

Phylogenetics

syntenet: an R/Bioconductor package for the inference and analysis of syntenic networks

Fabricio Almeida-Silva ^{1,2}, Tao Zhao³, Kristian K. Ullrich⁴, M. Eric Schranz ⁵ and Yves Van de Peer^{1,2,6,7,*}

¹Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium, ²VIB Center for Plant Systems Biology, VIB, 9052 Ghent, Belgium, ³State Key Laboratory of Crop Stress Biology for Arid Areas/Shaanxi Key Laboratory of Apple, College of Horticulture, Northwest A&F University, Yangling 712100, China, ⁴Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Biology, Ploen 24306, Germany, ⁵Biosystematics Group, Wageningen University and Research, Wageningen 6708, The Netherlands ⁶Department of Biochemistry, Genetics and Microbiology, Centre for Microbial Ecology and Genomics, University of Pretoria, Pretoria 0028, South Africa and ⁷College of Horticulture, Academy for Advanced Interdisciplinary Studies, Nanjing Agricultural University, Nanjing 210095, China

*To whom correspondence should be addressed.

Associate Editor: Pier Luigi Martelli

Received on August 16, 2022; revised on November 21, 2022; editorial decision on December 10, 2022

Abstract

Summary: Interpreting and visualizing syntenic relationships across several genomes is a challenging task. We previously proposed a network-based approach for better visualization and interpretation of large-scale microsyntenic analyses. Here, we present *syntenet*, an R package to infer and analyze syntenic networks from whole-genome protein sequence data. The package offers a simple and complete framework, including data preprocessing, syntenic detection and network inference, network clustering and phylogenomic profiling, and microsyntenic-based phylogeny inference. Graphical functions are also available to create publication-ready plots. Syntenic networks inferred with *syntenet* can highlight taxon-specific gene clusters that likely contributed to the evolution of important traits, and microsyntenic-based phylogenies can help resolve phylogenetic relationships under debate.

Availability and implementation: *syntenet* is available on Bioconductor (<https://bioconductor.org/packages/syntenet>), and the source code is available on a GitHub repository (<https://github.com/almeidasilva/syntenet>).

Contact: yves.vandepeer@psb.vib-ugent.be

Supplementary information: [Supplementary data](#) are available at *Bioinformatics* online.

1 Introduction

Gene and genome duplications provide organisms with the raw genetic material for biological innovations (Ohno, 1970; Panchy *et al.*, 2016; Van De Peer *et al.*, 2017). Thus, exploring the evolution of duplicated genes and genomes can help explain how new traits arise and diversify across taxa. Identifying collinear or syntenic regions (here used as synonyms, i.e. different genomic segments showing conserved gene content and order) within genomes has become standard practice to detect signatures of whole-genome duplications (WGD) and the genomic rearrangements that typically follow WGD events (Liu *et al.*, 2022; Ma *et al.*, 2021; Vanneste *et al.*, 2013; Wan *et al.*, 2021). Syntenic analyses can also be performed to compare different genomes to provide insights on population structure, species divergence and the evolution of gene families and traits (Jayakodi *et al.*, 2020; Li *et al.*, 2022; Tang *et al.*, 2022; Zhang *et al.*, 2021; Zhou *et al.*, 2017). However, when

comparing syntenic relationships among several genomes, interpretation and visualization is notoriously complex.

We previously proposed a network-based approach to analyze syntenic in large datasets that consists in treating anchor pairs (duplicates retained from a large-scale duplication event) from syntenic comparisons as connected nodes of an undirected unweighted graph (Zhao and Schranz, 2017). We have used such syntenic networks to study the evolution of MADS-box transcription factors in plants (Zhao *et al.*, 2017), explore the conservation patterns of syntenic clusters in mammalian and angiosperm genomes (Zhao and Schranz, 2019), and to provide insights into controversial phylogenetic relationships in angiosperms through a microsyntenic-based phylogeny (Zhao *et al.*, 2021). However, despite gaining wide interest, and its wide applicability, our method has not been incorporated in a distributable format. Here, we present *syntenet*, an R/Bioconductor package to infer and analyze

share a common gene onto a representative pair with the lowest *E*-value (see [Wang et al., 2012](#) for details on the synteny detection algorithm). It is noteworthy that synteny detection in R can also be performed with the Bioconductor package DECIPHER ([Wright, 2016](#)), which has its own synteny detection algorithm, but whose accuracy has not been benchmarked against existing tools.

