

Moving Forward with Pecan Genetics

Dr. Jennifer J. Randall

SEPGA

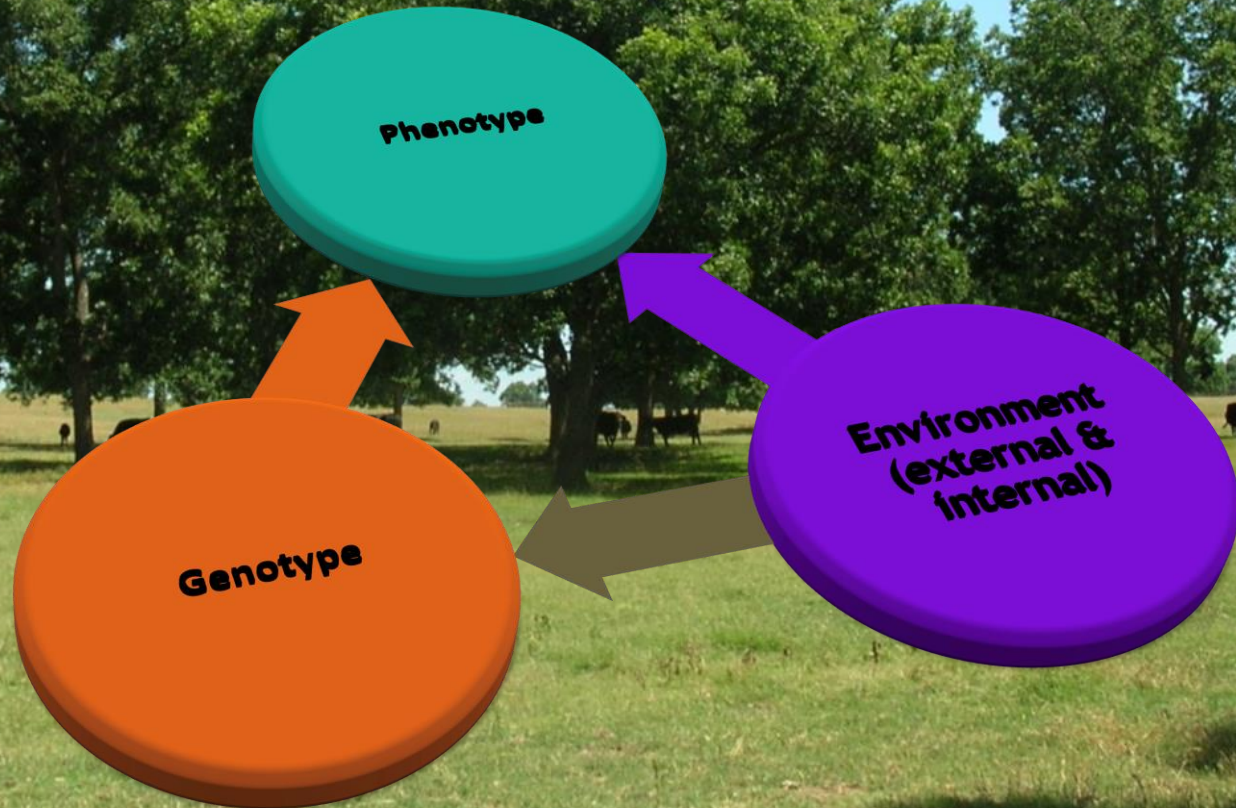
February 25, 2023



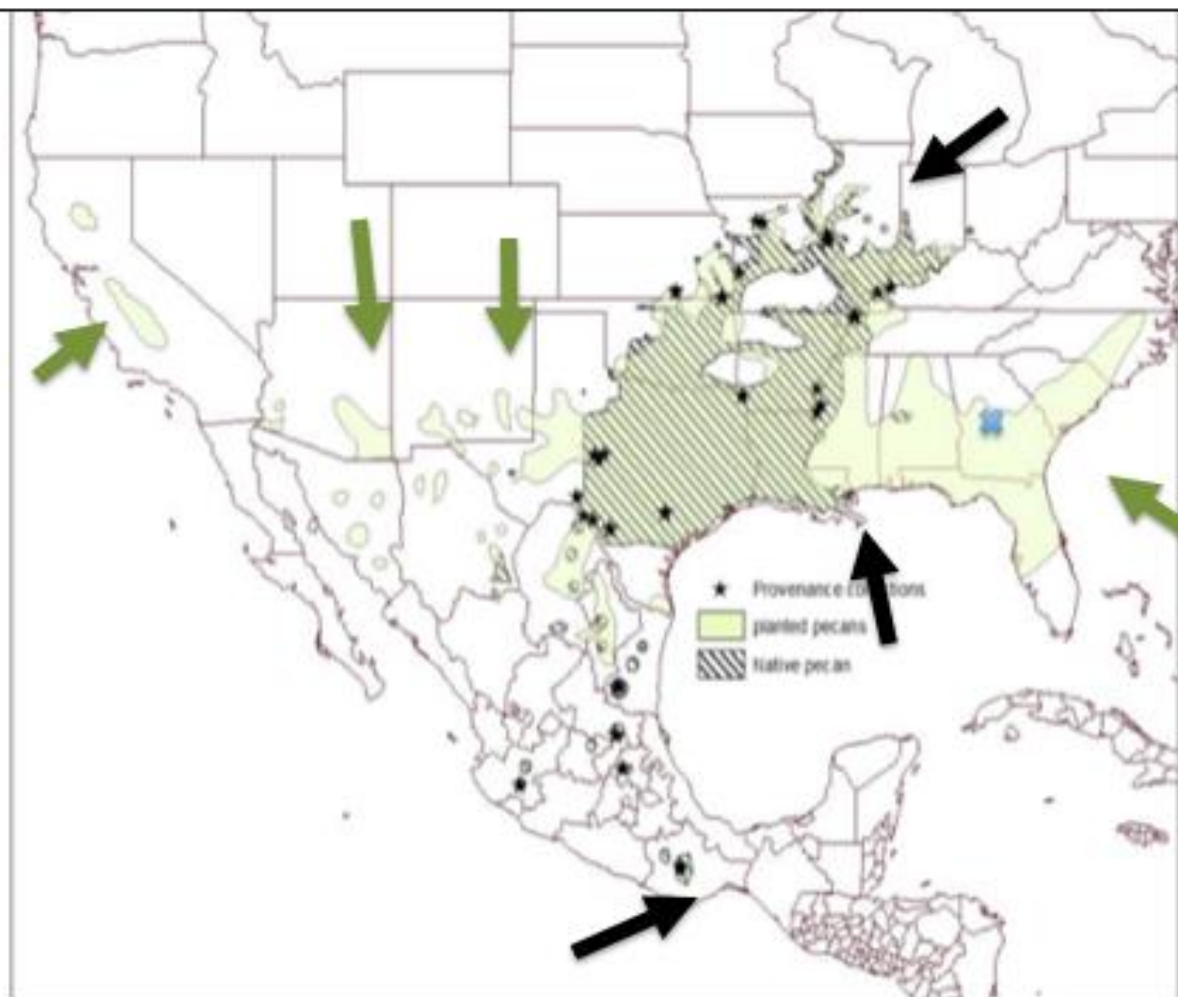
National Institute of Food and Agriculture

U.S. DEPARTMENT OF AGRICULTURE

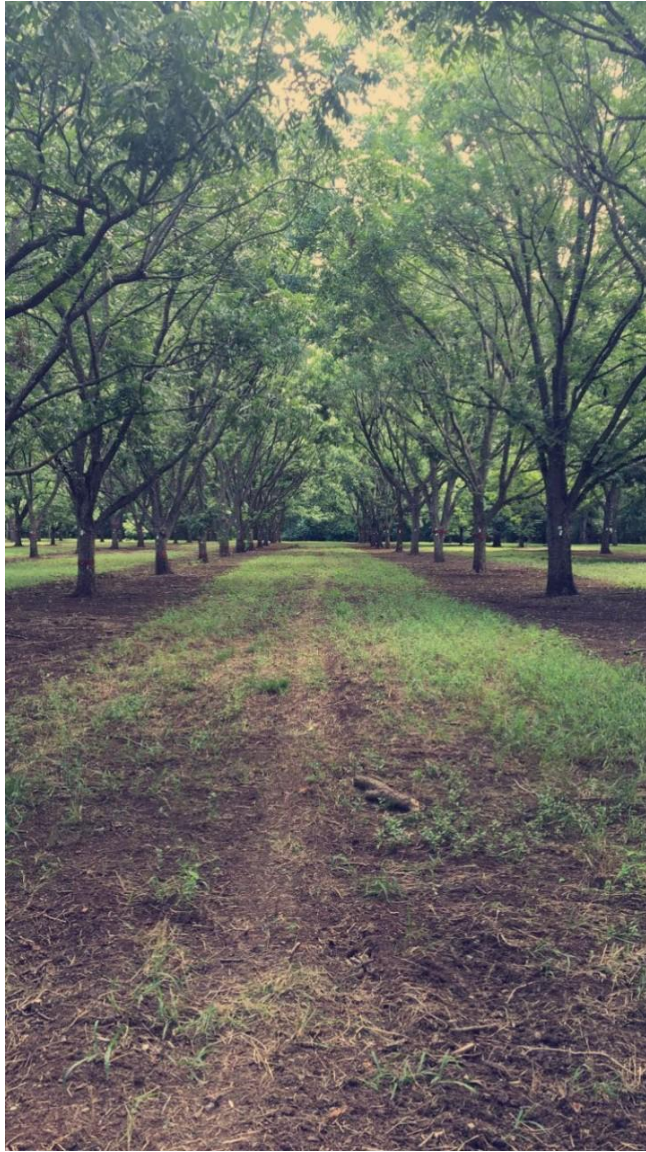
Genetics and Environment shape pecan crops.



