Direct Mapping of Response to Plant Density

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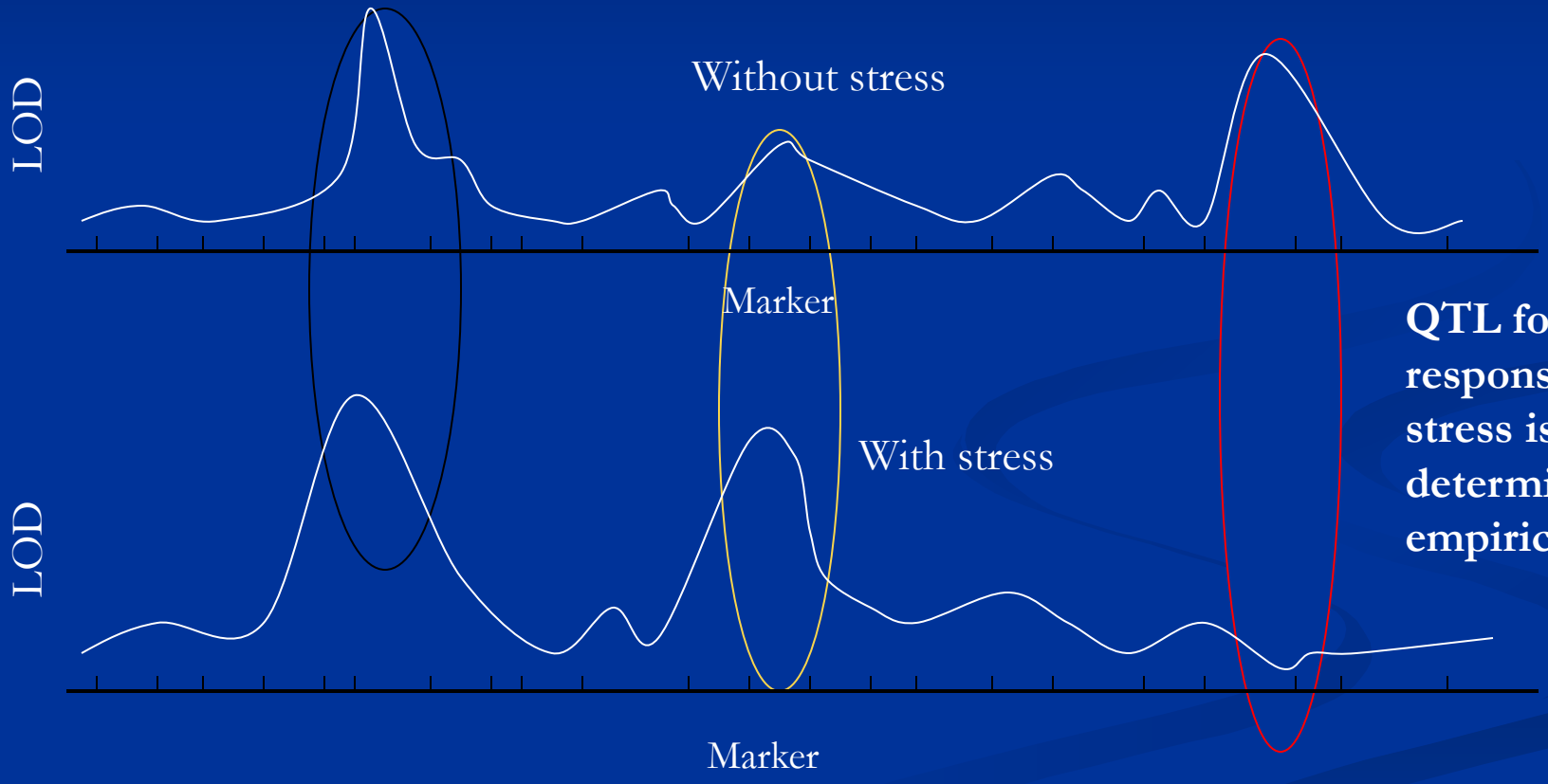
Are there loci with differential response to density?

- Or any other effect you wish to consider…
- Model this directly

Traditional Identification of QTL for response to some factor "stress"



Using a mixed model

- Include factors for the marker interval
- For the experimental design
- For the treatment condition
- Check mode fits
- Can be multivariate (multiple responses)

- Test the interactions between treatment (density) and marker interval

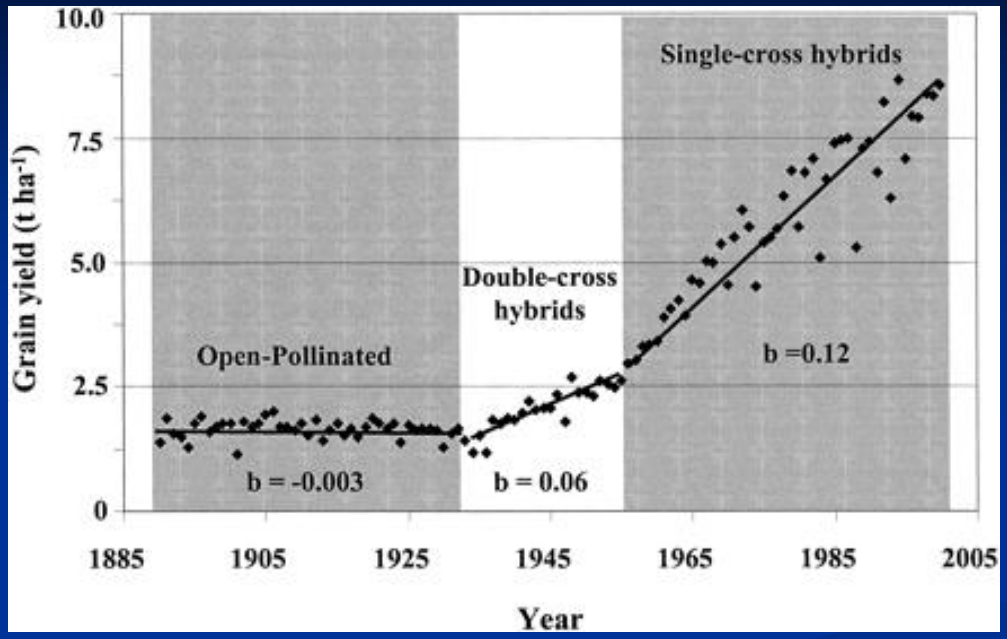
Model selection

- Divide the genome into linkage groups
- Choose the marker interval with the 'lowest' p-value for that linkage group (not necessarily significant)
- Fit all pairwise models, 3 way, 4 way…until the model is too big fit the FULL model all the time
- Look at the overall model fit- do not look at individual effects or interactions
- Choose the set of models that fit the best.

Coffman et. al. 2005 Genetics 170:1281-1297; Verhoeven et. al. 2010 Plos one 5(8): e12264

Acknowledgements

Evolution of maize yield during the last century



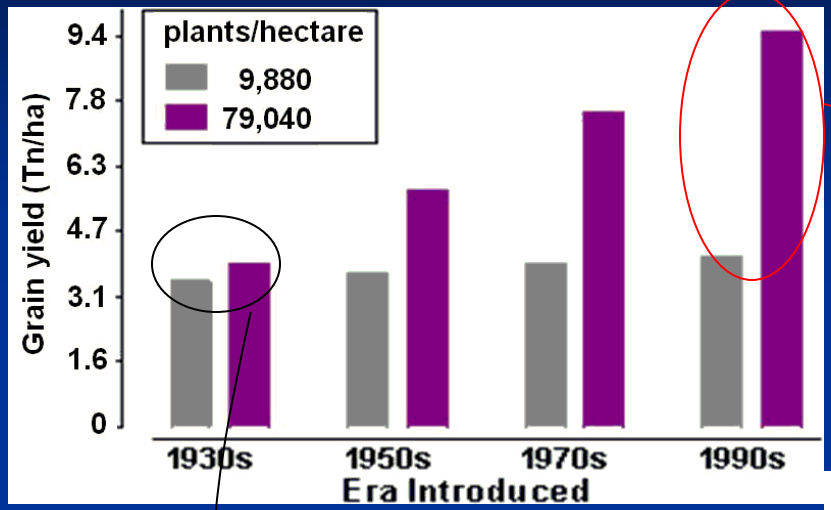
Adapted from Troyer, A 2006. Adaptedness and heterosis in corn and mule hybrids. Crop Science **46**, 528-543.

