Marker Development

Honey Locust / Northern Red Oak / Tulip
Poplar / Black Walnut / Other

Courtesy Jeanne Romero-Severson
Annual meeting Hardwood Genomics, Memphis September 21-23

MICHIGAN TECHNOLOGICAL UNIVERSITY

Contributor: Sandra Owusu (Ph.D. student on project).
Alexis Sullivan (Master student, other funds)
Oliver Gailing (Ph.D.)
<table>
<thead>
<tr>
<th>From</th>
<th>Total</th>
<th>Amplified (%)</th>
<th>Polymorphic (%)</th>
<th>Informative for mapping</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Medicago truncatula</em>¹,²</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ceratonia siliqua</em>³</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ceratonia siliqua</em>³</td>
<td>51</td>
<td>6 (~12)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Copaifera officinalis</em>⁴</td>
<td>24</td>
<td>11 (~46)</td>
<td>4 (~ 17)</td>
<td>0</td>
</tr>
</tbody>
</table>

¹- Eujayl et al. 2004; 2- Gupta and Prasad 2009; 3 - Caruso et al. 2008; 4 – National Center for Biotechnology Information (NCBI) database
### Table 2 Genomic SSR development in honey locust

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of marker</th>
<th>Markers tested</th>
<th>Amplified (%)</th>
<th>Polymorphic (%)</th>
<th>Informative for mapping (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. triacanthos</em></td>
<td>gSSR$^1$</td>
<td>72</td>
<td>72 (100)</td>
<td>5 (~7)</td>
<td>5 (~7)</td>
</tr>
<tr>
<td><em>G. triacanthos</em></td>
<td>gSSR$^2$</td>
<td>24</td>
<td>24 (100)</td>
<td>9 (~38)</td>
<td>9 (~38)</td>
</tr>
</tbody>
</table>

Genomic SSR development using Illumina sequencing of SSR-enriched libraries (Richard Cronn, Tara Jennings, PNW Research Station, USDA Forest Service, 3200 SW Jefferson Way, Corvallis, OR 97331)

1 - from original honey locust list of gSSRs
2 - from final honey locust gSSR list- Meg Staton
<table>
<thead>
<tr>
<th>Locus (fluorescent label)</th>
<th>Repeat motif</th>
<th>Forward primer sequence (5'-3')</th>
<th>Reverse primer sequence (5'-3')</th>
<th>Ta (°C)</th>
<th>Allele size range (bp)</th>
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</thead>
<tbody>
<tr>
<td>GLT002</td>
<td>(AT)$_9$</td>
<td>NED-taaaaagtaaccttaaagg</td>
<td>agtaaagaggtaacgattt</td>
<td>56</td>
<td>103-149</td>
</tr>
<tr>
<td>GLT021</td>
<td>(TC)$_{12}$</td>
<td>6-FAM-atatcaccaatttaagacc</td>
<td>gtacacaaaaccttcgagag</td>
<td>56</td>
<td>94-98</td>
</tr>
<tr>
<td>GLT026</td>
<td>(TA)$_{15}$</td>
<td>VIC-aagcttgattagagaaatt</td>
<td>agatagttctttcagttg</td>
<td>56</td>
<td>87-143</td>
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<tr>
<td>GLT4021</td>
<td>(TAAA)$_7$</td>
<td>6-FAM-ggagtacttttttaggttgatt</td>
<td>atcaacgttatagtgacctt</td>
<td>56</td>
<td>117-123</td>
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<tr>
<td>GLT4027</td>
<td>(TCCA)$_6$</td>
<td>6-FAM-aggaattattttctctaccaaa</td>
<td>cgaatctcattttatacaca</td>
<td>56</td>
<td>90-106</td>
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</tbody>
</table>
Table 4 Genetic variation at five microsatellite loci as characterized in 36 honey locust samples from 24 populations of a provenance test (Kellogg Farm, Michigan State University, MI)

<table>
<thead>
<tr>
<th>Locus</th>
<th>$N_a$</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>$F_{IS}$</th>
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</thead>
<tbody>
<tr>
<td>GLT002</td>
<td>19</td>
<td>0.886</td>
<td>0.914</td>
<td>0.031</td>
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<tr>
<td>GLT021</td>
<td>3</td>
<td>0.361</td>
<td>0.537</td>
<td>0.327</td>
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<tr>
<td>GLT026</td>
<td>13</td>
<td>0.861</td>
<td>0.880</td>
<td>0.021</td>
</tr>
<tr>
<td>GLT4021</td>
<td>4</td>
<td>0.556</td>
<td>0.671</td>
<td>0.172</td>
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<tr>
<td>GLT4027</td>
<td>5</td>
<td>0.676</td>
<td>0.729</td>
<td>0.072</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>8.800</strong></td>
<td><strong>0.668</strong></td>
<td><strong>0.746</strong></td>
<td><strong>0.125</strong></td>
</tr>
</tbody>
</table>

