Pathogen Genome Data

EMBL-EBI Bioinformatics of Plants and Plant Pathogens 23rd May 2016

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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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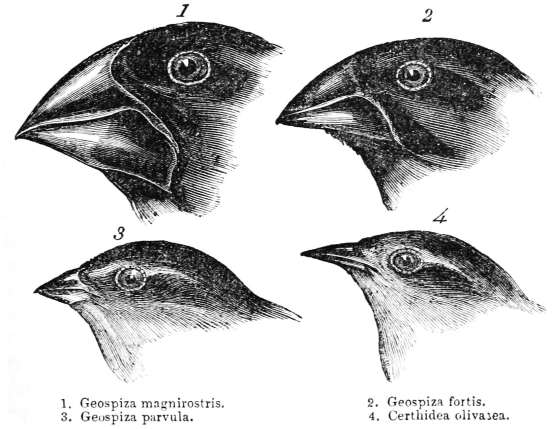
What can pathogen genome data do for you?

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

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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
GCF_000009125.1_ASM9../	17-May-2016 16:30	-
GCF_000197855.1_ASM1../	03-Sep-2015 06:48	-
GCF_000212635.3_ASM2../	17-May-2016 19:06	-
GCF_000215325.1_ASM2../	17-May-2016 21:15	-

Index of /genomes/genbank/bacteria/Ralstonia_solanacearum/latest_assembly_versions

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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 resources**
  - e.g. ftp://ftp.ensemblgenomes.org/pub/protists/
- **Ready-made comparative genomics!**
  - *Phytophthora* genomics alignments (Avr3a)
  - Gene trees (Avr3a)

Other Sources

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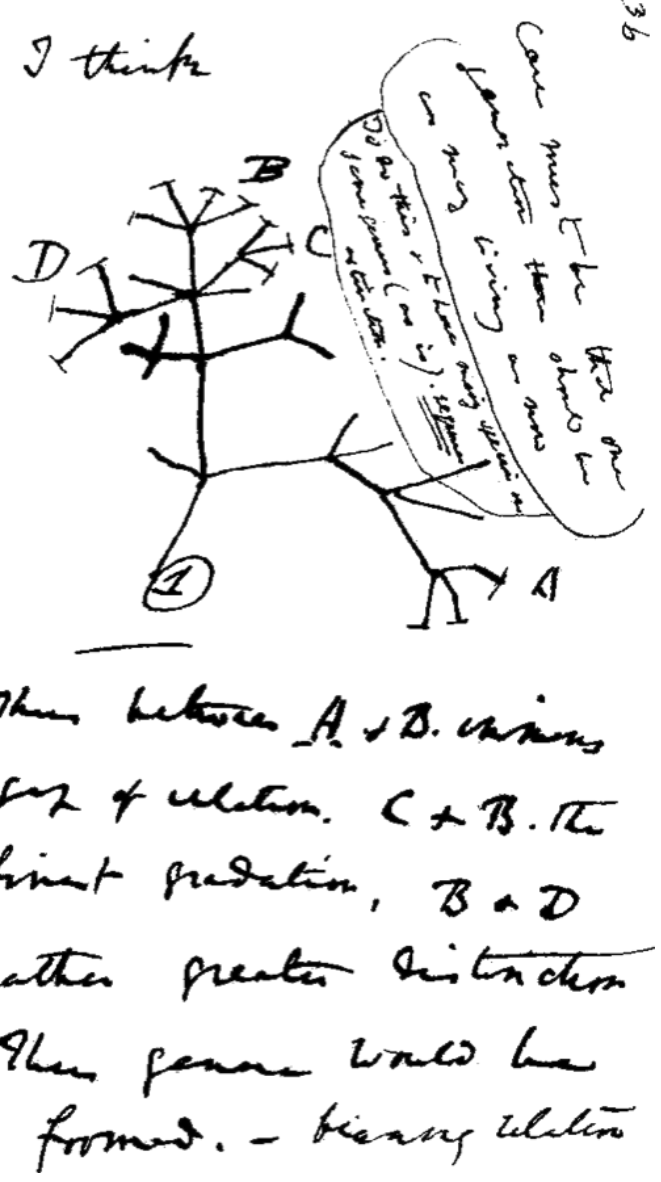
- Transfer functional information from model systems (*E. coli*, *A. thaliana*, *D. melanogaster*) to non-model systems
- Genome similarity $\propto$ phenotype? (*functional genomics*): virulence and host range
- Genome similarity $\propto$ relatedness? (*phylogenomics*): record of evolutionary processes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



Genomes aren't everything...

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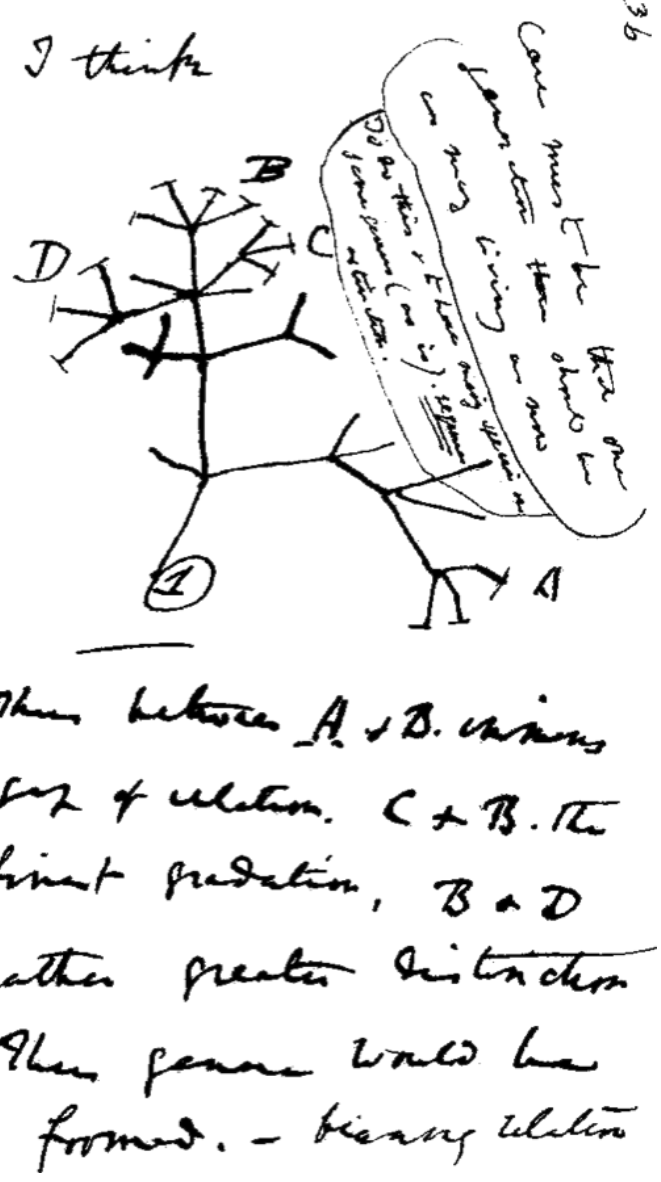
Context

- 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 spectra (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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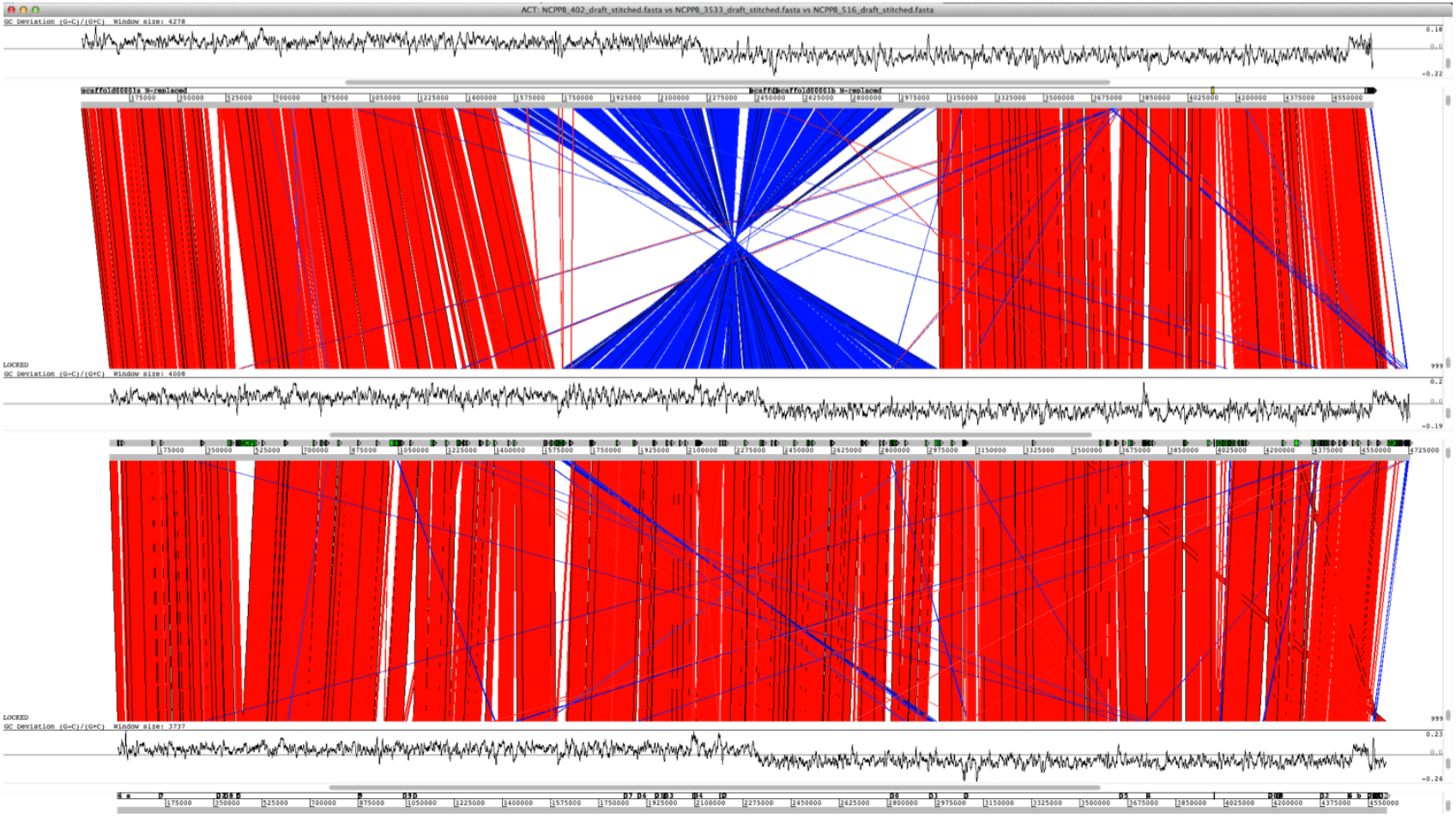
Pairwise comparisons produce alignments of similar regions.



Synteny and Collinearity

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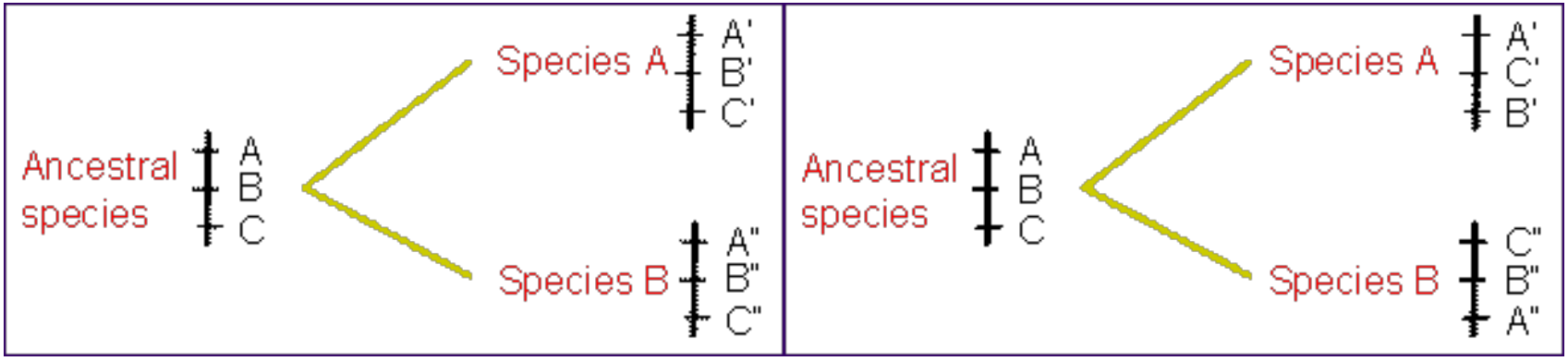
Genome rearrangements may occur post-species divergence

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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Chromosomes

- C-I: virulence genes.
- C-II: environmental adaptation

C-II has undergone extensive rearrangement; C-I has not.

