Biodiversity Bioinformatics: from large -scale phylogenomics to gene families and functions

DAY 2: August 31st 2023

Robert M. Waterhouse

Department of Ecology & Evolution, University of Lausanne, Swiss Institute of Bioinformatics, Switzerland

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Swiss Institute of Bioinformatics

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robert.waterhouse@gmail.com @rmwaterhouse www.rmwaterhouse.org

Instructor Biography -Introduction

2023-	Director, Environmental Bioinformatics Group SIB Swiss Institute of Bioinformatics
2017-23	SNF Assistant Professor University of Lausanne
2015-16	Marie Curie Fellow & Maître assistant University of Geneva *ZDOBNOV*
2013-14	Marie Curie Outgoing Fellow Massachusetts Institute of Technology *KELLIS*
2009-12	Postdoctoral Researcher University of Geneva *ZDOBNOV*
2005-09	Wellcome Trust PhD Imperial College London *CHRISTOPHIDES*
2004-05	Wellcome Trust MSc Bioinformatics Imperial College London
2000-04	MBioch Biochemistry University of Oxford

Teaching Assistants

Antonin
Thiébaut

Giulia
Campli

Goals for Today's Workshop

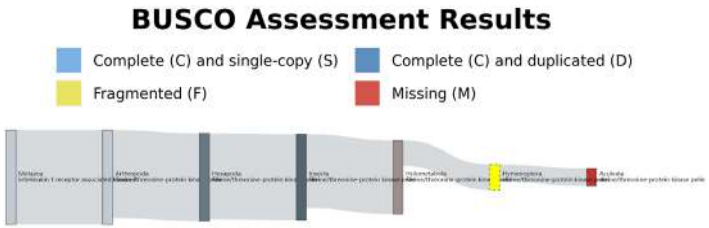
- ❑ Understand the principles of graph-based orthology delineation using OrthoDB as an example
- ❑ Learn how to browse and query OrthoDB
- ❑ Learn how to use BUSCO to assess genomics data
- ❑ Learn how to formulate comparative genomics questions, develop and apply approaches to address them (with a focus on using orthology data), and then critically interpret them, through case studies from arthropods

OrthoDB

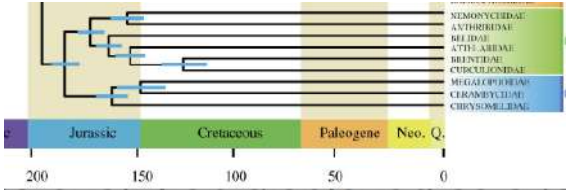
BUSCO

Comparative Genomics Hands -On: Concepts and Applications

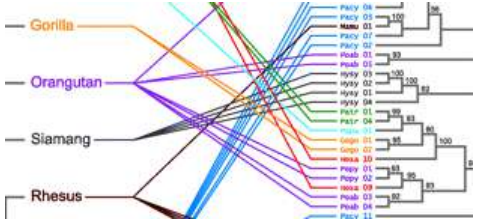
OrthoDB orthology and BUSCO quality



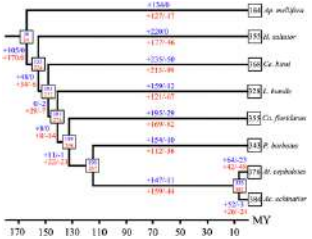
Species & gene tree estimation



Gene-tree-species-tree reconciliation



Gene ancestral state reconstruction



Quick Quiz

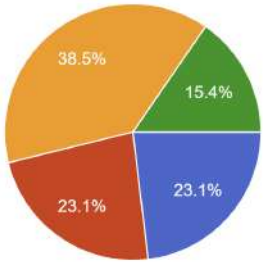
https://forms.gle/YwgAwDSsKwmJRRig7

SwissOrthology

How familiar are you with OrthoDB, the hierarchical catalogue of orthologues?

Copy

13 responses

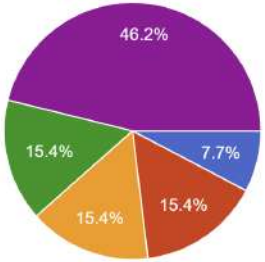


- I have never heard about OrthoDB
- I have heard about OrthoDB, but never visited the website
- I have visited the OrthoDB website, but not really used it much
- I have used OrthoDB data a bit in my research
- I have used OrthoDB data a lot in my research

How familiar are you with BUSCO, the Benchmarking Universal Single-Copy Orthologues?

Copy

13 responses



- I have never heard about BUSCO
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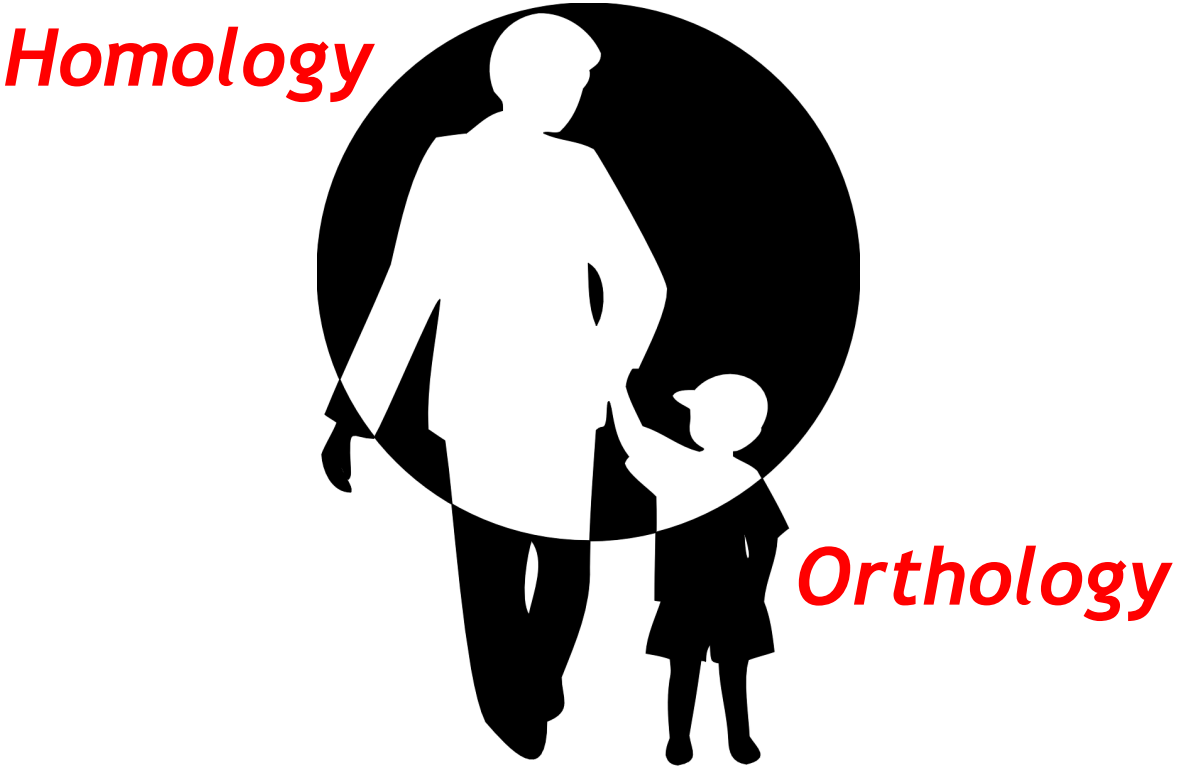
Orthology Delineation

What is orthology?

How do we delineate orthologs?

And why do we need to?
(species/gene trees/copy-number)

Orthology – what is it?



Orthology – what is it?

Homology

"designates a relationship of **common descent** between any entities, without further specification of the evolutionary scenario"

Orthologs, Paralogs, and Evolutionary Genomics[1]

Eugene V. Koonin

Annu. Rev. Genet. 2005. 39:309–38

Orthology – what is it?

"genes originating from a single ancestral gene in the last common ancestor of the compared genomes"

Orthology

Orthologs, Paralogs, and Evolutionary Genomics[1]

Eugene V. Koonin

Annu. Rev. Genet. 2005. 39:309–38

Orthology – what is it?

"paralogs are
genes related via duplication"

Paralogy

Orthologs, Paralogs, and Evolutionary Genomics[1]

Eugene V. Koonin

Annu. Rev. Genet. 2005. 39:309–38

Orthology – what is it?

Homologs

Common Ancestor

Orthologs

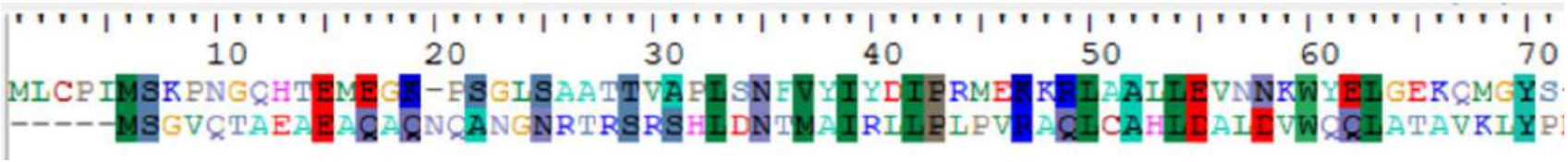
Speciation Event

Paralogs

Duplication Event

Sequence Homology – what is it?

Homology between protein or DNA sequences is typically inferred from their sequence similarity



Sequence homology search tools, e.g. BLAST, attempt to detect '**excess**' similarity

i.e. greater similarity or identity than expected by chance

=> statistically significant similarity

Sequence Homology – what is it?

"the link between **similarity** and **homology** is often misunderstood"

An Introduction to Sequence Similarity ("Homology") Searching

William R. Pearson[1]
[1]University of Virginia School of Medicine, Charlottesville, VA

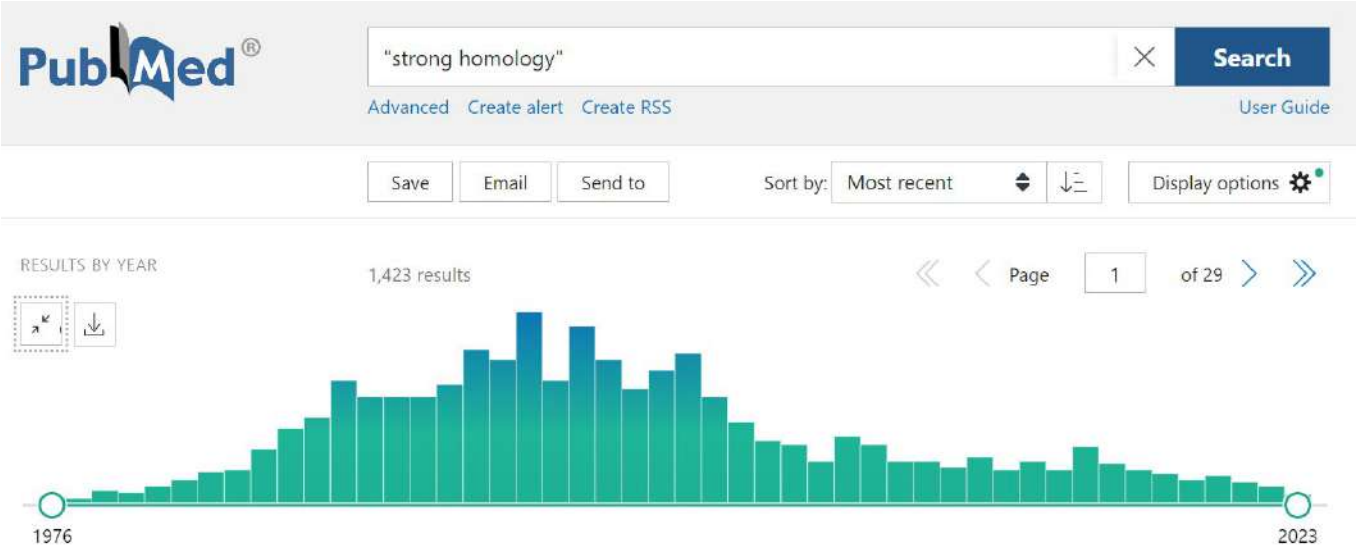
A pair of sequences can have **high** or **low** sequence similarity

But this does not translate to **strong** or **weak** homology!

Homology is the **conclusion**, i.e. given the level of similarity the sequences are likely to have arisen from a common ancestor

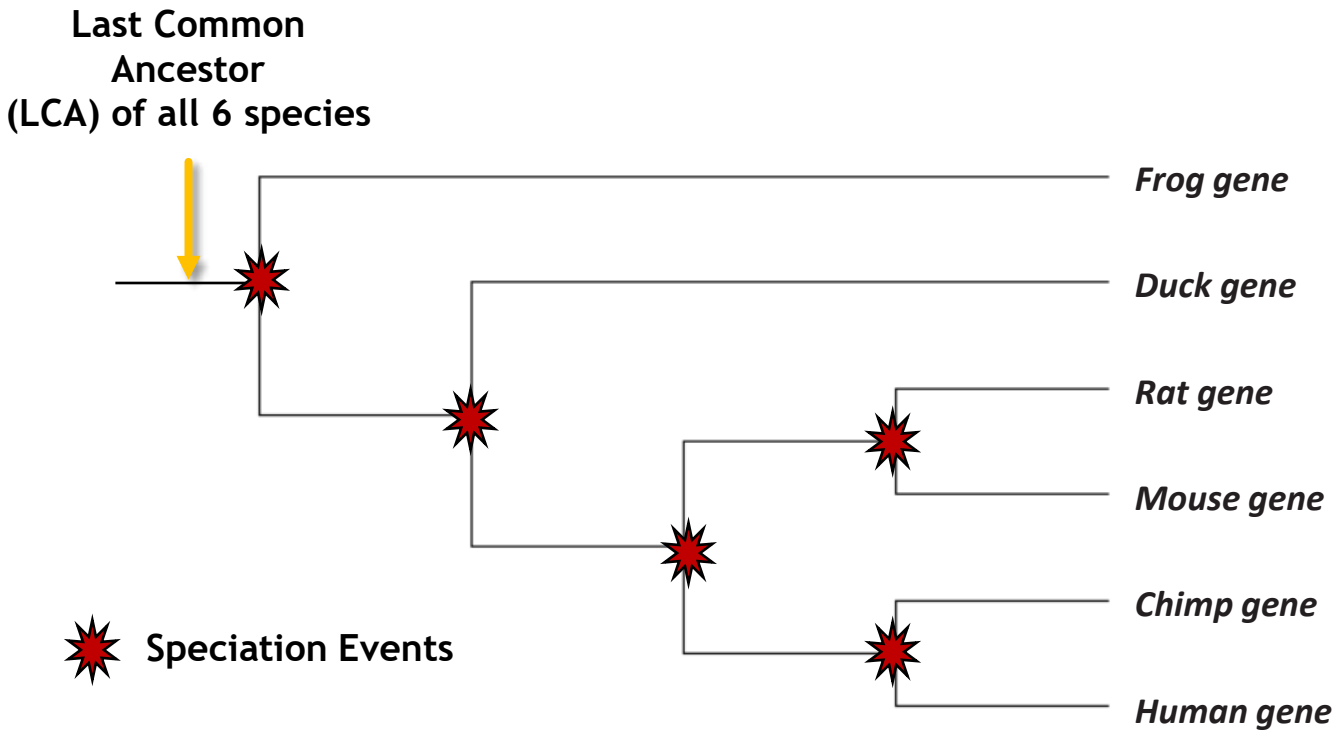
Sequence Homology – what is it?