Yield improvement can be attributed to a combination of improved crop management practices

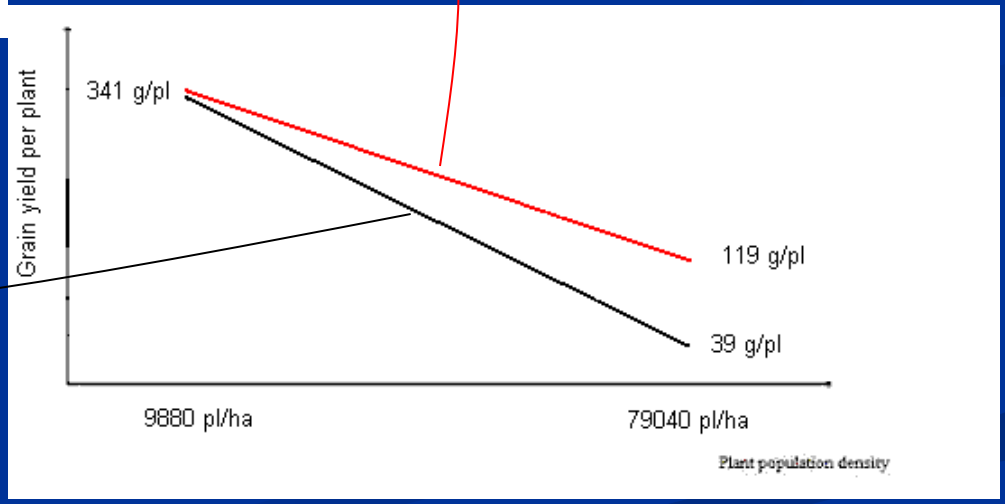
- Population density
- Fertilizer
- Pesticides
- Equipment efficiency
- Tillage

And improved genetics

Changes in maize yield across the last century



Adapted from Duvick, D 1997. What is yield? In G. Edmeades, M. Banziger, H. Mickelson, and C. Pena Valdivia (Eds.), *Developing drought- and low N-tolerant maize. Proceedings of a Symposium*, March 25-29, 1996, CIMMYT, El Batan, Mexico.

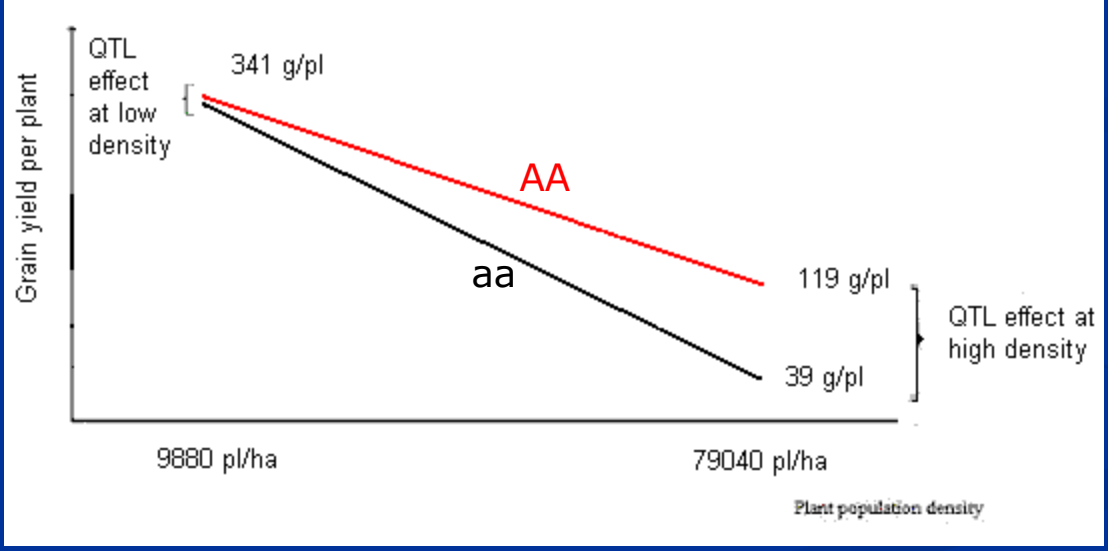


Implications for genetics

Assume there is 1 quantitative trait locus (QTL) with two alleles controlling grain per plant:

1990's genotype is AA at this locus

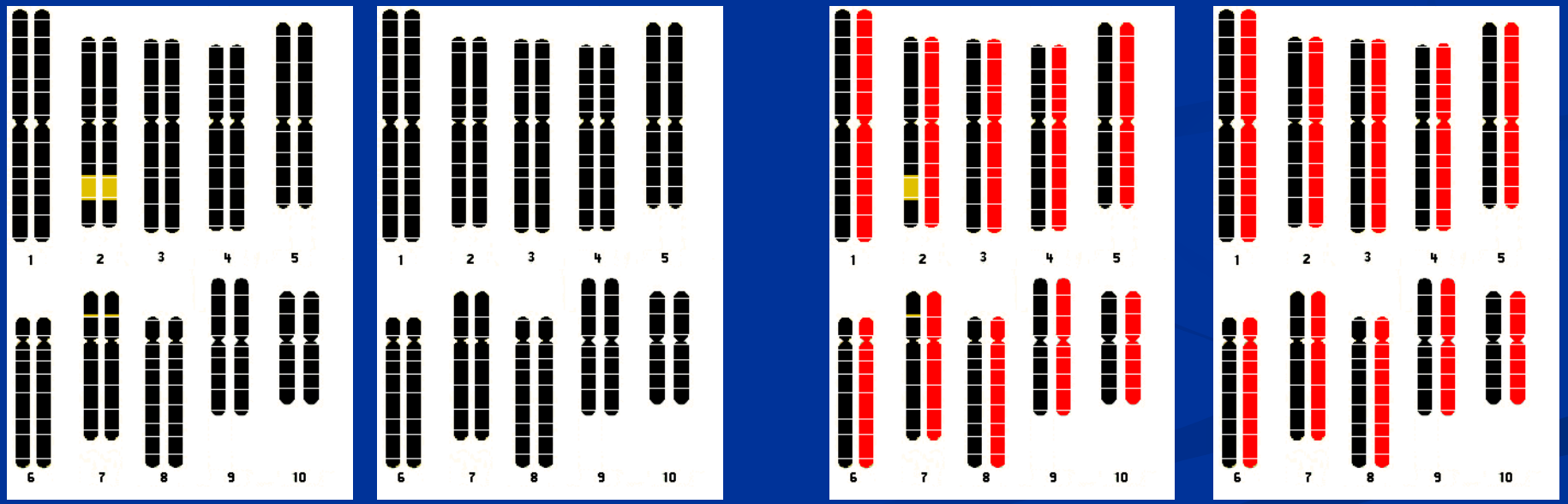
1930's genotype is aa at this locus



QTL by density interaction

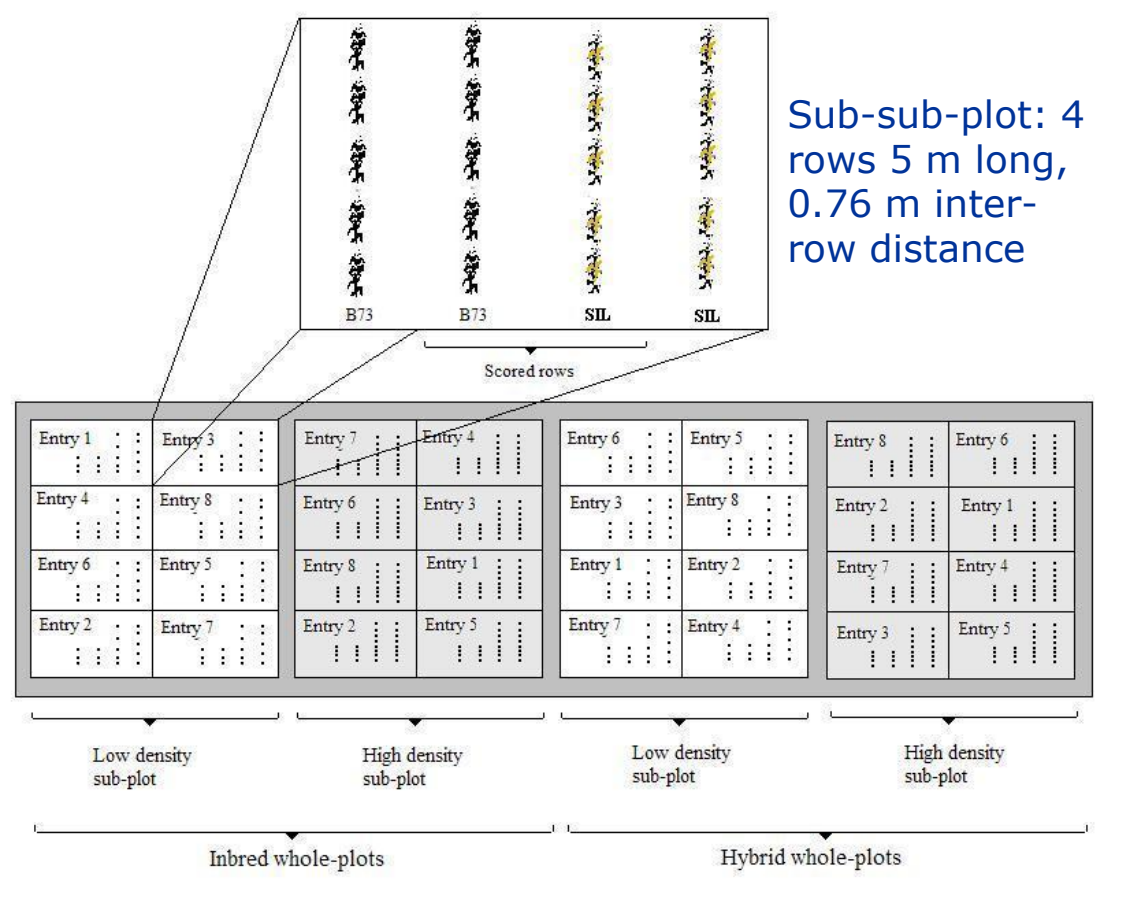
Experiment 1: SIL Thanks Jim!

- 8 Segmental Introgression Lines (SILs) and their hybrids to Mo17.
- Tx303 (subtropical line) introgressed into B73 background.
- Genetic background remains constant.



Gonzalo et. al. Genetics 173:331-348

Experimental design



Sub-sub-plot: 4 rows 5 m long, 0.76 m inter-row distance

Split-split-plot design

Whole-plot: inbred/hybrid

Sub-plot: density (50000 and 100000 pl ha^{-1})

Sub-sub-plot: Entry (8 SILs or their hybrids to Mo17)

5962 plants

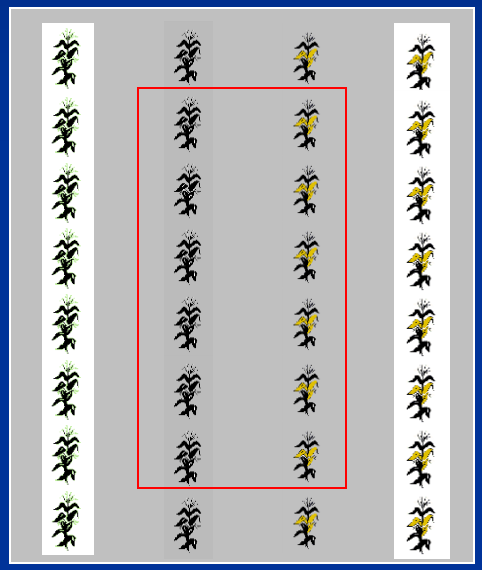
Phenotypic Measurements

- Plant height from at weeks 6, 7, 8 and 9 after planting (to the uppermost stretched leaf tip)
- Height to ear insertion
- Final height (to the collar of the flag leaf)
- Date of first visible anther
- Date of first visible stigma
- Kernel number per plant
- Grain yield per plant

- Days to anthesis/silking was calculated by subtracting the Julian date of planting from the Julian date of first visible anther/stigma
- Anthesis-to-silking interval (ASI) was obtained by subtracting the Julian date of first visible anther from the Julian date of first visible stigma

Estimation of the segment effects
Thanks Tony!

Sub-sub-plot



B73 SIL

Effect of the introgressed segment

$\mu_{SIL} - \mu_{B73}$

For final height (HCF), height of ear insertion (HEI), anthesis to silking interval (ASI), grain yield per plant (GY), kernel number per plant (KNP), and days to anthesis (DTA), an ANOVA model was used to analyze the data from each sub-sub-plot

$$z_{ij} = \mu + \tau_i + \epsilon_{ij}$$

The effect of the introgressed segment for each sub-sub plot was estimated by

$$\tau_{SIL} - \tau_{B73}$$

Plant heights were analyzed using a random coefficient model (week as the independent variable)

$$w_{ij} = \beta_0 + \beta_1(genotype) + \beta_2(x_{ij}) + \beta_3((genotype)(x_{ij})) + a_i + b_i(x_{ij}) + \epsilon_{ij}$$

Two effects of the introgressed segment were estimated:

initial growth rate (HW6) $\hat{\beta}_1$

mid-season growth rate (HGR) $\hat{\beta}_3$

Statistical Model

$$\mathbf{Y}_s = \begin{bmatrix} y_{ijklm}^{(HEI)} \\ y_{ijklm}^{(HCF)} \\ y_{ijklm}^{(ASI)} \\ y_{ijklm}^{(GY)} \\ y_{ijklm}^{(KNP)} \\ y_{ijklm}^{(HW6)} \\ y_{ijklm}^{(HGR)} \\ y_{ijklm}^{(DTA)} \end{bmatrix}$$

The effects of the introgressed segments estimated for each sub-sub-plot were analyzed using a multivariate mixed-effects model. For each sub-sub-plot *s*

$$\begin{aligned} y_{ijklm}^{(t)} &= \mu^{(t)} + L_i^{(t)} + C_j^{(t)} + (LC)_{ij}^{(t)} + \delta_{ik}^{(t)} + (C\delta)_{ijk}^{(t)} + D_l^{(t)} + (LD)_{il}^{(t)} + (CD)_{jl}^{(t)} \\ &\quad + (C\delta D)_{ijkl}^{(t)} + G_m^{(t)} + (LG)_{im}^{(t)} + (CG)_{jm}^{(t)} + (DG)_{lm}^{(t)} + (CDG)_{jlm}^{(t)} + \epsilon_{ijklm}^{(t)} \end{aligned}$$

for $t = \{\text{HEI, HCF, ASI, GY, KNP, HW6, HGR, DAT}\}$

$\mathbf{Y}_s$ is a vector with the 8 introgression effects for sub-sub-plot s

$y^{(t)}_{ijklm}$ is the effect of the introgressed segment *m* on trait *t* for a sub-sub-plot with density *l* in block *k* within location *i*

