Inflorescence QTL, Canalization, and Selectable Cryptic Variation

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What is “genetic architecture” …and why should we care?

GENETIC ARCHITECTURE (ANY_trait in ANY ORGANISM)

- QTL number
- QTL effect sizes
- QTL frequencies
- QTL gene action

Heritability ($h^2$)
Response to selection ($h^2_s$)
QTL discovery, mapping, cloning
Similar effect sizes across traits and species

**Mice:** 97 traits in 6 classes

**Humans:** 140 loci for 20 diseases

Flint J, Mackay T F Genome Res. 2009;19:723-733 “Genetic architecture of quantitative traits in mice, flies, and humans”
But plant and animal domesticates may be different

**Dogs:**
3 SNPs explain 38% of variance in body weight

**Tomatoes:**
Heterozygosity at SFT locus increases yield 60%

BUT... only true for purebreds

BUT... only true for determinate varieties

WT/WT    WT/sft    sft/sft
SFT (single flower truss) = FT (florigen)
What forces shape genetic architecture?

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*(ANY TRAIT IN ANY ORGANISM)*

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- QTL gene action

Heritability
Response to selection \((h_2s)\)
QTL discovery, mapping, cloning

Evolutionary history?
Biology?
Genetic “dissection” using 4892 RILs measured in 8 locations
Seven maize inflorescence traits measured

Teosinte

Maize

Inflorescence Traits

- Spike length (SL)
- Tassel length (TL)
- Branch zone (BZ)
- Branch number (BN)
- Cob length (CL)
- Cob diameter (CD)
- Ear row number (ERN)
Compare genetic architecture of 13 developmental traits in the NAM population

**TASSEL**
- Tassel length (TL)
- Spike length (SL)
- Branch zone (BZ)
- Branch number (BN)

**EAR**
- Cob length (CL)
- Cob diameter (CD)
- Ear row number (ERN)

**FLOWERING**
- Days to anthesis (DA)
- Days to silking (DS)
- Anthesis-silking interval (ASI)

**LEAF**
- Leaf length (LL)
- Leaf width (LW)
- Leaf angle (LA)

**DISEASE**
- Southern leaf blight resistance

This study.

Buckler et al. (2009) Science
The genetic architecture of maize flowering time.

Tian et al. (2011) Nature Genetics
Genome-wide association study of leaf architecture in the maize nested association mapping population.

Kump et al. (2011) Nature Genetics
Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population.
Outline

Effect sizes

Pleiotropy

Predictive ability of QTL models

Candidate genes

What affects genetic architecture?
# Two analyses: Joint linkage and GWAS

<table>
<thead>
<tr>
<th></th>
<th>Joint Linkage (JL)</th>
<th>Genome-wide association (GWAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model</strong></td>
<td>Phenotype = mean + pop + sum(QTL*pop effects) + error</td>
<td>Phenotype = mean + sum(SNP effects) + error</td>
</tr>
<tr>
<td><strong>Genetic information</strong></td>
<td>836 SNPs scored in 4892 RILs</td>
<td>836 SNPs scored in 4892 RILs, and 1.6 million SNPs scored in 27 parental lines</td>
</tr>
<tr>
<td><strong>Model fitting</strong></td>
<td>Stepwise regression, whole genome simultaneously</td>
<td>Stepwise regression, each chromosome separately</td>
</tr>
<tr>
<td><strong>Phenotypic data</strong></td>
<td>Best linear unbiased predictors (BLUPs)</td>
<td>Residuals calculated from a joint linkage model excluding QTL on the chromosome under consideration</td>
</tr>
<tr>
<td><strong>Significance threshold</strong></td>
<td>alpha = .001, determined by permutation</td>
<td>alpha = .05, determined by permutation</td>
</tr>
<tr>
<td><strong>Number of effects per QTL/SNP</strong></td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td><strong>Effect direction(s) of each QTL/SNP</strong></td>
<td>May be both positive AND negative</td>
<td>Either positive OR negative</td>
</tr>
<tr>
<td><strong>Resampling</strong></td>
<td>None</td>
<td>100 bootstrap samples, each composed of 75% of the RILs in each population sampled without replacement</td>
</tr>
</tbody>
</table>
Ear row number – chrom1
Joint linkage QTL have intermediate frequencies
GWAS SNP frequencies are much closer to neutral distribution.
Joint linkage: ear QTL have larger effects
GWAS: ear QTL have larger effects

Proportion of SNPs vs Effect size

- Tassel
- Ear
- Flowering
- Leaf

Inset: Effect size vs Proportion of SNPs
Effect sizes not strongly correlated with heritability

Joint linkage ($r^2=0.127$)  
GWAS ($r^2=0.233$)
Effect sizes not strongly correlated with deviation of B73 phenotype

Joint linkage ($r^2=0.067$)

GWAS ($r^2=0.043$)
Correction for B73-bias due to reference design of NAM

Problem: if B73 is an outlier, NAM QTL effects will be inflated

Solution: use inferred 26x26 matrix of QTL effects rather than 26x1 vector

<table>
<thead>
<tr>
<th></th>
<th>B73</th>
<th>Mo17</th>
<th>P39</th>
<th>Tzi8</th>
<th>CML52</th>
</tr>
</thead>
<tbody>
<tr>
<td>B73</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mo17</td>
<td>2.8</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P39</td>
<td>3.1</td>
<td>0.3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tzi8</td>
<td>3.0</td>
<td>0.2</td>
<td>0.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CML52</td>
<td>2.9</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0</td>
</tr>
</tbody>
</table>
Largest GWAS effects are at low frequency

- Tassel
- Ear
- Flowering
- Leaf
Largest GWAS effects have low BPP

Effects above 0.3 are most likely synthetic associations
Why are ear effects larger?