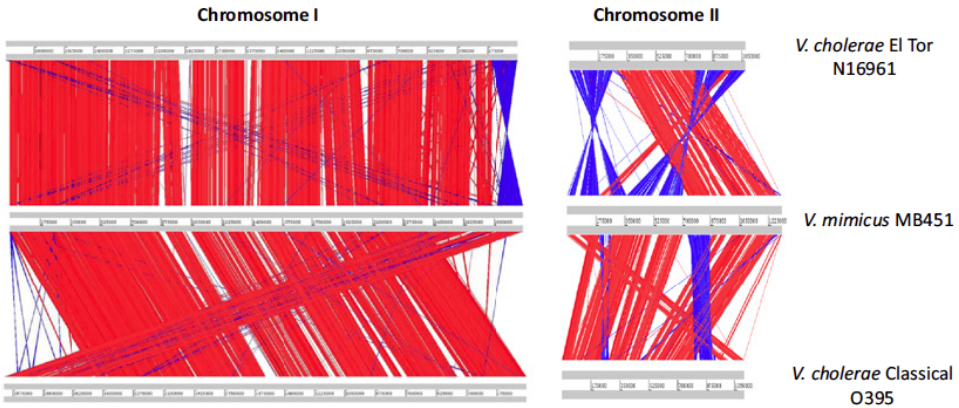


Fig. 2. Linear pairwise comparison of the *Vibrio mimicus* genome by Artemis Comparison Toll. Regions with similarity are highlighted by connecting red or blue lines between the genomes; red lines indicate homologous blocks of sequence, and blue lines indicate inversions. Gaps indicate unique DNA. The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

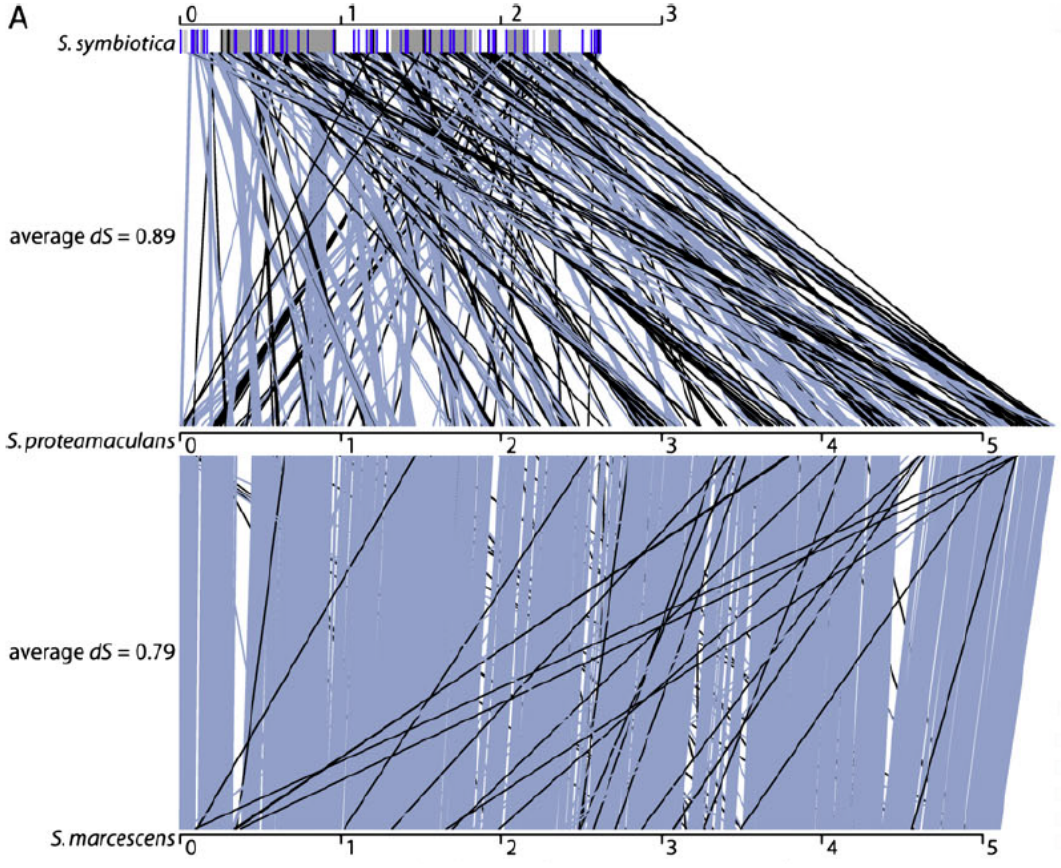
Suggests modularity of genome organisation, as a mechanism for adaptation (HGT, two-speed genome).

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



Whole genome classification [a] [b]

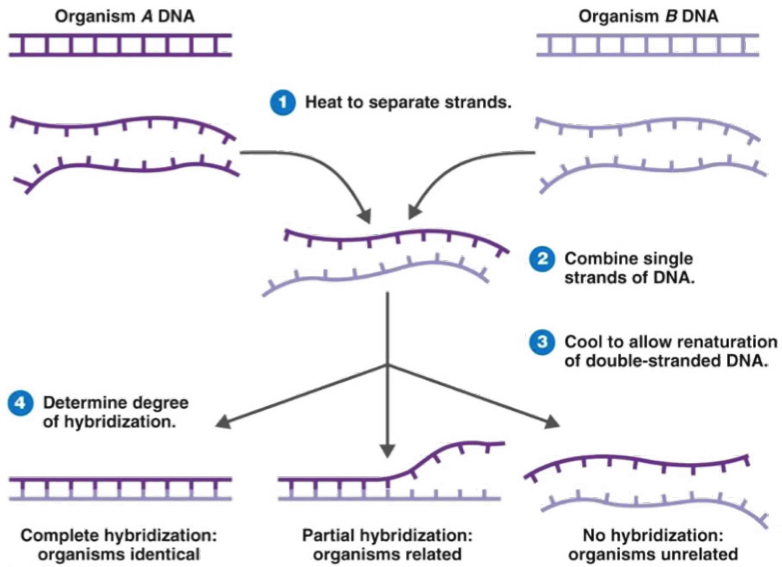
[a] Baltrus (2016) *Trends Microbiol.* doi:10.1016/j.tim.2016.02.004
[b] Pritchard *et al.* (2016) *Anal. Methods* doi:10.1039/c5ay02550h

- Widespread confusion about strain classification and nomenclature
  - Taxonomies contradicted by bioinformatic classification
  - Databases populated by non-taxonomists
- Philosophy and practice of taxonomy are in conflict
- Classification can be independent of existing nomenclature
  - The route from genotype to phenotype is complicated
  - Time to abandon traditional microbial species concepts?
- An unambiguous sequence-based classification scheme is possible

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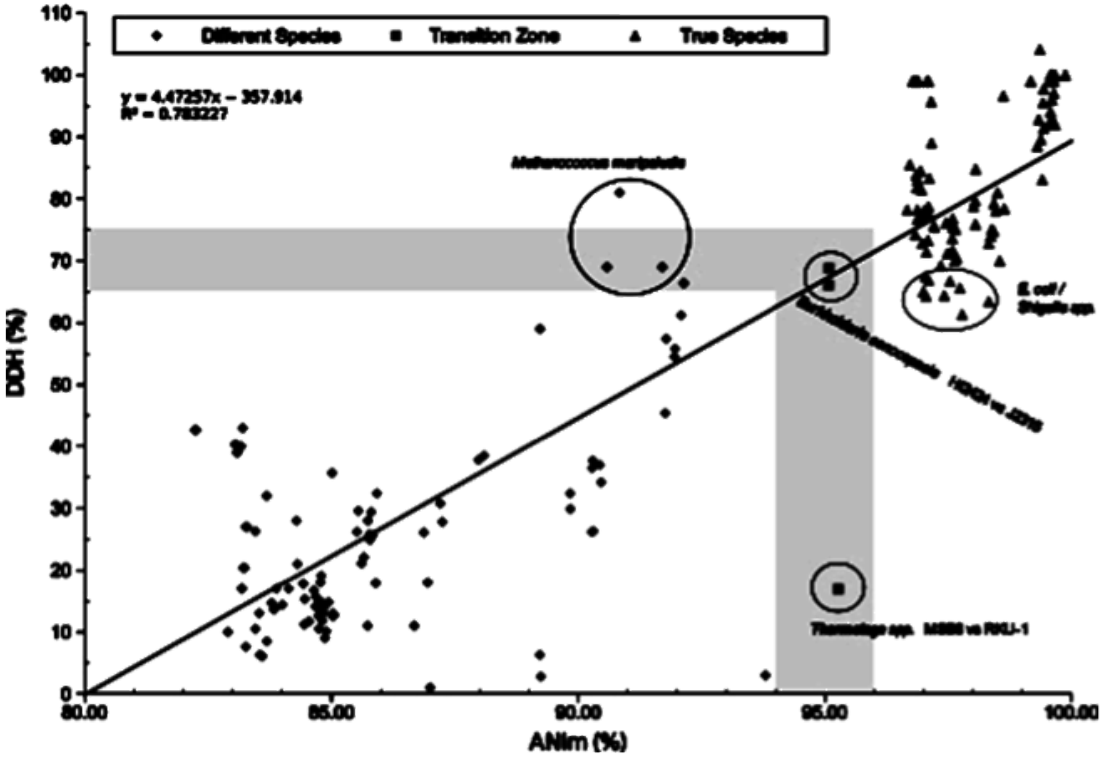
1. Align genomes (MUMmer)
2. **ANIm**: Mean % identity of all matches

