NAFGS 2024

Oaxaca, Mexico

NORTH AMERICAN FOREST GENETICS SOCIETY

As we approach the culmination of my tenure as President of the North American Forest Genetics Society (NAFGS), I am writing to reflect on our collective achievements and share some updates regarding our society's future.

Serving as your president for the past two years has been an honor and privilege. Since our establishment in 2022, we have made great strides in advancing the field of forest genetics across North America. Our inaugural meeting at the picturesque Asilomar Conference Grounds in Pacific Grove, California, marked a significant milestone in our journey. With the participation of approximately 70 delegates from Canada, the United States, and Mexico, we laid the groundwork for our society's organizational structure.

During my tenure, one of our primary objectives was to foster greater engagement and collaboration among our members. To this end, we have led the establishment of standing committees, which would serve as the foundation of our society. These committees will be vital in facilitating communication, networking, and knowledge exchange among researchers, practitioners, and enthusiasts in forest genetics.

I look forward to meeting our members at the upcoming biennial conference in Oaxaca, Mexico. This presents an excellent opportunity for our members to come together, share their latest research findings, and strengthen our community bonds. The participation of the members matters most to the success of this young society.

I will step down from my administrative role as part of my commitment to fostering continuity and innovation within our society. However, like our former president and founder, Dr. David Neale, I will continue to engage with society and support it.

In closing, I thank all of you for your support and commitment to the NAFGS. I would like also to thank the NAFGS Executive Committee members, Drs. Nathalie Isabel (vice president), Jill Wegrzyn (treasurer), and Patrick Cumbie (secretary). It has been a privilege to serve as your President, and I am confident that our society is poised for even greater achievements in the future.

Warm regards,
Fikret Isik
President, NAFGS

The success of this conference was a result of the hard work and dedication of

The Organizing Committee
Jill Wegrzyn, Christian Wehenkel, Jason Holliday, Laura Figueroa-Corona, Nicholas Ukrainetz, Pablo Martínez-Antúnez, Magdalena María Coello Castillo, Abel Santos Juárez Cortéz, and Megan Frye

The Scientific Committee
Juan Pablo Jaramillo, Alejandra Moreno Letelier, Alejandra Vázquez-Lobo, Charles Chen, Nathalie Isabel, Fikret Isik, and Carrie Pike

Acknowledgements and Sponsorships

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Time (CST)		Location
Friday, June 7th		
11:00am	TreeGenes / CartograPlant	Pre-Conference Virtual Classroom
Monday, June 10th		
9:00am - 5:30pm	Quantitative Genetics	Shedrom Room
9:00am - 12:30pm	Topics in Forest Genetics (Instruction in Spanish)	Pegaso Room
1:30pm - 5:30pm	Conservation Genetics: Intro to Variant Detection	Champagne Room
8:30am - 12:30pm	Conservation Genetics: Intro to Genome Assembly	Champagne Room
12:30pm - 6:30pm	Break until Evening	
6:00pm - 7:30pm	Registration and Welcome Mixer	Hotel Fortin
7:30pm	Dinner (on your own) - shuttles available	
Tuesday, June 11th		
7:00am - 8:30am	Light Breakfast and Registration	Hotel Fortin
8:30am - 8:35am	NAFGS Welcome	Plaza 2
8:35am - 9:00am	NAFGS Welcome	Plaza 2
9:00am - 9:45am	Keynote Speaker: Pamela Soltis (UF)	Plaza 2
9:45am - 10:00am	Coffee Break	Hotel Fortin
10:00am - 10:20am	NAFGS President Welcome: Fikret Isik (NCSU)	Plaza 2
10:20am - 12:30pm	**Session 1:** Population Genomics and Local Adaptation	Plaza 2
12:30pm - 1:45pm	Lunch	Hotel Fortin
1:50pm - 3:30pm	**Session 2:** Population Genomics and Local Adaptation	Plaza 2
1:50pm - 3:30pm	**Session 2:** Conservation Genetics	Plaza 3
3:30pm - 4:00pm	Keynote Speaker: Jessica Wright (US Forest Service)	Plaza 2
4:00pm - 4:15pm	Coffee Break	Hotel Fortin
4:15pm - 4:35pm	**Session 3:** Conservation Genomics	Plaza 2
5:00pm - 6:00pm	Light Refreshments	Hotel Fortin
6:00pm - 7:30pm	Performance: Casa de la Cultura Federico Flores Aguilar, San Pablo Huitzo, Oaxaca	Hotel Fortin
7:30pm	Dinner (on your own) - shuttles available	

Program at a Glance

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North American Forest Genetics Society

Time (CST)		Location
Wednesday, June 12th		
7:00am - 8:00am	Light Breakfast	Hotel Fortin
8:00am - 1:00pm	Local Field Trip – Monte Alban	Monte Alban
1:10pm - 3:00pm	**Session 4:** Quantitative genetics, tree breeding and forest management	Plaza 3
1:10pm - 2:50pm	**Session 4:** Genomics, transcriptomics and bioinformatics	Plaza 2
2:50pm - 3:00pm	Coffee Break	Hotel Fortin
3:00pm - 3:40pm	Panel Discussions	Plaza 2 / Plaza 3
3:45pm - 5:00pm	**Session 5:** Genomics, transcriptomics and bioinformatics	Plaza 2
5:00pm - 5:10pm	NAFGS Group Photo	
5:30pm - 7:00pm	Poster Session and Happy Hour	Plaza 1
7:00pm	Dinner (on your own) - shuttles available	
Thursday, June 13th		
7:00am - 8:50am	Light Breakfast	Hotel Fortin
9:00am - 10:00am	**Session 6:** Phylogenomics, phylogeography and demographic history	Plaza 2
10:00am - 10:30am	Keynote Speaker: Antonio González Rodríguez (UNAM)	Plaza 2
10:30am - 10:45am	Coffee Break	Hotel Fortin
10:45am - 12:00pm	**Session 7:** Forest Health	Plaza 2
12:00pm - 12:30pm	Keynote Speaker: Sam Yeaman (University of Calgary)	Plaza 2
12:30pm - 2:00pm	Lunch	Hotel Fortin
2:00pm - 3:30pm	**Session 8:** Quantitative genetics, tree breeding and forest management	Plaza 2
3:30pm - 4:00pm	Keynote Speaker: Hayley Tumas (UBC)	Plaza 2
4:00pm - 4:15pm	Coffee Break	Hotel Fortin
4:15pm - 5:15pm	**Session 9:** Phylogenomics, phylogeography and demographic history	Plaza 2
5:15pm - 5:45pm	NAFGS Committee Reports	Plaza 2
5:45pm – 6:00pm	Meeting Conclusion	Plaza 2
7:00pm	Final Banquet Celebration	Hacienda Santa Martha de Bárcena
Friday, June 14th		
6:00am - 3:00pm	Field Trip - Hot Springs	Hierve el Agua + Mitla
multi-day	Field Trip - 2-day trip	Analco

Friday, June 7th

11:00am	Virtual: TreeGenes / CartograPlant

Monday, June 10th

8:30am	Workshop: Conservation Genetics: Intro to Genome Assembly *Pre-registration required*
9:00am	Workshop: Quantitative Genetics *Pre-registration required*
9:00am	Workshop: Topics in Forest Genetics (Instruction in Spanish) *Pre-registration required*
1:30pm	Workshop: Conservation Genetics: Intro to Variant Detection *Pre-registration required*
6:00pm	Registration and Welcome Mixer
7:30pm	Dinner (on your own)

Tuesday, June 11th

7:00am	Light Breakfast and Registration
8:30am	NAFGS Welcome
8:35am	NAFGS Welcome
9:00am	**Keynote Speaker: Pamela Soltis (University of Florida)** Polyploidy and Innovation in Plants: Extending to Forest Trees
9:45am	Coffee Break
10:00am	NAFGS President Welcome: Fikret Isik (NCSU)

Session 1: Population Genomics and Local Adaptation
Moderator: Mengmeng Lu

10:20am	Genotype-environment associations reveal high adaptive potential in a montane conifer (*Abies religiosa*, *Pinaceae*) from central Mexico *Gustavo Ibrahim Giles-Pérez*
10:40am	Effects of two generations of breeding for growth in Douglas-fir (*Pseudotsuga menziesii*) on the frequency of climate-adaptive alleles *Rafael Candido Ribeiro*
11:00am	Allele frequency differences between Lost Pines and Piney Woods loblolly pines of Texas: Can they be used to select for drought tolerance? *Carol Loopstra*

| 11:20am | Black spruce vulnerability to climate change |
| | *Anna Fijarczyk* |

| 11:40am | Physiological and Transcriptomic Responses to Drought in Ponderosa Pine |
| | *Adam Gilewski* |

| 12:00am | Unraveling the genetic architecture of bud flush timing in two hybridizing *Populus* species in order to predict phenological responses to future climates |
| | *Baxter Worthing* |

| 12:30pm | Lunch |

Session 2a: Population Genomics and Local Adaptation
Moderator: Brandon Lind

| 1:50am | How to inform assisted migration for the keystone species *Populus tremuloides* |
| | *Melanie Zacharias* |

| 2:10pm | Local adaptation of *Pinus leiophylla* under climate and land use change models in the Avocado Belt of Michoacán |
| | *Vanessa Izaguirre Toriz* |

| 2:30pm | Predicting fitness under future climates across *Populus* trees from a natural hybrid zone |
| | *Alayna Mead* |

| 2:50pm | Sex-specific adaptation in Mexican *Populus tremuloides* Michx |
| | *Christian Wehenkel* |

| 3:10pm | Defining climate analogs for seed transfer and assisted migration |
| | *Bryce Richardson* |

Session 2b: Conservation Genetics
Moderator: Zane Smith

| 1:50pm | Conservation genomics in the eastern hemlock enabled through genomic, metabolomic, and population analyses |
| | *Karl Fetter* |

| 2:10am | First glimpse of genomic and candidate genes for local adaptation in an endemic oak from Oaxaca, Mexico. |
| | *Nelly Jazmín Pacheco Cruz* |

| 2:30pm | Can forest trees escape the extinction vortex? |
| | *Eduardo Mendoza-Maya* |

| 2:50pm | Is black maple a separate species? |
| | *Alix Pugeaut* |

3:00pm	Genetic identity of Agave peacockii Crouncher: microendemic species
	Paulina Montiel Castelán

3:10pm	Genetic diversity of *Agave salmiana* in plantations in Mexico
	Sonia Teresa Cruz-Vasconcelos

3:30pm	**Keynote Speaker: Jessica Wright (US Forest Service)** Climate change, forest genetics and postfire reforestation

4:00pm	Coffee Break

Session 3: Conservation Genomics
Moderator: Gustavo Perez Giles

4:15pm	Genomics-driven monitoring of *Fraxinus latifolia* (Oregon Ash) for genetic conservation and EAB-resistance breeding
	Anthony Eli Melton

5:00pm	Light Refreshments

6:00pm	Performance: Casa de la Cultura Federico Flores Aguilar, San Pablo Huitzo, Oaxaca

7:30p	Dinner (on your own)

Wednesday, June 12th

7:00am	Light Breakfast

8:00am	Local Field Trip – Monte Alban

4a: Quantitative genetics, tree breeding and forest management
Moderator: Melanie Zacharias

1:10pm	Enhanced Resistance Pines for Improved Renewable Biofuel and Chemical Production
	Gary Peter

1:30pm	Cytotype and genotype effects on the ecophysiology of quaking aspen (*Populus tremuloides*)
	Benjamin Blonder

1:50pm	Quantitative and population genetics of North American and European lineages of coastal Douglas-fir
	Brandon Lind

2:10pm	Resilience of *Picea martinezii* and *Picea mexicana* by analyzing the ring width and genome variations
	Carlos Alberto Segura-Sanchez
2:30pm	Additive and non-additive variance of clones and progenies of *Eucalyptus urophylla* at early age in Tabasco, México
	Liliana Hernández Hernández
2:40pm	Optimizing tree improvement programs: SNP genotyping for enhanced breeding values, genetic diversity assessment, and estimation of pollen contamination
	Esteban Galeano

Session 4b: Genomics, transcriptomics and bioinformatics
Moderator: Laura Figueroa Corona

1:10pm	Analyses of drought tolerance in Maritime pine using grafting as experimental system
	Lorenzo Federico Manjarrez Rodríguez
1:30pm	From Forest to Market: Genomic Tools for Ensuring Wood Traceability in Canada
	Pauline Hessenauer
1:50pm	Mobile and web applications for forest research from in field data collection to inventory and analysis.
	Margaret Staton
2:10pm	Genomic tools for breeding, conservation genomics, and pest/disease management of Christmas trees
	Ross Whetten
2:30pm	EASEL (Efficient, Accurate, Scalable Eukaryotic modeLs), a tool for the improvement of eukaryotic structural and functional genome annotation
	Cynthia Webster
2:40pm	Genomic linkage maps for shedding light on the rapid reproductive isolation of an *Abies* (*Pinaceae*) species pair from central Mexico
	Xochitl Granados-Aguilar
2:50pm	Coffee Break
3:00pm	Panel Discussions

	Session 5: Genomics, transcriptomics and bioinformatics
	Moderator: Pauline Hessenauer
3:45pm	Genomics of whitebark pine
	David Neale
4:05pm	Language models are whole genome representation learners for large and complex conifer genomes
	Charles Chen
4:25pm	Transcriptomics of seasonal needle abscission in a deciduous conifer (*Larix laricina*) demonstrates the evolution of deciduous behavior in seed plants
	Maurice Amee
4:45pm	Phylogenetic based method reveals the genetic basis of cold adaptation in maples
	Mengmeng Lu
5:00pm	NAFGS Group Photo
5:30pm	Poster Session and Happy Hour
7:00pm	Dinner (on your own)

Thursday, June 13th

7:00am	Light Breakfast

	Session 6: Phylogenomics, phylogeography and demographic history
	Moderator: Valentina Cardona Velasquez
9:00am	Rapid differentiation, ecological stasis, and edaphic adaptation during the temperate to tropical transition in Mexican firs
	Juan P. Jaramillo-Correa
9:20am	Differentiation of high altitude Mexican white pines
	Alejandra Moreno-Letelier
9:40am	Taxonomic revision of *Pinus* subsections *Ponderosae* and *Sabinianae* (*Pinaceae*)
	Ann Willyard
10:00am	**Keynote Speaker: Antonio González Rodríguez (*Instituto de Investigaciones en Ecosistemas y Sustentabilidad,* UNAM)** The oak conundrum: Disentangling divergence, reticulation and taxonomic delimitation in *Leucomexicana*

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10:30am	Coffee Break

Session 7: Forest Health

Moderator: Anthony Melton

10:45am	A plan to make American chestnut hybrid breeding work better
	Jared Westbrook

11:05am	Decodifying the quantitative resistance on White Pine Blister rust defense, through transcriptomics and epigenomics
	Laura Figueroa-Corona

11:15am	Tree Breeding including seed orchard management, progeny testing, Population and community ecology
	Jose Alberto Ponce Figueroa

11:35am	Cloning of *Pinus patula* through rooted cuttings: effect of stock plant etiolation and immersion time in a 5000 ppm IBA solution.
	Georgina Irasema Bautista Ojeda

12:00pm	Keynote Speaker: Sam Yeaman (University of Calgary) The genetic architecture of repeated local vs. global adaptation in plants

12:30pm	Lunch

Session 8: Quantitative genetics, tree breeding and forest management

Moderator: Benjamin Blonder

2:00pm	Tree genetic improvement methods and social acceptability *Carsten Kuelheim*

2:20pm	A drone's eye view of adaptive traits: multispectral vegetation indices from drone remote sensing quantify adaptive clines and hybrid status in provenance tests of Picea englemannii x glauca
	Samuel Grubinger

2:40pm	Sources of variance could tell us the chronicle of a death foretold by ozone pollution in a periurban forest of Mexico City.
	Verónica Reyes-Galindo

3:00pm	Association between dioecy and edaphoclimatic factors in Mexican populations of *Populus tremuloides* Michx.
	Javier Hernández-Velasco

3:10pm	Tree improvement and genetic diversity of *Pinus patula*
	Daniel Alejandro Rojas Hernandez

3:30pm	**Keynote Speaker: Hayley Tumas (University of British Columbia)** Genetic diversity in reforestation: By Ne means necessary
4:00pm	Coffee Break

Session 9: Phylogenomics, phylogeography and demographic history

Moderator: Alejandra Moreno-Letelier

4:15pm	Phylogeography of the *Juniperus deppeana* complex: speciation beyond morphology. *Rodrigo Martínez de León*
4:35pm	Historical demography of *Populus tremuloides* in Mexico by genomic analysis *Cecilia Nataly Gutiérrez Contreras*
4:55pm	Evolution and Ecology of Tropical Pines *Valentina Cardona Velasquez*
5:15pm	NAFGS Committee Reports
5:45pm	Meeting Conclusion
7:00pm	Final Banquet Celebration

Friday, June 14th

6:00am	Field Trip-Hot Springs
multi-day trip	Field Trip-Overnight / 2-day trip

Pamela Soltis
University of Florida

Polyploidy and Innovation in Plants: Extending to Forest Trees

Polyploidy is an important evolutionary force, especially in plants. The duplicate gene copies in a polyploid genome provide new genetic material for adaptation and innovation that may arise via processes such as neo- and sub-functionalization. In addition, nucleotypic effects, which result from increases in cell size due to an enlarged nucleus, may also confer novel features at cellular to ecological and evolutionary scales. We have been investigating the recent and recurrently formed allotetraploids Tragopogon mirus and T. miscellus (Asteraceae) as models for the study of polyploid evolution. These tetraploids originated less than 100 years ago in the Pacific Northwest of the USA following introduction of the parental diploid species from Europe. I will describe patterns of duplicate gene loss and expression as well as chromosomal diversity in the Tragopogon allotetraploids and how polyploidy has led to genetic and phenotypic novelty. Many forest trees are ancient polyploids, as first reported by Stebbins nearly 80 years ago, and more recent polyploidy is also evident in some of these species. I will explore what is known about polyploidy in forest tree species from North America, the challenges of sequencing, assembling, and annotating polyploid genomes, and the adaptive potential of polyploidy in these species. Finally, I will introduce the Polyploidy Integration and Innovation Institute, a new NSF-funded center to investigate polyploidy across biological scales from the cell to the ecosystem and across the Tree of Life, and I will invite participation in Institute activities from those working on polyploid trees.

Jessica Wright
US Forest Service

Climate change, forest genetics and postfire reforestation

In 1992 Ledig and Kitzmiller wrote, "Instead of following the general rule of thumb that says local seed sources are best, foresters may find that transfers across seed-zone boundaries are a better counter to climatic change. Faced with global

warming, they can either move seed northward from warmer climates or upward from lower elevations." This insight is over 30 years old and is now becoming an important tool in postfire reforestation projects in several jurisdictions. This idea of assisted gene flow, a form of assisted migration, is based on the concept that trees are adapted to the environment where they are found. Once established, trees cannot move to a better growing site. They do not migrate. One measure of evolutionary fitness is the number of grandchildren an individual produces. In forest trees, this can take hundreds of years, and requires not only that an individual tree survives, but that its offspring also survive and produce seeds. That process results in adaptation to the local environment, including climate. However, this process is disrupted when climate changes faster than the generation time of trees. Forest genetics has developed a number of tools for understanding adaptation, including provenance tests, climate modeling and genomic data. One new test coming online now is the Experimental Network for Assisted Migration and Establishment Silviculture (ENAMES). With over 30 experimental plots in Washington, Oregon and California, this project tests 4 sources of seed (local, early-, mid- and late- century climate) in a post-disturbance, operational landscape, as well as different silvicultural approaches (i.e. spacing, stock type, site prep) in a fully crossed design. Thirty years ago, Ledig and Kitzmiller said, "Foresters should not be too hasty in deploying seed among zones but must be ready to act if the signal for global warming becomes clearer." Research in Forest Genetics is helping to support these management decisions.

Antonio González Rodríguez

Instituto de Investigaciones en Ecosistemas y Sustentabilidad (IIES), UNAM

The oak conundrum: Disentangling divergence, reticulation and taxonomic delimitation in *Leucomexicana*

Antonio González Rodríguez, Saddan Morales Saldaña, Oscar De Luna Bonilla, Andrew L. Hipp, Ken Oyama and Susana Valencia.

The study of speciation in rapidly diversifying lineages that are also subject to introgression is challenging but essential for understanding biodiversity. Mexican white oaks (*Leucomexicana*) are a young and exceptionally diverse clade still

posing numerous questions regarding evolutionary relationships and species limits, complicated by their high richness in sympatry and frequent intercrossing. Solving such groups requires the integration of phylogenomics, ecology, quantitative morphology and taxonomy. Recently, we have adopted an approach based on broad sampling and bridging the micro- and macroevolutionary scales to disentangle particularly complicated species groups within *Leucomexicana*, such as the *Q. laeta* and *Q. microphylla* complexes. Our results suggest that oak species diversity in Mexico is underestimated and point to the need for phylogenetic network analyses. Additionally, ecological differentiation seems to be a part of evolutionary divergence among closely related oak species, either driving speciation or resulting from it. Finally, geometric morphometrics and quantitative micromorphological analysis should be incorporated into the taxonomic reappraisal of some species groups.

Sam Yeaman
University of Calgary

The genetic architecture of repeated local vs. global adaptation in plants

Understanding how species adapt to their environment is central to the study of evolution and predicting responses to climate change. The interplay between natural selection and migration is expected to shape the architecture of adaptation in different ways, depending on whether the direction of selection is spatially homogenous or heterogeneous. When different populations experience selection towards a similar phenotypic optimum, there is no tension with migration and "global adaptation" proceeds in manner similar to that predicted for a single population. By contrast, when populations are selected towards different optima, "local adaptation" occurs, which tends to favour architectures driven by fewer, larger, and more tightly clustered alleles than global adaptation. Despite this clear theoretical prediction, there have been few, if any, comprehensive tests in natural populations. Here, we bring together genome sequence data from thousands of individuals from 25 species of plants to compare signatures of repeated selective sweeps (global adaptation) with those of genotype-environment association (local adaptation). We deploy a common bioinformatic

pipeline to call variants in all datasets, reconstruct orthology relationships, and test for repeated signatures across species. In both the local and global adaptation analyses, we find a large number of genes with evidence for repeated signatures across multiple species, including many genes with previously established functions in response to biotic and abiotic stress. We then use RNAseq datasets to build gene co-expression networks, which provide an estimation of the pleiotropy of each gene (more connections/centrality == more pleiotropy). As pleiotropy is generally positively related to allele effect size, we would expect more pleiotropy for local adaptation and less pleiotropy for global adaptation due to the interplay between migration and selection. Our results are consistent with this prediction, with significant enrichment for high pleiotropy among the genes driving local adaptation, and low pleiotropy among the genes driving global adaptation. Despite high statistical evidence for repeatability, we also observe that much adaptation appears non-repeated and species-specific in its signature. To focus more deeply on adaptation within a single clade, we explore patterns of genomic differentiation in the hybrid zone between white and Engelmann spruce, and present some preliminary results on striking patterns of fine-scale adaptation to elevation and slope aspect.

Hayley Tumas
University of British Columbia

Genetic diversity in reforestation: By Ne means necessary

Hayley Tumas, Nicholas Ukrainetz, Alvin Yanchuk, Jon Degner, Trevor Doerksen, Dave Kolotelo, Marie Vance, Dragana Obreht Vidakovic, Erin Landguth and Sally Aitken

Centuries of harvest and reforestation have created a mosaic of planted and natural stands across forest landscapes in British Columbia, Canada. Over 60% of seed used in reforestation is now provided by breeding programs with policy setting minimum genetic diversity requirements for seedlots. However, as breeding programs continue to advance over generations and forest landscapes experience climate change as well as shifts in priorities, practice and governance, questions have arisen as to the impact of breeding and reforestation on genetic diversity at the landscape level. We use

effective population size (Ne) as a key metric to examine genetic diversity at three scales of forest management using genomic data for three widely planted conifers, lodgepole pine (Pinus contorta ssp. latifolia), interior spruce (*Picea glauca, P. engelmannii*, and their hybrids), and coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). We assess current methods for monitoring genetic diversity in seed used for reforestation, compare genetic diversity in selectively improved and natural seedlots, and examine the shift in genetic diversity from orchards to maturing stands on the landscape. Finally, we use the population genetic simulation model CDMetaPOP to understand the potential impacts of reforestation policy on landscape-level Ne. We find that current methods for monitoring genetic diversity in seed orchards based on estimates of cone crops are accurate. However, Ne is significantly lower in seed from breeding programs compared to natural stands. Natural regeneration of the same species substantially buffers genetic diversity in stands planted with selected material. Reforestation can have substantial impacts on landscape-level Ne, the magnitude of which depends heavily on the proportion of landscape harvested. Results will be used to evaluate current policy and practice.

