# Pathogen Genome Data

## EMBL-EBI Bioinformatics of Plants and Plant Pathogens 23$^{rd}$ May 2016

The James Hutton Institute

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These slides will be made available on SlideShare.

**These slides, and supporting material including exercises, are available at https://github.com/widdowquinn/Teaching-EMBL-Plant-Path-Genomics**

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# Introduction

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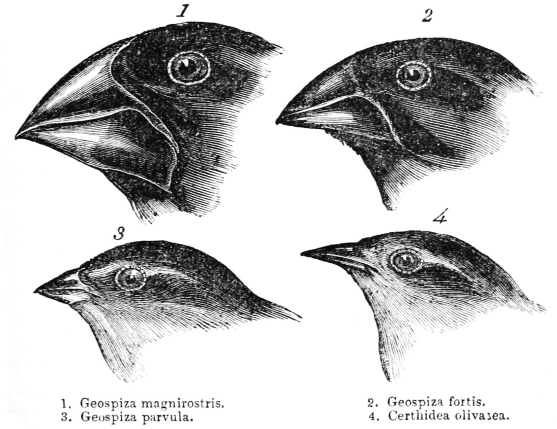
## What can pathogen genome data do for you?

Combining genomic data with comparative and evolutionary biology, addresses questions of pathogen evolution, adaptation and lifestyle.

"NOTHING IN BIOLOGY MAKES SENSE EXCEPT IN THE LIGHT OF EVOLUTION."

THEODOSIUS DOBZHANSKY



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# NCBI

NCBI

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## http://www.ncbi.nlm.nih.gov/

Repository of record for pathogen (and other) genome data

- Example: *Ralstonia solanacearum*
  - Browser interface
  - FTP repositories of genome data
    - RefSeq
    - GenBank

**Index of /genomes/refseq/bacteria/Ralstonia_solanacearum/latest_assembly_versions**

| Name | Last modified | Size |
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| GCF_000197855.1_ASM1../ | 03-Sep-2015 06:48 | - |
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| GCF_000215325.1_ASM2../ | 17-May-2016 21:15 | - |

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| GCA_000197855.1_ASM1../ | 13-Jun-2015 18:15 | - |
| GCA_000212635.2_ASM2../ | 17-May-2016 19:06 | - |

# GenBank vs RefSeq

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## GenBank

- part of International Nucleotide Sequence Database Collaboration (INSDC): EMBL/NCBI/DDBJ
- records 'owned' by submitter
- may include redundant information

## RefSeq

- not part of INSDC
- records derived from GenBank, 'owned' by NCBI
- stable non-redundant foundation for functional and diversity studies

# Ensembl

## http://www.ensembl.org

Automated annotation on selected genomes

- **Specialised sub-collections**
  - Ensembl Protists: http://protists.ensembl.org/
  - Ensembl Bacteria: http://bacteria.ensembl.org/
  - Ensembl Fungi: http://fungi.ensembl.org/
- **Downloadable resource**
  - e.g. ftp://ftp.ensemblgenomes.org/pub/protists/
- **Ready-made comparative genomics!**
  - *Phytophthora* genomics alignments (Avr3a)
  - Gene trees (Avr3a)

# Other Sources

- **Sequencing centres, e.g.**
  - JGI Genome Portals
  - Ensembl Bacteria: Broad Institute - now retiring their online resources
- **Specialist databases, e.g.**
  - FungiDB - fungi and oomycetes
  - CPGR - fungi and oomycetes (not recently updated)
- **Your friendly local sequencing centre!**
  - Aspera is commonly used to connect to your private data

# Optional Worksheet

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**`worksheets/01-downloading_data_biopython.ipynb`**

Downloading genome data from NCBI with Biopython

- MyBinder link

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# Why comparative genomics?