"the link between **similarity** and **homology** is often misunderstood"



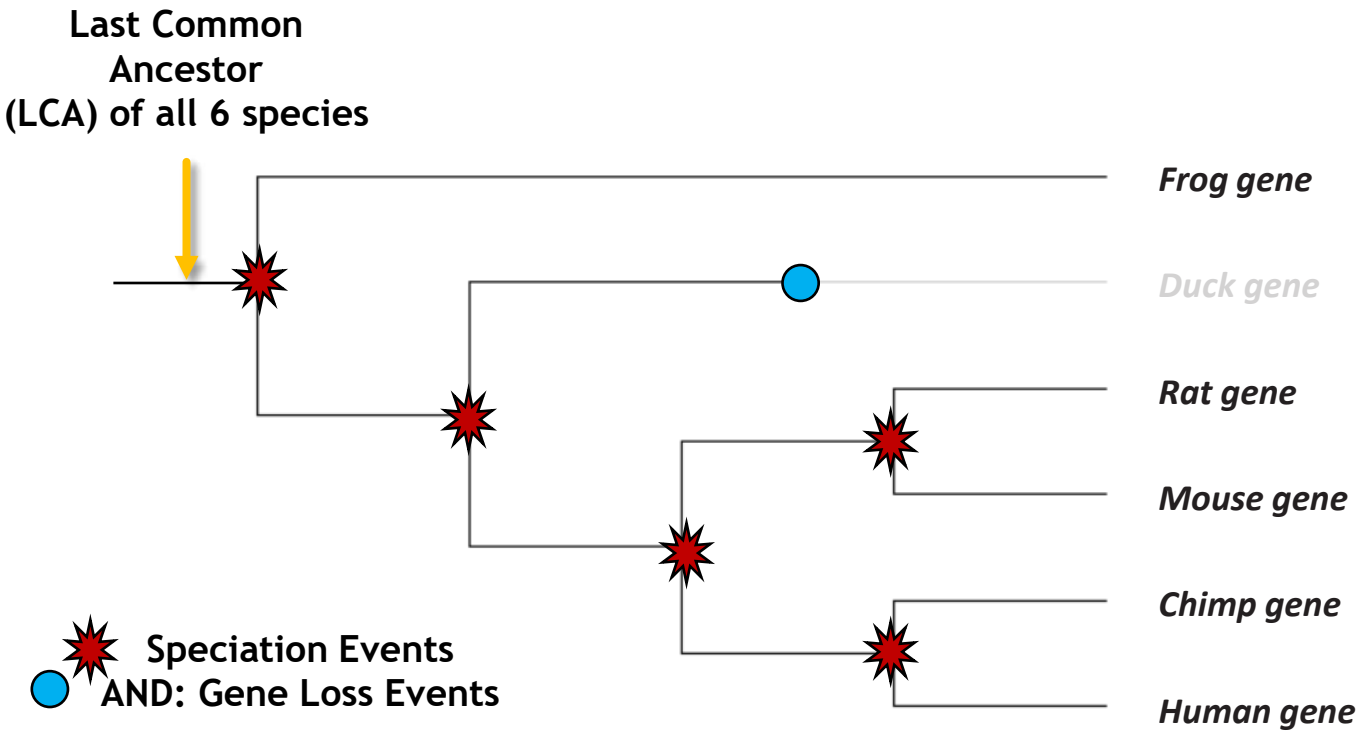
It is still worth pointing this out in 2023!

Orthology – simple scenario



Single-Copy Orthologs

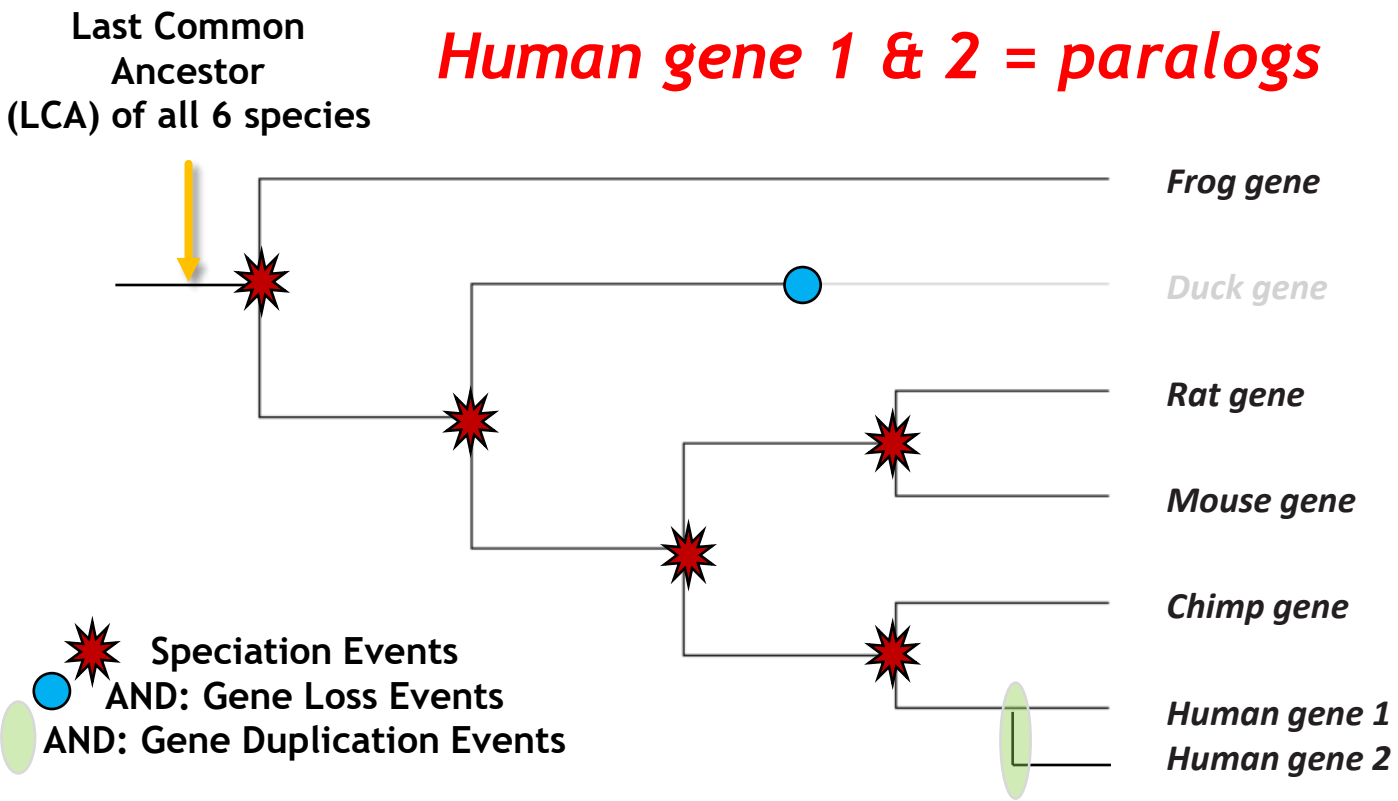
Evolution ≠ simple



Single-Copy Orthologs with Losses

Evolution ≠ simple

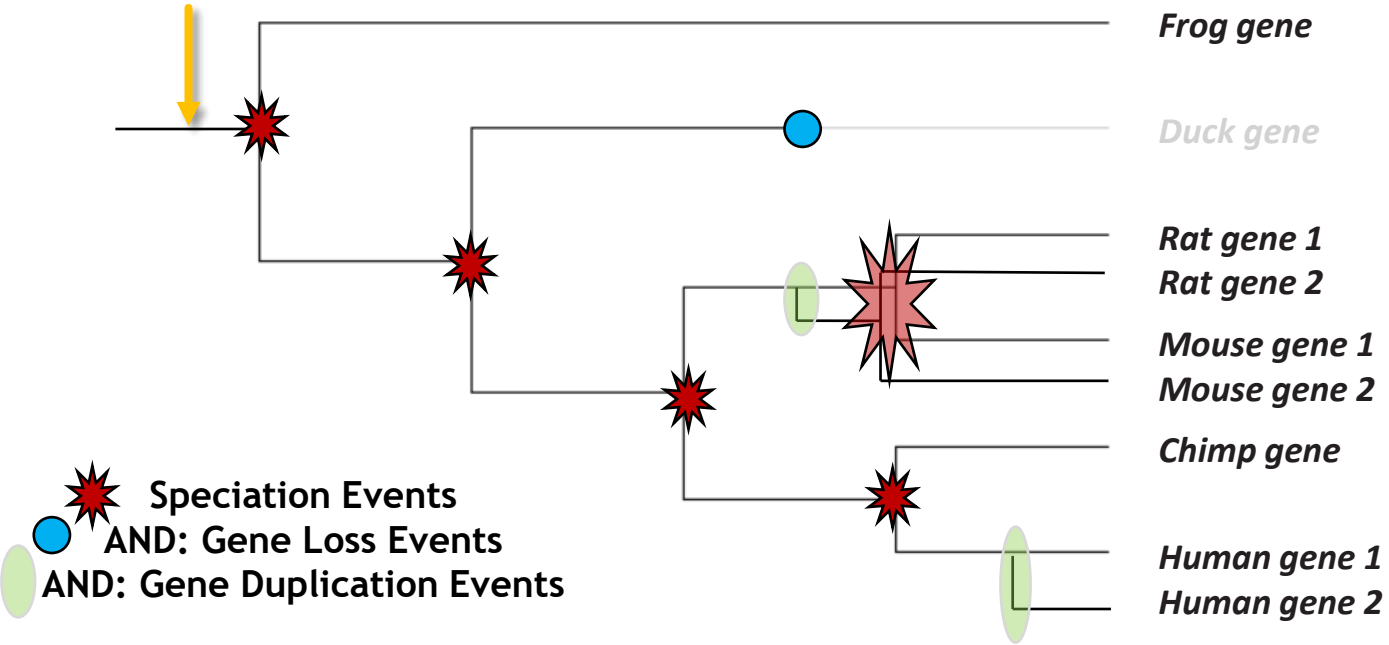
Human gene 1 & 2 = paralogs



Single-Copy Orthologs with Gains

Evolution ≠ simple

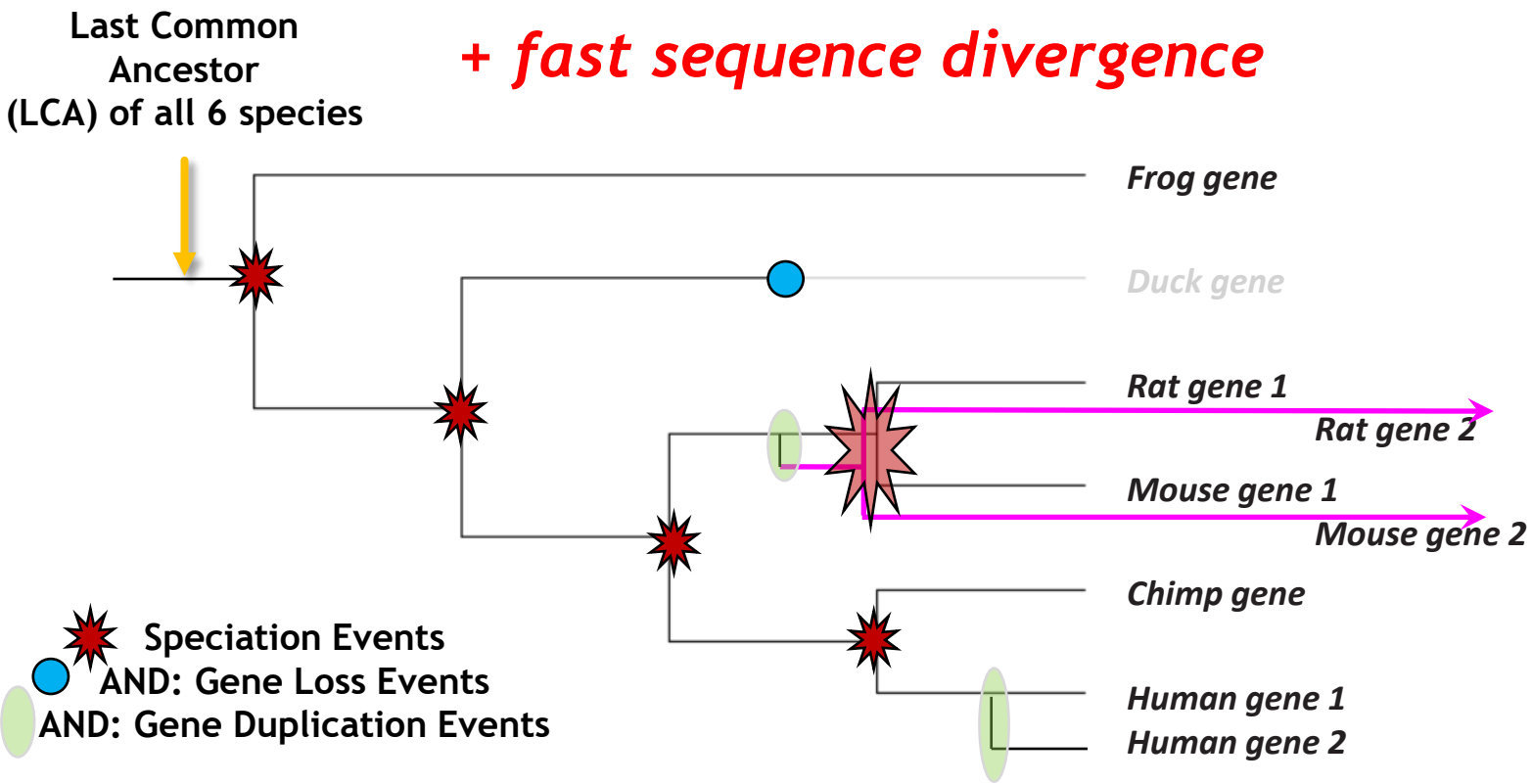
Rat gene 1 & 2 = paralogs
Mouse gene 1 & 2 = paralogs



Single-Copy Orthologs with Gains

Evolution ≠ simple

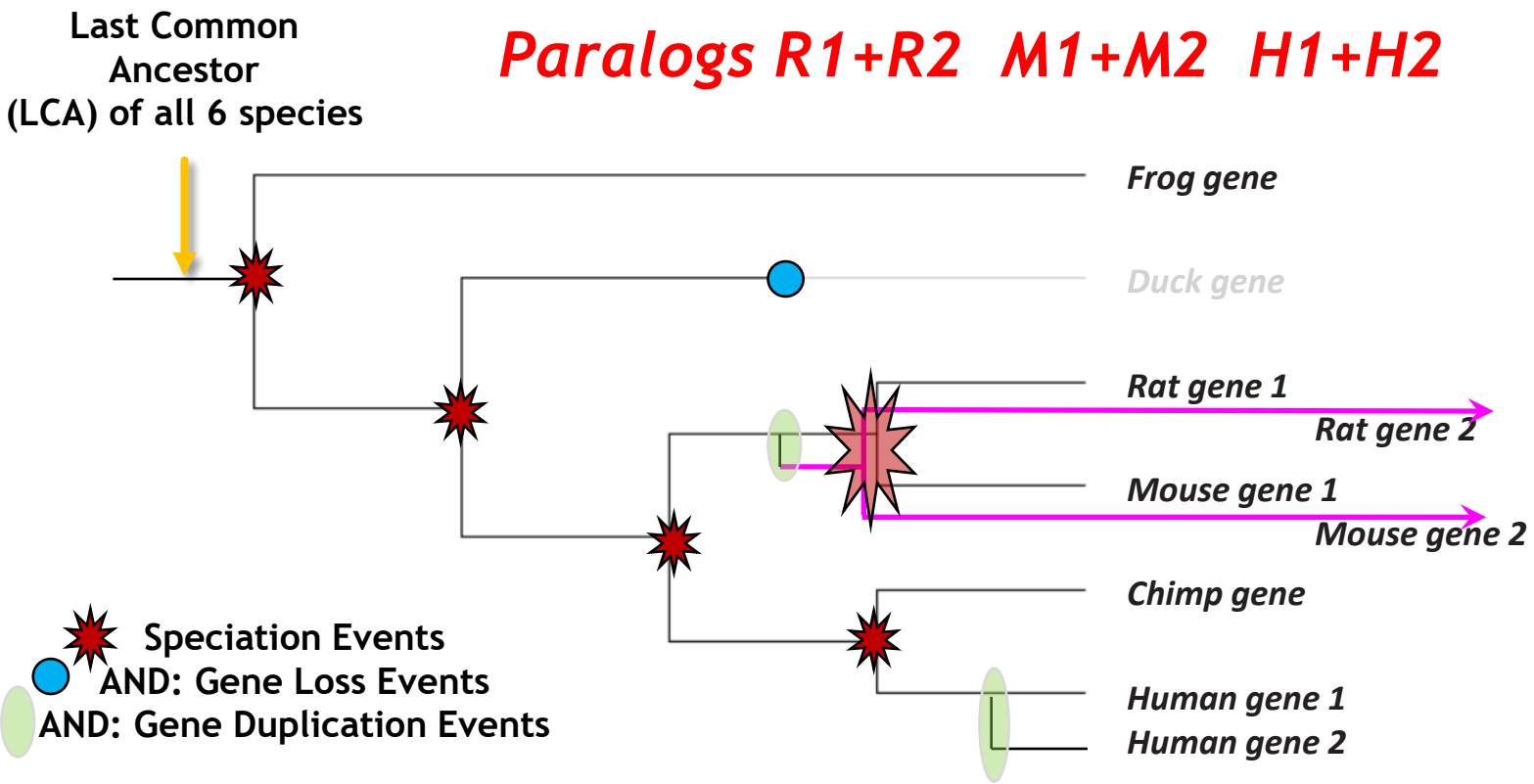
+ fast sequence divergence



Single-Copy Orthologs with Gains

Evolution ≠ simple

Paralogs R1+R2 M1+M2 H1+H2



Orthologs F+R1+R2+M1+M2+C+H1+H2

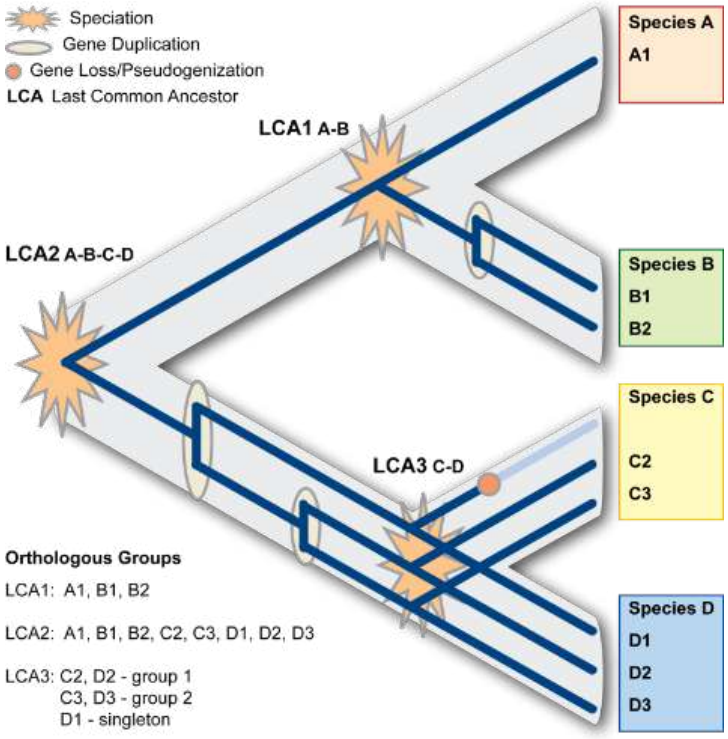
Orthology – what is it?

