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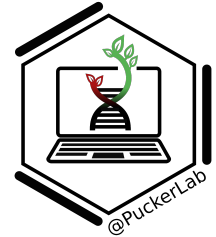


Prof. Dr. Boas Pucker

Synteny and Biosynthetic Gene Cluster

Availability of slides

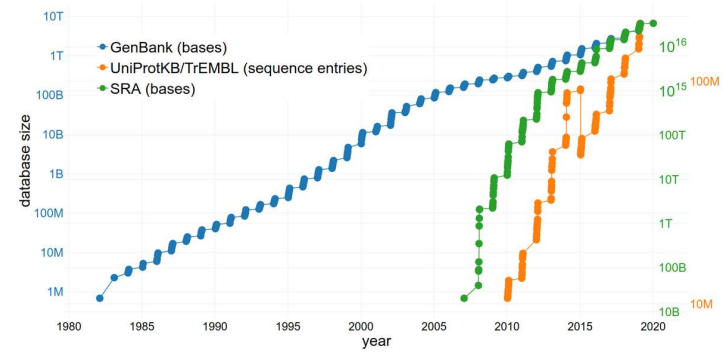
- All materials are freely available (CC BY) - after the lectures:
  - eCampus: WBIO-A-08
  - GitHub: https://github.com/bpucker/teaching

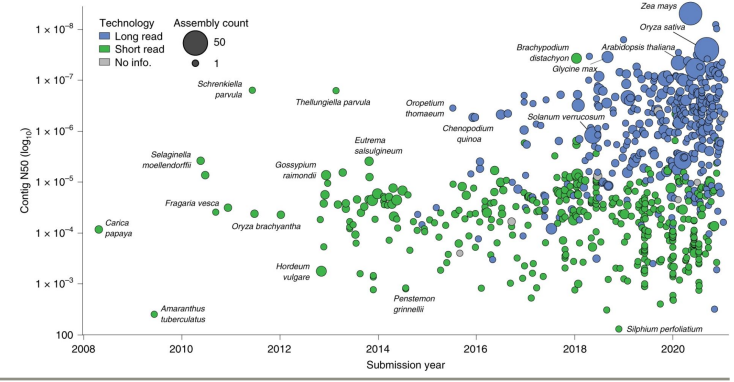
- Questions: Feel free to ask at any time

- Feedback, comments, or questions: pucker[a]uni-bonn.de

Progress in plant genomics

- Number of reference quality genome sequences is increasing
- Remaining challenge is separation of haplotypes
- Number of re-sequencing projects is increasing
- Focus is shifting towards investigation of intraspecies variation

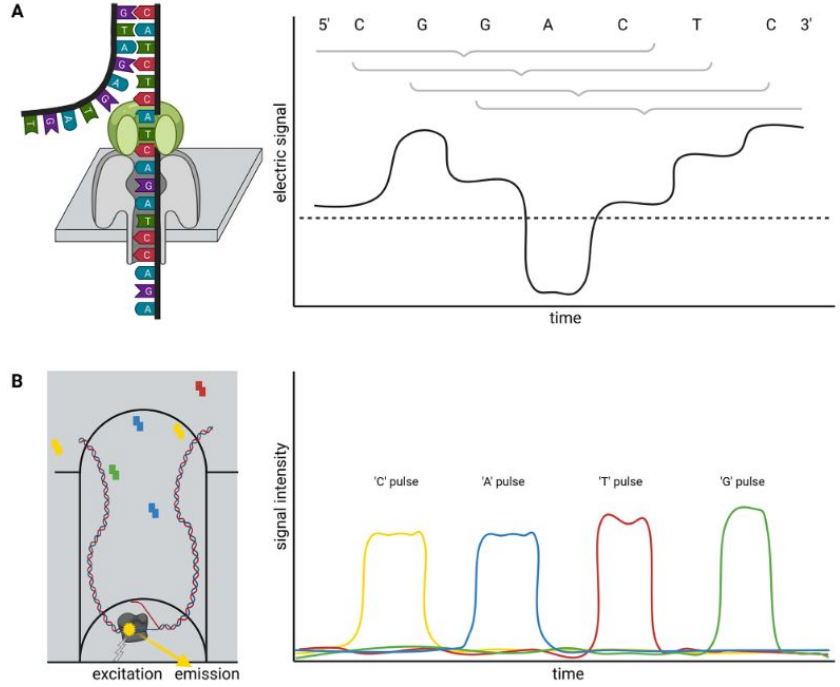




Sielemann et al., 2020: 10.7717/peerj.9954
Marks et al., 2021: 10.1038/s41477-021-01031-8

Long read sequencing

- Plant genomics is based on long read sequencing technologies:
  - ONT: Oxford Nanopore Technologies
  - PacBio: Pacific Biosciences
- Read length is >>20kb; up to million bases
- Read quality is continuously increasing (>99% accuracy)