L_i represents the location effect,

C_j represents inbred/hybrid effect

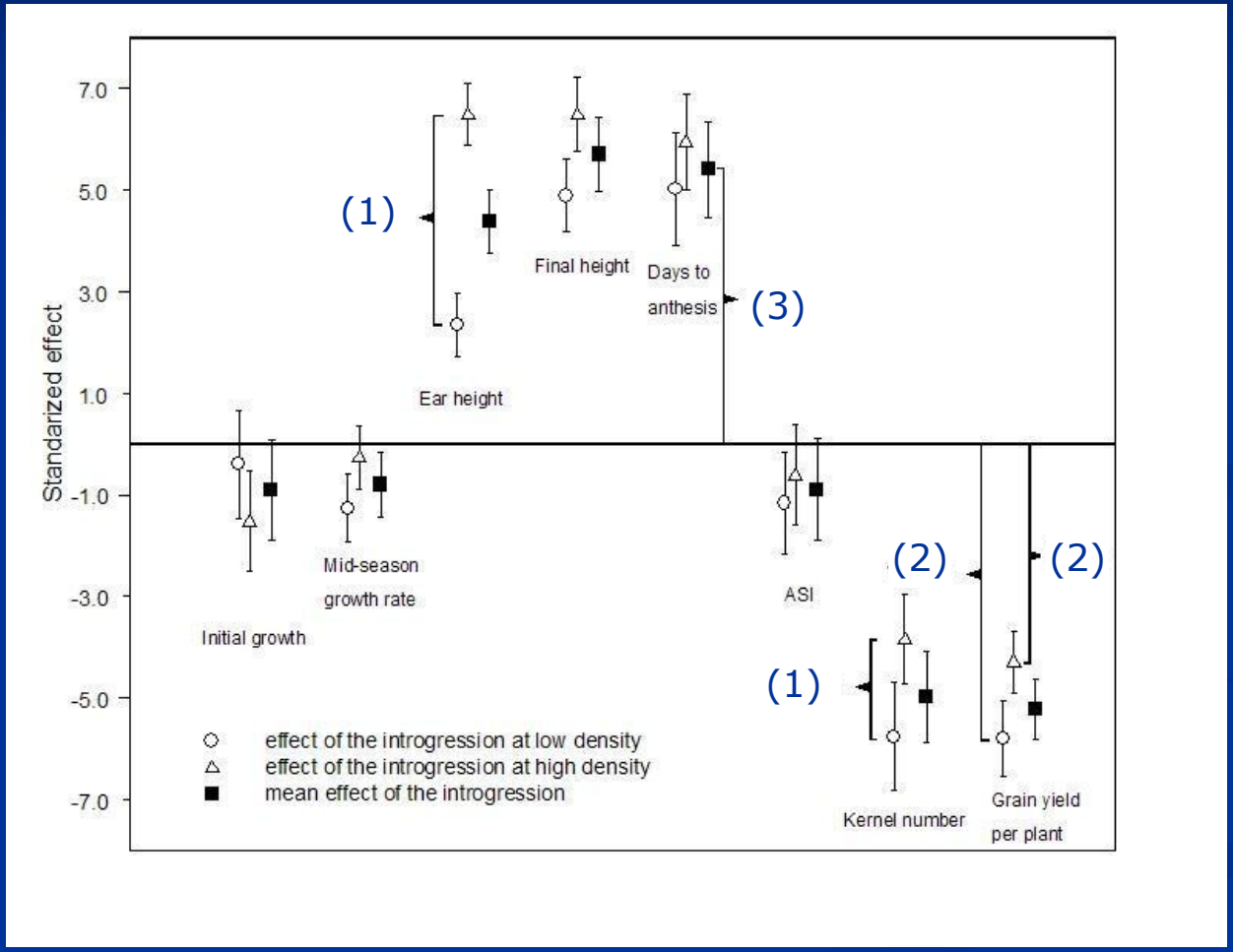
δ_{ik} represents block within location effect (random)

D_l represents the density effect

$$\mathbf{R} = Var(\mathbf{e}) = \begin{bmatrix} \mathbf{R}_1 & 0 & 0 & 0 & 0 \\ 0 & \mathbf{R}_2 & 0 & 0 & 0 \\ 0 & 0 & . & 0 & 0 \\ 0 & 0 & 0 & . & 0 \\ 0 & 0 & 0 & 0 & \mathbf{R}_n \end{bmatrix}$$

where $\mathbf{R}_1, \mathbf{R}_2, \ldots, \mathbf{R}_n$ are 8×8 unstructured variance-covariance matrices for the residuals from the effects of the introgressed segment measured on sub-sub-plot 1, 2, ..., n, respectively.

Testing QTL (segment) by density interaction



(1) Introgression effect by density interaction

$$\mu_{jlm}^{(t)} = \mu_{jl'm}^{(t)}$$

(2) Effect of the introgression at low and high density individually

$$\mu_{jlm}^{(t)} = 0$$

$$\mu_{jl'm}^{(t)} = 0$$

(3) Effect of the introgression across densities

$$\mu_{jm}^{(t)} = 0$$

Non-constant variance

- R_n, the 8x8 variance-covariance matrix of the residuals may vary across density and/or inbred/hybrid
- Different number of observations scored
- Other:
  - increased within-genotype variance at higher densities
  - increased within-genotype variance in homozygous genotypes

LRT and AIC: model (iii) provides the best fit

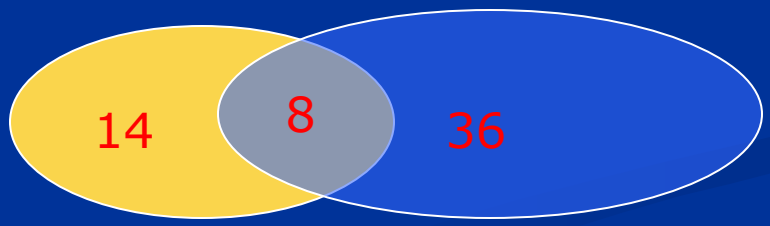
Table 5:

Model	Number of parameters in $\mathbf{R}$	-2 Log Likelihood	AIC	BIC
(i) Single $\mathbf{R}_n$ for all sub-sub-plots	36	3522.8	4400.8	5222.3
(ii) $\mathbf{R}_n$ varies across inbred/hybrid states	72	3254.0	4200.0	5085.1
(iii)$\mathbf{R}_n$ varies across densities and inbred/hybrid states	144	3057.2	4149.2	5170.9

Comparison with the overlying maps approach

- 14 significant segment by density interactions detected.
- 36 "significant" segment by density interactions detected using the traditional "overlying of maps" approach.

The locus was significant at both densities, but the size of the effect changed across densities

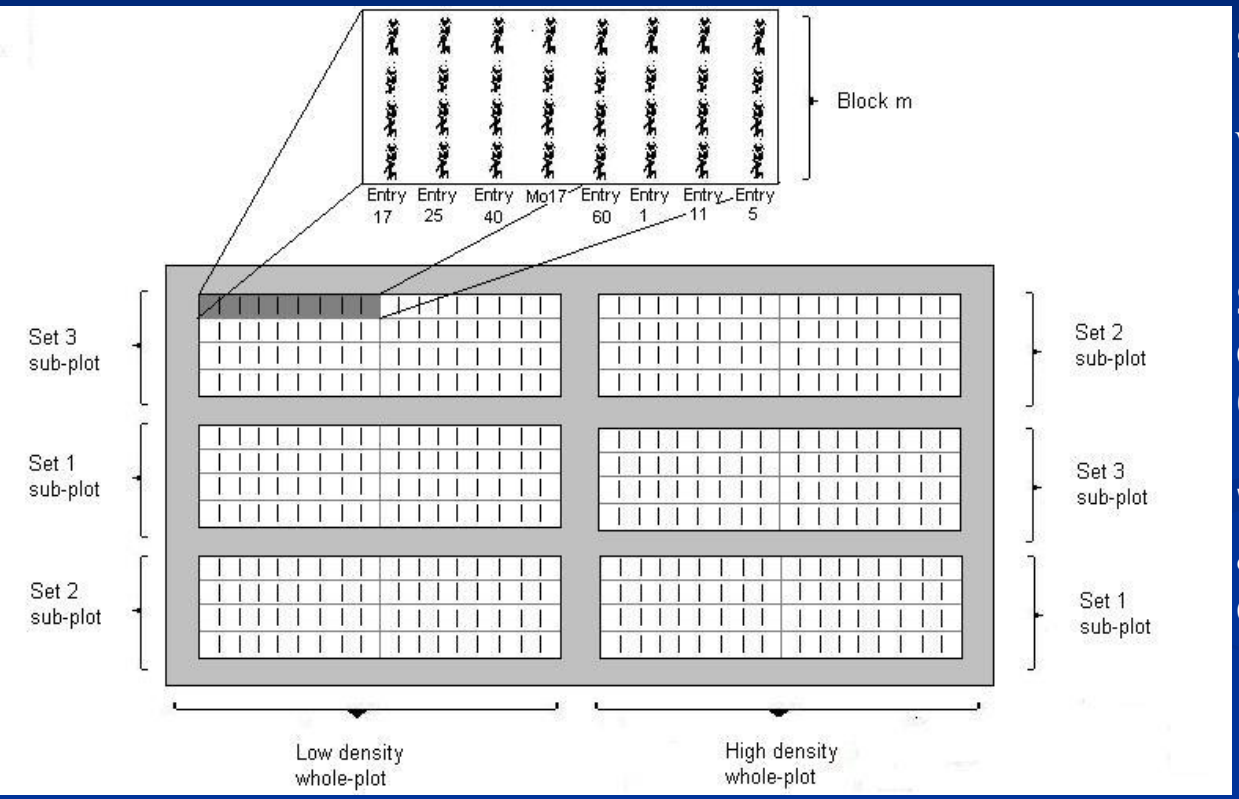


Loci with moderate to small constitutive effects (same size) with different error variances induced by the density

Are there loci with differential response to density?