Pecan native to North America Genetically Adapted to diverse environments



LJ
Grauke

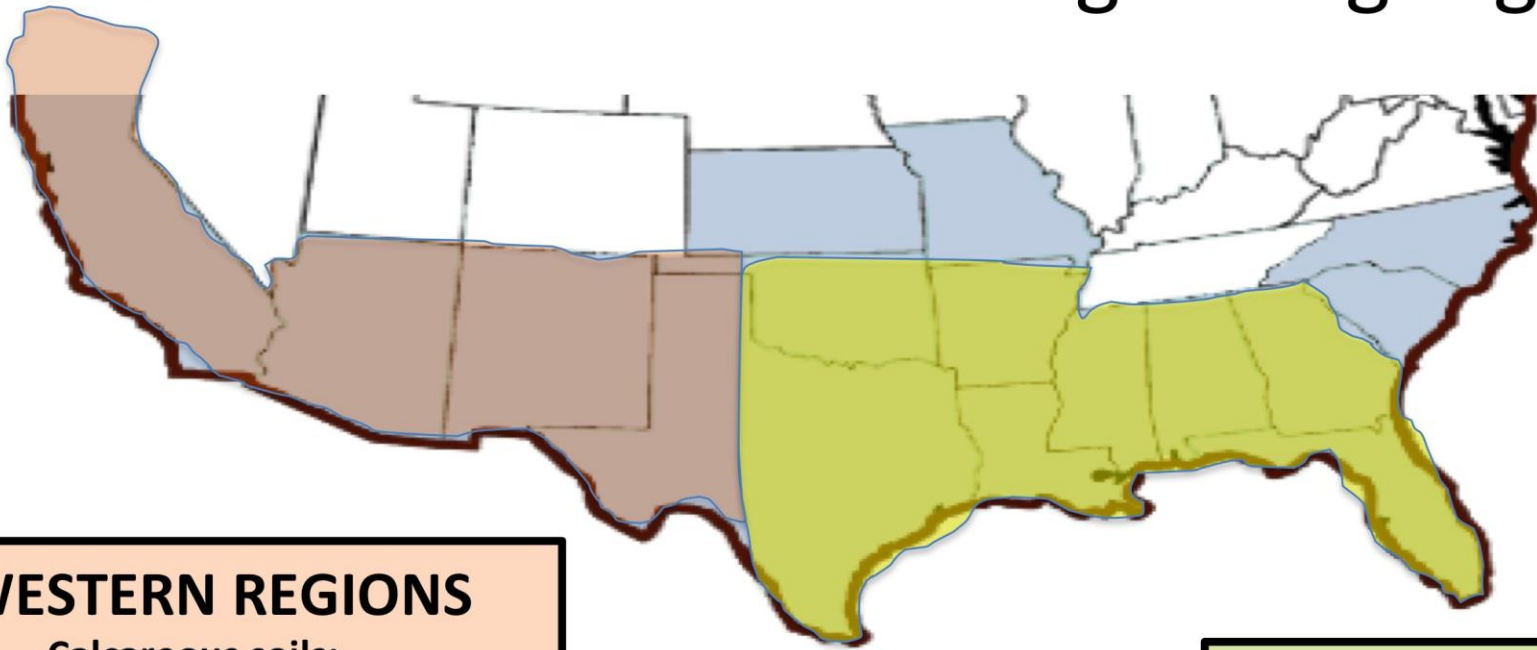


Picture A. Randall, 2017

USDA Pecan Germplasm Repository and Provenance Collections 'National Treasures' Resources at repositories

- Permanent collection of National Plant Germplasm System.
- Repository two locations in Tx (College Station; Brownwood)
- Provenance Collections: College Station, TX and Byron, GA
- Trees originating from Missouri-Mexico. More than 2,000 collections.

Critical Needs for US Pecan growing regions



WESTERN REGIONS

1. Calcareous soils: ←
 - a. High pH
 - b. Require micronutrients
2. Low water availability
3. Salinity Stress ←
4. High heat stress
5. *Phymatotrichopsis omnivora*: root rot.
6. Short growing season in North.

All US Regions

1. Alternate bearing ←
2. Pecan Bacterial Leaf scorch. ←
Xylella fastidiosa
3. Insect Pressures
4. Vivipary
5. Allergens ←

EASTERN/CENTRAL REGIONS

1. VARIABLE SOIL
2. HIGH DISEASE PRESSURE
 - a. Scab ←
 - b. Powdery mildew
 - c. Downy spot

Issues impacting pecans



Pecan nut exhibiting vivipary.



Pecan Scab caused by fungal organism *Ventura effusa*



Salinity Stress studies



Pecan bacterial leaf scorch; *Xylella fastidiosa*



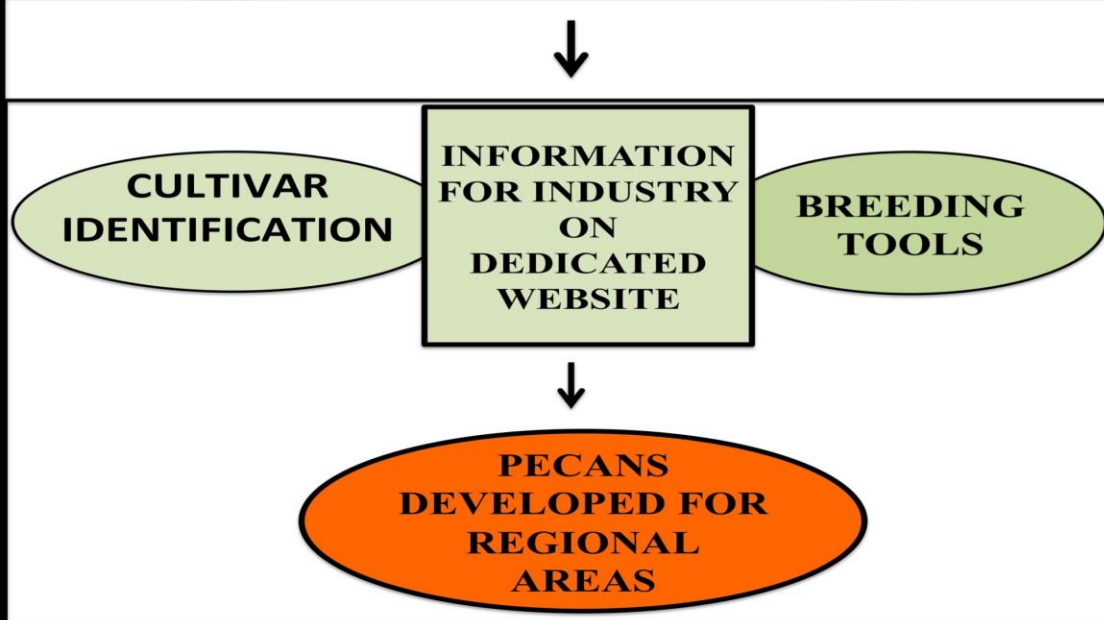
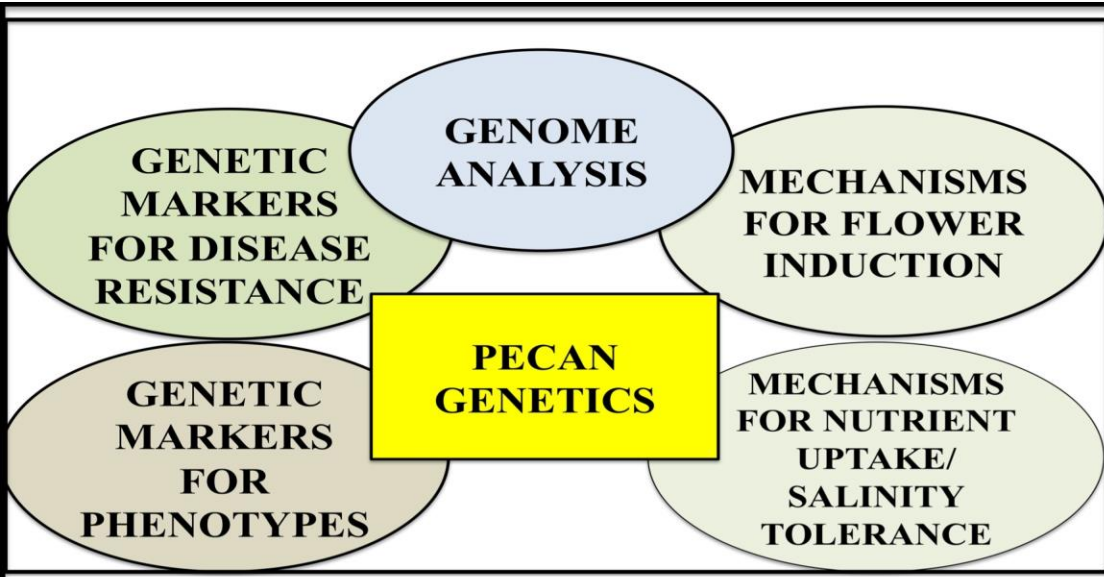
Nutrient uptake

The correct pecan genetics for its environment has the potential to reduce/eliminate inputs.



- Trees resistant to diseases.
- Trees resistant to insects.
- Water 'efficient' trees.
- Salinity tolerant trees.
- Increased and consistent yield.
 - Size Control
- Trees with appropriate nutrient acquisition (Zn, NI).

Coordinated Development of Genetic Tools for Pecans. 2016-2022. USDA NIFA 2016-51181-25408



Tools developed.

Long-term impact of tool development.

Accomplishments 2016-2022

'Coordinated Development of Genetic Tools for Pecans'

***Trained:**

- 20 undergraduate students
- 11 graduate students (5 PhD/ 3 MS)
- 5 Post-docs

***How Research Was Shared:**

- 71 Grower Presentations
- 68 Scientific Presentations (International and national conferences)
- 50 Peer Reviewed Publications
- 26 Trade Articles
- Pecantoolbox.nmsu.edu

— Pecan Genome Related Data Sets

NMSU HOUSING MORE THAN 18 TB

*High Resolution Chromosome Level Genomes

<http://phytozome.jgi.doe.gov>

pecantoolbox.nmsu.edu

*Re-Sequence Data (50X coverage): 864 accessions

*Rad-Seq Data Sets (25X coverage): 1000 accessions

Passport data sets for origin of these trees

Climate data for origin of trees

Phenotypic data set for these trees

*Mapping population(s): Multiple mapping populations, GBS

*Chloroplast Genomes

*MiSeq Sets (Fungal and bacterial organisms endophytes/associated).

*RNA-Seq Data Sets

*Proteomic data sets

*Trees for the future:
Coordinated development of
genetic resources and tools
to accelerate breeding of geographic and climate adapted
pecan trees.*

A: Main

31 of 238 • Page: A8



CARTOONIST'S TAKE



- **Funded by USDA-NIFA**
- **USDA NIFA 2022-51181-38332**

- **Grant awarded 'Center of Excellence'**
- **2022-2026**

Continual Grant \$8,000,000

“Trees for the Future” Funded by USDA-NIFA 2022-2026

Accelerate pecan breeding of geographic and climate

Collect phenotypes from all US geographical regions to determine genes controlling specific traits.