1. Heritability
2. “Extremeness” of B73 phenotype
3. Linked QTL

Deficiency of small ear effects

Biological, evolutionary explanation?
NAM design can distinguish linkage from pleiotropy

<table>
<thead>
<tr>
<th>DESIGN</th>
<th>OBSERVATION</th>
<th>CONCLUSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTL study,</td>
<td>Overlap between QTL for different traits</td>
<td>Maybe pleiotropy, maybe linkage</td>
</tr>
<tr>
<td>Single population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QTL study,</td>
<td>QTL overlap AND effect correlation across</td>
<td>Pleiotropy!</td>
</tr>
<tr>
<td>26 populations</td>
<td>26 populations at p &lt; 0.01 (r &gt; 0.495 in 2-tailed test w/ 24 df)</td>
<td></td>
</tr>
<tr>
<td>(NAM)</td>
<td></td>
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</tbody>
</table>
Common QTL control length of tassels, ears, and leaves

Joint linkage

GWAS
Tassel QTL pleiotropic with ear QTL also have larger effects

Mean effect sizes shown in standard deviations

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**Tassel**

0.108

**Ear**

0.134

**Other**

0.116

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* p < 0.05
** p < 0.01
*** p < 0.001
Using GWAS models to predict phenotypes

Sum of all GWAS SNP effects (weighted by BPPs) → Predict 27 parents → r² with phenotype (BLUPs) → Predict 4892 RILs
Ear trait models have lower predictive ability with no population term. 

\[
\begin{array}{cccccc}
\text{Trait} & \text{r}^2 (\text{obs,pred}) \\
\text{TL} & 0.0 & 0.2 & 0.4 & 0.6 & 0.8 & 1.0 \\
\text{SL} & 0.3 & 0.5 & 0.7 & 0.9 & 1.0 & 1.0 \\
\text{BZ} & 0.5 & 0.7 & 0.9 & 1.0 & 1.0 & 1.0 \\
\text{BN} & 0.4 & 0.6 & 0.8 & 1.0 & 1.0 & 1.0 \\
\text{CL} & 0.6 & 0.8 & 1.0 & 1.0 & 1.0 & 1.0 \\
\text{CD} & 0.7 & 0.9 & 1.0 & 1.0 & 1.0 & 1.0 \\
\text{ERN} & 0.8 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 \\
\text{DA} & 0.9 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 \\
\text{DS} & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 \\
\text{ASI} & 0.6 & 0.8 & 1.0 & 1.0 & 1.0 & 1.0 \\
\text{LL} & 0.5 & 0.7 & 0.9 & 1.0 & 1.0 & 1.0 \\
\text{LW} & 0.4 & 0.6 & 0.8 & 1.0 & 1.0 & 1.0 \\
\text{LA} & 0.3 & 0.5 & 0.7 & 0.9 & 1.0 & 1.0 \\
\end{array}
\]

Solid bars = 27 parents
Hashed bars = 4892 RILs
Candidate genes for maize inflorescence variation

1. Cloned maize inflorescence genes:
   - an1
   - ba1
   - bd1
   - bif2
   - Cg1
   - D8
   - dlf1
   - fea2
   - ids1
   - kn1
   - lg2
   - ra1
   - ra2
   - ra3
   - spi1
   - tb1
   - td1
   - te1
   - tga1
   - ts1
   - ts2
   - ts4
   - tsh1
   - tsh4
   - zfl1
   - zfl2

2. Gene families underlying maize domestication QTL:
   - **tga1**
     *(teosinte glume architecture1)*
     SBP-domain protein  →  17 genes
   - **tb1**
     *(teosinte branched1)*
     TCP-domain protein  →  24 genes
Testing QTL-candidate overlap

SBP-domain genes (n=17) within a given distance of BN QTL (candidates) versus random genes

SBP_2_4.21: 0.26cM; SBP_4_204.97: 0.48
SBP-domain genes are enriched for proximity to branch number QTL

Co-localization between SBP genes (n=17) and GWAS SNPs

SBP-domain genes are enriched for proximity to branch number QTL
Cloned mutants do not account for most natural variation

Co-localization between cloned maize inflorescence genes (n=26) and GWAS SNPs

Inflorescence genes within a given distance

Trait (# snps tested)

TL (10)  SL (10)  BZ (10)  BN (10)  CL (10)  CD (10)  ERN (10)  DA (10)  DS (10)  ASI (10)  LL (10)  LW (10)  LA (10)
Why so little overlap between inflorescence mutants and inflorescence QTL?

1. Genetic redundancy → mutants screens miss stuff

2. No natural variation left → sweep during domestication

3. Limitations of our study → not enough SNPs, recombination
Distinct genetic architecture for ear traits. Why?

GENETIC ARCHITECTURE
(ANY TRAIT IN ANY ORGANISM)

- QTL number
- QTL effect sizes
- QTL frequencies
- QTL gene action

Evolutionary history?

Biology?

Heritability

Response to selection ($h_s^2$)

QTL discovery, mapping, cloning
All maize variants for ear row number were cryptic variants in teosinte

Teosinte
ERN = 2

Maize
ERN = 8 – 20+
Why do cryptic variants alter genetic architecture in corn, tomato, and dogs?

tb1, tga1

SP

IGF1?

effect sizes

over-dominance

effect sizes
Canalization:
Organisms evolve robustness through accumulation of buffering mutations

Fixation of *tb1, tga1*

Domestication, Decanalization:
Release of cryptic variation

QTL Effects

Ear row number

Teosinte
Buffered

Maize
Unbuffered

Ear row number:
2 4 6 8 10 12 14 16 18 20
Hypothesis: Transgenesis may also decanalize, release cryptic variants.
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