Pucker et al., 2022: 10.1017/qpb.2021.18

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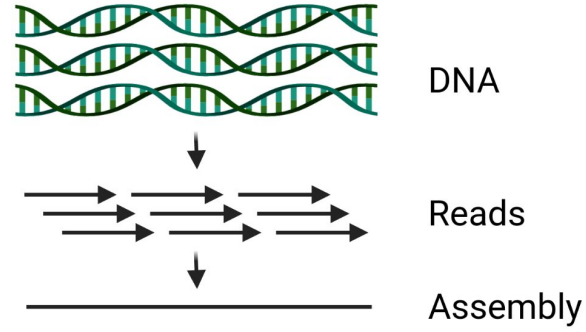
Genome sequence assembly

- Reads are shorter than chromosomes - even long reads
- Multiple copies of the genome (DNA exist that can be subjected to sequencing
- Assembly = putting sequence pieces together (finding the common string of all substrings)
- Genome = DNA in a cell
- Genome sequence = representation of the DNA in a cell; stored in FASTA files

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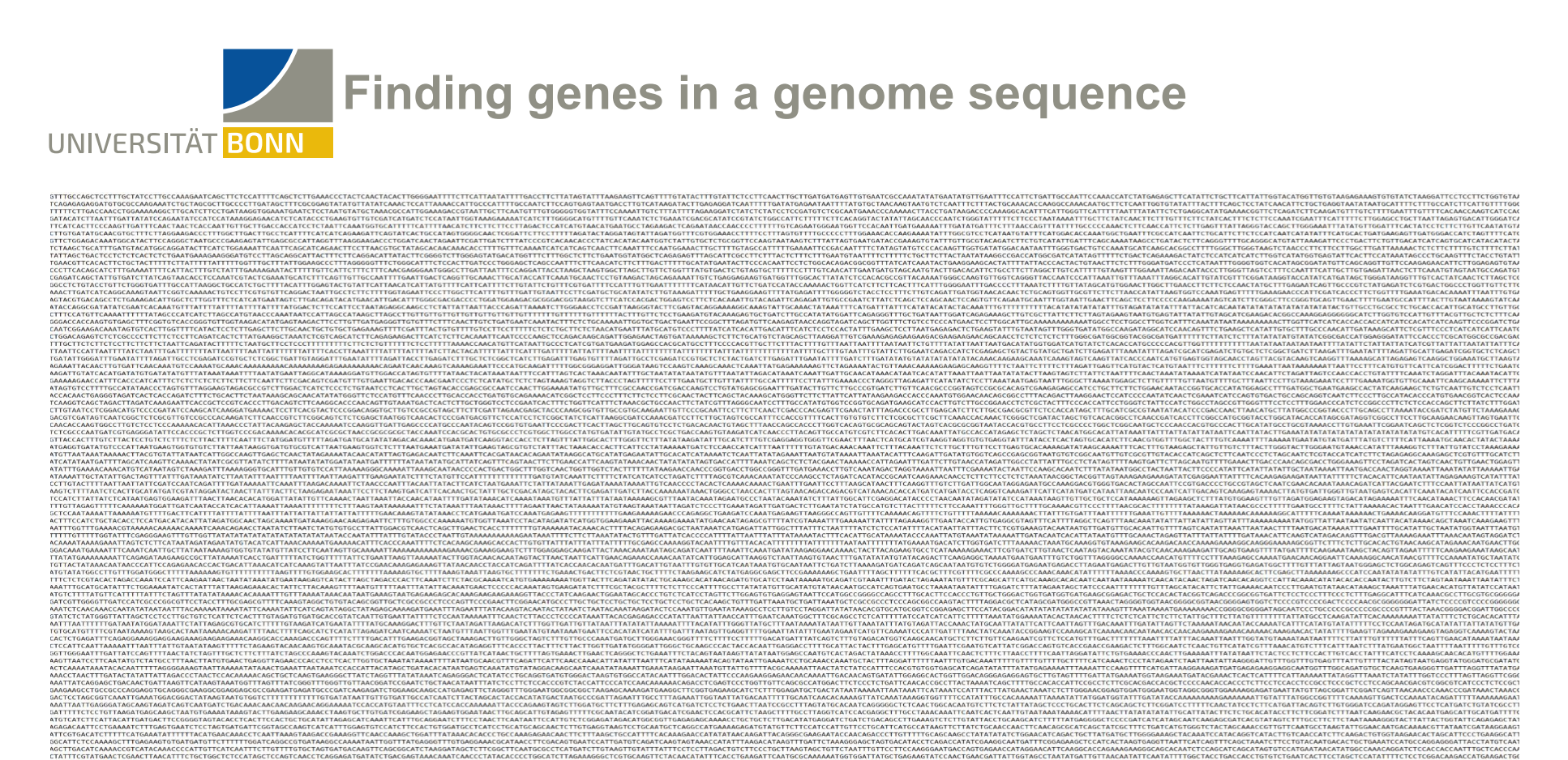
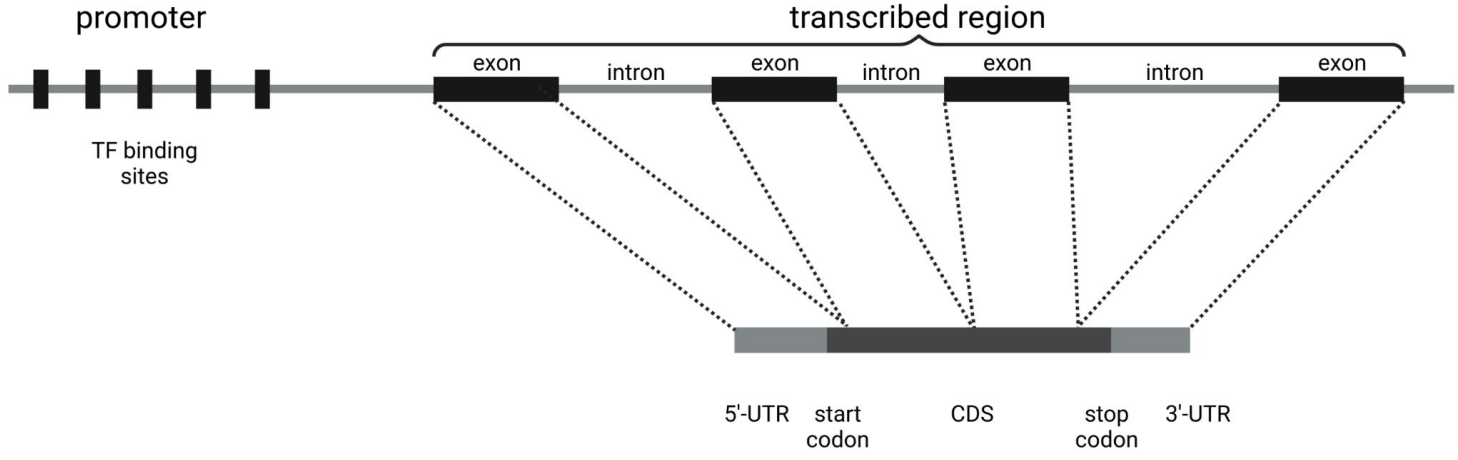
Finding genes in a genome sequence