Keynote Speakers

NORTH AMERICAN FOREST GENETICS SOCIETY

Day 1: Tuesday, June 11

Session 1: Population Genomics and Local Adaptation

Genotype-environment associations reveal high adaptive potential in a montane conifer (Abies religiosa, Pinaceae) from central Mexico

Presenting author: Gustavo Ibrahim Giles-Pérez

Co-authors: Erika Aguirre-Planter, Luis E. Eguiarte and Juan Pablo Jaramillo-Correa

During range expansion, forest tree populations at leading edges require evolving adaptations to face the newly selective pressures of colonized areas. Identifying the genomic basis of local adaptation during such range turnovers may shed some light on the capacity of species to mitigate the risk of demographic collapse and extinction in scenarios of abrupt environmental changes. Here, we explored the adaptive potential of *Abies religiosa*, a subtropical conifer that expanded its range during the last 220 Kya along an east-west environmental gradient in central Mexico; such a gradient is now composed of two different genetic pools. We genotyped 48 trees from nine populations that covered that gradient with ~5,200 SNPs derived from Genotyping- by-Sequencing (GBS). We then used Genotype-Environment Association (GEA) approaches to identify polymorphisms that were significantly correlated with climatic and edaphic population variation. The allelic frequencies of these SNPs was then fitted to a geographic cline that accounted for the divergence between genetic pools. We finally investigated whether the putatively advantageous alleles from the western genetic pool could have been originated from an ancient introgression from *A. flinckii*, a closely related species to *A. religiosa* that is likely preadapted to the warmer and drier conditions of western Mexico. To do so, we fitted genomic clines of inter-specific polymorphisms and pinpointed around 200 SNPs with an *A. flinckii* ancestry and a significant introgression in *A. religiosa* sympatric populations; one of these polymorphisms coincided with the previously retained candidates in the western *A. religiosa* genetic pool. These results suggest that local adaptation facilitated the east-west range expansion of *A. religiosa* across central Mexico; although such adaptation was likely fueled by standing genetic variation, there is some evidence pointing that ancient introgression from *A. flinckii* may also have contributed to that process. In a

changing climate, introgression from locally preadapted species should be considered as a genetic resource for the management and conservation of threatened, slowly-evolving tree species.

Keywords: Local adaptation, range expansion, *Abies religiosa*, Genotype-Environment Association, introgression

Effects of two generations of breeding for growth in Douglas-fir (Pseudotsuga menziesii) on the frequency of climate-adaptive alleles

Presenting author: Rafael Candido Ribeiro

Co-authors: Brandon Lind, Mengmeng Lu, Pooja Singh, Dragana Obreht Vidakovic, Sam Yeaman and Sally Aitken

Selective breeding has been used to improve growth in commercially important temperate tree species for decades, increasing the productivity of North American forests. However, climate change has intensified the effects of drought on these forests, driving tree mortality and reducing growth. Long-known patterns of local adaptation to cold temperatures in temperate species have also been disrupted. Yet, little is known about the genetic architectures of traits contributing to local adaptation to climate or how breeding for growth can affect adaptation to future climatic conditions. Here, we conducted a case-control genome wide association study of Douglas-fir (*Pseudotsuga menziesii*), with 20 provenances (80 seedlings/provenance) including both interior (var. glauca) and coastal (var. *menziesii*) Douglas-fir varieties to identify genetic variants associated with drought tolerance and cold hardiness. The frequencies of the top 2% of positive effect alleles (PEA) for both traits for seedlings from 53 natural provenances (32 var. *menziesii* and 21 var. glauca) were compared with 14 second-generation selectively bred seedlots (8 var. *menziesii* and 6 var. glauca) developed for the same regions as the natural provenances. Our results indicate that both drought tolerance and cold hardiness are highly polygenic. There is surprisingly little overlap in SNPs or genes between varieties. Sixteen genes were associated with both cold and drought for var. glauca but only one for var. *menziesii*, indicating different levels of pleiotropy in the varieties. A clear reduction in the frequency of PEAs for cold hardiness after two generations of selective breeding for growth was detected in both varieties, indicating a trade-off between growth and adaptation to low temperatures. In contrast, PEAs contributing to drought tolerance were not significantly altered by selection for growth. The implications of these results for the use of breeding versus climate-based seed transfer as adaptive strategies for climate change will be discussed.

Allele frequency differences between Lost Pines and Piney Woods loblolly pines of Texas: Can they be used to select for drought tolerance?

Presenting author: Carol Loopstra

Co-authors: Ian MacLachlan, Fred Raley, Claudio Casola, Mengmeng Lu and Jason West

Background: The commercial loblolly pine (*Pinus taeda* L.) forests of Texas are part of the Piney Woods (PW) of East Texas and Western Louisiana. Drought can be a problem with stand establishment and the summer dryness index is expected to increase. The Lost Pines (LP) are isolated populations of loblolly pine in central Texas and are more drought tolerant than trees in the PW. An understanding of the genes and variants that make the LP more drought tolerant may help with the breeding of trees in the PW.

Methods: We used the Pita50K genotyping array to genotype parents and progeny in a progeny test established by the Western Gulf Forest Tree Improvement Program (WGFTIP) as well as 28 trees from the LP. We compared allele frequencies (AF) for 33,368 SNPs and determined the difference between the LP and PW. Progeny height, diameter and survival data was provided by the WGFTIP. Percent carbon and nitrogen, ^{15}N and ^{13}C were determined at Texas A&M. Average AFs for the PW parents were used in regression analyses with the means for progeny phenotypic traits and mortality.

Results: Although AFs were usually within 0.2, there were large differences for a small proportion of SNPs. We selected SNPs with a difference between the populations of at least 0.4 and found that several were previously found to be differentially expressed in response to drought stress. We added SNPs with differences between 0.3 and 0.4 that were also differentially expressed and eliminated linked SNPs. We used 77 unlinked SNPs to determine AF differences for each of 49 female parents and their progeny. Fifteen of those SNPs are differentially expressed genes. Several of the other genes have functions that have been tied to drought stress responses but in some cases, we do not know gene function.

Within the PW maternal parents, we found large differences in the average frequencies of the "LP alleles" ranging from 0.14 to 0.51. We examined progeny

to see if maternal parents with allele frequencies more like LP trees have offspring that differ from those that are less similar.

We found no relationship between N, C, ^{15}N or ^{13}C and AF. We observed a slight but significant positive correlation between parental LP allele frequency and survival and a significant, negative correlation with both diameter and height.

Conclusions: PW parents that have AFs most like those found in LP trees have offspring with slightly greater survival that are smaller than PW trees that have AFs least similar to those of the LP trees. Unfortunately, we do not have genotype data for seedlings that died before foliage collections so we cannot compare individual tree allele frequency and mortality. The data was also affected by mortality due to factors besides drought including ants and fusiform rust. Controlled drought stress studies will be useful to further examine the relationship between allele frequencies, survival and growth.

Keywords: *Pinus taeda*, drought stress, adaptation, allele frequencies.

Black spruce vulnerability to climate change
Presenting author: Anna Fijarczyk

Co-authors: Nathalie Isabel, Etienne Robert, Patrick Lenz, Manuel Lamothe, Yves Bergeron, Benjamin Marquis and Martin Girardin

The Genomic Adaptation and Resilience to Climate Change (GenARCC) project, a collaborative effort across eight Canadian government departments, utilizes genomic tools to decode the genetic makeup of culturally, economically, and ecologically significant species and populations, including domestic and harvested species, as well as those at risk. With a focus on forecasting the impacts of climate change on biodiversity, ecosystem services, and emerging threats such as pests and pathogens, insights from GenARCC inform evidence-based decisions and policies to mitigate the adverse effects of climate change on both people and ecosystems in Canada.

Keywords: Dendroecology, adaptation, phenotype, GEA, phylogeography

Physiological and Transcriptomic Responses to Drought in Ponderosa Pine
Presenting author: Adam Gilewski

Co-authors: Jim Mattsson and Marie Vance

Ponderosa pine (*Pinus ponderosa*) is a drought-tolerant tree species of key importance for the British Columbian interior's increasingly hot and dry forests. British Columbia's only A-class seed orchard contains selections based on volume at rotation age, but their performance under drought conditions is unknown.

A 2-year drought experiment included ~670 one-year-old seedlings from 22 half-sib families. Individuals were planted in raised beds and a drought treatment was applied by installing rain-exclusion roofs each summer, allowing the beds to dry-down. Control beds were irrigated weekly to maintain soil moisture. Measured traits include height growth, mid-day needle water potential (WP), and water use efficiency (WUE; measured as δ13C). Additionally, needles collected from 80 individuals at the end of the first summer were RNA-sequenced. As roots could not be accessed in the beds, we instead measured the root growth of week-old seedlings from 25 families grown hydroponically in solutions with varying osmotic stress for 2-3 weeks.

Responses to well-watered and drought conditions in the raised beds revealed that: (1) families with greater height growth in the control treatment generally also grew faster under drought, (2) drought-stressed seedlings which grew well had higher WP and lower WUE, (3) fast-growing individuals within the drought treatment had weaker transcriptomic responses relative to trees that grew poorly under drought stress. (4) Genetic correlations from a preliminary multivariate model agreed with phenotypic correlations in that WP was positively correlated with height growth, as well as the tolerance of growth to drought. However, in contrast to the phenotypic correlations, WUE was instead positively correlated with tolerance, while being negatively correlated with growth under control conditions. The hydroponic experiments showed that mild osmotic stress stimulated rather than inhibited root growth, and that some families were not inhibited by stronger osmotic stress. Finally, we have generated an assembly of multiple ponderosa pine transcriptomes and identified drought-regulated genes, as well as associations between gene expression and traits under drought.

In summary, we found that some families previously selected for growth have higher drought tolerance and can therefore be used for deployment and further selection. Moreover, by incorporating WP measurements into selection, we may be able to increase both height growth and its tolerance to drought without a trade-off. In contrast, selection for increased WUE may further increase drought tolerance, but at the potential cost of height growth under ideal conditions. We hypothesize that genetic variation in WP (which was not genetically correlated with WUE) could be the result of increased access to water via an expanded root system, while the aspect of drought tolerance that

comes with increased WUE may stem from reduced stomatal conductance at the cost of photosynthesis.

Keywords: drought tolerance, physiology, genetic correlation, transcriptomics

Unraveling the genetic architecture of bud flush timing in two hybridizing *Populus* species in order to predict phenological responses to future climates

Presenting author: Baxter Worthing

Co-authors: Matt Fitzpatrick, Jill Hamilton, Jason Holiday, Raju Soolanayakanahally, Shawn Mansfield and Stephen Keller

Introduction:
In temperate forests, trees must break winter dormancy each spring, an event marked by the opening of dormant buds, or "bud flush". Bud flush timing is a phenotype of high adaptive importance, as the initiation of vegetative growth must be timed to maximize growing season length, while also minimizing potential exposure to spring frost events. As climate change threatens to shift the timing and variability of seasonal climatic transitions, accurate prediction of tree phenology under future climates is a key conservation concern. A reliable model of bud flush timing would therefore be a useful tool for forecasting the potential impacts of climate change on forests, and identifying specific tree populations that may be vulnerable to future maladaptation. Genetic variation, environmental variation, and their interaction (G×E) are all likely to influence bud flush timing, but the relative contributions of these factors to the genetic architecture of this trait remain unclear, as do the specific environmental variables and genetic variants involved. Here, we sought to better understand the genetic architecture of bud flush timing and predict shifts in spring phenology under future climates for two widely distributed and hybridizing North American poplar species – *Populus balsamifera* and *P. trichocarpa*.

Methods:
We compiled an unprecedented dataset of 15,636 total bud flush timing observations for 1,437 wild-sampled trees of *P. balsamifera*, *P. trichocarpa*, and their naturally occurring hybrids, planted in 5 geographically distinct common gardens across multiple years. We paired this rich phenotypic dataset with genetic data comprised of structural variants (SV) and single-nucleotide polymorphisms (SNP) for all observed trees. To reduce reference bias in admixed individuals, genotyping calls were made against our in-house pan-genome reference assembly. We use these data to train a genomic prediction

model of bud flush reaction norms across continuous environmental space for each genotype.

Results:
Extracting variance components from the genomic prediction models, we show that G×E interactions explain a significant portion of the variation in bud flush timing across our dataset, and suggest that bud flush reaction norms reflect adaptation to differing spring climates across the ranges of both species.

Conclusion:
We conclude that prediction accuracy across different environments and genetic ancestries highlights the applicability of this approach to predicting bud flush timing in unobserved environments, including future climate scenarios. Our predictions suggest that North American *Populus* populations vary in their potential for maladaptive phenological responses to future climates.

Keywords: phenology, prediction, plasticity, poplar

Session 2: Population Genomics and Local Adaptation

How to inform assisted migration for the keystone species Populus tremuloides

Presenting author: Melanie Zacharias

Co-authors: Roos Goessen, Nathalie Isabel, Christian Wehenkel and Ilga Porth
Background:

Quaking aspen (*Populus tremuloides* Michx.) is an important keystone species with a wide distribution range over North America. It exists in a wide variety of environmental settings and plays a crucial role for many ecosystem services. Therefore, it is crucial to explore the species vulnerability to climate change. Mexican populations are expected to become more interesting with global warming but this hypothesis was never tested.

Methods:
To investigate the genetic offset of the species when facing climate change, we analysed over 800 non-clonal, diploid individuals of *P. tremuloides* sampled across its wide distribution range. The sampled populations represent different ecological conditions, across Canada, the US down to central Mexico, including drier regions like the Prairies, western US or Texas. We use Gradient Forest, a random forest learning approach, to identify potential maladaptation under climate change in *P. tremuloides* by comparing its current genomic composition to the one predicted to be necessary in the future, called genomic offset. For identifying the environmental variables driving the genetic clustering, we use a full dataset of around 11,000 prefiltered SNPs and a subset of 355 SNPs which we already associated with environmental variables with two different climate datasets. For a better comparison among studies, we standardized the genomic offset with quantile normalization and sigma dissimilarity regarding future climate using different future climate scenarios. To reinforce the calculated genomic offset per population, we used diameter and height as phenotypic data from provenance trails of *P. tremuloides* to verify if a higher offset results in a lower growth performance. Additionally, we calculated transferability as well as donor and recipient importance of the populations to explore which populations in which areas are genetically best equipped for the future climate.

Results:
Precipitation variables have the highest influence on genetic structure across datasets. Genomic offset is highest in the Mexican populations presumably because of the most severe climatic changes predicted. Nevertheless, the

differences in phenotypic plasticity and ploidy among populations need to be discussed regarding the genomic offset results.

Conclusions:
The calculated offset indices can inform assisted migration programs, contributing to maintenance of healthy forests.

Keywords: genomic offset, aspen, Gradient Forest, genotype-environment analysis, climate change adaptation

Local adaptation of *Pinus leiophylla* under climate and land use change models in the Avocado Belt of Michoacán

Presenting author: Vanessa Izaguirre Toriz

Co-authors: Jonás A. Aguirre-Liguori, María Camila Latorre-Cárdenas, Eugenio Y. Arima and Antonio González-Rodríguez

Climate change and land use change are the main drivers of global biodiversity decline causing reductions in the distribution range of species and decreasing the amount of genetic diversity. Rapid climate change is altering the patterns of local adaptation by creating novel climates and disrupting genotype-environment associations. In consequence, populations may respond by adjusting via phenotypic plasticity, adaptive evolution or through migration. Methods as Gradient Forest can be used to estimate the genomic offset (GO) a measure of the vulnerability of populations to climate change. However, studies that have analyzed GO usually do not consider other processes that increase the loss of genetic diversity, such as changes in land use and habitat fragmentation. Michoacán´s pine oak forests have experienced a dramatic transformation to other land uses, most notably avocado cultivation which covered approximately 244,705 hectares by the year 2019. Moreover, it is projected that cultivation of avocado will continue to increase by approximately 178,534 additional ha by the year 2050. We selected *Pinus leiophylla*, a tree species in Michoacán´s temperate forest to simultaneously consider land use and climate change in GO scenarios. The aims were: 1) to assess current genotype-environment associations in *P. leiophylla* populations, 2) to evaluate the vulnerability of populations simultaneously considering GO on the face of climate change scenarios and the expansion of avocado crops, 3) to lay out assisted gene flow strategies for the species' management.

We obtain the DNA of 19 populations, estimated the genetic structure and diversity statistics, identified outlier SNPs and calculated connectivity metrics among forest fragments as mediated by both pollen and seed dispersal. We modeled how the turnover in allele frequencies across the landscape could

change under climate change and land use change scenarios in addition to evaluating assisted gene flow strategies. Priority conservation areas were identified that could be less affected by both disturbances.

We found that the adaptative genetic diversity of populations shows heterogeneous patterns across the landscape where there are populations that will be more vulnerable to climate and land use change. Some populations could serve as sources of diversity to reduce their risk of extinction. Connectivity patterns will also be modified by habitat loss. The areas with higher priority for conservation are those located in the Monarch Butterfly Biosphere Reserve. This area maintains populations that could be well adapted to future environmental changes and will be less susceptible to land use change, maintaining the connectivity.

This work is useful as a framework to incorporate distinct layers of information to provide a more robust representation of the response of the populations and serves as a way of designing better conservation strategies.

Keywords: genomic offset, local adaptation, conservation genomics, land use change, climate change.

Predicting fitness under future climates across *Populus* trees from a natural hybrid zone

Presenting author: Alayna Mead

Co-authors: Michelle Zavala-Paez, Matthew Fitzpatrick, Jason Holliday, Stephen Keller and Jill Hamilton

If tree populations are unable to adapt to changing climates, migrate to suitable climates, or survive changes through phenotypic plasticity, they will become extirpated without human intervention. Genetic diversity may allow populations to adapt to new conditions, and forest tree species often have a great deal of genetic variation originating from interspecific gene flow as well as local adaptation to heterogeneous environments. Conservation strategies can take advantage of this genetic variation through assisted gene flow, which introduces alleles that are pre-adapted to a population's future climate. However, because climate adaptation is complex and polygenic, it can be difficult to identify beneficial genetic variation. Common garden experiments that include many environments can be used to determine optimal climate conditions for genotypes originating from different climates, and to test whether accurate predictions can be made from genomic data alone. We have established 18 common gardens with *Populus* genotypes collected from multiple latitudinal transects spanning the natural hybrid zone of *Populus*

trichocarpa and *P. balsamifera* in western North America. Replicated genotypes were planted into common garden sites located across a wide range of environments and geographic localities in the United States. This experimental design allowed us to model the relationship between climate, phenotype, and genotype, and to identify the climate under which each genotype accumulated the greatest growth, a proxy for fitness in forest trees. Using this model, we determined the relative importance of genotype, garden environment, and climate of origin on growth. We also tested whether this model can accurately predict which genotypes have the greatest fitness in a particular environment. We find that genomic variation representing species ancestry and geographic region of origin are both important predictors of growth across common garden environments, indicating that genetic information can improve predictions of tree fitness under a particular climate. This information could be used to identify optimal seed sources and planting sites for planting *Populus* into restored or managed stands.

Keywords: *Populus*, hybridization, assisted gene flow, climate adaptation

Sex-specific adaptation in Mexican *Populus tremuloides* Michx

Presenting author: Christian Wehenkel

Co-authors: Javier Hernández-Velasco, J. Ciro Hernández-Díaz and Matthias Fladung

Background:
Between 4%-6% of angiosperm species are dioecious, including *Populus tremuloides*. In contrast to animals, dioeciousness in plants probably has its origin in recent evolution. At least two major genetic changes were necessary for the evolution of sexes: male sterility to produce female plants and female sterility to produce male plants. It is estimated that transitions from hermaphroditism to dioecy have occurred up to 5000 times during the evolutionary history of angiosperms. Such transitions are often explained by sexual dimorphism, which is observed in many plant species for a variety of phenotypic traits. This dimorphism is explained by the fact that males and females probably maximize their respective fitness by evolving different life-history traits that may enable sex-specific strategies for acquiring resources. In *Populus* spp., some studies have reported differences between the sexes in non-reproductive traits and adaptive strategies, while others have not. Our study aim was to identify sex-specific environmental adaptations in about 60 natural populations of *P. tremuloides* from the entire Mexican distribution area.

Methods:

For this purpose, 217 male and 217 female diploid individuals and about 36,810 filtered SNPs from genome resequencing were used. Gender assignment focused on detecting the Potri.019G047300 ("TOZ19") gen, which only occurs in male individuals of *P. tremuloides*.

Results:

Using BayeScEnv and pcadapt in R, there were different SNP outliers in male and female trees. There were significant sex-specific SNP associations with the environmental variables, such as mean annual temperature.

Conclusions:

Our results supported the hypothesis of differential plasticity: males and females differ in their plastic response to one or more environmental factors, leading to different degrees of sexual dimorphism along environmental gradients.

Keywords: SNPs, quaking aspen, evolutionary mechanisms, Potri.019G047300 ("TOZ19") gene

Defining climate analogs for seed transfer and assisted migration

Presenting author: Bryce Richardson

Co-authors: Gerald Rehfeldt, Cuauhtémoc Sáenz-Romero and Elizabeth Milano

Background:

Biodiversity is key to maintaining ecosystem function and resilience. However, limited understanding of many plant biological and genetic systems can limit adoption into restoration and reforestation programs. Moreover, given mid-century climate change projections, opportunities to develop a diverse array of restoration species could be fleeting, due to expected extirpation of uniquely adapted populations. Generalized, climate-based seed transfer uses climate partitioning to approximate adaptive differences. These seed transfer approaches can provide a stopgap when genetic knowledge is limited and timely decisions of collecting, bulking, and deployment of seeds are needed.

Methods:

We present a generalized seed transfer approach that uses Euclidean distance of 19 climate variables within North America. Euclidean distances are used to identify climate analogs from vegetation databases of about 685,000 plots. Analogs are classified into three thresholds (strong, moderate, and weak) that correspond to altitudinal climate gradients and are guided by the scientific

literature of observed adaptive variation of natural tree populations and seed transfer limits.

Results:
For strong threshold observations, about 97% of the analogs had climate distances equivalent to ≤ 300m elevation, whereas for the weak threshold observations, 53% had an elevation equivalence of ≤ 300m. On average 120m, 267m, and 293m elevation separated two points under strong, moderate, and weak thresholds, respectively. In total, threshold classification errors were low at 13.9%. We use examples of plot data identified from a reference period (1961–1990) and mid-century (2056–2065) analogs across North American biomes to compare and illustrate the outcomes of projected vegetation change and seed transfer.

Conclusions:
These examples showcase that mid-century analogs may be located in any cardinal direction and vary greatly in spatial distance and abundance from no analog to hundreds depending on the site. The projected vegetative transitions, predicted from vegetative plot analogs, will have substantial impacts on conservation programs and ecosystem services. Our approach highlights the complexity that climate change presents to managing ecosystems, and the need for predictive tools in guiding land management decisions to mitigate future impacts caused by climate change.

Keywords: assisted migration, decision support tool, Euclidean distance, novel climates, reforestation

Session 2: Conservation Genetics

Conservation genomics in the eastern hemlock enabled through genomic, metabolomic, and population analyses

Presenting author: Karl C Fetter

Co-authors: Vidya S Vuruputoor, Meghan Myles, David Baukus, Niharica S Kannan, Hamid Nodeh, Roland Kersten, Ben Smith, Sarah Johnson, John Butnor, Dana Nelson, Tim Cernak and Jill L. Wegrzyn

Background:
The eastern hemlock (*Tsuga canadensis*) is an imperiled forest tree species of eastern North America. The eastern hemlock is a keystone species providing numerous ecological benefits to forest systems in addition to its significant cultural importance for millions of people living inside its range. An invasive hemipteran insect, the Hemlock Woolly Adelgid (HWA), is threatening population viability of the eastern hemlock and is largely responsible for the collapse of hemlock populations. There is a pressing need to utilize conservation genomics principles and techniques to protect genetic resources. Here, we describe a conservation genomics program to create a chromosomal-scale reference genome, metabolomic investigation of terpenes putatively inhibiting or promoting HWA infestation, and a landscape genomics study to identify climate adaptive genomic variation. These studies aim to support future restoration efforts, evolutionary studies, and conservation of this imperiled species across academic, government, and non-profit organizational boundaries.

Methods:
A reference genome was created from short and long-read sequencing and assembled with multiple methods and scaffolded with Omni-C sequences. The genome was annotated using RNA evidence and gene models predicted and filtered with the EASEL genome annotation pipeline. The metabolomic study was conducted in three common gardens planted in 2014 of eastern hemlock accessions with variable tolerance to the HWA. Gardens were revisited for a year and leaf tissue sampled for paired terpene concentration and RNA expression analysis. To elucidate potential mechanisms of terpene induced HWA resistance or susceptibility, bayesian mixed models were fit to HWA counts, terpene concentration, and a design matrix. The landscape genomics study is supported by 800 wild samples of trees across the entire range of the species sequenced with RADseq. Climate adaptive genomic variation and genetic offsets were identified using LEA in R.

Results:

A 17 Gb assembly was created with high BUSCO and Merqury scores. Genome annotation identified gene models enabling comparison of gene families related to terpene chemistry and the plant immune system. Multiple regression of HWA counts and terpene concentrations identified camphene and D-Limonene as putative resistance molecules, while bornyl acetate promoted HWA infestation. Landscape genomic analyses identified genes with a genetic offset under future scenarios of climate change.

Conclusions:

These results lay the foundation for applied conservation genomic work in the eastern hemlock system. The reference enables comparative and population genetic analyses that will inform conservation decisions. Terpene analysis provides an important context for understanding variation of HWA infestation to metabolic associations with HWA infestation.

Keywords: reference genome, terpenes, landscape genomics, climate adaptation, conifers.

First glimpse of genomic and candidate genes for local adaptation in an endemic oak from Oaxaca, Mexico

Presenting author: Nelly Jazmín Pacheco Cruz

Co-authors: Cecilia Liana Alfonso Corrado, Ricardo Clark Tapia, Víctor Manuel Salazar Rojas, Juan Pablo Jaramillo Correa, Jorge Ciros Pérez, Andrea Olga Hernández Miranda, Claudia Verónica Granados Hernández and Tania Martínez León

Background:

Quercus macdougallii is an endemic oak distributed in the Sierra Juárez of Oaxaca, Mexico. It is a key species in the forest ecosystems where it occurs and is classified as threatened (NOM-059) and endangered (IUCN). In individuals from the northern part of its known distribution, low genetic diversity and differentiation have been reported through nuclear microsatellite analysis. Ecological studies have reported low growth and survival rates. It is distributed along altitudinal gradients from 2,600 to 3,100 masl and in a transition zone between two biogeographic provinces. Therefore, the objective of the study was to assess the genetic status of the species and identify potential candidate genes for local adaptation.

