

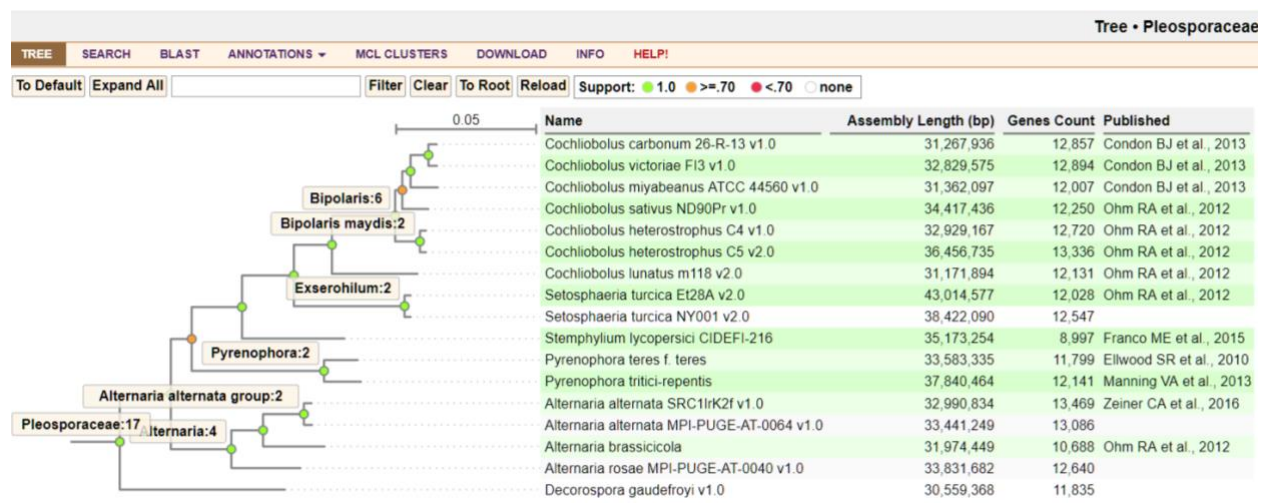
A short tutorial on SYNTENY as seen on MycoCosm.

The SYNTENY tab is used to for pairwise whole genome comparisons. Since this uses one genome as the comparator, the SYNTENY tab is only available on single genome portals (i.e: absent from groups). The application enables visual comparative analysis of complete genome assemblies at different levels of resolution, using pairwise and multiple genome alignments.

Objective: Explore genome synteny of *Cochliobolus heterostrophus* C5 with related genomes using the Pleosporaceae group page and the *Cochliobolus heterostrophus* C5 genome portal.

Go to the Pleosporaceae group page at genome.jgi.doe.gov/Pleosporaceae

Click on the TREE tab and locate *Cochliobolus heterostrophus* C5 in the tree.



Note the green selection box while mousing over the tree. A left-click collapses and expands the selection box. You can also use shift+click to zoom into the selection or ctrl+click to toggle select a node without collapsing it. The browser back button does not work on the tree page. Click the TREE tab again to restore the default view.

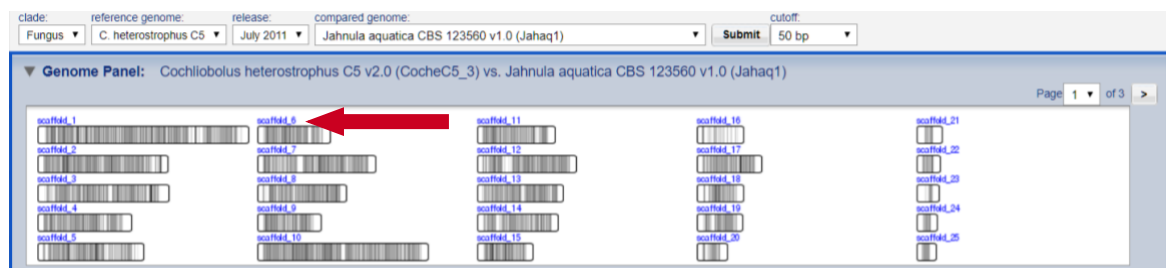
Click on “*Cochliobolus heterostrophus* C5” to go to the organism genome portal. Ideally, you should do this in another tab or window so that you can follow the exercises below keeping the phylogenetic placement of this organism in mind.

Click on the SYNTENY tab in the organism portal (*Cochliobolus heterostrophus* C5).

