Presentation and implementation of phylogenomics methods

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Outline

- Build the dataset:
  - What scale for infering species phylogeny ?
  - What are the possible errors ?
- Phylogenomics analysis
  - Whole genome features methods
  - Sequence based approaches:
    - Supermatrix
    - Supertree
- How to compare trees ?
- Conclusion

Why use more than one gene to reconstruct the evolutionary history of several species of interest ?

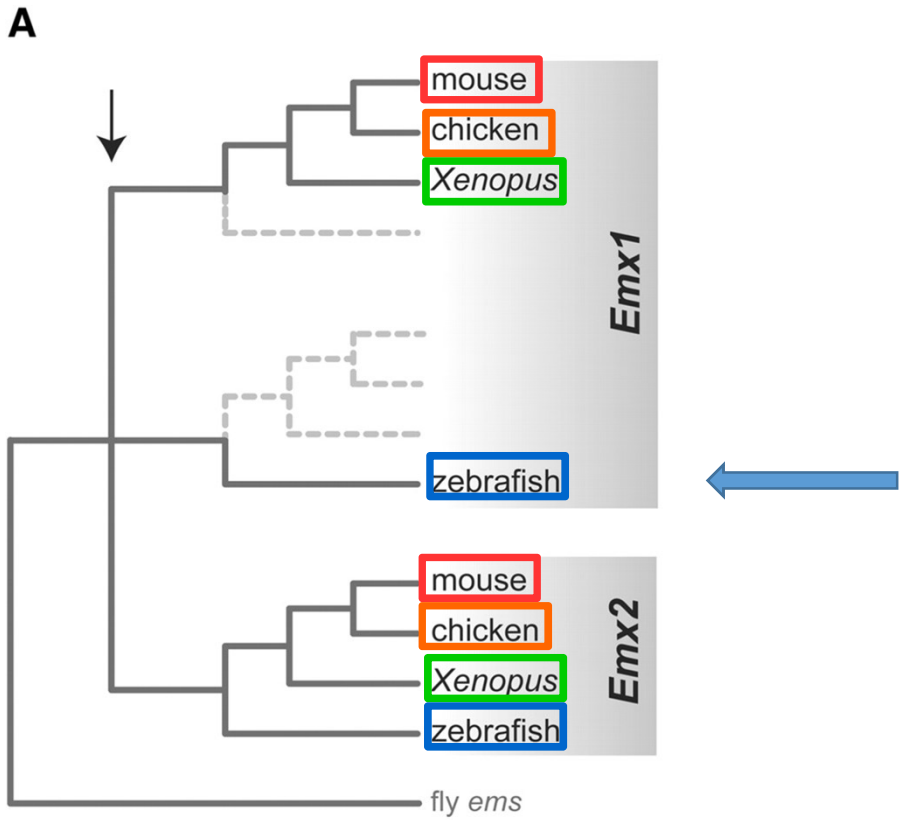
Limits of phylogenies based on a single gene

- Using a single gene allows to reconstruct the evolutionary history of the gene and not specifically of the corresponding OTU.
- The resolution can be poor.
- The evolutionary history of the gene may be different from that of the species because :
  - Hidden paralogy
  - Lateral gene transfer
  - Ancestral polymorphism

Sources of incongruence between the phylogeny of a gene and the evolutionary history of the species

- Hidden paralogy (gene duplication followed by a loss)
- Lateral gene transfer (LGT)
- Ancestral polymorphism :
  - Trans-specific polymorphism (TSP : These alleles have diverged prior to speciation and this diversity is maintained)
  - Incomplete Lineage sorting (ILS : selection or genetic drift may cause alleles to be lost over time in one lineage but not another when two populations diverge)

Sources of incongruence: Hidden paralogy



Hidden paralogy in Emx gene phylogeny.

Molecular phylogenetic trees of vertebrate Emx genes before the year 2000 (A) and now (B) are shown. Dotted lines indicate absences of relevant genes (gene loss or incomplete identification). Note that the zebrafish gene, initially recognized as emx1 in (A) (Morita et al. 1995), was later found orthologous to emx3 and renamed accordingly as shown in (B) (Kawahara and Dawid 2002). Arrows indicate gene duplications between gnathostome paralogs.

Kuraku (2010) Integr. Comp. Biol. 50 (1): 124-129.

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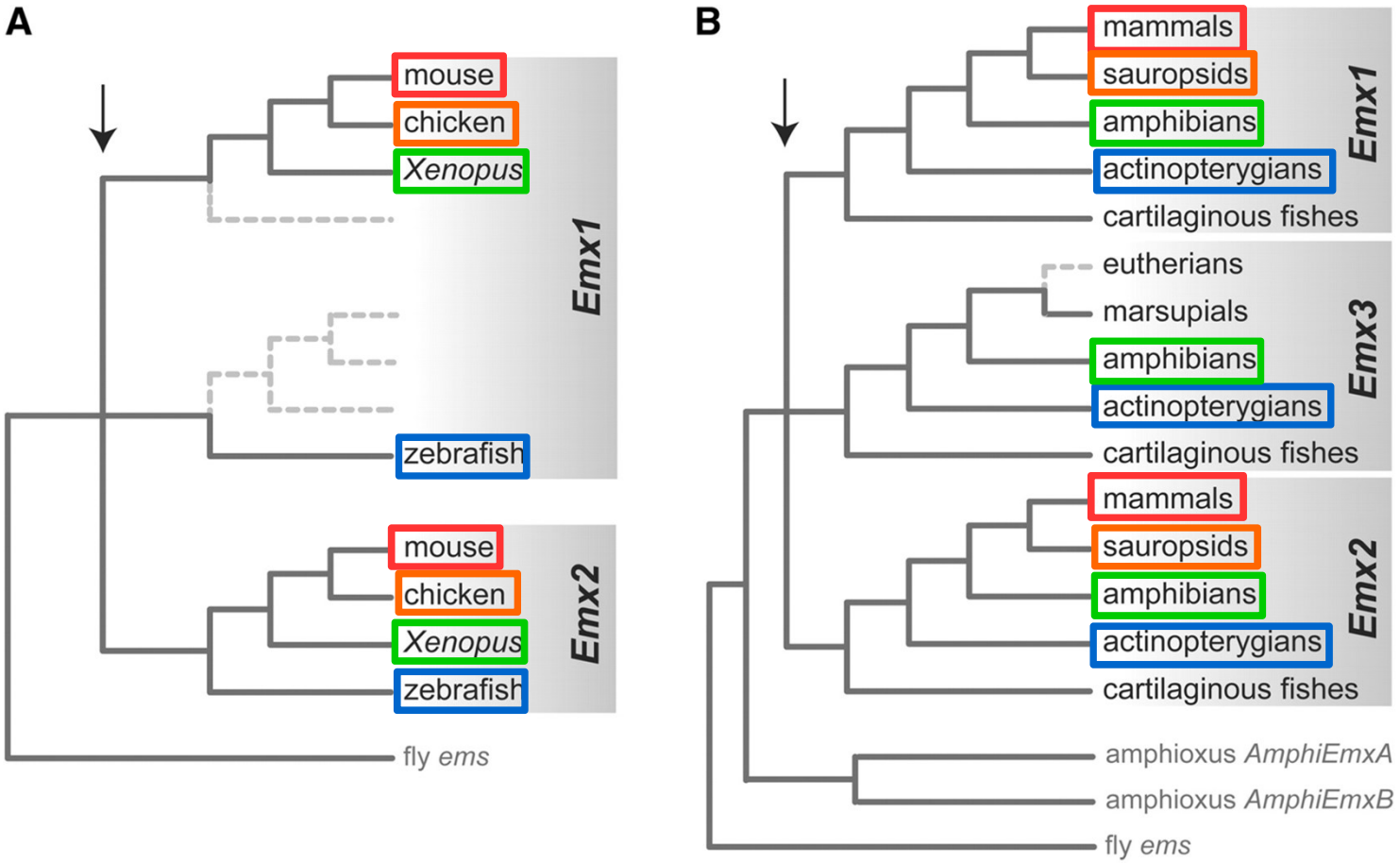

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Sources of incongruence: lateral gene transfer