Methodology:

Genotyping by sequencing (GBS) was performed on 79 individuals collected from nine sampling sites; five in the northern and four in the southern zone of

their known distribution during 2018. Bioinformatics analyses for the assembly and identification of single nucleotide polymorphisms (SNPs) were performed with ipyRAD using two approaches; 1) with reference genomes and 2) with reference transcriptomes, in both cases data from Quercus robur and Quercus lobata were used. This was done to compare and complement possible outlier SNPs. Population genetic analyses were performed on the SNPs identified with the reference genomes. SNPs outliers of both approaches were identified by PCAdapt analysis, for which a sequence homology search was performed in the UniProt database to identify the genes where SNPs outliers were found.

Results:
Between 5,383 and 5,415 SNPs were identified with the reference genomes. Low diversity (He ~ 0.12) and low genetic structure (ADMIXTURE and fastStructure) were found. Although the species behaves as a single population, a differentiation gradient was observed in the southern zone. Forty-one atypical SNPs were identified showing the presence of private alleles associated with the study areas. On the other hand, between 1,381 and 1,791 SNPs were identified with the reference transcriptomes. An analysis with PCAdapt identified nine atypical SNPs with the transcriptome of *Q. lobata* and 18 with that of *Q. robur*. Candidate genes with a large number of SNPs were identified, including those encoding disease-resistance proteins and those related to developmental and regulatory processes.

Conclusions:
The results will enable us to complement conservation and monitoring strategies for the species. The identification of potential candidate genes for local adaptation in an endemic oak will allow us to explore and consolidate knowledge of local adaptation in oaks. This is the first study of population genomics and functional annotation for the species and more important, for an endemic oak in Mexico.

Keywords: Conservation, SNPs outliers, endanger species, functional genes, *Quercus macdougallii*

Can forest trees escape the extinction vortex?
Presenting author: Eduardo Mendoza-Maya

Co-authors: Gustavo I. Giles-Pérez, J. Jesús Vargas-Hernández, Cuauhtémoc Sáenz-Romero, Miguel Martínez-Trujillo, María de los Ángeles Beltrán-Nambo, José Ciro Hernández-Díaz, José Ángel Prieto-Ruíz, Juan P. Jaramillo-Correa and Christian Wehenkel

Population bottlenecks has the potential to trigger the extinction vortex; i.e., a down-ward spinning cycle of increased endogamy and genetic drift followed by a reduction in the genetic diversity, individual fitness and population viability. However, it is not clear at what extent this model is fitted by organisms with long-life cycles and high reproductive outputs, such as forest trees. Specially, the synergistic effects of demographic histories and the throughout connection between all stages of the extinction vortex are not well understood in these taxa.

We used the Site Frequency Spectrum to simulate the demographic histories for two endangered forest trees with fragmented populations: *Picea martinezii* Patterson (n=89) and *P. mexicana* Martínez (n=83). We estimated: i) Ne as 10% individuals of total censuses in all seven known populations of both species; ii) genomic diversity (Ho); iii) fertility, with seed development traits (reduced to PC1 with a PCA) and germination; and iv) population viability, considering recruitment as the main indicator. We looked for evidence of the extinction vortex with linear regressions between: i) Ne vs population genetic diversity and fertility; and ii) individual genetic diversity vs fertility through Heterozygosity-Fitness Correlations (HFCs) to test the so-called general effect hypothesis (i.e., the additive effect of deleterious alleles distributed across the genome) as a genomic signature of the demographic histories.

P. martinezii showed an abrupt population decay from 22 to~1.8 thousand years ago (ka). A first population collapse occurred 900 ka for *P. mexicana*, with a long period of demographic stability and a second decay since ~40 to 0.6 ka. HFCs showed general effects for *P. martinezii* for one fertility variable (r[Ho vs PC1 = 0.27; p = 0.009) but not for any fitness-trait in *P. mexicana*. Regarding the effects of Ne over population genetic diversity and fertility, we observed a positive association with seed development traits (r[Ne vs PC1] = 0.79; p = 0.034), but not with germination or genetic diversity. The two smallest *P. martinezii* populations showed the lower genetic diversity and viabilities, but discordant fertilities.

Despite having less populations and adult trees than *P. martinezii*, genetic load purge is apparently more advanced in *P. mexicana*, which fitted the species' demographic histories. The two smallest *P. martinezii* populations are of conservation concern, but with unclear associations between the stages of the extinction vortex. This study highlights the utility of correlating genomic, demographic and reproductive data for the conservation of endangered taxa and suggests that traits related with fertility selection (i.e., early seed development traits) are a better proxy for individual fitness than economically-important traits. More evidence is needed to clarify the extinction vortex adjustment in forest trees.

Keywords: Fertility selection, Heterozygosity-Fitness Correlations (HFCs), *Picea martinezii*, *Picea mexicana*, population bottlenecks.

Is black maple a separate species?

Presenting author: Alix Pugeaut

Co-authors: Bautisse Postaire and Yann Surget-Groba (ISFORT)

In the current context of global changes, assessing the conservation status of species is fundamental to adopting effective conservation strategies. However, taxonomic uncertainties can undermine the quality of these assessments. We were interested in the case of the black maple in Canada: classified as a vulnerable species in Quebec, it is considered to not require any conservation status in Ontario. We assume that variations in the conservation status of the black maple come in part from the uncertainty of the taxonomic designation of the species: perceived by some as a full species and by others as a subspecies of sugar maple. Morphological dissimilarities between sugar maple and black maple support their designations as independent species. However, several genetic studies in the past have provided arguments pointing towards the absence of significant genetic divergence between the two types of maples, validating the black maple as a subspecies of the sugar maple. To clarify this situation, we genotyped with a high-throughput sequencing method (GBS) two stands in a continuous, undisturbed forest where the two maples are sympatric. Each stand contained three equidistant plots, two of sugar maple (N=20 individuals each) and one of black maple (N=20 individuals). We investigated genetic structure among these plots to test for hypothesis of species. The data obtained provided evidence of greater genetic differentiation between populations of the two morphospecies than between populations of the same morphospecies, in a restricted geographical area. In addition, we were able to show that black maple populations in Quebec display higher genetic differentiation compared to those of sugar maple at the same latitude. Overall, these results support the taxonomic designation of black maple as a species and constitute the first characterization of the genetic diversity of populations located at the northern limit of its distribution.

Keywords: conservation genetics, species delineation, integrative taxonomy

Genetic identity of Agave peacockii Crouncher: microendemic species

Presenting author: Paulina Montiel Castelán

Co-authors: Pablo Octavio-Aguilar, Deluce Maria Galván- Hernández, Javier López- Upton, J. Vargas-Hernández and Marcos Jiménez- Casas.

Given the primary objectives of the GenARCC project, which include quantifying genetic diversity, monitoring climate-associated changes in native species, and utilizing genomic tools to predict future vulnerabilities to climate change, we conducted a study focusing on black spruce (*Picea mariana*; BS). Leveraging dendroecological data collected from four BS common gardens established in the 1970s, encompassing 61 provenances (over 2,100 trees) representative of BS distribution, we investigated how intraspecific genetic variation influences BS' growth response to current and future climates. In parallel, we employed a Genomic Environment Association (GEA) approach to further assess the vulnerability of BS populations to future climates.

Keywords: hybridization, exogamy, microsatellite, parents, variation

Genetic diversity of *Agave salmiana* in plantations in Mexico

Presenting author: Sonia Teresa Cruz-Vasconcelos

Co-authors: Carlos Ramírez-Herrera, Martha Hernández-Rodríguez, Nicacio Cruz-Huerta, Armando Gomez-Guerrero and Valentín José Reyes-Hernández

Agave salmiana grows in the semiarid region in Mexico. It is divided in three taxa (*A. salmiana* subsp. *crassispina*, *A. salmiana* var. *salmiana* and *A. salmiana* var. ferox) so morphological variation is common in this species. However, little information is known about genetic diversity of *A. salmiana*. Genetic diversity is important to ensure the resilience of ecosystems upon changing climate factors. Information about genetic diversity and genetic structure of a species is important for conservation, management and genetic improvement. Thus, the objective was: to estimate the diversity and genetic structure in *Agave salmiana* in plantations in Temascalapa, State of Mexico, Mexico. Plant tissue was collected from 33 seven-year-old plants of *A. salmiana* subsp. *crassispina*, *A. salmiana* var. *salmiana* and *A. salmiana* var. ferox in three plantations. DNA extraction was done with the modified CTAB protocol. Approximately, forty-seven thousand SNPs (DNA single nucleotide polymorphisms) were identified in the CIMMYT laboratory Agricultural Genetic Analysis Service. Observed heterozygosity (Ho) and expected heterozygosity (He) were estimated. Also, the fixed indexes (FIT, FIS and FST) and a molecular analysis of variance (MANOVA) were computed. The Ho was 0.071 for *A. salmiana* subsp. *crassispina*, and it was 0.071 for *A. salmiana* var. ferox, while Ho was 0.078 for *A. salmiana* var. *salmiana*. The He varied from 0.178 for *A. salmiana* subsp. c*rassispina* to 0.198 for *A. salmiana* var. *salmiana*. It was 0.191 for *A. salmiana* var. ferox. The FIT

and FIS were 0.897 and 0.893, respectively while FST was 0.042. The MANOVA indicated that ninety six percent of genetic diversity was within taxa and only four percent among taxa. *Agave salmiana* has low genetic diversity. Low genetic diversity was found in *Agave salmiana*, and most of the genetic diversity was within three taxa. There was high level of inbreeding in this species.

Keywords: *Agave salmiana*, genetic diversity, genetic structure, SNP, fixed indexes

Session 3: Conservation Genetics

Genomics-driven monitoring of *Fraxinus latifolia* (Oregon Ash) for genetic conservation and EAB-resistance breeding

Presenting author: Anthony Eli Melton

Co-authors: Trevor M. Faske, Thomas L. Parchman and Jill A. Hamilton

Background:
Understanding the distribution of standing genetic variation for species of conservation concern is critical for designing conservation and restoration strategies. Genetic data can provide baseline knowledge needed to establish or prioritize conservation, restoration, or pre-breeding strategies.

Methods:
In this study, reduced representation sequencing was used to generate genomic data for over 1,000 samples from 61 populations across the range of Fraxinus latifolia, a foundational riparian tree native to the Pacific Northwest recently exposed to the invasive Emerald Ash Borer (*Agrilus planipennis*), to inform the establishment of genecological resources needed for proactive conservation and breeding.

Results:
Populations were genetically structured along a latitudinal gradient, with population connectivity largely maintained along central valley river systems and a center of genomic diversity within the Columbia River watershed of Washington and Oregon. Interestingly, putative polyploids were identified in southern California that may represent tetraploid hybrids. Despite evidence of gene flow, estimates of nucleotide diversity and effective population size estimates were low across all populations, suggesting that the patchy distribution of *F. latifolia* across parts of its range may impact long-term evolutionary potential of the species. In addition, range-wide genetic offset suggests populations may be at further genetic risk under climate change.

Conclusions:
Targeted ex situ collection strategies to preserve both core and peripheral populations across the range will capture a majority of genetic diversity, and propagation of germplasm from four recommended conservation units will preserve variation needed to initiate rangewide EAB-resistance breeding trials for *Fraxinus latifolia.*

Keywords: population genomics, landscape genomics, Fraxinus, threatened species, conservation

Day 2: Wednesday, June 12

Session 4: Quantitative Genetics

Enhanced Resistance Pines for Improved Renewable Biofuel and Chemical Production

Presenting author: Gary Peter

Co-authors: Mallory Morgan, Matthew Lane, Salvador Gezan, Chris Dervinis; Daniel Ence, David Kainer4, Mirko Pavicic, Manesh Shah and Daniel Jacobson

Background:
The constitutive and inducible oleoresin defenses in loblolly (Pinus taeda) and slash (Pinus elliottii var elliottii) pine provide physical and chemical resistance to insects and pathogens and the terpenes in oleoresin can be used as a renewable source of biofuels. Our focus is to increase constitutive oleoresin to enhance loblolly and slash pine resistance to pests and pathogens and to simultaneously increase biofuel feedstocks.

Methods and Results:
We conducted genome wide association analysis (GWAS) analyses of constitutive and inducible oleoresin flow, mono- and diterpene content were completed in our ADEPT2 population. In the ADEPT2 population, we simultaneously measured constitutive and induced oleoresin flow after treating clones with methyl-jasmonate (MeJA). We observed a strong genetic correlation (0.82) between induced and constitutive oleoresin flow, suggesting the genetic architecture of these traits is strongly shared. In the ADEPT2 population using linear mixed models and multi-locus linear mixed models with two sets of markers totaling ~2.28 million biallelic SNPs we identified 146 significant SNPs ($p<0.05$) for constitutive oleoresin flow, monoterpene composition and content. Two of the significant SNPs for wood limonene content are in an a-pinene synthase gene.

To identify genes regulating resin duct differentiation and function, we induced new axial resin canal formation in the cambial meristem by applying MeJA. We conducted a time course experiment with 78 RNAseq libraries from vascular cambial zone tissue collected from days 0, 1-14, 17, and 21 after MeJA treatment. Pooled libraries were sequenced to a 30x read depth and reads were mapped to a newly assembled, improved loblolly pine transcriptome that includes 64,671 genes. DESeq2 analysis identified significantly 1890 up and 4634 down differentially expressed genes across the time course compared with wild type controls. With these 6524 differentially expressed genes we created a Predictive

Expression Network (PEN) using iterative Random Forest Leave One Out Prediction to illustrate higher-order interactions between genes and to determine the gene-to-gene relationships that are the most highly predictive of each other. To identify and prioritize genes across the PEN, we applied random walk with restart (RWR) algorithms with selected seed genes. The RWR allows us to identify mechanistically associate genes that did not appear in GWAS due to a lack of statistical power or genetic variation but are still important for pine oleoresin traits. This identified 119 in the top 200 transcripts, and we are annotating the network to identify genes whose expression supports involvement in resin canal formation and terpene synthesis.

Conclusions:
We characterized the genetic architecture of 10 oleoresin traits to accelerate breeding and genetic engineering to enhance wood terpene composition.

Keywords: oleoresin, GWAS, pine, genetic architecture

Cytotype and genotype effects on the ecophysiology of quaking aspen (*Populus tremuloides*)

Presenting author: Benjamin Blonder

Background:
Quaking aspen (*Populus tremuloides*) is a widespread North American tree species. It occurs in diploid and triploid cytotypes. It is experiencing mortality in many portions of its range, often in areas where triploids are more common. Intraspecific cytotype variation may influence ecophysiology, with potentially greater drought mortality risk for faster growing triploids.

Methods:
We collected more than 300 genotypes from across the species range and grew out more than 2000 saplings via clonal propagation. After 1-2 years of growth we conducted a multi month drought and rewatering experiment. We also measured growth rates, defense chemistry, leaf and stem hydraulics, xylem anatomy, and reflectance spectra in undroughted conditions. All stems had their cytotypes identified via DNA sequencing.

Results:
In a common garden setting, there was limited effect of cytotype on most traits and drought responses. Exceptions occurred for stem xylem pit anatomy and growth rate. Reflectance spectra also varied consistently in shortwave infrared bands. Stronger effects of latitude of origin were found for most traits.

Conclusions:

Cytotype does not have a strong effect on most aspects of ecophysiology or drought response - in saplings in common environments. Interpretations include: allelic genetic variation is more important than cytotype variation for ecophysiology, cytotype effects occur at later life stages, or cytotypes differ in their environmental niches. We are growing these trees to maturity in multiple locations to distinguish these hypotheses.

Keywords: Cytotype, polyploidy, poplar, common garden

Quantitative and population genetics of North American and European lineages of coastal Douglas-fir

Presenting author: Brandon M. Lind

Co-authors: Leah Rettenbacher and Sally N. Aitken

The introduction of Douglas-fir (*Pseudotsuga menziesii*) to Europe in the early 1800s prior to the advent of provenance testing has led to the establishment of European lineages growing in environments both analogous and novel to those of their North American origins. Anecdotal evidence of phenotypic differences between European lineages and populations native to North America have been documented, suggesting local adaptation to European conditions may have occurred. Since this time, records of the North American origin of these lineages have been lost, though Oregon and Washington have been suspected as likely sources.

Keywords: adaptation, novel climates, genotype-environment associations, redundancy analysis

Resilience of *Picea martinezii* and *Picea mexicana* by analyzing the ring width and genome variations

Presenting author: Carlos Alberto Segura-Sanchez

Co-authors: José Villanueva-Díaz, José Ciro Hernández-Díaz, José Ángel Prieto-Ruíz and Artemio Carrillo-Parra

Dendrogenomics is a new interdisciplinary research field that combines dendrochronology, dendroecology, dendroclimatology, genetics, and genomics. This innovative approach provides a joint analysis of dendrological and genomic data, offering new opportunities to study the temporal dynamics of forest trees, describe the spatial and temporal population structure, above all,

to investigate the genetic adaptation potential of forest tree populations. The purpose of this study was to investigate differences in the resilience of rare and endemic Mexican *Picea martinezii* (10 trees) and *Picea mexicana* (14 trees) by analyzing … and … filtered SNPs from genome resequencing and … and … tree-ring width chronologies of the two species, before and after four severe drought events in the last 100 years. Significantly tree-ring width-associated SNPs ($|rs|$ = 0.51 – 0.89, $p < 0.05$) were found in several genes (in *P. martinezii*: pollen-specific leucine-rich repeat extension, isoform X1 similar to proline transporter 2, Reticulon B22, LisH/CRA/RING-U-box domains-containing protein, and Ornithine decarboxylase-like ; in *P. mexicana*: Cyclin-dependent kinase B1-1, Uncharacterized GPI-anchored At1g61900-like isoform X2, and Abscisic acid receptor PYL8-like genes). All of these genes were related to metabolic processes involved in cell wall architecture development during plant recovery processes. The results underline the strong influence of genetics on the withstanding and recovery of these spruces.

Keywords: SNP, tree-ring width chronology, recover, drought stress

Additive and non-additive variance of clones and progenies of *Eucalyptus urophylla* at early age in Tabasco, México

Presenting author: Liliana Hernández Hernández

Co-authors: Javier López Upton, Marynor Elena Ortega Ramírez, J. Jesús Vargas Hernández, Marcos Jiménez Casas and Cristiano Bueno de Moraes

Background:
Through selection processes, it has been possible to develop productive genotypes resistant to diseases, droughts and frosts in the genus Eucalyptus. The growth capacity of *Eucalyptus urophylla* has made it a species widely used in commercial plantations and genetic improvement and suitable for conducting research. Genetic parent/offspring interactions are rarely used in practice, since data from two generations is required and environmental conditions are often different. Therefore, it is relevant if parents and progeny are evaluated in the same environment. Thus, the additive and non-additive effects of the progeny are evaluated. Broad-sense heritability (H^2) indicates the genetic influence on the variation of a population considering the additive and non-additive genetic components. In contrast, narrow-sense heritability (h^2) is limited only to the additive variance and this is determined by observing the degree of similarity between relatives. In practice in forest trees the relationship between parents and offspring is not used, although data from two generations in the same environment are crucial to estimate how much of

the variation in a population is attributed to inheritance from parents and how much comes from recombination and dominance between them. Therefore, the objective of this work was to quantify the additive and non-additive variance of height and diameter of *Eucalyptus urophylla* after 24 months of plantation.

Methods:
They were established progeny and clonal trials that included 40 genotypes of *Eucalyptus urophylla* distributed in four sites of Tabasco. The genotypes were obtained through outstanding families of progeny assays and genetic material used by companies in southeastern Mexico. 22 genotypes are uncles-nephews, and 2 parent-offspring. A progeny trial and a clonal trial were established in each of the four sites, with a block design with 6 repetitions and 6 plants per plot, with a spacing of 3.8 x 2 meters. In total, each trial consisted of 1440 initially planted trees. Using approximately 965 to 1100 data points per trial.

Results:
The results presented high genetic control for progenies (h_i^2 = 0.34 and 0.38; h_f^2 = 0.80 and 0.82) and for clones (H_i^2= 0.25 and 0.22; H_c^2 =0.91 and 0.90) for total height and diameter, respectively. A non-additive variance V_na= 0.25 and 0.04 in the height and diameter were estimated, respectively. The average height was 14.9 m and diameter were 14.5 cm after 3 years of planting.

Conclusions: We conclude that it is possible to obtain significant gains through selection for height and diameter in fast-growing tropical species such as Eucalyptus. These studies will serve as a starting point in genetic improvement programs in Mexico.

Keywords: Additive variance, non-additive variance, Eucalyptus, forest plantations and heritability.

Optimizing tree improvement programs: SNP genotyping for enhanced breeding values, genetic diversity assessment, and estimation of pollen contamination

Presenting Author: Esteban Galeano

Co-authors: Eduardo Pablo Cappa, Jean Bousquet and Barb R. Thomas

Genotyping offers significant benefits in tree improvement (TI) programs, including enhancing breeding values, estimating genetic diversity, and detecting pollen contamination. Conventional methods, including pedigree-based models, are being enhanced by incorporating genomic data. The single-step GBLUP method, integrating phenotypic, pedigree, and genomic data,

excels for traits with low heritability and complex breeding programs. SNP genotyping can uncover unexpected impacts of roguing and pollen contamination. Despite its potential, few tree breeding programs fully integrate genomic data to simultaneously enhance breeding values, assess genetic diversity and pollen contamination. This study compared approaches and evaluated genetic parameters in a white spruce (Sw) breeding program. The Region I Sw TI program in central Alberta, Canada, was started in 1986. Five progeny trials were established in 2001 at five sites. The G354E site was chosen for its high survival and accessibility. A clonal seed orchard was also established in 1998. SNP genotyping was performed on samples from progeny trials, seed orchard seedlings, and parents. Pedigree-based (ABLUP), genomic-based (GBLUP), and pedigree and genomic-based (ssGBLUP) models were used to estimate breeding values and assess genetic parameters. Pollen contamination was monitored using pedigree reconstruction and pollen traps, with correlations assessed between pollen contamination levels and effective population size (Ne). ssGBLUP resulted in improved genetic parameter estimates and predicted breeding values compared to traditional ABLUP and GBLUP models. ssGBLUP led to a slight increase in heritability estimates for height and diameter compared to the ABLUP model. The accuracy of breeding values also improved with ssGBLUP. The rankings of families based on ssGBLUP identified discrepancies from ABLUP rankings. Genetic diversity remained stable, with no observed inbreeding. Pedigree reconstruction revealed consistent parental contributions across years and genotypes. Pollen contamination levels varied, showing strong correlations with Ne estimations. SNP and pollen trap methods displayed significant correlations in estimating pollen contamination levels and Ne estimations showed strong correlations among different methods. The implementation of ssGBLUP improved genetic parameter estimates and breeding value predictions for height and diameter in this Sw program. Genetic diversity in the seed orchard remained stable over eight years. Pedigree reconstruction revealed equal parental contributions within the orchard. Strong correlations were observed between pollen contamination levels and Ne estimations using molecular markers. This study underscores the efficiency of utilizing genomic tools for estimating pollen contamination, reconstructing pedigrees, and providing error correction. It advocates for continued adoption of modern approaches such as ssGBLUP in tree breeding practices.

Keywords: molecular markers, Picea glauca, tree breeding, effective population size, pollen Flow

Analyses of drought tolerance in Maritime pine using grafting as experimental system

Presenting author: Lorenzo Federico Manjarrez Rodríguez

Co-authors: Nuria de María, María Ángeles Guevara, María Dolores Vélez, José Antonio Cabezas, José Antonio Mancha, Paula Ramos, Alberto Pizarro, Endika Blanco-Urillo, Miriam López-Hinojosa, Irene Cobo-Simón, María Carmen Díaz-Sala, María Teresa Cervera.

Background:
Grafting is a propagation method widely used in fruit trees to propagate elite genotypes, enhancing their tolerance. In conifer, it is mainly used to establish seed orchards to produce high-quality seeds by grafting scions of elite trees onto unselected rootstocks. Multiple studies has pointed out grafting as a suitable system for studying the response to stresses such as drought. Conifers are a dominant component of Mediterranean dryland forests, where they are expected to become fiercer. The maritime pine (*Pinus pinaster* Ait.) is a highly valuable species, widely spread over the western Mediterranean basin, with a remarkable genetic plasticity that may enable it to thrive in very contrasting climate regions. Drought affects conifer growth, modifying wood quantity and quality, and increasing tree susceptibility to pests and pathogens. Therefore, knowing how forest trees respond to climate change disturbances is essential for selecting better-adapted trees to harmful conditions, maintaining genetic diversity to address sustainable development.

Methods:
We explored mRNA profiles from 48 stem samples of four *P. pinaster* graft constructs grown under well-water and water deficit conditions. These grafts combined four genotypes that showed contrasting response to drought in previous studies: Gal1056 (drought-sensitive) and Oria6 (drought-tolerant), used as scions and grafted onto either R1S (drought-sensitive) or R18T (drought-tolerant), used as rootstocks. The rootstock genotypes are F1 siblings from the controlled cross Gal1056 x Oria6 vegetatively propagated.

Results:
Our findings suggested that drought response in *P. pinaster* is associated with the establishment of drought tolerance mechanisms prior to water deficit, in which the provenance plays a relevant role. Thus, differences in drought tolerance strategies pointed at genetic trade-offs between growth and tree defense. Other tolerance approaches were associated with the metabolism of

compatible solutes such as starch and sucrose, the metabolisms of flavonoids, terpenoids and other ROS scavengers. In addition, our analysis of pine grafts allowed us to identify different expression patterns associated with responses to biotic and abiotic stresses as well as some metabolic pathways regulated by the interaction of genotypes in the grafts, which in turn are susceptible to be altered by water regime. Finally, these analyses allowed us to characterize differentially expressed genes that might be involve in the regulation of *P. pinaster* drought tolerance.