- 1. Climate: Phenotypes from US growing regions.**
- 2. Water Use efficiency**
- 3. Pecan tree interactions with beneficial and detrimental organisms for improved tree health.**
- 4. Gene networks for nut size, kernel composition, allergens and flowering.**
- 5. Pecan tree architecture, growth. Evaluate pecans for size control (vigor and height).**

Audiences



Growers



Industry



Schools



Public



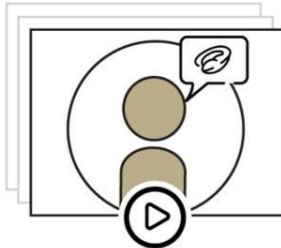
Interactive Gaming Simulation

Players can experience how climate change and genetics impact pecan orchards over long term.



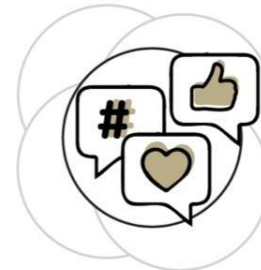
Suite of 12-15 Straight Talk Animations

Simple language and rich visuals make research concepts more accessible.



3-6 Impact Story Videos

Rich storytelling demonstrates the influence of the project.



Social Media Campaign

Creates shareable resources for coordinated national campaign with stakeholders.

PecanBoxToolkit Website

Single resource for all project partners and distribution of research and developed media campaigns.



Learning Games Lab
Innovative Media Research and Extension
learninggameslab.org

ACKNOWLEDGMENTS

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University of Georgia

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Dr. Lenny Wells

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USDA-ARS-LOUISIANA

Dr. Chris Mattison

University of Arizona

Josh Sherman



ACKNOWLEDGMENTS



USDA NIFA SCRI

'Coordinated Development of Genetic Tools for Pecans'

Advisory Board Members
2016-2022

Mike Adams

Dr. John Fowler

Mike Harvey

Dr. Randy Hudson

Lawton Pearson

David Salopek

Dr. Bruce Wood

USDA NIFA SCRI

'Trees for the Future' Advisory Board Members 2022-2026

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Deborah Walden Ralls

Mark Cook

Rafael Roviroso

Chad Selman

Dr. LJ Grauke

Buck Paulk

The logo for New Mexico State University, featuring the letters 'NM STATE UNIVERSITY' in white on a maroon background.



Composite Trees

Commercial orchards

Scion

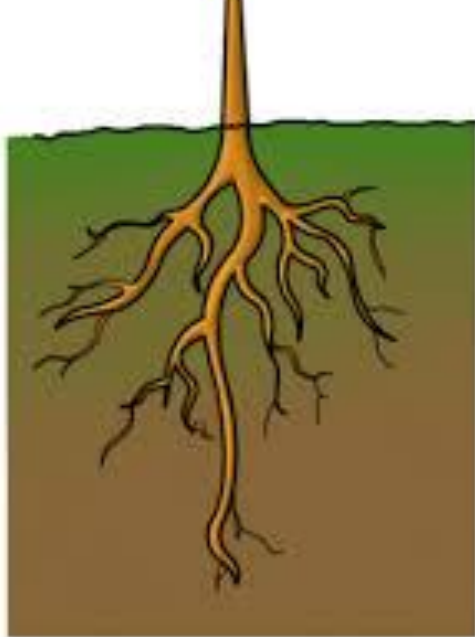
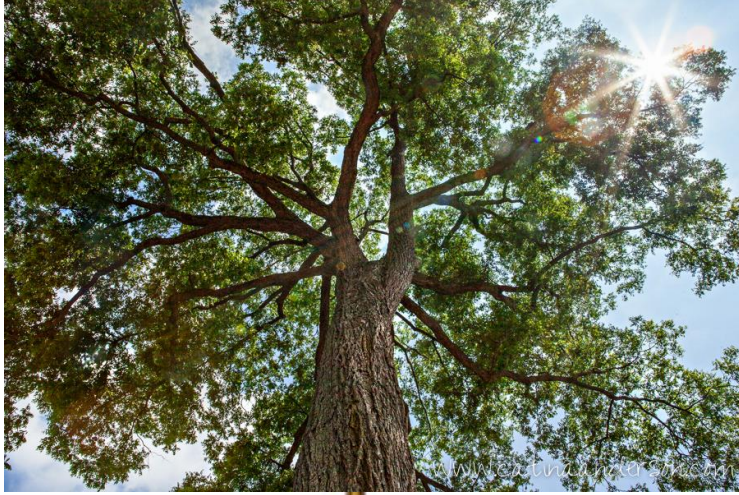
(Known Variety)
Western, Wichita,
etc.—Genetically
the same.

Rootstock

Father not
known
Genetically
different



Rootstocks influence tree performance



Nut quality; production
Disease Resistance



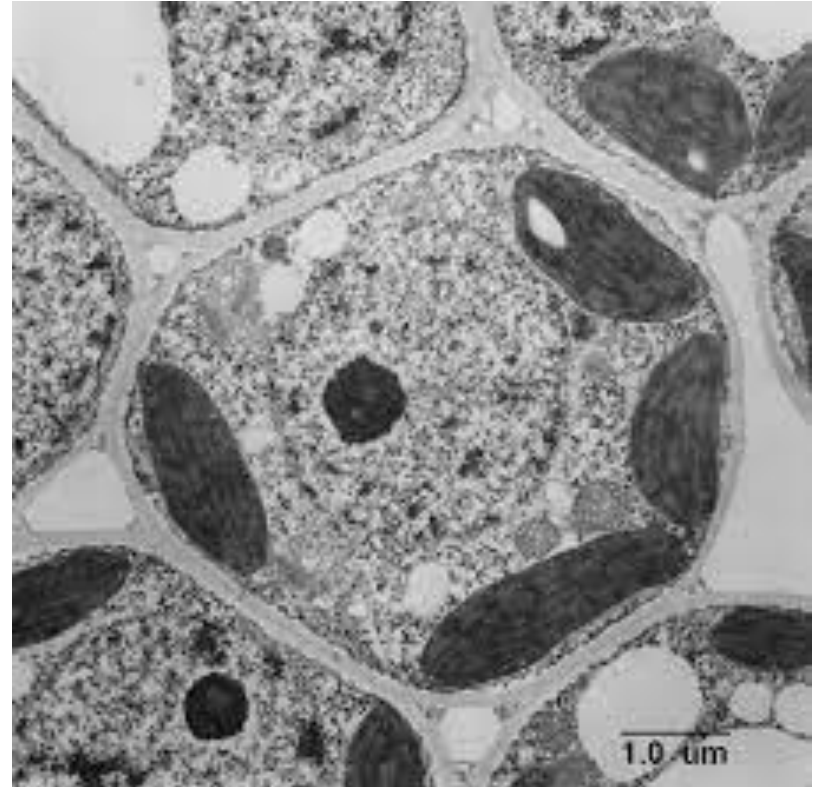
- Ability to grow in diverse environments (alkaline and salinity soils).
- Tree Architecture (Size Control)
- Nutrient uptake (Fe, Zn, Ni,).
- Disease Resistance

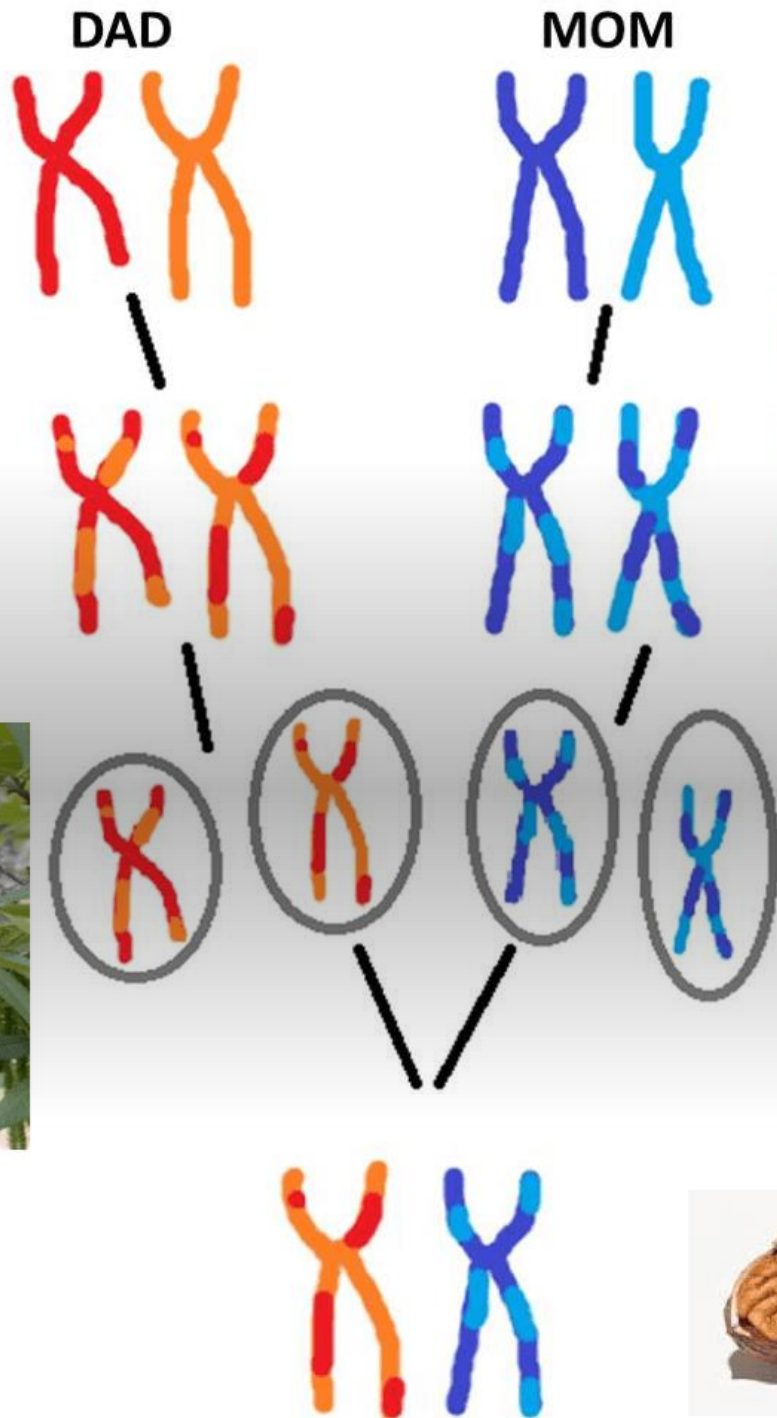
Genetic Studies on
Pecan need to focus on
both the rootstock and
scion



Genome

Complete set of genes or genetic material present in an organism.





Pecans have 16 pairs of chromosomes.