Phylogeny of HMG-CoA reductase in several kingdom

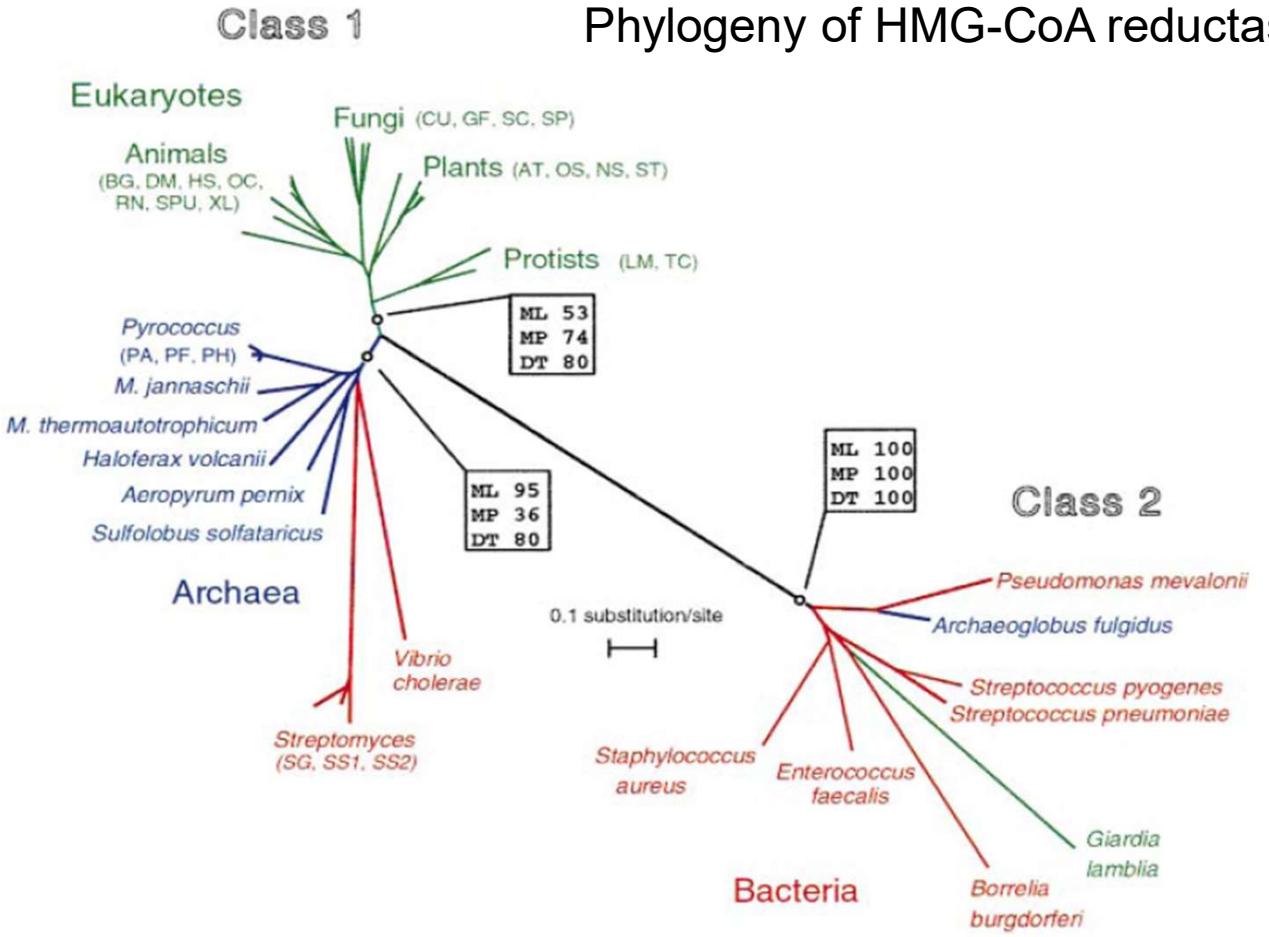
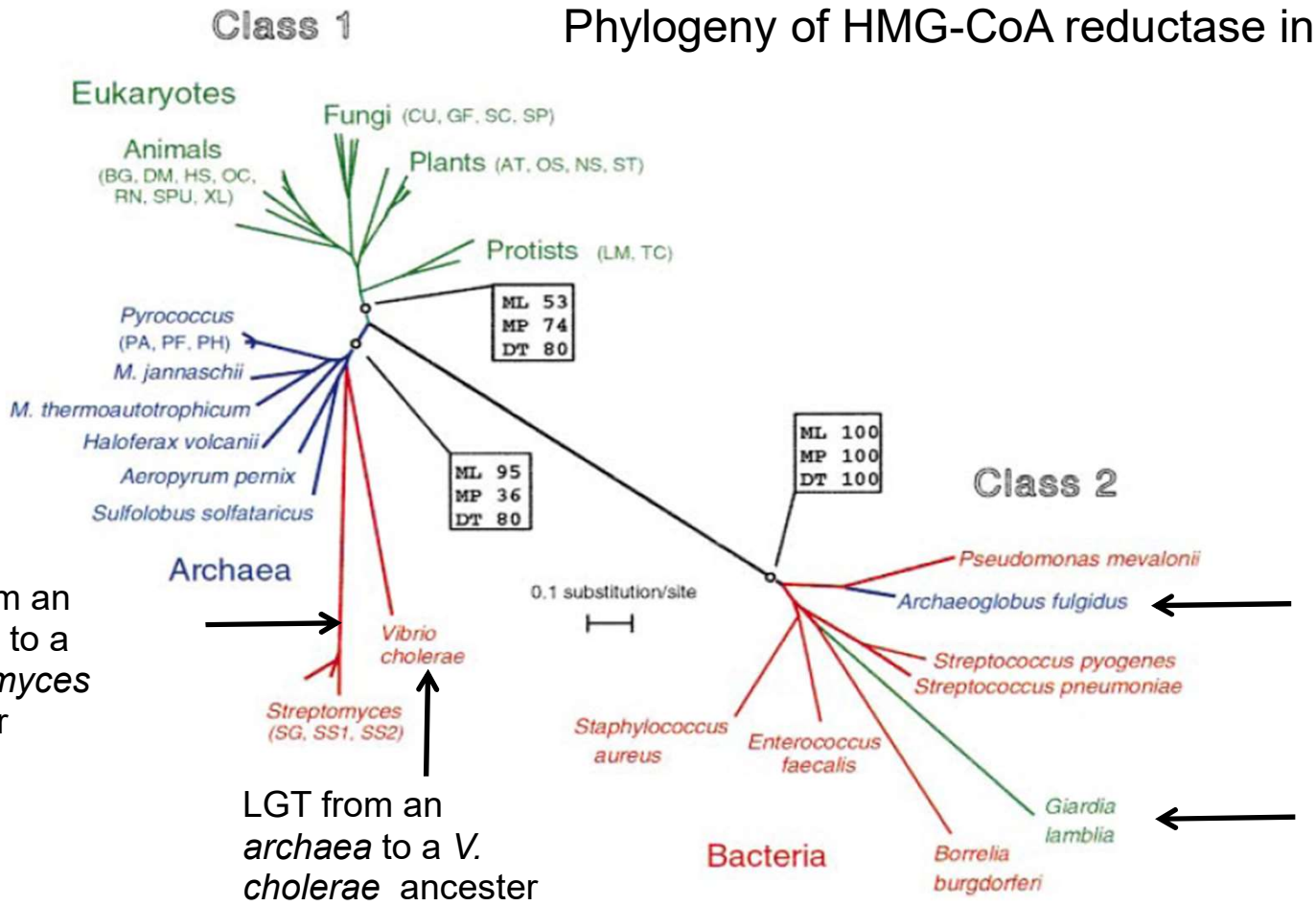


Fig. 3. Phylogeny of HMG-CoA reductase. A subset of 37 taxa from the alignment of all known HMGR protein sequences was used to carry out the analysis. The distance tree shown was determined using PROTDIST with PAM distances and branch lengths calculated with FITCH (PHYLIP 3.57; Felsenstein, 1993). The support values for important nodes of the tree are shown in boxes. (DT) percentage of distance bootstrap replicates supporting this topology using PROTDIST with PAM distances. SEQBOOT was used to generate 1000 bootstrap replicates, and the consensus tree was derived using CONSENSE. (ML) protML RELL values obtained using a quick-add search of 1000 trees and the JTT-F substitution model. (MP) bootstrap support for the consensus tree obtained from PROTPARS with 1000 bootstrap replicates. Organism names are

Boucher and Doolittle (2000) Molecular Microbiology 37 (4): 703-716.

Sources of incongruence: lateral gene transfer

Phylogeny of HMG-CoA reductase in several kingdom



LGT from an *archaea* to a *Streptomyces* ancestor

LGT from an *archaea* to a *V. cholerae* ancester

LGT from a *Pseudomonas* to a *A. fulgidus* ancester

Parasitic protozoan living in the mammalian intestine : acquiring a bacterial version of the gene by LGT

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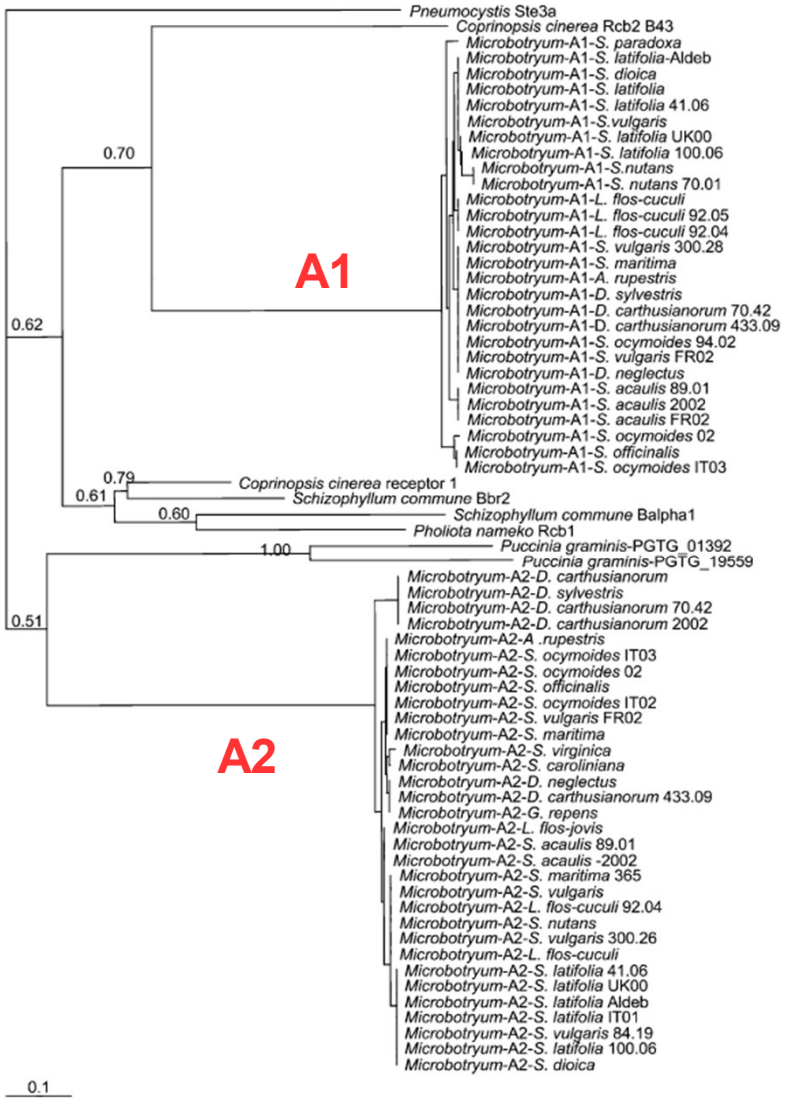
Sources of incongruence: trans-specific polymorphism

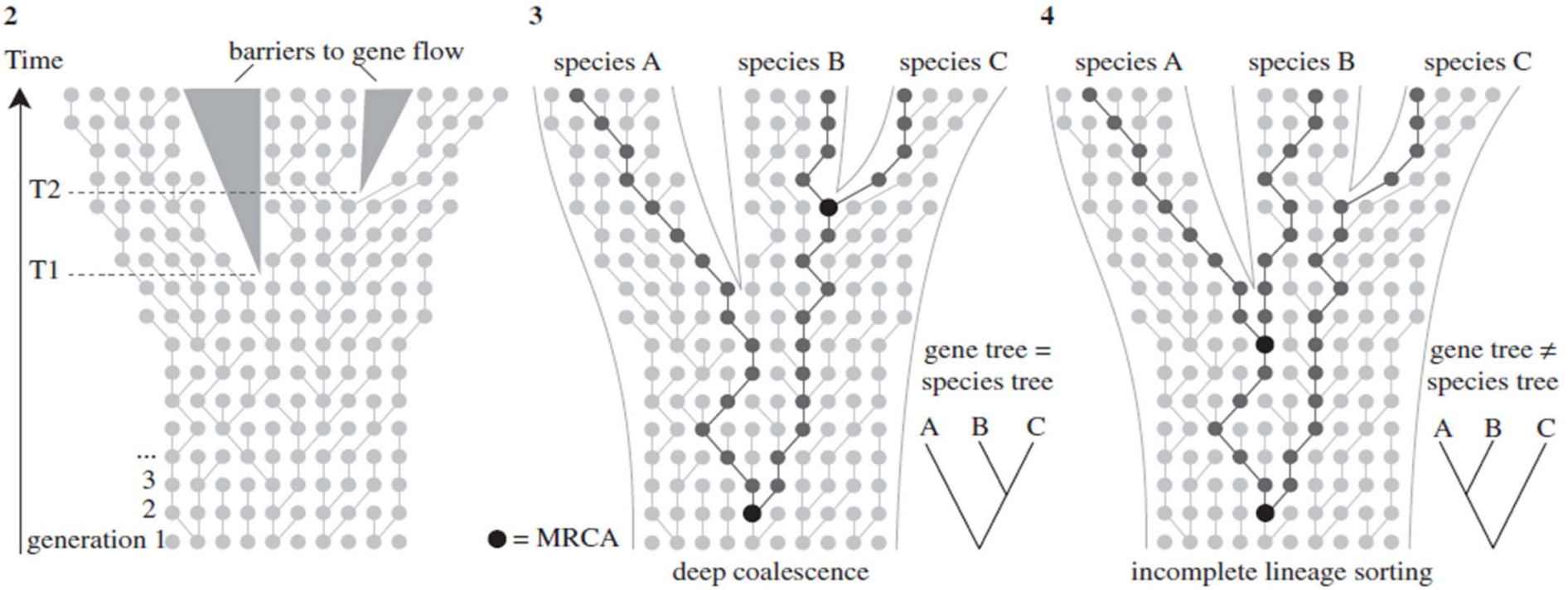


Phylogeny based on the pheromone receptor pr-MatA1 and pr-MatA2 of Mycrobotryum and other fungi.