- DDH:ANIm linear
- 70%ID ≈ 95%ANIb



55 Pectobacterium spp. ANIm [a]

[a] Pritchard *et al.* (2016) *Anal. Methods* doi:10.1039/c5ay02550h

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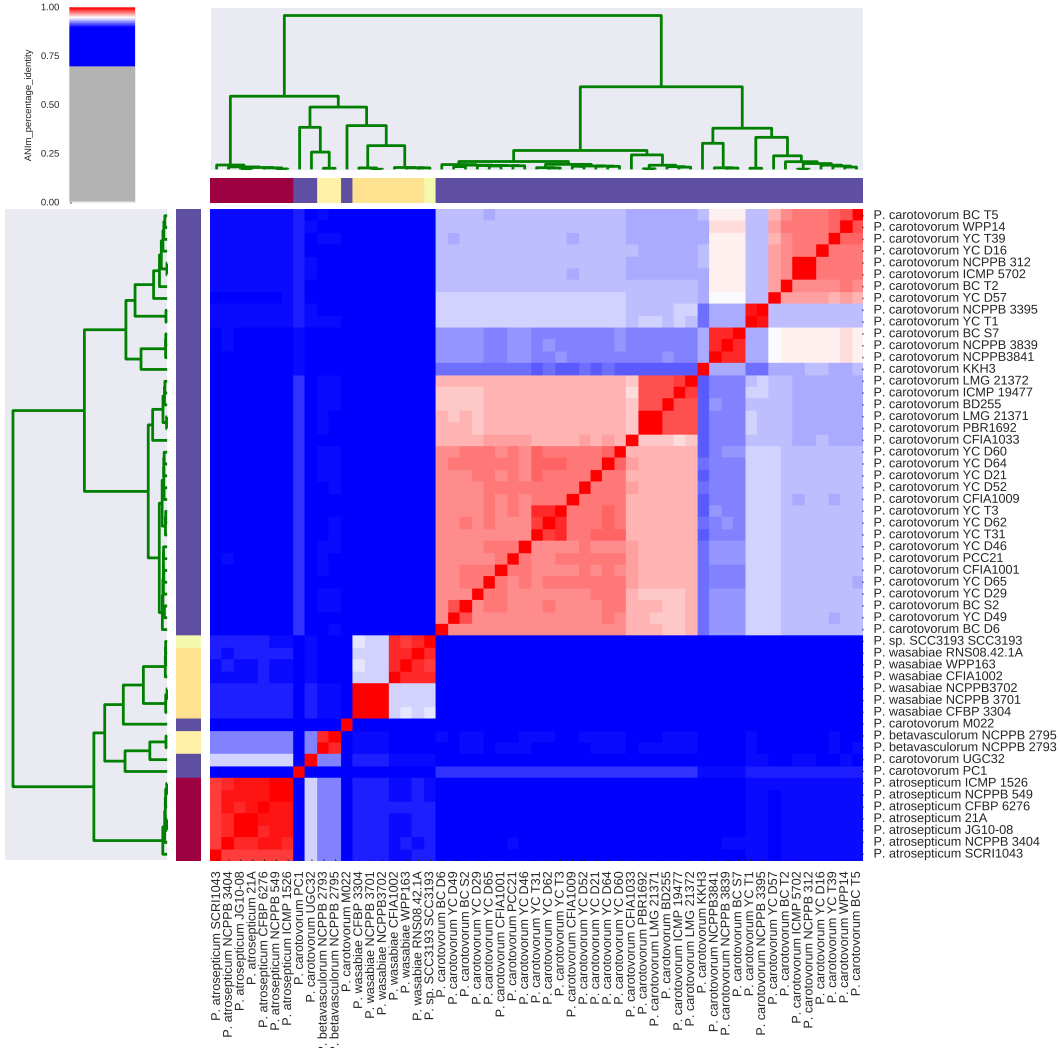
- Ten species-level groups (four novel)
- *P. carotovorum* split: several species
- *P. wasabiae* split: two species



55 Pectobacterium spp. ANIm[a]

[a] Pritchard *et al.* (2016) *Anal. Methods* doi:10.1039/c5ay02550h

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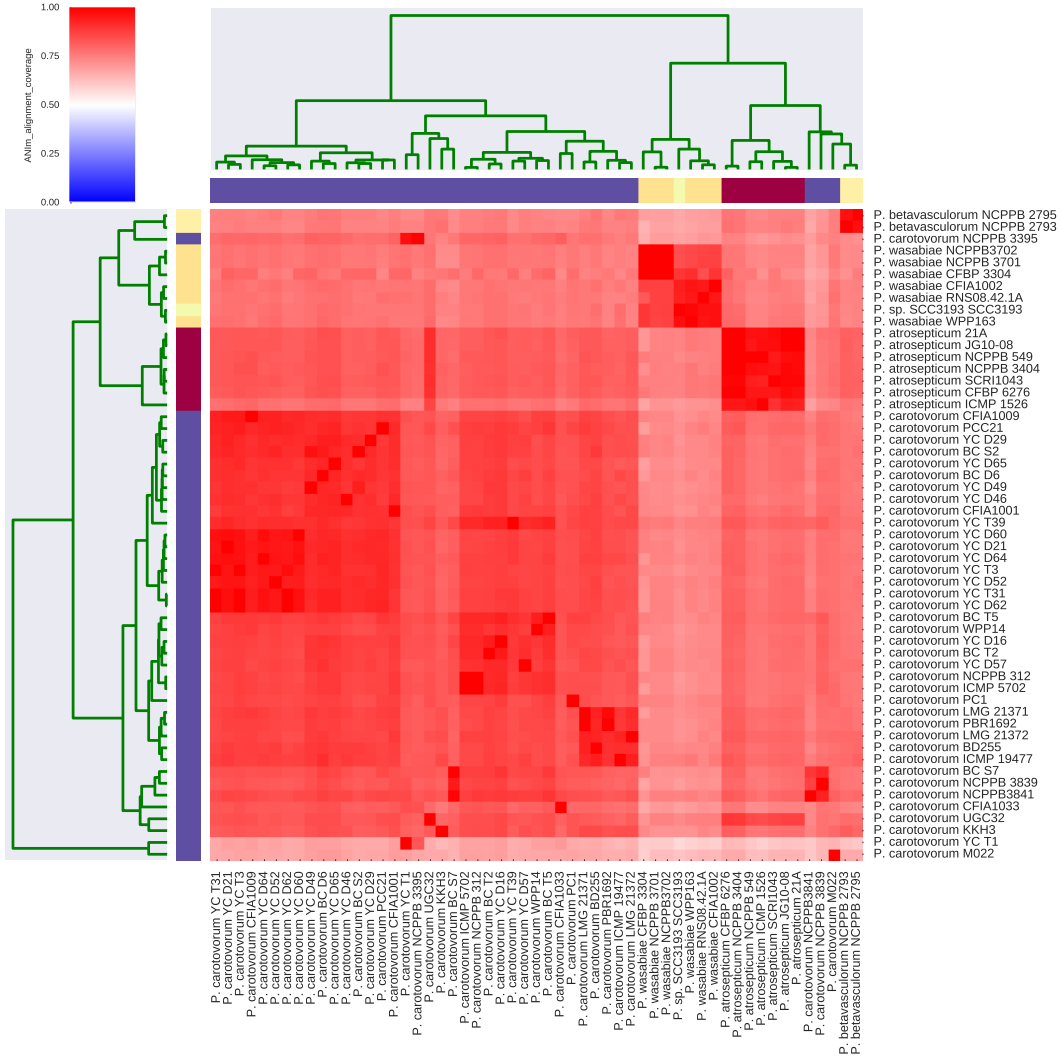
- All isolates align over >50% of whole genome



ANI

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Advantages

- Average identity of all ‘homologous’ regions
- Approximates limiting case of MLST/MLSA/multigene comparisons
- Adaptable to variable thresholding (LINS) classifications

Criticisms

- Thresholds ‘arbitrary’, based on homologous regions only
- 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

Chromosome painting [a]

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

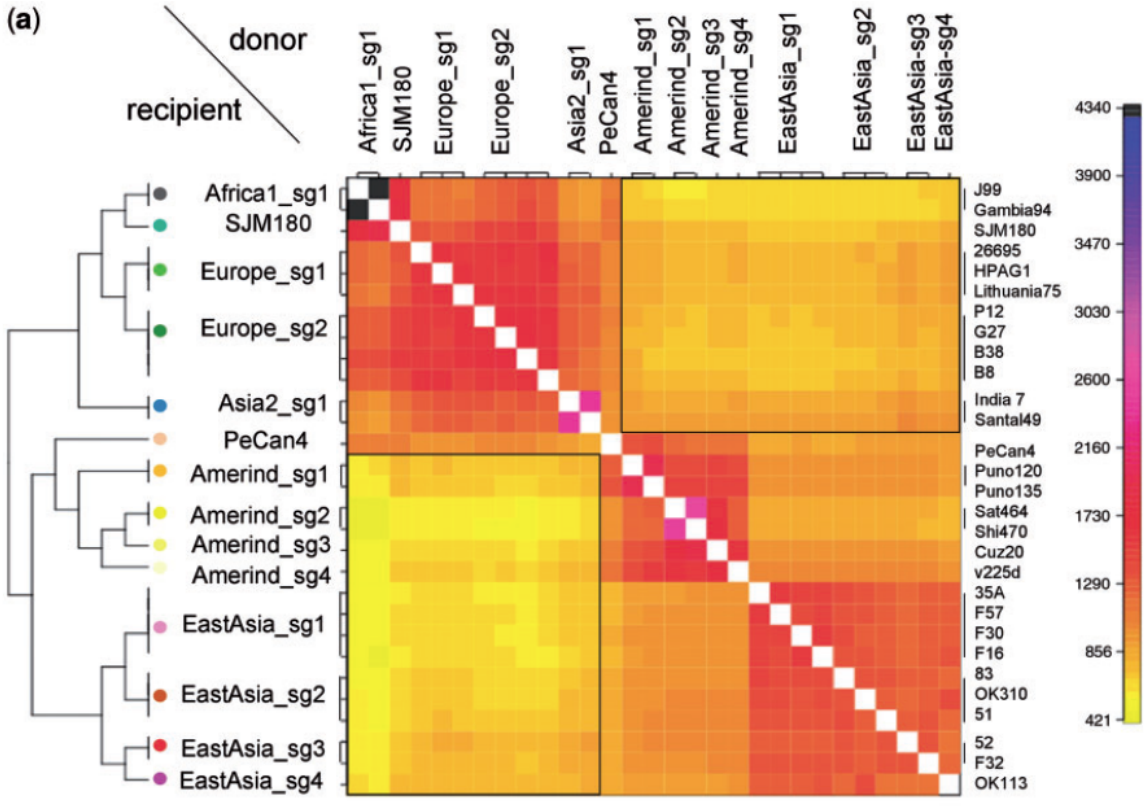
- "Chromosome painting" (FINESTRUCTURE) infers recombination-derived 'chunks'
- Genome's haplotype constructed in terms of recombination events from a 'donor' to a 'recipient' genome

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.



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Feature comparisons

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Feature comparisons

Comparisons of the annotated features of one genome with another (. . .or many others)

- gene features
- RNA features
- regulatory features

Equivalent features

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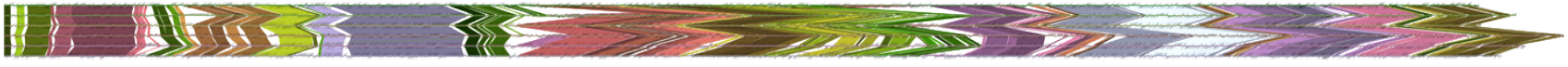
The power of genomics is comparative genomics!

- 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



Orthologues [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

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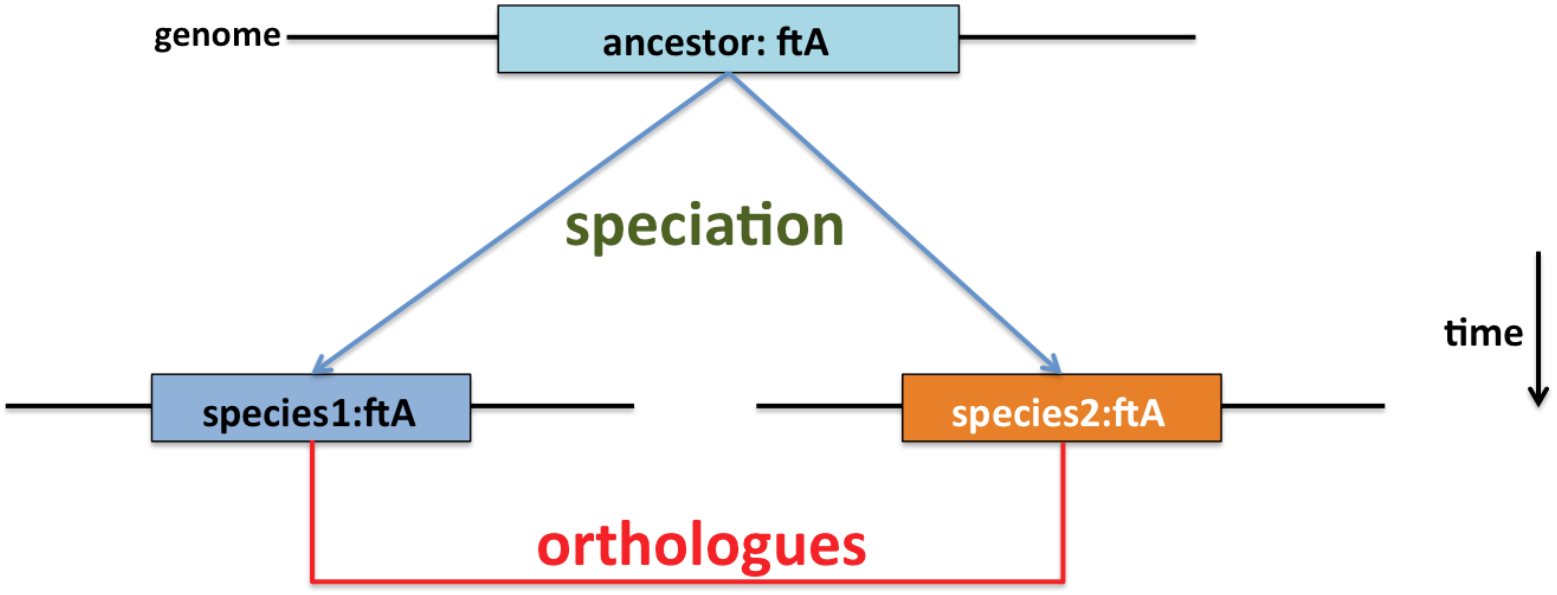
Orthologs/Orthologues

"Homologs that diverged through speciation" (orig.)
"Genes/products we think are probably the same thing" (mod. inform.)



