Examples of Genomic Data Used for Wood Developmental Biology and Woody Biomass Research

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What We Would Like to Know...

- **Developmental Biology:**
  - How is a cambium initiated and maintained?
  - How is cell differentiation regulated?
  - How are secondary vascular tissues patterned and how do they acquire polarity?

- **Evolutionary Biology:**
  - What are the evolutionary origins of secondary growth?
  - What are the ancestral mechanisms?
  - How was observed phenotypic variation generated?
Developmental Genetics Using Recombinant DNAs in *Populus*

- Recombinant DNAs produce dominant phenotypes that can be scored in primary transformants

- Reverse genetics
  - Misexpression
  - RNA interference, synthetic miRNAs

- Gene and enhancer traps
  - Provide precise descriptions of gene expression
  - Enable gene expression-based anatomical studies that can reveal surprising functional relationships among cell types, tissues, and organs
Testing Evolutionary and Functional Relationships Between the Shoot Apical Meristem and Cambium

Shoot Apex

- Patterning
- Polarity
- Recruitment & differentiation
- Meristematic cells

Stem

- Patterning
- Polarity
- Recruitment & differentiation
- Meristematic cells
An Example of Developmental Genetics in Trees: Functional Characterization of Small Gene Families Encoding Transcription Factors that Regulate Fundamental Shoot Apical Meristem Functions

• How are cambium cells specified and maintained in a meristematic state?
  - Class I KNOX

• How are secondary vascular tissues patterned and polarized?
  - Class III HD ZIPs
Class I KNOX-Regulation of the Cambium and Secondary Growth

- ARKs regulate differentiation of cambial daughter cells.
- ARKs regulate diverse genes to affect complex wood phenotypes.
- ARKs regulate lignin and secondary cell wall synthesis.
- ARK2 negatively affects polar auxin transport
Class III HD ZIPs Regulate Cambium Initiation, Tissue Patterning and Differentiation

- Class III HD ZIPs expression is not limited to presumed adaxial tissues in stems undergoing secondary growth.

- popREV promotes cambium initiation and patterning.

- popATHB15 negatively regulates tissue/cell differentiation

Image from Dr. David Webb
Reconstructing Transcriptional Networks Underlying Secondary Growth

• Changes in transcription highly correlated with $2^\circ$ vascular development

• Comprehensively model the complexity of regulatory networks

• Predictive models serve as a testbed for future research and applications

Regulatory Networks Controlling Hormone Signaling During Woody Growth of Forest Trees. USDA AFRI, Vladimir Filkov coPI.
Poplar hybrid crosses often produce dosage variants.
Triploid poplar are often robust


Right: TxD - triploid TTD, totally unaffected.

(Photograph courtesy of Cees van Ooysten)
Objectives

1. Survey genomic composition in commercial pedigrees of Populus F1 hybrids.

2. Manipulate gametic contribution for functional genomic and germplasm enhancement

3. Correlate variation in karyotype, gene dosage, and transcriptional modules with superior biomass production
Methods

Karyotyping and genotyping by sequencing
### Aim I: Progress to date

<table>
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<th>Genotype</th>
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<th>Triploid found</th>
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<th>Origin of 2N gamete</th>
<th>Aneuploid found</th>
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<td>TDD</td>
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<td>1 (+2 potential)</td>
<td>DMM</td>
<td>Pollen</td>
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<tr>
<td>D x N</td>
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</table>
**Aim II: produce and characterize karyotype variants**

- Pollen from male *P. nigra* tree (from Greenwood Res., OR)
- Pollen from male *P. nigra* tree (from NRRI, MN)

  **Gamma-irradiation of pollen, creation of a kill curve**

  - Cross to female *P. deltoides* branches in Placerville (CA)
  - Cross to female *P. deltoides* branches at Greenwood Res. (OR)
  - Cross to female *P. deltoides* branches at NRRI (MN)

  **Gamma-irradiation to create deletion variants**

- Pollen from 2 male TTD and branches from one female (TTD) triploid trees (from Greenwood Res., OR)

  **Cross to diploid T. deltoides and T. trichocarpa at Greenwood Res. (OR)**

  **Interploidy crosses to produce aneuploids**
Aim III: Planned experiments

Questions to address in aim III

• How does gene expression vary in response to karyotypic change?

• Can we identify genes similarly regulated in all heterotic hybrids, irrespective of their karyotype?

• Are there specific classes of genes that are non-additively regulated in Populus hybrids?

  • Can they explain the heterotic response?

• Is expressed biased towards alleles of one of the other parental species?
Acknowledgements

**Populus Gene and Enhancer Traps**
Gayle Dupper, US Forest Service
Rob Martienssen, Cold Spring Harbor
Rick Meilan, Purdue
Steve Strauss, Oregon State University

**Wood Development Transcriptional Networks**
Eriko Miura, US Forest Service
Lijun Liu, US Forest Service
Suzanne Gerttula, US Forest Service
Vladimir Filkov, UC Davis (coPI)

**Populus Hybrids**
Annie Mix, US Forest Service
Eriko Miura, US Forest Service
Isabelle Henry, UC Davis
Luca Comai, UC Davis (PI)