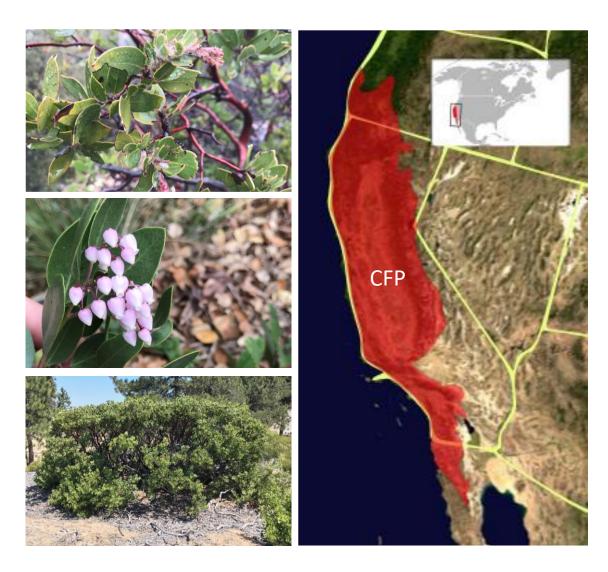
Preliminary annotation and analysis of the big berry manzanita (*Arctostaphylos glauca*) genome

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Amy Litt

Manzanitas, Arctostaphylos (Ericaceae)

- Evergreen shrubs and trees
 - Red and twisting branches
 - Urn-shaped flowers
- Most diverse woody genus in California Floristic Province (CFP)
 - Over 100 species and subspecies
 - 104 out of 105 species are found within CFP (center of manzanita biodiversity)



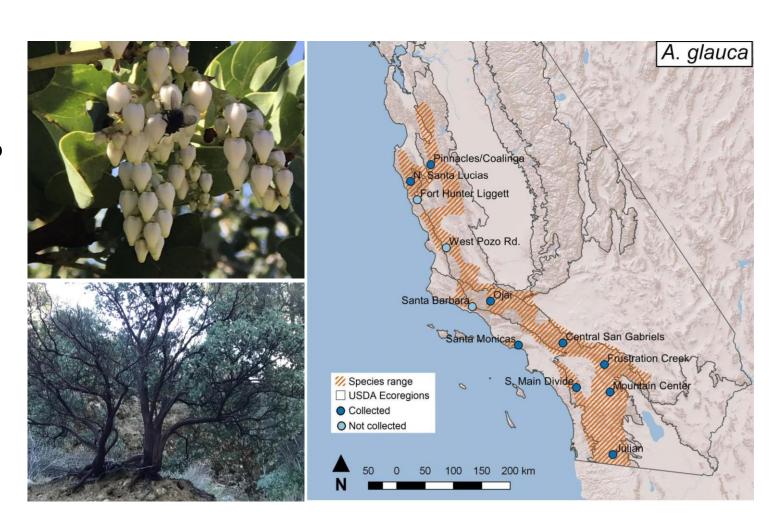
Project goal: Species delimitation in Arctostaphylos

- Complicated patterns of interspecific and intraspecific variation
 - Hybridization
 - Morphological overlap
- Use reduced-representation genomic sequencing to test species delimitation
- Facilitate the detection of genetic variants across the genus
 - 10x more SNPs with reference genome



Arctostaphylos glauca

- Diploid widespread manzanita species in CFP
 - 2n = 2x = 26
- Ecological importance
 - Pollination
 - Restore vegetation in firedisturbed area



Outline of methods

- De novo genome assembly
 - Oxford nanopore sequencing and short-read Illumina sequence data by Dovetail company
 - BUSCO to assess completeness
- Genome annotation
 - Identify and mask repetitive element: RepeatMasker
 - Structural annotation: Funannotate pipeline (https://funannotate.readthedocs.io/en/latest/index.html)
- Preliminary intraspecific and interspecific genome comparison with other Ericales:
 - Synteny: MCScanX (http://chibba.pgml.uga.edu/mcscan2/)