DNA is recombined for the next generation This gives genetic diversity.
MEIOSIS



Pecan Genetics

Website for Pecan Industry

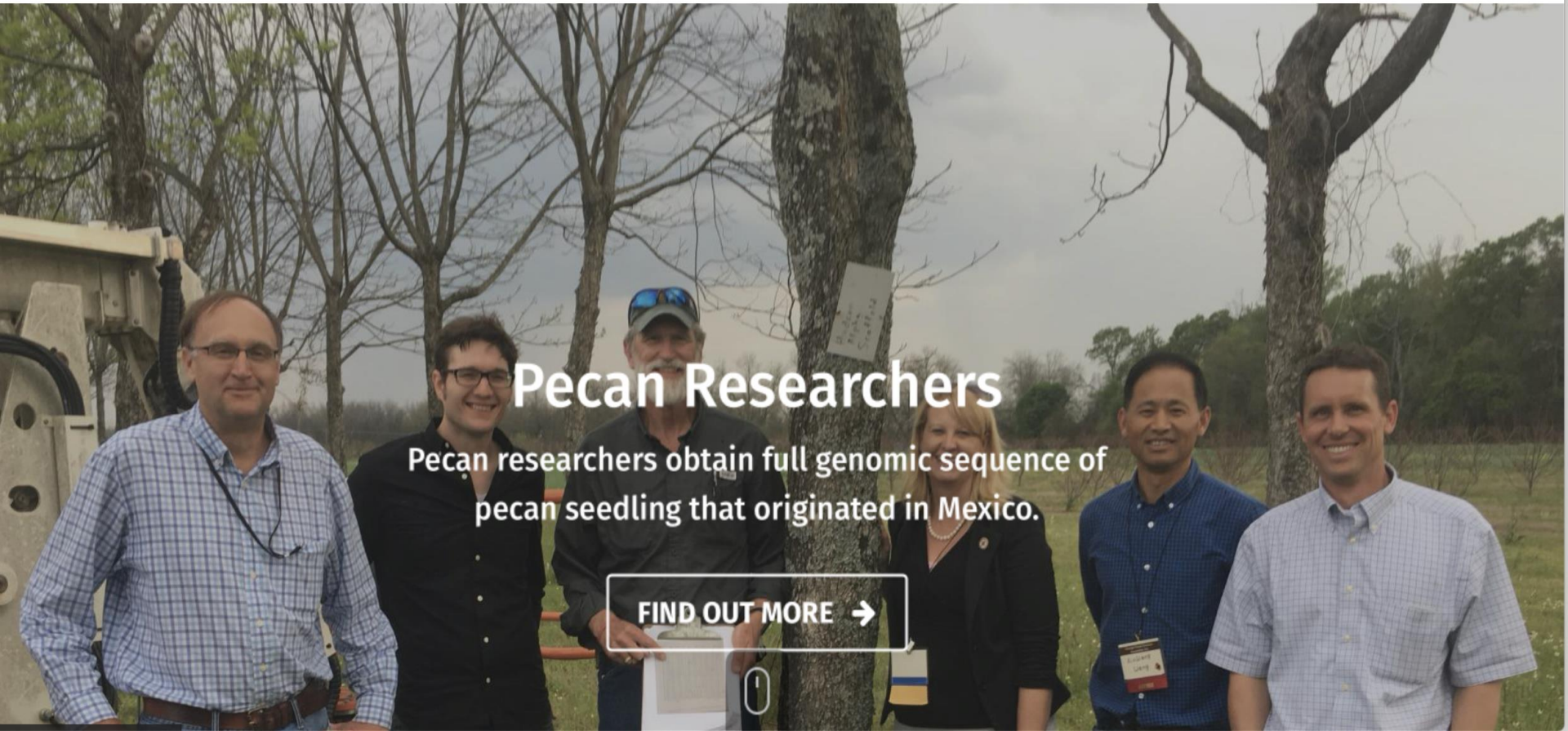
<http://pecantoolbox.nmsu.edu>



PECAN TOOLBOX

GENOMIC TOOLSET

HOME ▾ GENOME ▾ INDUSTRY/MEDIA BREEDERS/NUSERIES ▾ PECAN TREES ▾



Pecan Researchers

Pecan researchers obtain full genomic sequence of pecan seedling that originated in Mexico.

FIND OUT MORE →

Mapping Populations
'Lakota' x 'Oaxaca'
1072 Trees
College Station TX
Byron, GA
Tifton, GA

Microbiome
Studies

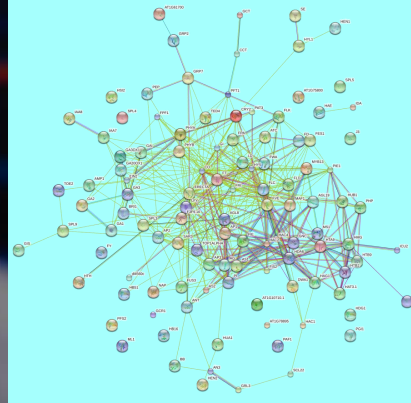
Core Microbiome
in Seedlings

Flowering Studies

Timing of genes
that control
flowers

Scab Studies

Genetics



Zinc Variability in
pecan cultivars

Phenology

Genetic Studies

Genome
Analyses
Cultivar
Identification

Salinity Studies

Rootstock Genetics
for salinity
tolerance

Pecan Bacterial
Leaf Scorch

Endemic
Seed Transmitted

Allergen Proteins

Timing of allergen
proteins in nuts

Market Tools for
Pecan Industry

Supply chain
Consumer Behavior



Chloroplast genome sequences of *Carya illinoensis* from two distinct geographic populations

Xinwang Wang¹ · Hormat Shadgou Rhein² · Jerry Jenkins³ · Jeremy Schmutz³ · Jane Grimwood³ · L. J. Grauke¹ · Jennifer J. Randall²

Received: 2 October 2019 / Revised: 12 March 2020 / Accepted: 7 April 2020
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Abstract

Pecan (*Carya illinoensis*) is the most economically important member of the across its broad geographic range in the process of crop improvement. In this sequences from two pecan genotypes, 87MX3-2.11 and the 'Lakota' cultivar. The chloroplast genome of *C. illinoensis* maintains the conserved structure a circular molecule that includes a large single-copy (LSC) and a small single-repeats (IRa and IRb). There were 124 genes found on the 87MX3-2.11 chl multiple copies of the same gene), with 108 and 107 unique genes, respectively genes are found among *C. illinoensis*, *C. sinensis*, and *Juglans* chloroplast g has fewer copies of some tRNA genes, with 'Lakota' lacking a start codon of The nucleotide divergence between the two pecan chloroplast genomes reflect populations of the species. Genomic divergence was also confirmed by the genome sequences representing Juglandaceae taxa. The complete chloroplast dation for understanding the influences of geographical adaptation, gene flc develop functional genomic tools for regional selection and pecan breeding.

Keywords Pecan · *Carya* · Chloroplast · Phylogeny · Adaptation · Breeding

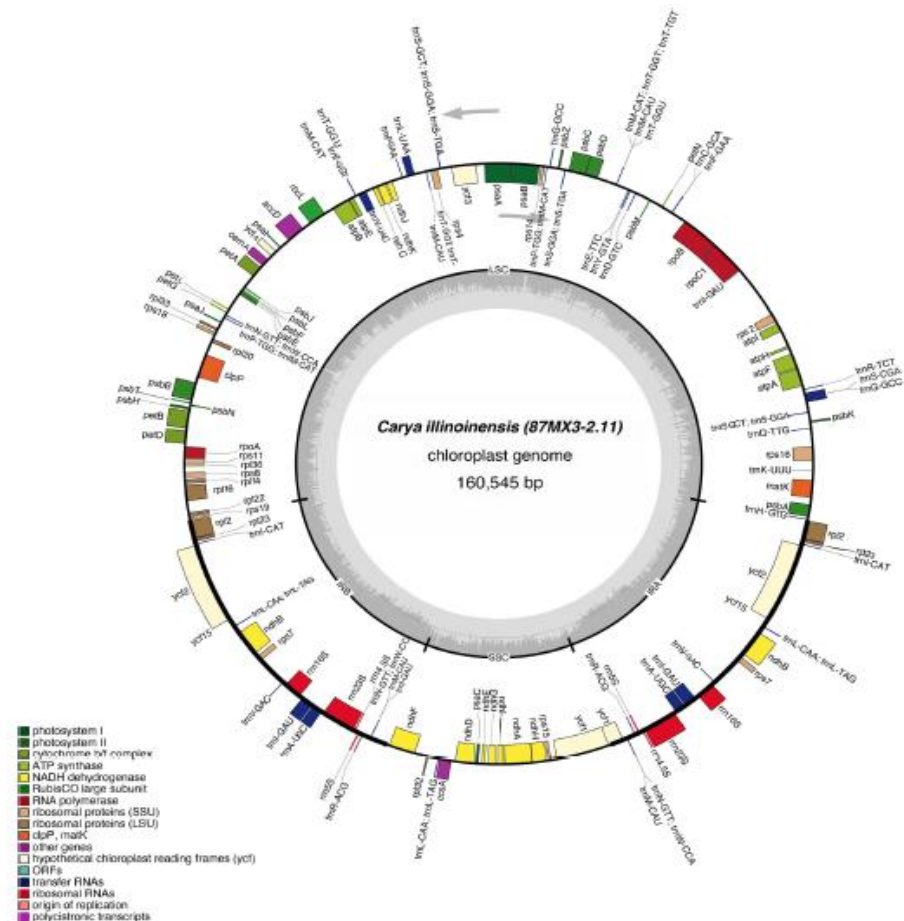


Fig. 1 Gene map of the 87MX3-2.11 chloroplast genome. The genes inside and outside of the circle are drawn clockwise and counterclockwise, respectively. The innermost circle (gray) is the GC

heat map of the whole genome. Two inverted repeats (IRa and IRb) are separated by a small single-copy (SSC) and large single-copy (LSC) regions