Trans-specific polymorphism: an allele sampled from a particular species can be more related of the same functional allelic class in other species than to members of different allelique classes in the same species (extrem case of balancing selection : i.e. heterozygote advantage).

B. Devier *et al*, Genetics 181: 209–223 (2009)

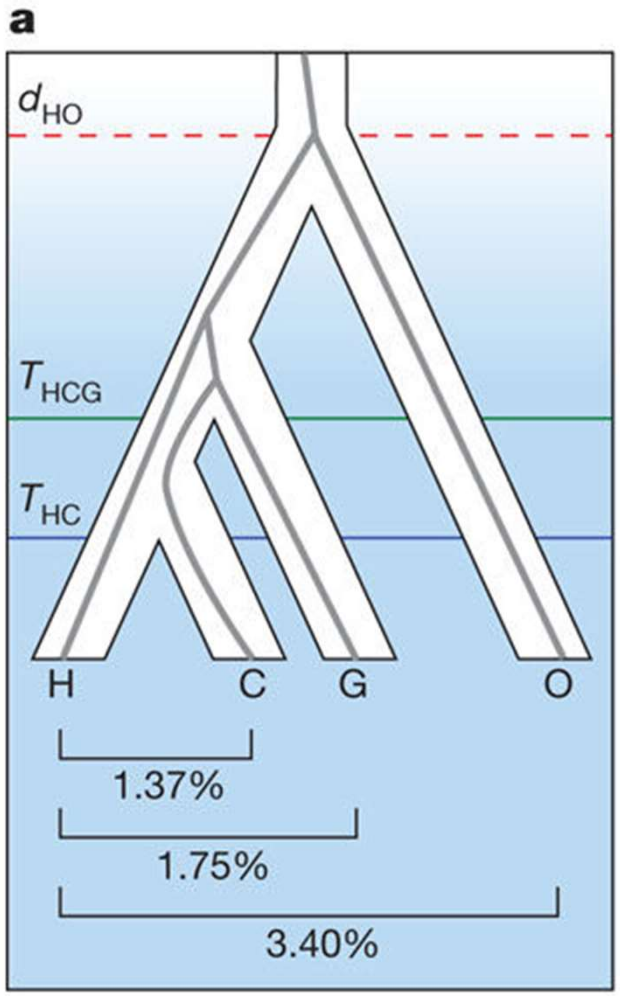
Sources of incongruence: incomplete lineage sorting



- **Lineage sorting**: the process by which alleles are inherited and lost over time
- **Deep coalescence**: coalescence of alleles occurring significantly earlier than the divergence of the species containing those alleles
- **Incomplete lineage sorting (ILS)**: the maintenance of genetic variation within a metapopulation lineage from one speciation event to the next, resulting in deep coalescence and gene tree–species tree incongruence (Baum & Smith, 2012)
- MRCA : Most Recent Common Ancestor

Leliart *et al.*, Eur. J. Phycol. (2014)

Sources of incongruence: incomplete lineage sorting



Phylogeny of the great ape family, showing the speciation of human (H), chimpanzee (C), gorilla (G) and orang-utan (O). Horizontal lines indicate speciation times within the hominine subfamily and the sequence divergence time between human and orang-utan. Interior grey lines illustrate an example of incomplete lineage sorting at a particular genetic locus—in this case (((C, G), H), O) rather than (((H, C), G), O). Below are mean nucleotide divergences between human and the other great apes from the EPO alignment.

The Chimpanzee and the Human are the most recently speciated. But the Gorilla and the Chimpanzee are the most recently diverged, in the flow of one particular gene.

Scally *et al.*, Nature 483 169-175 (2012)

Sources of incongruence: incomplete lineage sorting

- ILS: a persistence of polymorphisms across multiple successive speciation events followed by stochastic allele fixation in each descendant lineage.

- Scally *et al.* (Nature, 2012) found 30 % of bases exhibiting ILS between human, chimpanzee and gorilla across the genome.

- When speciation is more rapid than the sorting of genes (in large population for exemple), the sorting along species lines can be incomplete.

- ILS is more likely to occur if the distance between branchings is short (speciation temporally close).

- Balancing selection increases ILS.

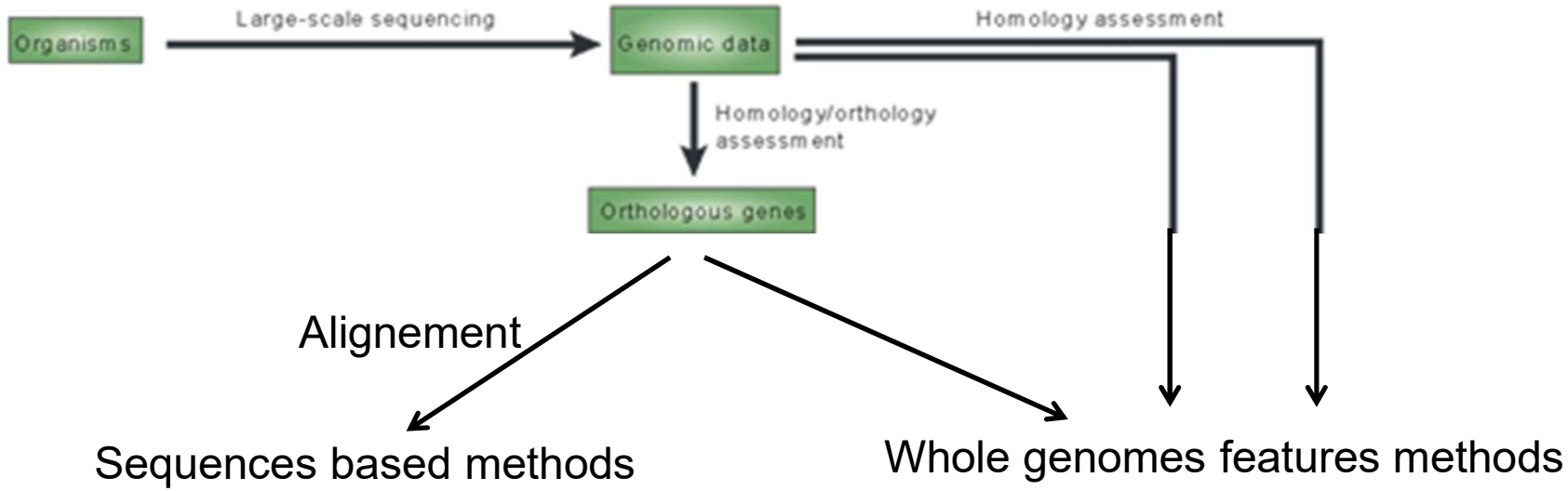
There is a lot of inconsistency sources in individual gene data, so in practice we integrate a lot of informations by assuming that the phylogenetic signal that we want is dominant.

Definition of possible errors

- **Stochastic errors** are sampling errors caused by a too small sample. To measure it, it's possible to use resampling method bootstrap or jackknife.

- **Systematic errors** appears when the evolutionary process violates the assumptions of model used for phylogenetic reconstruction.

Phylogenomic analysis : the type of methods



(From Delsuc *et al*, Nature reviews, 2005)

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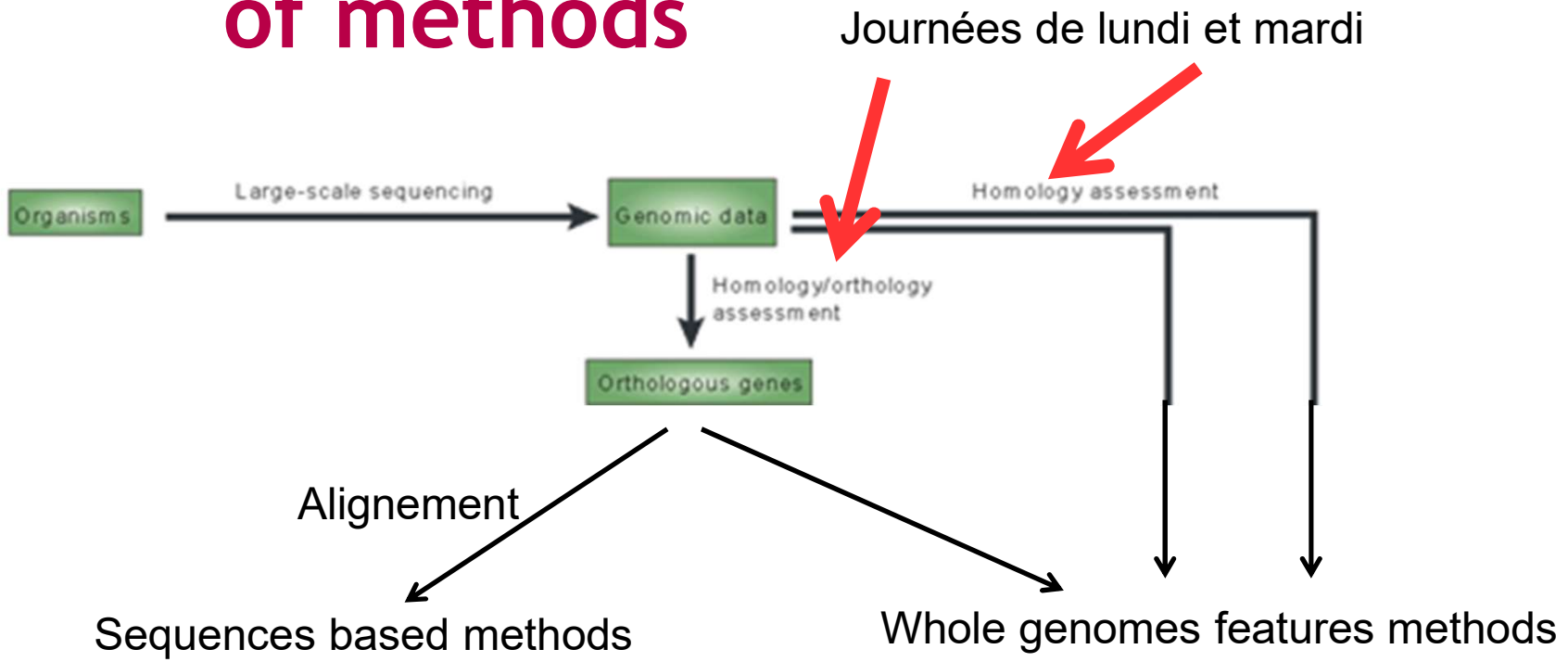

