “orthologue” allows analysis of groups of genes together by:

- synteny/collocation
- gene neighbourhood changes (e.g. *genome expansion*)
- pan genome (core/accessory genomes)

and of individual genes within those groups, by:

- multiple alignment
- domain detection
- identification of functional sites
- inference of directional selection (stabilising/positive selection)



Genome expansion ^a

^aHaas et al. (2009) *Nature* doi:10.1038/nature08358

- Mobile/repeat elements reproduce and expand during evolution
- Generates a “sequence laboratory” for variation and experiment
- e.g. *Phytophthora infestans* effector protein expansion and arms race

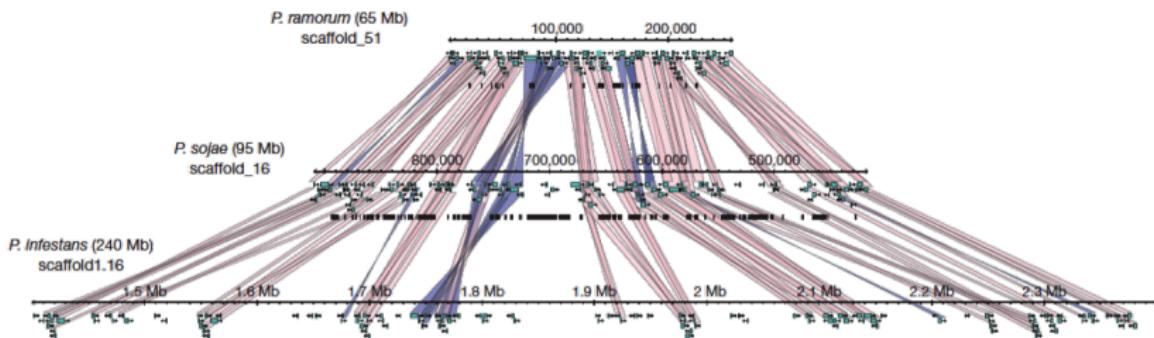


Figure 1 | Repeat-driven genome expansion in *Phytophthora infestans*. Conserved gene order across three homologous *Phytophthora* scaffolds. Genome expansion is evident in regions of conserved gene order, a

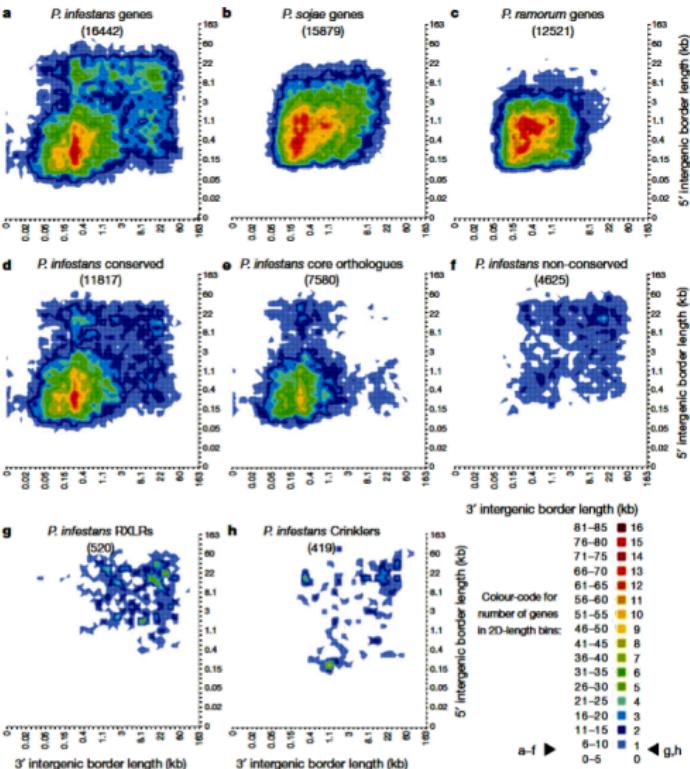
consequence of repeat expansion in intergenic regions. Genes are shown as turquoise boxes, repeats as black boxes. Collinear orthologous gene pairs are connected by pink (direct) or blue (inverted) bands.



Genome expansion ^a

^aHaas et al. (2009) *Nature* doi:10.1038/nature08358

- Mobile elements (MEs) are large, and duplicate/carry genes with them
- Larger intergenic regions in MEs
- Effector proteins found preferentially in regions with large gaps
- Two-speed genome associated with adaptability





The Pangenome

The Core Genome Hypothesis:
“The core genome is the primary cohesive unit defining a bacterial species”

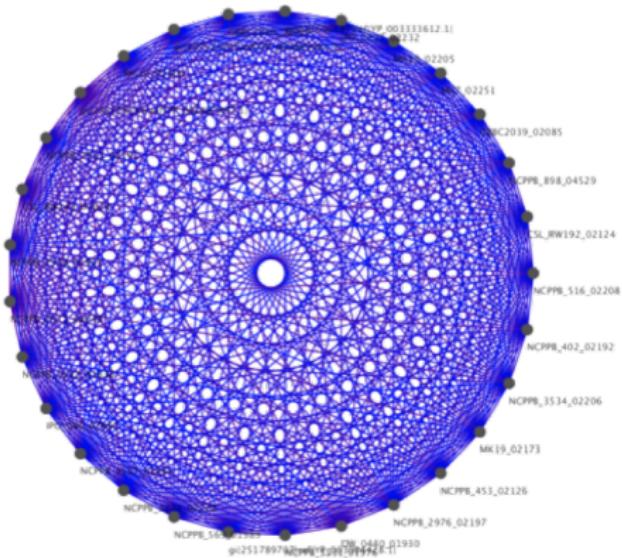


Core genome ^{a b}

^aLaing (2010) *BMC Bioinf.* doi:10.1186/1471-2105-11-461

^bLefébure *et al.* (2010) *Genome Biol. Evol.* doi:10.1093/gbe/evq048

Once equivalent genes have been identified, those present in all related isolates can be identified: **the core genome**.