Conclusions:
This study could contribute to enhance our understanding of conifer adaptation to environmental stimuli, in particular drought. Furthermore, it underscores grafting is a suitable systems for study the conifer transcriptomic profiles and comprehending how different genotypes may modulate the expression of transcripts, emphasizing the importance of selection both, scion and rootstocks genotypes to achieve desirable goals.

Keywords: *Pinus pinaster*, drought tolerance, stem transcriptome, grafting

From Forest to Market: Genomic Tools for Ensuring Wood Traceability in Canada

Presenting author: Pauline Hessenauer

Co-authors: Melanie Zacharias, Julien Prunier, Sam Yeaman and Ilga Porth

The traceability of forest products is crucial for climate change mitigation, promoting sustainable forest management and preventing deforestation and illegal exploitation. Traceability systems transparently track a product's journey throughout its value chain, enhancing the credibility of forestry practices. In Canada, wood traceability ensures compliance with laws like WAPPRIITA, and forest certification standards (e.g., FSC, SFI), ensuring legality, sustainability, and social responsibility of wood products. Established in 2019, the Canadian Experts Group on Wood Identification and Traceability aims to address commercial risks associated with the importation of illegal forest products and support market access in the forestry sector. Identification methods and traceability systems include anatomical, chemical, and genomic approaches, each presenting advantages and drawbacks. This project focuses on developing analytical pipelines to determine the geographic origin of wood products using genomic datasets from Canadian commercial species, with different evolutionary histories that have shaped their current geographical structure. Comparing a set of different species will thus provide a general overview of the possibilities of assigning forest species at the national level and developing a general assignment pipeline adaptable to any tree species. The

objectives of our project are (i) to develop and test a pipeline encompassing traditional and modern artificial intelligence models for precise assignment, (ii) to compare assignment accuracy between species (iii) to provide general guidelines for naïve assignment studies. To accurately predict geographic coordinates, we develop a bioinformatic pipeline encompassing various approaches: linear model, K-nearest neighbors, gradient boosting and random forest. We test our pipeline on three conifers (black spruce, lodgepole pine and eastern white pine) and two deciduous species (trembling aspen and black cottonwood), all presenting different population genetic structures and geographic distributions, ensuring the coverage of a vast portion of Canada. These diverse genomic datasets were independently acquired, involving different sampling and sequencing techniques. This diversity ensures the versatility of the developed pipeline and opens the possibility for its application to multiple species in the future. Preliminary results show that tree species evolutionary history and population genetic structure impact assignment accuracy and minimum number of markers required for accurate assignment. Additionally, our results indicate that while conventional classification techniques offer acceptable results, machine learning algorithms can outperform them, especially with fewer markers. This study aims to provide tools and guidelines that will have a positive impact nationally and internationally by strengthening wood traceability of Canadian tree species.

Keywords: Wood traceability, genomic data, machine-learning, sustainable forestry, geographic assignment

Mobile and web applications for forest research from in field data collection to inventory and analysis.
Presenting author: Margaret Staton

Co-authors: Noah Caldwell, Chance Stribling, Ellen Crocker and Jill Wegrzyn
Background

Forest research projects frequently involve collecting digital data and/or physical samples from forest trees with professional collectors and/or by leveraging citizen scientists. An integrated mobile app and web software system can guide users to produce high quality and consistent data, speed collection efforts, and help scientists manage and filter data.

Methods:
We have developed a suite of easy-to-use mobile apps and web-based databases to guide users in collecting observational data and physical samples from forests. This talk will focus on three applications, each with a free mobile application that utilizes GPS location services for all data collection points and

functions while the user is out of cellular range by saving data locally to the phone for later upload. After upload, the data can be accessed via a web interface that provides a public data overview and a private scientist dashboard for filtering, tracking, searching, and downloading research data.

Results:
For professional, international collection of both data and physical samples, we have developed FlorestaDB. The mobile app supports multiple collectors per project with barcode scanning of samples and is available in four languages. The web application offers the ability to manage project participants, plan expeditions, track sample shipping, and manage physical sample inventories. Only authorized users can access data via an interactive dashboard. The platform currently has over 300 users, 10,000 tree observations, and 37,000 physical samples from 47 different countries.

For citizen scientists, TreeSnap focuses on providing a simple interface that guides the public in scouting for trees affected by insects and diseases, including North American ash, chestnut, hemlock, and elm. In contrast to other reporting apps, we increase the utility of the collected data for our scientist partners by asking a set of customized questions for each species, with accompanying pictures of symptoms of pests and disease. With over 2,000 active users and over 16,000 tagged trees, TreeSnap is an important resource for scientists to find lingering trees that may harbor genetic resistance.

A third software system is in development to support plot monitoring. Research for forest health often needs data taken over years to record tree survival, changes in species composition, growth rates, and more. Our app will enable scientists to develop a custom plot monitoring system and work with users to take regular data measurements.

Conclusions:
Our 8 years of experience building apps that support data collection in forests has proven that excellent, easy-to-use software paves the way for higher quality data and participant satisfaction.

Keywords: Mobile app, database, sample collection, data management, data collection

Genomic tools for breeding, conservation genomics, and pest/disease management of Christmas trees

Presenting author: Ross W Whetten

Co-authors: Adarsha Devihalli, Caleb Cothron, Sai Karthik, Will Baldwin, Priya Rajarapu, Will Kohlway, Angela Chiang, and Justin Whitehill

Background:

Fraser fir (*Abies fraseri*) is endemic to the southern Appalachian Mountains of the eastern US, where the remaining populations are isolated to mountaintops in North Carolina, Tennessee, and Virginia. The exotic insect pest, balsam woolly adelgid (*Adelges piceae*), killed over 80% of mature trees in natural stands during the 1970s. Today, these natural populations are increasingly threatened by accelerated climate change. However, Fraser fir has become the premier Christmas tree in the US over the past 40 years. Fraser fir now accounts for 38% of all live Christmas trees sold in the US, with a market value estimated to exceed one billion US dollars annually. Methods The Christmas Tree Genetics (CTG) Program at NC State University began range-wide collections from natural stands in the 1980s and has since established first generation clone banks, seed orchards, and first and second generation progeny tests. The overall goal is to improve tree growth, Christmas tree quality, needle retention, and resistance to pests and diseases. Analysis of progeny test data has identified variation among Fraser fir families for growth, quality, degree of insect infestation, and susceptibility to deer browse. The spread and severity of a root rot disease, caused by a complex of Phytophthora and other oomycete pathogens, is causing increased concern amongst Christmas tree growers while the elongate hemlock scale (*Fiorinia externa*) - an exotic insect pest – is posing challenges to commercial production. The CTG program is now beginning the second cycle of mating and progeny testing, with the goal of integrating current breeding methods to accelerate genetic improvement of this economically important and ecologically endangered forest tree.

Results:

Modern breeding methods require genome information, so we constructed draft genome assemblies of both Fraser fir and *Phytophthora cinnamomi*, the most aggressive and wide-spread root rot pathogen on Fraser fir. The Fraser fir assembly now forms the basis for population resequencing experiments aimed at development of SNP markers for breeding applications. Genome annotation is ongoing, utilizing RNA-seq data, protein alignments, and de novo gene prediction to enable functional analysis of genes involved in pest and disease resistance, enhanced needle retention, and other economically and ecologically important traits. Conclusions Genomic information for Fraser fir and an important pathogen species will be utilized for functional studies to understand mechanisms, and incorporated into breeding strategies to accelerate the pace of improvement. Resistance to root rot and insect pests varies within and among Asian and Mediterranean fir species, and a comparative biology approach will facilitate a better understanding of resistance mechanisms in these species to help integrate disease and pest resistance into Fraser fir.

Keywords: Oomycete, disease resistance, insect resistance, root rot, molecular tree breeding, climate adaptation, forest pathology

EASEL (Efficient, Accurate, Scalable Eukaryotic modeLs), a tool for the improvement of eukaryotic structural and functional genome annotation

Presenting author: Cynthia Webster

Co-authors: Karl Fetter, Sumaira Zaman, Vidya Vuruputoor, Akriti Bhattarai and Vikesh Chinta

Background:
The emergence of affordable high-throughput sequencing technologies has facilitated new ways of exploring genetic variation in forest trees. Reference genomes are increasingly accessible, providing a strong foundation for next-generation breeding efforts. Despite these advancements, an efficient and accurate workflow for structural annotation of protein coding genes remains a challenge. Existing programs struggle with predicting less common gene structures (long introns) and finding the preferred TIS location. Plant genomes are especially difficult to annotate due to their larger size, often reflected by the number of repeats, pseudogenes, and polyploidy. We present EASEL (Efficient, Accurate, Scalable Eukaryotic modeLs), a genome annotation tool that leverages machine learning, RNA folding, and functional annotations to enhance gene prediction accuracy (https://gitlab.com/PlantGenomicsLab/easel).

Methods:
EASEL aligns RNA-Seq data and assembles transcripts via StringTie2 and PsiCLASS. Complete open reading frames are then predicted through TransDecoder using a gene family database. Gene models train AUGUSTUS, and the resulting predictions (with hints) are combined into a single gene set. Implicated gene structures are filtered by primary and secondary features with a random forest algorithm and clade-specific training set. Transcripts that have a predicted TIS and which score above a certain F1 threshold are retained and functionally annotated with EnTAP. BUSCO, mono:multi ratio and reciprocal BLAST annotation rate are metrics used to assess annotation accuracy. BUSCO classifies conserved genes and overall completeness, but often detracts from issues of fragmentation. A high mono:multi ratio (>0.20) can expose this problem. Fragmented models can also be identified by the rate of reciprocal similarity search alignments against well curated databases.

Results:
The level of genomic complexity varies greatly among forest trees. Some plant species, like *Juglans cinerea* (539 Mb), have relatively compact genomes, while

others may have variable ploidy, or large genomes like *Pinus albicaulis* (28 Gb). Despite differences between *J. cinerea* and *P. albicaulis*, EASEL was able to predict between 25,000-27,000 protein-coding genes with mono:multi ratios near 0.20. While embryophyta BUSCO completeness was higher in *J. cinerea* (99.6%) than *P. albicaulis* (73.9%), gymnosperms remain underrepresented in curated databases. This also explains the variation in reciprocal BLAST annotation rates (71-95%). Although runtime is largely dependent on genome size (*J. cinerea* ~ 5 days; *P. albicaulis* ~ 1 month), EASEL yields a highly accurate annotation and summary statistics.

Conclusions:
Existing genome annotation pipelines are often complex and time consuming. By wrapping EASEL into a publicly available Nextflow workflow, we provide more opportunities to non-genomics practitioners and academics with limited resources to generate high quality genome annotations.

Keywords: structural annotation, bioinformatics, nextflow, machine learning, reference genome

Genomic linkage maps for shedding light on the rapid reproductive isolation of an *Abies* (*Pinaceae*) species pair from central Mexico

Presenting author: Xochitl Granados-Aguilar

Co-authors: Gustavo I. Giles-Pérez, Erika Aguirre-Planter, Luis E. Eguiarte and Juan Pablo Jaramillo-Correa

Background:
Conifers are reputed for their large effective population sizes, rampant gene flow among populations and slow evolutionary rates, which often translates in poorly developed interspecific boundaries. In central Mexico, there is however an example of a sympatric species pair that shows evidence of reinforcement and fast speciation (*Abies religiosa – A. flinckii*). Reproductive isolation may evolve through various mechanisms and involve either neutral or adaptive forces that operate at specific genomic regions.

Methods:
We are investigating the basis of such rapid reproductive isolation using highly saturated linkage maps. Following next-generation sequencing, we identified SNP variation at 183 megagametophytes from two mother trees and used Lep-MAP3 to obtain the first linkage map for a Mexican fir.

Results:
The concatenated map was composed of 8,114 genetic markers distributed in 12 linkage groups that spanned 1560 cMs.

Conclusions:
This map will serve as an anchor for assembling the *A. religiosa* genome and its subsequent comparison with *A. flinckii*.

Keywords: reproductive isolation, genomic resources, conifers, speciation, Trans-Mexican Volcanic Belt

Session 5: Genomics, transcriptomics, and bioinformatics

Genomics of whitebark pine

Presenting author: David B.Neale

Co-authors: Aleksey V. Zimin, Amy Meltzer, Akriti Bhattarai, Maurice Amee, Laura Figueroa Corona, Brian J. Allen, Daniela Puiu, Jessica Wright, Amanda R. De La Torre, Patrick E. McGuire, Winston Timp, Steven L. Salzberg and Jill L. Wegrzyn

Whitebark pine (*Pinus albicaulis*) is a white pine of subalpine regions in western contiguous US and Canada. Whitebark pine has become critically threatened throughout a significant part of its natural range due to mortality from the introduced fungal pathogen white pine blister rust (WPBR, *Cronartium ribicola*) and additional threats from mountain pine beetle (*Dendroctonus Ponderosae*), wildfire, and maladaptation due to changing climate. Vast acreages of whitebark pine have suffered nearly complete mortality. Our goal is to develop a genomic screening tool to identify disease-resistant and climate-adapted seed sources for restoration in a faster, more cost-effective manner than traditional phenotype-based approaches. This will require discovering individual genes determining resistance and climate adaptation using genome wide association studies (GWAS) and environmental association analysis (EAA). In advance of GWAS and EAA studies, however, a deep enumeration and annotation of genes in whitebark pine is needed. To that end, we report here the sequencing of the whitebark pine genome. With deep-coverage Illumina short-reads of haploid megametophyte tissue and Oxford Nanopore long-reads of diploid needle tissue, followed by a hybrid, multistep assembly approach, we produced a final assembly containing 27.6 Gbp of sequence in 92,740 contigs (N50 537,007 bp) and 34,716 scaffolds (N50 2.0 Gbp). Approximately 87.2% (24.0 Gbp) of total sequence was placed on the twelve chromosomes. Annotation yielded 25,362 protein-coding genes, and over 77% of the genome was characterized as repeats. Whitebark pine has demonstrated the greatest variation in resistance to WPBR among the North American white pines. Candidate genes for quantitative resistance include disease resistance genes known as nucleotide-binding leucine-rich-repeat receptors (NLRs). A combination of protein domain alignments and direct genome scanning was employed to fully describe the three subclasses of NLRs (TNL, CNL, RNL). Our high-quality reference sequence and annotation provide a marked improvement in NLR identification compared to previous assessments that leveraged de novo assembled transcriptomes.

Language models are whole genome representation learners for large and complex conifer genomes

Presenting author: Charles Chen

Background:

The language of genetic code embodies a sophisticated grammar and rich syntax of interacting molecular elements, including genomic features of coding -genes, promoters, enhancers, exons and introns, and structural variants. Recent advances in self-supervision and feature learning suggest that statistical learning techniques can identify high-quality quantitative representations from inherent semantic structure. For example, in natural language processing (NLP), large language models (LLMs) including GTP and BERT, have exhibited groundbreaking proficiency in various language tasks such as: test taking, document analysis, and sentiment classification. However, comprehensive "DNA-language learning" faces unique challenges for its simple alphabet and long-range nucleic dependencies.

Methods:

This study presents a genomic feature-based language model capable of generating whole-genome representations through leveraging natural contrastive characteristics between genotyped individuals. We develop a set-based learning objective, AB learning, that compares the annotated genomic features of two population subsets for optimization, to achieve comprehensive whole-genome representation learning. Using this foundational objective, we trained a Transformer model to backpropagate information into dense genome vector representations and challenged our model to quantify and condense the genomes of 2,054 *Pinus* contorta (lodgepole pine) individuals sequenced in the RES-FOR project (https://resfor.ualberta.ca/), using only their short read sequences.

Results:

The resulting genome embeddings produced state-of-the-art accuracy in genomic prediction for five complex agronomic traits and self-organized onto a Euclidian space that can characterize population structure and reconstruct the pedigree. The simultaneously embedded short-sequence fragments identify locus clusters associated with water use efficiency, serving a foundation for language-based association models.

Conclusions:
Our self-supervised deep-learning model demonstrates the potential of language models to capture intricate genomic features and advancing genomics research in complex organisms (e.g. conifers).

Keywords: Natural language model, Self supervision, Transformer, Genomic prediction, Genomic association

Transcriptomics of seasonal needle abscission in a deciduous conifer (*Larix laricina*) demonstrates the evolution of deciduous behavior in seed plants

Presenting author: Maurice Amee

Background:
Although the majority of gymnosperms are non-deciduous, a few species are deciduous. *Larix laricina* is one such species that loses all needles in the Fall as an adaptive mechanism to extreme cold temperatures of the winter season. This process is well characterized in angiosperms, with virtually no work done to understand the molecular changes involved in seasonal senescence in conifers. Moreover, more must be done to explain the evolutionary history of deciduous behavior especially among tree species in temperate climates. This study first examines the transcriptomic architecture of *L. laricina* to characterize changes associated with seasonal needle abscission. To explain the evolutionary history of deciduous traits, we then perform a comparative analysis of orthologues of selected genes spanning the phylogeny of seed plants.

Methods:
We collected abscission zone tissue from six *L. laricina* trees across space and time. The sampling was guided by needle coloration and presence. Following bioinformatic analyses, we conducted differential expression analysis to identify candidate genes and pathways involved in seasonal needle abscission. Finally, I performed a comparative analysis of orthologues of selected genes spanning the phylogeny of seed plants and deciduous and evergreen trees.

Results:
We identified key genes and pathways involved including response to temperature, transport, lignin biosynthesis, and programmed cell death. We are currently carrying out a comparative analysis of the differentially expressed transcription factors (DETF). Initial analysis indicates that an ethylene-responsive transcription factor 2 (ERF-2) orthologue in *L. laricina* is more closely related to and forms a separate clade with the orthologues in angiosperms as opposed to those in gymnosperms. We hypothesize that

deciduous behavior may precede separation of gymnosperms from angiosperms and the trait may have been lost in the majority of gymnosperms over their evolutionary history.

Conclusions:
This study demonstrates the molecular mechanisms of seasonal needle abscission in a deciduous conifer using transcriptomics data and draws into a possible evolutionary history of deciduous behavior in temperate trees. First, we use molecular data to generate significant knowledge on the fundamental biology question of how deciduous trees respond to winter conditions. Despite the lack of a reference genome, we were able to assemble a reference transcriptome and carry out differential gene expression analysis, identifying key genes and pathways responsive to the environmental cues in the early Fall. Beyond the response mechanisms, we trace the evolutionary history of deciduous traits among trees in temperate climates.

Keywords: Deciduous, Transcriptomics, Larch, Evolution, Abscission

Phylogenetic based method reveals the genetic basis of cold adaptation in maples

Presenting author: Mengmeng Lu

Co-authors: Nathan G. Swenson

Maple (*Acer*) is a widespread and diverse genus in Northern Hemisphere with most species native to Asia. Maple species inhabit a wide range of climate zones, yet their genetic basis of climate adaptation is largely unknown.

In this study, we used the leaf RNA-seq samples of 29 maple species including both subtropical and temperate species to reconstruct the phylogeny and calculate the phylogenetic signals in the median climate variables within their natural distribution using two measures, Blomberg's K and Pagel's λ We correlated phylogenetic distance with Euclidean distance in climate variables between the pairwise studied species. To study the selective forces and genes that underlie climate adaption, we correlated the median values of selection strength and climate variables across the studied species and identified genes showing signals of positive selection, intensified selection, and relaxed selection, respectively.

Using 211 one-to-one ortholog groups derived from transcripts, we resolved the phylogeny amongst the studied species. We found that significant phylogenetic signals existed in minimum temperature of cold months, suggesting phylogenetic niche conservatism of cold tolerance. Cold adaption is correlated

with relaxation of selection in genes involved in heat response. Sugar accumulation and transport genes are enriched in intensified and positive selection genes.

Our study highlights a phylogenetic comparative approach to study the adaptive evolution of biological groups. The results suggest that cold tolerance and niche conservatism are two factors that shape the distribution in the studied maple species, and relaxation of selection and positive selection drive cold adaption in maples.

Keywords: Acer, Cold adaption, Phylogenetic niche conservatism, Relaxed selection, Positive selection

Day 3: Thursday, June 13

Session 6: Phylogenomics, phylogeography, and demographic history

Rapid differentiation, ecological stasis, and edaphic adaptation during the temperate to tropical transition in Mexican firs

Presenting author: Juan P. Jaramillo-Correa

Co-authors: Jorge Cruz-Nicolás, Sebastián Arenas and Gustavo I. Giles-Pérez

Understanding and disentangling the main drivers of local adaptation within and between species is a pre-requisite of any forest breeding, management, and conservation plan. Insights from past adaptive events may indeed help predicting future evolutionary trends and fine-tunning specific management actions. However, differentiating between adaptive and non-adaptive processes may be complex, particularly for long-lived and slowly-evolving species, like conifers. We explored which evolutionary processes occurred during the migration and diversification of firs (*Abies*, *Pinaceae*) between the Southwestern United States and Central America at various geographic and evolutionary scales. Using genomic data, we inferred a well-resolved phylogeny that showed four main fir lineages that fitted a North-South isolation by distance framework. Such lineages further had diminishing values of Ka/Ks towards the Equator, indicating reduced purifying selection efficiency. Groups showed a strong phylogenetic signal for morphological and climate variation, suggesting a random walk model of differentiation. However, early adaptation to tropical conditions was inferred in the ancestor of the southernmost firs, as all modern southern taxa were climatically differentiated from the northernmost species (*A. concolor*). Interestingly, autapomorphic traits were observed for soil properties, suggesting possible species-specific adaptations. We explored such adaptations in *A. religiosa* at the range-wide and local (within-population) scales. Genome-soil association analyses revealed a series of candidates correlated to various soil traits. Multilocus clines indicated a significant contribution of geographic proximity to both soil and candidate gene variation, which is consistent with the geological history of the mountains where this species is distributed. Altogether, our results show that a combination of non-adaptive and adaptive processes operated along different environmental and phenotypic axes during the southward migration of this plant lineage from North America, and its subsequent radiation in the Neotropics. Looking beyond climate change is thus advised when planning future forest management plans. For instance, integrating soil variation and the accumulation of partially deleterious variants should be a priority for such plans.

Differentiation of high altitude Mexican white pines

Presenting author: Alejandra Moreno-Letelier

Co-authors: Alfredo Ortíz-Martínez

Background:
High-altitude Mexican white pines (*Pinus strobiformis*, *Pinus ayacahuite* and *Pinus veitchii*) form a species complex with a contact zone in the Trans-Mexican Volcanic Belt. This has led to unstable taxonomic circumscription, which has led to problems in adequately assessing the conservation status and genetic diversity of these populations. In particular, the taxonomic status and number of populations of *P. veitchii* is contested, leaving it out of most endangered conifer lists despite being under extreme anthropogenic threats.

Methods:
To clarify species boundaries, we used morphological variables and genetic differentiation using SNPs of populations of white pines in the contact zone and adjacent areas.

Results:
Our results show that *P. veitchii* is morphologically similar to *P. strobiformis*, but has a significant level of admixture with *P. ayacahuite* in some of its populations, whilst exhibiting genetic differentiation.

Conclusions:
Both lines of evidence show conflicting results possible due to different evolutionary processes, but indicate the existence of *P. veitchii* as an independent lineage which requires its own monitoring and conservation program.

Keywords: white pines, species delimitation, conservation genetics, SNPs, multivariate methods

Taxonomic revision of *Pinus* subsections *Ponderosae* and *Sabinianae* (*Pinaceae*)

Presenting author: Ann Willyard

Co-authors: David S. Gernandt

Background:
We are writing a taxonomic revision of the important group of pine populations in subsections *Ponderosae* and *Sabinianae* that are native to southern British Columbia, the western United States, Mexico, and northern Central America.

Many of these taxa are considered taxonomically difficult, with nearly 300 species names and different combinations of infraspecific names published for what most botanists currently think are about 25 diagnosable taxa! While molecular phylogenetic results have resolved deeper relationships, incomplete lineage sorting and organelle capture (both plastid and mitochondrial) prevent many delineations at the species level. There are no floras that reliably diagnose many of these taxa across their geographic ranges using morphological characters, especially those that span national borders. This is an urgent issue because a continuing stream of useful research is being conducted, with many experiments based on an assumption that a "species" such as *Pinus* ponderosa (including populations east of the Great Basin) has been established as a biologically meaningful and morphologically diagnosable category. In fact, nuclear molecular evidence has shown that *Pinus* ponderosa is paraphyletic.

Methods:
We are identifying morphological traits that support resurrecting a few species and support some infraspecific taxa. Some specimens housed in herbaria and frequently cited by researchers were likely misidentified, and many of the oversimplifications of characters that are used in older flora are misleading. We are using fresh morphological observations of our own collections and over 6000 specimens stored in herbaria around the world for which high-quality images are now available. An important improvement will be describing the developmental stage of many structures, e.g., the length and color of fascicle sheaths when first formed compared to when the leaves are more mature. Another important aspect is noting the variability of some characters within some taxa.

Results:
We have created a list of the collections that we have examined, with cross-references to the taxonomic names used by historic experiments (e.g., common garden trials) and other frequently-used publications that cited the same collections under different taxonomic names. This list includes newly georeferenced coordinates for many of the older collections. Morphological characters have been identified and suitable photos selected to illustrate their shape and color.

Conclusions:
We plan to make this paper available later this year as a bilingual English-Spanish manuscript. It will include an annotated appendix of over 6000 collections and the herbaria where these specimens are housed and their images hosted. The appendix has geographic coordinates that will allow readers to easily map the collection sites for each taxon.