ARTICLE



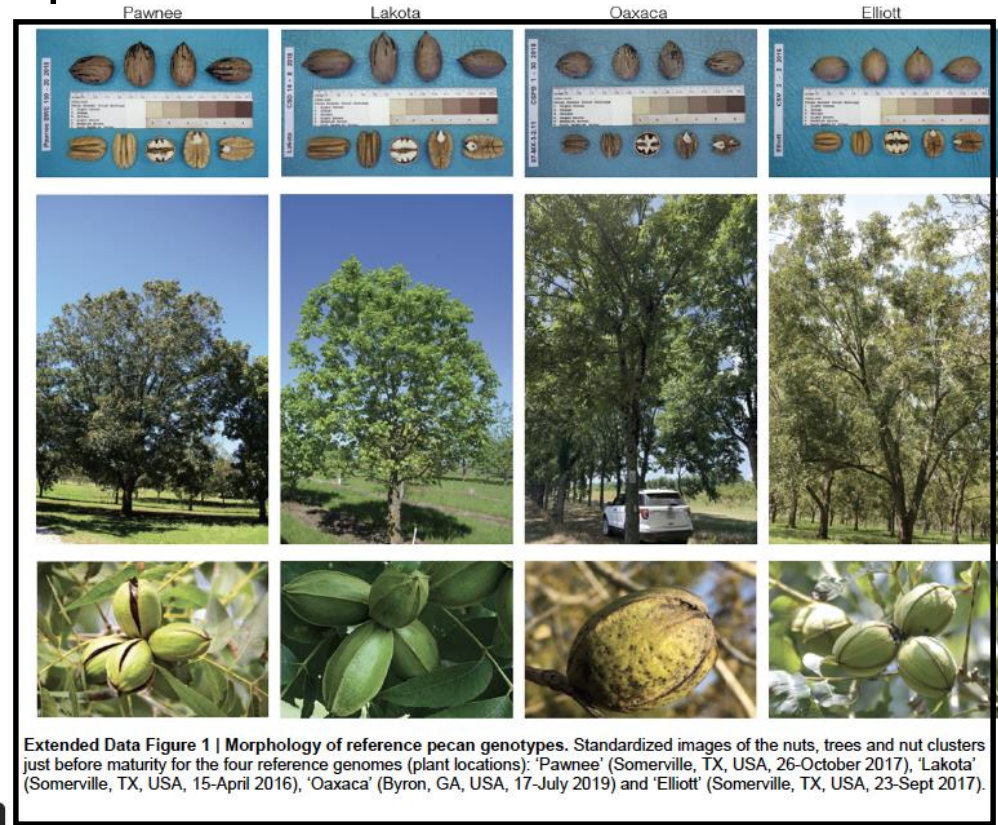
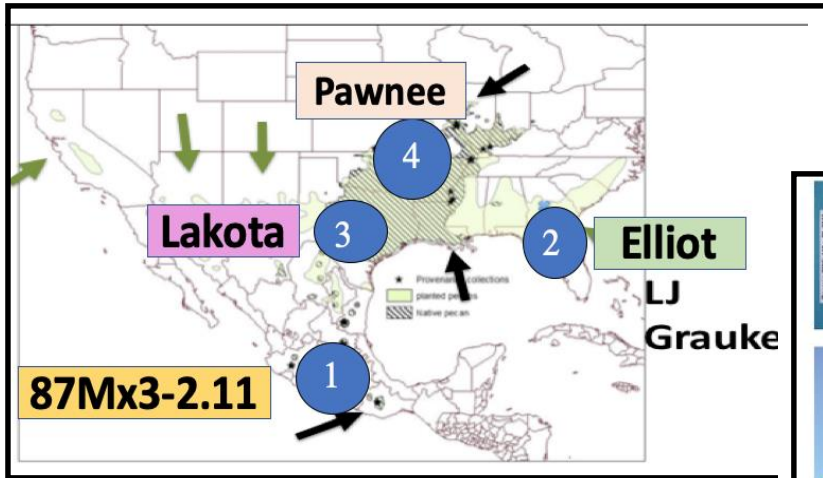
<https://doi.org/10.1038/s41467-021-24328-w>

OPEN

Four chromosome scale genomes and a pan-genome annotation to accelerate pecan tree breeding

John T. Lovell ^{1,15} , Nolan B. Bentley ^{2,15}, Gaurab Bhattarai^{3,15}, Jerry W. Jenkins ^{1,15}, Avinash Sreedasyam ^{1,15}, Yanina Alarcon ⁴, Clive Bock⁵, Lori Beth Boston¹, Joseph Carlson⁶, Kimberly Cervantes⁷, Kristen Clermont⁸, Sara Duke⁹, Nick Krom⁴, Keith Kubenka¹⁰, Sujan Mamidi¹, Christopher P. Mattison ⁸, Maria J. Monteros ⁴, Cristina Pisani⁵, Christopher Plott¹, Shanmugam Rajasekar¹¹, Hormat Shadgou Rhein⁷, Charles Rohla⁴, Mingzhou Song¹², Rolston St. Hilaire¹³, Shengqiang Shu ⁶, Lenny Wells¹⁴, Jenell Webber¹, Richard J. Heerema ¹², Patricia E. Klein ², Patrick Conner¹⁴, Xinwang Wang¹⁰, L. J. Grauke ¹⁰, Jane Grimwood ¹, Jeremy Schmutz ^{1,6}  & Jennifer J. Randall⁷ 

COMPLETE PECAN GENOMES HIGH RESOLUTION SEQUENCING



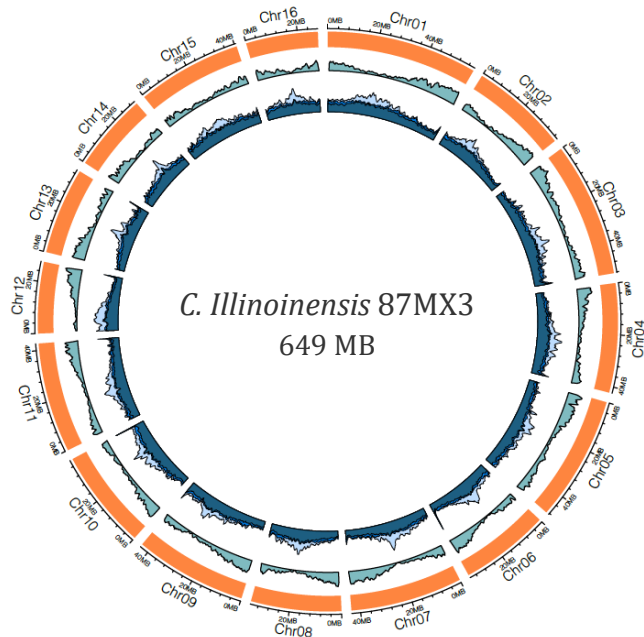
Lovell et al., 2021---*Nature Communications*

COMPLETE GENOMIC SEQUENCES

<http://pecantoolbox.nmsu.edu>

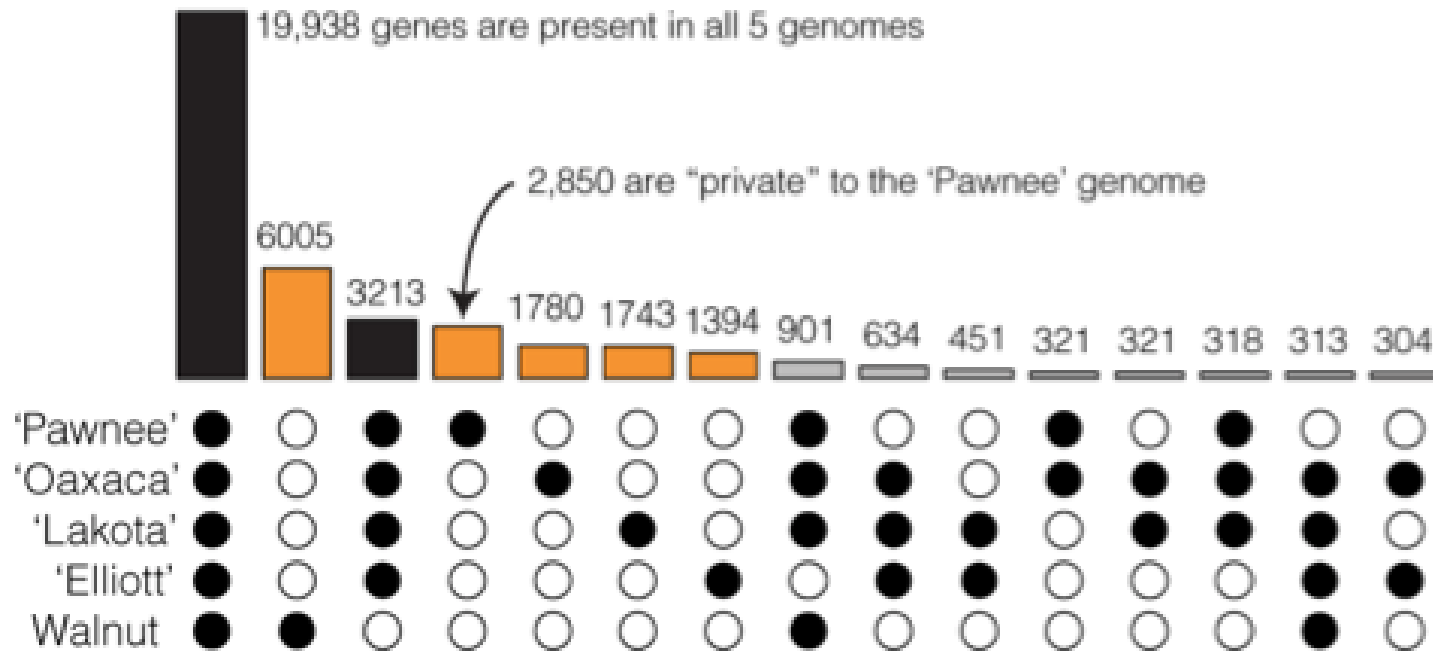
Genomic sequences were defined on chromosomes. This was done with linkage maps and sequencing tools. (Lovell et al., 2021).