- The effects of some loci depend upon the level of inter-plant competition.
- A large proportion of the observed response to density departs from additivity.
  - Dominance/epistasis may play an important role in the response to density in Maize.

Experiment 2: RILs Stuber set- Thanks Jim!



Split-plot design:

Whole-plot: density (50000 and 100000 pl ha^{-1})

Sub-plot: sets (each set consisted of 64 entries 62 RILs, B73 and Mo17)

Within a set, the 64 entries arranged in a 8 by 8 lattice design

4 locations (3 in Indiana, 1 in North Carolina), 2 replicates per location. Approx. 15360 plants

Gonzalo et. al. 2009 Heredity:1-17

Phenotypic measuments

On each row

- Final height (from the ground to the collar of the flag leaf)
- Date of 50% anthesis (50% of the plants in a row with visible anthers)
- Date of 50% silking (50% of the plants in a row with visible stigma)
- Number of ears (number of ears with more than 20 kernels in a row)
- Number of plants with no ears
- Weight of the ears from 5 plants that were not barren

Statistical model

Full model

$$y_{ijklmn} = \mu + L_i + D_j + (LD)_{ij} + \delta_{ik} + (D\delta)_{ijk} + S_l + (LS)_{il} + (DS)_{jl} + (LDS)_{ijl} + (\delta S)_{ikl} + (D\delta S)_{ijkl} + \gamma_{ijklm} + R_{ln} + (LR)_{iln} + (DR)_{jln} + (LDR)_{ijln} + \epsilon_{ijklmn}$$

y_{ijklmn} is the phenotypic value of the trait

L_i represents the location effect, (random)

D_j represents the density effect

δ_{ik} represents replicate within location effect (random)

S_l represents the set effect

γ_{ijklm} represents the block within location by replicate by density effect (random)

R_{ln} represents the entry within set effect (random)

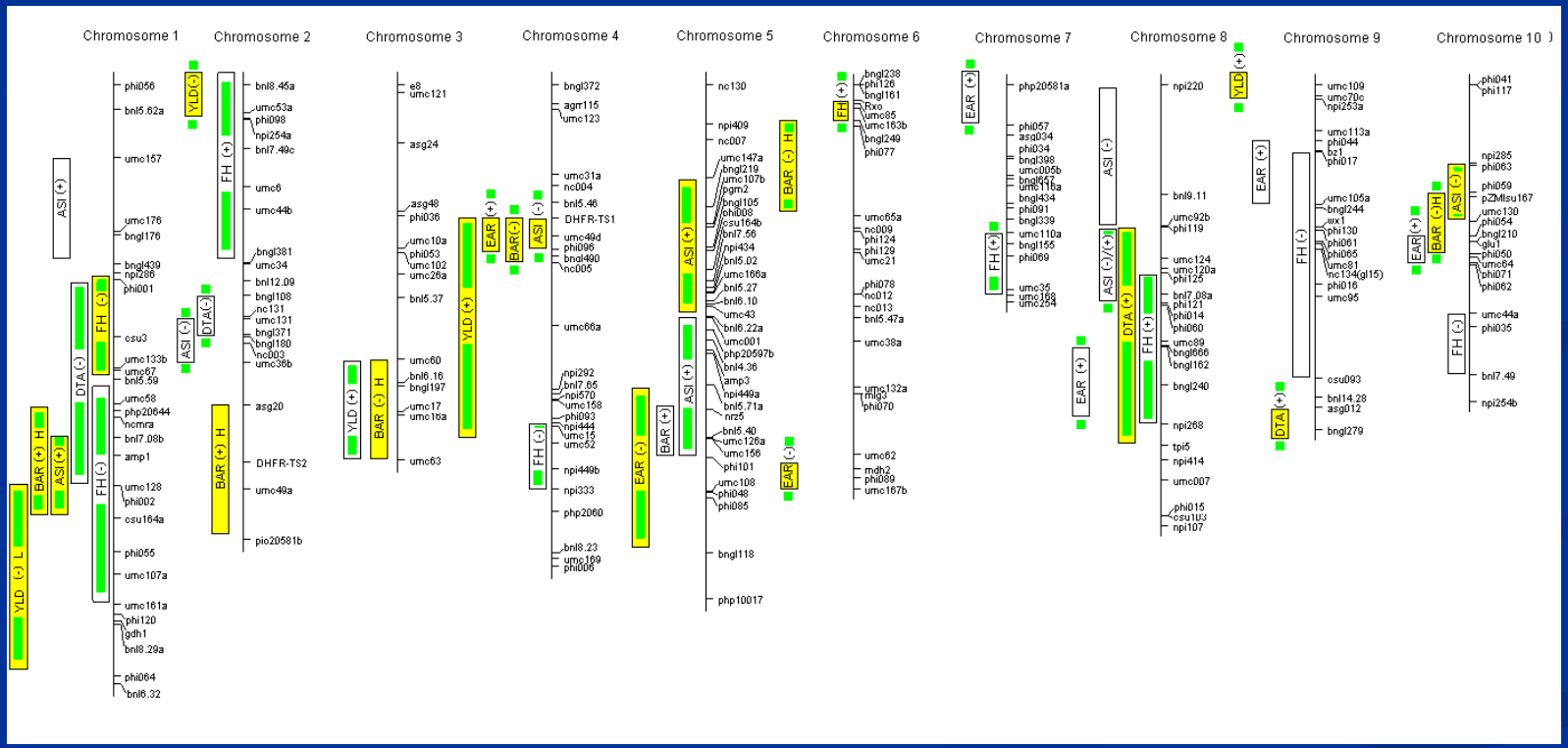
Reduced model: $(LDR)_{ijln}$ not significant for any trait (LRT and Wald test). Set (S_l) and the interactions involving S_l tested by fitting the model

$$y_{ijkmn} = \mu + L_i + D_j + (LD)_{ij} + \delta_{ik} + (D\delta)_{ijk} + \gamma_{ijkm} + R_n + (LR)_{in} + (DR)_{jn} + \epsilon_{ijkmn}$$

and comparing Bayesian Information Criteria (BIC) and standard errors for pairwise comparisons between entries. For traits where BIC for the reduced model was smaller and the standard errors did not change, the reduced model was used.

Summary of Significant QTL

Trait	QTL	QTL by density	Epistatic QTL
Final height (FH)	9	2	7
Days to anthesis (DTA)	4	2	4
ASI	9	4	7
Barrenness (BAR)	7	6	4
Ear per plant (EAR)	7	3	6
Yield per plant (YLD)	5	4	5
Total	41	21	33