Homology
Recognizing similarities as evidence of shared ancestry

Orthology
Orthologues arise by vertical descent from a single gene of the last common ancestor

Hierarchy
Orthology is relative to the species radiation under consideration

Orthologous Groups
All genes descended from a single gene of the last common ancestor



Nucleic Acids Research

OrthoDB: the hierarchical catalog of eukaryotic orthologs in 2011

Robert M. Waterhouse[1,2], Evgeny M. Zdobnov[1,2,3], Fredrik Tegenfeldt[1,2], Jia Li[1,2] and Evgenia V. Kriventseva[1,2,*]

Orthology Delineation

What is orthology?

How do we delineate orthologs?

And why do we need to?
(species/gene trees/copy-number)

Orthology Delineation

OrthoDB v11

UNIVERSITÉ DE GENÈVE FACULTÉ DE MÉDECINE

SIB Swiss Institute of Bioinformatics

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Text | e.g. hsp70, sex-lethal, "cytochrome c", kinase -serine | ▶ Advanced | Submit

The hierarchical catalog of orthologs
mapping genomics to functional data

Eukaryotes	Prokaryotes	Viruses	Genes
1,952	**18,158**	**7,962**	**100M**

EZlab BUSCO LEMMI miRmap NewickUtils

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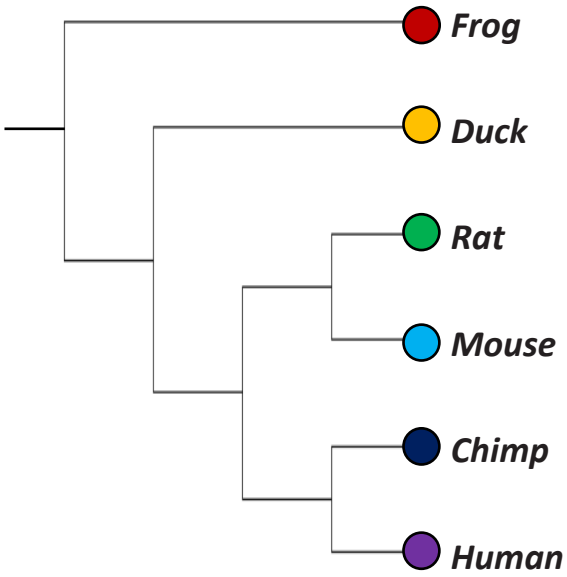
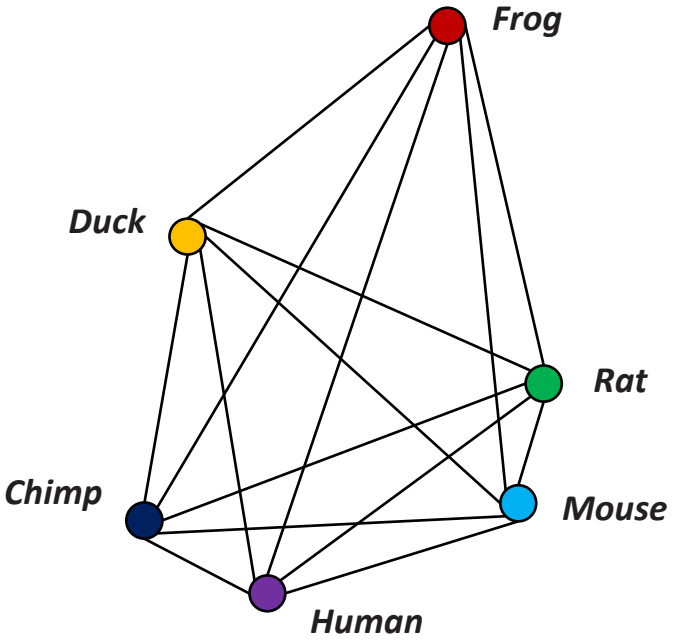
How do we delineate Orthology?

tree-based approaches

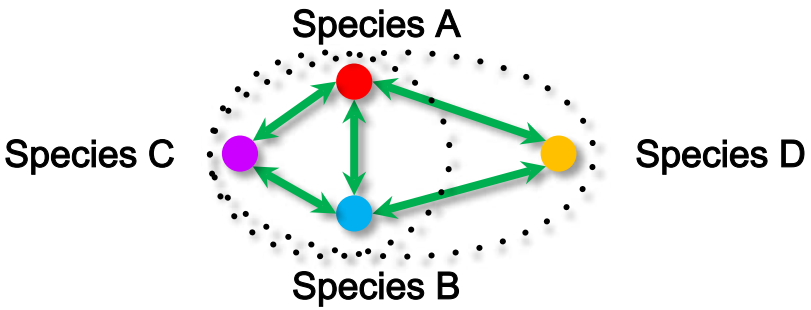


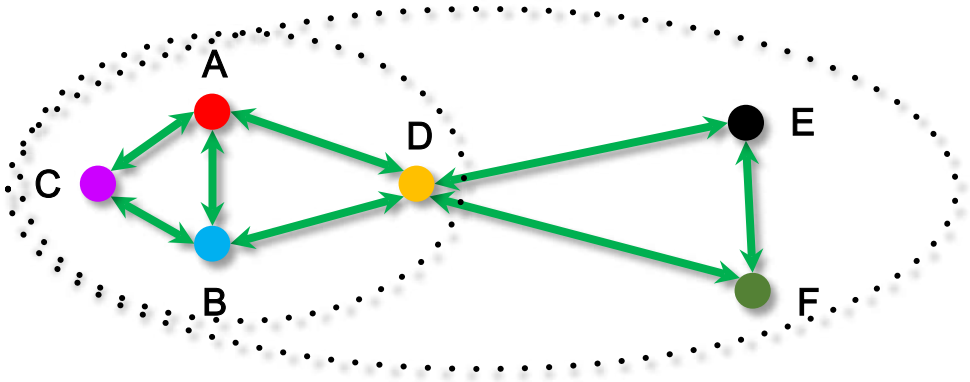
graph-based approaches



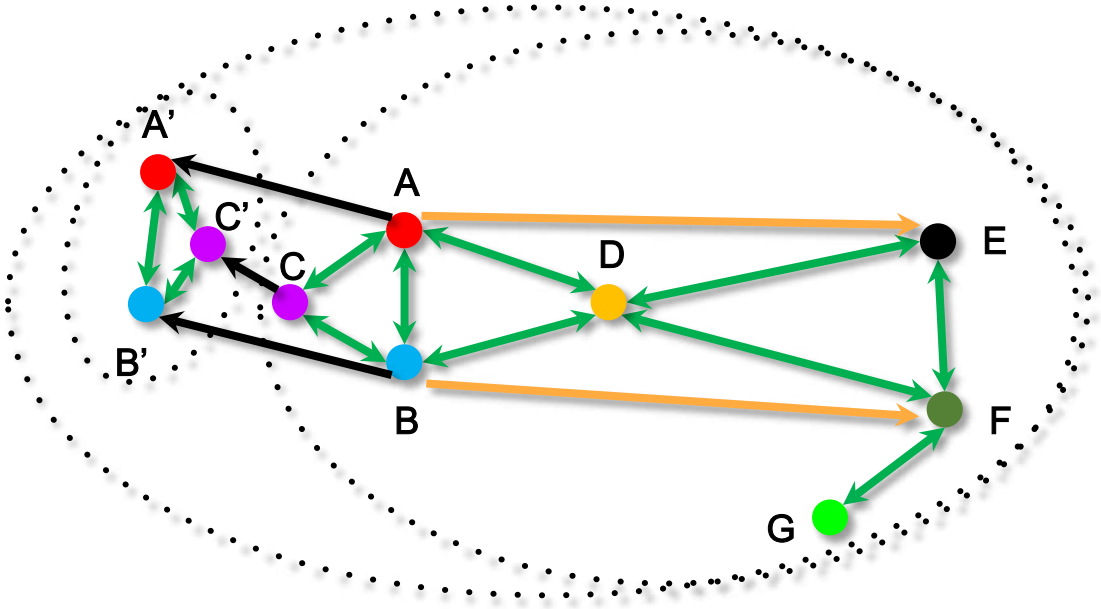
Single-Copy Orthologs

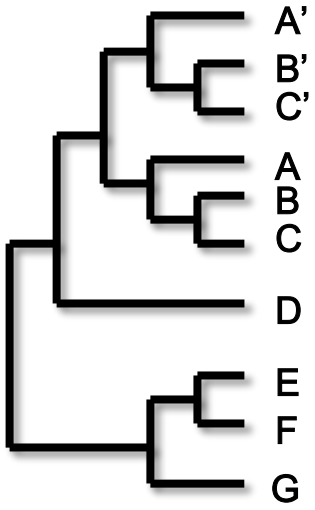
Graph-based best-reciprocal-hits





Within -clade duplications





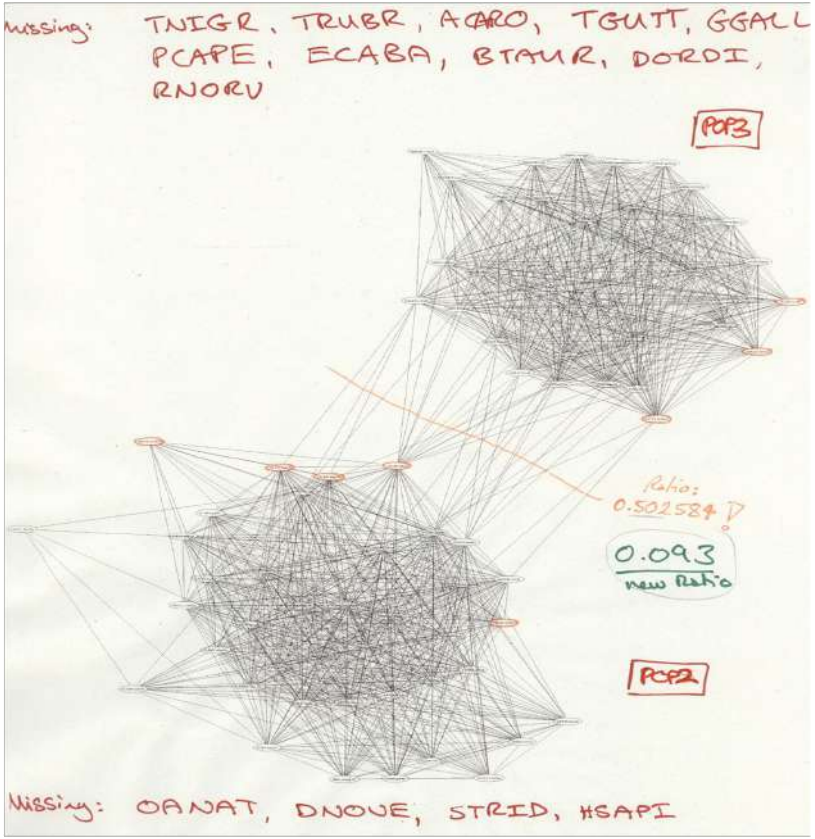
Real-world data can be messy!

Real example:

POP3 missing from 10 vertebrates

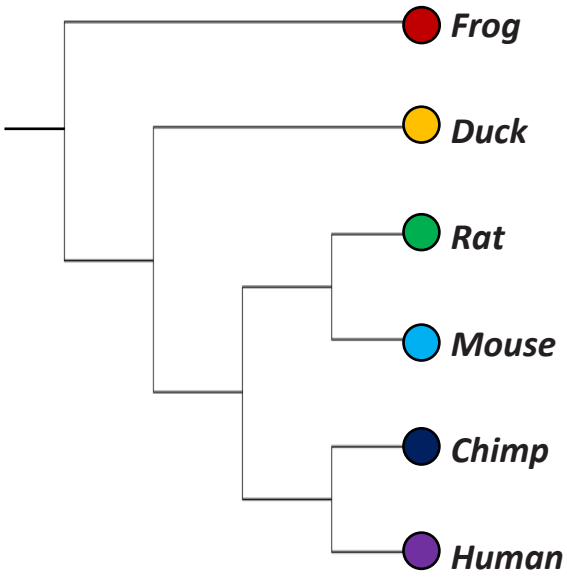
POP2 missing from 4 vertebrates

Two orthologous groups start to merge into one

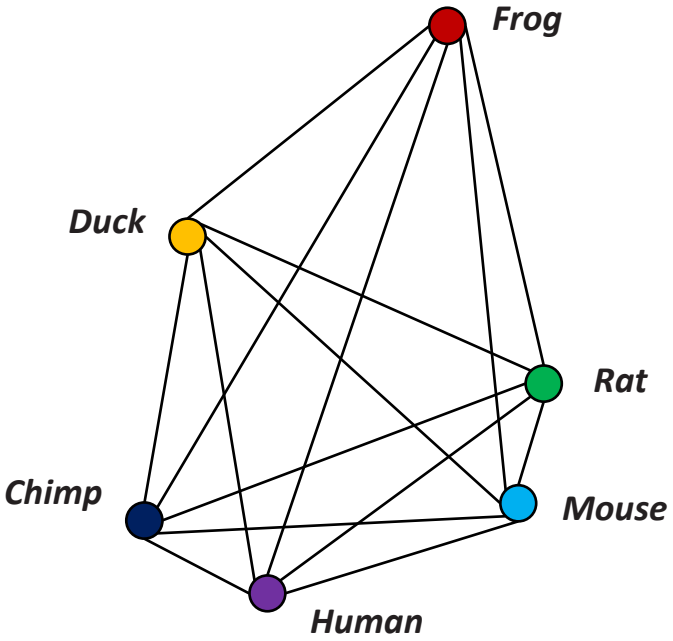


How do we delineate Orthology?

tree-based approaches



graph-based approaches



Single-Copy Orthologs

Orthology Delineation

What is orthology?

How do we delineate orthologs?

And why do we need to?
(species/gene trees/copy-number)

Quick Quiz

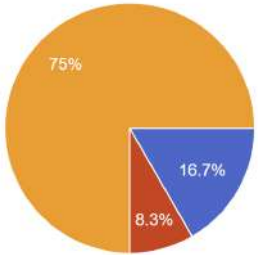
https://forms.gle/1nAyRyriwTNtuwvW6

SwissOrthology

Which description best describes your understanding of orthology? Orthologues are genes in different species ...

Copy

12 responses

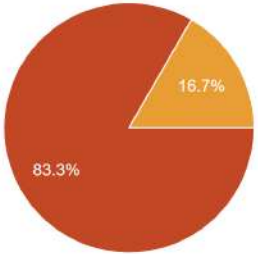


- ... that evolved from an ancestral gene without duplications or losses
- ... that perform the same specific biological function
- ... that evolved from a single gene in the last common ancestor
- ... that have the highest significant sequence homology
- ... that produce a gene tree that matches the species phylogeny

Which description best describes your understanding of how OrthoDB delineates orthology?