Keywords: ponderosa pine, taxonomy

Session 7: Forest Health

A plan to make American chestnut hybrid breeding work better

Presenting author: Jared Westbrook

Co-authors: Sara Fitzsimmons, Vasiliy Lakoba, Alexander Sandercock and Jason Holliday

Background:
The introduction of the pathogens that cause chestnut blight and phytophthora root rot (PRR) from Asia killed billions of American chestnuts in the last century. Today, American chestnuts persist as stump sprouts, but rarely reproduce. For 40 years, The American Chestnut Foundation (TACF) has backcrossed American x Chinese chestnut hybrids to American chestnuts to generate populations with improved disease resistance and > 95% American chestnut ancestry. The backcross program was based on the hypothesis that blight resistance is controlled by a few large effect genes. We now know that blight resistance is controlled by hundreds of genes. To increase blight resistance to levels sufficient for long-term survival, it will be necessary to generate hybrids that inherited greater fractions of their genome from Chinese chestnut without sacrificing the competitive growth potential of pre-blight American chestnuts.

Methods:
A two-generation plan will be presented to generate hybrids that inherited > 70% of their genome from American chestnut while inheriting intermediate to high levels of blight and PRR resistance. Key components of the plan are 1. regionalization of the breeding program to represent climate adaptive diversity from American chestnut, 2. controlled pollinations between parents that meet thresholds for disease resistance and American chestnut ancestry, and 3. genomic selection of progeny. To select parents, the long-term blight resistance of 5,530 hybrids was assessed at ~100 orchard locations. To assess American chestnut ancestry and estimate genomic breeding values, genotyping-by-sequencing was performed on 5,597 hybrids. A combination of progeny testing and genomic prediction were used to assess PRR resistance. Based on these data, thousands of potential crosses were selected where the average American chestnut ancestry of the parents exceeded 70%, blight resistance exceeded 40, and PRR resistance exceeded 25 on a 0 – American chestnut to 100 – Chinese chestnut scale. Genotypes of 6,000 progeny from 60 controlled crosses were simulated over two generations. Gains in disease resistance were predicted with a genomic selection intensity of ~10%.

Results:

Blight resistance of first-generation selections was predicted to vary from 40 to 57, PRR resistance from 25 to 55, and American chestnut ancestry from 70% to 81%. Second-generation selections were predicted to have blight resistance that varied from 50 to 75, PRR resistance that varied from 40 to 63, and American chestnut ancestry that varied from 70% to 89%.

Conclusions:

Controlled crosses and genomic selection are necessary steps to get the desired gains in disease resistance without increasing Chinese chestnut ancestry. If we take these steps, the hybrid breeding program has the potential to produce trees with sufficient disease resistance and competitive ability to resume reproducing in eastern forests.

Keywords: genomic selection, hybridization, tree breeding, chestnut blight, phytophthora root rot

Decodifying the quantitative resistance on White Pine Blister rust defense, through transcriptomics and epigenomics

Presenting author: Laura Figueroa-Corona

Co-authors: Akriti Bhattarai, Kailey Baesen, Angelia Kegley, Richard Sniezko, Jill Wegrzyn, Aleksey Zimin, Amanda De la Torre

Background: White pine blister rust is caused by the exotic pathogen Cronartium ribicola, affecting the North American five-needle pines with different degrees of susceptibility. In this study, we identified the quantitative resistance responses to this infection in sugar pine (P. lambertiana). Transcriptomic and epigenomic responses to fungal inoculation was evaluated in two year-old seedlings after four days of pathogen inoculation. Methods: Through the new genome version sequencing from haploid megametophyte tissue ~32 Gbp of sequence placed on the twelve chromosomes we characterized distinct profiles of transcription from 120 individuals before, 24h after and 96h after the infection. In addition to complete the genome dynamics the methylation patterns were obtained via Whole Genome Bisulfite Sequencing for three individuals characterizing the infection before and 96h after the inoculation. Results: Comparing the transcriptomic pattern before and 24h after the inoculation, 384 differential expression genes detection changes over the chitins catabolism and endochitinases signaling. After 96hrs inoculation, inner the 681 differential expression genes the intermediates to response to stress like jasmonic acid biosynthesis and flavonoid biosynthesis began to upregulated their transcription. Comparisons among susceptible and quantitative phenotypes after 24h of infection showed 492 DE genes were the susceptible individuals showed down-regulation of intermediates in the response to jasmonic acid and the terpen

biosynthesis. The methylation changes detected showed that quantitative resistance individuals had an increment from 60 to 70% of CG and CHG methylation types over chromosomes 6 and 10. In general, quantitative resistant individuals showed an increment of methylation in the DE set of genes after 96hrs. Conclusions: Our research sheds light on the first stage of infection and emergence of disease symptoms among sugar pine seedlings encoding the hypersensitive response. Cell wall modification, oxidative regulation signaling, programmed cell death, and plant innate immunity were all differentially expressed during the defense response against C. ribicola.

Tree Breeding including seed orchard management, progeny testing, Population and community ecology

Presenting author: Jose Alberto Ponce Figueroa

Co-authors: Christian Anton Wehenkel, Artemio Carrillo Parra, José Ciro Hernández Díaz, José Ángel Prieto Ruíz

This study focuses on the survival and development of seedlings from five species of Pinus and their hybrids under field conditions, emphasizing the influence of specific morphological characteristics such as root collar diameter (RCD) and height (H) prior to planting. It was conducted at two test sites in the Sierra Madre Occidental, Durango, Mexico, guided by the hypothesis that seedlings with a larger RCD in the nursery would have a higher probability of survival and growth when transplanted to the field.

Through a detailed statistical analysis, the study identified weak to moderate but significant correlations between RCD and height prior to planting and their respective measurements three years later, although no significant association was found between RCD and the probability of survival three years after planting. However, a positive correlation was observed between the height of the seedlings before planting and survival, suggesting the importance of the latter as a more reliable predictive indicator of survival under field conditions.

The results underline the need to consider a wider spectrum of factors, including genetic and environmental ones, in future research to improve forest management strategies. These findings point to the complexity of interactions between morphological, genetic, and environmental characteristics in the success of reforestations, highlighting that the survival of seedlings in natural conditions can be influenced by a variety of factors.

Keywords: pinus, reforestation

Selection of progenies of *Pinus caribaea* var. *bahamensis* for the production and quality of gum resin

Presenting author: Diego Torres Dini

Co-authors: Jefferson Gomes Lima, Osmar Vilas Boas, José de Arimatéia Rabelo Machado, Miguel Luiz Menezes Freitas, Marina Moura Morales and Ananda Virgínia de Aguiar

Tropical pine species such as *Pinus caribaea* var. *bahamensis* have adapted well to Brazil's tropical climate regions, such as the Southeastern region of the São Paulo state. The propose this study was to characterize the resin of *P. caribaea* var. *bahamensis* to identify superior progenies to commercial planting. The progeny test open-pollinated in the municipality of Assis, São Paulo, state in 1988. It was set up in a randomized block design, with 72 progenies, one plant per plot, and 28 repetitions. The spacing 6 x 6 meters. The Selegen program was used to analyze the data. Among the 72 progenies analyzed, the average production ranged from 6.48 kg. arv. -1 to 2,57 kg. arv. -1. Rosin and turpentine varied from 74.6% to 95% and 25% to 5%, respectively. Variations in turpentine, alpha-pinene, camphene, beta-pinene and total bicyclics for the 212 most productive trees ranged from 29.80% to 84.92%; 0.68% to 2.29%; 2.43% to 44.42%; 34.12% to 116.56%, respectively. This population shows genetic variation for resin characteristics that can be exploited to select individuals for commercial plantations.

Keywords: alpha-pinene, resin production, resin quality, tropical pine, turpentine

Cloning of *Pinus patula* through rooted cuttings: effect of stock plant etiolation and immersion time in a 5000 ppm IBA solution

Presenting author: Georgina Irasema Bautista Ojeda

Co-authors: J. Jesús Vargas Hernández, Marcos Jiménez Casas, Arnulfo Aldrete, María Cristina Guadalupe López Peralta, F. Victor Conde Martínez and Hilda Araceli Zavaleta Mancera.

Rooted cuttings is the most common macro-propagation technique used for mass production of clonal material for operational forest plantations. However, conifers have a low capacity to form adventitious roots. In a first trial, the effect of different immersion times (0, 15, 30, 60,120 and 240 seconds) of the base of the cuttings taken from 11-month-old *Pinus patula* mother plants, in a 5000-ppm liquid solution of indole-butyric acid (IBA), on their rooting capacity, was

evaluated. The trial was established in a rooting chamber with relative humidity between 85 and 100% and temperature between 20 and 24 °C. After 12 weeks, callus (CP) and rooting (RP) percentage, number of primary roots (NPR), length of the longest root (LR), and presence of secondary roots (PSR) were evaluated. Additionally, the early morpho-anatomical changes related to rooting formation were characterized. Significant effects ($p \leq 0.05$) of immersion treatments were found for RP, NPR, LR and PSR. Cutting survival was greater than 98% in all treatments; immersion in IBA solution for 120 seconds (2 min) produced the highest rooting percentage (83.6%), but the longest primary roots (up to 10.9 cm) were reached when IBA was applied for 240 seconds. Immersion times for 30 seconds or more in the IBA solution produced more primary roots; however, the presence of secondary roots was higher (76.6% of rooted cuttings) in the control treatment. In a second trial, 14-month-old stock-plant etiolation for three and six days, alone and combined with two-minutes immersion in a 5000 ppm of IBA solution, was evaluated. The highest rooting percentage was 64.4 in the treatment with six-days etiolation combined with IBA application. Etiolation causes differences in the percentage of rooting and changes in the anatomy of the cuttings. Morpho-anatomical analyzes show that adventitious roots in *Pinus patula* cuttings come from cells at the vascular cambium.

Keywords: adventitious roots, auxins, clonal forestry, etiolation, vegetative propagation.

Session 8: Quantitative genetics, tree breeding and forest management

Tree genetic improvement methods and social acceptability

Presenting author: Carsten Kuelheim

Co-authors: Swapan Chakrabarty, Zoe Ketola, Chelsea Schelly and Mark Rouleau

Genetic improvement of trees is an important tool for increasing the productivity and quality of forest products. Climate change may further endanger existing forest ecosystems through increases in frequency and intensity of biotic and abiotic stress events leading to reduced productivity, services, and carbon capture. Over the past 50 years, methods to improve trees have changed greatly. Additionally, improved trees may be perceived differently by society and many forest owners may be reluctant to utilize genetically manipulated trees. We problematize the idea of "social acceptance" when detached from the realities of adoption, implementation, and decision-making among relevant populations.

We first analyzed trends in tree improvement methods from 1990-2021 by analyzing over 1,500 scholarly publications that investigated tree improvements of any kind. Each paper was categorized by improvement method, tree species, species group, improvement target, and experimental approaches. We compared trends over time and relationships between the different categories. Next, we designed and conducted a survey that was sent to 1,500 forest landowners in Michigan, Minnesota, and Wisconsin to assess their perceptions of tree improvement methods and stated willingness of planting improved trees. The survey asked about their risk and benefit perceptions associated with levels of change in tree germplasm, including assisted migration scenarios, planting trees improved through classical breeding, marker-assisted or genomic selection breeding, or genetic modification, and their perceptions regarding example tree improvement scenarios.

We found that higher adaptability, productivity, and quality are the main objectives of tree improvement throughout time. Growth, product quality, and stress tolerance related traits are considered the most important. With the advent and development of state-of-the-art genetics, genomics, bioinformatics, and artificial intelligence tools, tree improvement has become more precise and rapid. Genomic selection and genetic modification of non-model tree species are more frequently adopted. The landowner survey response rate was 10%,

with respondents indicating the most concern about the impact of improved trees on their regional forests. Respondents perceived improvement methods like natural breeding and marker-assisted breeding to have lower risks than genetic manipulation.

Our analysis provides future directions and trends for selecting more efficient tree improvement techniques through a step-by-step selection process of the most effective method depending on species, target trait, and available resources. We conclude that tree improvement implementation can be limited by social acceptability from landowners and that communication is an important first step to allow informed decisions by landowners.

Keywords: tree breeding, genomic selection, genetic engineering, social acceptability of genetic modification

A drone's eye view of adaptive traits: multispectral vegetation indices from drone remote sensing quantify adaptive clines and hybrid status in provenance tests of *Picea englemannii*

Presenting author: Samuel Grubinger

Co-authors: Nicholas C. Coops, Gregory A. O'Neill, Jonathan C. Degner, Tongli Wang, Olivia J. M. Waite, José Riofrío and Tiziana L. Koch

Background:
The management of forest genetics is increasingly prioritizing climate adaptation and biodiversity conservation in the face of rapid environmental change. To identify uniquely adapted populations and select genotypes for future climates, it is crucial to link phenotypes, functional traits, and genetic architecture. Traits related to foliar structure, photosynthetic and photoprotective pigments, and waxes are important components of climate adaptation and have spectral signatures that can be quantified with drone remote sensing. Furthermore, seasonal changes in these traits can be assessed with repeated drone acquisitions to reveal adaptive differences in phenology.
Methods: We used multitemporal drone remote sensing and multispectral imaging in a provenance trial of interior spruce (*Picea engelmannii x glauca*) to assess patterns in multispectral vegetation indices over three years for 1363 individual trees from 88 populations from across western North America. First, we analyzed clines in multispectral vegetation indices for geoclimatic variables to detect signals of local adaptation. Second, we performed hierarchical clustering of populations by spectral traits to identify adaptively similar groups. We quantified mid-summer, late-summer, and late-winter

multispectral vegetation indices for each population and quantified changes in these indices from winter to summer and from mid summer to late summer.

Results:
Principal components of spectral trait variation described leaf-area greenness, the magnitude of winter-to-summer green-up, and late-summer decline in the red edge. We detected significant (P < .001) patterns of local adaptation in spectral traits to elevation, summer temperature, winter temperature, frost, snow, and aridity. Hierarchical clustering of these principal components identified eight geographically and climatically distinct clusters. These clusters distinguished major patterns in hybridization: allopatric P. glauca, allopatric *P. engelmannii*, a *P. sitechensis x glauca* introgression zone on the coast, and the P*. engelmannii x glauca* hybrid zone in interior British Columbia.

Conclusions:
Our findings suggest that seasonal dynamics in the spectral reflectance of vegetation in forest trees can identify important patterns in hybridization and adaptation to climate. We propose a framework for understanding functional traits through drone remote sensing and genetic correlations, leveraging data from mature common-garden trials and new analyses. We also discuss physiological and phenological interpretations of multispectral vegetation indices related to biomass allocation, nitrogen storage, and drought tolerance. Phenotyping using multitemporal drone remote sensing has the potential to bridge the gap between genomics and field assessments of common-garden trials to facilitate adaptation-focused selection of genotypes for future climates and genetic conservation.

Keywords: drone remote sensing, multispectral, phenology, adaptation, provenance testing

Sources of variance could tell us the chronicle of a death foretold by ozone pollution in a periurban forest of Mexico City

Presenting author: Verónica Reyes-Galindo

Co-authors: Verónica Reyes-Galindo, Juan Pablo Jaramillo-Correa, Ricardo Torres-Jardón, Eduardo Figueroa del Olmo, Alejandra Elizabeth De la Rosa González, Billy Emmanuel Ramírez Morales, César Zamora, Claudio Zamora Callejas, David Flores Flores, Karina Carrasco Nava, Luis Alberto Monroy de la Rosa, María del Pilar Rodríguez, Mauricio Martínez, Maurilio Salazar Zamora, Miguel Angel Morelos Zamora, Rafael Zamora Callejas, Oliver Tanui Ramírez Morales, Tonatiuh Zamora, Anabel Domínguez Reyes, Víctor Manuel

Domínguez Reyes, Jesus Gabriel Del Villar Caudillo, Estela Sandoval-Zapotitla, Omar Santiago Clemente, Rodriguez Salas Johana Valeria Amairan and Alicia Mastretta-Yanes.

Tropospheric ozone (O3) pollution is a major problem in the peripheral mountains southwest of Mexico City (CDMX); this pollutant is produced in high amounts because of the elevated number of cars in the city, and its effect is amplified by the topographic characteristics of its surrounding landscape. Such elevated levels of ozone have detrimental effects in plants, including needle reddening and diminished retention time. This decreases the overall plant vigor and increases their vulnerability to pests. Differences in symptom severity are however evident in sacred firs from these forests. Such differences translate in the contrasting expression of genes involved in carbohydrate metabolism, plant defense and gene regulation. However, the underlying cause of this differential expression still remains unclear. To delve deeper, we performed a large-scale phenotyping of 1,765 trees in the most affected area through a participatory monitoring with the local community. About 35% of the trees surveyed showed ozone damage symptoms, which were more pronounced in the older individuals ($p < 0.0001$) and apparently affected height-growth (R2c = 0.43, R2m = 0.27). A controlled experiment in open-top fumigation chambers with 3-year-old trees exposed to varying ozone concentrations (170-210 ppb, 0-50 ppb, and pure air) for three months revealed important phenotypic differences between treatments, particularly for plant height and diameter, needle anatomical damage, and photosynthetic rate. We are currently looking for genetic and epigenetic differences between symptomatic and asymptomatic individuals using GBS and EpiGBS analysis. We aim to elucidate the diverse mechanisms underlying ozone tolerance. The identification of ozone-tolerant individuals from the nursery stages can be a valuable tool for reforestation and forest restoration programs.

Keywords: *Abies religiosa*, ozone pollution, participatory monitoring, tolerant trees, controlled experiments.

Association between dioecy and edaphoclimatic factors in Mexican populations of *Populus tremuloides* Michx

Presenting author: Javier Hernández-Velasco

Co-authors: J. Ciro Hernández-Díaz, Matthias Fladung, J. Jesús Vargas-Hernández and Christian Wehenkel

Background:
Throughout its range, which extends from northern Canada to central Mexico, the species *Populus tremuloides* Michx. (quaking aspen) established in diverse

microhabitats, which has allowed it to implement diverse evolutionary and ecological mechanisms, to the point of being considered an extraordinarily "successful" species. However, these mechanisms have been little explored in Mexican aspen populations. Methods: We used data from 80 natural populations of *P. tremuloides* distributed in the main Mexican mountain systems (Sierra Madre Oriental and Occidental, Sierra de San Pedro Mártir and the Transmexican Volcanic Belt), to examine the association of dioecy (male/female gender ratio) with bioclimatic and soil variables. Gender assignment focused on detecting the Potri.019G047300 ("TOZ19") gen, which only occurs in male individuals of *P. tremuloides*. Results: It was found that the variables degrees-day below 0 °C (based on mean monthly temperature (dd0)), winter precipitation (winp) and soil sodium (Na), showed a higher influence on the distribution of sex ratio in the *P. tremuloides* populations under study. Conclusions: This result allows us to establish that sex ratios are associated to a greater extent with the occupation of different microhabitats, which results in differential resource acquisition due to differential reproductive costs incurred by the sexes, as has been reported for other dioecious species.

Keywords: SNPs, quaking aspen, evolutionary mechanisms, Potri.019G047300 ("TOZ19") gene

Tree improvement and genetic diversity of *Pinus patula*

Presenting author: Daniel Alejandro Rojas Hernandez

Co-authors: Andrea Giraldo, Carlos Rivera, Andrés J. Cortés, Esteban Galeano and Jorge D. Zea-Camaño

Background:
Pinus patula is a pantropical conifer species and an endemic pine tree of Mexico. So far, the tree improvement of this species has been stagnant in different Latin American countries for the last three decades. Therefore, it is essential to know the genetic relatedness (co-ancestry) among 47 *Pinus patula* individuals, and their genetic population structure of a stand belonging to a Colombian Forestry Company. Since all trees belong to the same population within 1 km2 (considered as a "seed orchard"), the project could potentially find low genetic diversity and some sort of inbreeding and LD.

Methods:
Fresh needles from 23-year-old *Pinus patula* trees were collected, in the Colombian tree stand (GUA033), for further DNA extraction of the 47 samples. Moreover, 5 samples of other stands were included as controls. Then, the Arbor Daicel company genotyped those samples using previous premanufactured targeted SNPs (D10101Pine3, custom myBaits 1-20K). We expected to obtain 2-8 million reads per

sample and between 5,000-10,000 informative SNPs after filtering, as done by Pelaez et al. (2020).

Preliminary results:
We obtain from 1.8-5.3 million read pairs. The percentage of duplicates ranged from 11 to 30 %, with a % GC between 38-40 %. The mean quality score was above 35, considered as adequate quality.

Expected results:
(1) Superior mothers of GUA033 will be suggested based on phenotypic and genetic data to subsequently guide a thinning, (2) the degree of inbreeding will be estimated in GUA033, configuring the baseline for the improvement program, (3) the population structure of the species will be better known by comparing the results with studies from the diversity center in Mexico, and (4) added value can be given to the seed coming from the stand, as it will have the necessary traceability.

References:
Peláez, P., A. Ortiz-Martínez, L. Figueroa-Corona, J. R. Montes, and D. S. Gernandt. 2020. Population structure, diversifying selection, and local adaptation in *Pinus patula*. American Journal of Botany. 107(11): 1555–1566.

Keywords: breeding program, seed orchard, pedigree reconstruction, genotyping, SNP marker

Session 9: Phylogenomics, phylogeography and demographic history

Phylogeography of the *Juniperus deppeana* complex: speciation beyond morphology

Presenting author: Rodrigo Martínez de León

Co-authors: Alejandra Moreno-Letelier, David S. Gernandt and Juan Pablo

The most recent glacial cycles of the Pleistocene affected the distribution, population sizes, and levels of genetic structure of temperate forest species in the main Mexican mountain systems. While the speciation process in tree species is not enough characterized, many research lines of investigation suggest a mayor role in adaptation process, specially to climatic conditions. The combination of climatic isolations and a complex topography make the Mexican highlands the prefect scenario for the study of climatic driven diversification. In this work we studied the genetic diversity of *Juniperus deppeana*, a conifer distributed from the Southwestern United States to the highlands of Central America. We used SNP data retrieved from GBS sequencing to infer phylogeographic structure, genetic diversity and demographic changes. We also characterized the climatic niche for each variety to infer the plausible area of suitability during past climatic conditions and to evaluate climatic niche discontinuities along with the species distribution. We found a marked genetic structure consisting of four different groups. Each corresponding to one of the mayor mountain systems across the Mexican highlands. According to our results the Chihuahuan desert, as well as the Tehuantepec Isthmus represent the main geographical barriers to gene flow in this species complex. Moreover, the climatic data suggest correspondence between genetic groups and climatic identities. So far our results support the idea of *Juniperus deppana* comprising a species complex with at least 4 different independent lineages. Nevertheless, it is possible that climatic conditions influenced this divergence. Therefor, future directions in this work will focus on the time of divergence and the characterization of candidate genes for the suggested adaptation process.

Keywords: Population genetics, temperate forest, xeric shrubland

Historical demography of *Populus tremuloides* in Mexico by genomic analysis

Presenting author: Cecilia Nataly Gutiérrez Contreras

Co-authors: Ilga Mercedes Porth, José Ángel Prieto Ruíz, José Ciro Hernández Díaz, Marcelo Barraza Salas and Marín Pompa García

Studying the demographic history of trembling aspen (*Populus tremuloides*), which occurs from northern Canada to central Mexico, can help understand how its populations have changed over time in terms of size and distribution, and shed light on the species' adaptability to significant climatic changes throughout history and into the future.

Our study was based on the investigation of Mexican populations of *P. tremuloides*, which were categorized into four metapopulations: Baja California, Sierra Madre Occidental, Sierra Madre Oriental and the Trans-Mexican Volcanic Belt. The analysis was carried out by about 36,810 filtered SNPs from genome resequencing of 520 individuals and with the help of tools SMC++ (Sequential Markovian Coalescent), MSMC2 (Multiple Sequential Markovian Coalescent) and Stairwayplot.

Results derived from the use of SMC++ indicate changes in effective population size (Ne) in response to climatic events dating back to 150,000 years ago, spanning the present interglacial period and the last glacial and interglacial periods. Likewise, decreases are recorded in the Sierra Madre Occidental approximately 70,000 years ago; in the Sierra Madre Oriental, they are detected between 80,000 and 100,000 years; in the Trans-Mexican Volcanic Belt, between 60,000 and 80,000 years; while in Baja California, they are observed in the periods of 60,000, 70,000, and 90,000 years. These cold and dry periods coincide in North America had a positive effect on the Ne in Mexico. However, the species has always been rare in Mexico since that time. The analyses with MSMC2 and Stairwayplot showed no correlation between the oscillations of Ne and the climate events up to 150,000 years ago. Only in the case of the Sierra Madre Occidental did Stairwayplot detect a decrease in Ne between 50 and 100 thousand years. The species was also very likely present in Mexico during the even warmer interglacial period 130000 years ago. Using SNPs and SMC+, we have finally succeeded in reconstructing the historical demography of *P. tremuloides* in Mexico up to 150,000 years ago, which is consistent with the North American climatic periods.

Keywords: Demographic history, *P. tremuloides*, SNP´s

Evolution and Ecology of Tropical Pines
Presenting author: Valentina Cardona Velasquez

Co-authors: Daniel Alejando Rojas Hernandez, Esteban Galeano Gomez and Andres Javier Cortes Vera

Tropical pines dominate the plantation forests of South America and Africa. These species have a wide range of uses, from timber production, reforestation of degraded areas and non-timber forest product. However, since the early 1980s, academy and industry mainly investigated the genetics of timber yield traits. Therefore, exploring the basis of tropical pine population genetics would be relevant for use in conservation and forest tree improvement. In this study, we conducted a systematic review of the population genetics of five species of the genus *Pinus* - *P.patula*, *P.oocarpa*, *P.tecunumanii*, *P.caribaea* and *P.maximinoii* - distributed in Mexico, Central America and the Caribbean. Our objective was to identify and understand the sources of genetic variation in the species. Through a systematic review of five databases, we analyzed the phylogeny, ecology, geographic distribution, patterns of variation according to current and future environmental conditions, genome, and genetic structure of the species. In general, the species evaluated have strong interspecific differentiation, large genomes, abundant repeated regions, and high genetic diversity. Their natural distribution is in the tropical and subtropical zones with the highest species richness of the genus *Pinus*, shorter divergence time than template pine zones, and high climatic and geographic variability, which favors local adaptation. On the other hand, in the natural populations is evidence of low inbreeding and greater genetic variability within populations than between populations, indicating high gene flow. Despite the lack of information on the complete pine genome, pine genetics knowledge evolved according to the development of genetic markers, from allozymes and isoenzymes, RAPD, SSR, pDNA, nDNA to SNPs. From a broad perspective, this review analyzes the evolutionary history of tropical pines and identifies the current state of genetic diversity of natural populations, and variations that are novel in terms of diversity, adaptability to climate change and response to pathogens. Therefore, these efforts will allow to continue genetic selection schemes with tools from genomics, that can be able to respond to new environments or industry challenges.