Assembly statistics



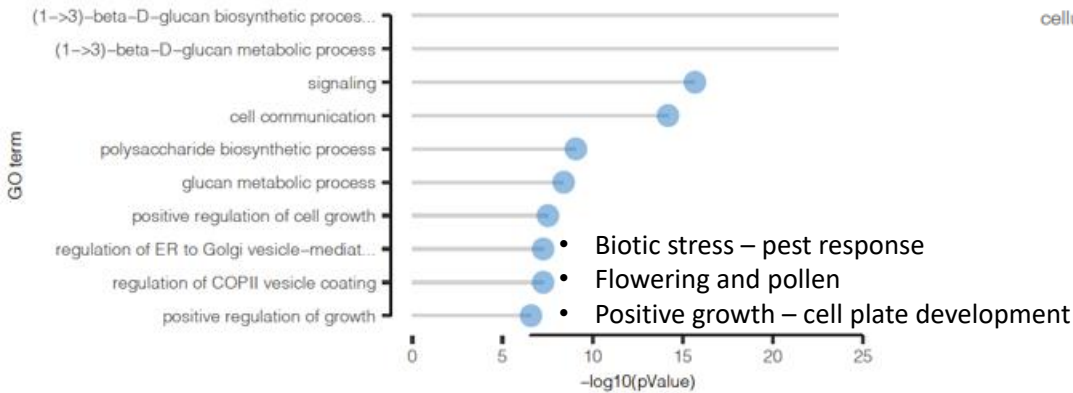
Genomic features	Oaxaca	Lakota	Elliott	Pawnee
Assembly size (Mb)*	649.96	668.99	656.69	674.27
Number of scaffolds	298	261	431	16
Number of contigs	552	499	829	34
Gap content (%)	0.4%	0.4%	0.6%	0.0%
Contig N50 (Mb)	4.4	3.7	4.4	26.5
Genome in chromosomes (%)	98%	96.1%	95.5%	100%
Number of annotated genes	31,911	33,280	31,042	32,267
Repeat sequences (%)	46.5%	33.8%	32.3%	49.7%

c Shared gene presence (and absence) in the pangenome

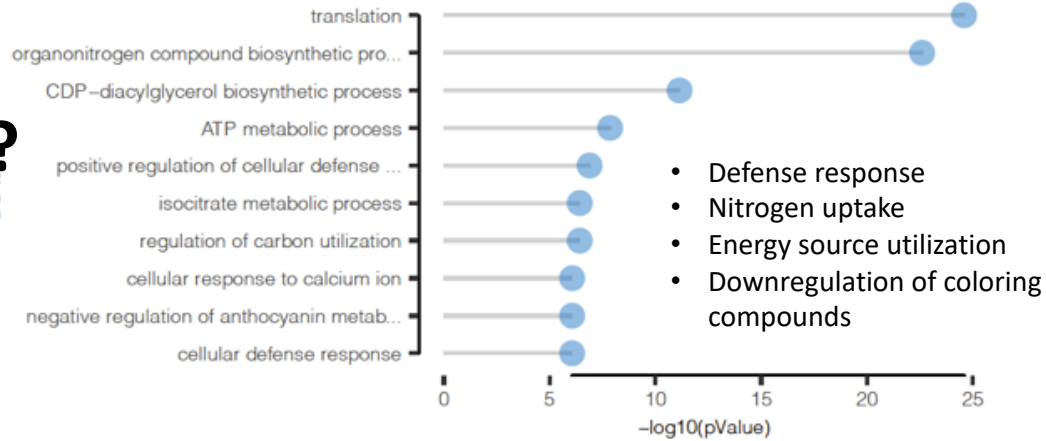


GENOME ANALYSIS-WHAT DOES THE GENOME TELL US ABOUT EACH TREE?