Copy

12 responses

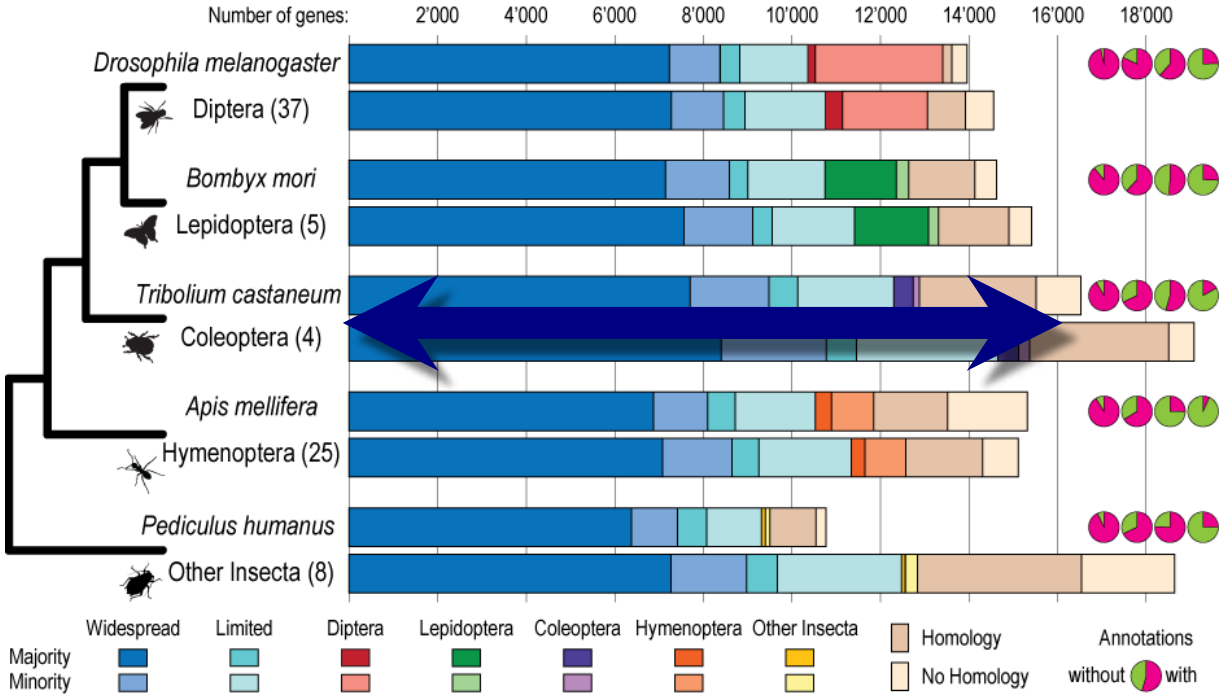


- Gene trees are reconciled with the known species tree to define speciations and duplications
- Best-reciprocal-hits determine how genes are progressively added to form orthologous groups
- The full all-against-all best-reciprocal-hit graph is progressively split to define groups of orthologues

Orthology – why do we need it?

1) Tracing the **Evolutionary Histories** of all genes in extant species

2) Building **Hypotheses on Gene Function** informed by evolution



Orthology ≠ Function … BUT …

By tracing the **Evolutionary Histories** of all genes in extant species

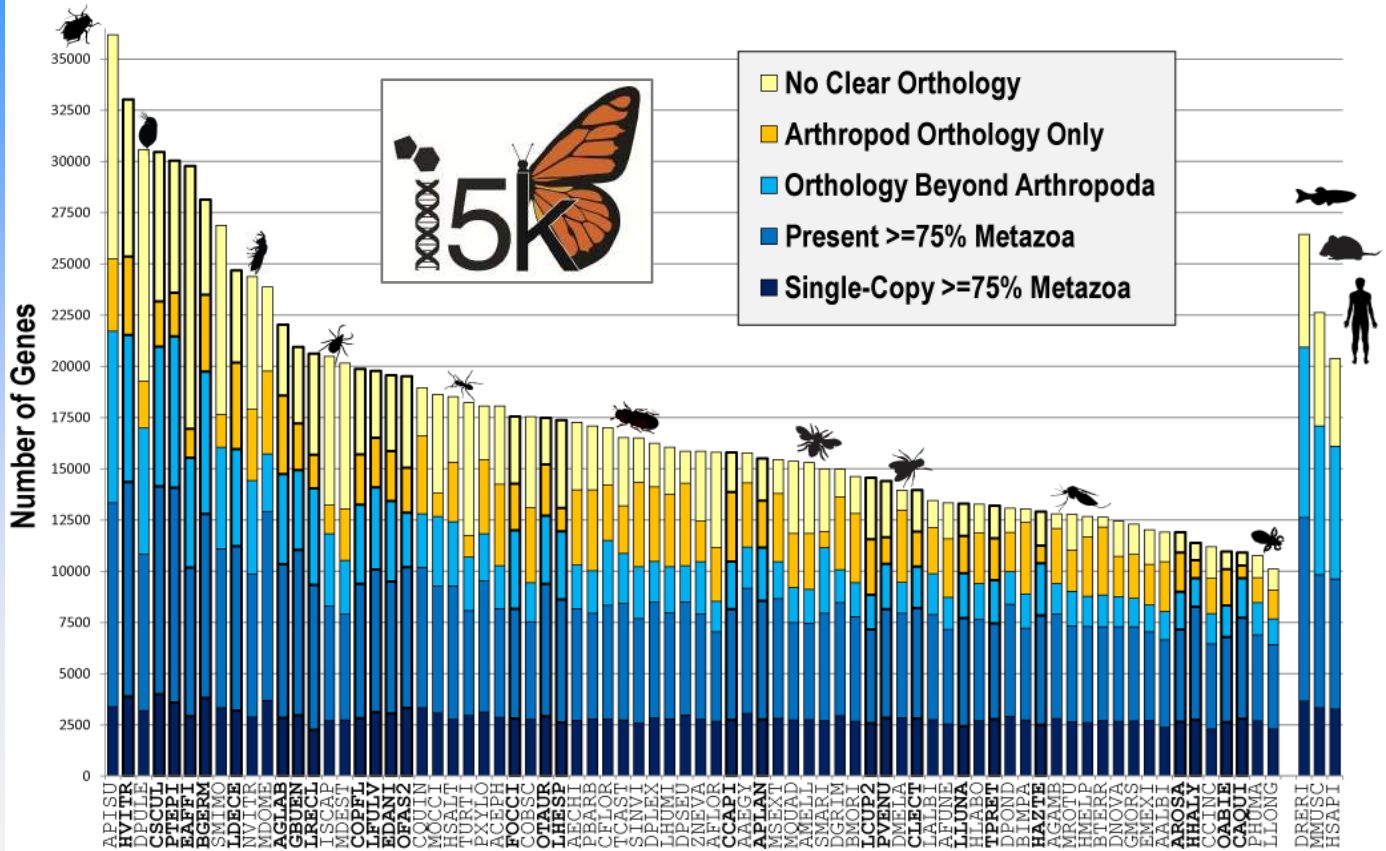
We can build **Hypotheses on Gene Function** informed by evolution

"validity of the conjecture on **functional equivalency** of orthologs is crucial for reliable annotation of newly sequenced genomes and, more generally, for the progress of functional genomics.

The huge majority of genes in the sequenced genomes will **never be studied experimentally**, so for most genomes **transfer of functional information** between orthologs is the only means of detailed functional characterization."

Annu. Rev. Genet.
2005. 39:309–38

Evolutionary histories: classes



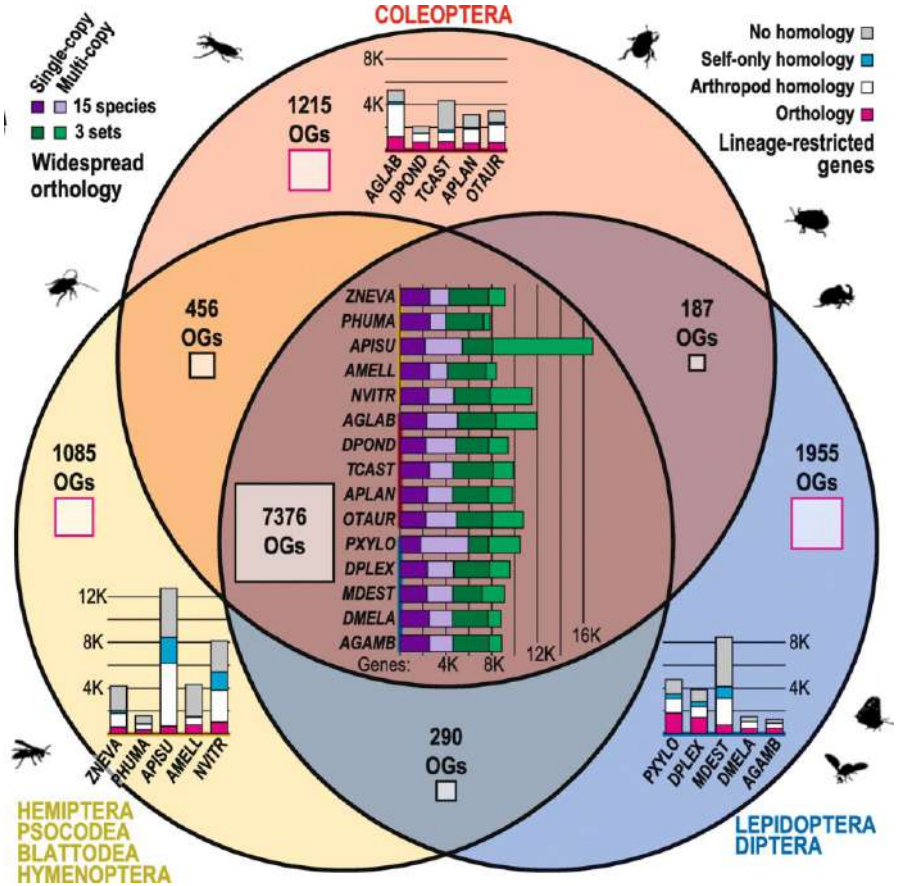
Unique

Variable

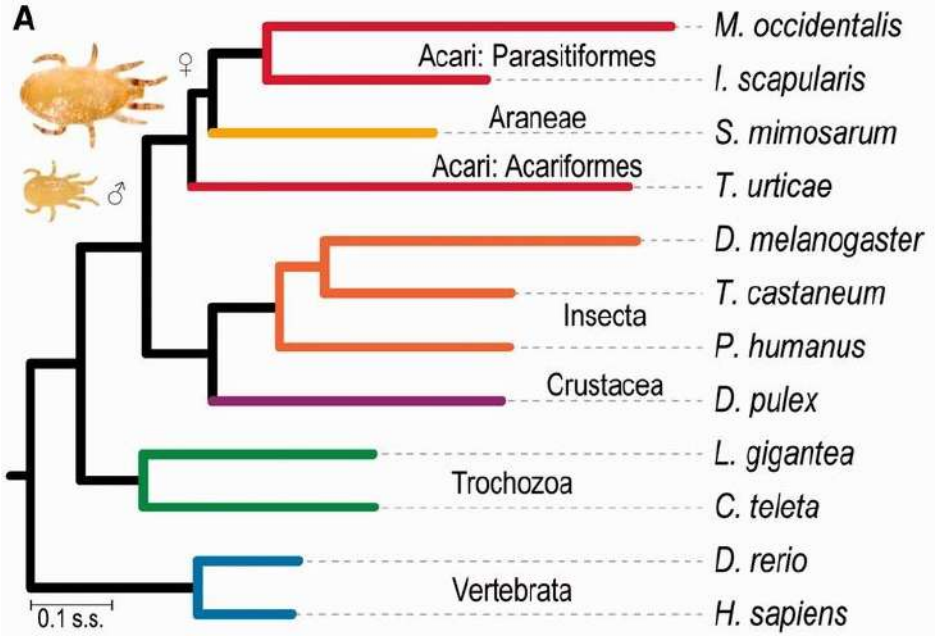
Common

Evolutionary histories: classes

Clade-specific & variable - count orthologues



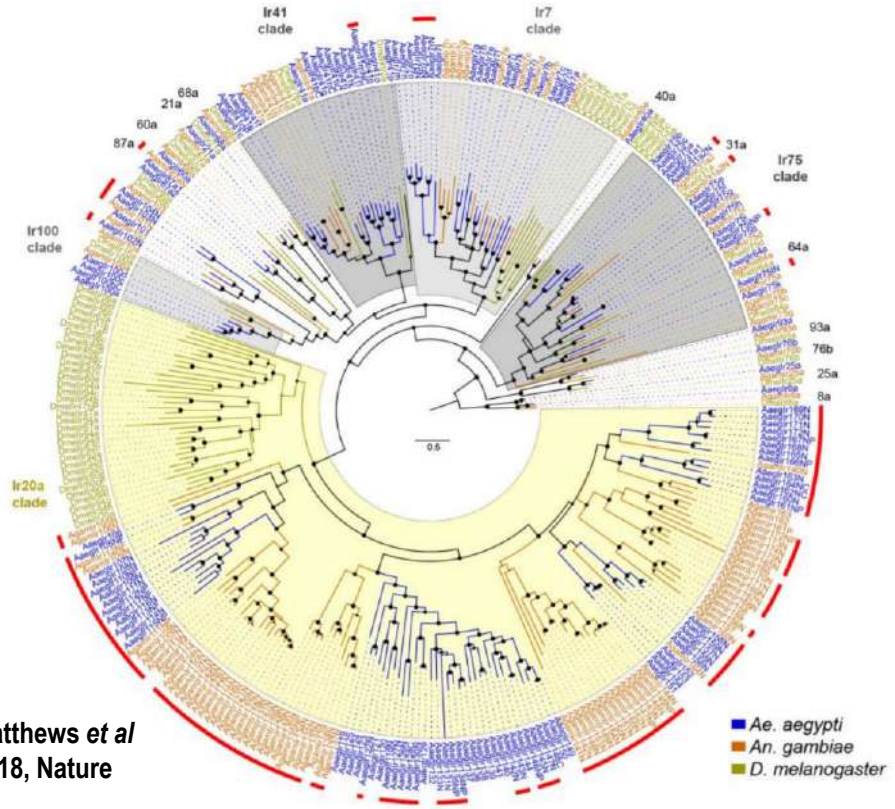
Species Tree Estimation



Phylogenomics with single -copy orthologues

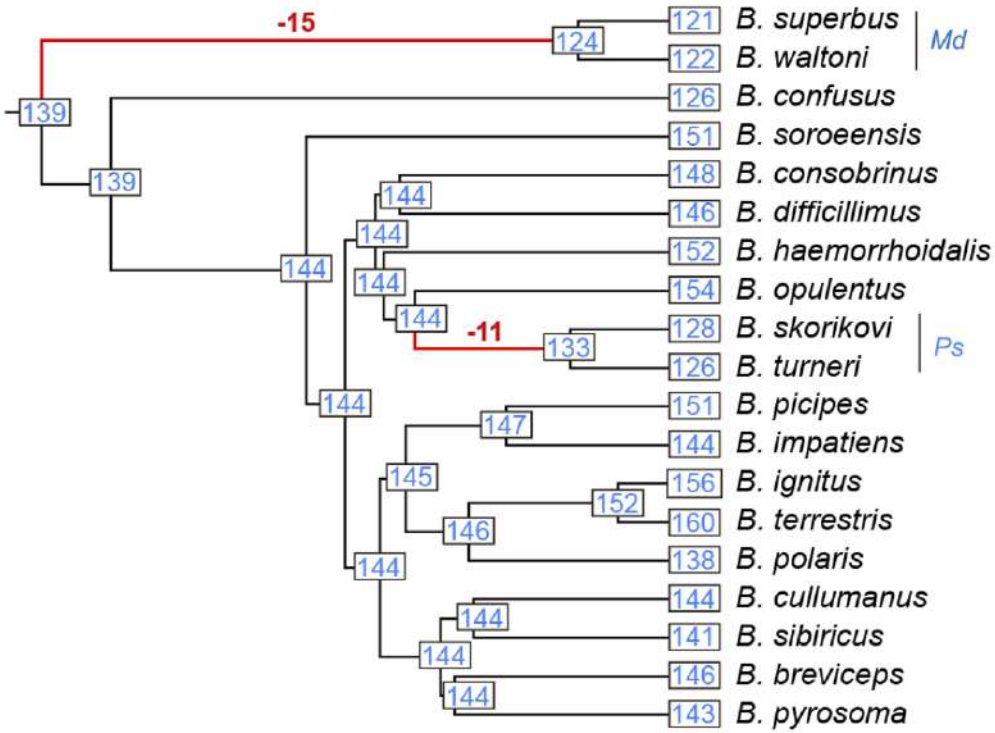
Gene Family Tree Building

All Ionotropic Receptors OrthoGroups in three species: conserved and dynamic IR OGs