Siadjeu & Pucker et al., 2020: 10.3390/genes11030274

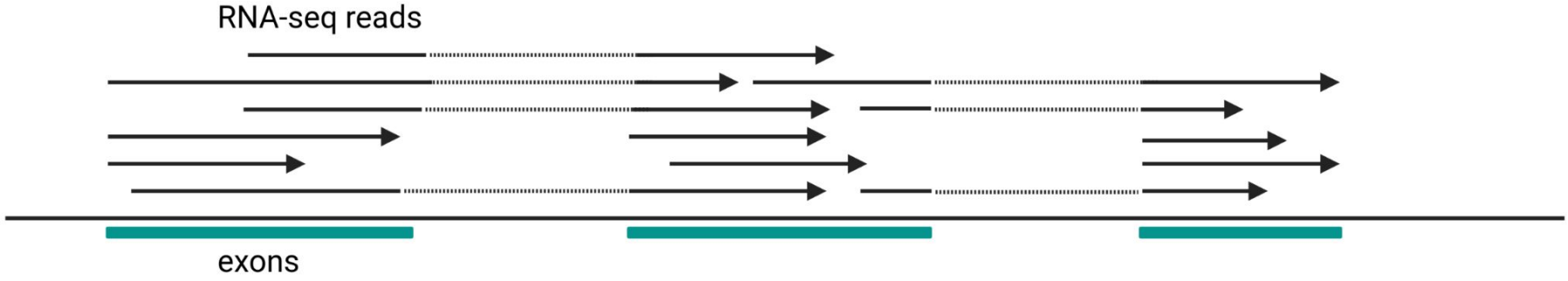
Plant gene structure

- CDS = (Protein) Coding Sequence
- ORF = Open Reading Frame
- UTR = UnTranslated Region
- TF = Transcription Factor



Gene prediction (structural annotation)

- Aligned RNA-seq (cDNA-derived) reads indicate exon positions
- Splitting of reads indicates intron positions
- CDS can be identified as ORF within the covered regions

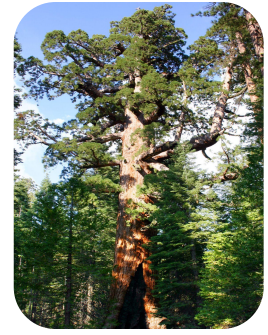


Gene function prediction (functional annotation)

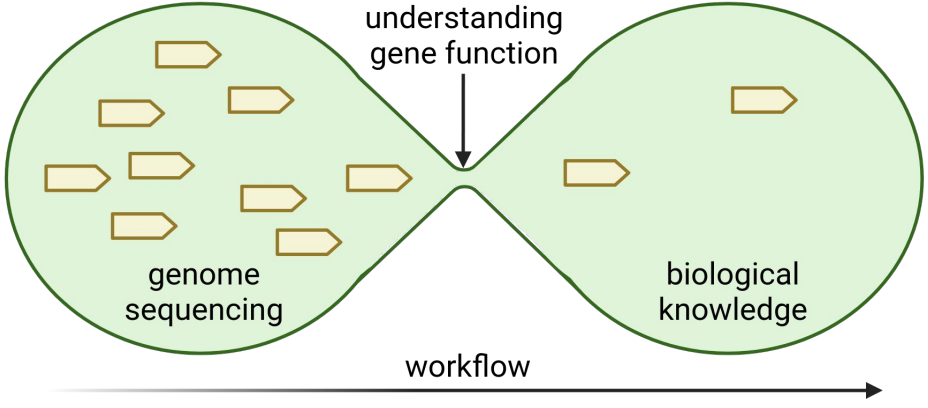
- Thousands of genes per plant species; only a minority of *Arabidopsis thaliana* genes studied