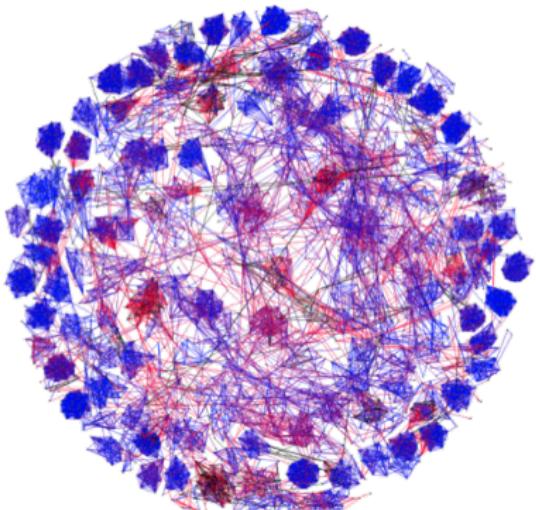


Accessory genome ^a ^b

^a Laing (2010) *BMC Bioinf.* doi:10.1186/1471-2105-11-461

^b Lefébure *et al.* (2010) *Genome Biol. Evol.* doi:10.1093/gbe/evq048

The remaining genes are **the accessory genome**, and are expected to mediate function that distinguishes between isolates.



Accessory genome ^{a b}

^a Croll and McDonald (2012) *PLoS Path.* 8:e1002608 doi:10.1371/journal.ppat.1002608

^b Baltrus et al. (2011) *PLoS Path.* 7:e1002132 doi:10.1371/journal.ppat.1002132.t002

Accessory genomes are a cradle for adaptive evolution
 This is particularly so for bacterial pathogens, such as
Pseudomonas spp.

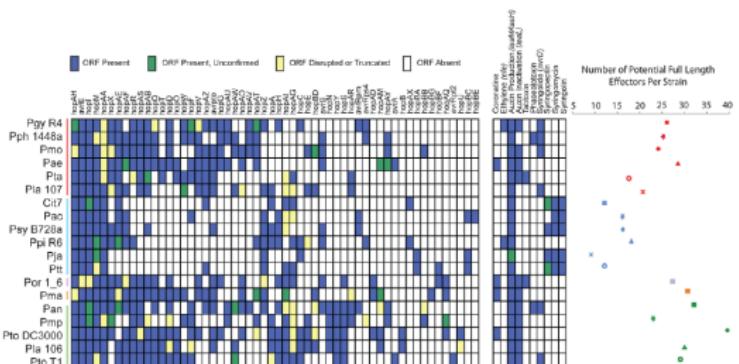


Figure 3. *P. syringae* isolates harbor extensive diversity in virulence gene repertoires. TTE, toxin, and plant hormone biosynthesis genes are listed across the top. *P. syringae* genomes, color-coded by phylogenetic group as in Figure 1. At the left, a blue box indicates presence of full-length ORFs or complete pathways within each genome. Green boxes indicate that genes or pathways are present by similarity searches, but the presence of full-length genes could not be verified by PCR, or the pathways are potentially incomplete. Yellow boxes indicate that genes are either significantly truncated or are disrupted by insertion sequence elements. White boxes indicate absence of genes or pathways from the strains based on homology searches. At the far right, the total number of potentially functional TTE proteins is shown for each genome and displayed according to the color-coded strain and group symbols shown in Figure 1.
 doi:10.1371/journal.ppat.1002132.g003



What didn't I get to?

- Genome-Wide Association Studies (GWAS)
 - Try <http://genenetwork.org/> to play with some data
- Prediction of regulatory elements, e.g.
 - Kellis *et al.* (2003) *Nature* doi:10.1038/nature01644
 - King *et al.* (2007) *Genome Res.* doi:10.1101/gr.5592107
 - Chaivorapol *et al.* (2008) *BMC Bioinf.* doi:10.1186/1471-2105-9-455
 - CompMOBY <http://genome.ucsf.edu/compmoby>
- Detection of Horizontal/Lateral Gene Transfer (HGT/LGT), e.g.
 - Tsirigos & Rigoutsos (2005) *Nucl. Acids Res.* doi:10.1093/nar/gki187
- Phylogenomics, e.g.
 - Delsuc *et al.* (2005) *Nat. rev. Genet.* doi:10.1038/nrg1603
 - AMPHORA <https://phylogenomics.wordpress.com/software/amphora/>



Messages to take away

- Comparative genomics is a powerful set of techniques for:
 - Understanding and identifying evolutionary processes and mechanisms
 - Reconstructing detailed evolutionary history of a set of organisms
 - Identifying and understanding common genomic features of organisms
 - Providing hypotheses about gene function for experimental investigation



Messages to take away

- Comparative genomics is comparisons
 - What is *similar* between two genomes?
 - What is *different* between two genomes?
- Comparative genomics *is* evolutionary genomics
 - Lots of scope for improvement in tools
- Tools that 'do the same thing' can give different output
 - BLAST vs MUMmer
 - RBBH vs MCL
 - The choice of application matters for correctness and interpretation



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