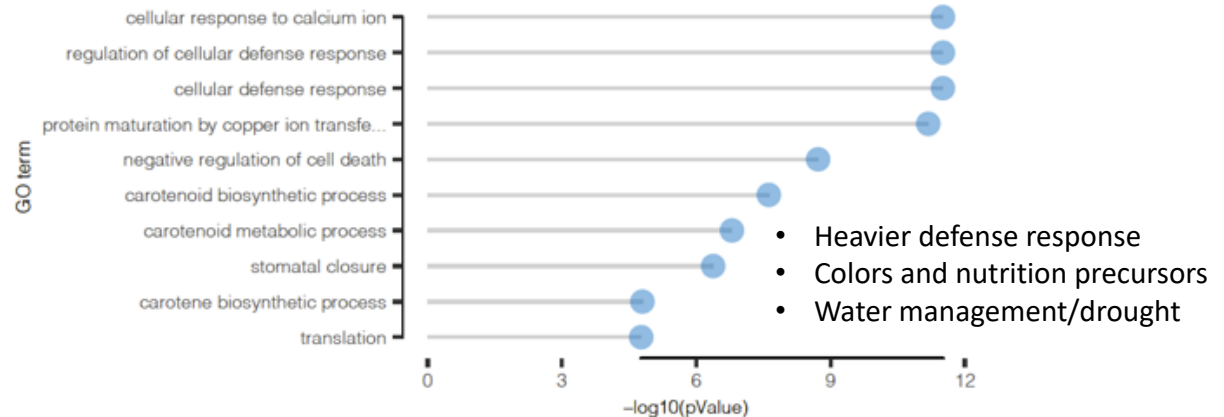
87-Mx—3-2.11



Lakota

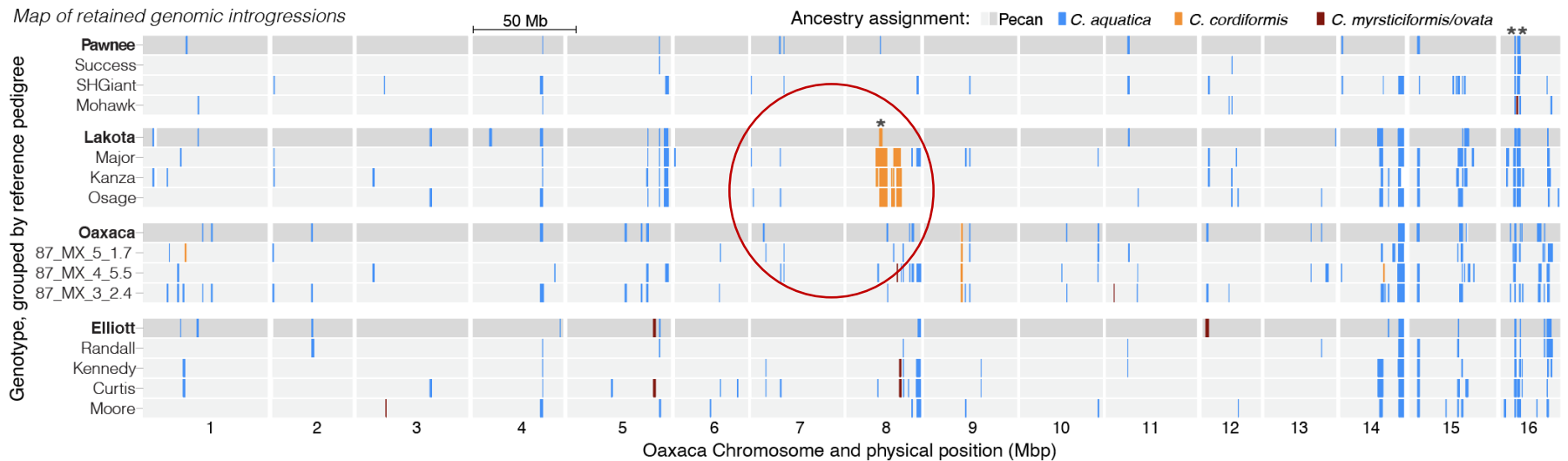


ELLIOT



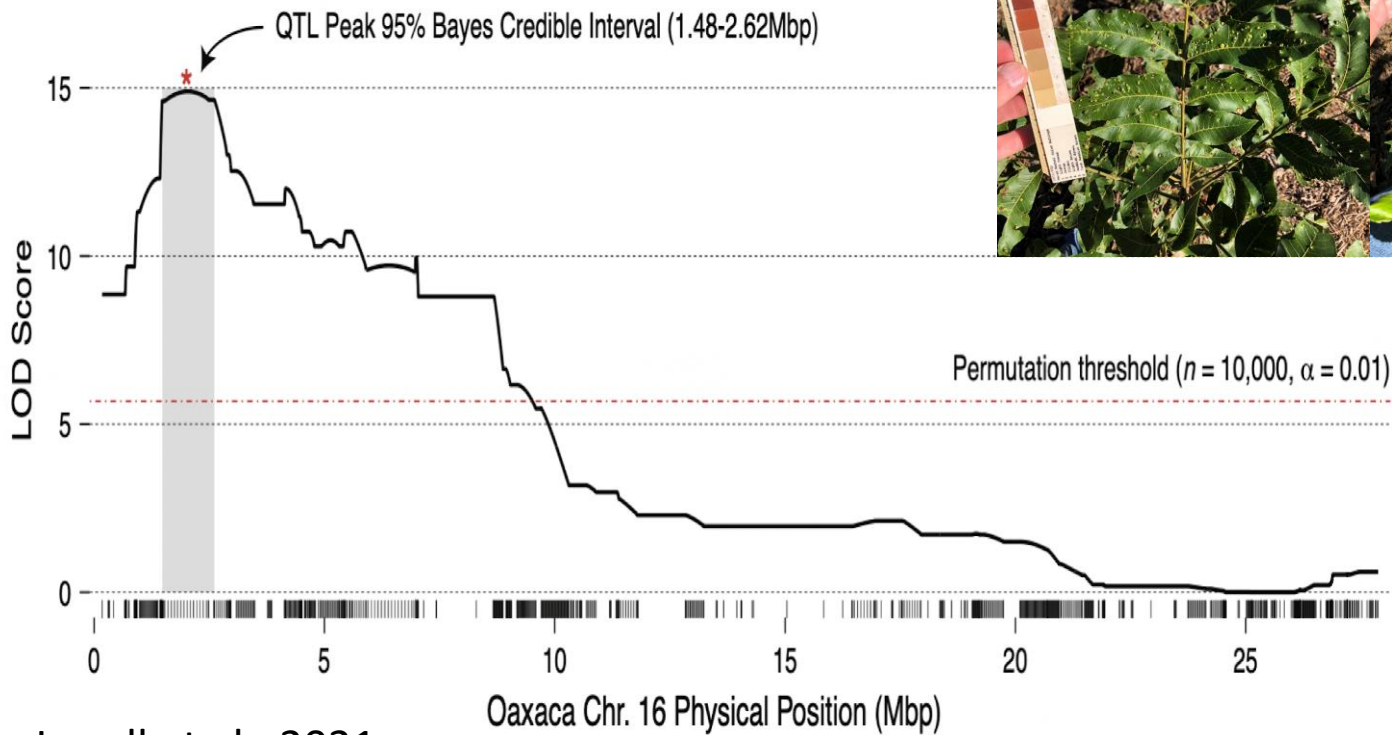
GENETIC MAP OF PECAN GENOMES

SPECIFIC AREAS OF ANCESTRY



Lovell et al., 2021

Phylloxera Resistance QTL identified from 'Lakota' x '87Mx3-2.11' population



Lovell et al., 2021

Several Sequence Studies

Genome Wide Association Study
NMSU-USDA-University of Tokyo

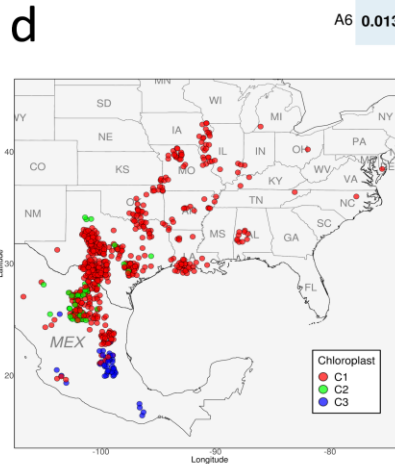
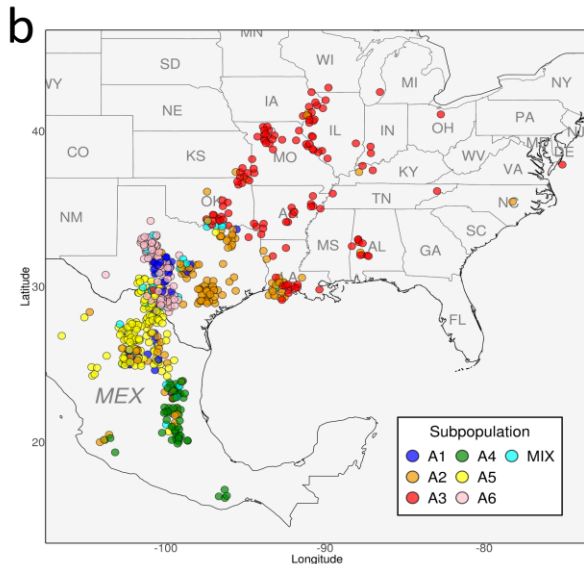
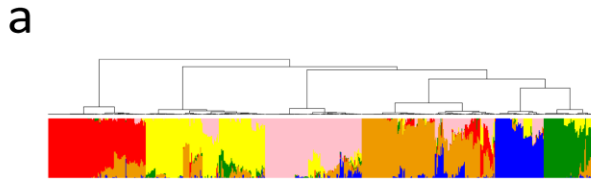


Re-Sequence Data
USDA-NIFA Grant

Pecan Trees: 820

Carya and Hybrids: 180

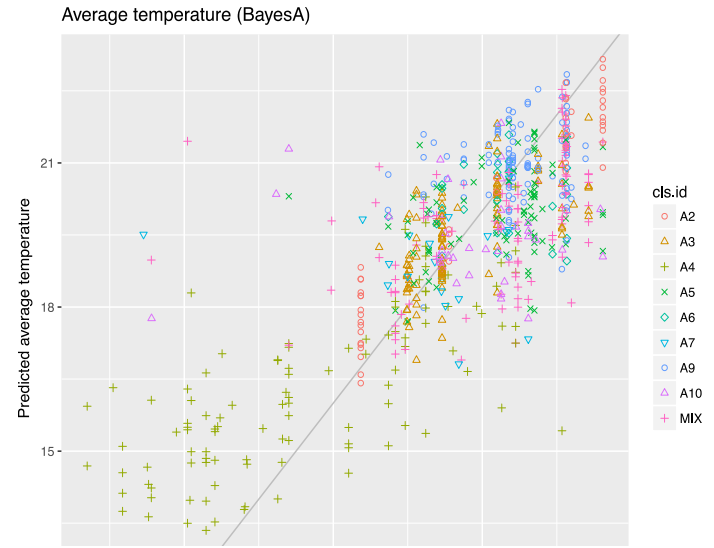
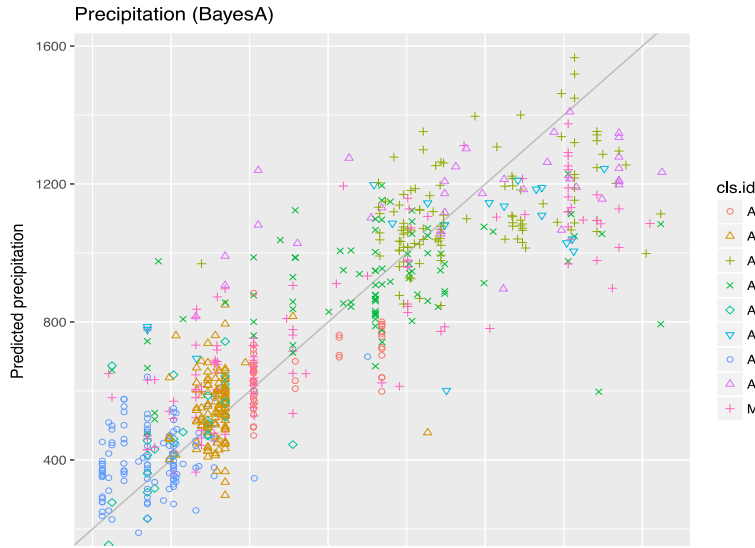
	Mean	Median	Range
Coverage	51.26x	50x	16x-116x
Mapping %	82.0%	83.4%	49% - 90%



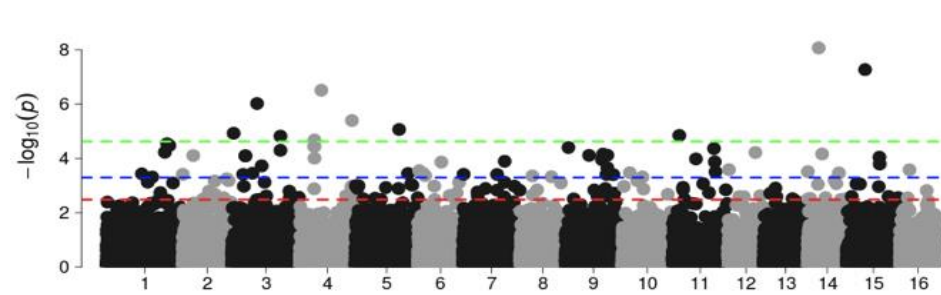
Will be able to mine genomes for:

1. Areas in genome for climate
2. Population genetic studies
3. Disease resistance/Insect resistance
4. Desired traits

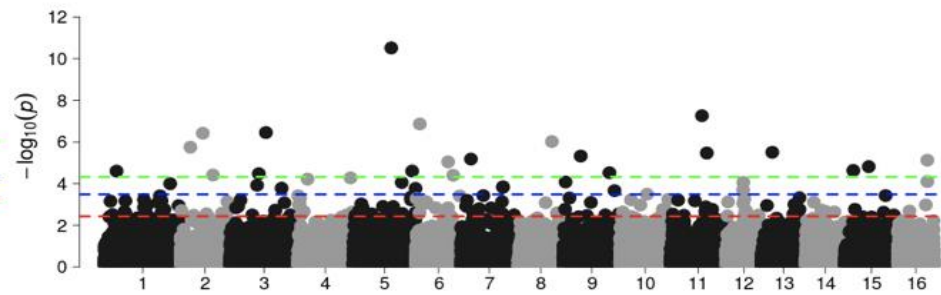
Genome wide polymorphisms association to climate variables. Prediction accuracy of annual average of precipitation and temperature.



PC1 of climate variables



PC2 of climate variables

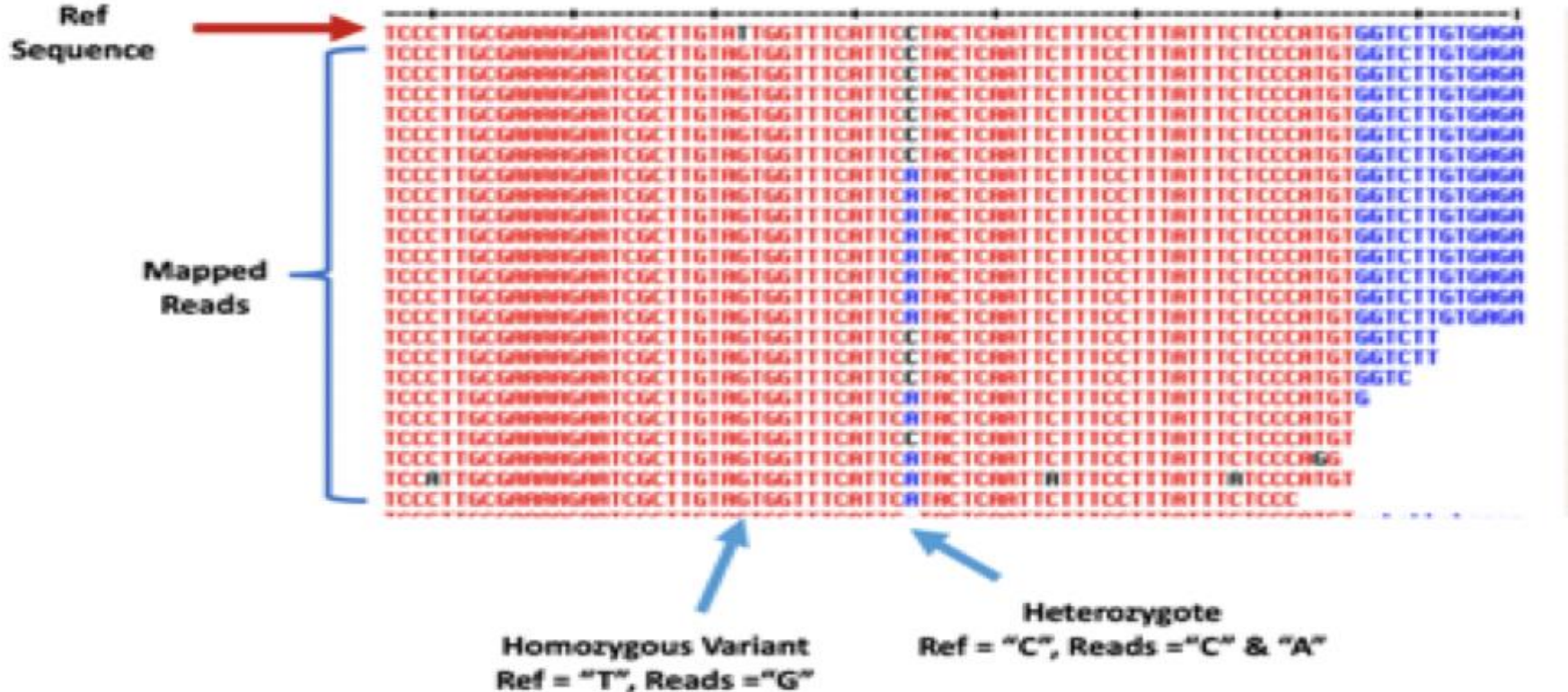


Ishimori et. al. in progress.