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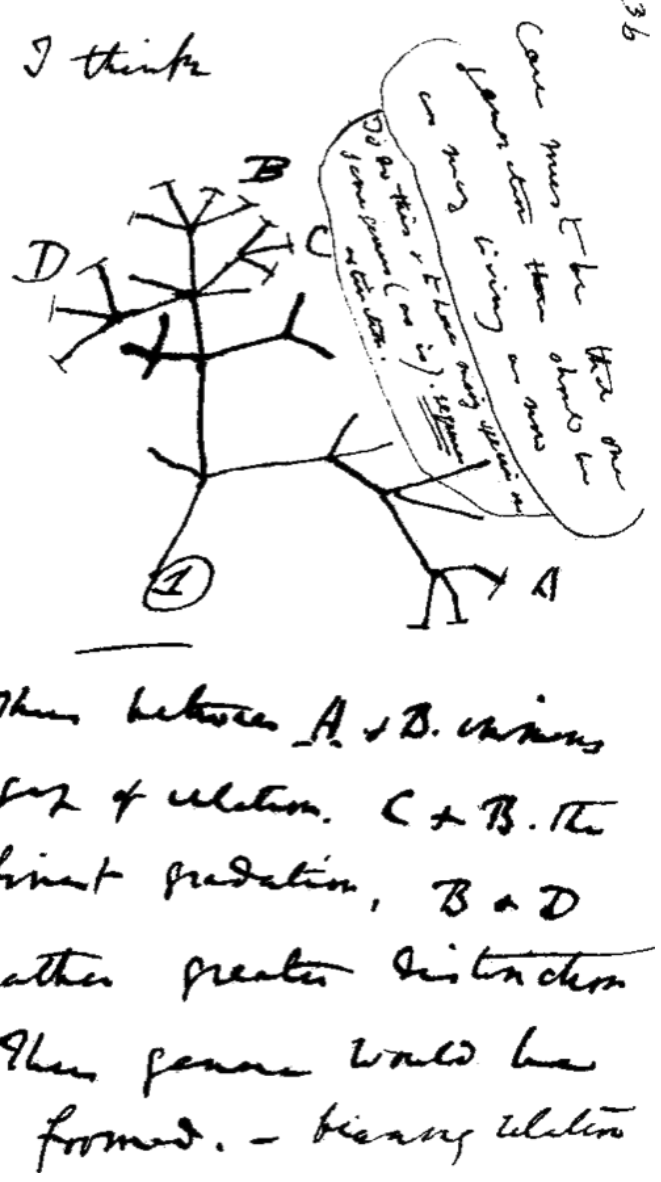
- Transfer functional information from model systems (*E. coli*, *A. thaliana*, *D. melanogaster*) to non-model systems
- Genome similarity $\propto$ phenotype? (*functional genomics*): virulence and host range
- Genome similarity $\propto$ relatedness? (*phylogenomics*): record of evolutionary processes and constraints



# Genomes aren't everything...

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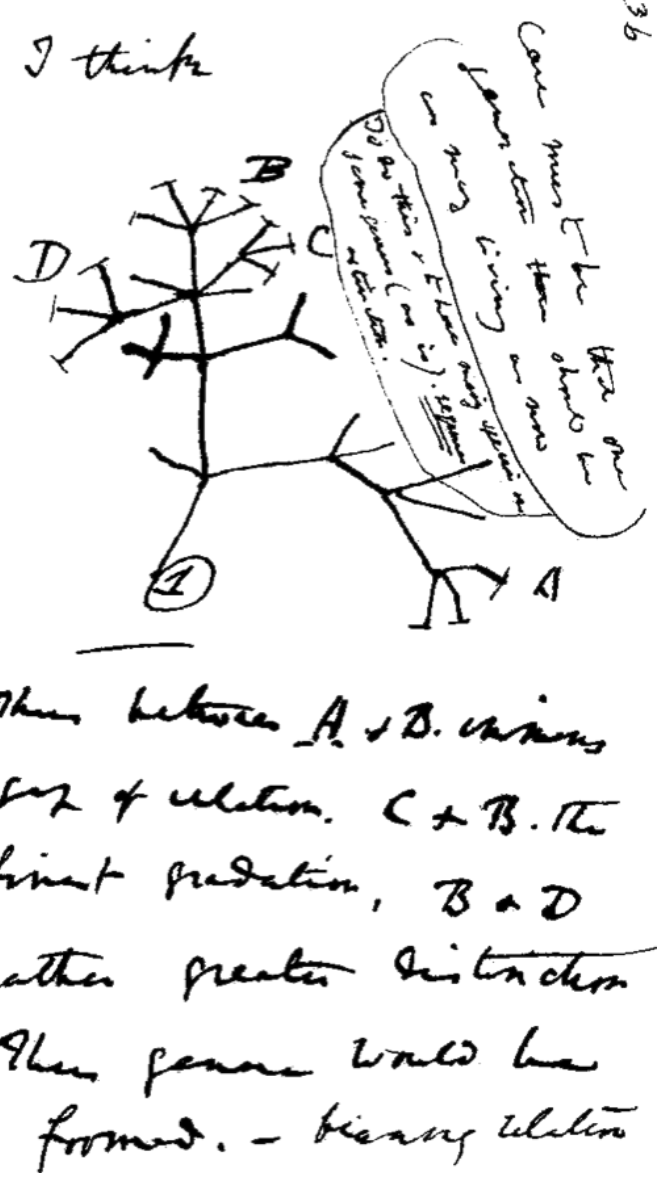
## Context

- epigenetics
- tissue differentiation/differential expression
- mesoscale systems, etc.