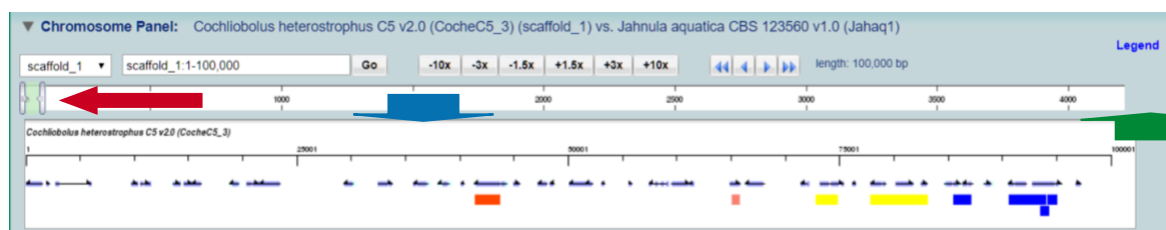


Genomic synteny is displayed in three collapsible panels in the Synteny Browser: the Genome Panel, the Chromosome Panel and the Comparison Panel.

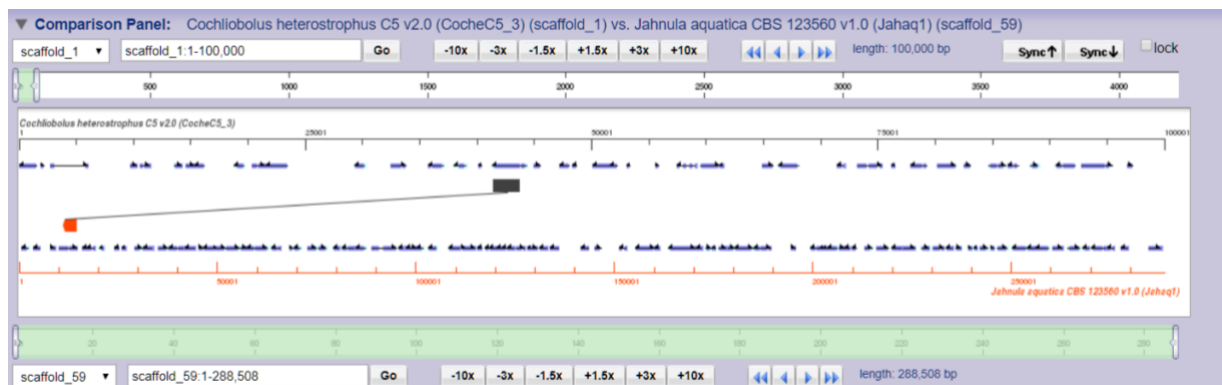
The Genome Panel depicts alignment density for all scaffolds in the reference genome against all chromosomes in the compared genome. Here, alignment density is defined for a region in the reference genome as the number of syntenic regions in the compared genome. Darker regions in the image have higher density of coverage. Clicking on a particular scaffold selects that for the Chromosome and Comparison panels below.²



The Chromosome Panel shows all of the alignments in the compared genome to a particular interval on a single chromosome in the reference genome. Synteny is depicted as "blocks" along the reference-genome interval. Each block represents an alignment of two sequences, where the position of the block indicates the alignment's location on the reference genome and the color of the block indicates the chromosome where the match is found on the compared genome. Click on Legend (green arrow below) to reveal the color-coding schema. The blocks appear stacked on top of each other when a fragment of the reference genome has synteny with multiple locations in the compared genome. The navigation buttons along with the chromosome slider (red arrow below) allow for zooming and panning along the interval of the reference chromosome. The protein models (blue arrow) leads to the protein page which links to its annotation and the genome browser.



