Genetic and QTL Analysis of Maize Kernel Composition Traits

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Martin Bohn  
Steve Moose  
Torbert Rocheford
Maize grain is a Great Source of Energy with Many Uses
- Human Food
- Animal Feed
- Industrial Use
  - Starch
  - Sweeteners
  - ETHANOL

➢ Starch Protein Oil
Rationale

- Ninety Generations of Selection for Low Protein Resulted in Strains
  - Approximately 75% Starch & Only 4% Protein

- Evaluate for ILP QTL Alleles that improve Starch concentration in a historically elite background
Objectives

- Map QTL for Starch, Protein, Oil, and kernel Weight in a (ILPXB73)B73 S$_1$, S$_3$ Populations and in the Two Populations Combined

- Perform Principal Components analysis (PCA) on the traits and Map QTL using the Principal Components as Traits

- Compare QTL Across Two Different Generations

- Compare QTL with Other Studies
Material and Methods

Genetic materials

- **150 (ILP X B73) B73 S$_1$ families**

- **138 (ILP X B73) B73 S$_3$ families**
  - 2 Reps in 2002 and 2005

Experimental design

- South Farms of Urbana
- Randomized Alpha (0,1)
- 15 ft plots, 15 plants/row

Diagram:

```
ILP$^{90}$ X B73
  ↓
F1 X B73
  ↓
BC$_1$
  ↓
  ⊗
  ↓
  ↓
BC$_1$S$_2$
  ⊗
  ↓
BC$_1$S$_3$
  ↓
  ⊗
  ↓
  ↓
  ....150 (BC$_1$S$_1$)
  ↓
  ⊗
  ↓
  ↓
  ↓
  ....138 (BC$_1$S$_3$)
```
Material and Methods

- **Phenotypic Data Collection**
  - NIR ~ Starch, Protein & Oil
  - 100K weight

- **Genotypic Data Collection**
  - \(BC_1S_1\)
    - Map 142 markers
      - (1282.5 cM)
  - \(BC_1S_3\)
    - 114 markers
### Means

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Starch</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BC₁S₁</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILP</td>
<td>757.2</td>
<td>55.1</td>
</tr>
<tr>
<td>B73</td>
<td>677.4</td>
<td>115.9</td>
</tr>
<tr>
<td>BC₁S₁</td>
<td>701.1</td>
<td>95.7</td>
</tr>
<tr>
<td><strong>Range (BC₁S₁)</strong></td>
<td>660.9 – 718.5</td>
<td>83.5 – 126.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Starch</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BC₁S₃</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILP</td>
<td>745.0</td>
<td>63.8</td>
</tr>
<tr>
<td>B73</td>
<td>674.3</td>
<td>125.3</td>
</tr>
<tr>
<td>BC₁S₃</td>
<td>689.6</td>
<td>111.6</td>
</tr>
<tr>
<td><strong>Range (BC₁S₃)</strong></td>
<td>656.8 – 709.9</td>
<td>91.1 – 147.1</td>
</tr>
</tbody>
</table>

### Transgressive Segregation

- Families with **Lower Starch** than both parents
- Families with **Higher Protein** than both parents
QTL detected for Individual Traits in BC$_1$S$_1$ Population

- Additive Effects were significant

<table>
<thead>
<tr>
<th>Trait</th>
<th>N*</th>
<th>$R^2$ (%)</th>
<th>p (%)</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>10</td>
<td>39.4</td>
<td>46.8</td>
<td>Most B73</td>
</tr>
<tr>
<td>Oil</td>
<td>9</td>
<td>38.2</td>
<td>44.4</td>
<td>Both</td>
</tr>
<tr>
<td>Starch</td>
<td>8</td>
<td>35.2</td>
<td>54.7</td>
<td>Most ILP</td>
</tr>
<tr>
<td>KWeight</td>
<td>10</td>
<td>38.8</td>
<td>63.1</td>
<td>Most ILP</td>
</tr>
</tbody>
</table>