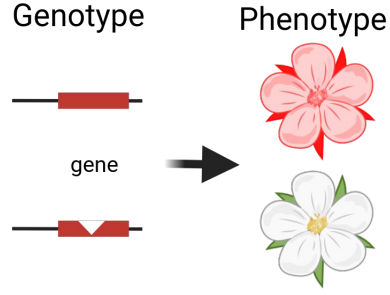
- Not all species are genetically accessible

Arabidopsis thaliana



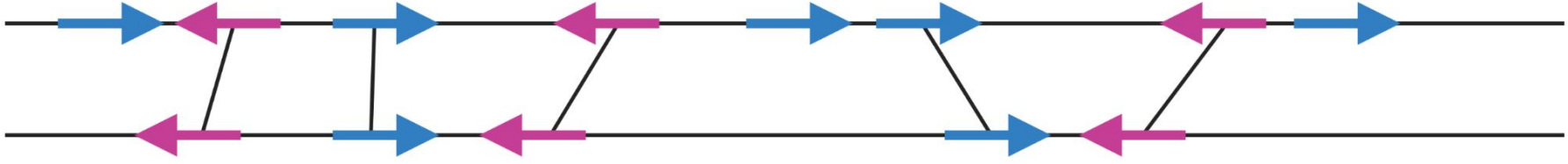
Sequoiadendron giganteum





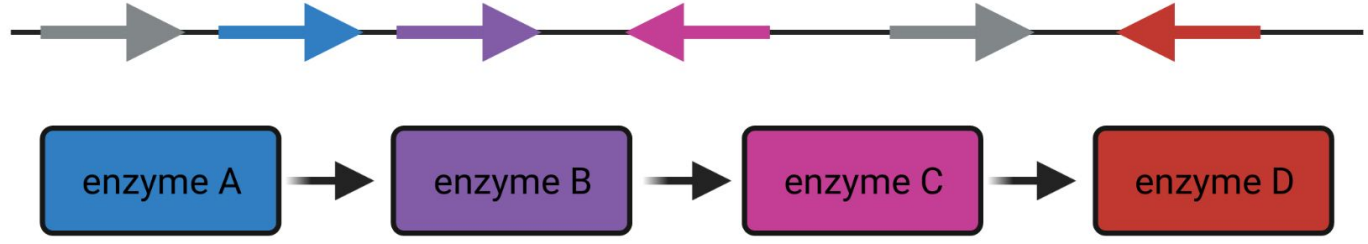
Synteny

- Synteny = same order of genes in different species
- Shared ancestry is responsible for synteny
- Changes during evolutionary times reduce synteny



Biosynthetic gene cluster

- Genes involved in one biosynthetic pathway
- Genes located in the same gene cluster
- Genes belong to different gene families
- Evolutionary benefits of clustering?



Biosynthetic gene cluster - example 1

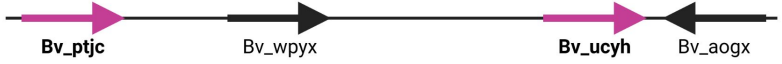
- Thalianol gene cluster

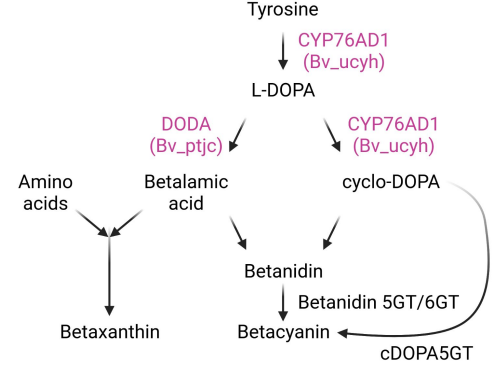
- Marneral gene cluster

Biosynthetic gene cluster - example 2

- Betalain biosynthesis gene cluster in sugar beet

- Evolution of betalain and anthocyanin biosynthesis is a model system for evolutionary questions