Parameters calculated: observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), inbreeding coefficient ($F_{IS}$) and number of alleles ($N_a$). All parameters were calculated in GENALEX v.6.41 (Peakall and Smouse 2006).
### Table 5 Transferability of *Q. robur* EST-SSRs (Durand et al. 2010) to other *Quercus* species

<table>
<thead>
<tr>
<th>From</th>
<th>To</th>
<th>Type of marker</th>
<th>Total</th>
<th>Amplified</th>
<th>Polymorphic</th>
<th>Linkage groups covered</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Q. robur</em></td>
<td><em>Q. alba</em></td>
<td>EST-SSR</td>
<td>149</td>
<td>125</td>
<td>54</td>
<td>1 - to - 12</td>
</tr>
<tr>
<td><em>Q. robur</em></td>
<td><em>Q. coccinea</em></td>
<td>EST-SSR</td>
<td>90</td>
<td>73</td>
<td>38</td>
<td>1 - to - 12</td>
</tr>
<tr>
<td><em>Q. robur</em></td>
<td><em>Q. ellipsoidalis</em></td>
<td>EST-SSR</td>
<td>90</td>
<td>73</td>
<td>38</td>
<td>1 - to - 12</td>
</tr>
<tr>
<td><em>Q. robur</em></td>
<td><em>Q. rubra</em></td>
<td>EST-SSR</td>
<td>167</td>
<td>150</td>
<td>52</td>
<td>1 - to - 12</td>
</tr>
<tr>
<td><em>Q. robur</em></td>
<td><em>Q. velutina</em></td>
<td>EST-SSR</td>
<td>90</td>
<td>73</td>
<td>38</td>
<td>1 - to - 12</td>
</tr>
</tbody>
</table>


20 EST-SSR markers developed for *Q. robur* (Durand et al. 2010) and 8 gSSR markers (Sullivan et al. 2012), have been characterized in 262 samples from *Q. ellipsoidalis, Q. velutina, Q. rubra* and *Q. coccinea.*
Contribution to goals and anticipated activities

Goals
Honey locust: gSSR development for paternity exclusion (10 markers)
Red oak: transfer of EST-SSRs from *Q. robur*

Anticipated activities Year 3:
Honey Locust: identification of full-sib mapping pedigree, development of framework map
Additional activities: gSSR development in sugar maple, test of EST-SSRs in *Q. alba*


Marker Development & Genotyping:

Yellow-poplar (*Liriodendron tulipifera*)

Red bay (*Persea borbonia*)

Ralph Zhang  Dr. Victor Xu
Yellow-poplar cDNA sequence assembly and marker identification

33,745 isotigs (putative individual transcripts)

Of 9,471 SSRs identified with primers: 3,177 show evidence of polymorphism in the sequence data.

27,159 SNPs were identified.

Dr. Meg Staton
Clemson University
# of informative SSR markers for framework linkage map: n x 10=19 x 10=190

Total of EST SSR markers tested: 220
size: 200~350 bp
Repeats: 8-30 nt
Tm: >= 50 °C

# of markers resulted in good PCR amplifications after anneal temperature optimization: 170

[Image of gel electrophoresis with marker numbers and temperature settings]
Construction of yellow-poplar framework linkage map

Full sib seedlings needed: 19 x 20 = 380

629 control pollinated seedlings
685 open pollinated seedlings

Dr. Scott Schlarbaum’s group, University of Tennessee at Knoxville

(by E Schlarbaum)
11 markers for full sib screening
### 7 markers: ABxCC, ABxCD, AAxBB
553 CP seedlings

<table>
<thead>
<tr>
<th>7 markers</th>
<th>6 markers</th>
<th>5 markers</th>
<th>4 markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>vvvvvvvv</td>
<td>vvvvvv/x</td>
<td>vvvvvxx</td>
<td>vvvvxxx</td>
</tr>
<tr>
<td>46 seedlings</td>
<td>7 seedlings</td>
<td>4 seedlings</td>
<td>1 seedlings</td>
</tr>
</tbody>
</table>

X: failed allele calling

97 seedlings with one marker in wrong configuration
66 seedlings with two markers in wrong configuration
### Genetic Map Construction

| Year | Q4 | Q3 | Q2 | Q1 | Q4 | Q3 | Q2 | Q1 | Q4 | Q3 | Q2 | Q1 | Q4 | Q3 | Q2 | Q1 | Q4 | Q3 | Q2 | Q1 |
|------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| -1   | Q4 | Q3 | Q2 | Q1 | Q4 | Q3 | Q2 | Q1 | Q4 | Q3 | Q2 | Q1 | Q4 | Q3 | Q2 | Q1 | Q4 | Q3 | Q2 | Q1 |
| Year 1 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |
| Year 2 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |
| Year 3 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |
| Year 4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |

HD = High Density Map; F = Framework Map

<table>
<thead>
<tr>
<th>Genetic Map Construction</th>
<th>Percent Task Completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Red Oak (HD)</td>
<td>50%</td>
</tr>
<tr>
<td>Black Walnut (HD)</td>
<td>100%</td>
</tr>
<tr>
<td>Sweetgum (F)</td>
<td>100%</td>
</tr>
<tr>
<td>Honey Locust</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Yellow-poplar</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Comparison of genetic diversity between two yellow-poplar seed orchards (Clemson, SC and Knoxville, TN)
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Min # Reps</th>
<th>Max # Reps</th>
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</thead>
<tbody>
<tr>
<td>Base Pairs in Motif</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 bp (Dinucleotides)</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>3 bp (Trinucleotides)</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>4 bp (Tetranucleotides)</td>
<td>6</td>
<td>20</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>SSRS</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Sequences with at least 1 SSR</td>
<td>64,528</td>
<td></td>
</tr>
<tr>
<td>Number of SSRs identified</td>
<td>68,994</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Motif Pattern Length</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2 bp (Dinucleotides)</td>
<td>61,697</td>
<td></td>
</tr>
<tr>
<td>3 bp (Trinucleotides)</td>
<td>6,121</td>
<td></td>
</tr>
<tr>
<td>4 bp (Tetranucleotides)</td>
<td>1,176</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SSRs with PRIMERS</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Sequences with at least 1 SSR with primers</td>
<td>19,014</td>
<td></td>
</tr>
<tr>
<td>Number of SSRs identified with Primers</td>
<td>20,046</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Motif Pattern Length</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2 bp (Dinucleotides)</td>
<td>17,777</td>
<td></td>
</tr>
<tr>
<td>3 bp (Trinucleotides)</td>
<td>1,975</td>
<td></td>
</tr>
<tr>
<td>4 bp (Tetranucleotides)</td>
<td>294</td>
<td></td>
</tr>
</tbody>
</table>

Red Bay gSSRs

Dr. Meg Staton
Clemson University
Goal: Testing of 96 red bay SSR markers

Leaves from 25 red bay trees collected by Oliver Bukles

Testing of 48 markers are being conducted
Red Oak Genetic Mapping

Q. rubra map v.1 (near completion)
- 503 full sibs
  - 50 gSSR’s
  - 22 EST-SSR’s (FGP)
  - 12 AFLP’s
- Goal is 120 markers

QTL Study
- 2 traits
  - Bud burst
  - Marcescence

QTL study and v3 map goals
- 2 years field observations
- 1500 additional markers
  - Penn State EST-SRRs
  - SNP chip SNPs

SM1 (female parent)

Mapping parents in winter.
Note marcescence in SM2
v2: Comparative mapping

Done

Q. robur mapped EST-SSRs
  • Screened: 72 markers from O. Gailing
  • Verified informative: 20

Real Gene informative markers
  • 10 candidate genes (Cold, drought, freeze tolerance)
  • 2 SSR and 8 SNP in informative configurations

Resources under development

Q. robur unmapped EST-SSRs
  • ~ 81,000 ESTs at NCBI.
  • 1552 Q robur EST-SSRs selected by TM criteria

Additional Q. rubra EST-SSRs
  • 568 EST-SSRs (FGP) by TM Criteria

Other Fagaceae EST-SSRs (FGP)
  • 2962 C. mollissima EST-SSRs selected by TM criteria

Goal
  • 300 framework markers and comparative map with Q. robur
Black Walnut Genetic Mapping

- Mapping Population (done)
  - 395 Sparrow x Schlesser
  - 298 full-sibs in ground
  - 162 Sparrow x Emma K

- Parentage Study Information (in progress)
  - Multi-year parentage study
  - Phenology data
  - Pollination neighborhood size

Goals
- Three papers on parentage study done
- Fund genetic and genomics 1000 cankers projects
- Initiate QTL studies on mapping population
Black Walnut Marker Development

Marker Development gSSR’s

- Existing gSSR’s (30)
  - 15 in mappable configuration
  - 15 optimized to $T_m$
- New gSSR’s
- ~14,463 gSSR’s from Oregon State
  - ~8000 usable
  - So far 100 optimized

Marker Development EST’s

- 7996 $J. \text{hindsii} \times J. \text{regia}$
  - 265 potential markers identified
- 78 $J. \text{nigra}$
  - 5 potential markers identified
- 5213 $J. \text{regia}$ EST’s
- 3279 Castanea mollissima ESTs