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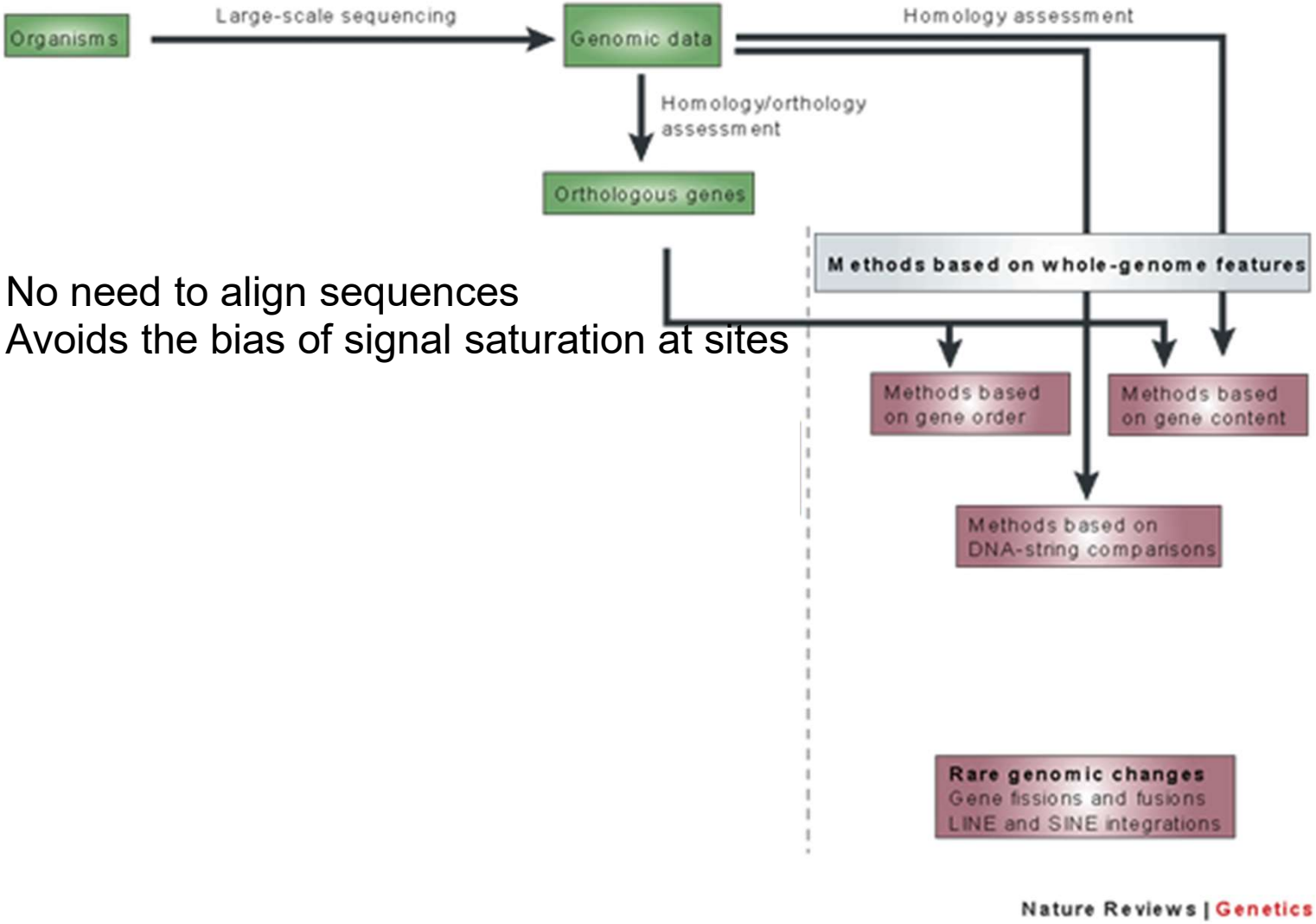
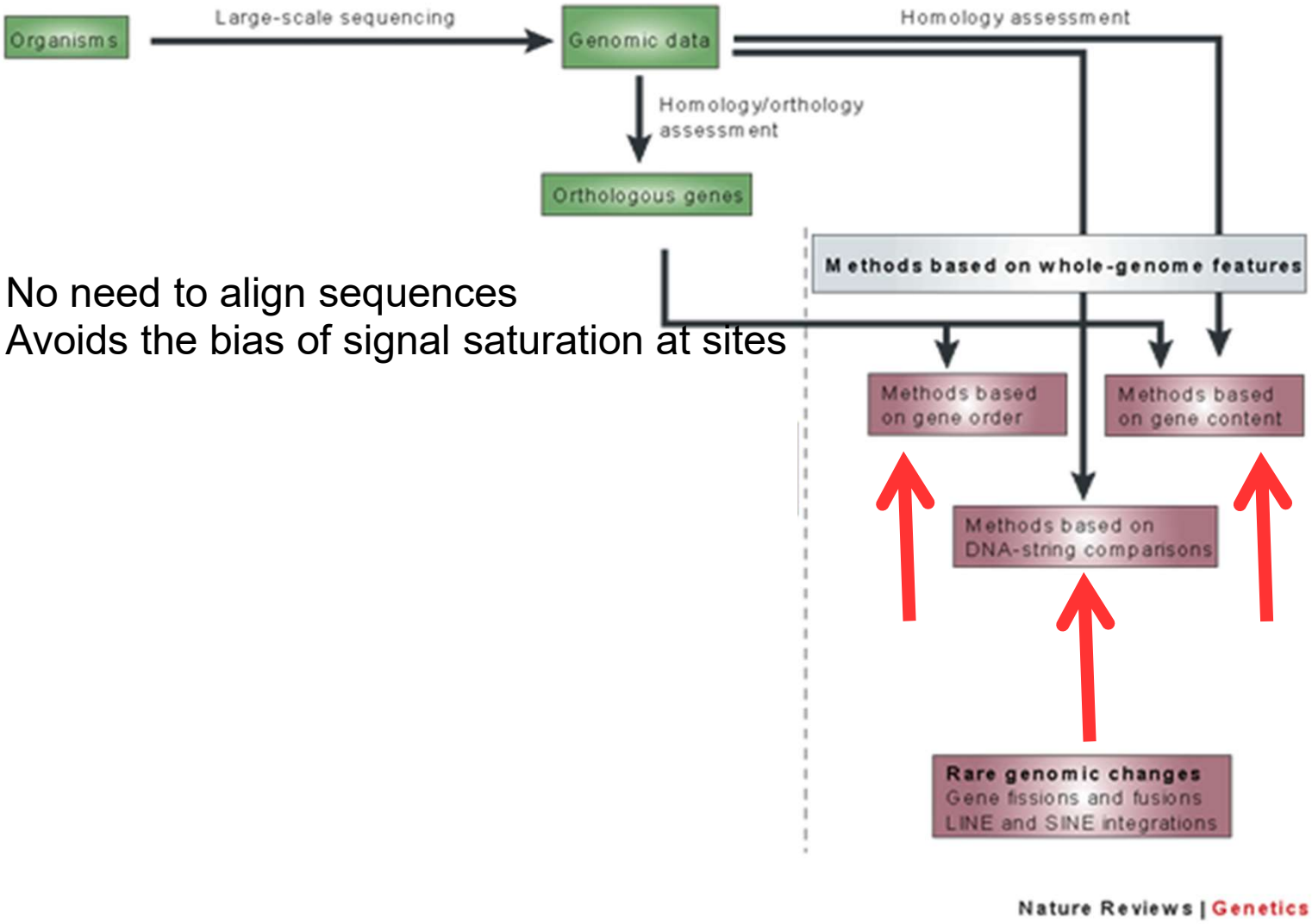


No need to align sequences
Avoids the bias of signal saturation at sites

(From Delsuc *et al*, Nature reviews, 2005)

Phylogenomic analysis : the methods



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(From Delsuc *et al*, Nature reviews, 2005)

Whole genome features methods

- Gene content
- Gene order approach
- DNA-string approach

Comparison of gene content

- Find the potential orthologous genes
- Write the presence/absence matrix

	Species 1	Species 2	Species 3	...
Gene 1	0	1	1	
Gene 2	0	0	0	
Gene 3	1	1	0	
...				

  - And build the tree with maximum parsimony
- Or compute the distance matrix (normalized by the number of genes in each genome involved)
  - And build the tree with NJ
- Disadvantages: big/small genome attraction

Comparison of gene content

Table 1• Common gene content in genomes

	AF	MT	MJ	PH	AQ	SY	BS	MG	BB	EC	HI	HP	SC
AF	**2,407**	*48.1*	*50.1*	*40.2*	*38.2*	*26.3*	*26.8*	*33.3*	*25.2*	*28.1*	*26.4*	*23.6*	*23.1*
MT	900	**1,871**	*55.7*	*37.4*	*35.3*	*31.1*	*30.9*	*30.3*	*24.8*	*32.0*	*24.2*	*22.3*	*27.9*
MJ	870	966	**1,735**	*43.7*	*32.7*	*29.2*	*28.1*	*31.2*	*22.2*	*31.1*	*22.4*	*22.3*	*27.8*
PH	829	699	759	**2,061**	*30.9*	*23.8*	*27.2*	*31.4*	*24.0*	*26.1*	*21.7*	*20.1*	*23.7*
AQ	582	537	497	471	**1,522**	*52.5*	*53.8*	*54.5*	*44.6*	*59.0*	*44.0*	*43.7*	*31.1*
SY	632	581	506	491	799	**3,168**	*30.5*	*58.8*	*48.1*	*35.9*	*44.6*	*41.0*	*19.1*
BS	645	578	488	561	819	967	**4,100**	*70.7*	*56.5*	*33.6*	*51.3*	*42.0*	*16.1*
MG	156	142	146	147	255	275	331	**468**	*50.4*	*62.2*	*57.5*	*52.1*	*40.4*
BB	214	211	189	204	379	409	480	236	**850**	*52.2*	*46.2*	*43.8*	*29.4*
EC	676	598	539	538	898	1,138	1,376	291	444	**4,290**	*77.8*	*49.9*	*17.1*
HI	453	416	384	372	669	766	880	269	393	1335	**1,717**	*41.1*	*28.8*
HP	375	355	354	320	665	652	668	244	372	793	653	**1,590**	*22.2*
SC	555	522	482	488	474	606	659	189	250	735	494	353	**6,296**

The numbers of genes shared (see Methods) between genomes (lower left triangle), the percentage of genes shared between genomes (the total number divided by the number of genes in the smallest genome; upper right triangle) and the numbers of genes per genome (bold). HI, *H. influenzae*[16]; MG, *M. genitalium*[17]; SY, *Synechocystis* sp. PCC 6803 (ref. 18); MJ, *M. jannaschii*[19]; EC, *E. coli*[20]; MT, *M. thermoautotrophicum*[21]; HP, *H. pylori*[22]; AF, *A. fulgidus*[23]; BS, *B. subtilis*[24]; BB, *B. burgdorferi*[25]; SC, *S. cerevisiae*[26]; AQ, *A. aeolicus*[27]; PH, *P. horikoshii*[28].