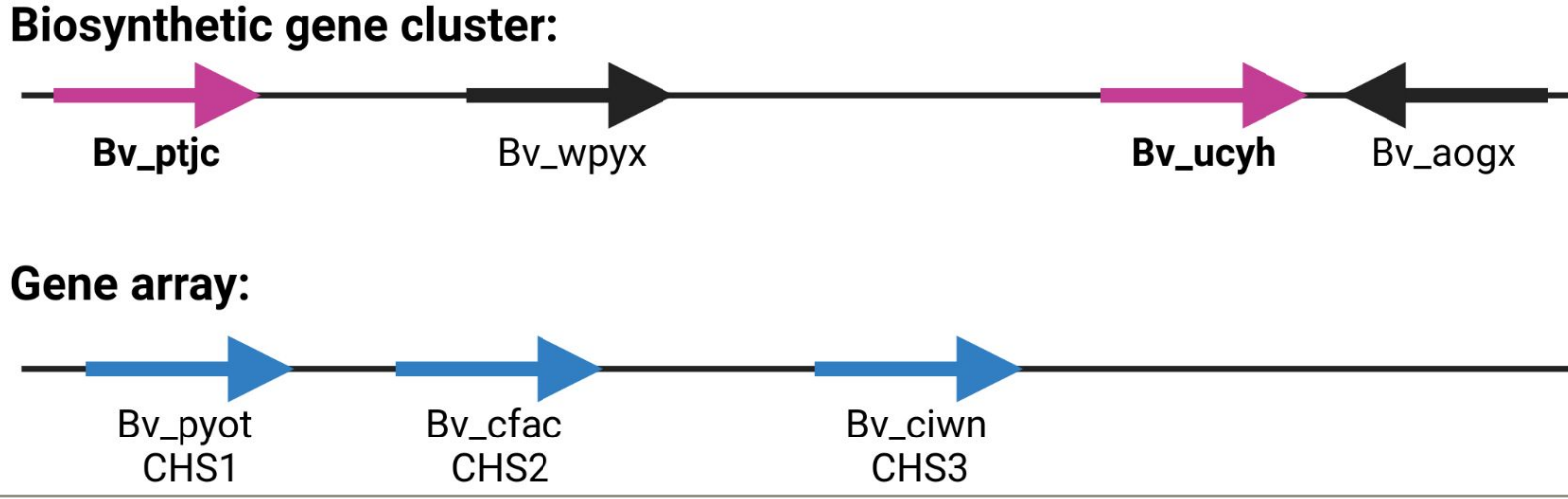


Brockington et al., 2015: 10.1111/nph.13441
Timoneda et al., 2019: 10.1111/nph.15980

Biosynthetic gene clusters vs. gene arrays

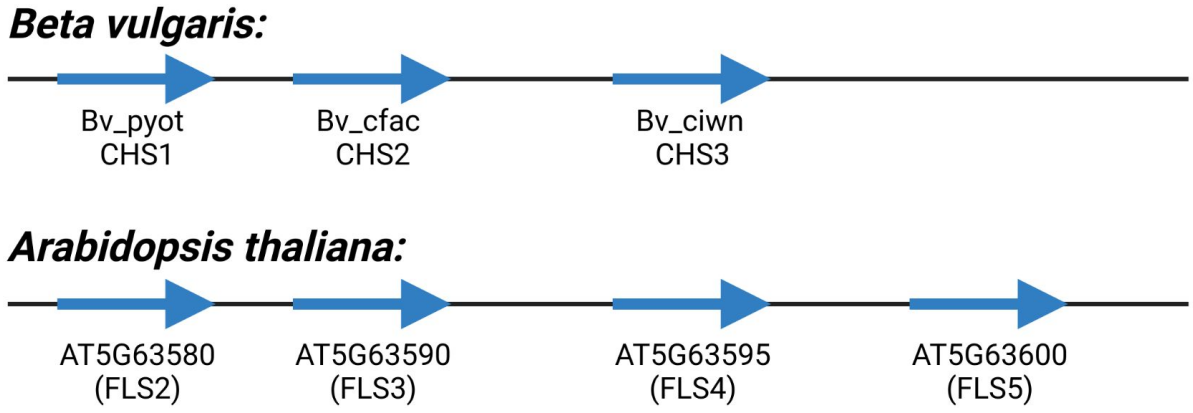
- Biosynthetic gene clusters are characterized by genes encoding enzymes with different functions

- Gene arrays comprise copies of the same gene (result of tandem duplications)



Gene array examples

- Gene arrays are frequent, but less interesting
- CHS gene array in sugar beet (*Beta vulgaris*)
- FLS gene array in *Arabidopsis thaliana*



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Identification of biosynthetic gene clusters

- Neighbouring genes must have different functions
  - Assign functional annotation (InterProScan, Mercator, RBHs)

- Neighbouring genes must be involved in the same pathway
  - Assign pathway to genes (KEGG, GO, MetaCyc)

- Co-expression of clustered genes is additional evidence
  - Analyse gene expression across numerous samples

- Conservation across some species can be additional evidence
  - Synteny analysis (JCVI)