Keywords: local adaptation, phylogenetic, population genetic, tropical pines, diversity

Presentation Details

Poster Abstracts

Bursera linanoe population structure

Iris Jacaranda Cruz-Larios

Bursera linanoe (*Burseraceae*) is an endemic species of the seasonal dry tropical forest and has great cultural importance in Mexico. Its wood and essential oils are used to make the well known Olinalá boxes. The exploitation is artisanal and does not implement any management methods. This has negatively impacted natural populations of this tree and the entire plant community. Therefore, it is important to evaluate the diversity and genetic structure of *B. linanoe* in order to provide tools for policy design and conservation. We genotyped 92 individuals from 4 populations using ISSR markers to determine genetic diversity, isolation by distance and genetic structure and connectivity. Our results show a relatively high genetic diversity that is similar across population, regardless of conservation status. Most of the variation is found within populations, but it shows a moderate pattern of isolation by distance, and a marked population structure between the population between Oaxaca and the rest of the populations. Our results highlight the need to consider population structure to define management plans in order to maintain the local variation and genetic connectivity.

Resilience of *Picea martinezii* and *Picea mexicana* by analyzing the ring width and genome variations

Carlos Alberto Segura-Sanchez

Dendrogenomics is a new interdisciplinary research field that combines dendrochronology, dendroecology, dendroclimatology, genetics, and genomics. This innovative approach provides a joint analysis of dendrological and genomic data, offering new opportunities to study the temporal dynamics of forest trees, describe the spatial and temporal population structure, above all, to investigate the genetic adaptation potential of forest tree populations.. The purpose of this study was to investigate differences in the resilience of rare and endemic Mexican *Picea martinezii* (10 trees) and *Picea mexicana* (14 trees) by analyzing ... and ... filtered SNPs from genome resequencing and ... and .. tree-ring width chronologies of the two species, before and after four severe drought events in the last 100 years.

Significantly tree-ring width-associated SNPs (|rs| = 0.51 – 0.89, $p < 0.05$) were found in several genes (in *P. martinezii*: pollen-specific leucine-rich repeat extension, isoform X1 similar to proline transporter 2, Reticulon B22, LisH/CRA/ RING-U-box domains-containing protein, and Ornithine decarboxylase-like ; in

P. mexicana: Cyclin-dependent kinase B1-1, Uncharacterized GPI-anchored At1g61900-like isoform X2, and Abscisic acid receptor PYL8-like genes). All of these genes were related to metabolic processes involved in cell wall architecture development during plant recovery processes. The results underline the strong influence of genetics on the withstanding and recovery of these spruces.

Taxus globosa somatic embryogenesis

Jeniffer Ivette Vargas Abonce

Taxus globosa Schltdl., is under ""special protection" in NOM-059-SEMARNAT-2010, due to its small populations, caused by a reduced reproductive success reflected in limited, poor, and scattered seed production, high latency, low viability and germination rates.

It has been verified that somatic embryogenesis technique can be used to obtain viable somatic embryos to counteract the effect of dormancy requirement of several Taxus species seeds, using excised zygotic embryos as explants. However, little work has been done using vegetative material as explant source, and information for *Taxus globosa* under this technique is still scarce.

In this work, needle explants from male (ME) and female (FE) cuttings, and zygotic embryos excised from seeds, were tested to obtain Embryogenic Calli (EC) and Globular Embryos (GE). 5 embryos were placed per Petri dish (5 dishes per treatment), as for the needles, the plate was divided into two sections, 6 females and 6 males segments were placed separately in each section (two dishes per treatment).

All, cultured on half strength MS or WPM medium, both, with and without active carbon, and supplemented with different hormones mixes and concentrations (2,4-D (1.8, 8mg/l), BA (0.225, 0.5, 1.125, 2.25mg/l), NAA (0.9, 4.5mg/l), KIN (0.5mg/l) / Originating treatments: OT1 to OT10 per medium).

For zygotic embryos, only WPM OT2 produced calli. For needle explants, non-carbon MS medium was better than WPM, with 33% and 5% callogenesis rate, respectively. In both mediums, best treatment was OT2, with 6 and 5 calli average production, respectively. For carbon medium, there were no significant differences in callogenesis rate nor in calli average production between mediums. In all treatments, there was no significant difference between calli production from ME and FE based on the medium.

Two morphological types of embryogenic calli were obtained. There were no significant differences in the average number of EC produced depending on the

type of medium, neither on sex nor their interaction; however, there were differences depending on the OT, e.g. MS and WPM OT2 produced 97 and 89 EC vs OT5 with 7 and 6 EC, respectively.

There were no significant differences in the average number of GE produced depending on the medium, sex, nor their interaction. However, differences were observed depending on the OT, with OT2 again being the best (MS: 16 (ME) and 18 (FE); WPM: 17 (ME) and 21 (FE)).

For *Taxus globosa* EC and GE production, needle explants performed better than zygotic embryos. MS was better than WPM medium. OT2 produced the highest average of calli, EC, and GE. There were no significant differences depending on the sex of the explant; however, it is important to control the sex of the produced embryos to counteract sexual segregation when establishing the seedlings in natural populations."

Gene expression differences attributed to species-specific interactions across varying belowground geologies in PA

Denise Alving

Climate adaptation has emerged as a priority in forest management as projected changes in climate are expected to impact forest ecosystems. In Pennsylvania, climate change projections suggest some species will thrive under changed conditions, others may decline, and new species may establish. However, these projections do not account for interactions with belowground environments, including nutrient availability, pH, and water availability which may impact the success of species in addition to climatic factors. In Pennsylvania, the two main underlying geologies, sandstone and shale, determine minerals available to trees through weathering, soil texture, and chemistry. Previous studies have suggested that some trees accumulate more biomass on sites with higher or lower nutrient availability depending on their survival and growth strategies. Consequently, to manage species in a changing climate requires quantifying belowground impacts for current and potential future species to assist in the pro-active management of native forests to limit potential loss of forest ecosystem services.

This experiment explores the interactions of two prevalent Pennsylvania bedrocks, sandstone and shale, with native and introduced tree species. One thousand seedlings from a mixture of five native species and four novel species projected to thrive in Pennsylvania in the next 100 years were planted across four different common garden sites, two overlying sandstone and two overlying

shale bedrock. We measured seedling health survivorship and growth twice a year for three growing seasons between spring 2021 to spring 2024. In August 2021, leaf material was collected for gene expression analysis from three native oak species northern red oak (*Q. rubra*), black oak (*Q. velutina*) and white oak (*Q. alba*), and one novel, future climate adapted species southern red oak (*Q. falcata*), at one site overlying shale and one site overlying sandstone. The goal of this comparison was to evaluate gene expression differences that may be attributed to species-specific interactions across varying belowground geologies. A Weighed Gene Correlation Network Analysis was performed to evaluate patterns of co-expression among the most differentially expressed genes. Across the two bedrock types genes associated with regulation of cellular processes and stress responses, as well as mechanisms for regulation of ion-binding and photosynthesis in response to site-specific challenges were differentially expressed. Quantifying variation in gene expression across different species under varying geology will aid in identifying the genetic mechanisms underlying adaptation to belowground environments. Ultimately, this work will be critical to development of assisted seed transfer guidelines under climate change.

Does Environment Predict Telomere Attrition in Long-Lived Forest Trees?

Jordan Basalyga

Forest management in a changing climate aims to predict how different species respond to varying environmental conditions. Biomarkers sensitive to the environment may provide valuable information needed in prediction that may be applied to species management and restoration decision-making. Telomeres, repetitive sequences of non-coding DNA that protect the tips of chromosomes from degradation may be valuable biomarkers for predicting species' life history or fitness across abiotic or biotic environments. However, to assess the role of telomeres as a potential biomarker there is a need to establish longitudinal studies to evaluate telomere length variation across spatially varying environments. Previous research has indicated telomeres shorten in response to aging, somatic cell division, and may vary in response to abiotic or biotic stress. However, the enzyme telomerase counteracts shortening adding telomeric repeats to chromosome tips to maintain telomere length. Substantial telomere length variation persists within and among populations across different species and previous observations from Arabidopsis suggests the environment of origin may predict telomere length and timing of life history. Given the relationship between telomere length and fitness, this indicates natural selection may play a large role influencing telomere length variation. Despite this observation, very few studies to date have assessed the relationship between environment, life history variation, and telomere length variation in

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plants. Ecologically and economically important Populus serves as an excellent biological model for examining if environmental variation predicts telomere length and its rate of change. In this study, we used ten clonally-replicated Poplar common garden experiments planted across North America to assess the impact of the environment, genomic ancestry, and life history on telomere length and rate of attrition over the growing season. The common garden experiments include genotypes from *Populus trichocarpa*, *P. balsamifera*, and their hybrids. To quantify variation in telomere length, we are using quantitative polymerase chain reaction (qPCR) on DNA extracted from Populus leaves sampled at two time points across the growing season. We are asking 1) Does climate transfer distance predict rate of telomere length change within a growing season for *P. trichocarpa*, *P. balsamifera*, and their hybrids? 2) Does telomere length predict timing of bud burst and bud set across genotypic classes? and 3) What is the contribution of genomic ancestry, genotypic variation, and common garden environment to telomere length variation? The results from this study will provide insight into the relationship between telomere length and the environment with potential applications to species management and breeding longer term in forest trees

Sugar pine genome assembly
Akriti Bhattarai

Background:
The white pines comprise the subsection *Strobus* of the genus *Pinus*, with species native to North and Central America, Europe, and Asia. They are susceptible to a devastating disease known as white pine blister rust (WPBR) caused by the fungus *Cronartium ribicola*. Major resistance loci (MGR) to WPBR have been identified in four North American species, one of which is the locus Cr1 in sugar pine (*P. lambertiana*). In species that lack MGR, the focus has been identifying quantitative resistance (QR) which can vary greatly between and within species. Some candidate genes for the MGR loci have been characterized as nucleotide-binding leucine-rich-repeat receptors (NLR), a large class of plant disease resistance genes. NLRs are additionally candidates for QR genes providing partial resistance.

Methods:
The genome annotations of the recently assembled whitebark pine genome and re-assembled sugar pine (*P. lambertiana*) genome provide an improved resource for the identification of putative NLR genes. The improved contiguity and completeness of the re-scaffolded sugar pine genome allow for a deeper exploration of candidate genes associated with the MGR locus Cr1.

Results:

The re-assembled sugar pine genome was 26.4 Gbp, of which 23.9 was anchored to 12 pseudo chromosomes with a BUSCO completeness of 65.5%. The scaffolded genome annotation contained 30,110 genes with a BUSCO completeness of 85.5%. The improved whitebark pine annotation contained 27,555 genes with a BUSCO completeness of 73.9%. In the sugar pine genome, an amplicon based on the marker Cr1lB associated with the Cr1 locus was aligned to the genome and this region was explored for candidate genes, some of which were identified as NLR type genes.

Conclusions:

The improved chromosomal level genome assemblies and more complete gene annotations for sugar pine and whitebark pine have allowed for greater exploration of candidate genes associated with loci for resistance against white pine blister rust (WPBR).

Quantitative trait loci of beech bark disease resistance in American beech (*Fagus grandifolia* Ehrh.)

Mitchell Calvin

Beech bark disease is a disease complex of beech trees (genus *Fagus*) caused primarily by the invasive beech scale insect (*Cryptococcus fagisuga* Lind.) and subsequent infection by either *Neonectria faginata* or *Neonectria ditissima* fungi. Beech bark disease can cause up to 50-85% mortality in American beech 10 years post-invasion leading to ecologically significant changes in forest species richness and stand structure. American beech trees are very susceptible to beech bark disease, but a small number have been confirmed to be resistant. Previous work, using a genome-wide association study, identified SNPs within a major gene associated with beech bark disease resistance. However, a linkage map made with a mapping family of 115 individuals was used to perform QTL analysis using 46 phenotyped members of the mapping family. These results identified two QTLs for resistance but failed to validate the results of the GWAS study. With increasing genomic resources for American beech the ability to further characterize quantitative disease resistance has improved. To address these needs, this study will construct a linkage map to identify quantitative trait loci.

The mapping family for this study consists of 92 American beech individuals that were bred from a reciprocal cross between two BBD resistant parents. The progeny were exposed to a known quantity of beech scale eggs, after which the inoculation sites were covered and left to develop for approximately a year. After this period, counts of beech scale adults and egg clusters were taken to

assess the ability of the insect to complete its life cycle on each beech genotype and to quantify the degree of susceptibility of non-resistant individuals. Genomic DNA will be extracted from leaf tissue of the phenotyped progeny to undergo genomic sequencing. The sequence data will then be used to construct a linkage map and perform QTL analysis to identify loci associated with beech bark disease resistance in this family. These loci will be compared against QTLs derived from a previously mapped family (unpublished). A BLAST search will be performed with the identified loci against relevant genomes in an attempt to reveal potential gene function.

While this work is still underway, this research intends to identify additional genomic loci in American beech that contribute to quantitative resistance to beech bark disease.

Knowledge of genomic markers that confer resistance can be used to develop marker assisted selection strategies to identify resistant parents and progeny increasing the efficiency of breeding efforts and accelerating the development of improved seed sources that can be used for reforesting areas affected by beech bark disease. Additionally, understanding the genomic basis of resistance can be used to predict the vulnerability of American beech populations naïve to beech bark disease.

Exploring genotype-by-environment interactions: impacts on genome-wide association and genomic prediction for 30 white spruce traits related to productivity, defense, and climate-adaptability

Eduardo Pablo Cappa

Background:
White spruce (*Picea glauca* (Moench) Voss) is considered a keystone species in boreal forest ecosystems and a valuable resource for the commercial timber industry. White spruce is also known for its adaptability to diverse environments. Understanding the genetic basis of traits related to productivity, growth, and stress resilience is essential, especially in the context of a changing climate. Genome-wide association studies (GWAS) and genomic prediction (GP) are potent tools for understanding complex traits in forest trees. However, their application to genotype-by-environment interactions in white spruce remains limited, highlighting a research gap in forest tree genetics. We conducted a comprehensive multi-environmental GWAS and GP analysis for 30 productivity, defense, and climate-adaptability traits assessed on 1,540 white spruce trees from central Alberta, Canada, genotyped for 467,224 SNP markers, and growing across three environments.

Methods:

Multi-environment GWAS was performed to obtain estimates of marker effects, and their associated p-values were obtained by back solving for SNP effects based on the breeding value predictions. A ten-fold cross-validation analysis was conducted on 30 traits using a multi-environment model. Predictive ability (PA) was assessed by correlating predicted breeding values obtained by fitting the full data set with those from validation set values, accounting for narrow-sense heritability, while prediction bias (PB) was determined through regression coefficients.

Results:

A total of 563 significant associations (p-value $< 1.07 \times 10\text{-}05$) were found across the 30 traits and three environments. Among them, 105 SNPs showed overlapping associations over two or three environments. Wood density, myrcene, total monoterpenes, α-pinene, and catechin had the highest overlap (>50%) across environments. Gas exchange traits, intercellular CO_2 concentration, and intrinsic water use efficiency had the highest number of significant associations (>38%), while showing less stability across environments (<1.2%). Among the 30 traits, 20 showed a significantly different PA (0.03-0.41) across environments. Stable carbon isotope ratio had the highest average PA (0.36), while gas exchange traits had the lowest PA (0.07). However, only two traits showed differences in PB across environments. The majority (80%) of the site-trait PB values fell within a narrow range (0.90 to 1.10), indicating low bias across traits and sites.

Conclusions:

We demonstrated that the integration of GWAS and GP techniques offers valuable insights into the complex interplay between genotype, phenotype, and environment in white spruce trees grown in central Alberta, Canada.

Conservation genomics in a pine species: *Pinus chiapensis* (Martínez) Andresen

Karla Vianey Martínez Conde

Pinus chiapensis (Martínez) Andresen plays a crucial role in the canopy of Mesoamerican cloud forests. Nevertheless, its conservation status is cause for concern due to its limited genetic variation. Recently, there has been a growing interest in developing strategies and tools for the conservation and sustainable management of *P. chiapensis*. However, the conservation and management of natural resources are intricate processes that necessitate integration of diverse information and analytical tools. Presently, genomic analyses provide insights into the variability and genetic structure of populations and species, aiding in forecasting and detecting signs of local adaptation to environmental

conditions. In this study, we examined the genetic variation and population structure across the natural range of *P. chiapensis* using ddRADseq and a referenced assembly. Population diversity parameters indicate moderate genetic variation within populations and low levels of structuring. Given that fragmented and isolated populations may suffer from inbreeding depression, a criterion for identifying populations at risk is the fixation coefficient. However, many plant species employ biological mechanisms to prevent inbreeding by promoting outcrossing and are considered exogamous. Thus, it appears that the effects of population reduction and fragmentation have not yet significantly impacted diversity patterns, likely due to the reproductive system of pines.

Selection of *Pinus taeda* progenies and installation of a clonal seed orchard

Diego Torres Dini

Background

The *Pinus taeda* provenances installed in Uruguay come from various points in the United States and South Africa and were established by more than a dozen companies during the 1990s. To optimize the production of wood or cellulose and conservation of *P. taeda*, it is essential to establish seed orchards containing trees of high genetic quality. Seed orchards allow the obtaining of seeds of uniform and improved quality, thus guaranteeing the production of superior plants and the transmission of desirable characteristics to future generations. These seed orchards are established through the selection of the best trees that are outstanding in terms of growth, pest and disease resistance, and wood quality. The aims of this work were the selection of plus trees and the installation of a clonal seed orchard that produces improved seed adapted to the edaphoclimatic conditions of Uruguay.

Methods

The morphological selection of 124 plus trees was carried out in 2003 through a survey in 9 forestry companies covering an area of 2068 ha. These selected plus trees were propagated by grafting and used to establish a clonal seed orchard in 2004, totaling about 124 clones represented by 2,220 plants (about 17 ramets per clone). Two progeny tests were also installed (in 2004), using open-pollinated seeds collected from the 124 selected plus trees. One trial was established in the state of Rivera in the company FYMSA and the other in the state of Paysandú in the company LUMIN. The two trials were established using a randomized complete block design with, each one with 124 open-pollinated families, one plant per plot, with 20 and 25 repetitions, respectively. The plantings followed the spacing of 4×2.5 m, totaling an area of 10 m2 per plant.

The diameter at breast heigh (DBH, cm) and height (m) traits were measured and the individual volume (m3) was calculated in Rivera and Paysandú progeny tests at the ages of 9 and 8 years, respectively. For the quantitative genetic analysis of the progeny tests, the SELEGEN software was used.

Results

From the joint analysis of variance we observed that the genotypic correlation between environments was high (rgloc> 0.9) for the evaluated traits, indicating a simple genotype-environment interaction. The estimates of individual heritability in the strict sense (h2a) were 0.428 for DBH, 0.469 for height and 0.511 for volume. To estimate genetic gain (G), the individual volume trait was chosen, using three different selection intensities to determine the best strategy for optimizing genetic gains. Selecting 25% of the plus trees (33 clones) gives G=13%, where if we increase the selection intensity to 135% (16 clones), we obtain a G=17%.

Conclusions

Through selection, it was possible to install an orchard for the production of seeds with genetic gains for wood production and adapted to the soil and climate conditions of Uruguay.

Genetic variation and phenotypic plasticity in shoot growth and needle traits with adaptive value to drought in *Pinus pseudostrobus*

Sebastian Escobar-Alonso

Global warming has intensified the challenges experienced by natural populations of forest species. The primary mechanisms for these species to endure the impacts of fluctuating environments, including more frequent stressful events, are genetic variation (GV) and phenotypic plasticity (PP) of adaptive traits. *Pinus pseudostrobus* is an ecologically and economically important tree species in Mexico, primarily found in the Trans-Mexican Volcanic Belt. Due to its wide geographical distribution and the varied climatic conditions where it prevails, this species is an appropriate biological model to investigate the relevance of GV and PP in adaptively important traits. We evaluated the level of GV and PP in shoot growth and bud phenology traits in 44 open-pollinated progenies of *Pinus pseudostrobus* from central Mexico established in a progeny trial replicated at two sites with different soil and climate conditions. In parallel, we evaluated other morphoanatomical traits in needles, and the sapwood/leaf area ratio (Huber Index [HI]) in a subsample of 30 progenies. Results showed wide GV in almost all evaluated traits and strong genetic control in bud phenology traits. Bud burst, bud set and shoot growth during wintertime showed an adaptive relationship with climate conditions at

origin site of progenies, particularly with moisture availability and aridity index (AI). A significant correlation between the number and mean length of growth units, stomatal density, needle thickness, specific leaf area and HI with AI was also found, showing the adaptive value of those traits. Significant variation in adaptive PP was also found between families for most traits associated with productivity, in terms of height growth, and mean annual temperature at the place of origin. These findings indicate that the observed GV and PP could help *Pinus pseudostrobus* populations in central Mexico to cope with global warming. This knowledge can also be used to design appropriate conservation and management strategies for the genetic resources of the species in the face of climate change

Genome of hemlock woolly adelgid

Karl C Fetter

Background:
The hemlock woolly adelgid (HWA, *Adelges tsugae*) is a gall-forming hemipteran insect which alternates between a hemlock and spruce host in its native range in eastern Asia. The HWA was introduced to eastern North America in the 1920's to 1950's on ornamental cuttings of Japanese hemlocks and has subsequently devastated eastern hemlock (Tsuga canadensis) populations. Introduced HWA are not known to form galls in eastern North America, potentially as a result of a bottleneck during introduction or an inability to find a suitable spruce host. HWA forms quote-unquote wool from waxes to protect them during sedentary stages of their life cycle. Here, we present a chromosome-scale reference genome for *A. tsugae* and identify bicycle genes related to gall formation and wax synthesis.

Methods:
400 adelgids were sampled in September 2023 from a hedge of cloned accession of eastern hemlock growing at the Mountain Research Station in Waynesville, NC. Adelgids were collected into an extraction buffer and high molecular weight genomic DNA extracted from 300 individuals. Libraries were prepared for ONT sequencing and a single flow cell was used to obtain 32.5 M reads and 100.5 Gb of called bases. Multiple assembly tools were run, Canu being preferred after removing contaminants with centrifuge, reducing heterozygosity with Purge Haplotigs, and RagTag scaffolding chromosomes against a relative. Genome annotation was performed with EASEL and Stringtie2 using RNA sequences.

Results:
The ONT sequences were assembled into a genome of 220.7 Mb scaffolding to 9 chromosomes with a high hemipteran BUSCO completeness of 99.3%. Bicycle

genes important for gall formation were identified and showed increased expression in salivary glands. Wax synthesis genes were identified, with increased expression in the thorax.

Conclusions:

Studying the genomes of invasive pests is critically important for understanding their evolution and physiology. This adelgid genome provides important insights into the biology of the adelgid and its evo-ecological relationships to forest trees. The genome sheds light on the evolution of gall-forming and wax biosynthesis genes, and is an important resource for conservation genomics in the hemlock-adelgid system.

Survival and development of seedlings from five species of *Pinus* and their hybrids under field conditions in Mexico

Jose Alberto Ponce Figueroa

This study focuses on the survival and development of seedlings from five species of *Pinus* and their hybrids under field conditions, emphasizing the influence of specific morphological characteristics such as root collar diameter (RCD) and height (H) prior to planting. It was conducted at two test sites in the Sierra Madre Occidental, Durango, Mexico, guided by the hypothesis that seedlings with a larger RCD in the nursery would have a higher probability of survival and growth when transplanted to the field.

Through a detailed statistical analysis, the study identified weak to moderate but significant correlations between RCD and height prior to planting and their respective measurements three years later, although no significant association was found between RCD and the probability of survival three years after planting. However, a positive correlation was observed between the height of the seedlings before planting and survival, suggesting the importance of the latter as a more reliable predictive indicator of survival under field conditions.

The results underline the need to consider a wider spectrum of factors, including genetic and environmental ones, in future research to improve forest management strategies. These findings point to the complexity of interactions between morphological, genetic, and environmental characteristics in the success of reforestations, highlighting that the survival of seedlings in natural conditions can be influenced by a variety of factors."

Conservation zones for forest genetic resources of *Pinus leiophylla* Schiede ex Schltdl. et Cham.

Andrés Flores

Background:
Facing continuous environmental changes, genetic conservation is a key to maintaining forest resources for the future. However, conservation activities are limited by the financial, temporal, and technical resources available. For Mexico, there is genetic information for most Pinus species, but their conservation efforts are limited, and their proposals to conserve them are few. In particular, there are no in situ genetic conservation efforts for pine species, which guarantee its continuity over time in future climate scenarios. Therefore, it is necessary to define sites to preserve the genetic material within its natural distribution. The objective of this work was to define the genetic conservation units (GCU) of *Pinus leiophylla* Schiede ex Schltdl. et Cham. and determine the use of its genetic resources.