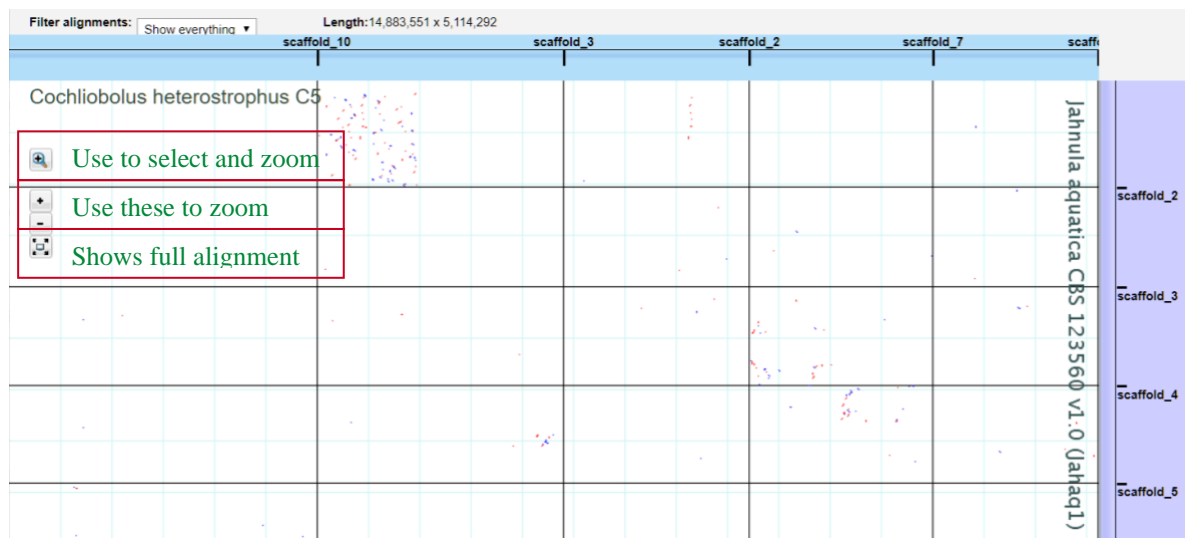
The Comparison Panel zooms further to depict synteny between a specific interval on the reference genome and a specific interval on the compared genome. In this view, each aligned region is depicted as a pair of blocks, one along the reference chromosome (grey) and one along the compared chromosomes (colored), connected by a line. Also displayed in the Compared Panel are gene model tracks (if available) for the reference and compared chromosomes.



Syntenic blocks and gene models are both interactive, as described above for the Chromosome Panel. Navigation controls allow the user to switch chromosomes, zoom and pan independently over the reference and compared genomes. The SYNTENY page also allows whole genome pairwise comparison and comparison of one-to-many using the ‘Dot Plot’ and ‘Vista Point’ views respectively.



'Dot Plot' (VistaDot) is an interactive tool that enables users to look at the DNA conservation between two genome assemblies at different levels of resolution and across multiple chromosomes/scaffolds. In the main view window, DNA coordinates of the reference genome are presented on the X axis, and DNA coordinates of the compared genome are presented on the Y axis. All chromosomes or scaffolds are concatenated together, usually in a descending order by size. The diagonal lines in the image display the homologous regions between the two genomes. If the line is blue, the regions are on the same strand. If the line is red, the regions are on opposite strands. The grid in black lines indicates scaffold/chromosome boundaries. Use the toolbar on the left to zoom or select specific regions on the plot. The map can also be navigated using click+drag similar to google maps. A cutoff control above the main window allows you to filter alignments to show only syntenic regions greater than a specified length. Dot Plot' hides the genome portal navigation bar. You can click the “Synteny” view to restore it. ‘Vista Point’ shows multiple genome alignment using "peaks and valleys" graph as seen on the genome browser. The thresholds that determine what gets colored, as well as minimum and maximum percentage bounds can be adjusted by the user. The order of the curves and the zoom can be adjusted using drag-and-drop and click-and-drag respectively.



Exercise:

1. Study the phylogenetic tree of the Pleosporaceae.
2. Use the SYNTENY tab in the *Cochliobolus heterostrophus* C5 genome portal and compare it to the genome of *Cochliobolus heterostrophus* C4. Compare the *Cochliobolus heterostrophus* C5 genome with other genomes of increasing phylogenetic distance like *Cochliobolus sativus*, *Setosphaeria turcica* and *Alternaria brassicicola*. Increase the viewed area by dragging the slider to cover a greater percentage of the scaffold. Note how increasing the cutoff from the default (50bp) can remove spurious alignments often caused by repeats.
3. Use the 'Dot Plot' view to study the high congruence between the two *Cochliobolus heterostrophus* assemblies. Compare the *Cochliobolus heterostrophus* C5 genome with other genomes of increasing phylogenetic distance as above. Note the breakdown of large scale synteny with increasing phylogenetic distance into mesosynteny as described by Ohm et al. (2012). In mesosynteny, genes are conserved within homologous chromosomes (scaffolds), but with randomized orders and orientations. Mesosynteny becomes more pronounced moving further phylogenetically to *Stagonospora nodorum* (Phaeosphaeriaceae). Ohm et al. showed that this type of genome evolution can be explained by repeated intra-chromosomal inversions.

Reference:

- Ohm RA, Feau N, Henrissat B, Schoch CL, Horwitz BA, et al. (2012) Diverse Lifestyles and Strategies of Plant Pathogenesis Encoded in the Genomes of Eighteen Dothideomycetes Fungi. PLOS Pathogens 8(12): e1003037.