Matthews *et al* 2018, Nature

Ancestral Copy -Number Reconstruction

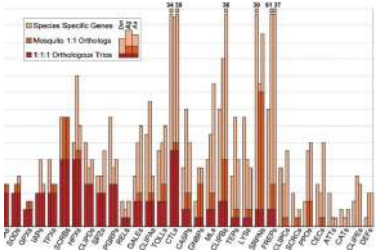


Bumblebee Odorant Receptors : two major gene loss events

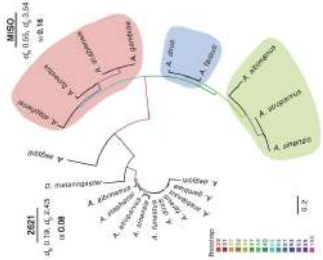
Dynamically evolving families

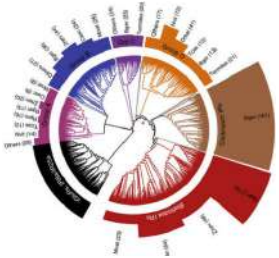
Many of the most biologically interesting genes and gene families show highly dynamic evolutionary histories

IMMUNITY



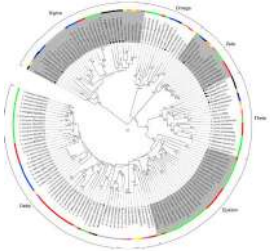
REPRODUCTION





CHEMOSENSATION

DETOXIFICATION



Goals for Today's Workshop

- ❑ Understand the principles of graph-based orthology delineation using OrthoDB as an example
- ❑ Learn how to browse and query OrthoDB
- ❑ Learn how to use BUSCO to assess genomics data
- ❑ Learn how to formulate comparative genomics questions, develop and apply approaches to address them (with a focus on using orthology data), and then critically interpret them, through case studies from arthropods

OrthoDB

BUSCO

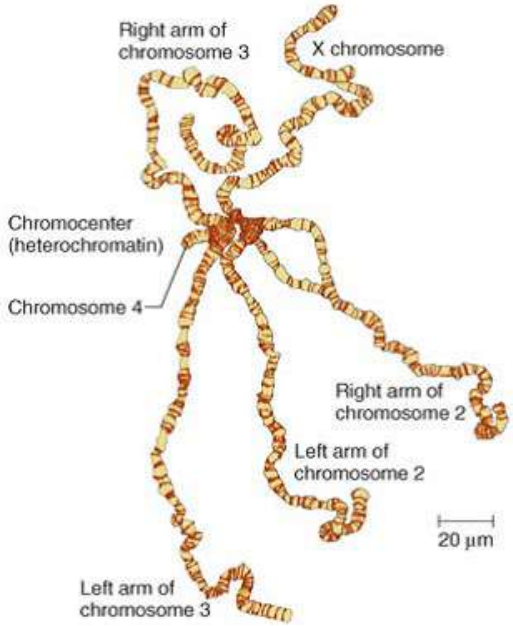
Assessing genomics data quality: BUSCO

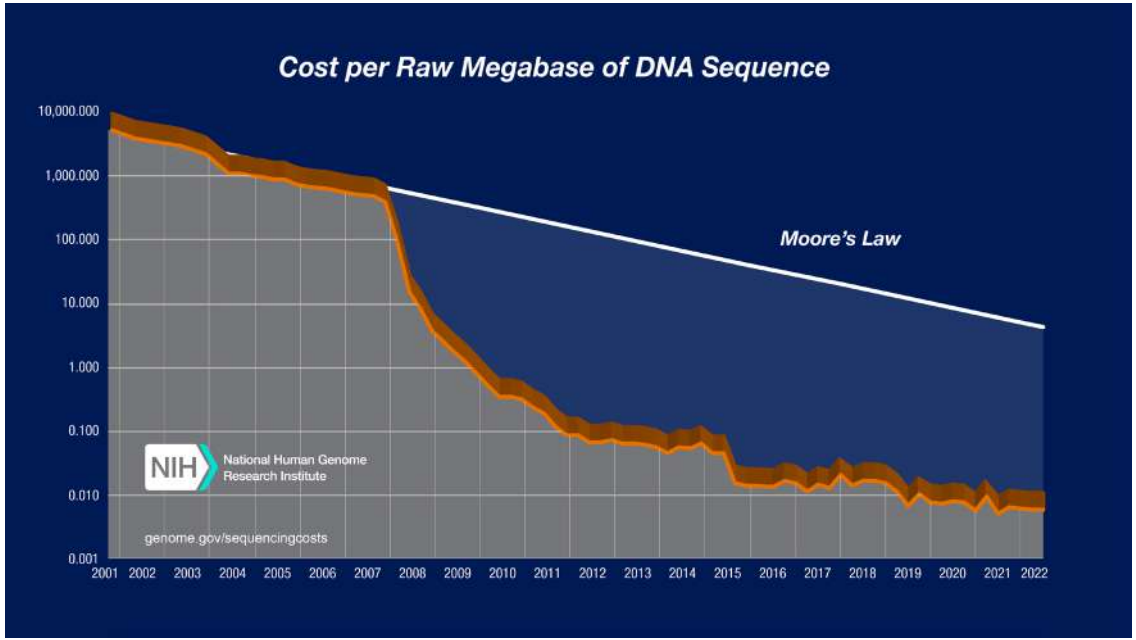
What is BUSCO?

How does BUSCO work?

Why do we need BUSCO?
(BUSCO in action)

Genomics for Everyone!





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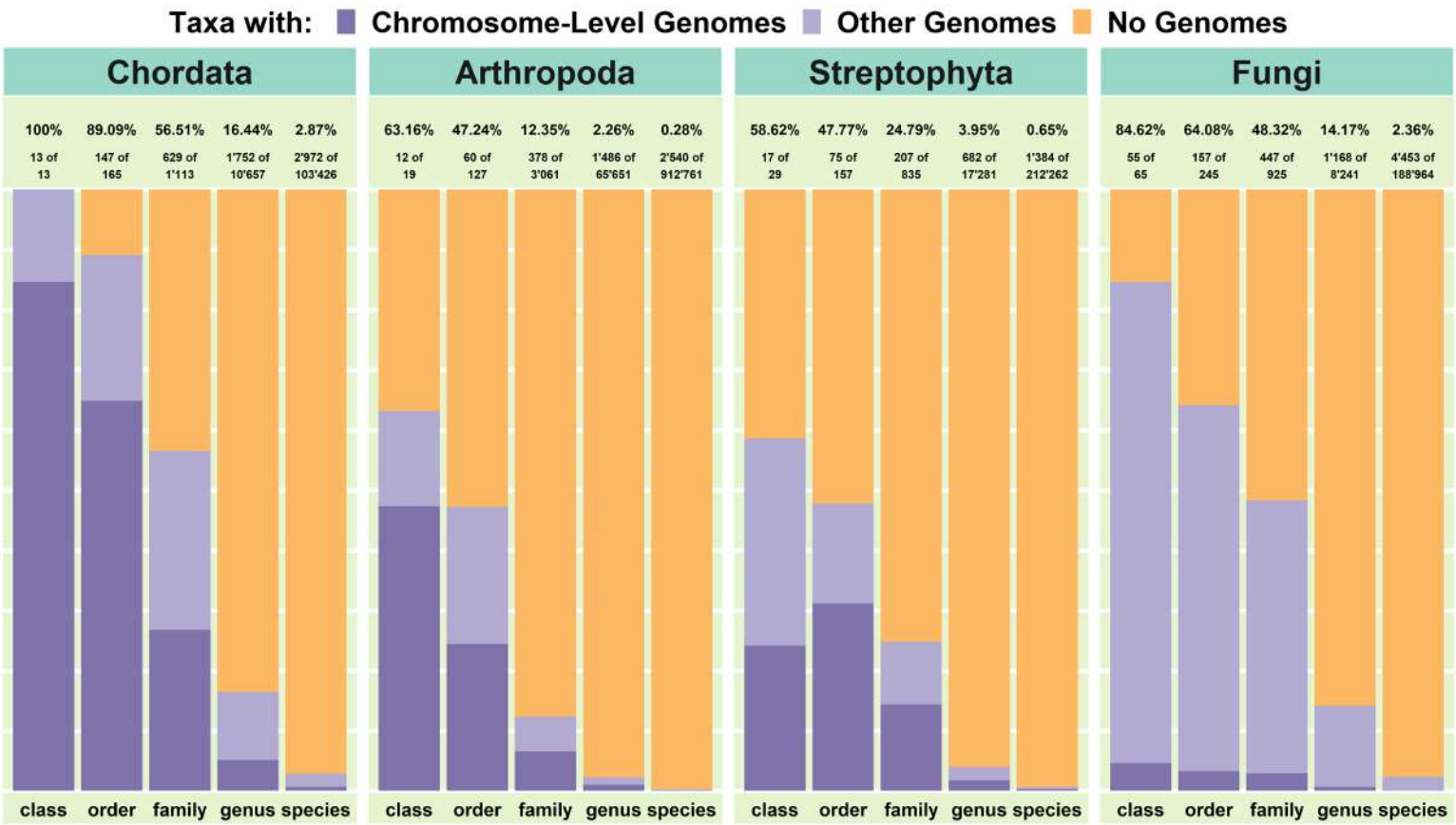
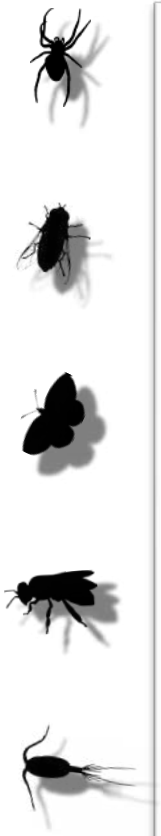
TGCCAAAGTAAAC [illegible] CCAGCACAACGG
CAACGGAGGAGATACCCAGCCTGGAGTCCCTGGGCAATATACACATTAGCACCGTACAGCGGGCAGCCGAATCCTTGCTG
GAGATCGATTATGCGGAGCTAGAAAACGCCACGGACGGCTGGAGTCCGGATAATCGACTGGGACAGGGCGGATTCGGAGA

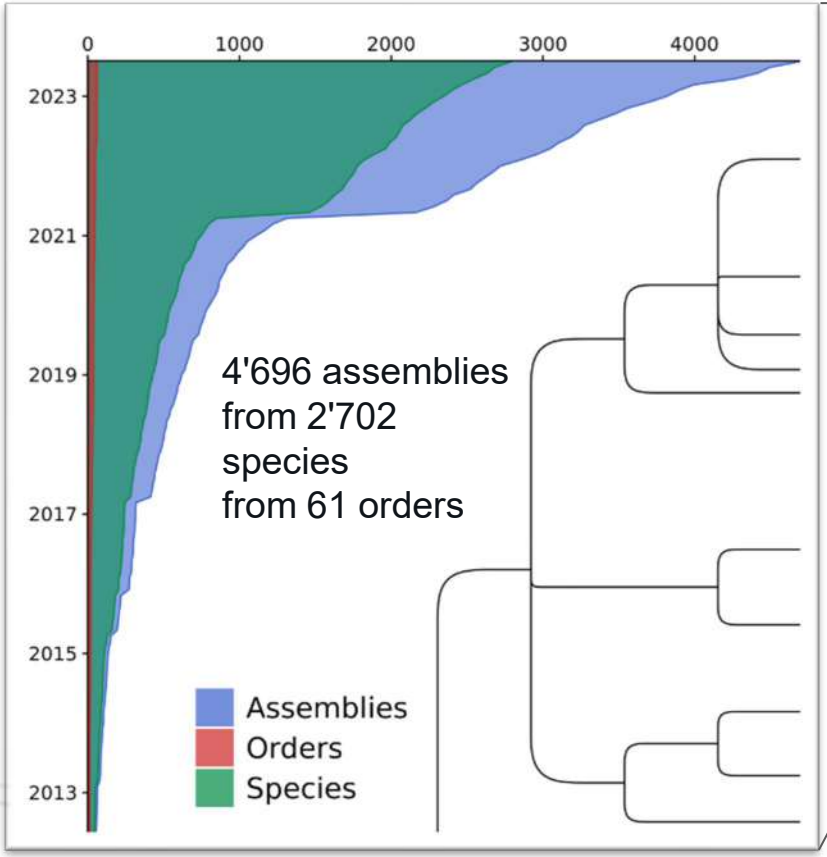
Nucleotide-level resolution

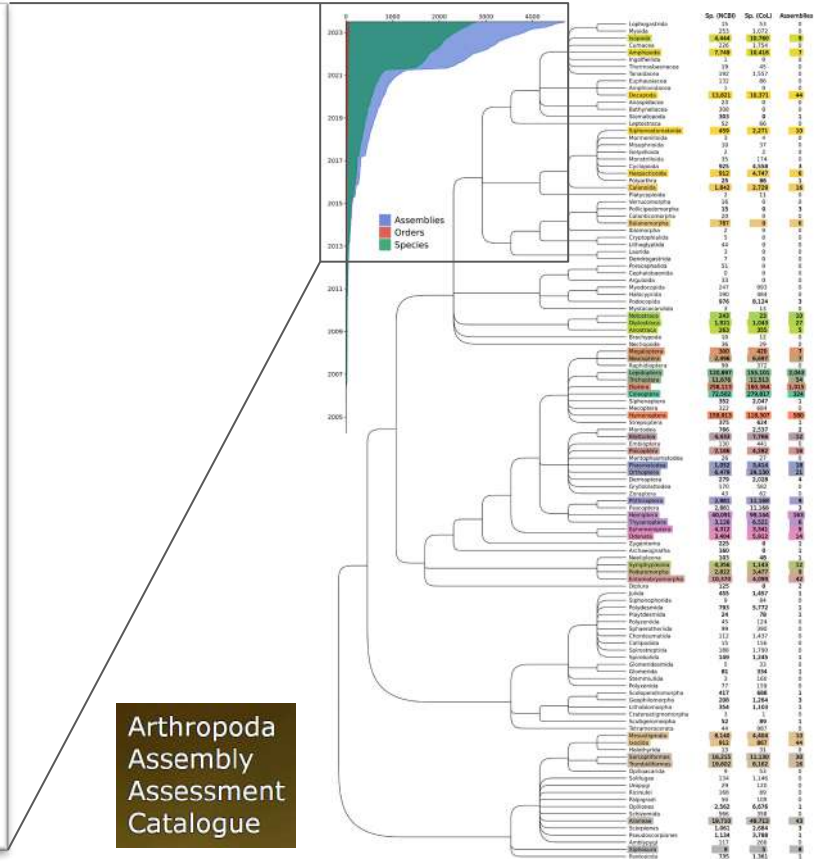
Rapidly increasing numbers of genomes



Rapidly increasing numbers of cool genomes



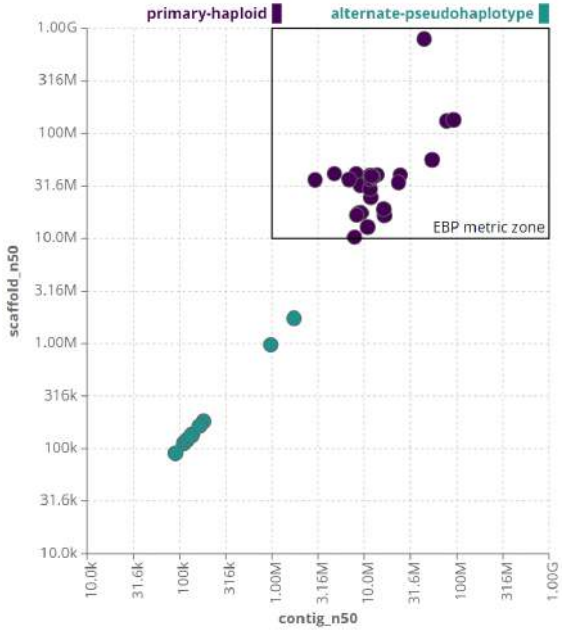




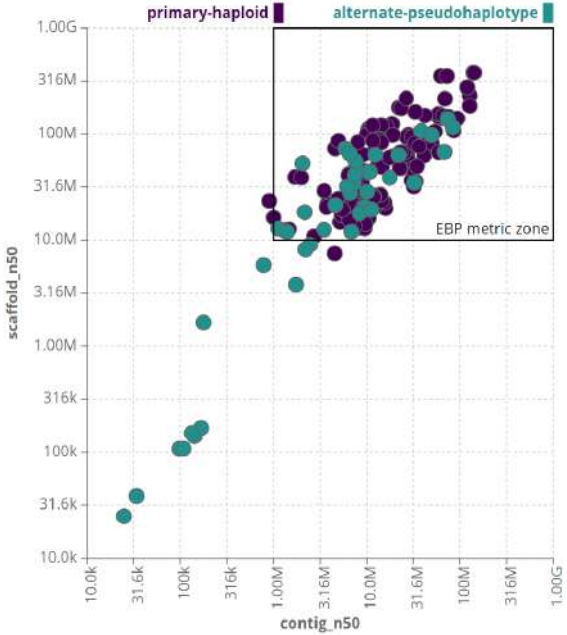
Arthropoda
Assembly
Assessment
Catalogue