Methods:
The distribution of the species in genetic zones was determined based on the National Forest and Land Inventory (NFLI) latitude and longitude records. In contrast, the genetic conservation units were defined based on population size, management, monitoring, and ownership. Finally, for the use of the genetic resources of the species in each genetic zone, the number of trees sampled by the NFLI, the number of populations with molecular data, the number of populations with seed stands, the number of individuals selected for progeny tests, the number of provenance tests, and the number of seed orchards were taken into account.

Results:
Twelve genetic zones were identified in which the species was present, but only eleven (III.1, III.2, III.3, III.4, X.1, X.2, X.3, XII.1, XII.3 XII.4, and XII.5) satisfied the criteria for the establishment of 20 GCU (91%). Of these zones, 90% had existing genetic information (molecular characterization and provenance trials). 11,556 trees were sampled for the utilization of forest resources; of them, 27 and 2 populations underwent molecular characterization and seed stands, respectively. Progeny, provenance, and seed orchard tests for this species were 1, 2, and 1, respectively.

Conclusions:
The UCGs are expected to help maintain the target species' genetic resources in situ for their future use and exploitation in forest management programs.

Witches broom in loblolly pine
Pinar Guner

Witches' broom, an aberration characterized by the proliferation of short, densely packed branches, is occasionally observed on isolated branches in the crowns of Pinus spp. Some witches' brooms are apparently caused by somatic mutations and result in dwarfism segregating in progeny from their cones (Grimshaw and Bayton , 2022). Our study seeks to characterize the genomics of the witches' broom mutation in *Pinus taeda* (loblolly pine).

We sampled needle tissue and cones from more than 20 trees with witches' brooms, as well as from the trees' normal branches. DNA was extracted from needle tissues and genotyped using the Pita50K SNP array (Caballero et al. 2021) to investigate marker associations. Nutrient profiles, cone morphology, hormone levels, and tissue anatomy via microscopy of afflicted and normal branches were compared. Seeds were extracted from open-pollinated cones collected from brooms and their neighboring typical branches for sowing in the greenhouse. Open-pollinated seeds from the dwarf *P. taeda* var nana were collected from the JC Raulston Arboretum at NC State University (which originated from witches' broom cones).

Mutant foliage exhibited lower levels of potassium, calcium, magnesium, and manganese compared to normal foliage. However, mutant foliage displayed significantly higher levels of phosphorus compared to normal foliage. Twenty-one out of 42 Nana open-pollinated seedlings exhibited dwarfism and were on average 20 cm at 20 weeks compared to 30 cm at 20 weeks for their non-dwarf half-siblings.

A practical application of dwarfism would be the development of rootstock for grafted pine seed orchards, which presently require heavy bucket-lift equipment to harvest cones for reforestation due to their tall stature. While dwarfing rootstock is common in fruit trees, there is no forestry analog, which could facilitate safer and faster cone harvest.

Feasibility of breeding programs for genetic improvement of *P. pseudostrobus*
Eduardo Hernández Hernández

Genomic analysis revealed three major genetic clusters dividing populations in western, central, and eastern Canada. Climate variables, including mean annual climate moisture index, mean annual total precipitation, and total annual temperature range, were strongly associated with genome-wide variation and

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overlapped a major allelic transition between Central and Eastern genetic clusters. Additionally, our growth model highlighted mean summer and autumn temperatures as the most relevant climate factors for future adaptation to climate change, with high temperatures proving detrimental to growth. Projecting growth until 2100 under different climate change scenarios, preliminary results suggest that black spruce is likely headed toward a general climate maladaptation, potentially impacting its carbon sequestration potential on the long term.

Population genomics of race non-specific disease resistance in the endemic Pin*us taeda-Cronartium quercuum* f. sp. *fusiforme* pathosystems

Molly Irvin

Fusiform rust disease, conferred by the fungus *Cronartium quercuum* f. sp. *fusiforme*, is the most significant threat to pine farming in the southern U.S. Our group previously discovered the first evidence for race-non-specific resistance in a mapping population of *Pinus taeda*. In this research, the goals are to study virulence variation in single-spore isolates of the pathogen and understand how they interact with two known QTL in the host. A population of 6,000 half-sib seedlings was developed from a maternal parent, carrying two resistant QTL linked in repulsion on linkage group 2. The population was inoculated with a mixed basidiospore inoculum (112,333 spores/mL) of the pathogen. About 12% of the progeny developed disease symptoms. Megagametophytes from seedling progeny were harvested and will be genotyped to determine the genotypic classes of progeny segregating for two QTL. Artificial induction of haplotype pycniospore production via cold treatment will be conducted this summer and pycniospores will be collected and DNA extracted. Illumina sequencing will be utilized to discover SNPs in the fungal genome, and these SNPs will be used to identify genomic regions exhibiting signatures of selection (selective sweeps). Genes in these regions will be examined to determine whether common fungal effector(s) may be responsible for non-race-specific resistance conferred by the resistance alleles.

Camcore's Pine Hybrid Testing Program: Results from 43 Trials

Romeo Jump

Camcore, an international tree breeding and gene conservation program, part of the Department of Forestry and Environmental Resources in the College of Natural Resources at NC State University, was founded in 1980 to conserve the

genetic diversity of coniferous forests in Mexico and Central America. The program has been continuously focused on seed collection and ex-situ conservation, in addition to the development of tree improvement programs in collaboration with private forest companies, government agencies, non-profit institutions, and research partners. Camcore initiated a pine hybrid program in 2003, aiming to assess the commercial viability of various hybrids. These hybrids potentially offer benefits like higher tolerance to drought and frost, better disease resistance, good wood properties, or higher volume growth. Over 90 trials have been conducted in Africa and Latin America. In 2015, the focus shifted to characterizing the wood properties of hybrids, and evaluations were carried out in multiple countries at age 8. Wood traits were assessed in a subset of 12 tests, measuring cellulose, lignin, MOE and wood resistance. Thirty trees per treatment (hybrid or control lot) were randomly selected in each test. The data analysis involved fitting generalized linear models for various traits, including volume at 5 and 8 years, percentage of cellulose and lignin, core resistance, and MOE. The significance of each modeled trait was assessed. If variation between treatments was observed, LSMEANS comparisons were made, and confidence intervals were calculated and plotted. For volume modeling, treatments with less than 60 percent field survival were excluded, and study sites were grouped based on climate and/or hybrid overlap. Wood properties were modeled separately for Argentina/Brazil and Colombia/South Africa.

Volume analysis revealed significant differences between treatments at 5 and 8 years, indicating variations in mean volume, with promising hybrids including CARxTEC, PATxTEC and GRSxTEC in Argentina, Brazil, Colombia, Kenya, Mozambique and South Africa. Hybrid rankings derived from 8-year data were similar to those observed at age 5. For pure species, only *Pinus patula* and *P. tecunumanii* ranked well for growth at 5 years; *P. taeda* and *P. oocarpa* ranked intermediate, and *P. pseudostrobus* ranked low in all South African trials. Wood quality analysis showed that PATxTEC, PATxGRS and GRSxTEC excelled in all wood traits at age 8, while TECxCAR performed well only in resistance in Colombia/South Africa. Among pure species, *P. patula*, *P. tecunumanii*, *P. taeda*, *P. oocarpa*, *P. pseudostrobus* and *P. radiata* showed good wood properties. A multi-national full-sib breeding program for three specific hybrids with *P. tecunumanii* has been initiated. The encouraging performance of a number of pine hybrids should motivate additional efforts to investigate other hybrid combinations and pursue additional full-sib breeding efforts with hybrids showing potential.

Population and community ecology

Liliana Elizabeth Rubio Licona

Environmental conditions influence plasticity of functional traits as it has been reported for several taxa; however, there is relatively little information about the inter-specific variation in leaf and wood functional traits for co-occurring Quercus species. Our goal was to examine the inter-specific differential expression in leaf and wood anatomical traits for co-occurring Quercus species, along elevation gradients on two mountains differing in climatic conditions. We collected foliage and wood samples from three trees for each co-occurring species at 100 m altitude intervals on Alcaparrosa and Las Ánimas mountains, in the State of México. Through conventional anatomical techniques we measured stomatal size and density (SL, SW, SD), leaf thickness (LT), palisade and spongy parenchyma ratio (PPR, SPR) and epidermis ratio (ER) in five leaf samples per tree; additionally, we determined specific leaf area (SLA), intensity of leaf fall (ILF) during the drought season, and nitrogen and phosphorous content. On wood samples we evaluated vessel frequency (VF), vessel diameter (VD) and wood density (WD) per tree for all Quercus species. Analyses of variance were performed to explore differences among species, considering mountain and altitude factors, on the expression of functional traits. Furthermore, a principal component analysis was done to identify functional differences between species. We found that: 1) species showed plasticity on different traits; 2) wood traits plasticity was found almost exclusively at mountain level; 3) leaf plasticity was expressed at landscape (mountain) and microsite (altitude) scale; 4) the greater phenotypic plasticity was found on a) foliar traits related to carbon assimilation and gas exchange (PC 1= 30.5%; SD and SPR); and b) on traits concerning hydraulic safety (PC2=12.6%; VF and ILF). Our results show that higher temperature and lower humidity conditions at Alcaparrosa mountain promote leaves with smaller but more dense stomata and wood with smaller but more frequent vessels in these Quercus species; SD, SPR and ER are labile traits since showed variation both between mountains and along the elevation gradient. Co-occurring Quercus species on these mountains showed different ecological strategies to face drier environments; some taxa showed plasticity in particular leaf traits while others exhibited it on both leaf and wood traits. Our findings indicate that co-occurring Quercus species in these mountains differ in strategies and potential for adjustment in a context of rising temperatures and increased drought of the temperate climate zones.

Germination of *Pinus coulteri*

Fatima López López

Background:

Pinus coulteri is naturally distributed in Mexico and is represented by only six small populations, limited to the State of Baja California. The species is listed as near threatened and endangered. Germination and seed viability parameters are taken into account as criteria to categorize species that may be at risk. To know the seed viability status in the populations, seed viability, germination percentage G_max, uniformity U_8416 and the time required for 50% of viable seeds to germinate T_50 were evaluated in seeds from Rancho Nuevo (RN), San Faustino (SF), Laguna de Hanson (LH), Santa Catarina (SC) and Sierra Blanca (SB).

Methods:

Seeds from each population were soaked in distilled water for 18 hours, to separate empty seeds from full ones. Next, the filled seeds were treated with 1% tetrazolium chloride, to corroborate their viability, viable if the embryonic tissue was stained red and nonviable without staining. Prior to germination, each seed was soaked in a 2% hydrogen peroxide solution for 24 h. Later, the seeds were placed in Petri dishes with absorbent paper, 15 per box representing a replica; Depending on seed availability, per population, 48 to 72 replicates were used. Germination was considered complete once the radicle reached at least the size of the seed. Germination was evaluated every two days for 140 days and the database was analyzed by non-linear regression based on the Hill function of four parameters (y, a, b, and c), with which G_max, U_8416and T_50.

Results:

The seeds of the five populations showed high viability and germination capacity, averaging 88% and 68%, respectively. Viability was between 96% for LH and 73% in RN; and G_max between 83% in SC and 52% in SB. The shortest U_8416 value that generated the fastest germination was 22 days for RN, 54 days shorter than SF, the population with the slowest U_8416. The T_50 for RN occurred on day 20 of the establishment of the experiment and was the shortest time recorded for the five populations and 56 days was the longest for SF.

Conclusions:

The results show that the embryos are in good condition and have high potential to germinate, despite the fact that the seed was collected more than 10 years ago. Differences found between populations may be due to some physical characteristics of the seed, such as the size and thickness of the testa, which influence the speed of germination. Aspects of depression due to inbreeding can be ruled out, although it is necessary to carry out a molecular analysis of genetic variation to know the effect of evolutionary forces on Mexican populations *of P. coulteri*.

Presentation Details

Assessing range-wide genetic diversity for targeted conservation, restoration, and breeding efforts in Black ash (*Fraxinus nigra*)

As one of the most destructive pests ever introduced to North America, the emerald ash borer (EAB) has decimated eastern ash species impacting forest-based ecosystems, economies, and indigenous culture. Despite the extensive damage to eastern forests, there are opportunities yet to conserve genetic resources and identify genetic groups that will be critical to the development of restoration and breeding efforts. Of North American ash species, black ash (*Fraxinus nigra*) is one of the most vulnerable to EAB. Given this susceptibility and the continued expansion of EAB, recent projections suggest this species could be functionally lost from the forest in the next 20-30 years. Black ash is unique across ash genera as it inhabits low lying areas and river terraces where there is high seasonal flooding and wet mineral soil. In acidic bogs and swamp-like ecosystems, black ash can comprise 95% of the canopy cover where there is standing water, outcompeting many other tree species in these marginal environments due to its hydrophilic nature. The loss of black ash due to EAB poses not only a threat to the foundational ecology of these unique environments, but also represents a substantial threat to a tree that is keystone to the cultural heritage of many tribal communities across the species' native range. Given the functional and cultural importance of black ash, it is imperative to evaluate range wide genetic variation to inform ex situ and in situ conservation efforts. The genomic resources we have established through range-wide population collections allow us to measure proxies of genetic health across the species' native range. We are assessing genetic variation, effective population size, and genetic differences within and among populations across the range of Fraxinus nigra. We are also using genotype by environment associations (GEAs) to identify climate factors influencing rangewide variation to project allele frequency change needed in future climates. In addition, we are establishing a common garden of maternal families sampled range wide to quantify variation in traits important to climate adaptation. The common garden will allow us to partition the contributions of genetic and environmental variation to phenotypic variance, an essential resource to guide restoration in a changing climate. Combining genomic data with common garden experiments will be foundational to further conservation, restoration, and breeding efforts for this critically endangered species."

Enhancing Genomic Selection for Black Spruce (*Picea Mariana*): Building and Validating a Genomic Selection Model for Open-Pollinated Families

Jesse Milani

Background:
This study focuses on the growth and survival of black spruce seed sources from various regions, examining their performance across assisted migration trials in Northern and Central Ontario. The background information includes the geographic scope of the study and the motivation behind investigating seed source variability in response to changing climate conditions.

Methods:
The study utilized four assisted migration trials in Northern and Central Ontario to assess the growth and survival of black spruce seed sources from Ontario, Quebec, Minnesota, Wisconsin, and Michigan. Multiple linear regression models were employed to analyze the relationship between seed source responses and 85 independent climate variables.

Results:
The results highlight considerable variability in survival and growth responses among the black spruce seed sources across the trial sites. Certain climate variables were found to have a strong correlation with the performance of the seed sources, as indicated by the regression analysis. Notably, local seed sources exhibited below-average performance, suggesting potential challenges for future reforestation efforts under changing climatic conditions.

Conclusion:
The findings suggest that sourcing seeds from southern regions may offer advantages for future reforestation endeavours in northern areas, given the below-average performance of local seed sources. This conclusion emphasizes the importance of identifying suitable seed sources to mitigate the risk associated with maladapted sources. It provides valuable insights for managers grappling with the complexities of assisted migration strategies in the context of climate change.

Contextualization of the *Pinus patula* scenario in Brazil and general analysis of the data through Selegem

Diego Torres Dini

The *Pinus patula* is a species of pine widely cultivated in Brazil, especially in the southern and southeastern regions of the country. Its introduction aimed

primarily to meet the commercial demands for wood, given the quality and versatility of its raw material. Widely used in forest plantations for reforestation of degraded areas, the *Pinus patula* stands out for its rapid growth and adaptability to different environmental conditions. In the Brazilian scenario, it significantly contributes to the timber industry, being employed in furniture making, paper production, packaging, and construction. Its economic cultivation is driven by its ability for rapid growth, making it a viable option for large-scale production. However, recently, a growing challenge caused by attacks of capuchin monkeys has been observed. During periods of native fruit scarcity, these animals begin to feed on the phloem, cambium, and sap of some pine varieties, such as *Pinus taeda*, resulting in significant damage to the trees. These damages include girdling, characterized by partial bark removal, and ring-barking, leading to the death of the affected plant. This situation presents challenges in breeding programs and can result in losses of up to 50% in plant growth. Given this scenario, studies indicate that *Pinus patula* may be a promising alternative. It is observed that the attack of these monkeys is less frequent in this species, making it an attractive option for both planting and forming borders of *Pinus taeda* plantations, for example. An analysis of data from two plantations of *Pinus patula* from FRP revealed a very favorable performance of this species in the southern and southeastern regions of Brazil. With an average DAP (Diameter at Breast Height) of 35.3373 and a Mass Area Index (DAP) of 40.4443, the results suggest good adaptation and growth of these trees in this region. Additionally, the analysis of baguettes, which measure basic density in three distinct parts represented by the CV% index: bark 13.4629, intermediate 10.4499, and pith 18.3253, allows for an evaluation of planting differences by location and climate, under different conditions. These data indicate a promising potential for the cultivation and use of *Pinus patula* in reforestation programs and the timber industry in Brazil.

Keywords: *Pinus patula*, Reforestation, Capuchin monkeys, Rapid growth, Data analysis.

Optimal age selection in breeding *Tectona grandis*
Olman Murillo

Background: Teak is one of most widely planted species in the tropics due to its high wood value and moderate growth. Development of breeding programs in several countries and organizations has been motivated in last decades. Two main questions motivated this research, how early can be possible performing genetic selection and which is the optimal age selection in clonal Tectona grandis. Methods. A clonal test was established in 2006 at Nicoya, northern pacific region of Costa Rica. The trial had an initial spacing of 4x4m and arranged in a complete random block design with 32 clones. Measurements and

data were gathered at ages 3.2, 3.6, 4.4, 4.9, 5.9, 8.9, 9.7, 10.6, 11.8, and 12.9 years old for diameter at breast height (DBH), commercial height (estimated), tree quality (assessed) and commercial volume. Genetic parameters and genetic correlations at all ages for all variables were obtained through SELEGEN software (REML/BLUP). Results. Individual DBH heritability (H2gi) ranged from 0.02 to 0.11, while clonal mean heritability (H2mc) ranged from 0.43 to 0.82. Highest mean clonal heritability for commercial volume was registered at 9.7 years. Significant genetic correlations were found (rg = 0.74) between DBH at 4.9 years and commercial volume at 8.9 years. Spearman rank correlation and graphical analysis determined high clonal ranking stability at the age of 4.9. Conclusions. The optimum selection age was determined at 9 years of age, while early selection can be practiced with low risk at 5 years of age, supported by a genetic correlation of rg = 0.7.

Double Tree Plot design for your progeny tests

Olman Murillo

Background:
Double Tree Plot is proposed as a variation of the conventional Single Tree Plot, with the main advantage in accelerating genetic testing.

Method:
The design is based on planting two ramets or seedlings per accession in each of 16 blocks. The trees are established in pairs in reduced spacings like 4 x 2,5m, 4 x 2m or even 4 x 1,5m, depending on the tree species. This reduced spacing in one of the two axes, allows for testing large number of materials in small areas, thus, the possibility of establishing genetic tests in more homogenous environmental conditions. Other benefit is related to an earlier competition effect, that allows for a rapid detection and elimination of the tree with lowest adaptation within each pair. This first phenotypically based thinning produces a 50% elimination of the original trees in the trial, and after that, the genetic test continues as a regular Single Tree Plot with all its benefits, but now with the best lucking 16 trees per accession. In tropical tree species, this first phenotypical thinning is being performed as early as at the age of 2 or 3. This test design is especially relevant in young tree improvement programs where there is not enough expertise in the production of good plants for testing, or in field tests planning and establishment, or in initial tests maintenance. Planting two seedlings or ramets per block per accession reduces future imbalance due to mortality effects. After first thinning, the new Single Tree Plot remains at a 4 x 4m spacing. The next thinning will be performed at age 4 or 5 by rouging another 50%, which will take 8 out of 16 remaining trees per accession based on their genetic value.

Results:
Examples of results with Double Tree Plot tests in the Tree Improvement Cooperative GENFORES are shown.

Conclusion:
This Double Tree Plot design allows a more efficient procedure and accelerated genetic testing of larger amounts of materials. Especially relevant with progeny tests.

CartograPlant
Brandon Lind

Background:
CartograPlant (https://cartograplant.org) is a novel web-based application that allows for the storage and analysis of genotypic, phenotypic, and environmental data for georeferenced plant populations. It facilitates the integration of these three data types, allowing for the performance of population genomics, quantitative genetics, and spatial analysis on stored plant data. With the increasing availability of large biological data sets, it is important to ensure that data are appropriately stewarded for future use. The FAIR data principles are a common guideline used to allow data reuse for future research. They state that data should be Findable, Accessible, Interoperable, and Reusable. Prevalent challenges when working with large and varied biological data sets include insufficient storage space, insufficient RAM for analysis, and lack of standardized data formatting. CartograPlant facilitates the FAIR storage and integrated analysis of genotypic, phenotypic, and environmental plant data.

Methods:
Genotypic and phenotypic data are collected both through direct author submission of studies to the TreeGenes database (https://treegenesdb.org/) and through biocuration efforts of published studies (via supplemental datasets) as well as those affiliated with Dryad. These data are stored in standardized formats and with descriptive metadata. TreeGenes utilizes Tripal (tripal.info), an open source genomic database website management tool. Tripal allows developers the flexibility to create, share, and reuse functionality with other data repositories. Analysis pipelines were developed using the Galaxy framework and support diversity estimation, calculations of population structure, and performance of association mapping and landscape genomics.

Results:
To date, CartograPlant hosts genetic and/or phenotypic data from 429 different plant species across 181 genera. This currently amounts to over 771 million

genotype calls and over 1.8 million phenotypes. CartograPlant also offers 977 regional and global environmental layers with which users can interact. The centralization of this genotypic, phenotypic, and environmental data allows for the possibility of meta-analysis across studies. Users can select from thousands of plants based on individual interests (traits, region, species, marker types).

Conclusions:
CartograPlant allows for both the FAIR storage and analysis of biological data. It provides an accessible and extensive data repository, offering representation for both model and non-model plant species from around the world. It allows researchers to ensure that their data can be used for future research. It also allows researchers limited by computational power or knowledge to easily perform analyses.

New resources for understanding old pathogens: Lessons from *Discula destructive*

Israel Shade Niece

Background:
Fungal pathogens of forest trees have dramatically altered forest biodiversity across North America. Understanding the genomic underpinnings of pathogenicity, e.g., evasion of host defenses or horizontal gene transfer, is crucial to predicting and preventing the potential for future epidemics, especially under anthropogenic climate change. Dogwood anthracnose, a disease caused by the ascomycete fungus, *Discula destructiva* Redlin, has decimated native flowering and pacific dogwoods (*Cornus florida* L. and *C. nuttallii* Aud., respectively) in North America since its first report in the mid-1970s. It survives as an anamorphic hemibiotroph and is one of many notable pathogens of forest trees in the order *Diaporthales*. Related *Diaporthales* pathogens include *Cryphonectria parasitica* (Murrill) Barr, the causal agent of chestnut blight, and *Ophiognomonia clavigignenti-juglandacearum* (Nair, Kostichka, & Kuntz) Broders & Boland, the causal agent of butternut canker. Dogwood anthracnose typically manifests as necrotic lesions on leaves and bracts, canopy dieback, and perennial cankers that result in eventual mortality. The extensive impacts of *Diaporthales* pathogens on forest ecosystems are undeniable, yet scant genomic resources (i.e., annotated, chromosome-scale reference genomes) exist to help understand the traits that dictate virulence and pathogenicity. Development of such resources is crucial to understanding historical epidemics to apply past lessons to novel pathogen invasions. Yet, challenges such as limited funding and unique secondary metabolite profiles of filamentous fungi complicate the extraction of high molecular weight (HMW) DNA required for high-quality genome assembly. Thus, the goal of this research is to develop and characterize foundational genomic resources for *D.*

destructiva, in the form of an annotated, chromosome-scale reference genome, to enhance our understanding of the hypervirulent tendency of the *Diaporthales* pathogens.

Methods:
HMW DNA was extracted from the mycelium of isolate, AS111, using a fungal cell wall degrading enzyme (yatalase) with an optimized protocol of the Qiagen MagAttract Kit. Library preparation and long-read sequencing via the PacBio HiFi platform were complemented by high-throughput chromosome conformation capture (Hi-C) sequencing, to enable the assembly of a high-quality chromosome-scale genome.

Results:
The assembly resulted in a 46.13 Mb genome with a BUSCO genome completeness of 92.3%. RNAseq reads from sporulating and nonsporulating isolates were utilized in genome annotation to identify genes involved in infection.

Conclusions:
Our fine-tuned DNA extraction protocol will help improve accessibility to long-read sequencing technologies in recalcitrant fungal pathogens. Additionally, our high-quality reference genome will enable fine-scale profiling of the virulence genes within *D. destructiva*, as well as serve as a resource for comparative genomics with other notable *Diaporthales* pathogens.

Genetic diversity of a seed orchard of *Pinus patula*
Carlos Ramírez-Herrera

Pinus patula Schiede ex Schltdl. & Cham., grows in natural populations in the Sierra Madre Oriental, Eje Neovolcánico and Sierra Madre del Sur in Mexico. This species is important in the lumber industry. Thus, seeds are required for plant production for tree plantations. Seed orchards are important seed sources for establishing plantations with improved seeds, so it is important to know the genetic diversity in seed orchards. Genetic diversity is key to generating new varieties that are better adapted to environmental changes. Thus, the objective was to estimate the genetic diversity of a seed orchard of *Pinus patula*. The seed orchard is in Cuaunepantlan, Acaxochitlan, Hidalgo State, Mexico. The seed orchard is formed with selected trees from a progenies test in which thirty-six families from the natural population (first improved cycle), and another thirty-six families from progenies tests from South Africa and Colombia (second improved cycle) were included. Foliage was collected from a total of 89 trees. Fifty trees were sampled for the first improved cycle while thirty-nine trees were samples for the second improved cycle. The Charge-Switch PlantMini Kit

(Invitrogen) was used to extract genomic DNA from the foliage. Nine microsatellite markers ((SSR) were analyzed in the DNA sequences after PCR reaction. Alleles per locus (A), observed heterozygosity (Ho) and expected heterozygosity (He) were estimated for the seed orchard and each group of improved trees using the macro GenAlEx v6.5. Also, the fixation indexes (FIT, FIS and FST) were calculated. In the seed orchard, considering the 89 trees, A was 8.556, while Ho and He were 0.768 and 0.627, respectively. The first improved cycle trees: A was 0.711; Ho and He were 0.771 and 0.611. For the second improved trees group, A was 0.722; Ho and He were 0.768 and 0.623. The FIT and FIS were -0.243 y -0.252. The FST was 0.008 ± 0.002. Thus, the *Pinus patula* seed orchard had a high genetic diversity. The genetic diversity was very similar between the improved tree groups. The FIT and FIS indicated endogamy in the seed orchard but with excess heterozygotes. FST showed small differences among the two groups of trees in different improved cycles.