## Phenotypic plasticity, responses to

- temperature
- stress
- community, etc.

...and therefore systems biology...



# Levels of comparison

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## Bulk Properties

e.g. *k*-mer profiles (MaSH, MetaPalette, etc.)

## Whole Genome Sequence

- sequence similarity (BLAST, BLAT, MUMmer, etc.)
- structure and organisation (Mauve, ACT, etc.)

## Genome Features/Functional Components

- numbers and types of features: genes, ncRNA, regulatory elements, etc.
- organisation of features: synteny, operons, regulons, etc.
- functional complement (KEGG, etc.)

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# Whole genome comparisons

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**Whole genome comparison**

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

Minimum requirement: **two genomes**

- Reference Genome
- Comparator Genome

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

# Pairwise genome alignments

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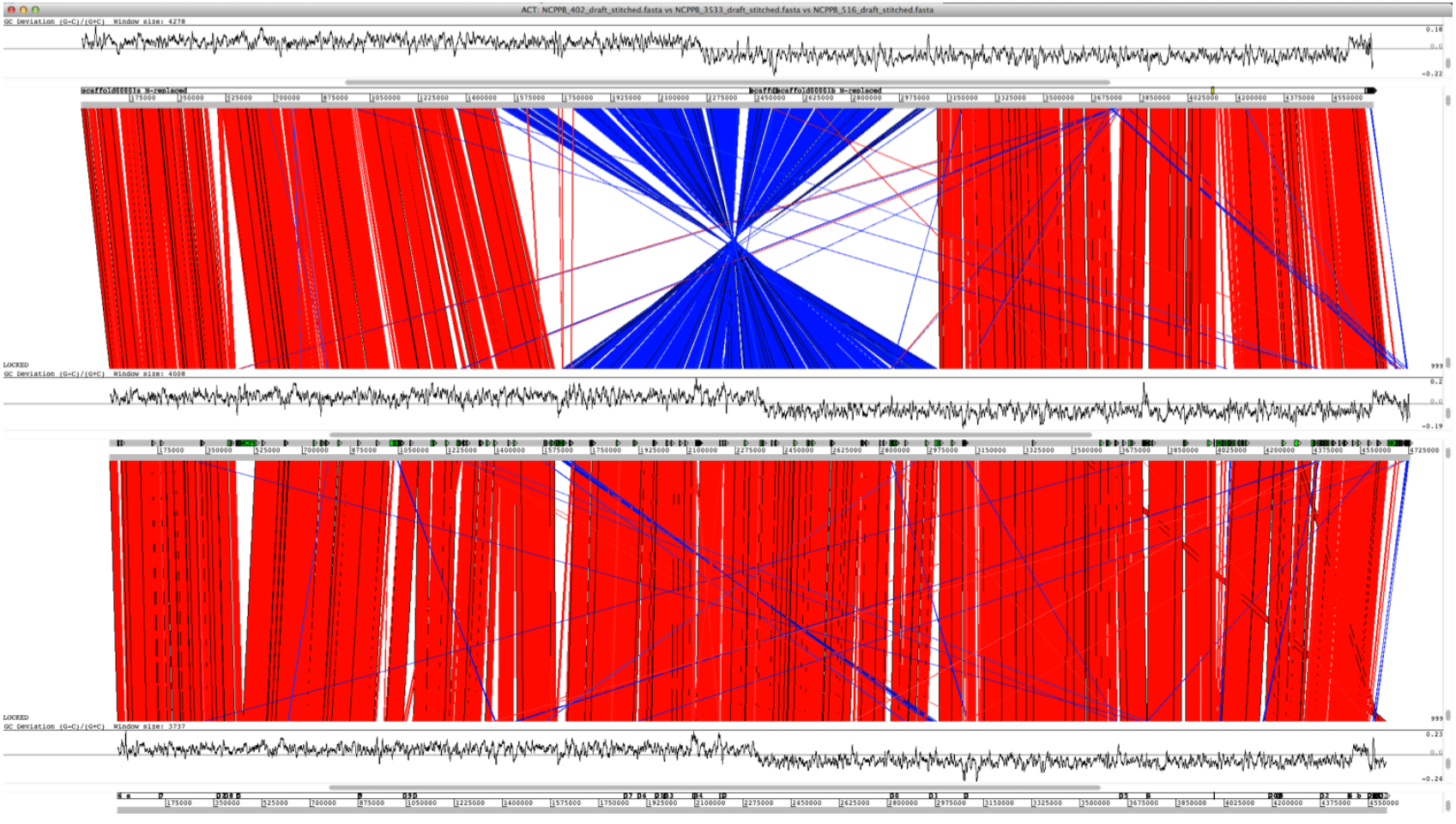
Pairwise comparisons produce alignments of similar regions.



