Bioinformatics, 39(1), 2023, btac806
https://doi.org/10.1093/bioinformatics/btac806
Advance Access Publication Date: 20 December 2022
Applications Note

Phylogenetics

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

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

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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). Synteny 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 synteny relationships among several genomes, interpretation and visualization is notoriously complex.

We previously proposed a network-based approach to analyze synteny in large datasets that consists in treating anchor pairs (duplicates retained from a large-scale duplication event) from synteny comparisons as connected nodes of an undirected unweighted graph (Zhao and Schranz, 2017). We have used such synteny networks to study the evolution of MADS-box transcription factors in plants (Zhao *et al.*, 2017), explore the conservation patterns of synteny clusters in mammalian and angiosperm genomes (Zhao and Schranz, 2019), and to provide insights into controversial phylogenetic relationships in angiosperms through a microsynteny-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

synteny networks from whole-genome protein sequences. *syntenet* integrates our entire previously developed method in an easy and simple framework, from data preprocessing to network analyses, and visualization.

2 Implementation

For seamless integration with other Bioconductor packages, the input objects for *syntenet* are base R or core Bioconductor classes (Fig. 1A). The complete pipeline requires the external software tools DIAMOND (Buchfink *et al.*, 2021) and IQTREE2 (Minh *et al.*, 2020). Users must input: (i) protein sequences for each species, stored in a list of *AAStringSet* objects and (ii) genomic coordinates for each gene, stored in a *GRangesList* object.

2.1 Data preprocessing and similarity searches

The input data are automatically preprocessed to clean up gene names, add unique species identifiers to gene and chromosome names, and to make sure that only translated sequences from primary transcripts are included. The processed data are used as input to sequence similarity search programs, such as BLASTp (Altschul *et al.*, 1997) or DIAMOND (Buchfink *et al.*, 2021). Although users can run DIAMOND or BLAST on the command line, *syntenet* has a wrapper function named *run_diamond* that runs DIAMOND from the R session and reads the tabular output as a data frame (Fig. 1A).

2.2 Synteny detection and network representation

The function *infer_syntenet* integrates gene coordinates with the DIAMOND output to detect anchor pairs (Fig. 1A). Synteny detection is performed using a native version of the popular MCScanX algorithm (Wang *et al.*, 2012), which has been ported to R with the Rcpp framework to integrate C++ code in R packages (Eddelbuettel and François, 2011). Hence, users have access to the same accuracy and speed of MCScanX without having to install it. As in the original MCScanX algorithm, anchor pairs and the syntenic blocks to which they belong are stored in a .collinearity file, which is parsed by *syntenet* into a 2-column data frame (i.e. an edge list representation of a network). The MCScanX algorithm automatically corrects artificially large numbers of collinear gene pairs due to tandem arrays by collapsing multiple consecutive homologs that 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.

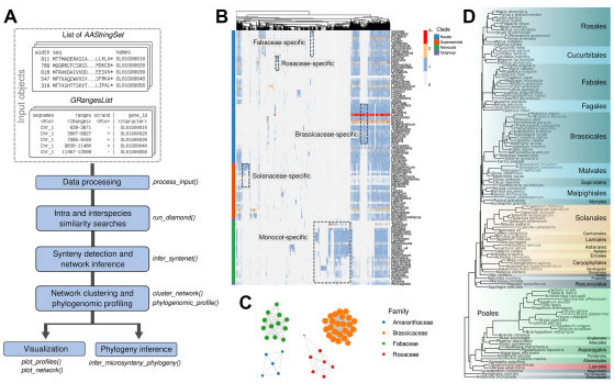

Fig. 1. Workflow and possible applications of *syntenet*. (**A**) Schematic model of the whole pipeline for synteny network inference and analysis. Boxes represent the different steps of the pipeline, with the corresponding function names in italicized fonts on the right and below. (**B**) Example heatmap of phylogenomic profiles that can be created with the function *phylogenomic_profile()*. Dashed boxes highlight family-specific synteny clusters. Interestingly, species with recent whole-genome duplications (e.g. *Malus domestica* and *Glycine max*) have a greater number of genes in most synteny clusters. Data were obtained from Zhao and Schranz (2019), and code to create the figure is available on Supplementary Data S1. (**C**) Network representation of some family-specific synteny clusters from Figure 1B produced with the function *plot_network()*. (**D**) Microsynteny-based phylogeny of angiosperms. Data were obtained from Zhao *et al.* (2021), and code to reproduce the figure is available on Supplementary Data S2

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 syntenet, 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.

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