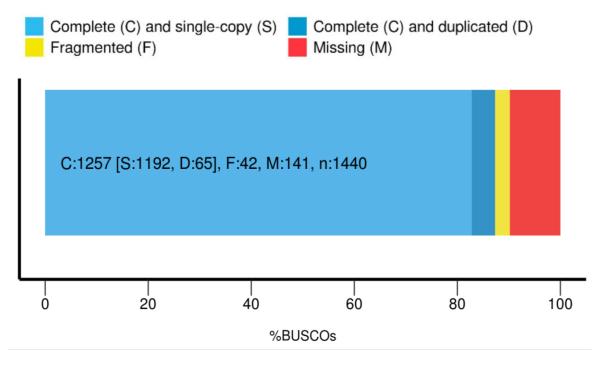


De novo genomic assembly of A. glauca

- Good contiguity after assembly
- 87.3% completeness using embryophota_odb9 lineages in BUSCO

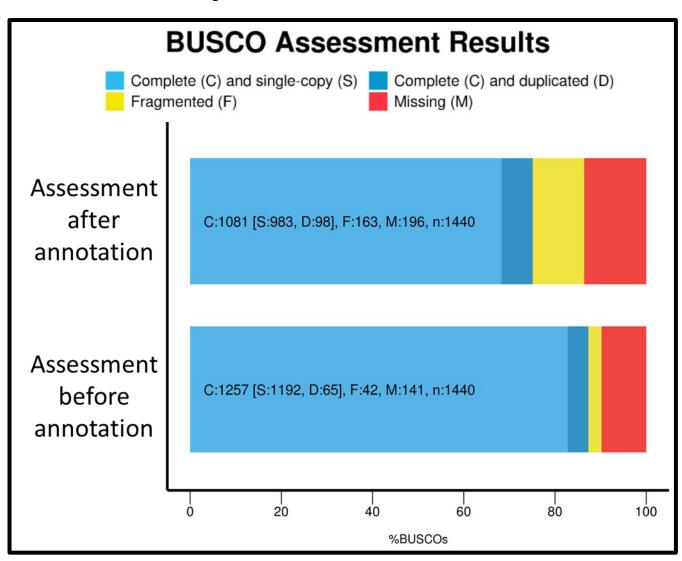
Statistics of Assembled Genome			
Genome size	567.62Mb		
N50	38.767Mb		
N90	33.601Mb		
Chromosome-level assembly	13 scaffolds > 1 Mb		
Percent of genome in gaps	0.09%		

BUSCO Assessment Results



Genome annotation summary

- Repetitive elements: 45.62% of the genome
- Number of protein coding genes: 40,069
- Average length of protein coding genes: 3024.83bp
- BUSCO assessment: 75.1% completeness



Pattern of collinearity within A. glauca genome

Statistics of Synteny Analysis within A. glauca genome				
Number of Collinear Genes	8387			
Number of All Genes	40,069			
Percentage of collinear genes	20.93			

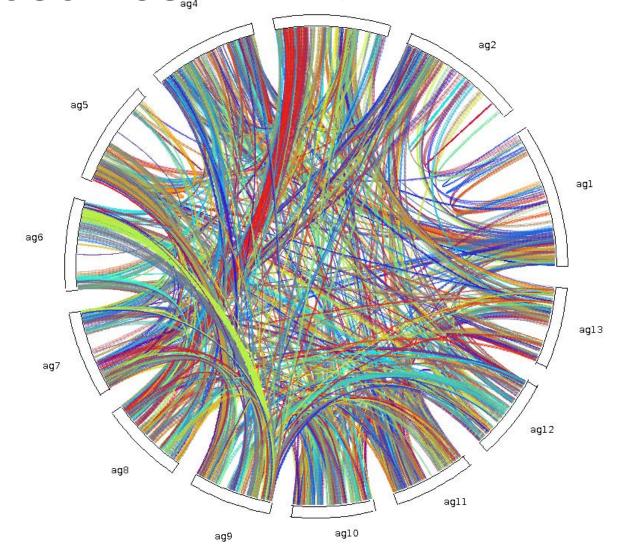
MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity

Yupeng Wang, ^{1,2} Haibao Tang, ^{1,3,4} Jeremy D. DeBarry, ⁵ Xu Tan, ^{1,3} Jingping Li, ^{1,2} Xiyin Wang, ^{1,6} Tae-ho Lee, ¹ Huizhe Jin, ^{1,2} Barry Marler, ¹ Hui Guo, ^{1,3} Jessica C. Kissinger, ^{2,5,7} and Andrew H. Paterson ^{1,2,3,7,8,*}