# Synteny and Collinearity

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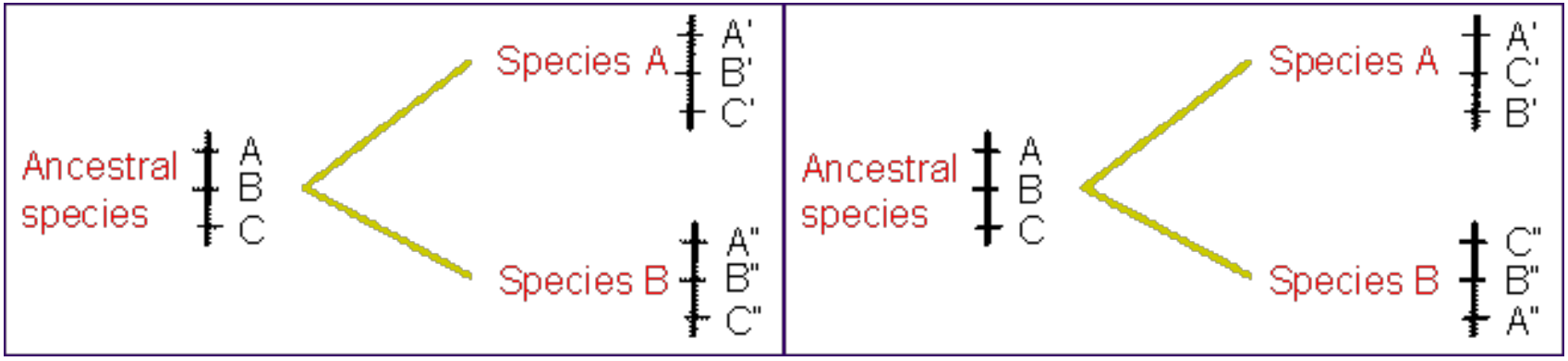
Genome rearrangements may occur post-species divergence

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 functional constraint (and help determine *orthology*)

# 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: environmental adaptation; C-I: virulence genes.
C-II has undergone extensive rearrangement; C-I has not.

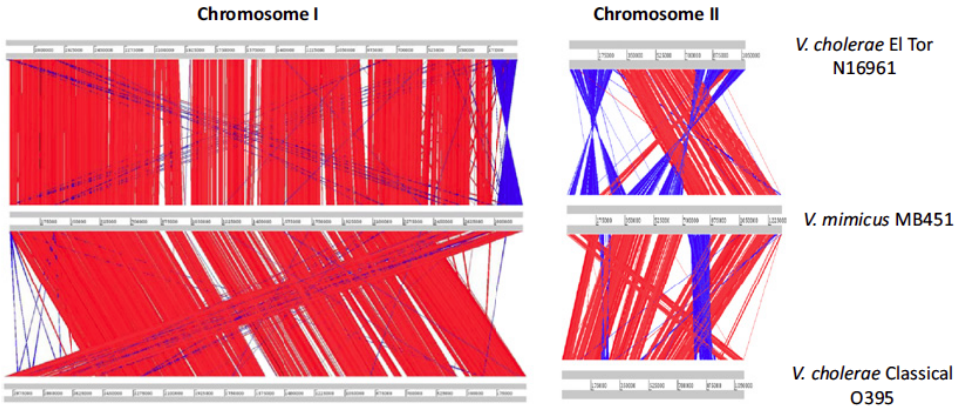



**Fig. 2.** Linear pairwise comparison of the *Vibrio mimicus* genome by Artemis Comparison Toll. Regions with similarity are highlighted by connecting red or blue lines between the genomes; red lines indicate homologous blocks of sequence, and blue lines indicate inversions. Gaps indicate unique DNA.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