Goals

- 360 framework markers with Penn State EST-SSRs (v1)
- 1500 SNP chip and Penn State EST-SSR markers (v2)
Black Walnut Genetic Mapping

• Mapping Population (done)
  • 395 Sparrow x Schlesser
  • 298 full-sibs in ground
  • 162 Sparrow x Emma K

• Parentage Study Information (in progress)
  • Multi-year parentage study
  • Phenology data
  • Pollination neighborhood size

Goals
• Three papers on parentage study done
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• Initiate QTL studies on mapping population
Black Walnut Marker Development

Marker Development gSSR’s

- Existing gSSR’s (30)
  - 15 in mappable configuration
  - 15 optimized to $T_m$
- New gSSR’s
- ~14,463 gSSR’s from Oregon State
  - ~8000 usable
  - So far 100 optimized

Marker Development EST’s

- 7996 *J. hindsii* X *J. regia*
  - 265 potential markers identified
- 78 *J. nigra*
  - 5 potential markers identified
- 5213 *J. regia* EST’s
- 3279 *Castanea mollissima* ESTs

Goals

- 360 framework markers with Penn State EST-SSRs (v1)
- 1500 SNP chip and Penn State EST-SSR markers (v2)
Sweetgum Marker Development

Penn State University:
   John Carlson,
   Teo Best,
   Nicole Zembower,
   Charles Addo-Quaye
Sweetgum Marker Development

No previous publications on SSR or SNP markers for Liquidambar

gSSR’s

- 10943 gSSRs identified
  - 2469 loci with primers (potential markers)
    - 2 bp, 2173
    - 3 bp, 234
    - 4 bp, 62
  - Testing not yet started

EST-SSR’s

- RNA sequencing not yet underway for Sweetgum
- Commences year 3
Sweetgum Marker Development

Goal: Test 96 Sweetgum gSSR markers

Leaves collected from 25 sweetgum trees collected from Kim Steiner’s Sweetgum provenance trial DNA to be extracted in October, and screening of markers to commence by November
Sweetgum Marker Development

<table>
<thead>
<tr>
<th>RESEARCH ACTIVITIES</th>
<th>Q4</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q1</th>
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<tbody>
<tr>
<td>Sweetgum</td>
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<td>Treatments; RNA Isolate</td>
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<tr>
<td>Marker Development and Parent / Progeny genotype</td>
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<tr>
<td>Framework genetic map</td>
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</tbody>
</table>

Anticipated activities Year 3 Activities:

- Have gSSR markers ready by end of 2013 for seedling screening
- Conduct RNA isolation and EST sequencing of parent tree tissues
Marker Development for additional species

Sugar Maple – EST database species
Green Ash – EST database species
Black Gum – additional species

<table>
<thead>
<tr>
<th>EST Sequencing</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q4</td>
<td>Q1</td>
<td>Q1</td>
<td>Q1</td>
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<tr>
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<tr>
<td></td>
<td>Q4</td>
<td>Q4</td>
<td>Q4</td>
<td>Q4</td>
</tr>
</tbody>
</table>

Percent Task Completion

- Northern Red Oak, Black Walnut, Sweetgum, Honey Locust, Sugar Maple, Green Ash
Marker Development for additional species

Sugar Maple – EST database species

**gSSR’s**
- 10,329 gSSRs identified
  - 1330 loci with primers (potential markers)
    - 2 bp, 1112
    - 3 bp, 232
    - 4 bp, 53

**EST-SSR’s**
- RNA sequencing not yet underway
- Parent tree RNASEq commences year 3

**SCREENING**
- Testing not yet started
- Seedlings germinating for testing in 2013-14
Marker Development for additional species

Green Ash – EST database species

**gSSR’s**
- 5,690 gSSRs identified (to date)
  - 965 loci with primers (potential markers)
    - 2 bp, 823
    - 3 bp, 123
    - 4 bp, 19

**EST-SSR’s**
- Sequencing of Parent tree and Oxone-treated seedlings RNAs is underway
- Sequencing of other stressed seedlings soon

**SCREENING**
- Testing not yet started
- DNAs extracted from > 100 trees in provenance trial
Marker Development for additional species

Black Gum (*Nyssa sylvatica*)
Representative of Cornales

**gSSR’s**
- 12,207 gSSRs identified
  - 1323 loci with primers (potential markers)
    - 2 bp, 1275
    - 3 bp, 42
    - 4 bp, 6

**EST-SSR’s**
- RNA sequencing not yet underway
- Parent tree RNASeq commences year 3

**SCREENING**
- Testing not yet started
- 200 + seedlings available soon

http://www.hardwoodgenomics.org