(Snel B. *et al.*, Nature genetics, 1999)

Comparison of gene content

- Used for large evolutive scale, no problem with:

=> LGT

=> Duplication

=> Sites saturation

- Other distances have been proposed:
  - SHOT distance (Korbel et al., 2002)
  - Huson and Steel's model (Huson and stell, 2004)
  - Gu and Zhang's method (Gu and Zhang, 2004)

Whole genome features methods

- Gene content
- **Gene order approach**
- DNA-string approach

Comparison of gene order

- Find the genes families (homologies).
- Compute distance matrix based on breakpoint between genomes (inversions, transpositions, deletion, duplications).
- Software example : GRAPPA, DCM-GRAPPA (Tang & Moret, 2003)

Comparison of gene order

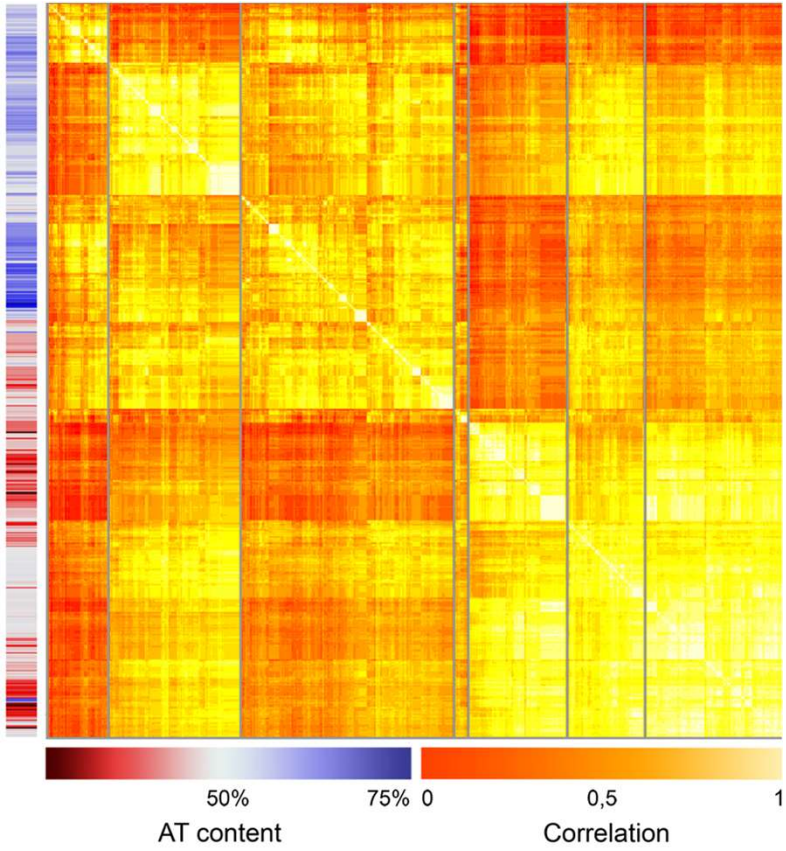
- Used for mitochondries and chloroplasts genomes
- Low error rate
- Rare events in eucaryotes genomes (large evolutionary scale)
- Problems :
  - Very limited data (mostly organelles)
  - Mathematics complex
  - Evolutionary models not well known

Whole genome features methods

- Gene content
- Gene order approach
- **DNA-string approach**

DNA string approach

- No need to orthology / homology
- Frequency matrix of words in sequences.
- Compute distance matrix (difference in the use of words).

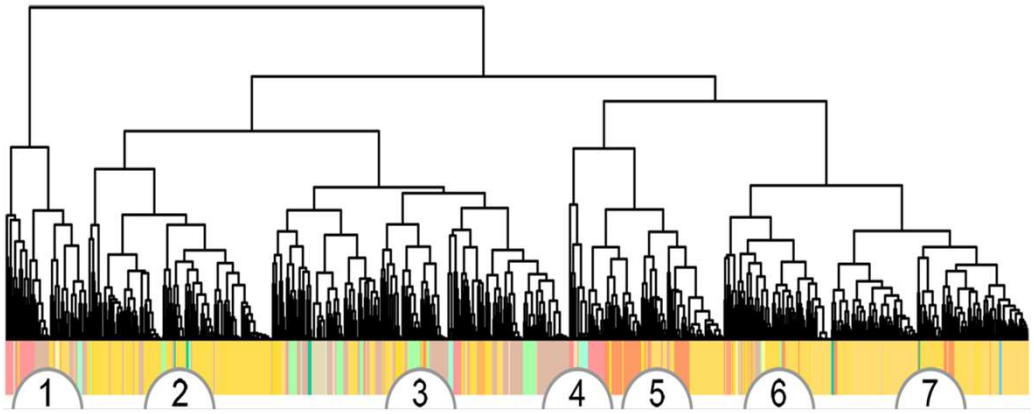


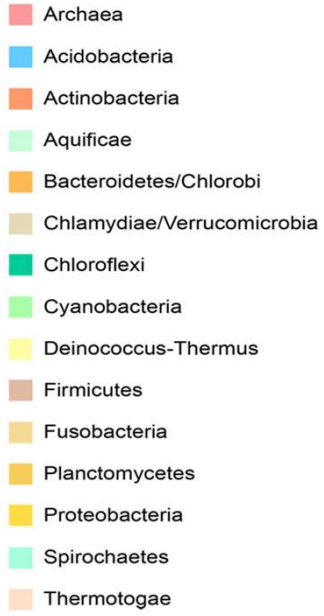
867 prokaryotic genomic DNA sequences compared pair-wise using hexanucleotide-based genomic signatures.

(Bohlin J. *et al.*, BMC genomics, 2009)

DNA string approach

- Build trees with clustering or NJ.
- Using of species known to have benchmarks to locate the analyzed species





Cluster diagram of 867 prokaryotic genomic DNA sequences compared pair-wise using hexanucleotide-based genomic signatures

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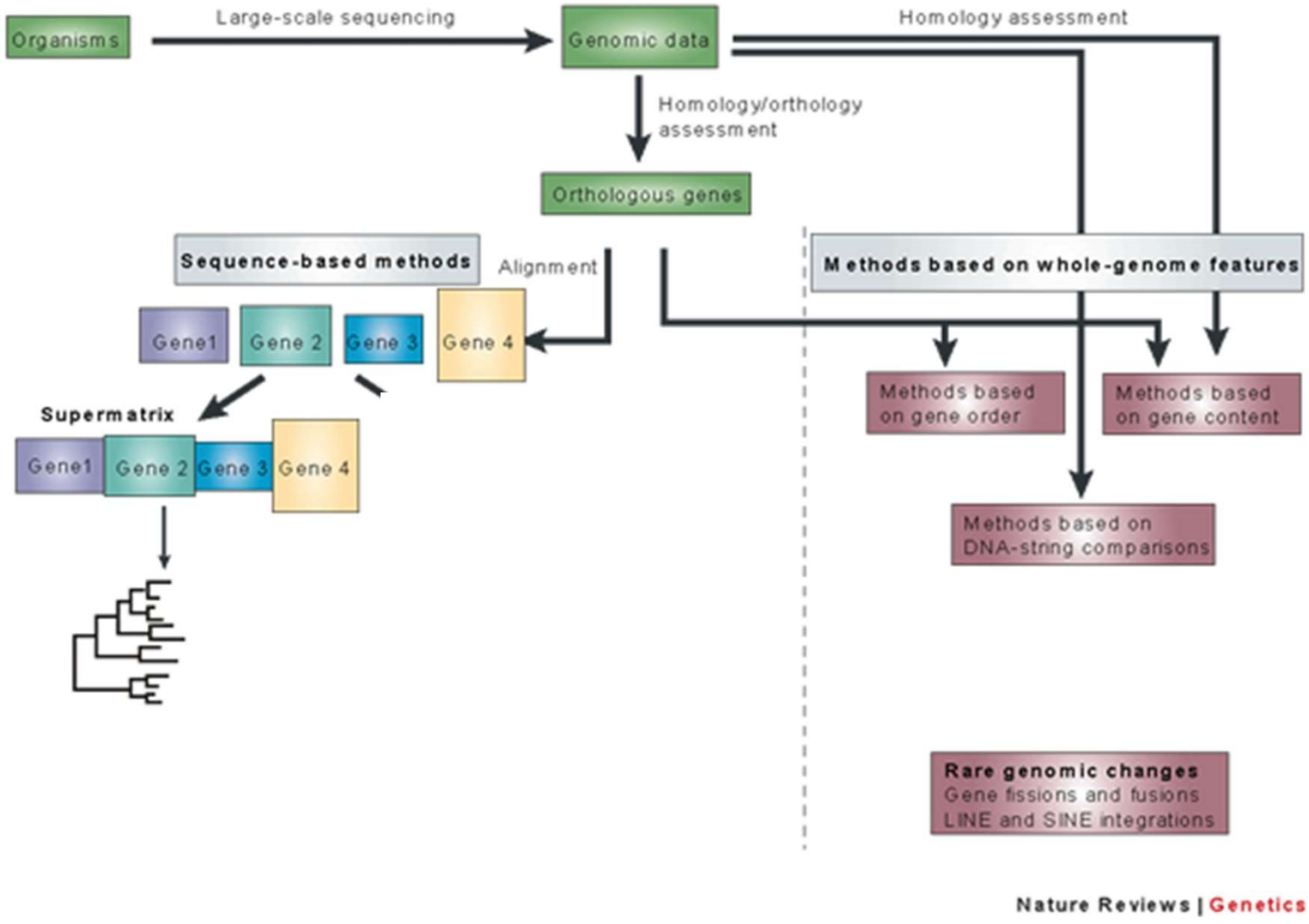
Phylogenomic analysis : the methods



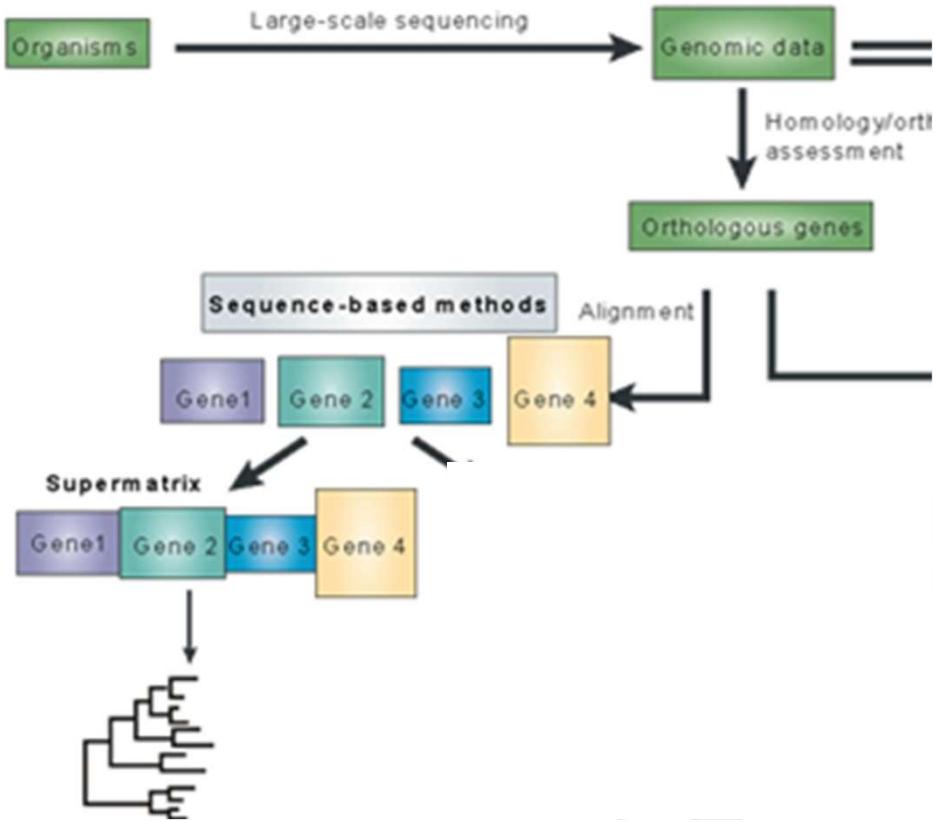
(From Delsuc *et al*, Nature reviews, 2005)

Sequence-based methods

- Supermatrix approach
- Consensus
- Supertree approach

The supermatrix approach

- The basic assumption is that the desired phylogenetic signal is dominant.
- Super alignment: concatenation of individual genes alignment
- Using « standard » methods of phylogeny (ML and bayesian if it's possible).