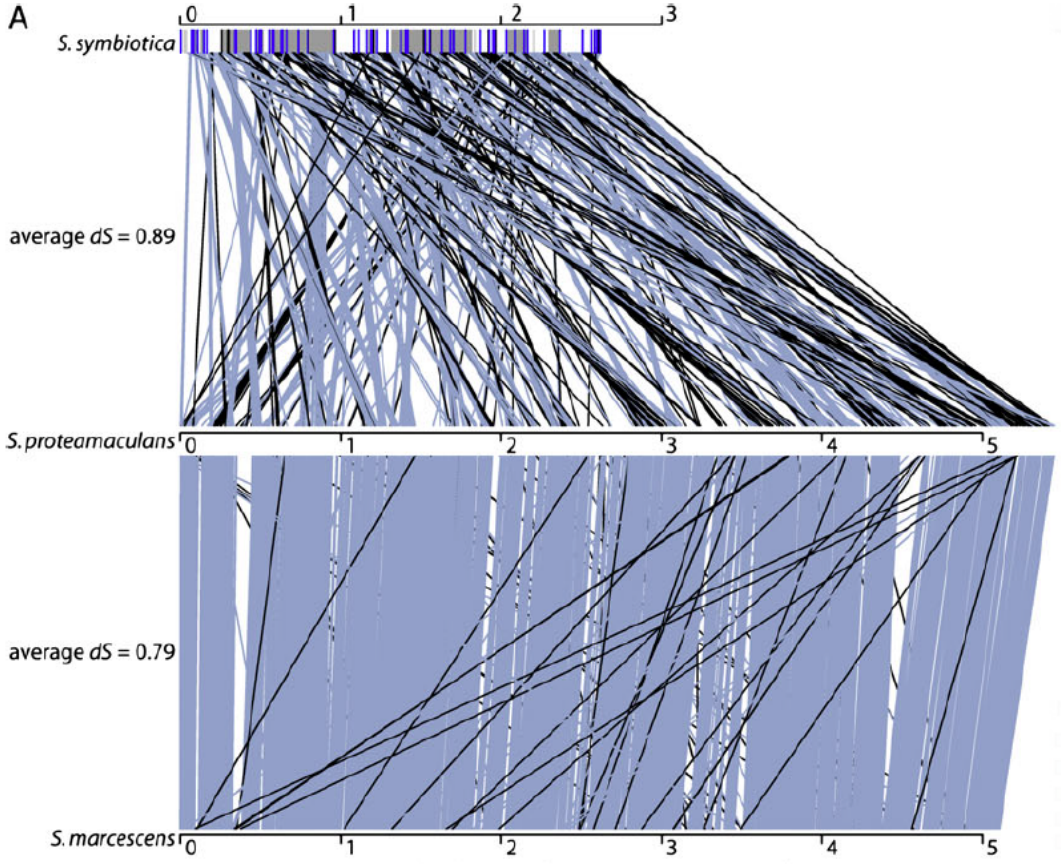
Suggests modularity of genome organisation, as a mechanism for adaptation (HGT, two-speed genome).

# 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

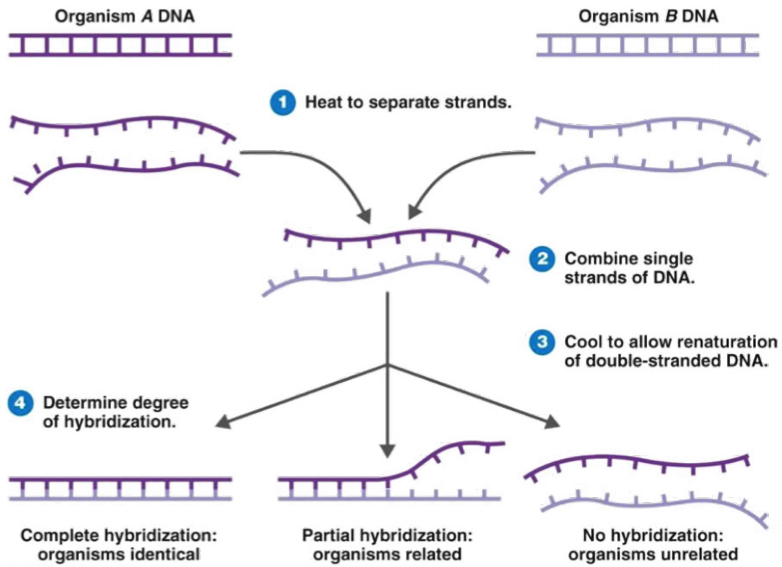
Massive genomic decay: consequence of adaptation



# DNA-DNA hybridisation[a]

[a] Morello-Mora and Amann (2001) *FEMS Micro. Rev.* doi:10.1016/S0168-6445(00)00040-1

- "Gold Standard" for prokaryotic taxonomy, since 1960s. "70% identity ≈ same species."
- Denature DNA from two organisms.
- Allow to anneal. Reassociation ≈ similarity, measured as $\Delta T$ of denaturation curves.