Why orthologues? [a] [b] [c]

[a] Chen and Zhang (2012) *PLoS Comp. Biol.* doi:10.1371/journal.pcbi.1002784

[b] Dessimoz (2011) *Brief. Bioinf.* doi:10.1093/bib/bbr057

[c] Altenhoff and Dessimoz (2009) *PLoS Comp. Biol.* **5**:e1000262 doi:10.1371/journal.pcbi.1000262

- Formalise the idea of *corresponding genes* in different organisms.
- Suggest two relationships:
  - **Evolutionary equivalence**
  - **Functional equivalence** ("The Ortholog Conjecture")

The Ortholog Conjecture

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.

Finding orthologues [a]

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

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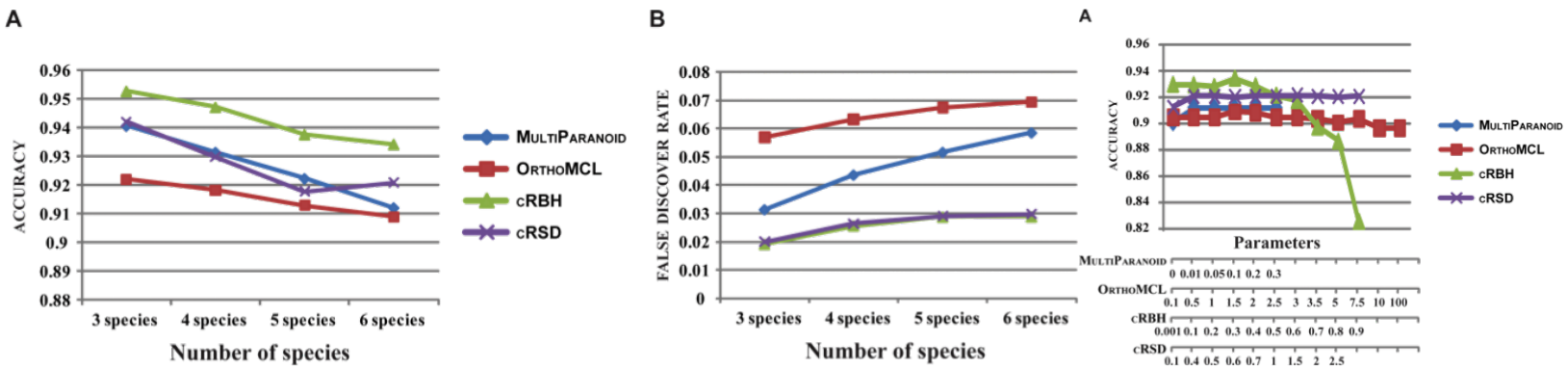
Which discovery method performs best?

- 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.



EXERCISE

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`exercises/02-cds_feature_comparisons.ipynb`

- RBBH analysis of *Pseudomonas* CDS feature annotations

- MyBinder link

One-way BLAST vs RBBH

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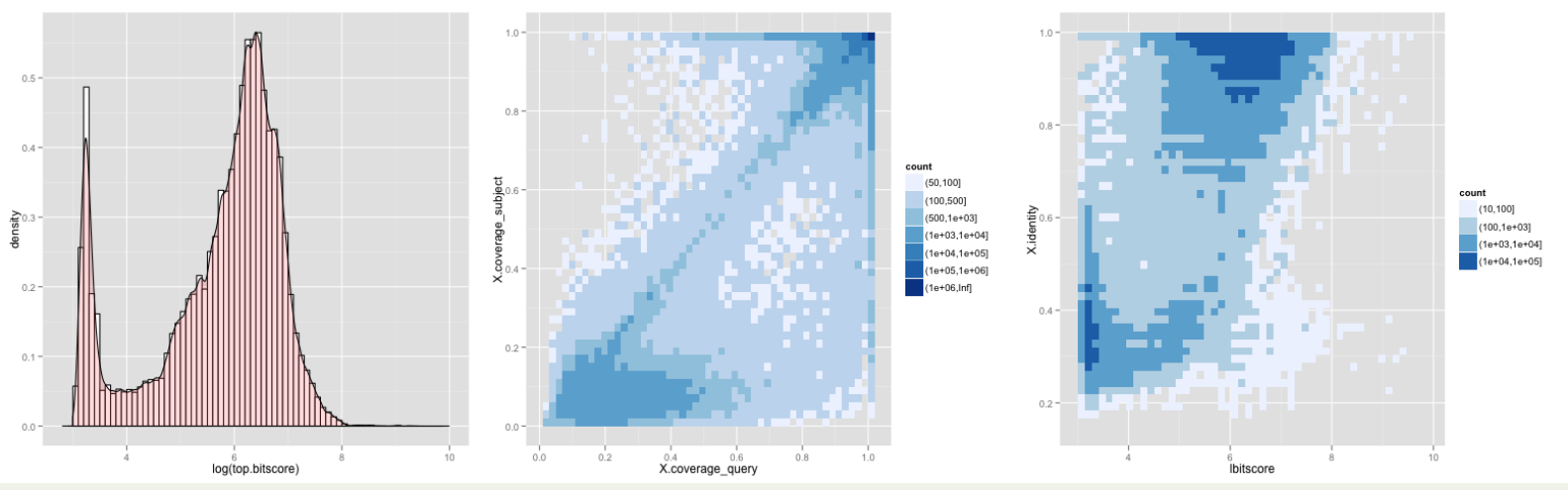
One-way BLAST includes many low-quality hits



One-way BLAST vs RBBH

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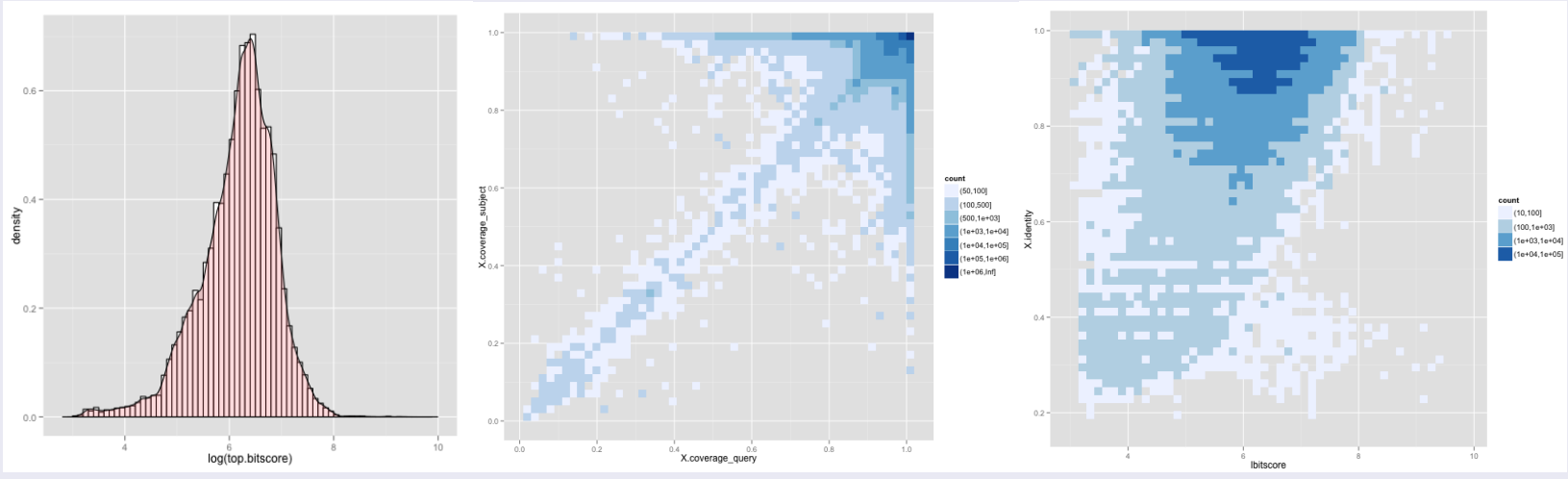
Reciprocal best BLAST removes many low-quality matches



The Pangenome

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The Core Genome Hypothesis

"The *core genome* is the primary cohesive unit defining a bacterial species"

- Once equivalent genes have been identified, those present in all related isolates can be identified: **the core genome**.
- The remaining genes are **the accessory genome**, and are expected to mediate function that distinguishes between isolates.

`Roary`: Rapid large-scale prokaryote pan-genome analysis - works on a desktop machine.

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

Accessory genomes

A cradle for adaptive evolution, particularly for bacterial pathogens, such as *Pseudomonas* spp.

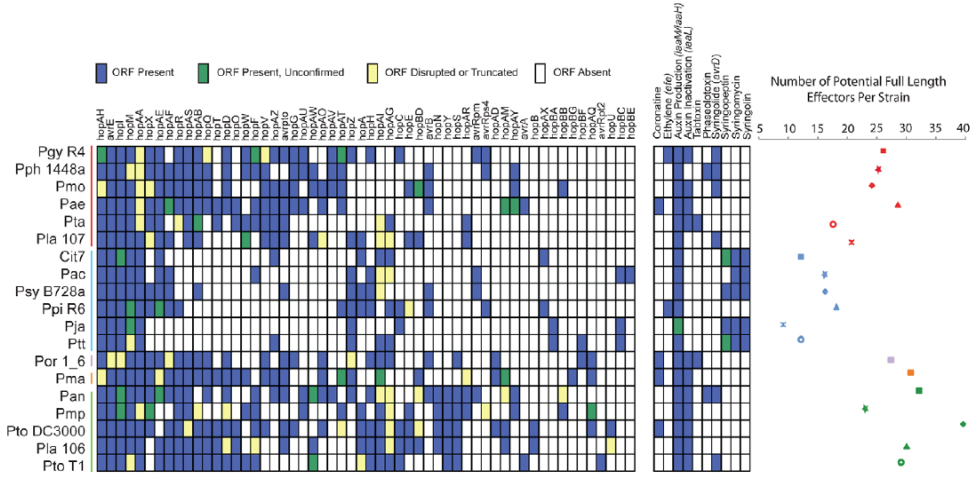

Figure 3. ***P. syringae*** **isolates harbor extensive diversity in virulence gene repertoires.** TTE, toxin, and plant hormone biosynthesis genes are listed across the top, *P. syringae* genomes, color-coded by phylogenetic group as in Figure 1. At the left, a blue box indicates presence of full-length 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