The supermatrix approach (2)

Gene 1

OTU 1 _____
OTU 2 _____
OTU 3 _____

Gene 2

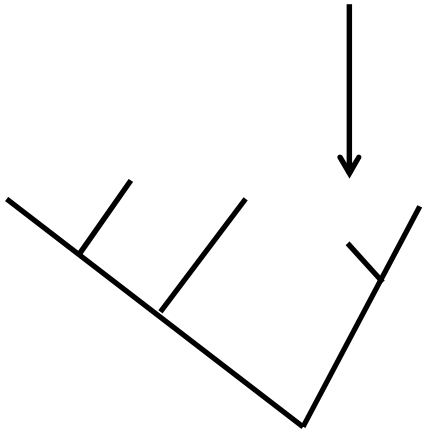
OTU 1 _____
OTU 4 _____
OTU 5 _____

…Gene n

OTU 2 _____
OTU 3 _____
OTU 4 _____

OTU 1 _____...??????????
OTU 2 _____????????...____
OTU 3 _____????????...____
OTU 4 ????????????_____...____
OTU 5 ????????????_____...??????????

1 model fixed
1 set of parameters inferred
ML or bayesian methods



The supermatrix approach (3)

- May mix phylogenetic signal from different evolutionary histories
- Will require an evolutionary model with a lot of parameters (+ heterogeneity of sub. Rate, invariable site: gamma law or FreeRate model that generalize it + pInv) or a mixture model (Lartillot & Philippe, Mol Biol Evol, 2004), partitioned model, heterotachy models (explained later)
- Missing data are represented with ???? => The impact of missing data is relatively low if the alignment is sufficiently large (Roure *et al*, Mol Biol Evol, 2013)
- Works relatively fine when the sampling (genes and species) is good.

The supermatrix approach (4)

- Advantages/disadvantages :
  - (+) Minimize stochastic errors
  - (-) Long computation time and high memory usage for very large datasets
  - (-) It only sets a model and parameters for this model for all the superalignment
  - (-) Even the most complex model of sequence evolution cannot yet account for the complexity in superalignments (increases the systematic bias)
  - (-) Sensible to the relative sizes of datasets. For instance, if two data sets conflict, the supermatrix is dominated by the signal of the biggest one

Mixture model (for proteins)

- Mixture model allowing that amino-acid replacement pattern at different site of a protein alignment to be described by distinct substitution processes.

A Mixture model: CAT Model (Lartillot & Philippe, 2004)

- Distinct classes (categories) differing by their equilibrium frequencies over the 20 residues.
- The number of classes, their respective amino-acids profiles and the affiliation of each site to a given class are variables in the models.
- CAT model is designed to better capture the heterogeneity in the substitution pattern
- → introduced only in a Bayesian context. In addition, these mixture models only perform well on large alignments.

A Mixture model: CAT Model adaptation (Le at al, 2008)

- Adapted to ML
- Adapted to small alignment
- C10 to C60 (number of classes). 20 is often enough in the ML framework.
- Describes better saturated data
- Decrease systematic errors by relaxing the assumption of substitutional homogeneity along the sequence

A Mixture model: GHOST Model (Crotty at al, 2017)

- The General Heterogeneous evolution On a Single Topology (GHOST) model
- More specifically, GHOST is an *edge-unlinked mixture model* consisting of several site classes, each having a separate set of model parameters and edge lengths on the same tree topology.
- The GHOST model does not require the *a priori* data partitioning, a possible source of model misspecification.

Partitioned / mixed models

- The user partitions the supermatrix, these methods applies appropriate models and their specified parameter estimates to each data partition and subsequently incorporate this into a single tree search.

- Like ModelFinder can be used to find the best-fit model for the data, PartitionFinder2 (Cognato AI, Vogler AP, 2001) can be used to find the best-fit partition model.

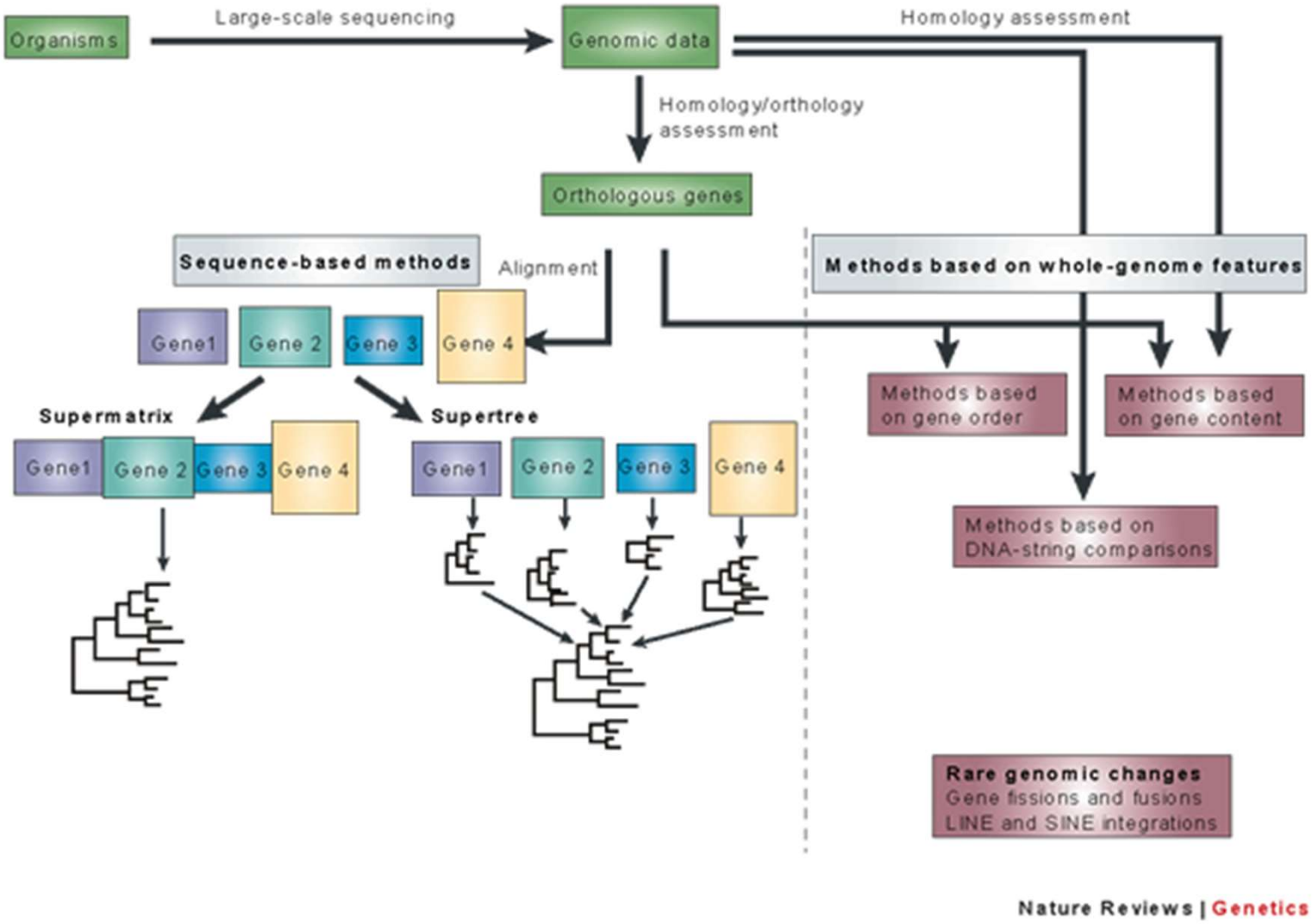
Partitioned / mixed models

- They introduce a huge number of parameters and this may result in over-parametrized models as unadapted as the under-parametrized “concatenate” one.

⇒ implemented in MrBayes 3 and in IQ-TREE (ML)

- Bayesian analysis is able to deal with higher dimensional models than ML.

Phylogenomic analysis : the methods



(From Delsuc *et al*, Nature reviews, 2005)

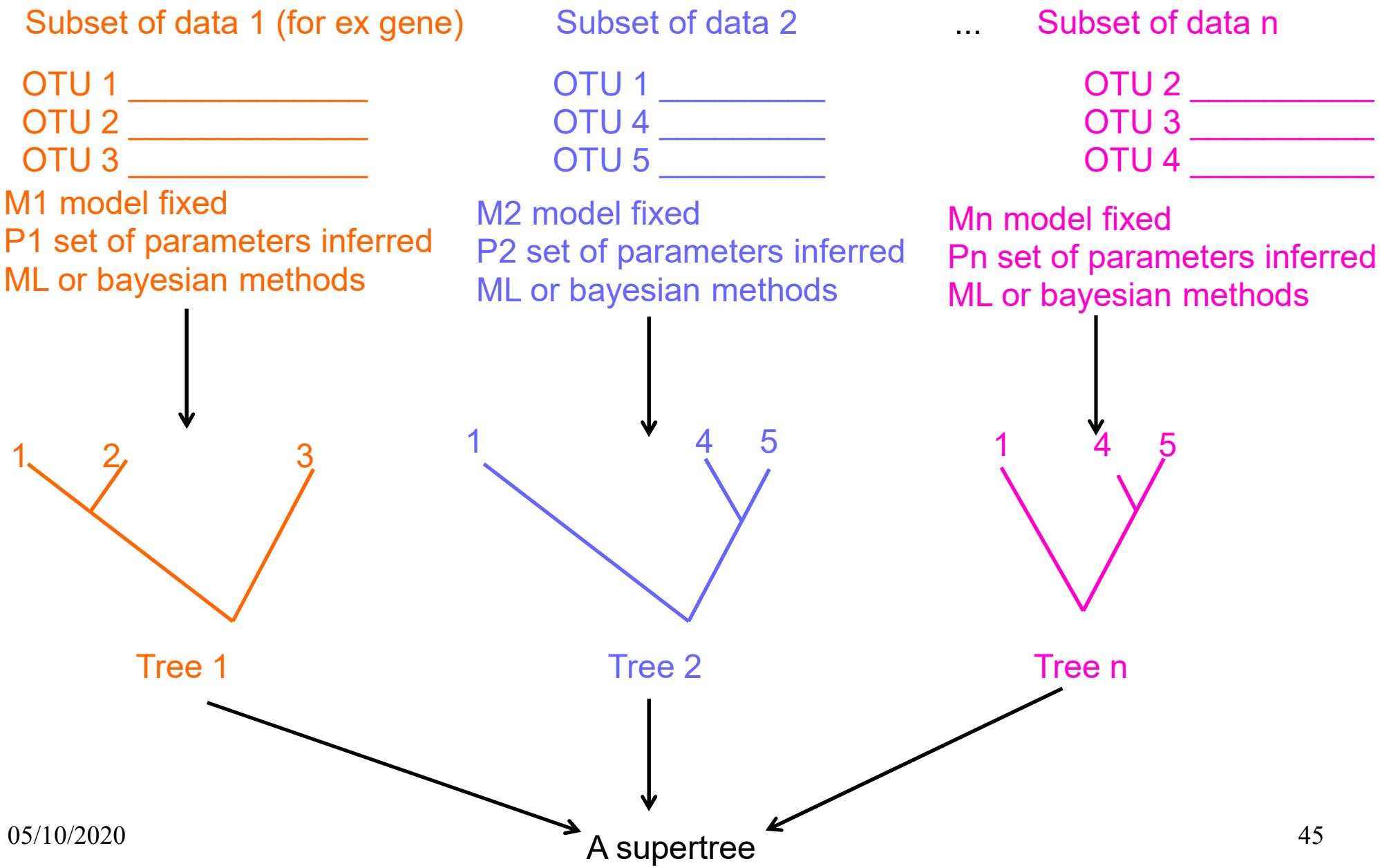
Sequence-based methods

- Supermatrix approach
- **Supertree approach**
  - **Consensus**
  - Other supertree approach