BioGenome Projects producing new data

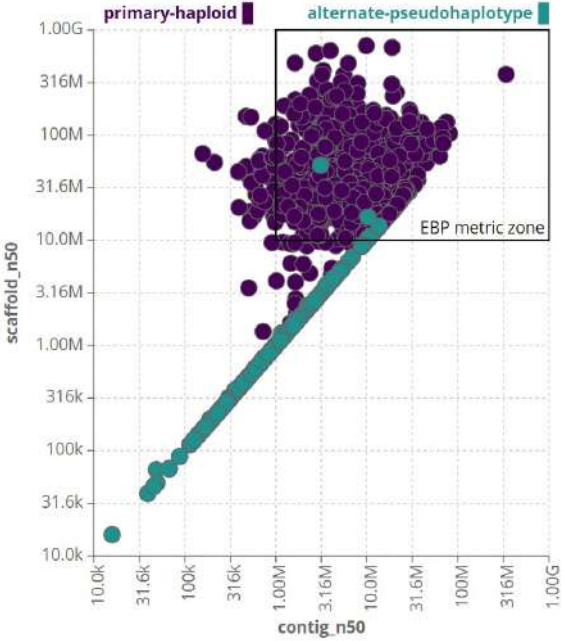
Ag100Pest Initiative (Ag100Pest)



The California Conservation Genomics Project (CCGP)



European Reference Genome Atlas (ERGA)

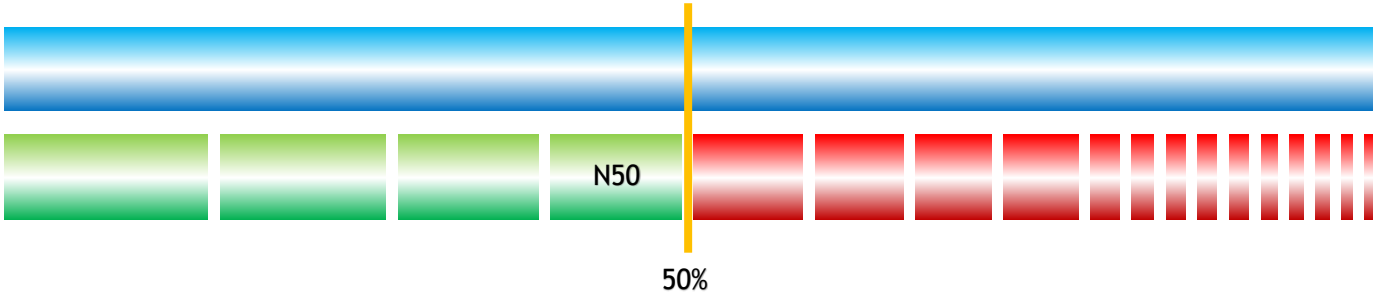


How can we gauge the quality of these resources?

1) Does the assembly size match the expected genome size?

2) How fragmented is the assembly?

Assembly contig or scaffold N50 size:
half the assembly is found on contigs/scaffolds of length N50 or greater



3) How ‘gappy’ is the assembly?

4) Does the assembly contain all the genes it is expected to?
How much of a multi-life-stage transcriptome maps back to the assembly?
How many of the ‘expected’ genes are actually in the assembly?

BUSCO: evolutionarily expected genes

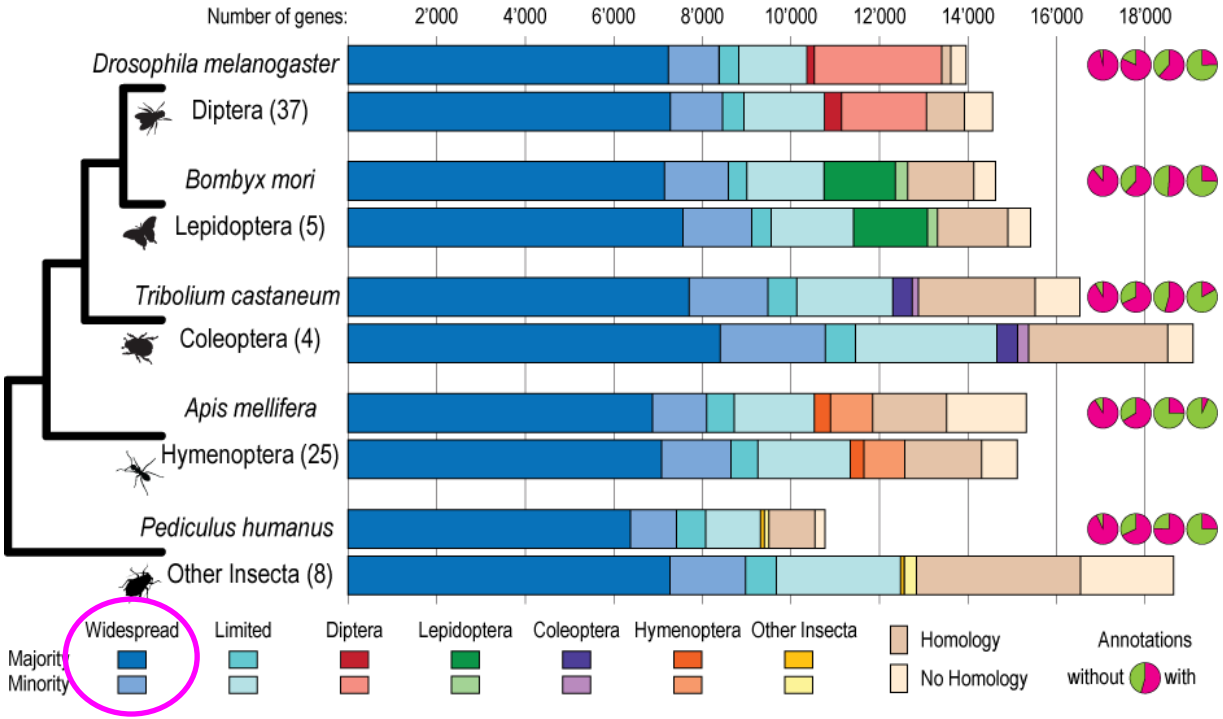
Widespread genes in extant species from a given taxa should be present in any newly sequenced species

Features in common:
6 legs
2 compound eyes
1 pair of wings
Etc.

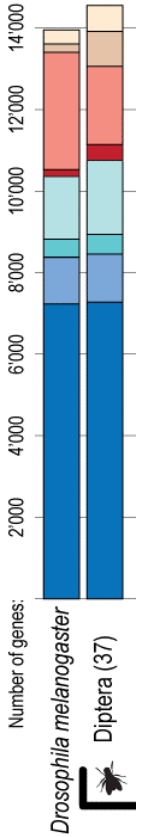
Therefore we EXPECT:
6 legs
2 compound eyes
1 pair of wings
Etc.

BUSCO: evolutionarily expected genes

Widespread genes in extant species from a given taxa should be present in any newly sequenced species



BUSCO: looking for widespread & unique genes



Widespread ⇔ Restricted

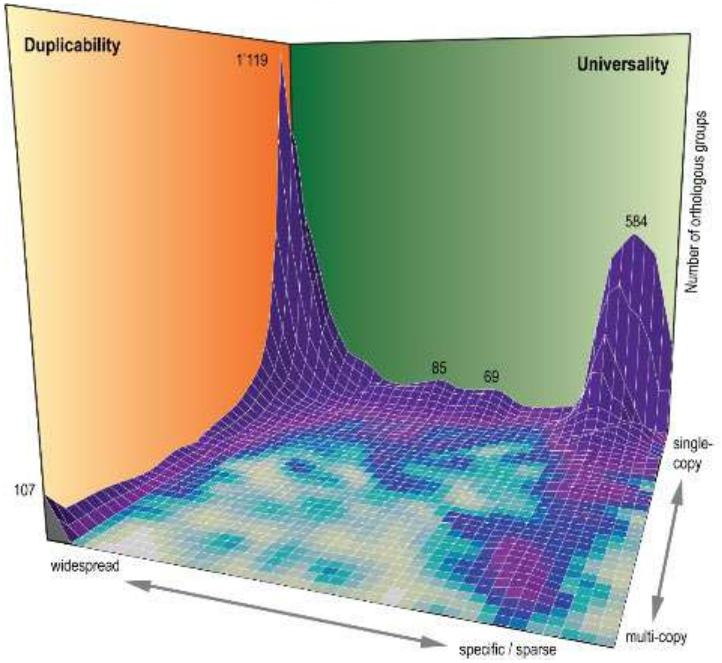
Drosophila melanogaster

orthology with genes from 80 insects

From mostly single-copy to mostly multi-copy

From present universally to present sparsely

Orthology Landscape



Current Opinion in Insect Science 2015, 7:15–23

A maturing understanding of the composition of the insect gene repertoire

Robert M Waterhouse[1,2,3,4]

BUSCO: looking for widespread & unique genes

Benchmarking Universal
Single-Copy Orthologues

QUEST FOR QUALITY

"BUSCO CALIDAD"

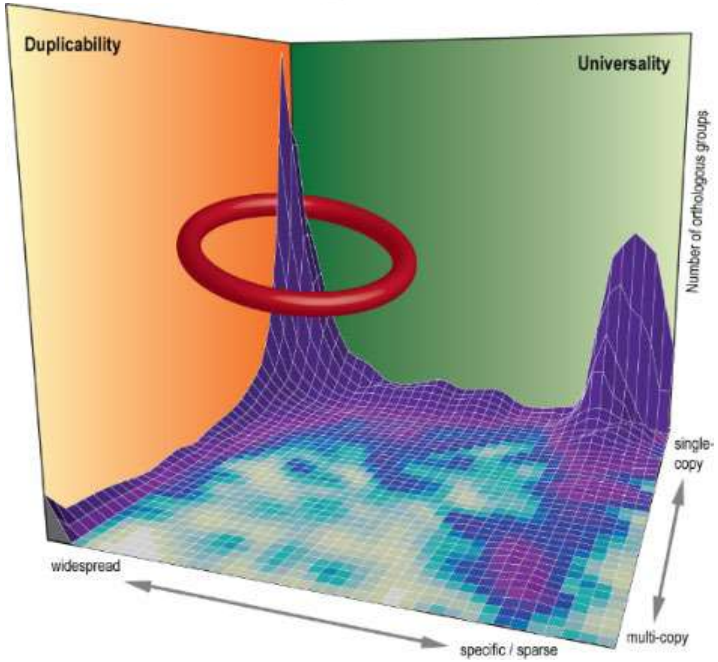
"BUSCO QUALIDADE"

Genome analysis Applications Note Bioinformatics

BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs

Felipe A. Simão*, Robert M. Waterhouse*, Panagiotis Ioannidis, Evgenia V. Kriventseva, Evgeny M. Zdobnov*

Orthology Landscape



http://busco.ezlab.org

BUSCO: looking for widespread & unique genes

Ortho-Groups with genes found in the majority of species as single-copy orthologues

Evolutionary Expectation for them to be found in any newly-sequenced genome

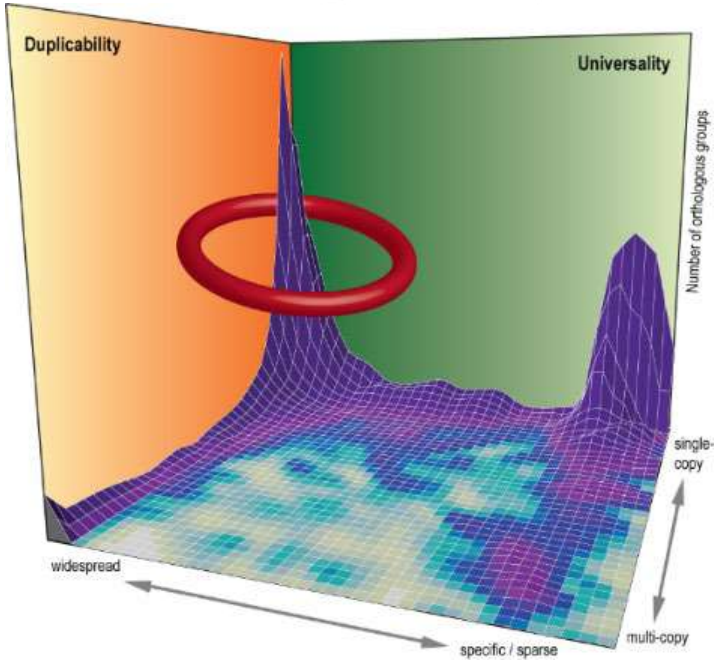
Implemented Assessments

Gene Content Completeness
genome assemblies
annotated gene sets
assembled transcriptomes

Bonus Features

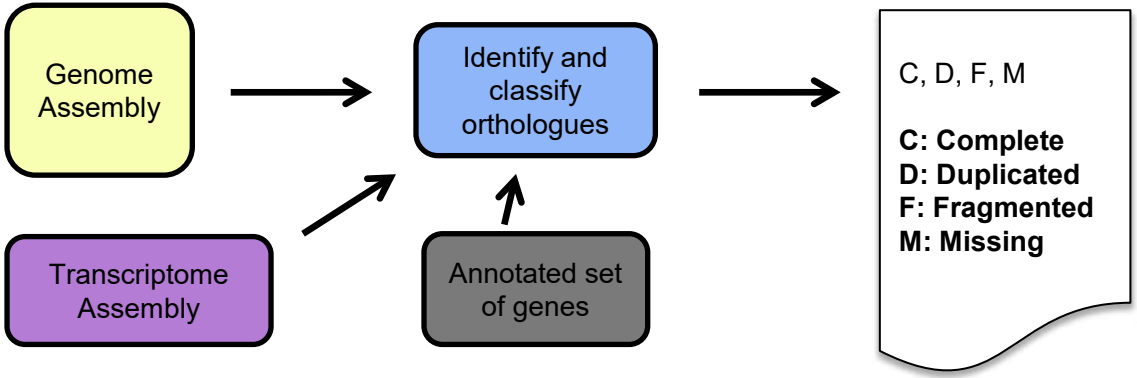
genes for phylogenomics
gene predictor training

Orthology Landscape

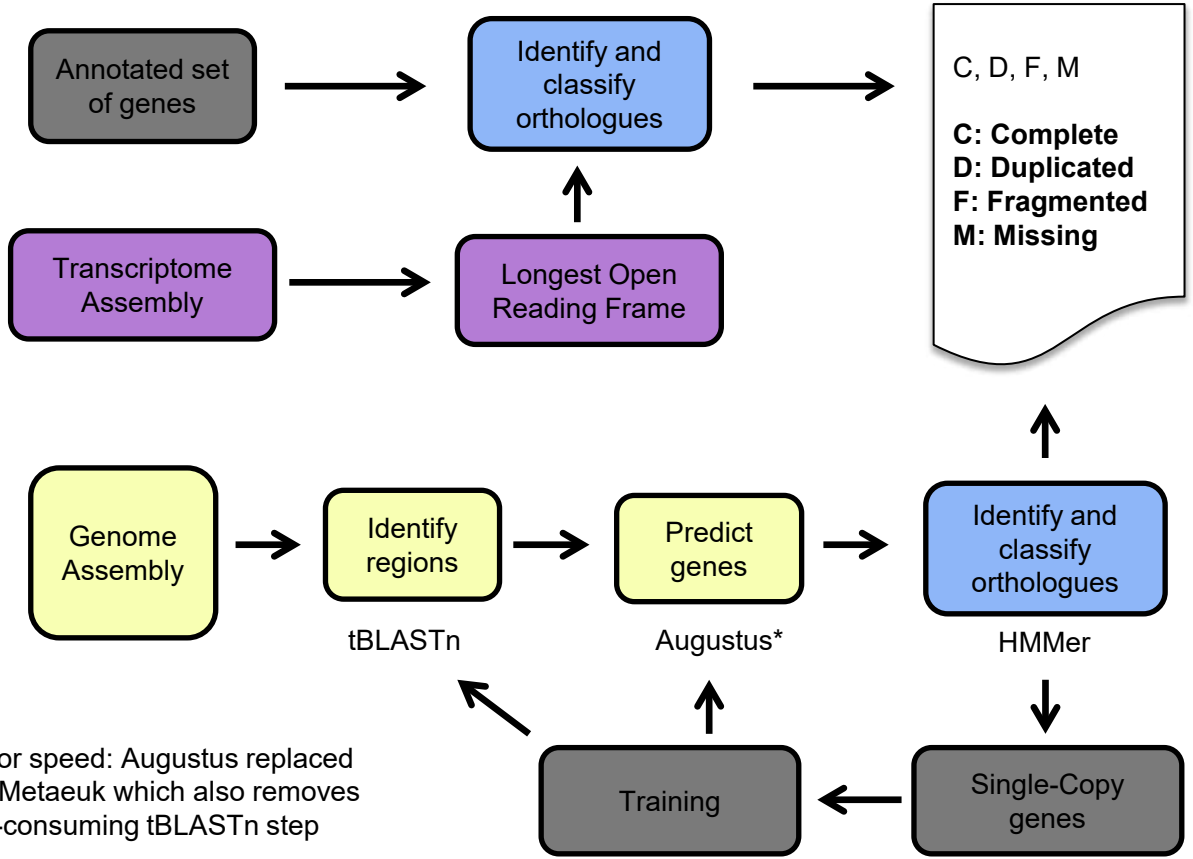


http://busco.ezlab.org

BUSCO completeness assessments



BUSCO completeness assessments



*v5 for speed: Augustus replaced with Metaeuk which also removes time-consuming tBLASTn step

Identify and classify orthologues – HOW?