Population Re-Sequence

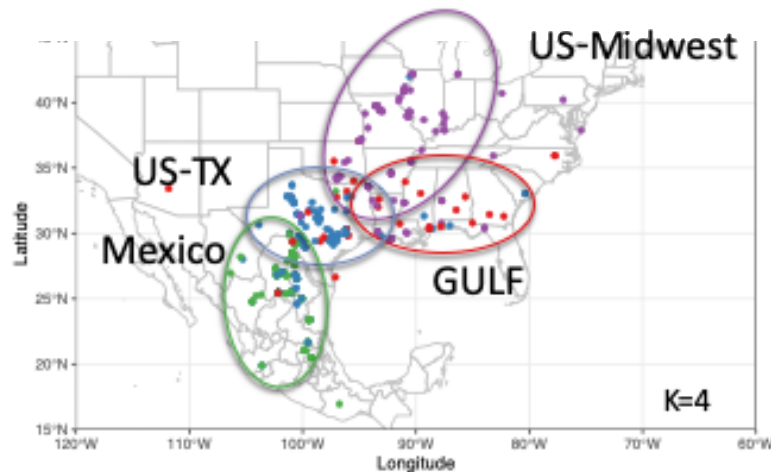
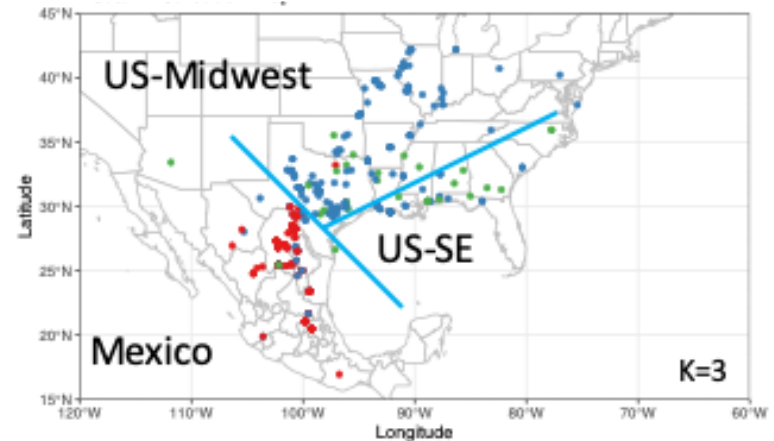
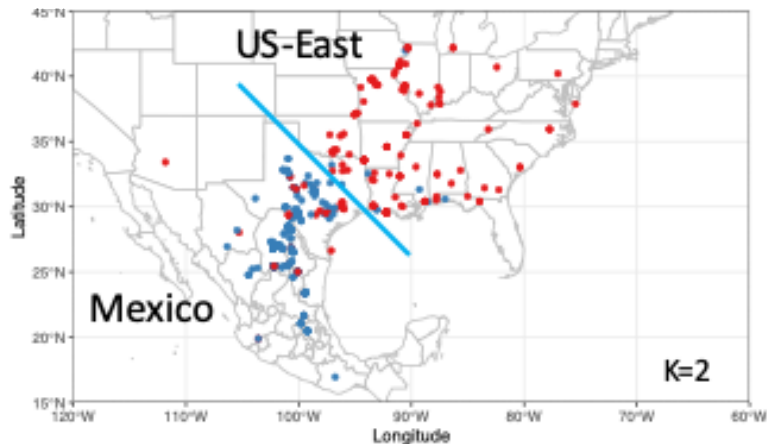
Pecan Trees: 820 Native Trees
Carya and hybrids: 180 Trees

Chr01 : 7200 – 7280



	Mean	Median	Range
Coverage	51.26x	50x	16x-116x
Mapping %	82.0%	83.4%	49% - 90%

Initial Population View (440 *C. illinoensis*)



- We can mine these data for adapted genes
 - Temperature/Rainfall or other climate
 - Population specific genes
 - Whole genome association analysis with agronomic phenotypes to ID useful genes for breeding

J. Schmutz

Summary

- Genomes sequenced to the chromosome level and annotated.
- Phenotypic data from Carya repository and from US being collected.
- Pecan has a continuous genetic differentiation over geographic distribution of the species
- Environmental conditions of the locations could also be predicted accurately by genomic prediction.
- Need to model what genetics will be required in geographical locations 10, 20, 50, and 100 years from now.



Funding for Pecan Work



SCBG 2013
SCBG 2015
SCBG 2017
SCBG 2020
SCBG 2021



USDA NIFA 2016-51181-25408
USDA NIFA 2022-51181-38332




San Simone Research Group



Development of Mapping Population

'Lakota' x 'Oaxaca'
2016 and 2017 Crosses
1072 Trees



'Lakota' x 87Mx3-2.11 Mapping Population

1. Original cross using 'Oaxaca' pollen from GA with 'Lakota' trees in GA and TX.
2. All seed from the cross was numbered and measurements recorded.
3. Seed was stratified and planted in pots (some was also sent to NM for introduction into tissue culture).
4. Trees planted in College Station TX, Byron GA, and Tifton GA.

'Lakota' x 'Oaxaca' Mapping Population

2016 and 2017 population

- Test systems located in Texas and Georgia (2 locations).
- Phenotypic data will be collected. Segregation of traits observed.
- **Genomic data collected for each tree. Data will be used to develop markers.**
- Microbiome data collected for subset of seedlings from GA and Tx.



Stages of the 'Lakota' X 'Oaxaca' Mapping Population.



Dr. Patrick Conner with cross in Tifton, GA.



Scab screening nursery in College Station, TX.



'Lakota' x 87Mx3-2.11 Mapping Population 2017 population

TX



2019

TX



Planted at pecan
germplasm repository.
Picture Sept 2021 (Tx).



GA



Planted in Byron, GA

Sent to GA Feb 2020

'Lakota' x 'Oaxaca' Mapping Population 2017 population



All seeds from cross were measured and recorded.



Seeds were planted in pots (or sent to NM for TC).



Growth parameters were measured for each tree.



After one year of growth seedlings in GA were sent to Tx for screening of scab.

Pecan Rootstock /Genetic Test System

- Micropropagation of hundreds of genotypes.
- Genotypes evaluated for ability to propagate and root.
- In-vitro and Greenhouse screenings. Salinity; tree nutrient requirements, etc.
- Ability to 'clean' plants from pathogens.
- Genetic System for tests with large numbers.



Micropropagated Pecan Trees in Green House



Clonal Rootstock Field Trials

Arizona (planted 2018)



New Mexico (2019 and 2021)

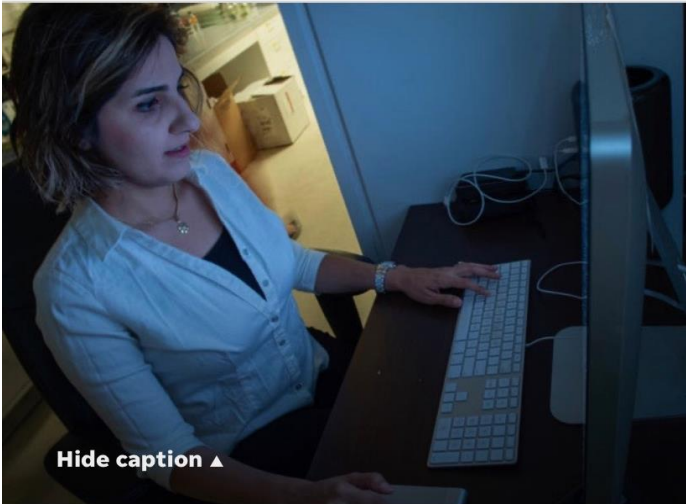




BE BOLD. Shape the Future.
New Mexico State University
aces.nmsu.edu

RNA-Seq Studies of protandrous and protogynous pecan trees.

Las Cruces Sun News. [Subscribe](#) [Sign In](#)

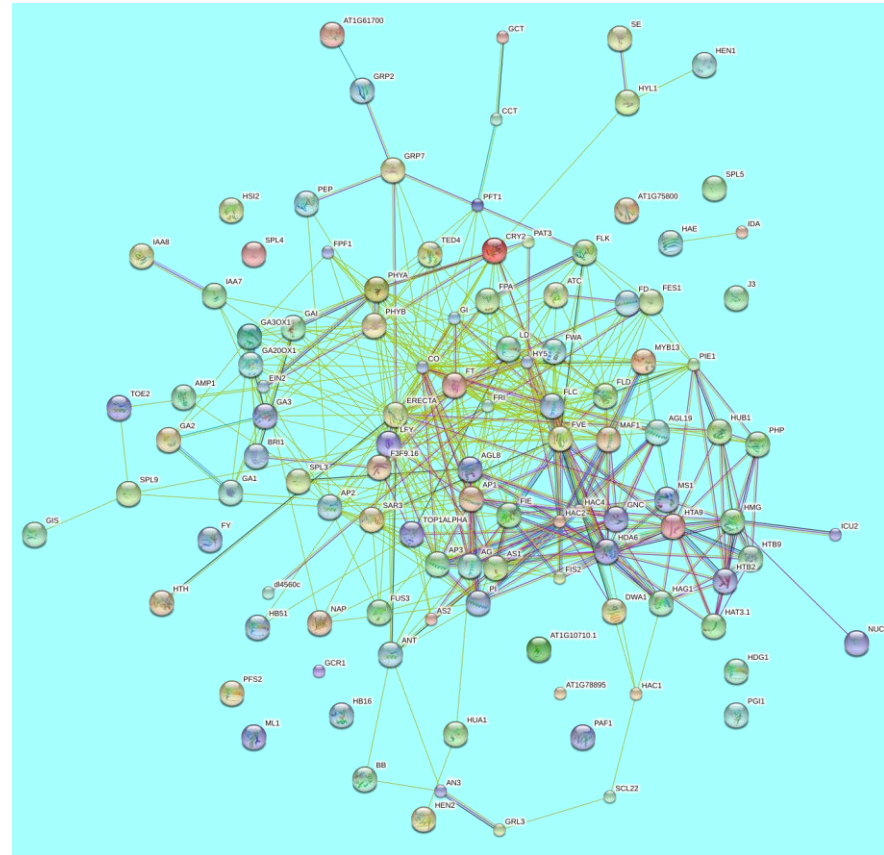


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Hormat Shadgou Rhein, a Ph.D. student of Micro Biology, look over large datasets in the Randall Lab with Jennifer Randall, a professor in the Department ... [Show more](#) ▼

JOSH BACHMAN / NEW MEXICO STATE UNIVERSITY

NMSU researchers drive agriculture into the future with big data



Genes identified for catkin and pistillate flowers. Gene networks involved in floral initiation.



Microbiome Analysis Seedlings

Wichita, Western, Pawnee, Mandan, Burkett