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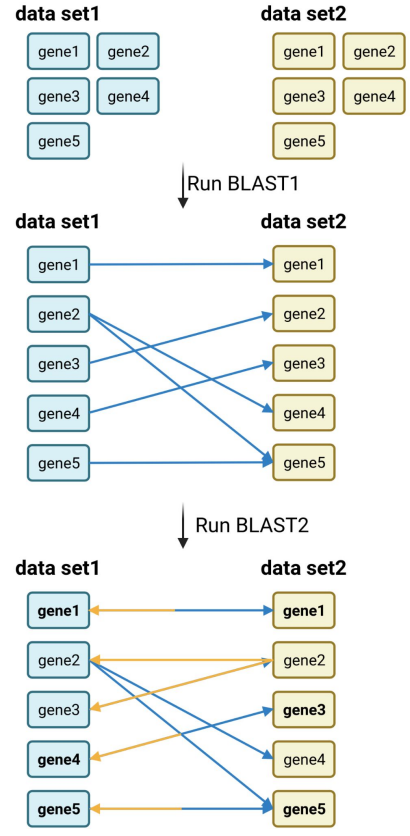
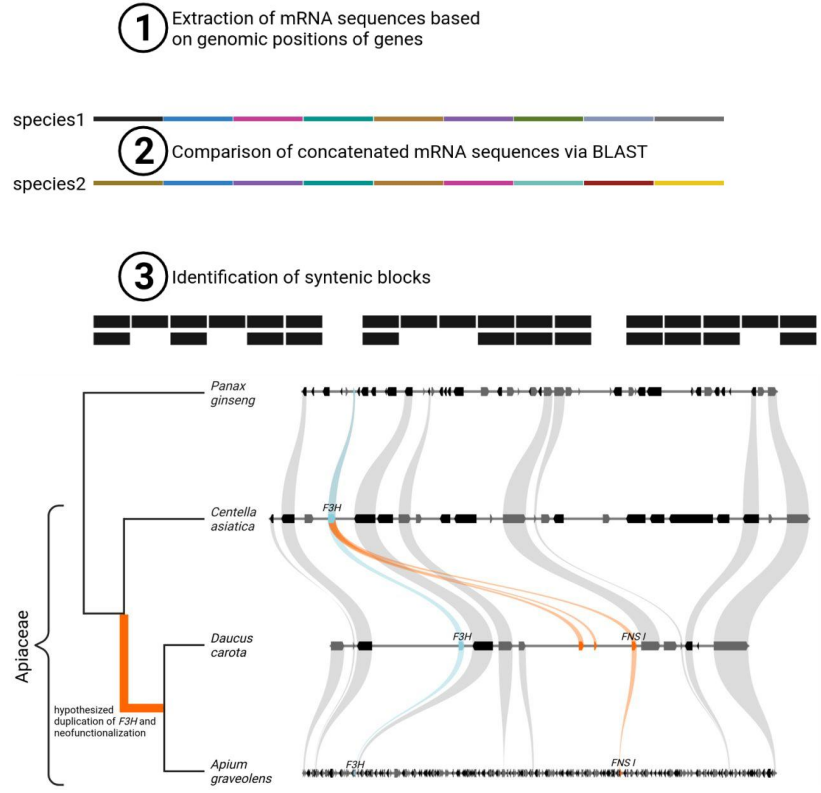
Reciprocal Best BLAST hits (RBHs)

- Run BLAST of data set 1 against data set 2
- Run BLAST in opposite direction
- Check for bidirectional (reciprocal) hits; reciprocal hits add reliability to the pairing
- Functional annotation can be transferred from data set 1 to data set 2
- Computationally efficient, but not perfect method



JCVI (tool for synteny analysis)

- Required input: FASTA file with mRNA sequences and GFF3 file with gene positions
- BLAST of concatenated mRNA sequences is used to connect gene across species
- Identification of syntenic gene blocks
- Extension of syntenic blocks

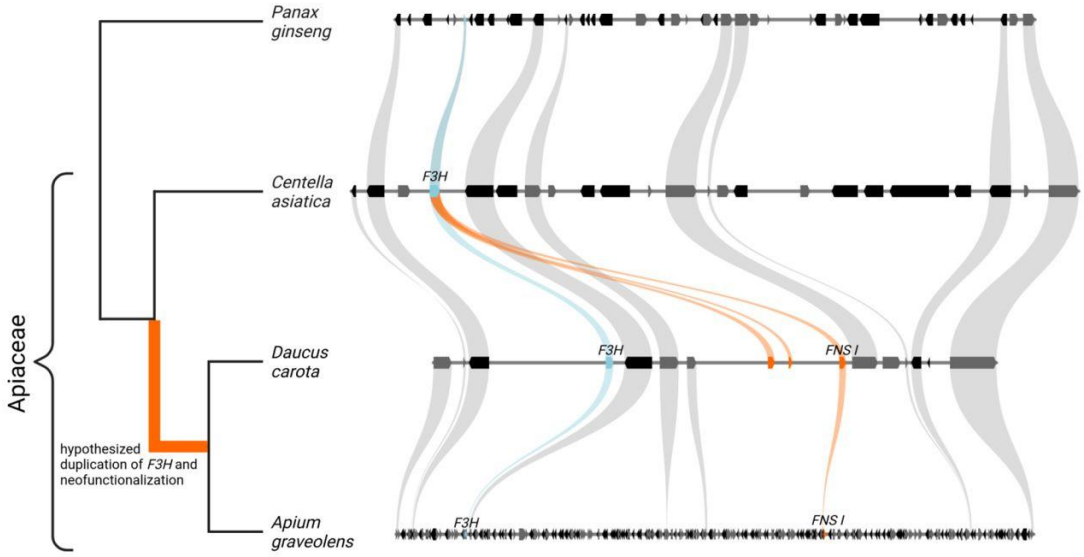


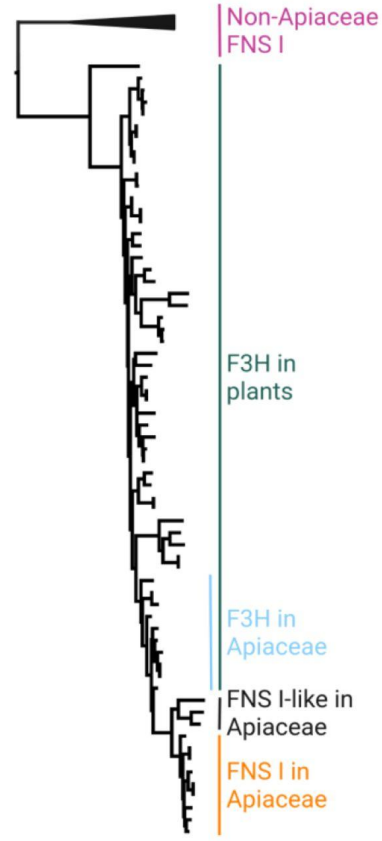
Pucker & Iorizzo, 2022: 10.1101/2022.02.16.480750