Species filtering to select best representatives from each clade

- Avoiding biasing the alignments with closely-related species

BUSCO completeness assessments

For each clade/lineage …

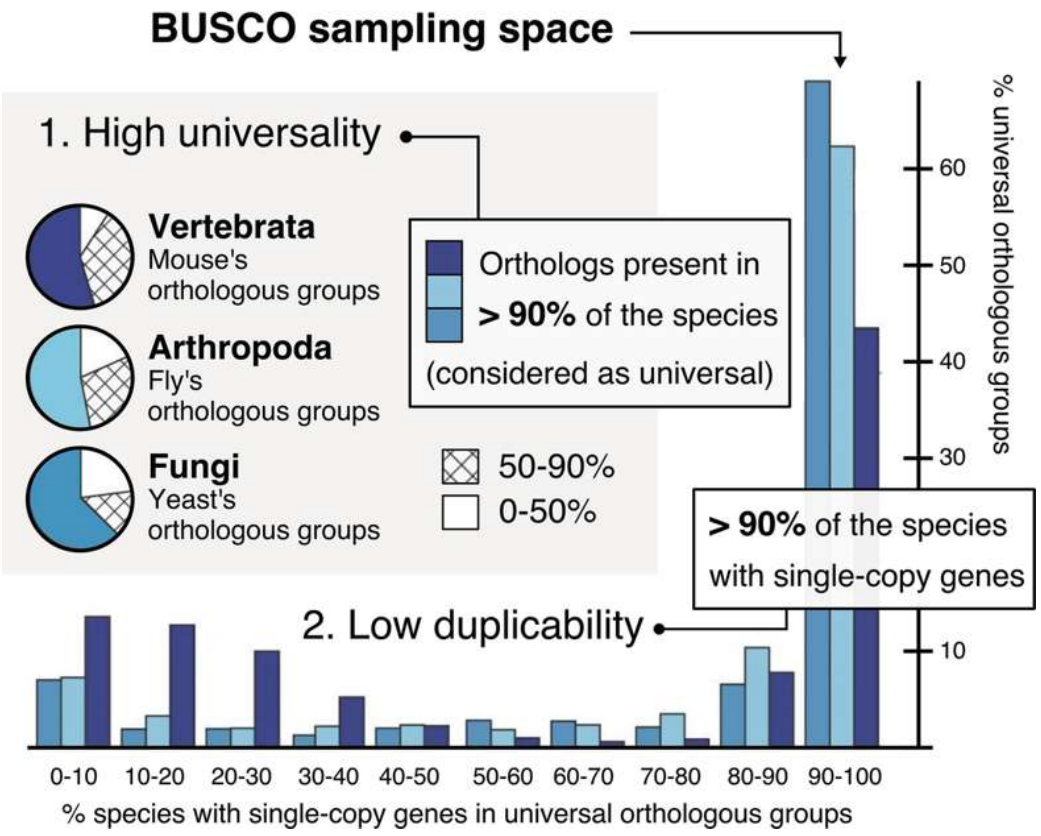
E.g. vertebrates, arthropods, or fungi

Filter OrthoGroups to retain those:

- Present in >90% species
- Single-Copy in >90% species

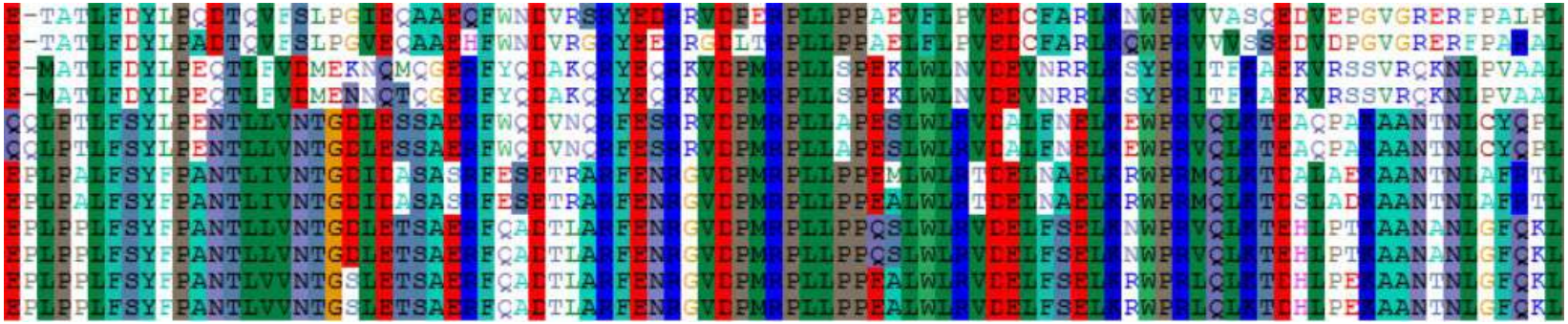
To obtain lineage datasets of

Benchmarking Universal Single-Copy Orthologues



Building BUSCO lineage datasets

1) Multiple protein sequence alignments for each orthologous group



2) HMM profiles from alignments for searching protein sequences

3) Consensus sequences for searching genome assemblies

4) Consensus sequence variants for searching genome assemblies

5) Augustus block profiles for predicting gene models

Building BUSCO lineage datasets

Filtering of initial BUSCO sets

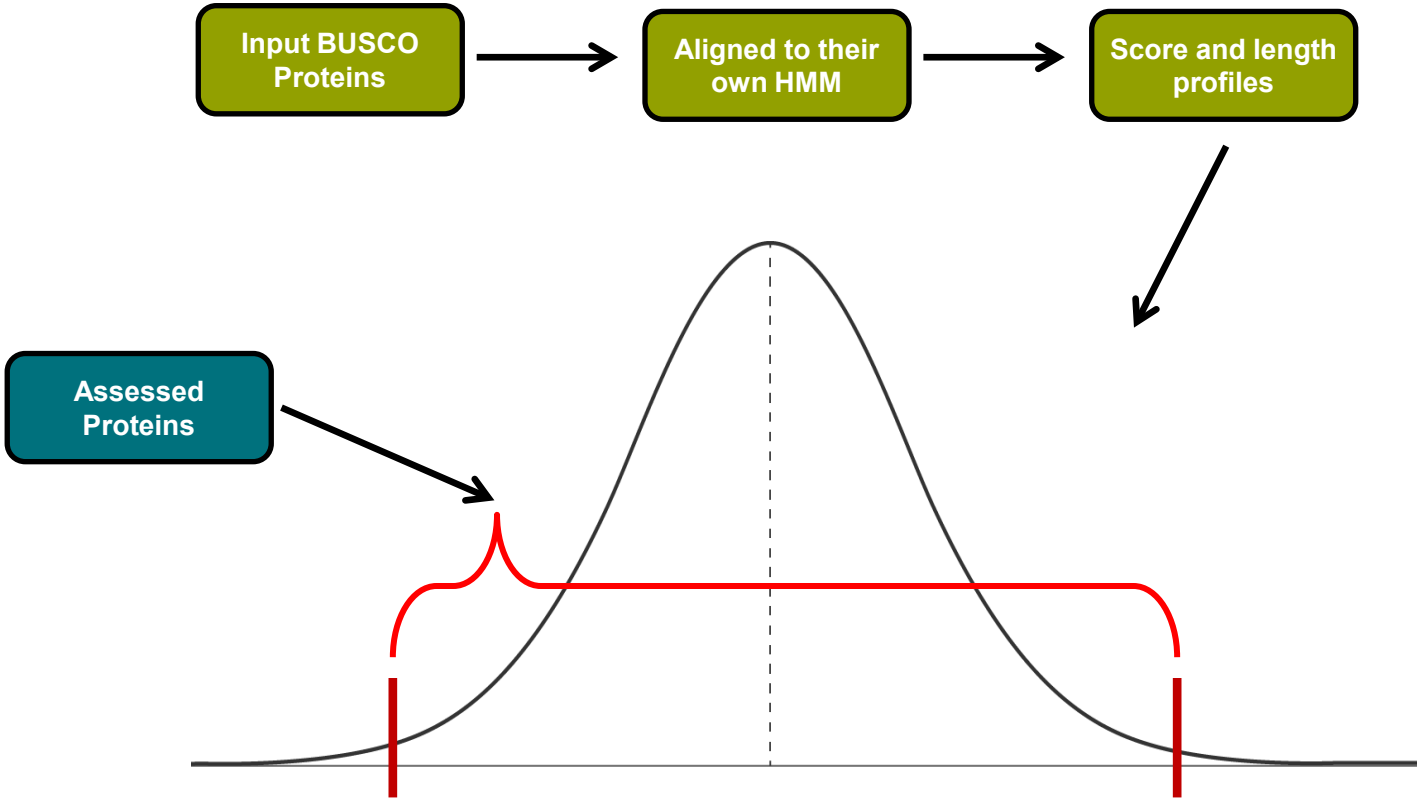
HMM profiles run against all proteins from all input species

Score and length cut-offs fine-tuned for each BUSCO to maximise sensitivity and specificity

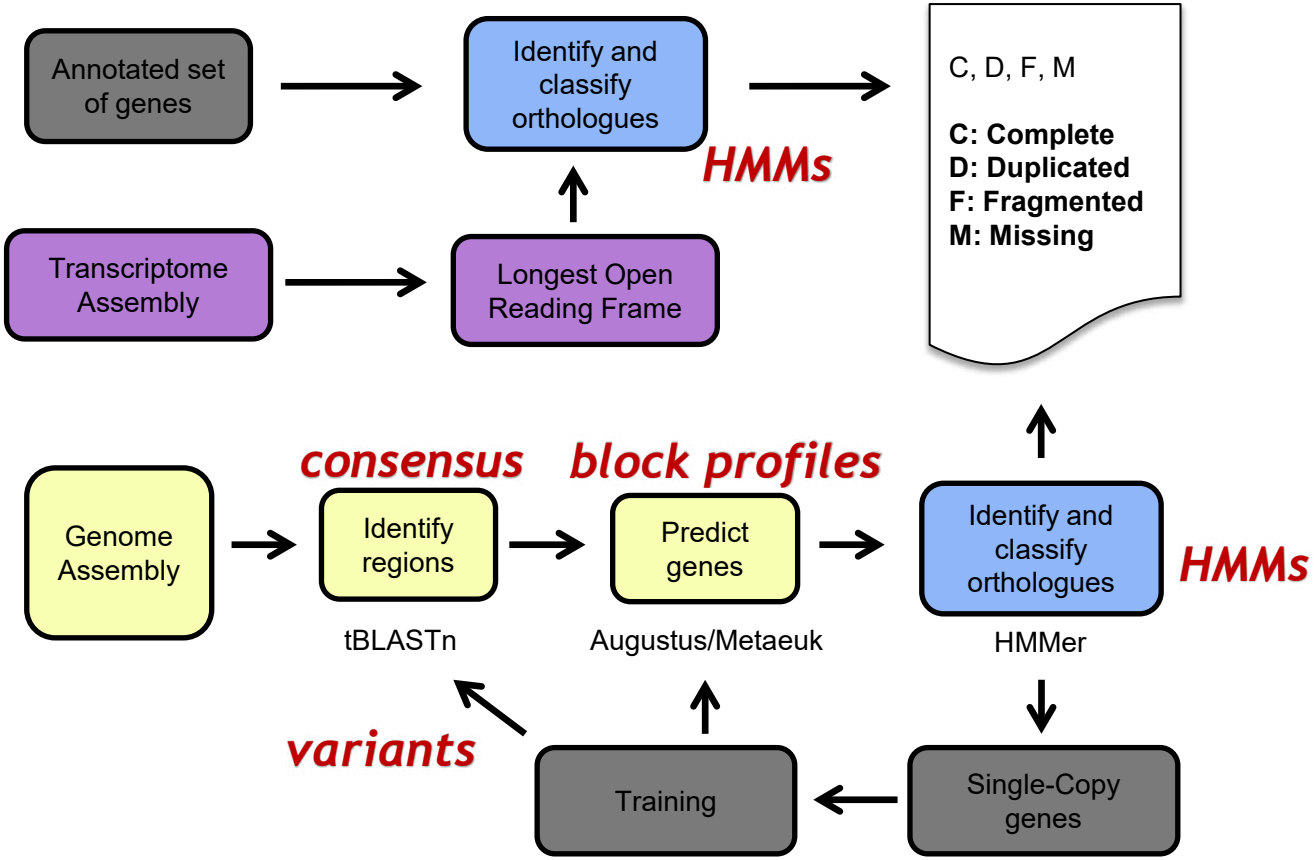
Only BUSCO profiles with high sensitivity and specificity are kept

Testing of BUSCO profiles on non-input species remove BUSCOs whose Augustus/MetaEuk step fails

Identify and classify orthologues – HOW?



BUSCO completeness assessments



Quick Quiz

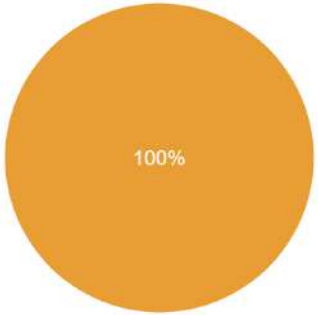
https://forms.gle/Wd7ZZTfUqpoVsPsB9

SwissOrthology

Which description best describes your understanding of what BUSCO aims to achieve?

Copy

13 responses



- To assess the sequencing quality of genomic data including genomes, gene sets, and transcriptomes
- To identify and score all highly conserved genes in a newly sequenced and annotated genome or transcriptome
- To estimate completeness of genomic data including genomes, gene sets, and transcriptomes in terms of expected gene content

Assessing genomics data quality: BUSCO

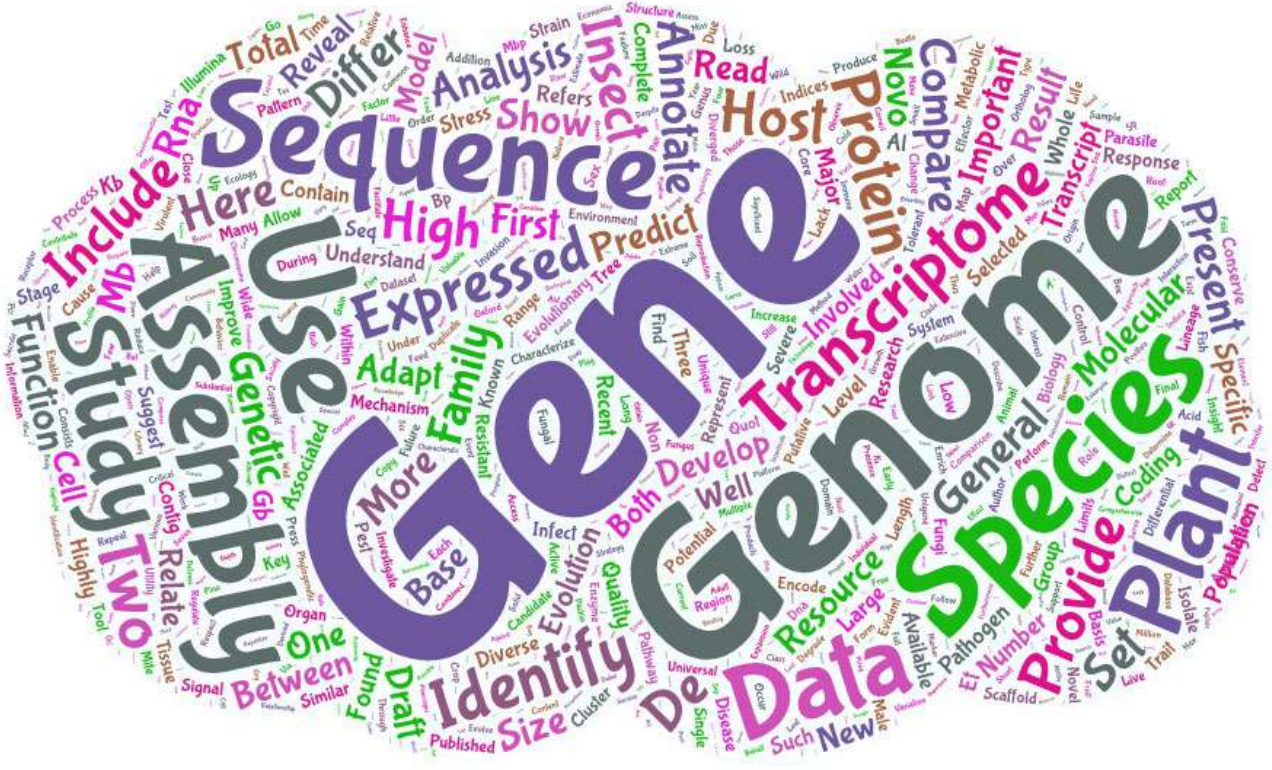
What is BUSCO?