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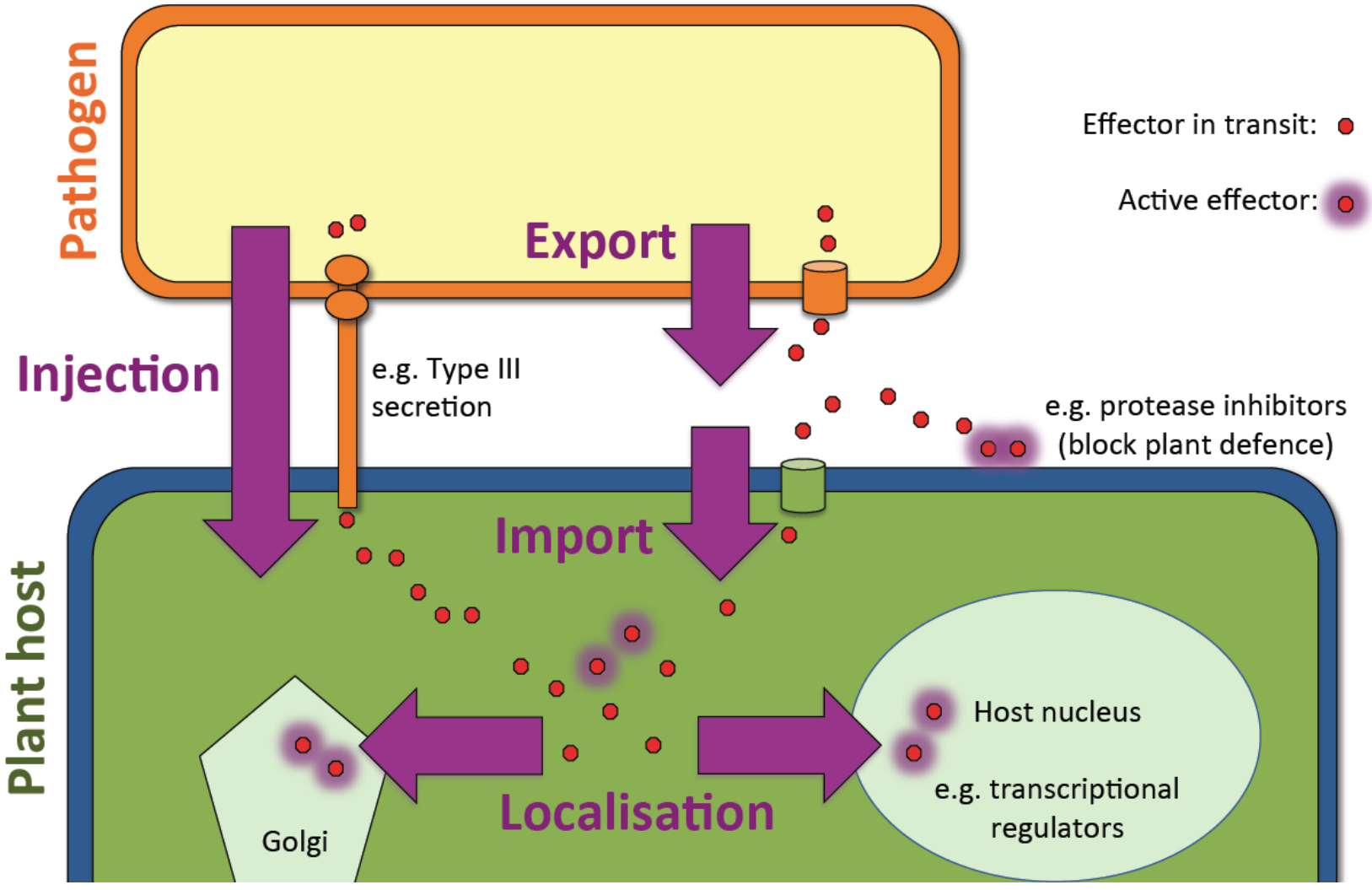
What is an effector?



What is an effector?

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Effector

A molecule produced by pathogen that (directly?) modifies host molecular/biochemical 'behaviour'

- Inhibits enzyme action (e.g. *Cladosporium fulvum* AVR2, AVR4; *Phytophthora infestans* EPIC1, EPIC2B; *P. sojae* glucanase inhibitors)
- Cleaves a protein target (e.g. *Pseudomonas syringae* AvrRpt2)
- (De-)phosphorylates a protein target (e.g. *P. syringae* AvrRPM1, AvrB)
- Retargeting host system such as E3 ligase (e.g. *P. syringae* AvrPtoB; *P. infestans* Avr3a)
- Regulatory control (e.g. *Xanthomonas campestris* AvrBs3)

What is an effector?

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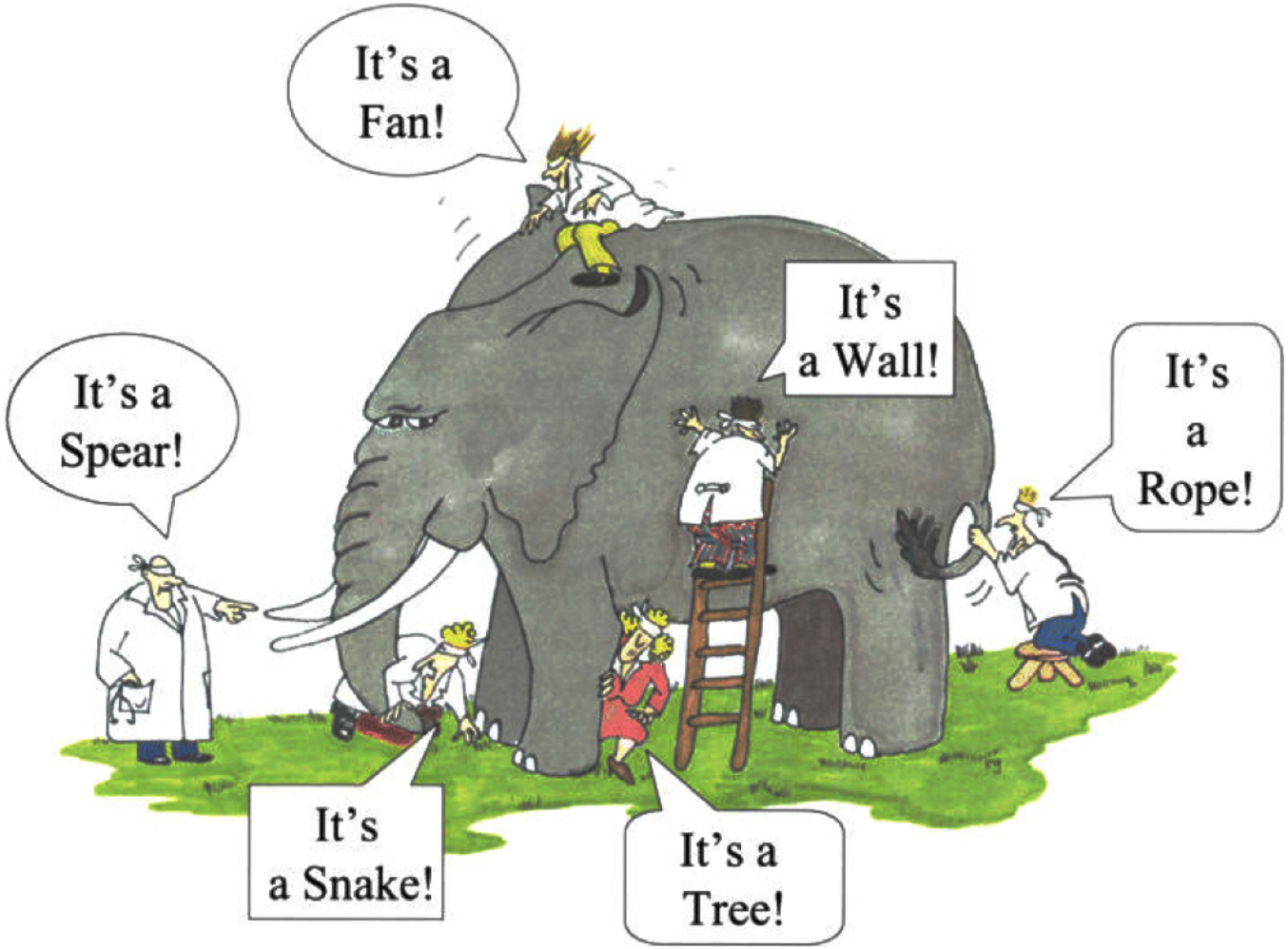
No unifying biochemical mechanism

No single test for 'candidate effectors', even in one organism



Effectors are modular [a] [b]

[a] Greenberg & Vinatzer (2003) *Curr. Opin. Microbiol.* doi:10.1016/S1369-5274(02)00004-8
[b] Collmer *et al.* (2002) *Trends Microbiol.* doi:10.1016/S0966-842X(02)02451-4

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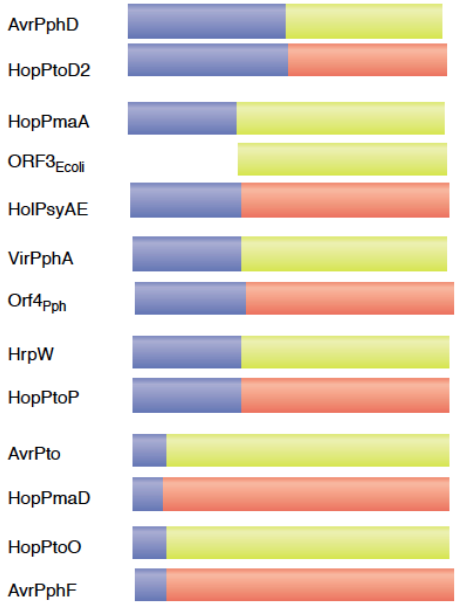
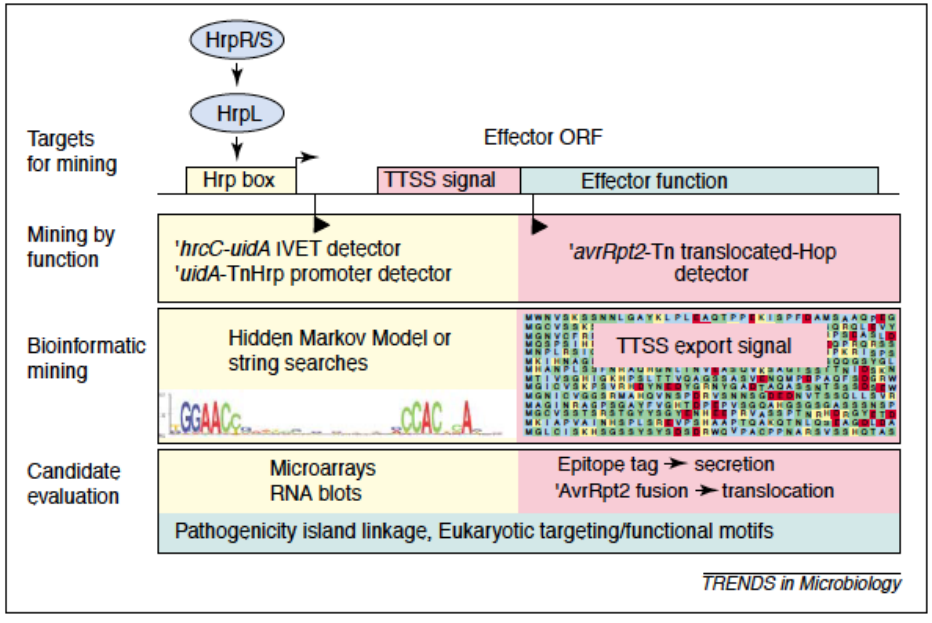
Delivery

N-terminal localisation/translocation domain

Activity

C-terminal functional/interaction domain





Effectors are modular [a] [b]

[a] Dong *et al.* (2011) *PLoS One* doi:10.1371/journal.pone.0020172.t004

[b] Boch *et al.* (2009) *Science* doi:10.1126/science.1178811

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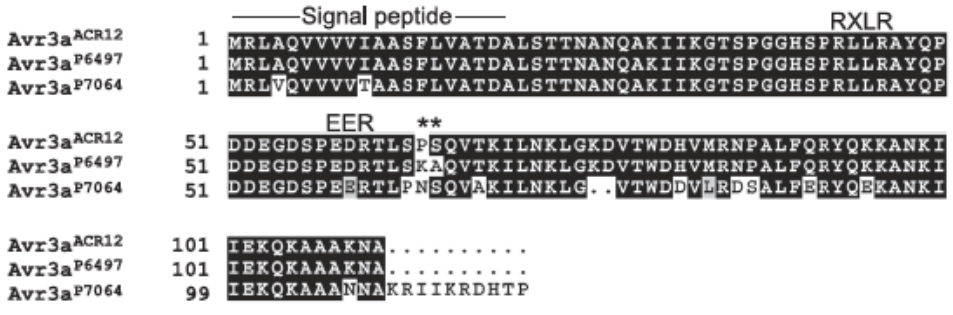
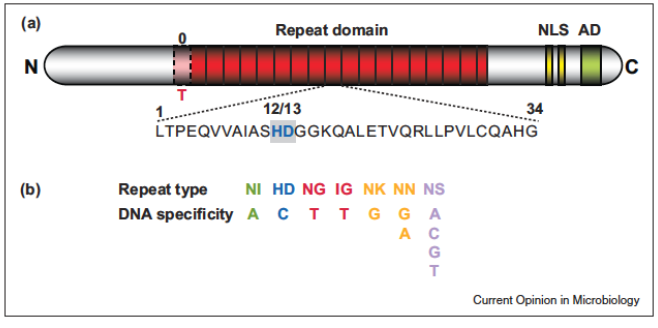
Delivery

Typically common to effector class: RxLR, T3E, CHxC

Activity

May be common (TAL) or divergent within effector class (RxLR, T3E)





Effector prediction tools (online) [a] [b]

[a] Sperschneider *et al.* (2015) *PLoS Pathogens* doi:10.1371/journal.ppat.1004806
[b] Sonah *et al.* (2016) *Front. Plant Sci.* doi:10.3389/fpls.2016.00126

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Bacterial Type III Effectors

- EffectiveT3
- modlab
- T3SEdb

Fungal/Oomycete Effectors

- EffectorP
- Galaxy Toolshed RxLR predictor

What do we look for? [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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What if someone hasn't built a classifier for your protein family?