2.3 Synteny network clustering and phylogenomic profiling

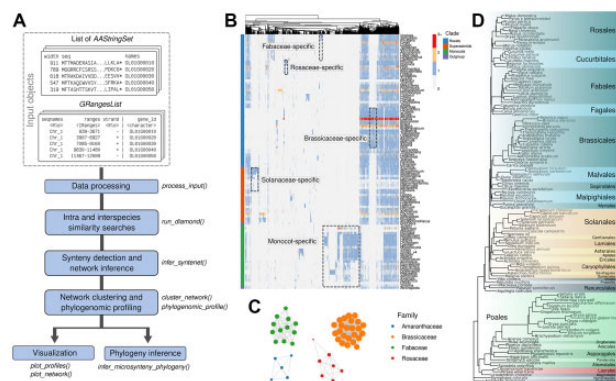
The synteny network inferred with *infer_syntenet* is represented as an edge list. Network clustering is performed with the Infomap algorithm by default, which has been demonstrated as the best clustering technique for synteny networks (Zhao *et al.*, 2021), but users can also specify other network clustering algorithms implemented in the *igraph* package (<https://igraph.org>), such as Leiden, label propagation, Louvain and edge betweenness. Synteny clusters are used for phylogenomic profiling, which consists in obtaining a matrix m_{ij} representing the number of genes from cluster j that can be found in species i (Fig. 1B). This analysis can reveal synteny clusters that are deeply conserved across taxa, and taxon-specific clusters (e.g. family-specific synteny clusters in Fig. 1B). Clusters can be visualized either as a heatmap (Fig. 1B) or as a network plot (Fig. 1C). For the heatmap visualization, phylogenomic profiles are clustered using Ward's clustering on a matrix of Euclidean distances, but both the distance measure and the clustering algorithm can be modified by users.

2.4 Microsynteny-based phylogeny reconstruction

For phylogeny inference, the matrix of phylogenomic profiles is binarized, transposed and exported as a PHYLIP-formatted file. The function *infer_microsynteny_phylogeny* passes this PHYLIP file to IQTREE2, which infers a phylogeny from binary data using the MK+FO+R model with 1000 bootstrap replicates and 1000 replicates for the SH-like approximate likelihood ratio test. Users can also choose a different substitution model, if it is suitable for binary data.

3 Application to real datasets

We demonstrated the effectiveness of *syntenet* by reproducing results from previous works on synteny networks. We recreated phylogenomic profiles for angiosperm genomes from [Zhao and Schranz \(2019\)](#), and we reconstructed the microsynteny-based phylogeny of angiosperms from [Zhao et al. \(2021\)](#) ([Supplementary Data S1 and S2](#)). We have also inferred synteny networks from 16 algae genomes (phylum Chlorophyta) available on Pico-PLAZA 3.0 ([Van Bel et al., 2018](#)). Synteny detection and network inference for 16 Chlorophyta genomes took 29 s on an Ubuntu 20.04 laptop with an Intel i5-1135G7 processor (2.40 GHz; 8 GB RAM) ([Supplementary Data S3](#)). A detailed runtime benchmark of how *syntenet* scales with increasingly large datasets is available in [Supplementary Data S3](#).



4 Known limitation

Synteny detection in highly fragmented genomes is challenging, so the MCScanX algorithm might fail to detect some syntenic blocks in such genomes (Liu *et al.*, 2018). Thus, when selecting species to use in synteny, we recommend using genomes with at least 85% complete BUSCOs to avoid bias. However, with the fast advancement in sequencing technologies and resequencing of low-quality reference genomes, genome completeness will likely cease to be a concern in the near future.

Funding

Y.V.d.P. acknowledges funding from the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Program [833522]. Y.V.d.P. and F.A.-S. acknowledge funding from Ghent University.