How does BUSCO work?

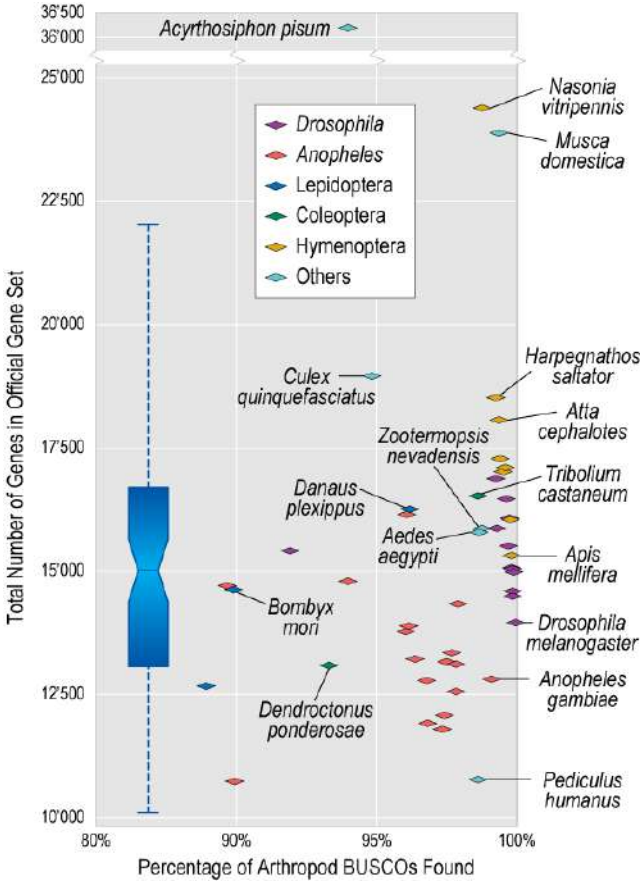
Why do we need BUSCO?
(BUSCO in action)

BUSCO research applications

Many genomics studies now using BUSCO – what for?



BUSCO in action: insect gene sets



Large gene sets are not necessarily complete

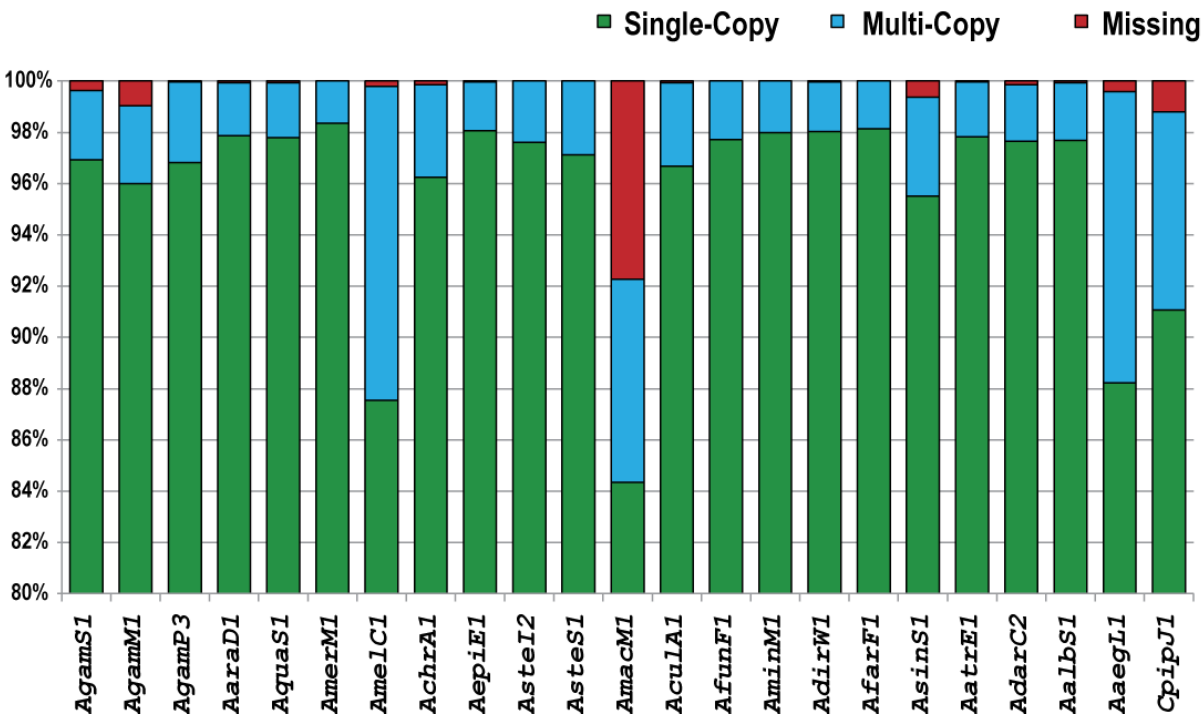
Current Opinion in Insect Science 2015, 7:15–23

A maturing understanding of the composition of the insect gene repertoire

Robert M Waterhouse[1,2,3,4]

Small gene sets are not necessarily incomplete

BUSCO in action: Anopheles gene sets

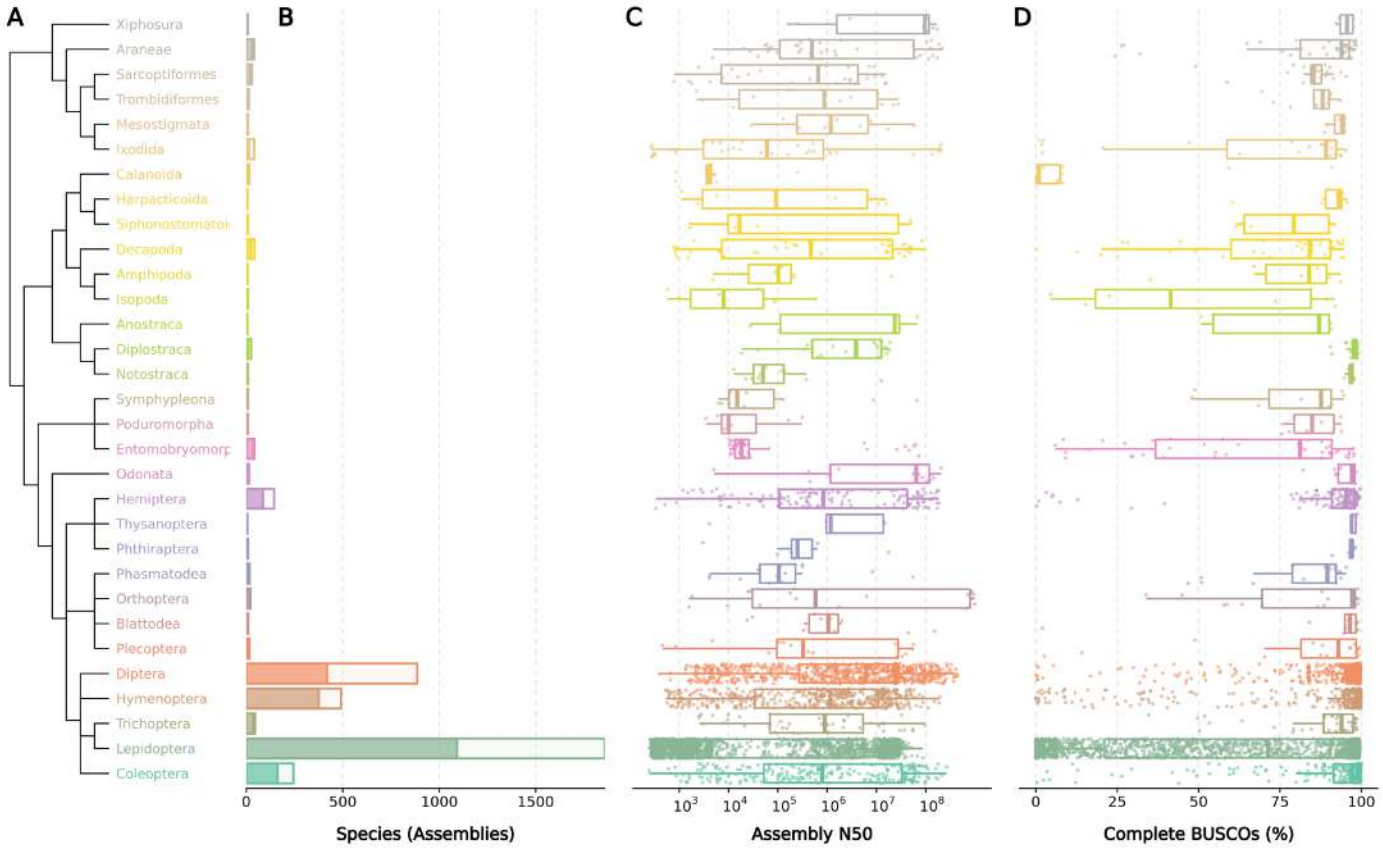


- Most remarkably complete in terms of genes
- Missing: *An. maculatus* - fragmented assembly
- *An. christyi*, also fragmented, but few missing
- Duplicates: *An. melas* - assembly haplotypes (fixed)

BUSCO utilities beyond Quality Control

- Comparative Genomics
- Gene Predictor Training
- Phylogenomic Analyses

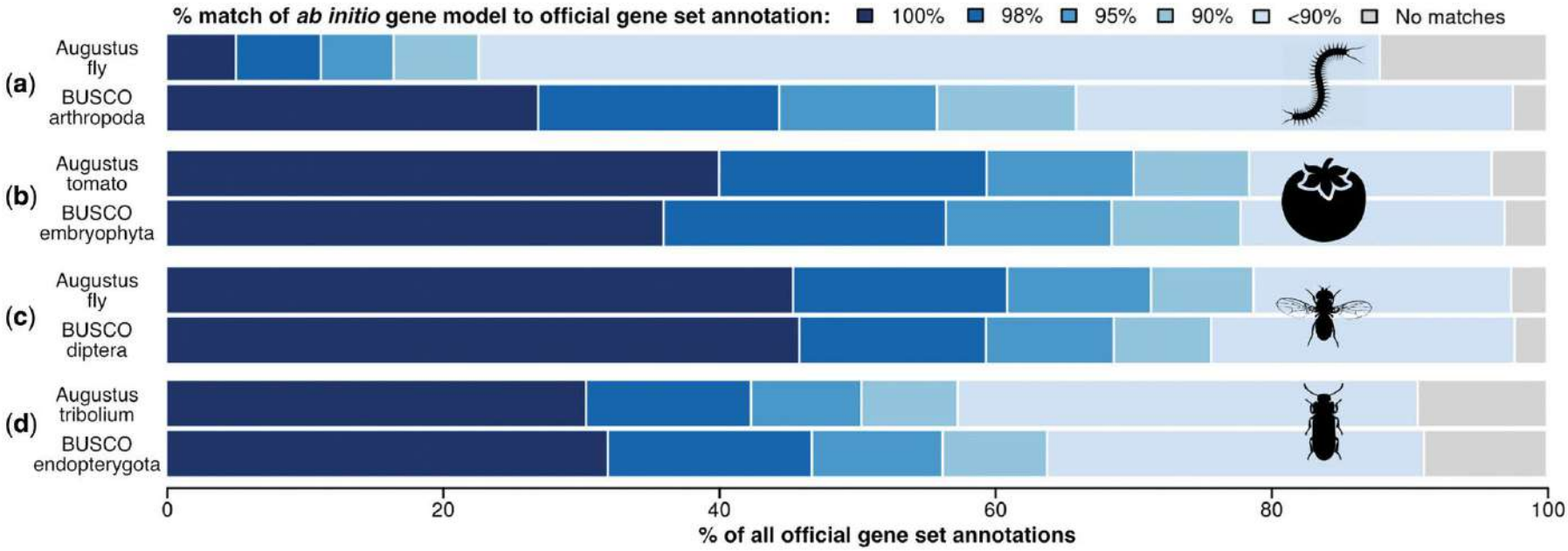
BUSCO in Comparative Genomics



Analyses sensitive to incomplete datasets

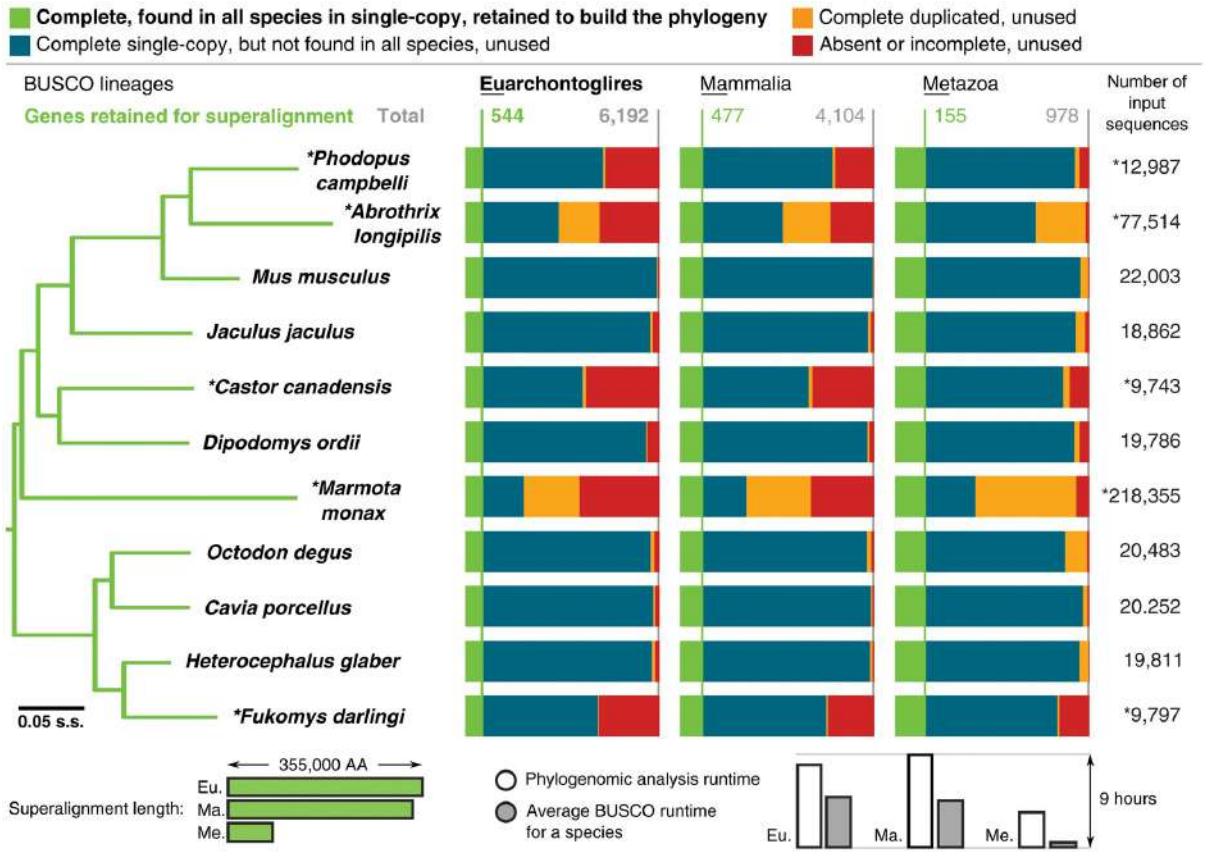
Quantitative assessments with BUSCO offer logical selection criteria to help focus on the most complete genomic resources available

BUSCO in Gene Predictor Training



BUSCOs, being generally widely- and well-conserved genes, offer ideal predefined sets for such training procedures, even without the need to first perform RNAseq

BUSCO in Phylogenomic Analyses



Estimating true phylogenetic relationships among organisms is a prerequisite to almost any evolutionary study

BUSCOs represent predefined sets of reliable markers where assessments of genomes, annotated gene sets, and/or transcriptomes can identify shared subsets from different types of genomic data for phylogenomics studies

BUSCO utilities – BUSCO in action

- Quality Control
- Comparative Genomics
- Gene Predictor Training
- Phylogenomic Analyses

Goals for Today's Workshop

- ❑ Understand the principles of graph-based orthology delineation using OrthoDB as an example
- ❑ Learn how to browse and query OrthoDB
- ❑ Learn how to use BUSCO to assess genomics data
- ❑ Learn how to formulate comparative genomics questions, develop and apply approaches to address them (with a focus on using orthology data), and then critically interpret them, through case studies from arthropods

OrthoDB

BUSCO