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Visualize collinear regions between *A. glauca* chromosomes

- Circle plot from MCScanX suggests substantial WGD events
 - 13 white bars on the edge of plot represent 13 chromosomes of A. glauca
 - Each colored bundle represents a collinear block of a syntenic gene pair



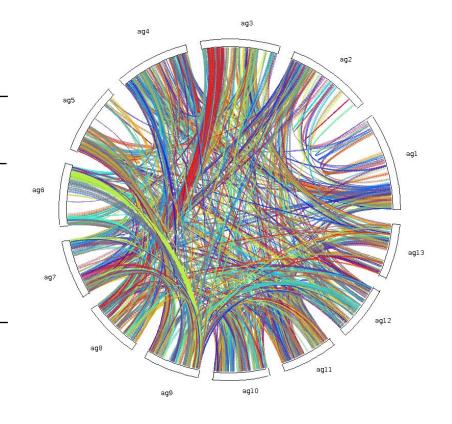
Complicated pattern of collinearity may result from the large number of total genes

Statistics of S	Synteny Ana	lysis within	A. glauca	genome
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Number of Collinear Genes 8387

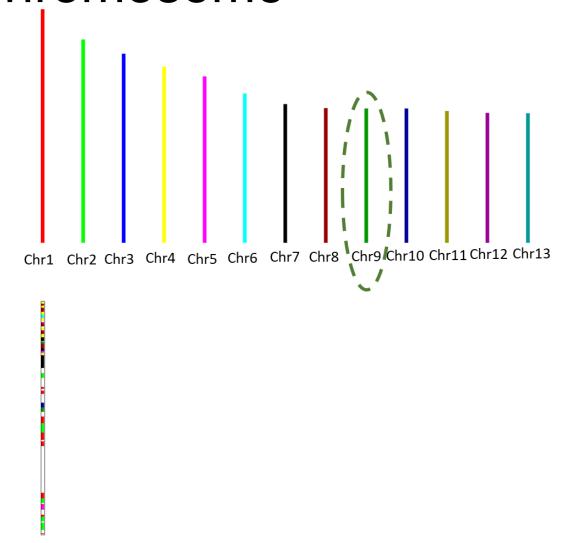
Number of All Genes 40,069

Percentage of collinear genes 20.93



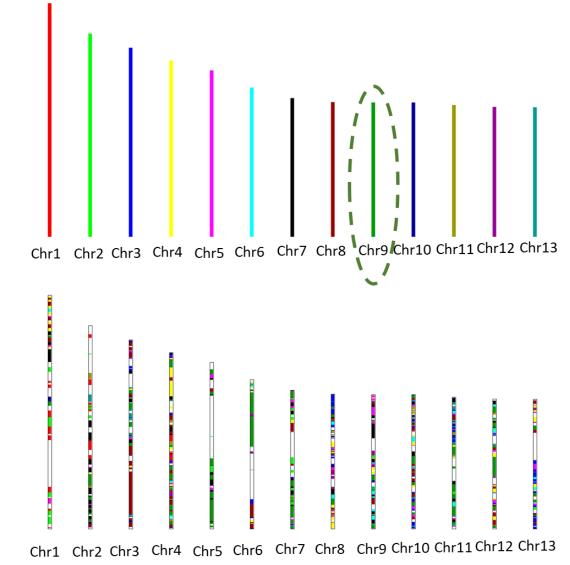
The estimated proportion of segmental duplications on each chromosome

- Bar plot from MCScanX also suggests substantial duplication events within A. glauca
 - The corresponding origin for each chromosome is indicated in color
 - Segmental regions of chromosome 9 have been observed across other chromosomes