(Methusalem funding, BOF.MET.2021.0005.01). K.K.U. acknowledges institutional funding through the Max Planck Society. T.Z. acknowledges the Chinese Universities Scientific Fund [2452021133].

Conflict of Interest: none declared.

References

- Altschul,S.F. *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, **25**, 3389–3402.
- Buchfink,B. *et al.* (2021) Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nat. Methods*, **18**, 366–368.
- Eddelbuettel,D. and François,R. (2011) Rcpp: seamless R and C++ integration. *J. Stat. Softw.*, **40**, 1–18.
- Jayakodi,M. *et al.* (2020) The barley pan-genome reveals the hidden legacy of mutation breeding. *Nature*, **588**, 284–289.
- Li,M.H. *et al.* (2022) Genomes of leafy and leafless platanthera orchids illuminate the evolution of mycoheterotrophy. *Nat. Plants*, **8**, 373–388.
- Liu,D. *et al.* (2018) Inferring synteny between genome assemblies: a systematic evaluation. *BMC Bioinformatics*, **19**, 1–13.
- Liu,Y. *et al.* (2022) The cycas genome and the early evolution of seed plants. *Nat. Plants*, **8**, 389–401.
- Ma,X. *et al.* (2021) A chromosome-level *Amaranthus cruentus* genome assembly highlights gene family evolution and biosynthetic gene clusters that may underpin the nutritional value of this traditional crop. *Plant J.*, **107**, 613–628.
- Minh,B.Q. *et al.* (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.*, **37**, 1530–1534.
- Ohno,S. (1970) *Evolution by Gene Duplication*. Springer Science & Business Media, Heidelberg, Germany.
- Panchy,N. *et al.* (2016) Evolution of gene duplication in plants. *Plant Physiol.*, **171**, 2294–2316.
- Tang,D. *et al.* (2022) Genome evolution and diversity of wild and cultivated potatoes. *Nature*, **606**, 535–541.
- Van Bel,M. *et al.* (2018) PLAZA 4.0: an integrative resource for functional, evolutionary and comparative plant genomics. *Nucleic Acids Res.*, **46**, D1190–D1196.
- Van De Peer,Y. *et al.* (2017) The evolutionary significance of polyploidy. *Nat. Rev. Genet.*, **18**, 411–424.
- Vanneste,K. *et al.* (2013) Inference of genome duplications from age distributions revisited. *Mol. Biol. Evol.*, **30**, 177–190.
- Wan,T. *et al.* (2021) The *Welwitschia* genome reveals a unique biology underpinning extreme longevity in deserts. *Nat. Commun.*, **12**.
- Wang,Y. *et al.* (2012) MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res.*, **40**, e49–14.
- Wright,E.S. (2016) Using DECIPHER v2.0 to analyze big biological sequence data in R. *R J*, **8**, 352.
- Zhang,H. *et al.* (2021) Comparison of Brassica genomes reveals asymmetrical gene retention between functional groups of genes in recurrent polyploidizations. *Plant Mol. Biol.*, **106**, 193–206.
- Zhao,T. *et al.* (2017) Phylogenomic synteny network analysis of MADS-box transcription factor genes reveals lineage-specific transpositions, ancient tandem duplications, and deep positional conservation. *Plant Cell*, **29**, 1278–1292.
- Zhao,T. *et al.* (2021) Whole-genome microsynteny-based phylogeny of angiosperms. *Nat. Commun.*, **12**, 1–14.
- Zhao,T. and Schranz,M.E. (2017) Network approaches for plant phylogenomic synteny analysis. *Curr. Opin. Plant Biol.*, **36**, 129–134.
- Zhao,T. and Schranz,M.E. (2019) Network-based microsynteny analysis identifies major differences and genomic outliers in mammalian and angiosperm genomes. *Proc. Natl. Acad. Sci. USA*, **116**, 2165–2174.
- Zhou,P. *et al.* (2017) Exploring structural variation and gene family architecture with De novo assemblies of 15 *Medicago* genomes. *BMC Genomics*, **18**, 14.