Some characteristics of supertrees

- Meta-analysis: analyses of smaller datasets are combined
- Input trees can be based on different kinds of data (e.g. morphology, DNA-DNA hybridization) and they can be obtained by different methodologies
- Can be used to build very large phylogenies for partially overlapping analyses

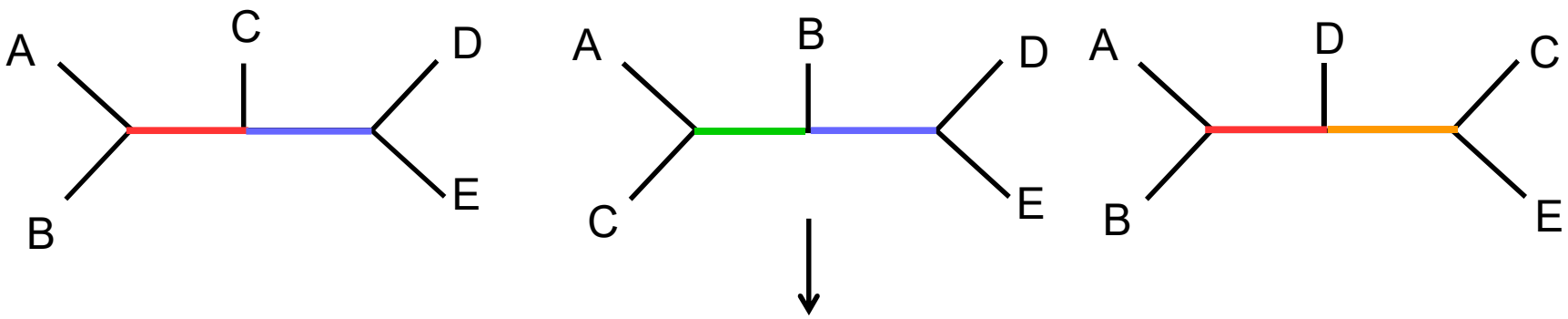
The supertree approach



Consensus Tree

- Used to test the tree robustness and for the bootstrap
- For example :
  - Strict consensus tree: a bipartition will be included if it's present in all input trees (cannot handle incompatible source trees)
  - Majority consensus tree: a bipartition will be included if it's present in more than half of the input trees (conflict resolved by vote method)
  - Majority Rule (extended) tree: Do a majority consensus tree. Then the other sets of species in order of the frequency with which they have appeared, adding to the consensus tree any which are compatible with it until the tree is fully resolved.

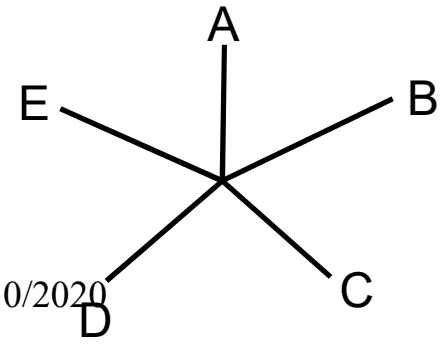
Consensus Tree (2)



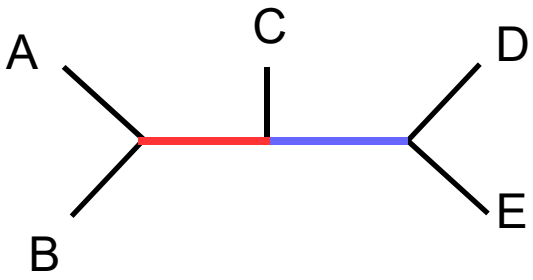
Weighted bipartitions

A, B | C, D, E 2
A, B, C | D, E 2
A, C | B, D, E 1
A, B, D | C, E 1

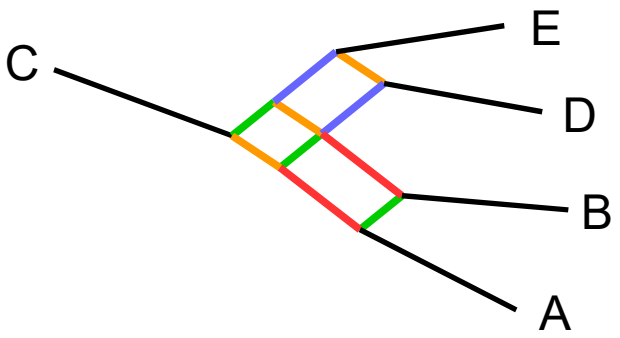
Strict consensus (100%)



Majority consensus (50%)



Consensus networks (≥ 33%)



(Holland & Moulton, Algorithms in bioinformatics, 2003)

Diapositive 47

CH1 Claire Hoede; 19/10/2019

Network Tree

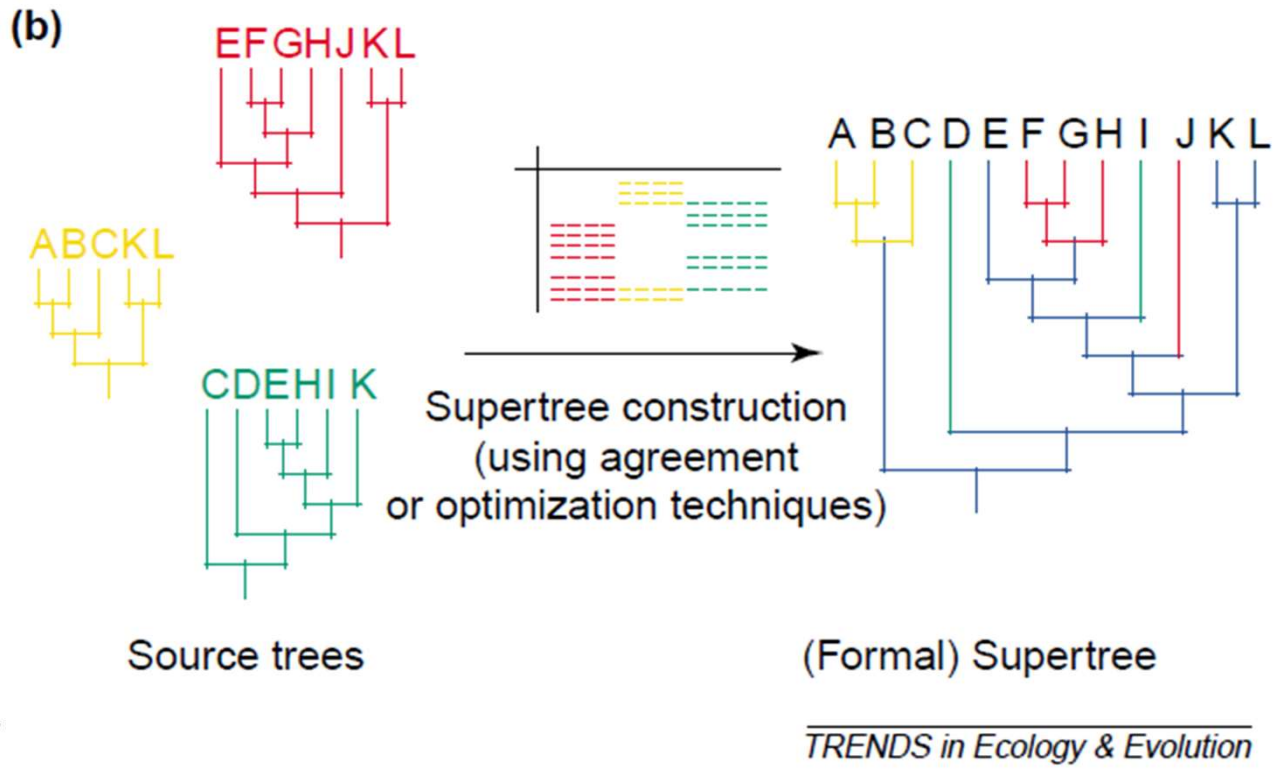
- Consensus network is one method to build network tree.
- Splitstree, for example, is a program for computing unrooted phylogenetic networks from molecular sequence data http://www.splitstree.org/, (Huson & Bryant, 2006).
- Phylogenetic networks should be looked when hybridization, horizontal gene transfer, recombination or gene duplication and losses are involved (could be induce split incompatibility).

Sequence-based methods

- Supermatrix approach
- **Supertree approach**
  - Consensus
  - **Other supertree approaches**

Supertree methods

- Identical taxons sets are not needed (# consensus).
- Start with a set of trees constructed independently and not with an alignment (# super matrix method)



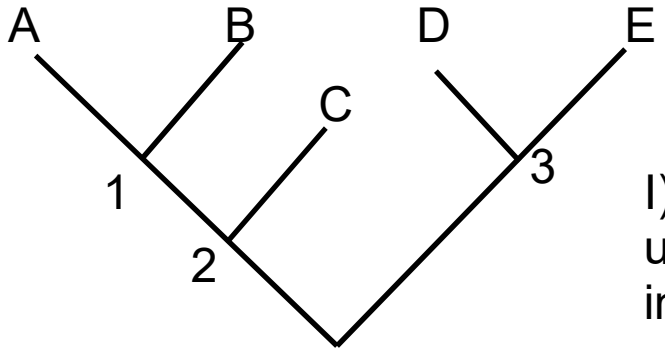
(Bininda-Emonds O., 2004)

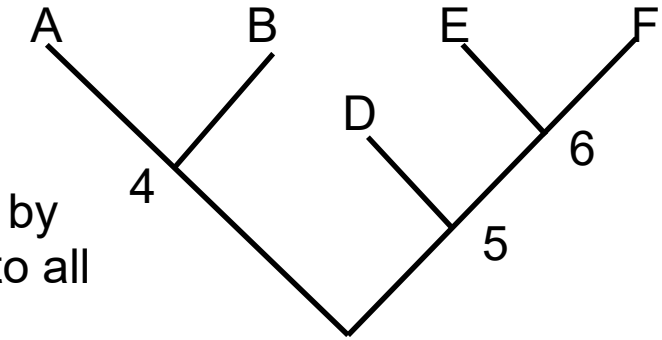
Matrix representation using parsimony (MRP)

- This is the most common method
- It's a vote method :
  - The hope is that each taxon is erroneously placed in only few source
  - Trees are highly resolved and accurate, but can lead to propose supertrees containing clades that contradict all source trees
- MRP needs a matrix representation

(Bininda-Emonds O., Trends in ecology and Evolution, 2004)

Build a super-tree MRP



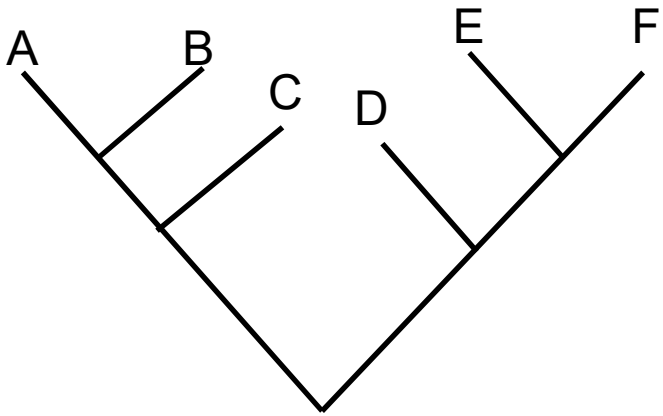


I) Input trees are rooted by using a taxon common to all input trees

II) Binary matrix representation (Baum and Ragan, 1992)

	1	2	3	4	5	6
A	1	1	0	1	0	0
B	1	1	0	1	0	0
C	0	1	0	?	?	?
D	0	0	1	0	1	0
E	0	0	1	0	0	1
F	?	?	?	0	1	1

III) Super-trees MRP (Parsimony)



1: species share a common node
0: species do not share a common node
?: species not present in tree

PhySIC & PhySIC_IST

- It's a veto method :
  - the phylogenetic information of every source topology have to be respected,
  - and the supertree is not allowed to contain clades that a source tree would vote against
  - these methods remove conflicts either proposing multifurcations in the supertree or pruning rogue taxa

Ranwez V., et al. ; Systematic Biology. 2007 56(5):798-817.
Scornavacca C., et al. ; BMC Bioinformatics. 2008, Oct 4;9:413.