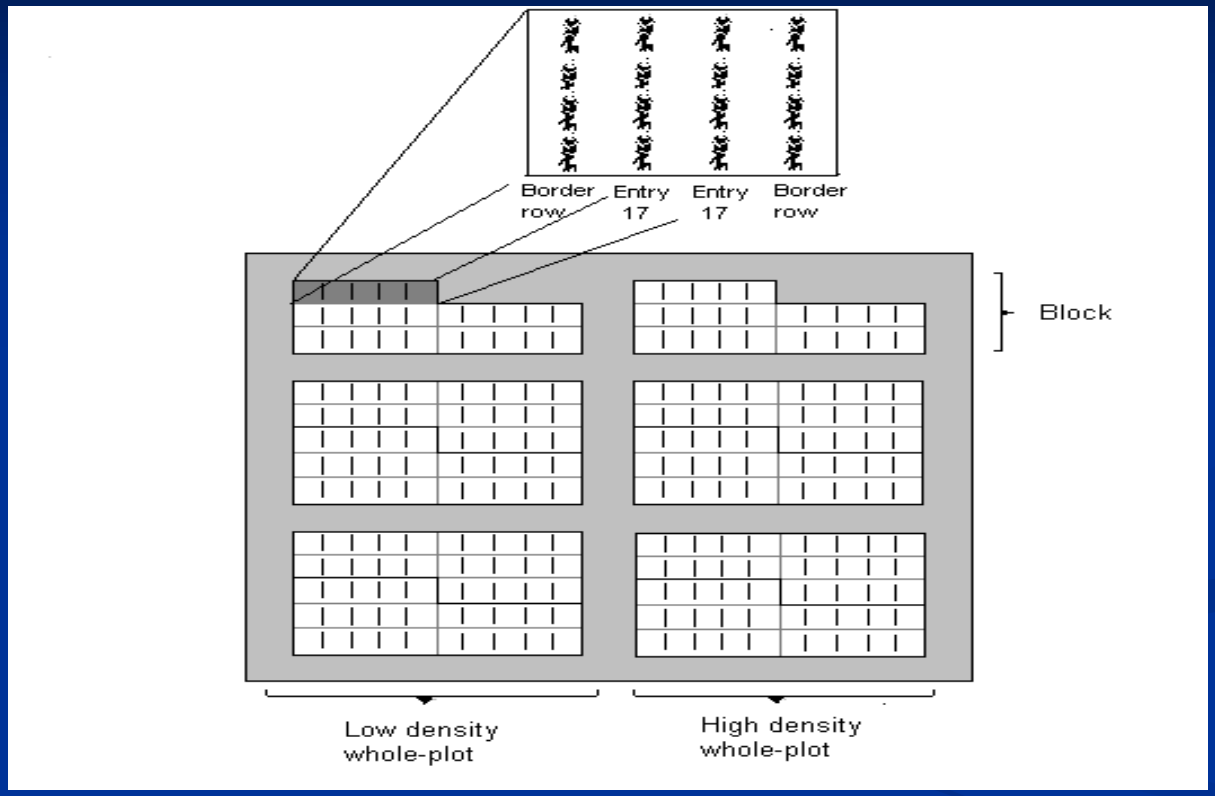


Validation of a 4-locus model for barrenness

Thanks Tony!

14 RILs with the favorable allelic combination (Mo17, B73, B73, Mo17)

10 RILs with the unfavorable allelic combination (B73, Mo17, Mo17, B73)



Yield trial in 3 locations, 3 replicates per location in 2005.

Experimental design: Split-plot: Whole-plot: density (50000 and 100000 pl ha^{-1})

Sub-plot: entries (24 RILs and check line Mo17). Entries arranged in 5 by 5 lattice.

Measurements (on center 2 rows):

- Number of ears with more than 20 kernels
- Number of plants with no ears
- Grain yield per plot (harvested with combine)

Validation of a 4-locus model for barrenness

	Yield (Tn ha^{-1})		Barrenness		Ears per plant	
	Low density	High density	Low density	High density	Low density	High density
Mo17	2.77	3.33	5.54%	9.22%	0.96	0.91
RIL mean	1.69	1.90	16.05%	28.83%	0.86	0.72
Unfavorable allelic combination	1.39	1.55	25.67%	42.27%	0.75	0.58
Favorable allelic combination	1.90	2.15	9.18%	19.23%	0.94	0.82
LSD (5%) favorable vs. unfavorable	0.29		4.27%		0.05	

- RILs with unfavorable allelic combination had significantly higher percentage of barrenness and fewer ears per plant at both densities.
- Differential response to density for ears per plant and barrenness
- RILs with the unfavorable allelic combination yielded significantly less than RILs with favorable allelic combination, but these difference was not affected by density treatments

What is the genetic architecture for response to density in temperate germplasm?

- QTL associated with Barrenness and Yield per plant (potential) are strongly affected by density
- Epistasis plays an important role in the genetic control of all phenotypes measured
- QTL for Barrenness were verified to impact Yield per unit surface area in yield trials

How does plant population density influence reciprocal effects?

Gonzalo et. al. 2007 Heredity 99:14-30

Motivation

- Maize geneticists and breeders have recognized reciprocal effects as one source of genetic variability and the presence of reciprocal differences has been documented since early days
- Reciprocal effects may account for a large portion of the genetic variance in certain forms of resistance to insect feeding
- Reciprocal differences are generally not consistent across environments and do not have a uniform sign for all hybrids tested between two germplasm groups

- Current molecular work is limited to the study of the gametophyte- are the sporophytic differences in reciprocals due to epigenetics?

- If reciprocal differences have a heritable genetic component, these differences should be able to be modeled and mapped in an appropriate population

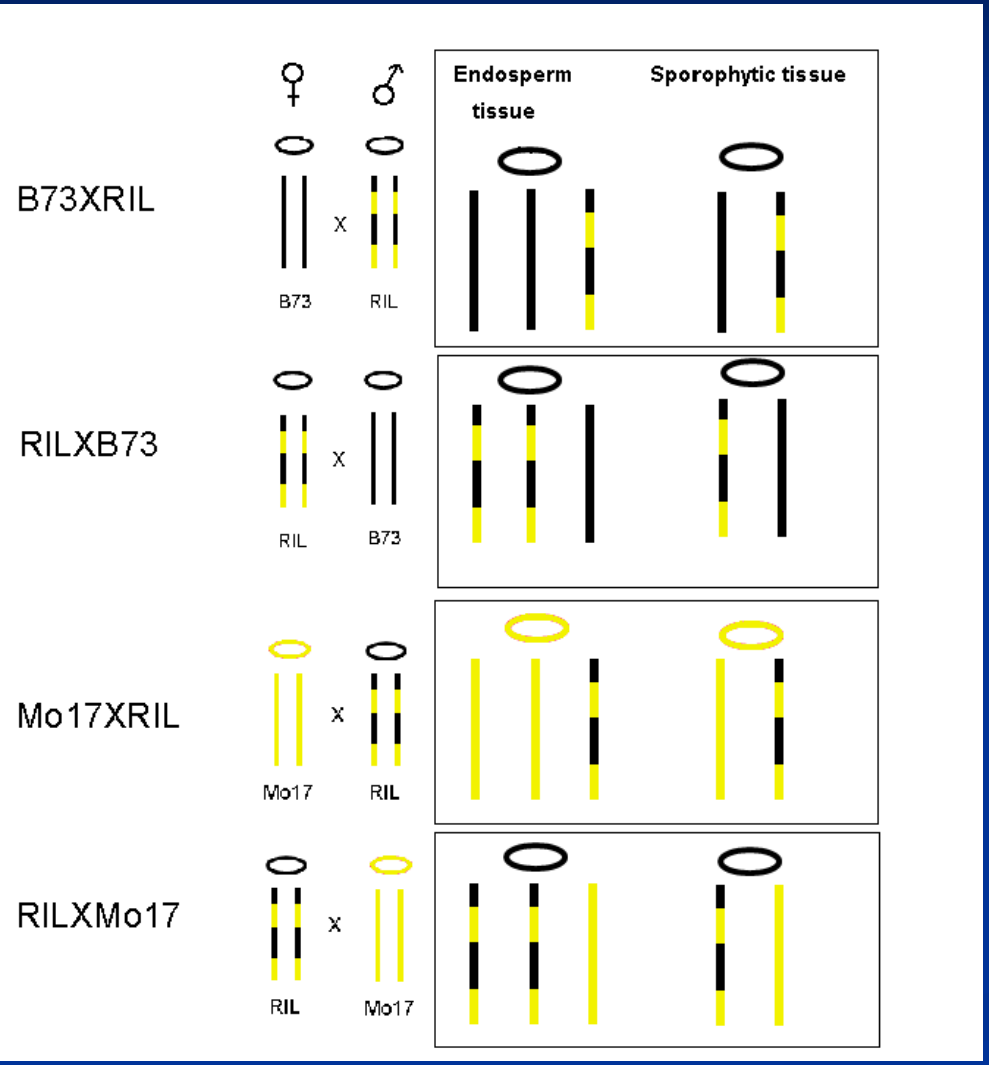
Why would reciprocal crosses be different?

