Contrasting Mechanisms of Sex Determination in the Salicaceae

Steve DiFazio
PAG
1/11/2015

Introduction

- The Salicaceae are a large family of primarily woody species with worldwide commercial and ecological importance
- Two important genera in temperate environments: *Populus* and *Salix*
- Extensive genomic and genetic resources are available for both genera
- Excellent opportunity to use comparative genomics to apply comparative genomics approaches

<table>
<thead>
<tr>
<th>Populus</th>
<th>Salix</th>
</tr>
</thead>
<tbody>
<tr>
<td>~30 species</td>
<td>~400 species</td>
</tr>
<tr>
<td>Wind pollinated</td>
<td>Primarily insect pollinated</td>
</tr>
<tr>
<td>Trees</td>
<td>Mosaically shrubs</td>
</tr>
</tbody>
</table>

Key Shared Characteristics
- Woody
- Fast-growing
- Dioecious: separate males and females
- Phenolic glycoside defense compounds
- Riparian pioneer species
- 1N=19 chromosomes (plus polyploidy)
- Extensive hybridization
- Relatively small genomes: 300 Mb to 500 Mb
- Shared whole genome duplication

*Salix purpurea* Genome Sequence

- Based on 125X coverage of paired Illumina GAII and HiSeq sequences plus 10X sequence coverage from mate-pair libraries (4500-6500 bp)

<table>
<thead>
<tr>
<th></th>
<th>Total length</th>
<th>L50</th>
<th>Min</th>
<th>Max</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contigs</td>
<td>348,752,128</td>
<td>45,586</td>
<td>129</td>
<td>783,829</td>
<td>24,653</td>
</tr>
<tr>
<td>Scaffolds</td>
<td>392,048,189</td>
<td>190,822</td>
<td>542</td>
<td>2,215,764</td>
<td>9,287</td>
</tr>
</tbody>
</table>

- High haplotype diversity (SNPs and indels) likely caused very fragmented assembly
- Dense genetic mapping for chromosomal assembly

Crossing Design for F₂ Family

<table>
<thead>
<tr>
<th>Map</th>
<th>Markers</th>
<th>Lbs</th>
<th>Total Length</th>
<th>Max LG Length</th>
<th>Mean LG Length</th>
<th>Mean inter-marker length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male BC</td>
<td>2211</td>
<td>22</td>
<td>2159.6 cM</td>
<td>545.1 cM</td>
<td>216.3 cM</td>
<td>2.2 cM</td>
</tr>
<tr>
<td>Female BC</td>
<td>2623</td>
<td>22</td>
<td>4219.3 cM</td>
<td>379.1 cM</td>
<td>201.8 cM</td>
<td>1.6 cM</td>
</tr>
<tr>
<td>Intersexual</td>
<td>3637</td>
<td>30</td>
<td>3418.8 cM</td>
<td>301.6 cM</td>
<td>113.9 cM</td>
<td>0.91 cM</td>
</tr>
</tbody>
</table>

- Overall high collinearity with *Populus*, despite the fact that *Populus* was not used as a guide in producing the assembly
- One striking difference is a chromosomal fusion/fission event involving chromosomes 1 and 16
Why Sex?

- It is a key adaptive trait of central evolutionary importance
- Commercial importance
  - Some evidence of sex dimorphism in the Salicaceae: Males more stress-tolerant? Faster-growing?
  - Males are generally preferred horticulturally
  - Breeding programs can balance their sex ratios at the seedling stage
  - Sterility is an important trait for transgene containment and may affect productivity and horticultural value

Sex in Plants

- Dioecy has evolved independently in many plant lineages
- Mostly autosomally determined
- Some evidence of sex chromosomes in a few taxa (e.g., Carica, Cannabis, Rumex, Silene, Populus)
- Main characteristics of sex chromosomes:
  - sex determination loci,
  - suppressed recombination,
  - structural complexity (divergent haplotypes),
  - high repeat content