Accelerating Breeding of Durable Rust Resistance in Southern Pines

Makayla Rutski

Background:
As the challenge of global climate change intensifies, plant pests and pathogens will increasingly impede growers, threatening forest resource sustainability. Southern pines face significant threats from exotic and native pests and fungal diseases, particularly fusiform rust caused by *Cronartium quercuum* f.sp. *fusiforme* (Cqf). Traditional breeding methods are time-consuming and labor-intensive, taking ≈ 12 years to yield results. With the advent of genomic selection models, there is potential to expedite this process significantly. This study aims to leverage host and pathogen genomic information to accelerate breeding and selection for durable qualitative and quantitative disease resistance to fusiform rust in southern pines.

Methods:
This study proposes a novel paradigm for breeding for durable fusiform rust resistance by incorporating comprehensive phenotypic and genomic methodologies. Objectives include the development of advanced marker identification techniques, integrating host-pathogen genomic data into predictive models, and applying these models to accelerate breeding and selection. We plan to identify markers associated with virulence and resistance by genotyping a wide array of pathogen isolates and 1,000 full-sib progeny from different loblolly pine families. We aspire to generate a robust framework for selecting rust-resistant pines across various geographic regions and against

diverse host-pathogen combinations by integrating qualitative and quantitative resistance traits.

Results:
Initial results have validated the use of gall volume and lesion length as heritable measures of quantitative resistance. A preliminary GWAS has pinpointed potential markers linked to quantitative resistance in *Pinus taeda*, and screening experiments have begun correlating host resistance data with pathogen genomic information.

Conclusions:
This project represents an advancement in forest tree breeding, particularly in adapting breeding programs to climate change. This research aims to elucidate the genetic underpinnings of quantitative disease resistance and evaluate the durability of previously identified resistance genes in loblolly pine. This study fills a critical knowledge gap regarding quantitative resistance to fusiform rust and sets a new standard for breeding programs targeting fungal diseases in long-lived woody perennials. This research offers broad societal benefits, including enhanced economic gains for forest landowners and durability of forest resources to fungal diseases in the face of climate change.

Influence of landscape characteristics on the diversity and genetic structure of two pine species distributed in the Meseta Purepecha eco-region, Michoacan, Mexico

Agustin Molina Sánchez

La fragmentación del hábitat está considerada la principal causa de la crisis global de biodiversidad y está relacionada directamente con la reducción del hábitat, lo que provoca aislamiento genético y reducción del tamaño efectivo de las poblaciones, con la consecuente pérdida de variación genética e incremento de la endogamia. Por tanto, las poblaciones con tamaños pequeños son más susceptibles a desaparecer localmente. Actualmente se asocia la información ambiental con la variación y estructura genética de las poblaciones, bajo la perspectiva de la genética del paisaje, con el fin de determinar la dinámica de la fragmentación del hábitat y su repercusión en la distribución de la variación genética, lo cual tiene fuertes implicaciones para el manejo y conservación de las especies. La eco-región Meseta Purépecha en Michoacán, se encuentra bajo elevadas presiones antrópicas, debido a cambios de uso de suelo con fines agrícolas, principalmente para producción de aguacate y frutillas. Bajo este contexto, el presente estudio analizó el efecto de la fragmentación del hábitat sobre la distribución de la variación genética de dos linajes de Pinus (Pinus montezumae y Pinus pseudostrobus), distribuidos en la eco-región Meseta

Purépecha en Michoacán, con el uso de marcadores moleculares microsatélites nucleares (SSRn). Se estimaron parámetros de la diversidad genética y estadísticos de la estructura genética, los cuales se asociaron con variables ambientales bajo las hipótesis de aislamiento por distancia (IBD) y aislamiento por ambiente (IBE) mediante pruebas de Mantel. Los resultados muestran que las poblaciones de los dos linajes contienen niveles de variación genética intermedios (He=0.413), con un índice de endogamia significativo en todas las poblaciones (0.565-0.533) y con tamaños efectivos históricos reducidos (300.0-452.5). Las poblaciones presentan una estructura genética marcada (RST=0.129, P<0.05), dividida en cinco grupos genéticos, donde la mayoría de las poblaciones de P. pseudostrobus están diferencialmente incluidas dentro de dos grupos genéticos (K3 y K4). El sentido de la migración fue heterogéneo: tres grupos comparten migrantes entre sus poblaciones (K1, K2 y K5), mientras que fue bidireccional para los otros dos grupos, siendo el grupo K4 el que recibe más migrantes del resto de los grupos. Se proveen evidencia de que la hipótesis de IBE es la causal más importante de la estructura genética observada, donde las variables de mayor influencia fueron: elevación, Bio7, Bio5, y la variable estructural Importancia del fragmento (dPC) para mantener la conectividad. Éstas variables limitan el flujo genético entre las poblaciones y promueven la divergencia genética y ecológica de los linajes de pinos estudiados. Se recomienda conservar las poblaciones con números censales bajos y con poca endogamia, así como hacer uso de los grupos genéticos para realizar acciones de restauración, de tal forma que se obtenga germoplasma idóneo para cada área.

Re-evaluating the phylogenomic landscape of *Fraxinus* section *Melioides*

Zane Smith

Introduction

In 2022, emerald ash borer (EAB) was first reported in the western United States (US). This long-feared expansion of the invasive range of EAB now threatens countless western ash trees (*Fraxinus* spp.) after two decades of devastating mortality throughout the eastern US. Despite extensive efforts to slow the advance of EAB, no scalable solutions have been identified. While morphological characteristics have been the primary method of species identification for germplasm conservation and a lingering ash breeding program, these efforts have been impeded by overlapping phenotypic diversity among ash species. This trend, acknowledged even among morphology experts, is exacerbated by cryptic polyploidy and hybridization. The rapid decline of eastern Fraxinus, and soon western Fraxinus, creates urgency to investigate the complex species relationships represented within the genus and delineate species to support much needed conservation and breeding.

Methods:

To streamline the application of conservation effort, *Fraxinus* (n=190; primarily from *Fraxinus* section *Melioides*) were sampled throughout the continental US, morphologically evaluated, and vouchered by experts in *Fraxinus* taxonomy and morphology. We then employed reduced representation sequencing (RADseq) and flow cytometry to re-evaluate species relationships among North American (NA) *Fraxinus* using a maximum likelihood phylogenetic reconstruction based on 51,400 genome-wide SNPs.

Results:

Using both genotype and cytotype information, we propose that several cryptic polyploid *Fraxinus* are on unique, stable, evolutionary trajectories from their closely related diploid counterparts, including pumpkin ash (*F. profunda*; 8n), Biltmore ash *(F. biltmoreana*; 6n), and a novel species in the southeastern United States (F. sp. nov.; 4n). Despite evidence for ploidy series in the western US, there is substantial remaining taxonomic ambiguity, evidencing the need for further sample profiling in this region. While initial morphological surveys were broadly successful, hybridity and phenotypic plasticity introduced potential confounding factors in species identification in several specimens.

Conclusions:

This study supports that genome duplication events have impacted the evolutionary trajectory of *Fraxinus* section *Melioides*, as exemplified by a currently undescribed tetraploid species in Eastern NA. While ambiguities remain, particularly in the Western NA species, this study offers substantial evidence that ex situ germplasm efforts should be re-evaluated and that collections of conservation concern should be genotyped to accurately identify species relationships. Furthermore, the preliminary sequencing efforts undertaken here serve as a valuable foundation to guide higher resolution sequencing efforts (whole-genome resequencing) and secondary morphological evaluation to further clarify the taxonomy of *Fraxinus* section *Melioides*."

Unveiling leaf ontogeny in pines: Integrating morphoanatomical analysis with differential gene expression patterns in *Pinus cembroides* (*PINACEAE*)

Lluvia Giselle Álvarez Soto

Pine trees undergo significant changes in leaf development throughout ontogeny. The juvenile (primary) leaves exhibit distinct differences from adult leaves in anatomy, shoot organization, and physiological characteristics. Differential gene expression analysis between juvenile leaves and needles has revealed the upregulation of genes associated with lignin, epicuticular waxes,

and cell wall synthesis in juvenile leaves. In this study, we conducted a morphoanatomical examination of the leaves of *Pinus cembroides*, evaluating lignification levels and epicuticular wax coverage in photosynthetic leaves (both juvenile and adult), fascicular leaves, and cataphylls. Juvenile leaves show lower lignification levels and reduced epicuticular wax coverage compared to needles. We interpreted developmental observations considering previously reported gene expression patterns and physiological differences between juvenile and adult leaves in pine trees. This comprehensive analysis contributes valuable insights into the nature of juvenile leaves and underscores the intricate process of leaf development within the genus.

Leveraging replicated common garden experiments to quantify phenotypic plasticity in stomatal traits in *Populus*

Alden J Stone

The capacity to grow, persist, and thrive across a range of environments is a key requisite for many forest tree species. Understanding how trees respond to different environmental conditions can assist in predicting responses to climate change and extreme weather events, such as heat waves and droughts. One way trees respond to local environments is by opening and closing stomata to regulate gas exchange and water loss. Variation in stomata size, density, and frequency on adaxial (upper)- and abaxial (lower)- leaf surfaces can impact gas exchange and influence physiological function across environments. In this study, I am examining stomatal trait variation for 48 genotypes of *Populus trichocarpa*, *P. balsamifera*, and their hybrids sampled across their native distribution. *P. balsamifera* and *P. trichocarpa* are two riparian species with expansive geographic ranges. P. balsamifera spans much of the drier, continental environments of the upper Alaskan Boreal to the sub-Boreal forests in Maine and Canada. Closely related *P. trichocarpa* persists in more maritime climates of the Pacific Northwest. Given this, we expect that P. balsamifera has evolved higher water-use efficiency relative to *P. trichocarpa*, particularly as *P. trichocarpa* exhibits amphistomaty–stomata occurrence on both adaxial and abaxial leaf surfaces–a trait less prevalent in P. balsamifera. The two species are sympatric and hybridize along river valleys throughout northwestern U.S. and Canada. Genotypes spanning both parent species and their hybrids were collected across repeated latitudinally distributed hybrid zones. Plants were then clonally propagated and planted in 12 common garden experiments distributed across North America. We are comparing stomatal morphology of replicated clones across these novel environments. Leaves were sampled and scanned from individuals across all 12 common gardens. Using ImageJ software

and Stomata Counter, stomatal size and density for adaxial and abaxial leaf surfaces was measured. We predict variation in size and density can be affected by the local environment during leaf development, therefore we expect substantial phenotypic plasticity for stomatal traits across genotypes. Quantifying the effect of localized environments on stomatal trait plasticity, we may be able to use our understanding of stomatal trait variation to predict physiological responses across environments. Ultimately, predicting how trees will respond to environmental changes will be critical to forest management in a changing climate.

Tale of the terpenes: analysis of molecular mechanisms across hemlock species

Vidya Vuruputoor

Background:
Introduced in the 1920s, the hemlock woolly adelgid (HWA- *Adelges tsugae*), an adelgid native to East Asia, has caused massive destruction to *Tsuga canadensis* (Eastern hemlock) forests. By feeding on the phloem, HWA can kill hemlock individuals within five to ten years of infestation. While *T. canadensis* and *T. caroliniana* (Carolina hemlock) are the most susceptible to HWA, East Asian hemlock species – *T. diversifolia*, and *T. ulleungensis* – are tolerant, and *T. chinensis* (Chinese hemlock) resistant. The level of tolerance exhibited by each hemlock species is a consequence of (i) ecological factors – sun, shade, and temperature fluctuations play a major role in the success of Tsuga tolerance towards HWA; and (ii) physiology – Asian species have, on average, thicker cuticles compared to *T. canadensis* and *T. caroliniana*, thereby physically obstructing the HWA to feed on the phloem, likely due to co-evolution with HWA. In this study, we seek to understand patterns of molecular evolution in terpenoid biosynthesis pathway genes across the Tsuga phylogeny using transcriptomic and metabolomic analyses.

Methods:
We paired terpenoid profiles generated through GC/MS and across three hemlock species (*T. canadensis*, *T. ulleungensis*, and *T. heterophylla*) with gene expression to assess trends of terpenoid production. For our broader analysis, we generated transcriptomes for all nine key hemlock species and 10 other gymnosperms. Key terpenoid pathway genes, including terpene synthases (TPS) and genes regulating the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways, were detected in the transcriptomes using HMMER, and HyPhy was used to detect signatures of positive selection.

Results:

Preliminary GC/MS data showed that *T. canadensis* produced the highest amounts of isobornyl acetate and lower levels of beta-caryophyllene and alpha-humulene. Transcriptomics correlated with this metabolomic profile, indicating gene family contractions/expansions underlie terpenoid biosynthesis. Extending across hemlock species and gymnosperms will elucidate patterns of lineage-specific terpenoid biosynthesis pathways across *Tsuga*.

Conclusions:

The integrated genomic and metabolomic data generated from this study directly informs a complementary project involving a year-long effort to assess terpenoid metabolite production across the hemlock species comprehensively.

Differential expression of βglu1 gene in *Picea glauca* and *P abies*

Ernest Ting Yu Wu

Background:

Forest trees are attacked by many species of insect pests, making their ability to defend against their attacks important to ecosystem function. In *Picea glauca*, white spruce, a defence-related gene, βglu1, is responsible for releasing phenolic compounds (acetophenones) as a chemical defence against an insect defoliator, *Choristoneura fumiferana*, eastern spruce budworm,. βglu1 is also expressed in *Picea abies*, Norway spruce, although *C. fumiferana* is not present within the species natural range. We aim to compare the range-wide variation of βglu1 transcript levels in North America for *P. glauca* and Europe for *P. abies* and understand if adaptive forces drive this variation.

Methods:

We obtained foliage samples of populations spanning the entire range distribution of *P. glauca* and *P. abies* from common gardens in Calling Lake, Alberta, Canada and Saldus, Latvia, respectively. We quantified βglu1 transcript levels using RT-qPCR, and on a subset of samples, targeted transcriptome sequencing was conducted to quantify the expression levels of each of the five and three full-length βglu1 gene forms present in *P. glauca* and *P. abies*, respectively.

Results:

βglu1 transcript levels are correlated between RT-qPCR and targeted transcriptome sequencing results, both at the individual gene form level and at the total expression level of all gene forms, especially in *P. glauca*. Overall, wide

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ranges of variation in βglu1 transcript levels were observed both within and between populations in both species. However, only in *P. glauca*, a significant longitudinal gradient was observed, with one βglu1 gene form being differentially expressed across populations, but not in *P. abies*. Eastern *P. glauca* populations, which historically had higher number of *C. fumiferana* outbreaks, have higher βglu1 transcript levels than western *P. glauca* populations, with fewer to no outbreaks. A linear mixed effects models suggest that *C. fumiferana* outbreak class was a significant explanatory variable for βglu1 transcript levels in *P. glauca*, whereas climate per se was not a significant explanatory factor in either species.

Conclusions:
Our understanding of the adaptive variation of the acetophenone defences is important given the changes in the frequency and intensity of insect outbreak due to climate change. The large range of variation in our data suggests that βglu1 expression levels may still be under active selection, with both the population demographic history of the species and external selective forces modulating this variation. The geographic pattern of βglu1 transcript level in *P. glauca* corresponds well with the current patterns of defoliation of *C. fumiferana*. However, for *P. abies*, we need a better understanding of the causes of variation in βglu1 expression level.

Mass selection of *Pinus patula* in Brazil for wood production and quality

(NEW)

Ananda Virginia de Aguiar1, Fernanda Neves Lima, Wanderley dos Santos, Julio Cesar Chalegre Munhoz, Laura Orloski de Andrade Moura Castro[5], Bruno Marchetti Souza, Jorge Luis Monteiro de Matos[5], Ivan Venson, José Guillherme Prata[5], Diego Torres Dini[6], Jarbas Yukio Shimizu.

Pinus patula is a species of pine that is few planted in Brazil. However, plantations and trials with this species have shown rapid growth and adaptability to different environmental conditions. Therefore, the species emerges as an option for plantations where there is a higher frequency of attacks by monkeys on species of the genus *Pinus*. Attacks by these monkeys are less frequent in this species, making it an attractive option both for planting and for forming borders of *Pinus taeda* plantations, for example. The damage caused by the monkeys includes girdling, characterized by partial removal of the bark, and ring peeling, leading to the death of the affected plant. Thus, the proposal was to carry out a mass selection in a forest plantation of *P. patula* based on stem shape and growth traits. The plantation was established in the region of Lages, SC. After selecting the 50 plus trees in 5 ha, evaluations of

growth traits (diameter at breast height) and stem quality (straightness, branch angle and thickness), and wood density were carried out. Trees with calluses, bifurcation and other defects were not considered in the evaluation. The data were subjected to descriptive analysis and phenotypic raking. The general means and coefficients of variation for the traits were, respectively, 40.44 cm and 13.68 % for DBH (diameter at breast height), 9.05 and 12.28 % for branch angle, 8.77 and 18.26% for thickness, 0.46 g/cm³ and 32.67% for wood density in the bark-to-pith direction, respectively. The selected trees present expressive phenotypic variation for the traits of growth and wood quality and may be part of the base population for genetic improvement of the species.

Date	Time	Shuttle Schedule
	7:30pm	Hotel Fortin to Central Oaxaca
June 10	9:30pm	Central Oaxaca to Hotel Fortin (first bus)
	10:30pm	Central Oaxaca to Hotel Fortin (last bus)
	7:30pm	Hotel Fortin to Central Oaxaca
June 11	9:30pm	Central Oaxaca to Hotel Fortin (first bus)
	10:30pm	Central Oaxaca to Hotel Fortin (last bus)
	7:30pm	Hotel Fortin to Central Oaxaca
June 12	9:30pm	Central Oaxaca to Hotel Fortin (first bus)
	10:30pm	Central Oaxaca to Hotel Fortin (last bus)
	7:00pm	Hotel Fortin to Hacienda Santa Martha Etla
June 13	11:00pm	Hacienda Santa Martha Etla to Hotel Fortin
	11:00pm	Hacienda Santa Martha Etla to Central Oaxaca

Shuttle Schedule

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Hotel Fortin Plaza Map

SALON PEGASO

GRAN SALON

PLAZA I

PLAZA II

PLAZA III

SALON PLATINO

PORTAL

SALON JADE

ALBERCA
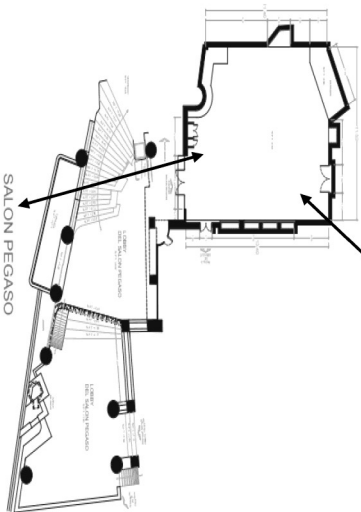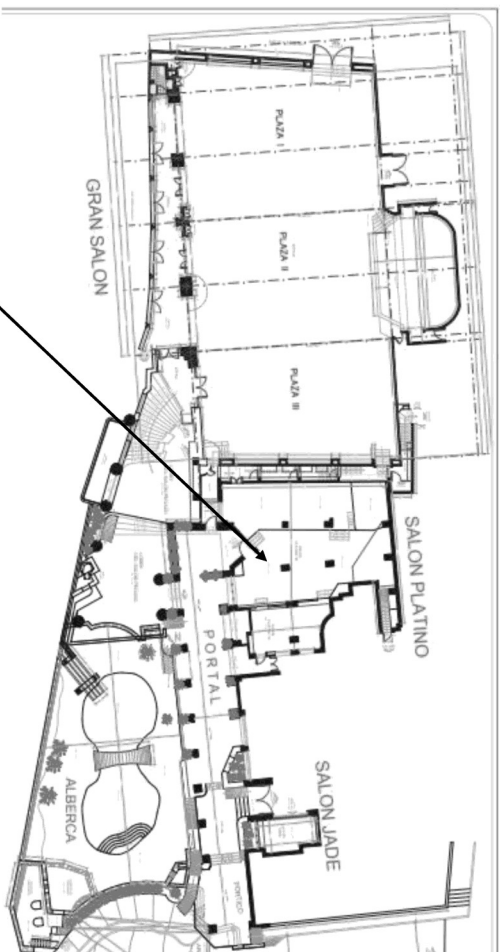
Salón Shedron Y Champagne 2do Nivel, Junto a restaurante.

SALON CHAMPAGNE

TERRAZA

SALON SHEDRON

VESTIBULO DEL RESTAURANTE
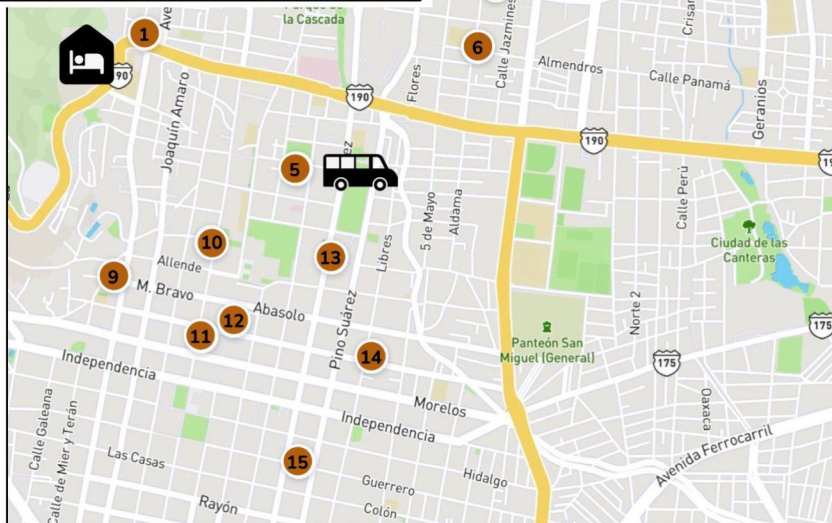
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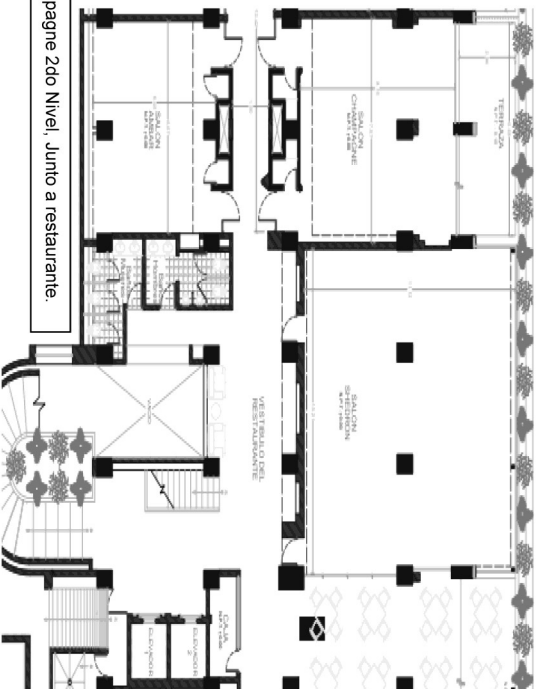
Oaxaca Area Map
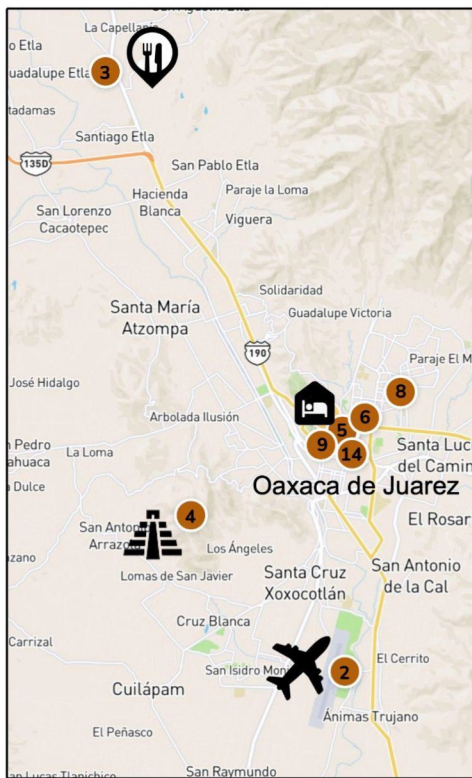
1. Hotel Fortin
2. Oaxaca International Airport ✈
3. Hacienda Sta. Martha de Barcena (Banquet)
4. Monte Alban
5. Shuttle Pickup (Hotel Casa Conzatti)

Restaurants
6. Sirilo Cocina Folk
7. Itanoní
8. Don Juanito
9. Espacio Luvina
10. La Popular & Zandunga Sabor Istmeño
11. La Matatena Pizzería
12. Casa Mayordomo
13. Taniperla
14. Lechoncito de Oro
15. In Situ Mezcalería

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Notes

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Notes

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