- Same nuclear DNA

- Endosperm (flowering plants)
- Cytoplasm (plants and animals)

- Epigenetics (☺)

Genetic Material

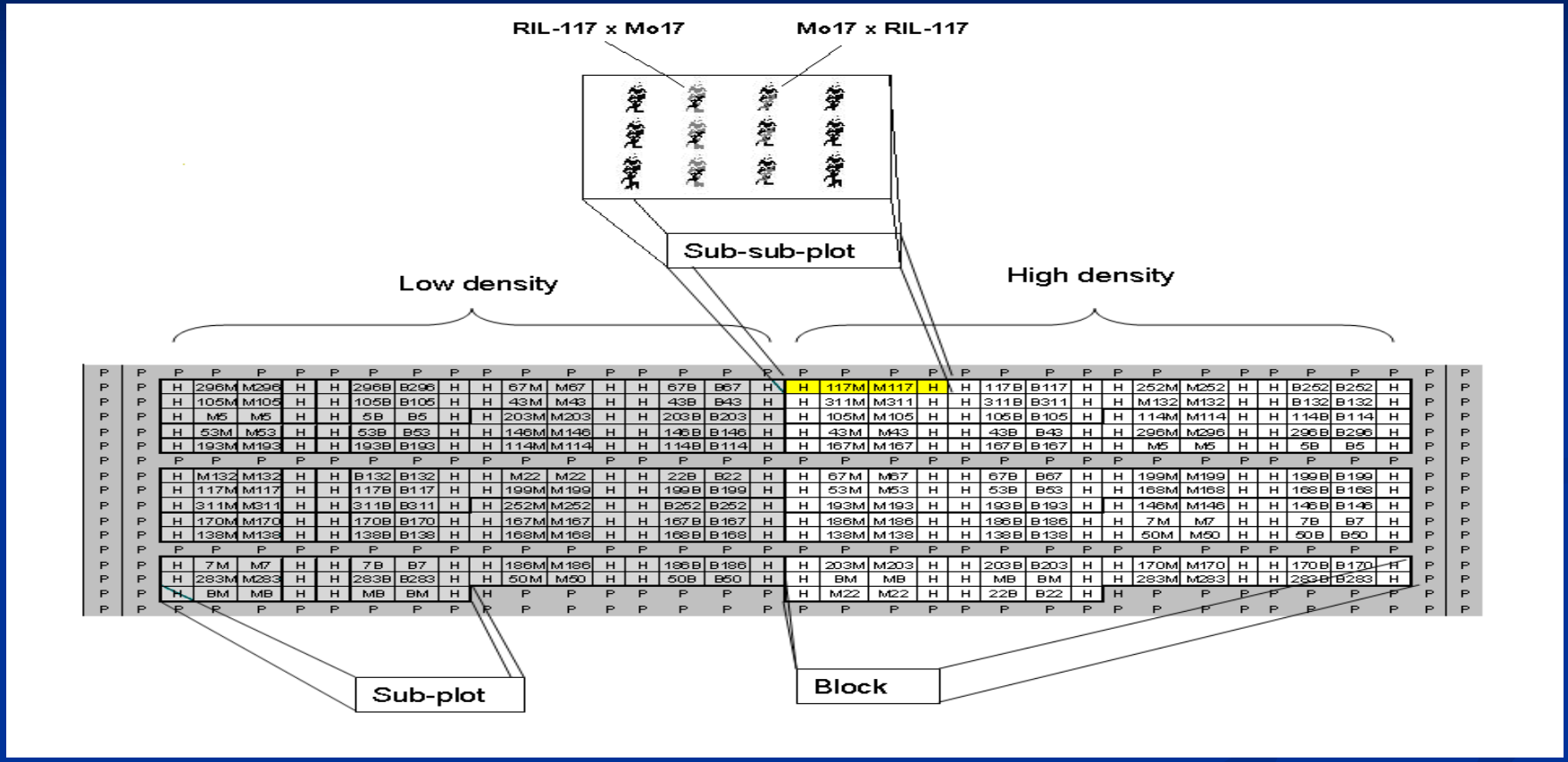


B73xMo17 RILs backcrossed to both parental lines in both directions:

- 23 RILxB73
- 23 B73xRIL
- 23 RILxMo17
- 23 Mo17xRIL

Seed for these backcrosses produced in the same nursery in 2004.

Experiment



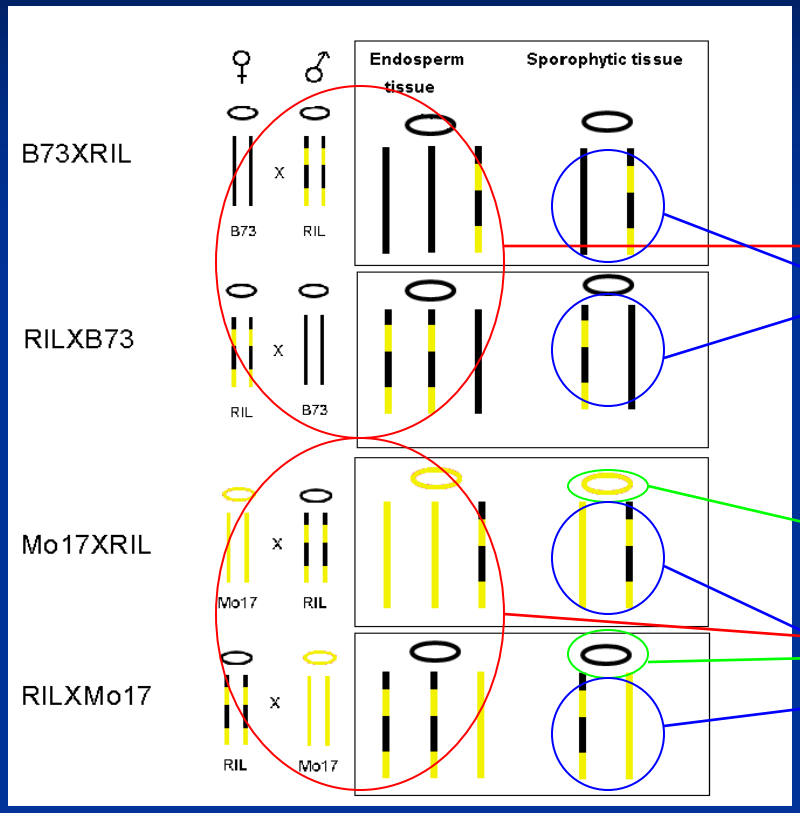
Split-split-plot:
Whole-plot: density (50000 and 100000 pl ha-1)
Sub-plot: entries (23 RILs and two check lines). Entries arranged in 5 by 5 lattice.
Sub-sub-plot: backcross parent (B73 and Mo17)

Experiment

- 2 replicates
- 3 locations
  - Approximately 50,000 plants measured EACH time

- Measurements:
  - Weight of 100 kernels (5 samples)
  - Plant height at the V7 and V12 stages (to the uppermost stretched leaf tip)
  - Final plant height (to the collar of the flag leaf)
  - Date of 50% anthesis and silking (50 % of the plants with visible anthers and stigma)

Genetic model



For a backcross with seed parent *i*, pollen parent *j*, and nuclear DNA *k*, the value of the phenotype can be written as

H0: RILxB73=B73xRIL

$$Y_{RIL_l,B73,RIL_lxB73} - Y_{B73,RIL_l,B73xRIL_l} = (S_{RIL_l} - S_{B73}) + (P_{B73} - P_{RIL_l}) + (SN_{RIL_l,B73xRIL_l} - SN_{B73,B73xRIL_l}) + (PN_{B73,B73xRIL_l} - PN_{RIL_l,B73xRIL_l})$$