Ortholog assignment confirmation

- Phylogenetic tree for ortholog assignment confirmation

- Evolutionary signal can support synteny analysis results





Pucker & Iorizzo, 2022: 10.1101/2022.02.16.480750

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Chromatin structure influences gene expression

- Chromatin structure can determine gene expression
- Shared chromatin structure in gene cluster
- Shared regulation of all genes in cluster
- Genes acting in the same pathway need co-expression

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Evolutionary benefits of gene clusters

- Biosynthetic gene clusters are often evolutionary young pathways
- Many biosynthetic gene clusters lead to pathogene resistances
- Clustered genes ensure joint inheritance
- Clustering might prevent toxic intermediates
- Clustering could support shared regulation

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Biosynthetic gene cluster - examples

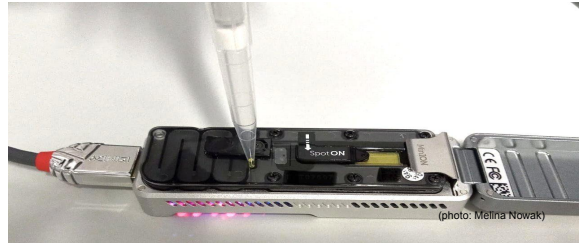
S.no	Species	Metabolite	Compound class	Chromosome (core genes)	Function	Tissue of expression	References
1	*Arabidopsis thaliana*	Thalianol	Triterpenes	5	Unknown physiological function, unregulated expression of cluster genes leads to dwarfing of plant. Modulate root microbiome content	Roots	Field and Osbourn, 2008; Field et al., 2011; Chen et al., 2019a
2	*A. thaliana*	Marneral	Triterpenes	5	Unknown physiological function, unregulated expression of cluster genes leads to dwarfing of plant	Roots	Field and Osbourn, 2008; Field et al., 2011
3	*Avena strigosa*	Avenacins	Triterpenes	1	Defense against pathogens	Roots	Li et al., 2021a
4	*Cucumis sativus*	Cucurbitacins	Triterpenes	6	Insect deterrent properties and possess medicinal value	Leaves and fruits	Shang et al., 2014
5	*Ricinus communis and Jatropha curcas*	Casbenes	Diterpenes	1	Possess medicinal value and used in treating cancers and HIV infection	Constitutive expression in leaves, roots and stems	King et al., 2014, 2016
6	*Zea mays*	2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA)	Hydroxamic acids	4	Defense related activities	Mainly expressed during seedling stages and in roots.	Frey et al., 1997
7	*Oryza sativa*	Momilactones	Diterpenes	4	Insect deterring properties and anti-fungal properties	Induced expression during pathogen attack	Shimura et al., 2007; Wang et al., 2011
8	*O. sativa*	Phytocassanes Oryzalides	Diterpenes	2	Defense related activities	Induced expression during pathogen attack	Swaminathan et al., 2009; Wu et al., 2011
9	*Lotus japonicus*	Linamarin Lotuastralin	Cyanogenic glucosides	3	Herbivore deterrent activities	Above ground plant parts	Takos et al., 2011
10	*Sorghum bicolor*	Dhurrin	Cyanogenic glucosides	1	Herbivore deterrent activities	Above ground plant parts	Takos et al., 2011
11	*L. japonicus*	20-hydroxy-betulinic acid	Triterpene	3	Possible role in plant development and nodule formation	Elevated expression in roots and nodules	Krokida et al., 2013

Bharadwaj et al., 2021: 10.3389/fpls.2021.697318

EXAMPLE: Withanolides

- Antiproliferative activity (potential cancer drugs)
- Biosynthesis is largely unknown except for first step