- Tests are for protein family membership and/or 'effector-like' functional signal
- The same as any sequence classification problem (functional annotation)
- Many possible approaches
- (Supervised) machine learning problem:
  - train
  - test
  - validate

Sequence space [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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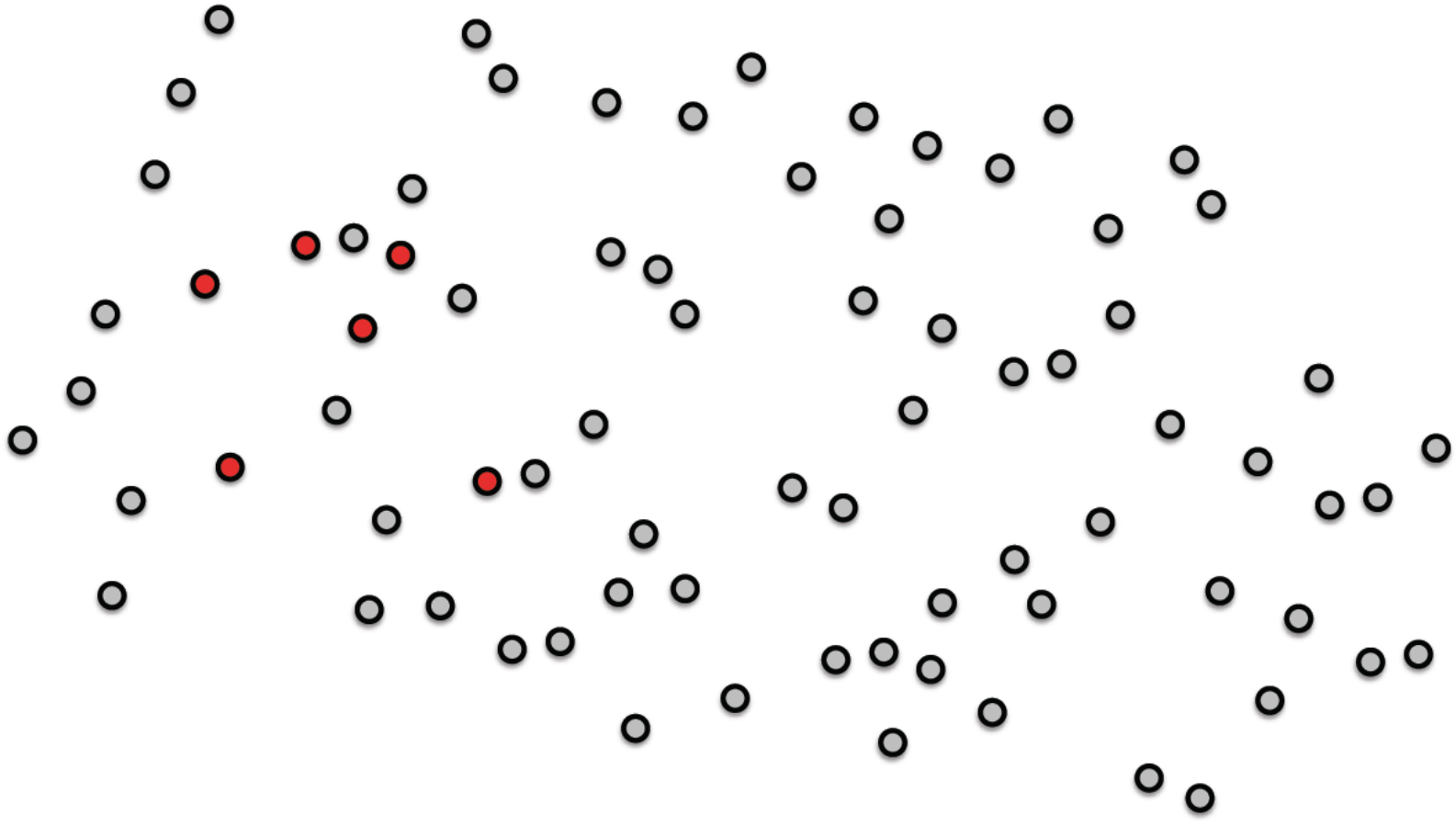
Known members of our effector class are in red



Similarity distance [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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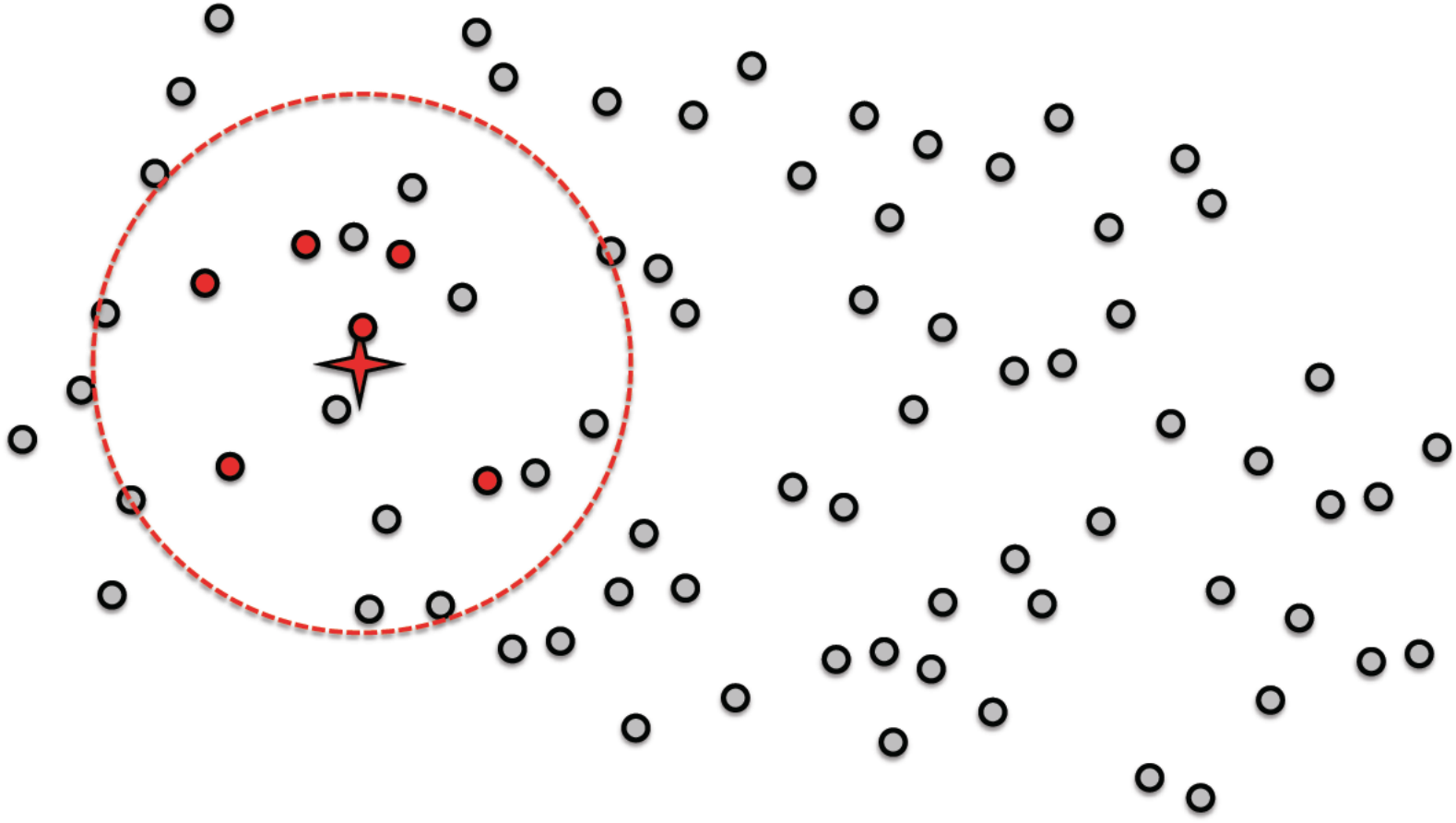
Define a representative *centre*, and a distance from it that includes known effectors



Classify candidates [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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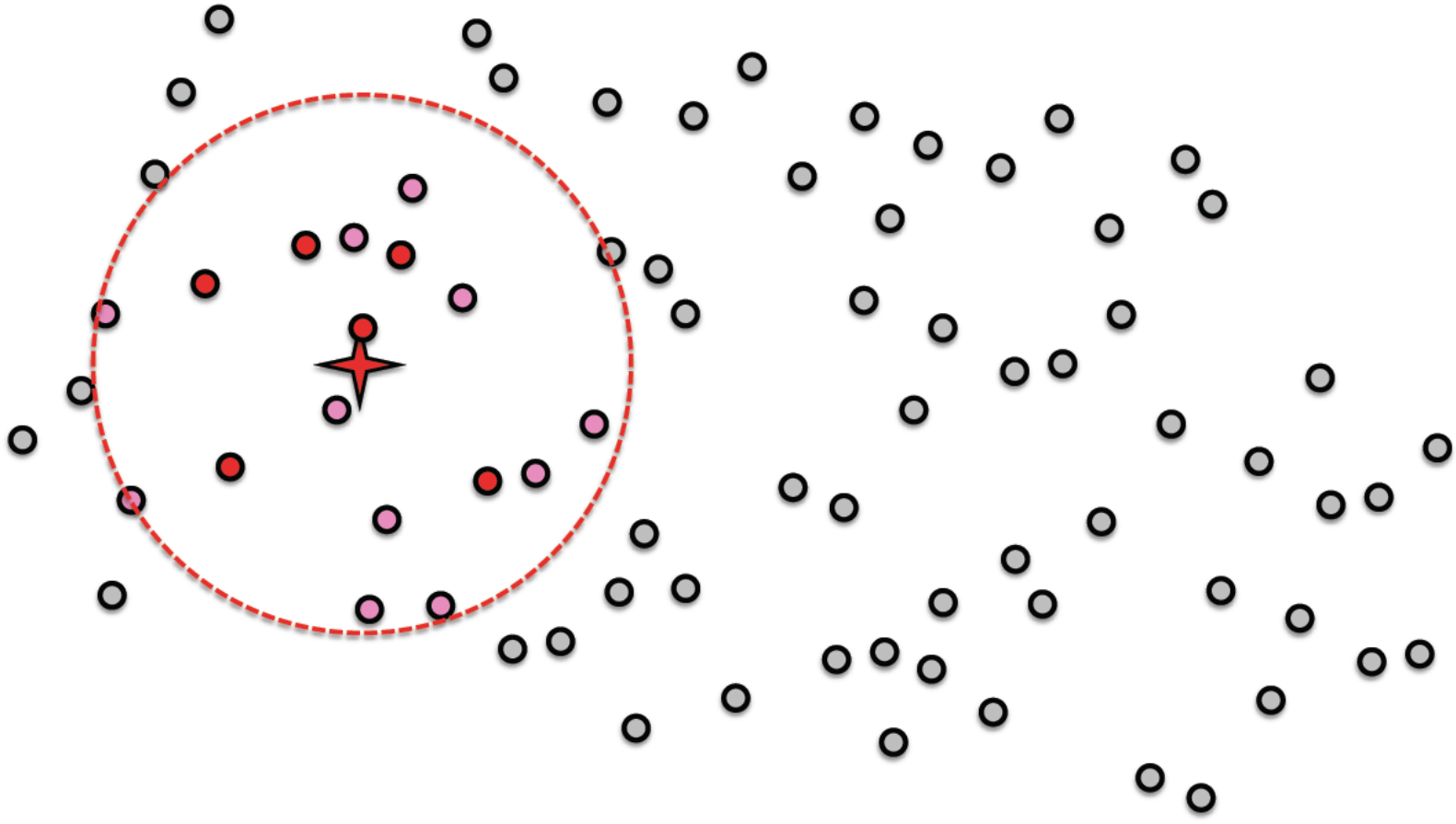
Classify sequences within the distance as similar