2 QTL in common between Starch, Protein and Oil
5 QTL in common between Starch and Protein
4 QTL in common between Starch and Oil
## Principal Component Analysis (PCA) BC$_1$S$_1$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue ($\lambda$)</td>
<td>2.19</td>
<td>0.93</td>
<td>0.74</td>
</tr>
<tr>
<td>% of total variation</td>
<td>54.7</td>
<td>23.1</td>
<td>18.6</td>
</tr>
<tr>
<td>eigenvalue (λ)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kernel traits loadings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>-0.63</td>
<td>-0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>Protein</td>
<td>0.58</td>
<td>0.43</td>
<td>-0.20</td>
</tr>
<tr>
<td>Oil</td>
<td>0.41</td>
<td>-0.28</td>
<td>0.87</td>
</tr>
<tr>
<td>Kernel weight</td>
<td>-0.31</td>
<td>0.84</td>
<td>0.43</td>
</tr>
</tbody>
</table>

### Detected QTL

<table>
<thead>
<tr>
<th>PC</th>
<th>N</th>
<th>$R^2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>7</td>
<td>27.7</td>
</tr>
<tr>
<td>PC2</td>
<td>4</td>
<td>24.9</td>
</tr>
<tr>
<td>PC3</td>
<td>5</td>
<td>34.3</td>
</tr>
</tbody>
</table>

**10 QTL** identified for **PCs**

common to QTL found for **Individual Traits**
Kernel weight
Protein Oil Starch

QTL detected for Traits & PCs in BC₁S₁ Population
BC\textsubscript{1}S\textsubscript{3}
QTL detected for Individual Traits in BC$_{1}$S$_{3}$ Population

<table>
<thead>
<tr>
<th>Trait</th>
<th>N</th>
<th>partR$^2$ (%)</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>5</td>
<td>7.7 – 9.4</td>
<td>Most B73</td>
</tr>
<tr>
<td>Oil</td>
<td>2</td>
<td>7.2 ; 8.3</td>
<td>ILP</td>
</tr>
<tr>
<td>Starch</td>
<td>3</td>
<td>7.3 – 12.5</td>
<td>ILP</td>
</tr>
<tr>
<td>KWeight</td>
<td>7</td>
<td>7.7 – 23.2</td>
<td>Both</td>
</tr>
</tbody>
</table>

ILP contributed All the Alleles for Increasing Starch Concentration and Both Alleles for Increasing Oil

Most of the Additive and Dominance Effects on Increasing Protein came from B73 Alleles

No Epistatic Interactions were detected for any of the Traits
Principal Component Analysis (PCA) $BC_{1}S_{3}$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue ($\lambda$)</td>
<td>1.97</td>
<td>0.99</td>
<td>0.94</td>
</tr>
<tr>
<td>% of total variation</td>
<td>48.65</td>
<td>25.21</td>
<td>24.17</td>
</tr>
</tbody>
</table>

Kernel traits loadings

<table>
<thead>
<tr>
<th>Trait</th>
<th>PC</th>
<th>PC</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>0.67</td>
<td>0.12</td>
<td>-0.24</td>
</tr>
<tr>
<td>Protein</td>
<td>-0.68</td>
<td>0.11</td>
<td>0.19</td>
</tr>
<tr>
<td>Oil</td>
<td>0.23</td>
<td>-0.68</td>
<td>0.69</td>
</tr>
<tr>
<td>Kernel weight</td>
<td>0.20</td>
<td>0.72</td>
<td>0.66</td>
</tr>
</tbody>
</table>

11 QTL identified for the PCs were common to QTL identified for Individual Traits

<table>
<thead>
<tr>
<th>PC</th>
<th>N</th>
<th>$PartR^{2}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>8</td>
<td>6.8 – 10.3</td>
</tr>
<tr>
<td>PC2</td>
<td>7</td>
<td>6.8 – 26.3</td>
</tr>
<tr>
<td>PC3</td>
<td>6</td>
<td>7.2 – 13.4</td>
</tr>
</tbody>
</table>
QTL detected for individual traits for the $BC_1S_1$ and $BC_1S_3$ combined analysis

