


University of Missouri  
**IPG** Interdisciplinary  
 Plant Group



Division of  
**Biological Sciences**  
 University of Missouri

## Phylogenomics and Placement of Polyploidy with Transcriptomes in Brassicaceae

Dustin Mayfield  
 Wednesday, July 11<sup>th</sup>, 2012  
 University of Missouri-Columbia  
 J. Chris Pires Lab

## Family History

- Three major lineages of ~3700 extant species
- Family-wide origin
  - Pre-Miocene (molecular) or
  - Miocene ~23-5MYA (paleo-  
 botanical/ecological)
- Rapid colonization of new dry environments
- Radiation and diversification rates among  
 highest reported for angiosperms

## Phylogenomics Transcriptomes in Brassicaceae

RNA (normalized) for phylogenomic resolution  
 with 100s-1000s of markers is cost effective

**Phylogenomic analysis of transcriptome data elucidates co-occurrence of a paleopolyploid event and the origin of bimodal karyotypes in Agavoideae (Asparagaceae)**

Michael R. McKain<sup>1</sup>, Norman Wickett<sup>1,2</sup>, Saravanaraj Aravamudan<sup>3</sup>, W. Richard Mark W. Chase<sup>3</sup>, J. Chris Pires<sup>4</sup>, Claude W. Leebens-Mack<sup>1,2</sup>

NATURE | LETTER

**Leveraging skewed transcript abundance by RNA-Seq to increase the genomic depth of the tree of life**

Chris Todd Hittinger<sup>1,2</sup>, Mark Johnston<sup>1,2</sup>, John T. Tossberg<sup>1</sup>, and Antonis Rokas<sup>1,2</sup>

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**Phylogenomics reveals deep molluscan relationships**

Kevin M. Kocot, Johanna T. Cannon, Christiane Todt, Mathew R. Citarella, Andrea B. Kohn, Achim Meyer, Scott R. Santos, Christoffer Schander, Leonid L. Moroz, Bernhard Lieb & Kenneth M. Halanych

Report

**Transcriptomes of the Parasitic Plant Family Orobanchaceae Reveal Surprising Conservation of Chlorophyll Synthesis**

Norman J. Wickett<sup>1,2</sup>, Loren A. Honas<sup>1</sup>, Eric K. Wafar<sup>1</sup>, Malay Das<sup>2,3</sup>, Kan Huang<sup>3</sup>, Biao Wu<sup>4</sup>, Lena Lanzhen<sup>1</sup>, Michael P. Timko<sup>5</sup>, John Yoder<sup>6</sup>, James H. Westwood<sup>2</sup>, Claude W. dePamphilis<sup>1</sup>

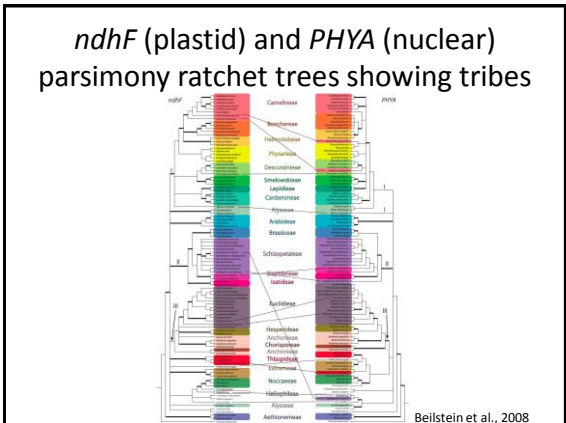
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## Method

- 1: Make libraries
- 2: Sequence
- 3: Assemble transcripts
- 4: Identify orthologs
- 5: Align
- 6: Make trees and networks
- 7: Placement of polyploidy

## Method

- 1: Make libraries

For RNA isolation we use PureLink RNA minikits  
 For RNA-seq library preparation, we use TruSeq RNA Sample Preparation Kits v2

*What have we learned?*

Wet lab is easy, assembly/analysis is harder, typos,  
 RNA quantity, Brassica vs Grasses, **Quality RNA!**

Workflows for Illumina library preparation (RNA and DNA) and suggestions soon @ <http://dustinmayfieldjones.wordpress.com/> under the 'For Collaborator's tab

**Dustin Mayfield-Jones**  
 Graduate Student at the University of Missouri-Columbia

### Method

- 2: Sequence – ~190M, 170M filtered

### Method

- 3: Assemble transcripts - Trinity

### Method

- 4: Identify orthologs

Identified set of 959 single copy genes that are shared single copy genes for *Arabidopsis thaliana*, *Populus trichocarpa*, *Vitis vinifera*, and *Oryza sativa*, or APVO SSC genes

Taxonomic Group	Number of single copy APVO SSC Plant Tribes present
Eurosids	913
Asterids	519
Core Eudicots	76
Basal Eudicots	189
Monocots	948
Basal Angiosperms	48
Gymnosperms	502
Vascular Plants	438
Green Algae	190

**DFG HaMStR**  
 (Hidden Markov Model based Search for Orthologs using Reciprocity)

Modified from Duarte et al., 2010

### Method

- 5: Align – MUSCLE

MUSCLE: multiple sequence alignment with high accuracy and high throughput by Robert C. Edgar

Alternatives include ClustalW, T-coffee, or other multiple sequence aligner.

### Method

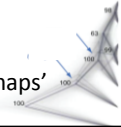
- 6: Make trees and networks – RaxML / PAUPRat

Dunn et al., 2008

## Trees vs Networks

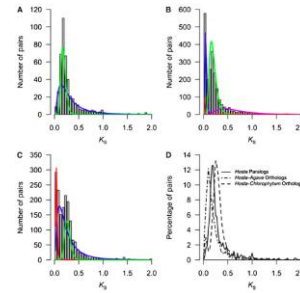
- Unlike a phylogenetic tree, which shows the most likely or most parsimonious tree, a network shows many (or all) feasible trees in one 2D representation. In effect, it allows us to visually represent the ambiguity we have about which of the feasible trees is the true tree.

I dream of beautiful trees as 'heat maps'



## Method

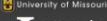
- 7: Placement of Polyploidy – Ks plots




McKain et al., 2012

## Summary


- Use RNA-seq to help **resolve the complex phylogenomic and WGD history** of Brassicaceae, a family with high radiation and diversification rates, multiple rounds of WGD, and hybridization.
- Inform those interested in the evolutionary relationship of both the **model plant, Arabidopsis, and crops, such as the Brassica oilseed and vegetable crops** including agronomically important cultivars such as canola, broccoli, cauliflower, cabbage, kale, Kohlrabi, and Brussels sprouts
- We expect to **identify previously unknown WGD events** that will further our understanding about their genetic consequences.



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**Pires Lab –**  
 Chris Pires, Pat Edger, Tatiana Arias, Madison Knapp, Avinash Karn




Marcus Koch, University of Heidelberg  
 Martin Lysák, Masaryk University


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