EXERCISE

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`exercises/03-effector_finding.ipynb`

- Downloading annotated *Pseudomonas* AvrPto1 effectors from a public sequence repository
- Building a (HMM) model from this training set
- Searching public genome annotations with the model

- MyBinder link

Choosing a distance [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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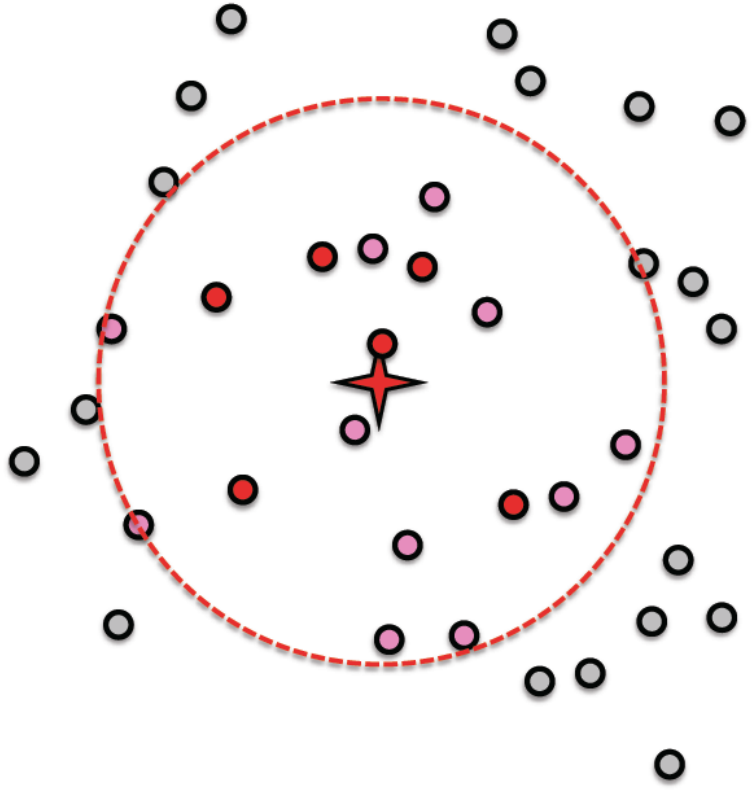
- How do we define distance?
- How large a distance should we take?
- How do we know if we chose well?



Are you in or out? [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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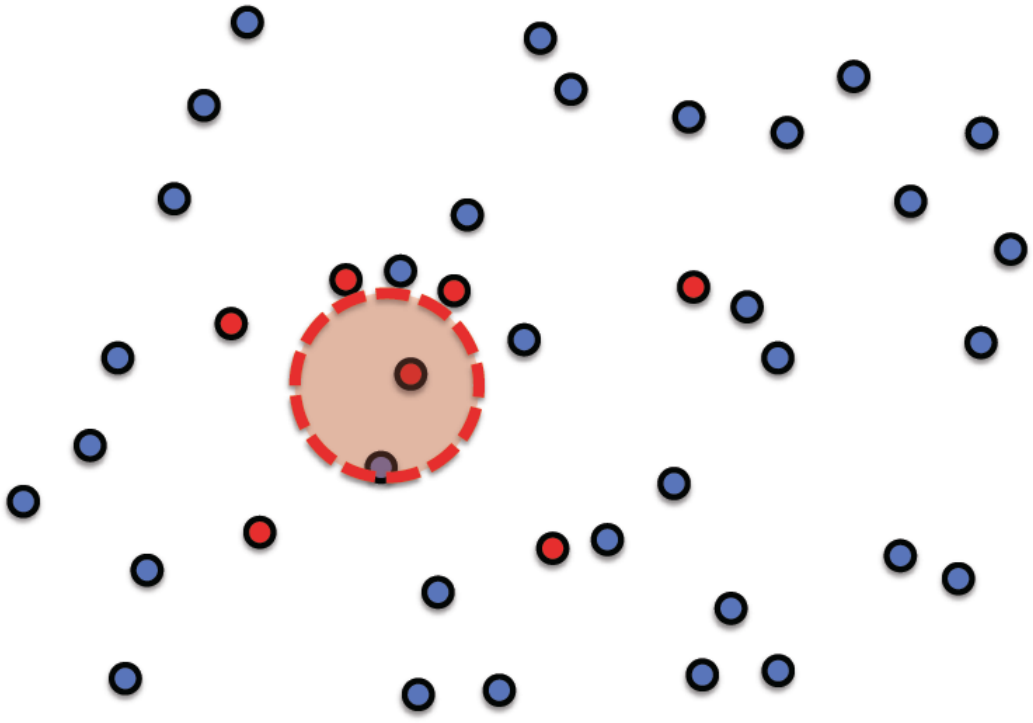
- The boundary (distance) classifies sequences as 'in' or 'out'
- Sequences are predicted to be *either* in the class *or* not in the class
- Changing distance/boundary changes classification



Confusion matrix:

	IN	OUT
Red	1	5
Blue	1	36

TP/TN/FP/FN [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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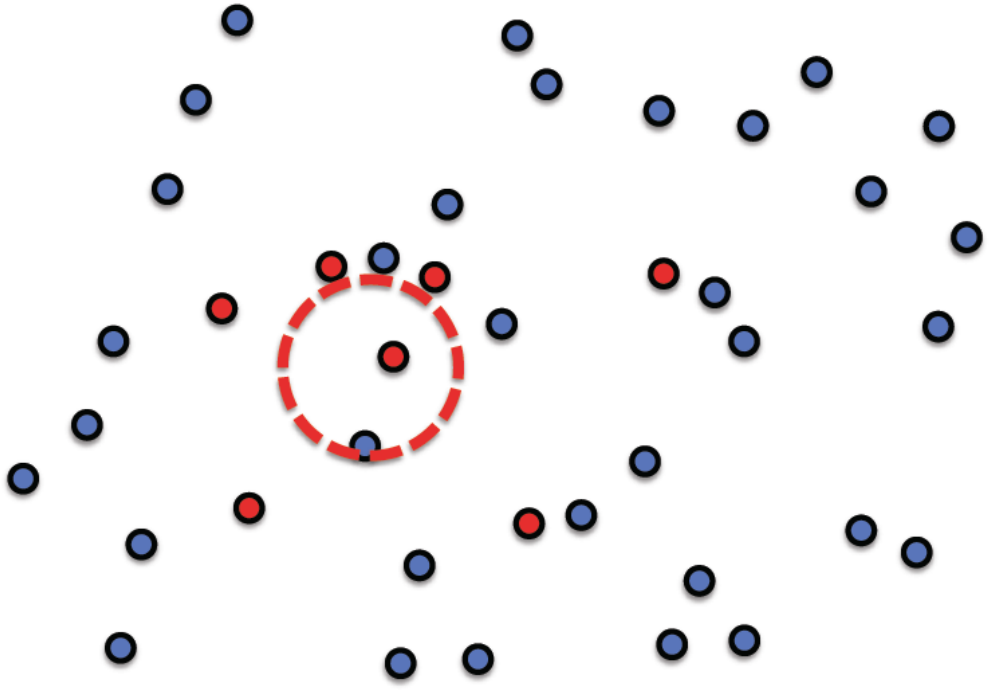

True positive

False negative

	IN	OUT
Red	1	5
Blue	1	36

False positive

True negative

FPR/FNR/Sn/Sp/FDR [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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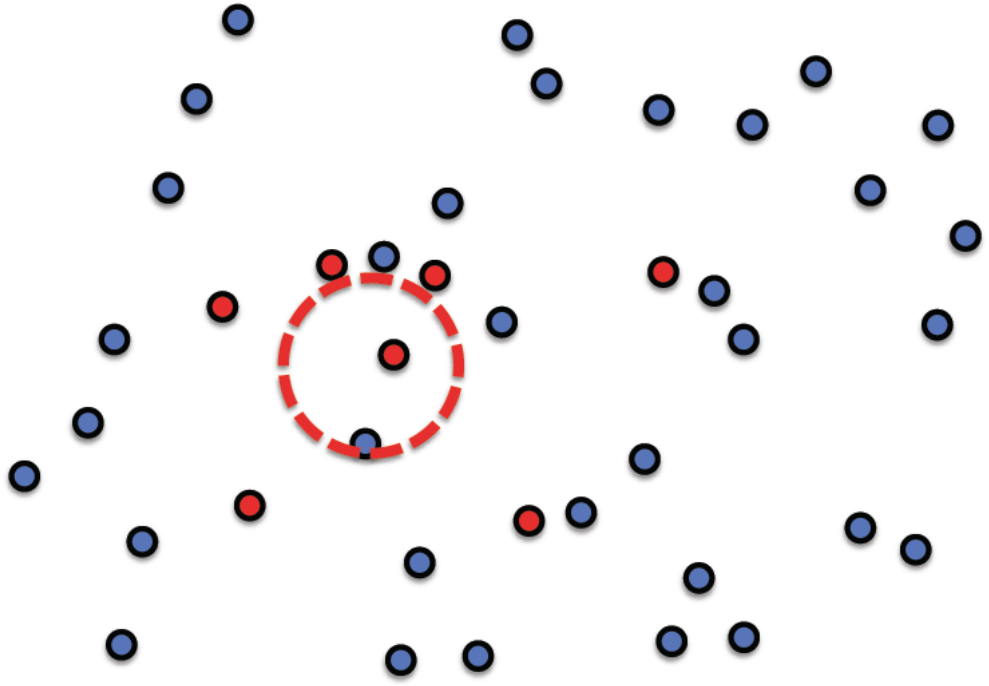

	IN	OUT
Red	1	5
Blue	1	36

False positive rate	FP/(FP+TN)
False negative rate	FN/(TP+FN)
Sensitivity	TP/(TP+FN)
Specificity	TN/(FP+TN)
False discovery rate	FP/(FP+TP)