<table>
<thead>
<tr>
<th>Trait</th>
<th>N</th>
<th>$\text{partR}^2$ (%)</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>13</td>
<td>3.2 – 7.7</td>
<td>Most B73</td>
</tr>
<tr>
<td>Oil</td>
<td>7</td>
<td>3.7 – 4.5</td>
<td>ILP</td>
</tr>
<tr>
<td>Starch</td>
<td>15</td>
<td>2.1 – 7.2</td>
<td>Most ILP</td>
</tr>
<tr>
<td>KWeight</td>
<td>14</td>
<td>2.4 – 10.8</td>
<td>Both</td>
</tr>
</tbody>
</table>

ILP contributed Additive Effects for Increasing Starch
Most Additive Effects on Increasing Protein came from B73 Alleles
All Additive and Dominance Effects for Increasing Oil came from B73 Alleles
QTL detected for individual traits in the combined analysis

1.01
1.02
1.03
1.04
1.05
1.06
1.07
1.08
1.09
1.10
1.11
1.12

1 2 3 4 5 6 7 8 9 10

sh1
wx1
sus
du1

bt1
bt2
ae1
su1

sh2

starch protein oil Kernel weight

++
++
+}

+
Summary & Conclusions

BC$_1$S$_1$  BC$_1$S$_3$ and Combined Analysis

- ILP Improved B73 for Starch Composition
- Some QTL were Significant for More than one Trait
  ↓
  Pleiotropic or linked QTL?

- Only One QTL identified for Starch mapped near the location of a Gene known to affect Starch Biosynthesis ($bt\ 1$)
  - Other Genes not in the Pathway Influencing % Starch
    (Transcription Factors ?)
Summary & Conclusions

Comparison of QTL in the Three analysis

- 2 QTL for starch
- 4 QTL for protein
- 2 QTL for kernel weight

Comparison of QTL in this Study with Other Studies

- 11 QTL detected for starch across different genetic backgrounds

MAS

New Gene Discovery
Microarray Experiment
Microarray Data Analysis

- Top families **High & Low Starch** selected
- **BC1S1 & Testcross**
- **Ear and Leaf tissue collected**
  - 10, 15 and 20 DAP

### Conditions

<table>
<thead>
<tr>
<th>BC1S1</th>
<th>Testcross</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004_11(12,13)DAP Kernel</td>
<td>2004_11(12,13)DAP Kernel</td>
</tr>
<tr>
<td>2004_20DAP</td>
<td>2004_20DAP</td>
</tr>
<tr>
<td>2005_15DAP</td>
<td>2005_10DAP</td>
</tr>
<tr>
<td>2005_20DAP</td>
<td>2005_15DAP</td>
</tr>
</tbody>
</table>

**& Leaf**
Some Results

2004 vs 2005 (UP, Union in DAPs, Intersection in years)

15DAP vs 20DAP with 2005 Data

Gene Selection

\[ y_{ijkl} = \mu + \alpha_i + \beta_j + \lambda_k + \delta_l + \lambda \delta_{kl} + \varepsilon_{ijkl} \]

135 Genes selected
Next

- Quantify ESTs - qPCR
- Map ESTs Associated Expression Differences
- Relate EST Map Position to QTL for Starch concentration
- Next Generation Testcross Progenies with Grain Yields Reasonably Similar to Commercial Checks, MAS
Acknowledgements

Dr. Troyer – Troyer - Darwin Fellowship

Field Technicians
Jerry Chandler
Don Roberts

All the members of the lab

Undergrad and High School Students

Questions?
Genetic Materials

(ILP×B73)B73 S₃ × FR616

138 Testcross Lines

<table>
<thead>
<tr>
<th>Testcross</th>
<th>yield Bu/acre</th>
<th>starch g Kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>142.72</td>
<td>653.2</td>
</tr>
<tr>
<td>Range</td>
<td>114.5 - 156.5</td>
<td>645.4 – 655</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Checks</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>105.8 - 178.7</td>
<td>588.0 - 646.6</td>
</tr>
</tbody>
</table>

- Evaluated in 1999, 2001 and 2002
- 12 Reps
- 10 hybrid checks