Proxy for sequence similarity - replace with genome analysis[1]?

[1] Chan *et al* (2012) *BMC Microbiol.* doi:10.1186/1471-2180-12-302

# Average Nucleotide Identity (ANIm)[a]

[a] Richter and Rossello-Mora (2009) *Proc. Natl. Acad. Sci. USA* doi:10.1073/pnas.0906412106

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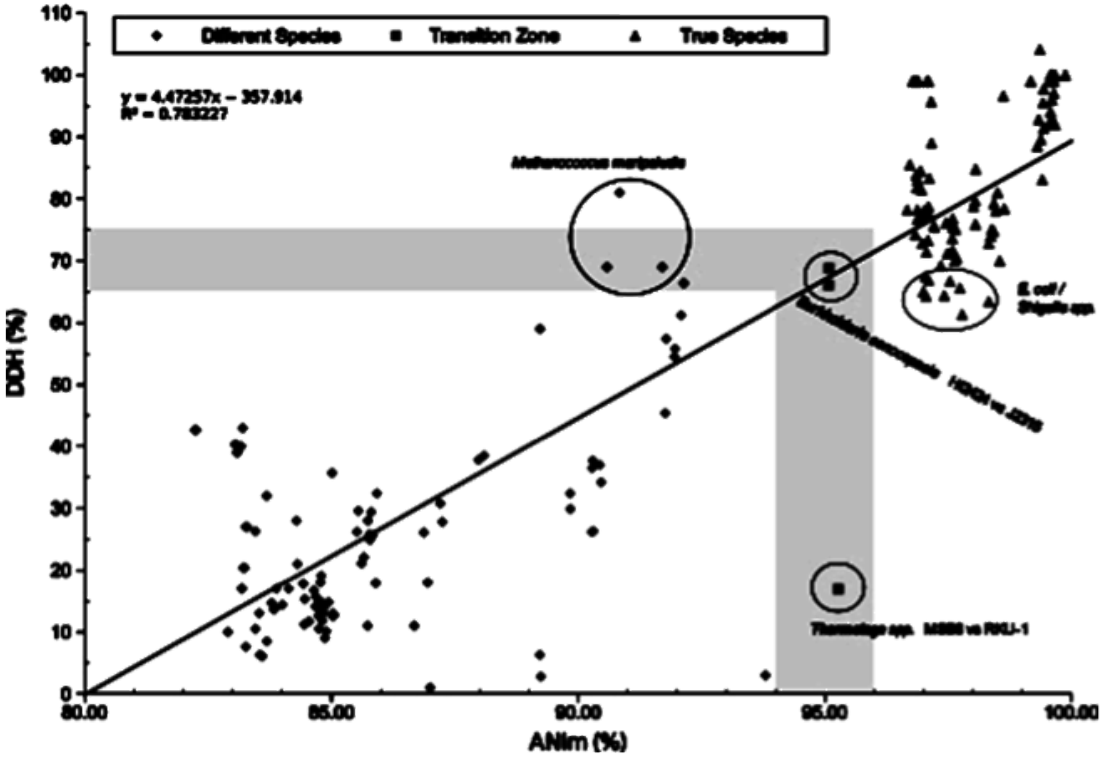
1. Align genomes (MUMmer)
2. **ANIm**: Mean % identity of all matches

- DDH:ANIm linear
- 70%ID ≈ 95%ANIb



# ANI

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## Advantages

- Average identity of all 'homologous' regions
- Approximates limiting case of MLST/MLSA/multigene comparisons
- Classification not dependent on dataset composition (unlike tree methods)

## Criticisms

- 95% threshold 'arbitrary'
- Taxonomic classification, not phylogenetic reconstruction
- No functional (or gene-based) interpretation; still need pangenome classification and analysis

# EXERCISE

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`exercises/01-whole_genome_comparisons.ipynb`

- Pairwise comparison of *Pseudomonas* genomes
- ANIm classification of *Pseudomonas* isolates

- MyBinder link

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