Small Boundary [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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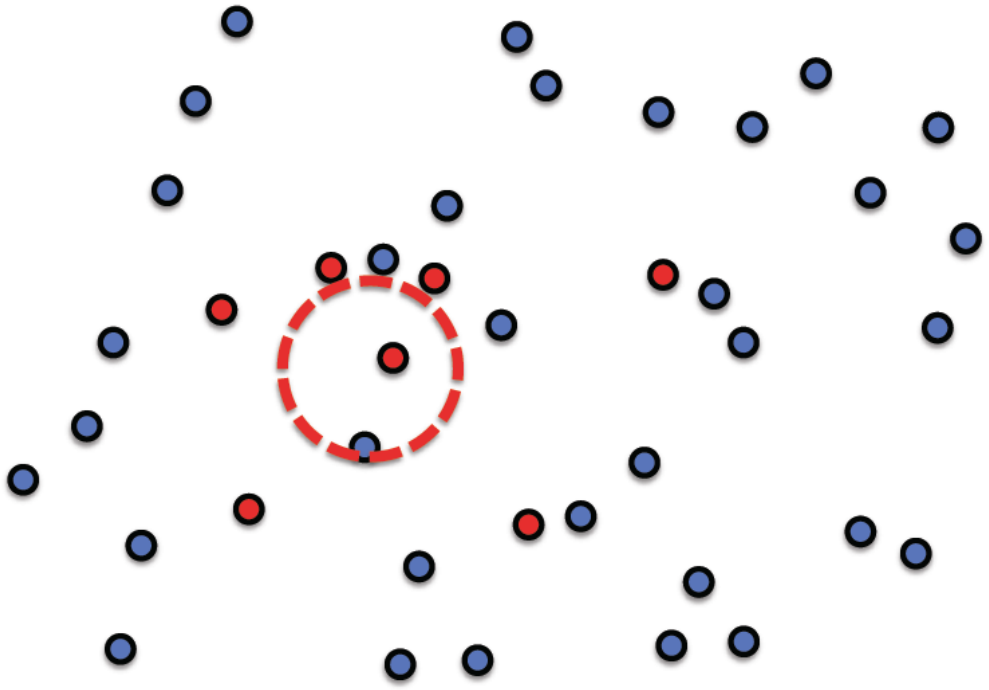
- Changing distance/boundary changes classification



	IN	OUT
Red	1	5
Blue	1	36

False positive rate	1/37 = 0.03
False negative rate	5/6 = 0.83
Sensitivity	1/6 = 0.17
Specificity	36/37 = 0.97

Medium boundary [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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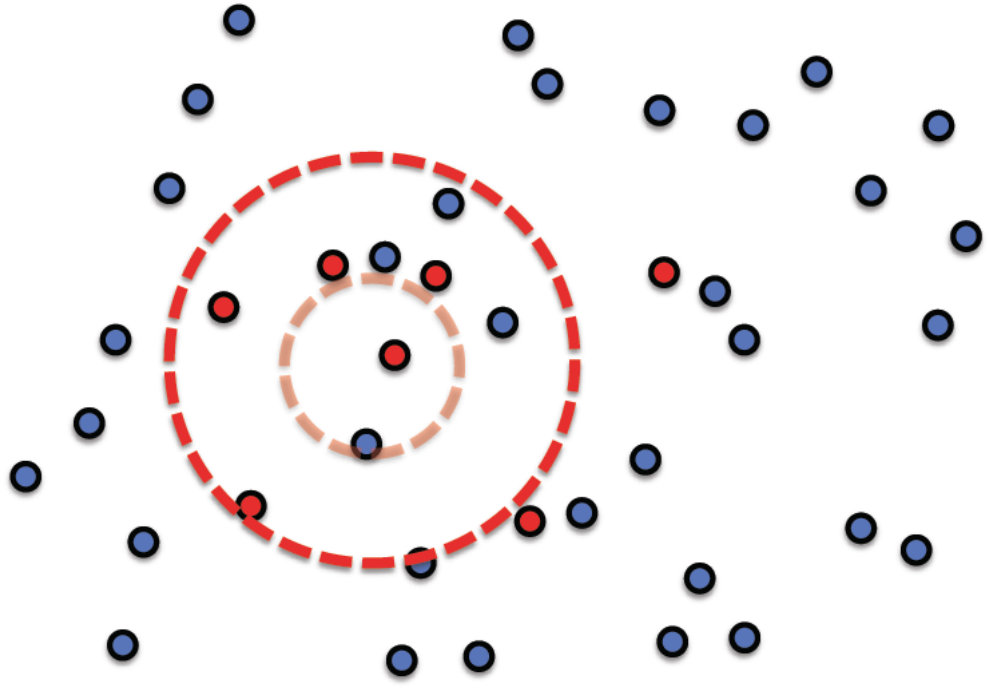
- The boundary (distance) classifies sequences as 'in' or 'out'
- Sequences are predicted to be *either* in the class *or* not in the class
- Changing distance/boundary changes classification



	IN	OUT
Red	5	2
Blue	4	33

False positive rate	0.11
False negative rate	0.29
Sensitivity	0.81
Specificity	0.89

Large boundary [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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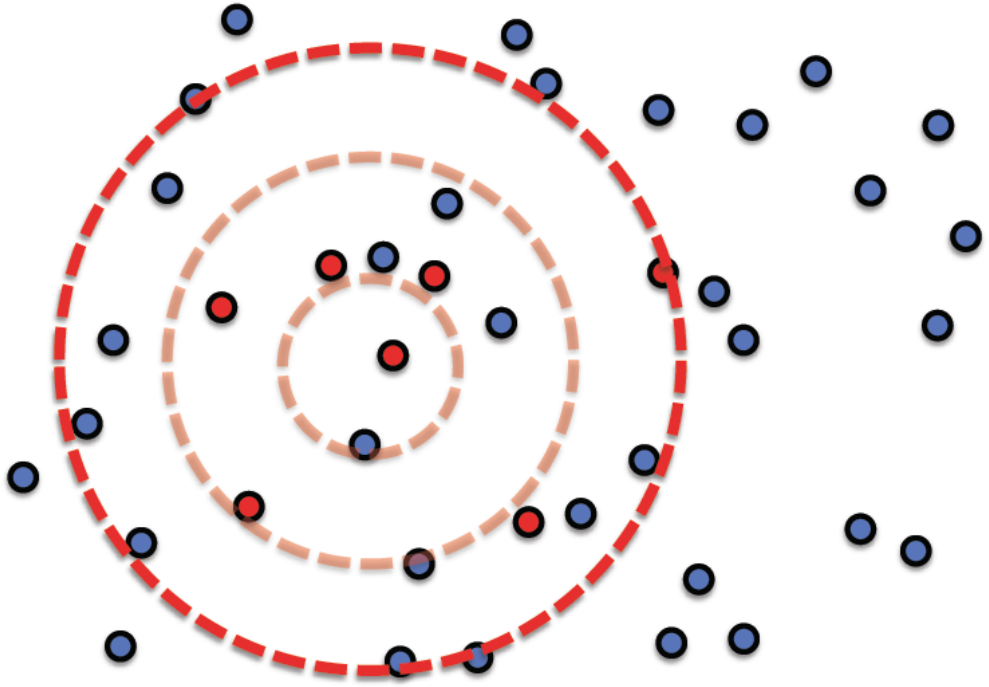
- The boundary (distance) classifies sequences as 'in' or 'out'
- Sequences are predicted to be *either* in the class *or* not in the class
- Changing distance/boundary changes classification



	IN	OUT
Red	7	0
Blue	14	23

False positive rate	0.38
False negative rate	0
Sensitivity	1
Specificity	0.62

Choosing a boundary [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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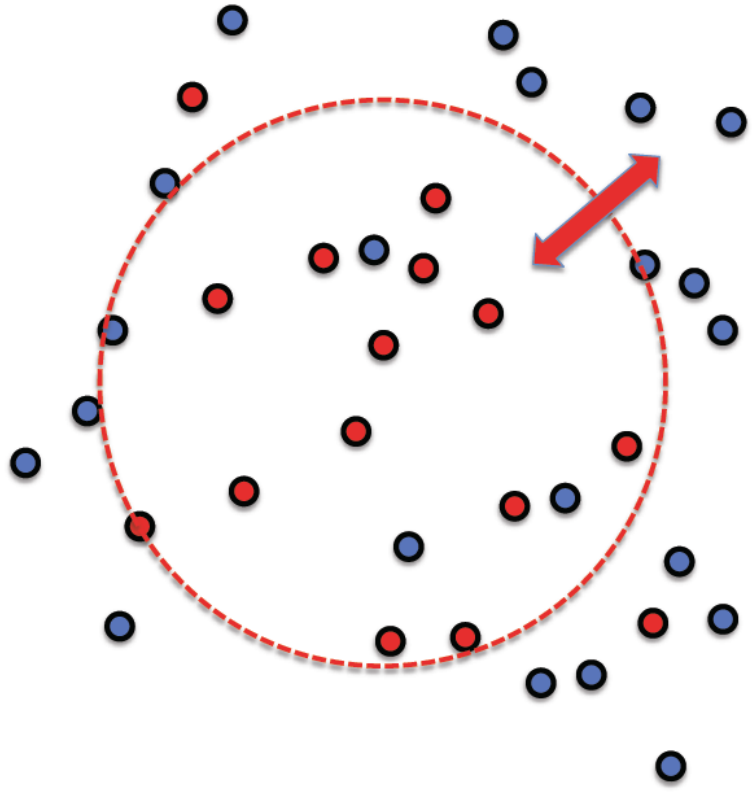
- Assign known 'positive' and 'negative' examples
- Vary 'distance' and measure predictive performance (F-measure, AUC, ...
- Choose the distance that gives the 'best' performance



Crossvalidation [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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- Estimation of classifier performance depends on:
  - Boundary choice/distance measure
  - Composition of training set ('positives' and 'negatives')
- Cross-validation gives objective estimate of performance
- Many strategies (beyond today's scope), including:
  - leave-one-out (LOO)
  - *k*-fold crossvalidation
  - repeated (random) subsampling
- Always test against a *hold-out set* (not used to train the classifier)

Post-crossvalidation [a]

[a] Pritchard & Broadhurst (2014) *Methods Mol. Biol.* doi:10.1007/978-1-62703-986-4_4

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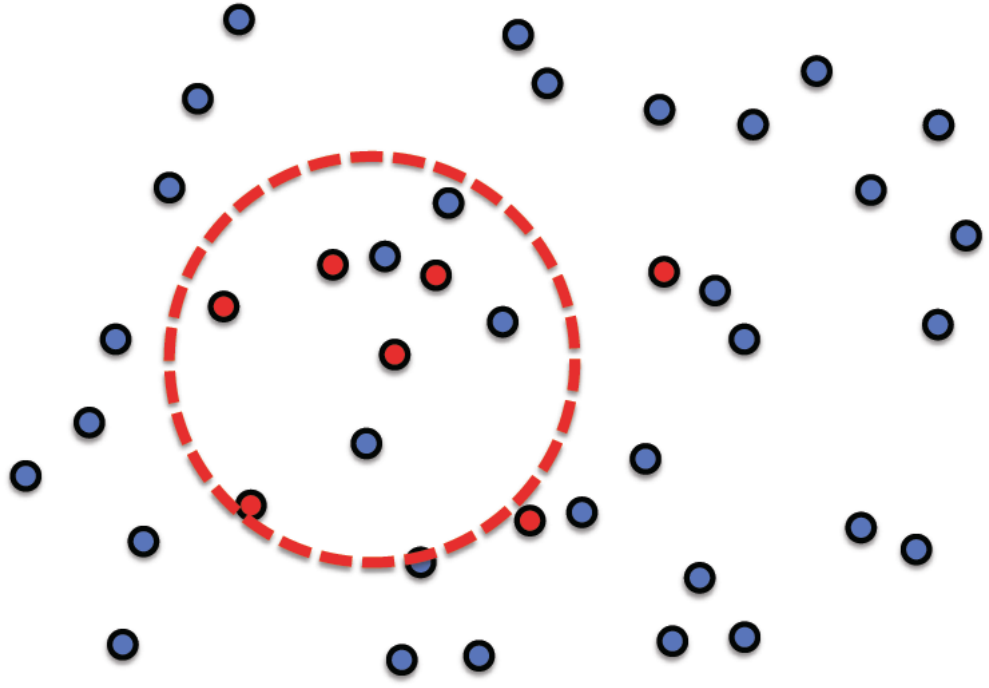
- Crossvalidation gives 'best' method & parameters
- Apply 'best' method to complete dataset for prediction



False positive rate	0.11
False negative rate	0.29
Sensitivity	0.81
Specificity	0.89
Precision	0.56

BEWARE THE BASELINE EFFECT!

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By: Leighton Pritchard