PhySIC & PhySIC_IST

- **Non-contradiction property:** supertrees must not containing clusters that conflict either directly with a source tree or indirectly with a combination of them (PC)

- **Induction property**: every piece of phylogenetic information displayed in the supertree is present in one or several source topologies, or induced by their interaction (PI)

PhySIC & PhySIC_IST

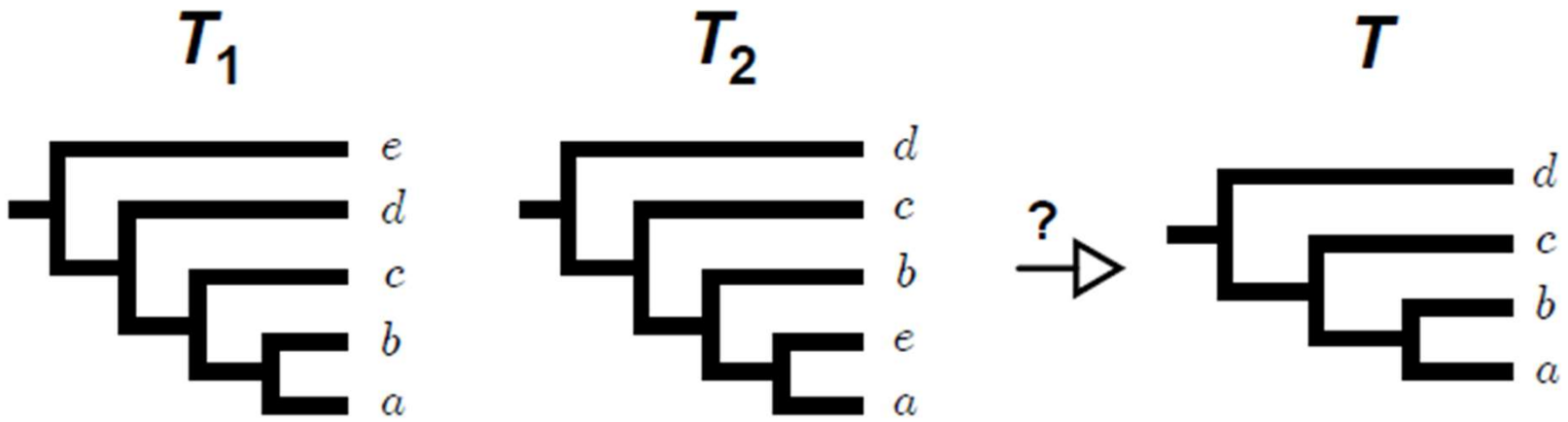


Figure 4.1: **An example of informative non plenary supertree for a forest of two rooted trees** - Excluding rogue taxa from the analysis can lead to more informative supertrees.

Phylogenetic Signal with Induction and non-Contradiction

- The aim of PhySIC is to infer supertrees that satisfy PI and PC and that resolve as many triplets as possible. It consists in two steps:
  - given a forest of rooted trees F, a supertree T_{PC} satisfying PC for F is computed by the $PhySIC_{PC}$ algorithm.
  - some branches of T_{PC} are eventually collapsed by the $PhySIC_{PI}$ algorithm until the so-modified T_{PC} satisfies also property PI.

Super Tree methods: advantage / disadvantage

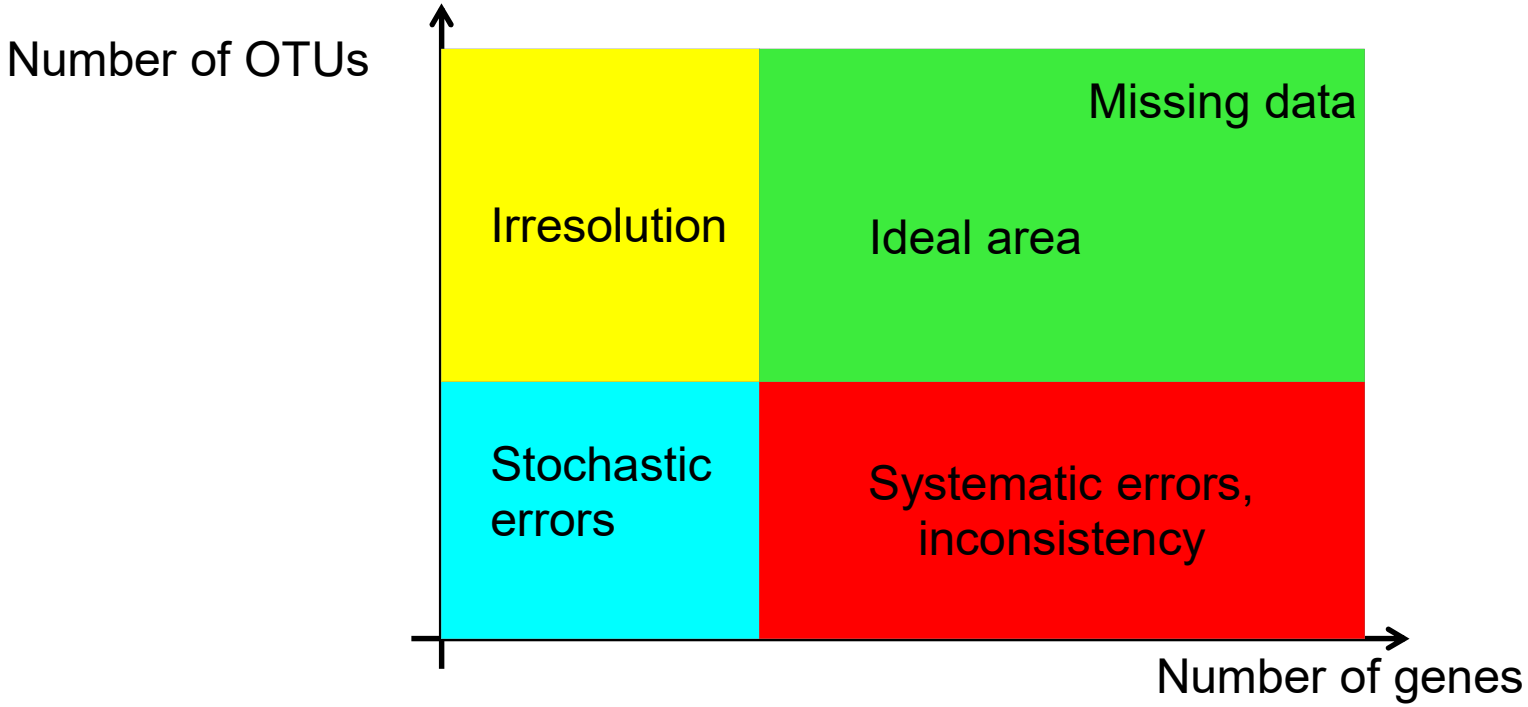
- (-) The length of branches are not directly interpretable in terms of evolutionary distance
- (-) Most methods weigh poorly-supported and well-supported input trees equally
- (-) Input trees must be rooted properly
- (-) If input trees are clashing in their topologies ⇒ supertree resolution is too low
- (+) It's faster for very large dataset that super matrix approach
- (+) Phylogeny of each gene is made with the appropriate model and parameters and/or methods

Compare trees with metrics

- Robinson & Foulds (symmetric difference metric): Sum of the specific bipartitions for each two trees (treedist)
- Branch score distance: using the branch length (treedist)
- In a likelihood framework (tree-puzzle, RaxML, CONSEL, IQ-TREE) :
  - The SH test (Shimodaira and Hasegawa, 1999)
  - Two-sided KH test (Kishino and Hasegawa, 1989), the one-sided KH test (Goldman et al., 2000)
  - Expected likelihood weights (Strimmer and Rambaut 2002)
  - AU tests (Shimodaira, 2002)

To conclude

- The phylogenomic is still a research domain (methods and analysis)
- Test several models and methods for testing the robustness of the tree produced (computationally intensive)
- Be aware of sampling problems



Stochastic and systematic errors

- Stochastic errors are sampling errors caused by a too small sample. To measure it, it's possible to use resampling method bootstrap or jackknife.

- Systematic errors appears when the evolutionary process violates the assumptions of model used for phylogenetic reconstruction.

  ⇒ To reduce it we need to reduce the non-phylogenetic signal: eliminate species with rapid evolution, remove positions saturate with multiple substitutions, make a recoding or try to use a more complex model (partitioned and / or mixture) ...

Methods and use cases

Class Methods	Methods	Use Case
Based on whole genome features => No need to align sequences => Avoid the signal saturation at sites	Genome signature	Unknown species
	Gene Content	Large evolutionary scale Doesn't need orthology inference
	Gene Order	Large evolutionary scale in Eucaryotes Used for organelles
Based on sequences => need to align sequences	Supermatrix	Individual genes have not enough signal Phylogenetic signal is assumed majority
	Supertree	Individual genes have enough signal Heterogeneous dataset Very big dataset if you're using simple methods

References

- Scientifique articles cited in the slides
- Presentation :
  - M2 – Phylogénomique. Frédéric Delsuc : Equipe de Phylogénie et Evolution Moléculaire, Institut des Sciences de l'Evolution de Montpellier
- Thèses :
  - Béatrice Roure soutenue en 2011 : « Amélioration de l'exactitude de l'inférence phylogénomique »
  - Céline Sconavacca soutenue en 2009 : « Supertree methods for phylogenomics »

Différences ML et bayèsien

http://genoweb.toulouse.inra.fr/~formation/M2_Phylogenomique/2018_supports/CoursPhylogenie2018.pdf

Le maximum de vraisemblance (page 64 et suivantes) est la probabilité d'observer les données sachant le modèle et l'arbre.

L'inférence bayésienne (page 72 et suivantes) calcule une probabilité postérieure de l'arbre et des paramètres du modèle sachant les données. Et donc, c'est plus intuitif comme probabilité.

Le théorème de Bayès explicite bien la différence (page 73).