The estimated proportion of segmental duplications on each chromosome

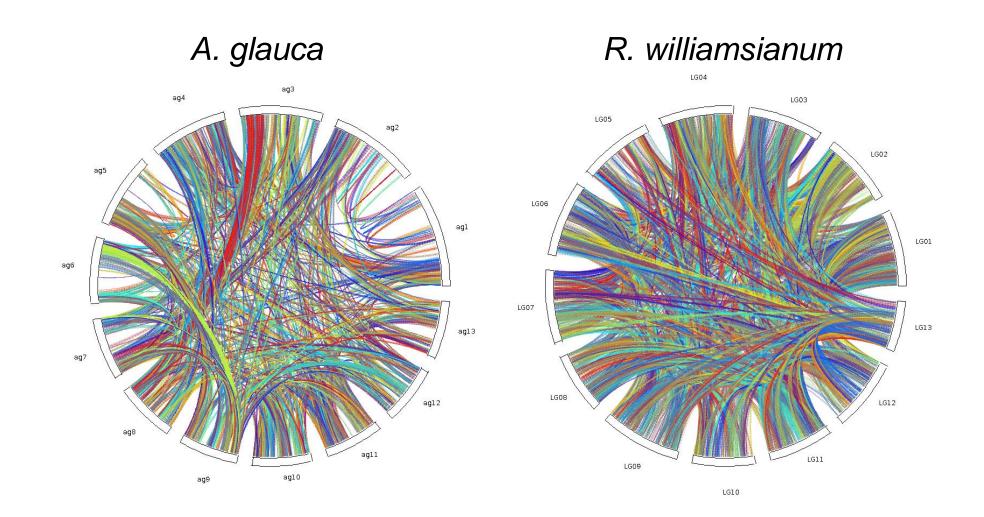
- Bar plot from MCScanX also suggests substantial duplication events within A. glauca
 - The corresponding origin for each chromosome is indicated in color
 - Segmental regions of chromosome 9 have been observed across other chromosomes



Differences in duplicate gene origins among Ericales

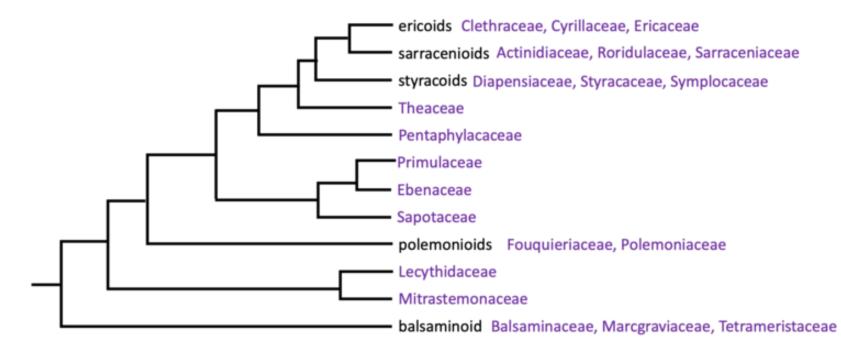
Species	No. of duplicate	No. of genes from different Origins (percentage)				itage)
	genes	Singleton	Dispersed	Proximal	Tandem	WGD
Arctostaphylos glauca	39,807	7,306 (17.7)	13,544 (34.0)	5285 (13.3)	5285 (13.3)	8387 (21.1)
Rhododendron williamsianum	21,375	3,037 (14.2)	6,608 (30.9)	651 (30.5)	2,558 (12.0)	8,521 (39.9)
Actinidia chinensis var. chinensis	33,116	1,600 (4.8)	2,295 (6.9)	274 (0.8)	1,655 (3.5)	27,292 (82.41)
Actinidia eriantha	42,943	3,802 (8.9)	8,381 (19.5)	2,072 (4.8)	2,078 (4.8)	26,655 (62.1)
Primula veris	18,285	2,871 (15.7)	13,319 (72.8)	385 (2.1)	1,126 (6.17)	584 (3.0)

Visualization of WGD in *A. glauca* and *R. williamsianum*



Limitation and future work

- Too many genes
 - RNA-seq data to facilitate structural and functional annotation
 - Additional genome sequencing to reduce the gap
- Include more genomes of Ericales



Acknowledgement

- PI: Amy Litt
- Glen Morrison
- Sarah Wilson
- Alex Rajewski
- Zheng Li
- Michael Barker











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Self-genome comparison and cross-genome of A. glauca & R. williamsianum

Reference genome	Target genome	Maximum duplication depths	Duplication depth with maximum number of genes
A. glauca	R. williamsianum	37	2
R. williamsianum	A. glauca	26 Difference	ce: 9 3 small scale
R. williamsianum	R. williamsianum	46	4 chromosomal
A. glauca	A. glauca	22	0 duplications