Withania somnifera



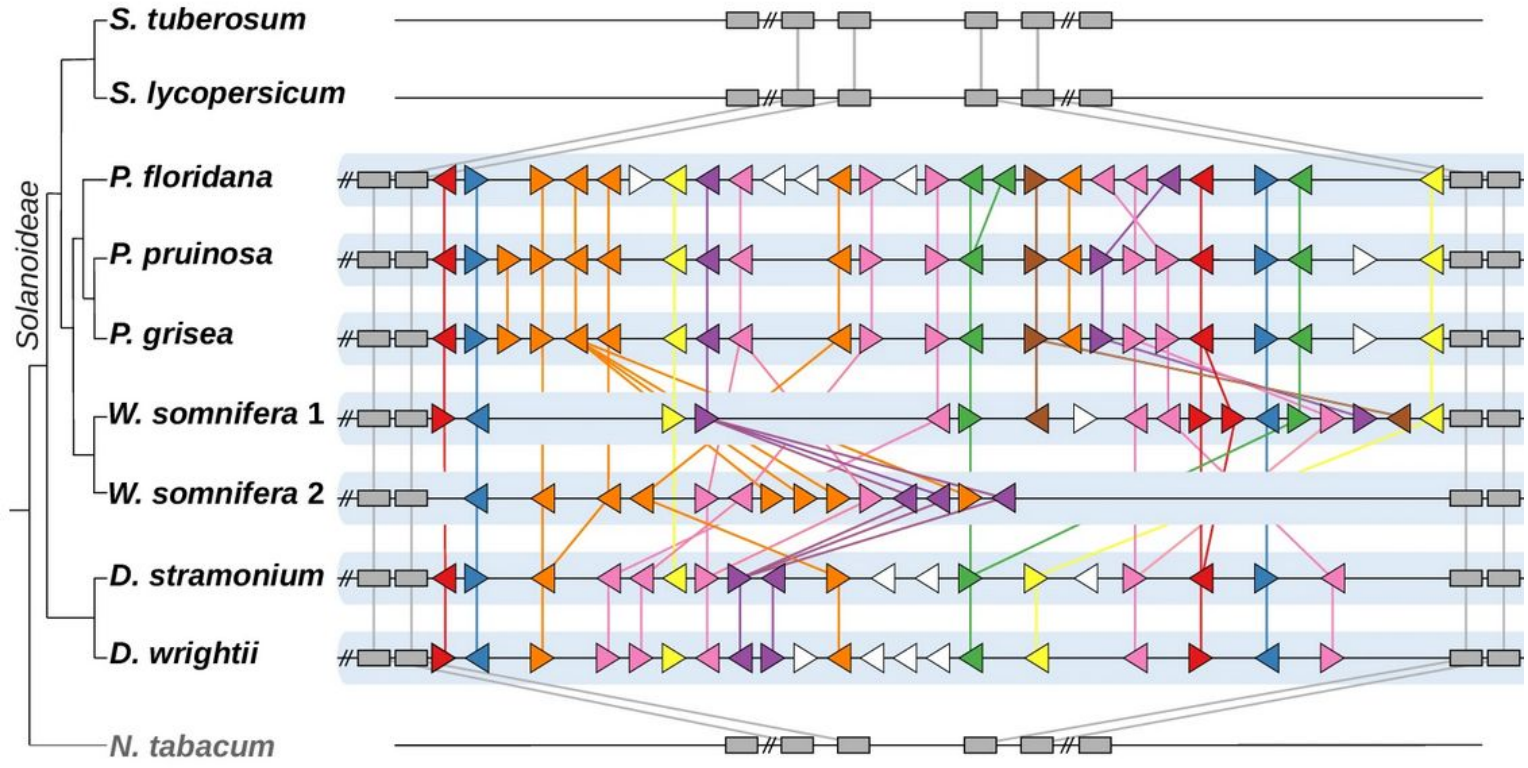
- ONT sequencing, genome sequence construction, annotation

Withania somnifera
(Wowbobwow12, CC BY-SA 3.0)

Datura innoxia
(Flobbadob, CC BY-SA 4.0)

Nicandra physalodes
(Flobbadob, CC BY-SA 4.0)

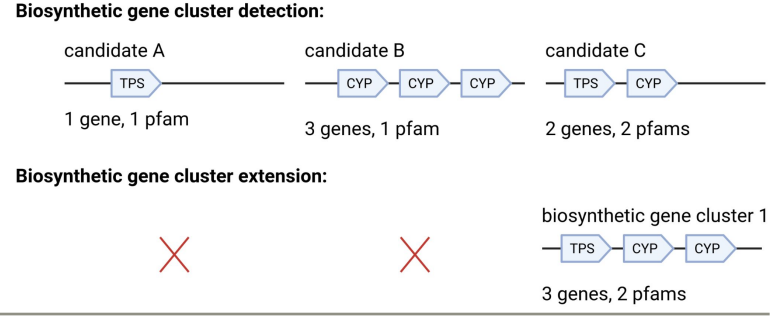
EXAMPLE: Withanolide biosynthesis gene cluster



Hakim, Choudhary, Malhotra, Peng et al., 2024: 10.1038/s41467-025-61686-1

Tools for gene cluster identification - plantiSMASH

- Required input: FASTA file and GFF3 file
- plantiSMASH is plant version of antiSMASH (bacteria tool)
- Many integrated tools: NCBI BLAST+, Diamond, HMMer3, GlimmerHMM, CD-HIT
- Web server available: http://plantismash.secondarymetabolites.org/



Kautsar et al., 2017: 10.1093/nar/gkx305

Summary

- Long read sequencing technologies
- Genome sequence assembly and annotation
- Synteny
- Biosynthetic gene clusters in plants

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Time for questions!

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Questions

1. Which sequencing technologies are used to analyze plant genomes?
2. What is synteny?
3. What are the characteristics of a biosynthetic gene cluster?
4. What are evolutionary benefits/forces that cause clustering of genes?
5. Which plant biosynthesis pathways are clustered? (examples)
6. You read about a specific metabolite that is only produced by 10 species of a plant family. How would you approach the identification of underlying genes?