Sex Determination in *Populus*

Chr19 as a sex chromosome in *Populus*

Tuskan et al. 2012. TGG 8:559
Populus trichocarpa x deltoides Gender QTL

- Gender mapped for 35 males and 83 females using R/QTL binary trait mapping
- Same region identified on Chr19 as found P. deltoides and P. nigra
- Suggestive peaks on Chr5 and Chr10, also supported by association analysis

Association Analysis of Sex Determination in P. trichocarpa

- Sex determination mapped in 124 unrelated individuals using genome-wide SNPs (69 males and 55 females)
- Multiple loci associated with sex determination
- Assembly artifacts? Some of these regions were originally on Chr19 in the v1 assembly

Salix purpurea Gender QTL

- Gender mapped for 153 progeny using same methods as for Populus
- Sex mapped as a single primary locus in all three maps (intercross shown above)
- Sex determination appears to be localized to Chr15 in this family

Map positions of sex determination QTL

Sex Determination in Salix

- Gender mapped for 35 males and 83 females using R/QTL binary trait mapping
- Same region identified on Chr19 as found P. deltoides and P. nigra
- Suggestive peaks on Chr5 and Chr10, also supported by association analysis

Whole Genome Association Analysis for Gender in Salix

- 39 males and 34 females
- 23,000 GBS markers
- Mixed linear model with PCs and kinship
Summary: Genetic Control of Sex

- *P. trichocarpa* clearly has sex determination on Chr19, with some possible accessory loci.
- In contrast, sex determination maps to Chr15 in *Salix* both in controlled crosses as well as in an unrelated association population.
- Are there shared mechanisms of sex determination or is dioecy independently evolved?

Comparing the Salicaceae Sex Chromosomes

Comparison of Orthologous Chromosomes

Comparison of Sex Determination Regions
Genes in Translocated Segment

- Shoot meristem
  - Shoot apex
  - Axillary bud
- Leaves
  - Leaves (LPI #1)
  - Leaves (LPI #2)
  - Leaves (LPI #5)
- Floral
  - Female floral bud
  - Male floral bud
  - Female flowers
  - Male flowers
  - Seed capsules
- Seed
  - Seed
  - Germinating seedling (24 hr)
  - Germinating seedling (48 hr)
- Root
  - Roots (tissue culture)
  - Roots (field)
- Stem
  - Xylem
  - Xylem + pith
  - Phloem/cambium
  - Phloem/cambium + cortex

Microarray Expression, Poplar

- Three of the candidate genes had high expression in floral tissues
- Need data from differentiating floral meristems
- JGI Community Science Project in 2015

XY vs ZW

Genotypic Differences between Females and Males in Sex Determination Regions of *Salix* and *Populus*

*Salix* Genotypic Differences between Females (top) and Males (bottom) (snapshot of region)

Loci Associated with Gender
Female is the Heterogametic Sex in Salix

• There is a preponderance of female heterozygous genotypes and male homozygous genotypes at sex determining loci
• Many loci are null in males
• The reference genome is from a female and likely consists of a mix of Z and W chromosome pieces
• *Populus trichocarpa* is an XY system

Conclusions

• The *Salix purpurea* genome provides tremendous potential for comparative genomics with *Populus*
• Sex determination offers a powerful case study
• Sex chromosomes are at an extremely early stage of development, and are apparently labile
• Sex heterogamety is also variable, with a ZW system in place in *Salix* and an XY system in *Populus*
• This provides opportunities to study the evolutionary consequences of heterogamety
  - How prevalent is Haldane’s rule? Is the heterogametic sex typically less fit in hybrid crosses?
  - How does sex dimorphism and sexual selection differ between XY and ZW systems?
Acknowledgements

• Ran Zhou, Eli Rodgers-Melnick, Luke Evans

• Larry Smart, Fred Gouker, Craig Carlson

• Haibao Tang, Chris Town, Vivek Krishnakumar

• Jerry Tuskan and the JGI and Phytozome teams

• Funding: USDA SUN Grant and USDA CAP project, NEWBio