For RIL *l*, the models for all 4 backcrosses are

H0: RILxMo17=Mo17xRIL

$$Y_{B73,RIL_l,B73xRIL_l} = S_{B73} + P_{RIL_l} + N_{B73xRIL_l} + C_{B73} + SN_{B73,B73xRIL_l} + PN_{\cdots}$$

$$Y_{RIL_l,Mo17,RIL_lxMo17} - Y_{Mo17,RIL_l,Mo17xRIL_l} = (S_{RIL_l} - S_{Mo17}) + (P_{Mo17} - P_{RIL_l}) + (C_{B73} - C_{Mo17_l}) + \cdots$$

$$Y_{RIL_l,Mo17,RIL_lxMo17} = S_{RIL_l} + P_{Mo17} + N_{Mo17xRIL_l} + C_{B73} + SN_{RIL,Mo17xRIL_l} + PN_{Mo17,Mo17xRIL_l}.$$

"Parent-of-origin" effect

"Maternal effect"

Cytoplasmic effect

General results

Backcross	Height V7 stage		Height V12 stage		Final height		Days to Anthesis		Days to Silking	
	High	Low	High	Low	High	Low	High	Low	High	Low
RIL-5 x B73	-2.53	-2.90	-7.23	-7.47	3.13	-1.83	1.81	1.81	0.91	0.91[1]
RIL-22 x B73	-6.68	-7.73	-5.93	-8.03	0.63	-1.17	0.98	1.51	1.39	1.91
RIL-53 x Mo17	-5.00	-6.47	-11.83	-4.80	4.26	2.37	1.29	0.82	1.55	0.92
RIL-67 x B73	4.26	4.36	13.30	11.30	1.93	-2.55	-0.79	-0.49	-1.36	-0.47
RIL-105 x B73	-9.80	-5.30	-4.53	-9.40	9.63	1.47	1.44	1.64	1.69	0.79
RIL-105 x Mo17	-12.07	-11.53	-7.40	-8.36	3.90[2]	3.94	1.66	1.17	2.38	1.43
RIL-114 x Mo17	-5.40	-8.07	4.5	-12.20	-0.97	-5.22	0.67	1.18	0.65	2.10
RIL-117 x Mo17	-7.73	-8.90	-4.43	-12.53	-0.70	-5.19	0.98	0.98	0.93	1.56
RIL-138 x B73	-3.18	4.26	-0.27	12.20	-3.47	4.1	0	-0.65	-0.16	-0.16
RIL-146 x Mo17	-1.90	-10.47	-2.00	-11.07	-2.67	-7.03	0	0.65	0.16	1.08
RIL-167 x B73	-3.23	-6.73	-0.60	-10.87	-3.57	-3.93	0.66	1.49	0.48	0.79
RIL-168 x B73	-6.80	-9.53	-4.90	-10.40	-1.43	-2.07	0.95	1.95	1.23	1.90
RIL-168 x Mo17	-8.79	-6.30	-8.90	-2.23	-7.70	-0.98	0.16	0.99	0.31	0.97
RIL-170 x Mo17	-4.46	-2.07	-13.07	-4.26	-10.63	-3.52	0.95	-0.32	1.22	-0.15
RIL-186 x B73	-9.40	-10.20	-2.47	-12.10	-4.13	-9.70	0	1.17	0.8	1.31
RIL-186 x Mo17	-3.93	-6.53	-5.60	-9.40	-4.13	-4.46	-0.33	0.5	0	0.95
RIL-193 x B73	-2.40	-9.90	-4.70	-14.10	-2.60	-15.47	0.34	0.32	0.33	0.8
RIL-193 x Mo17	-6.40	-5.50	-4.77	-8.27	-2.13	-4.73	1.32	1.49	1.59	1.62
RIL-203 x B73	-1.83	-12.90	-3.70	-12.50	-0.07	-5.33	0.16	1.15	0.62	1.90
RIL-252 x Mo17	-7.67	-12.53	-5.23	-14.07	-1.5	-5.46	1.46	1.82	1.37	1.08
RIL-296 x B73	6.83	-0.20	9.7	-0.33	-5.03	-6.74	-0.46	-0.63	-0.73)	0.14

Differences in plant height diminish earlier at high density and eventually disappear.

Some of the reciprocal differences in days to anthesis and silking are the results of differences in early development.

R^2 days to anthesis vs. V7 height: 0.44 high density, 0.57 low density

Results

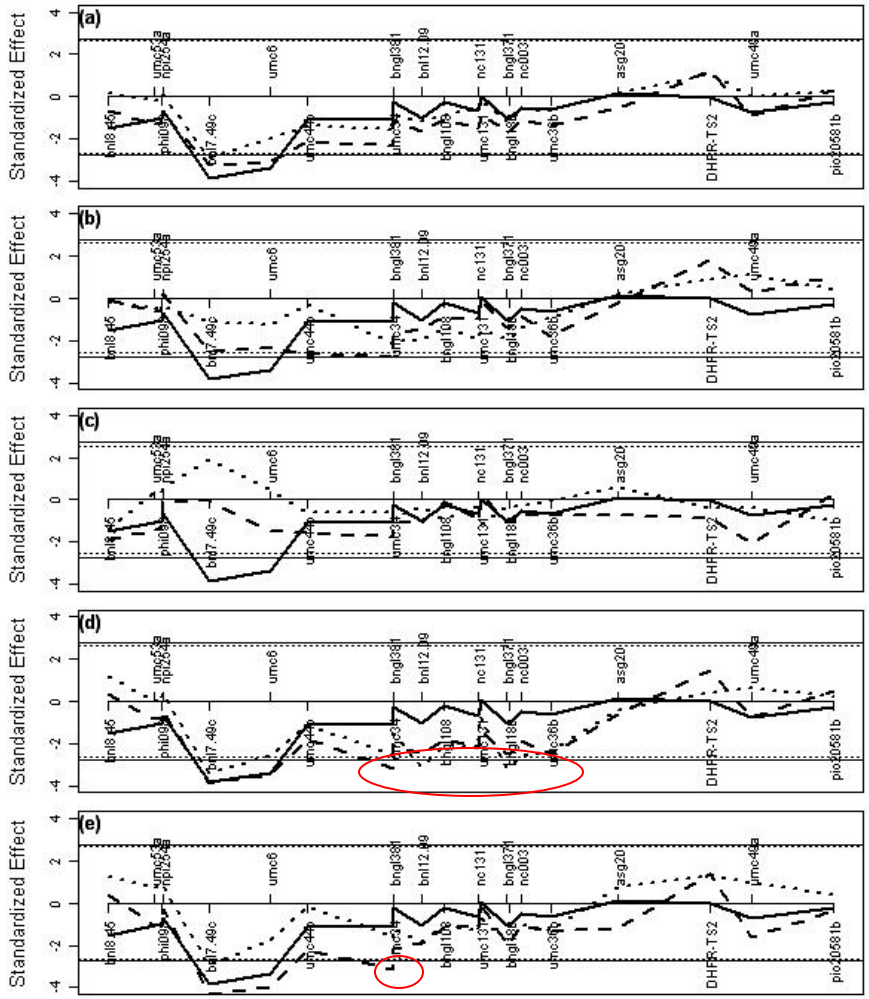
- Impact of density was pronounced and the density by cross interaction term was significant
- Relationship between reciprocals was affected by density
- Consistent with the methlyation hypothesis

Mapping

- 92 estimated reciprocal differences into the four groups depending on the marker genotype of the RIL parent

$$\Lambda_{ijk}^{m} = \mu + G_i + D_j + (GD)_{ij} + \epsilon_{ijk}$$

Contribution to the reciprocal differences for markers on chromosome 2



Height at the V7 stage

Height at the V12 stage

Final height

Days to anthesis

Days to silking

Other markers not significant for kernel weight and significant for other traits:

Marker **umc16a** on chrom. 3 (final height), marker **phi069** on chrom. 7 for days to anthesis and silking, marker **phi015** on chrom. 8 for days to silking

Also did an anlysis where we included reciprocal differences in kernel weight as a covariaate

Conclusions

- Model for reciprocal effects
- Method for mapping these reciprocal effects
- Beginning to understand how to separate the components 'maternal effect' , 'cytoplasmic effects' and 'parent of origin' effects.
- Evidence for reciprocal effects in the sporophyte in Maize
- Consistent with what we know about methylation
  - Epigenetics may indeed affect more than the endosperm

Summary

- Detection of loci responsible for adaptation to higher density requires the study of loci by density interactions.
- For QTL mapping of these loci, **direct** testing of QTL by density interaction is of importance.
- A large proportion of responses to density depart from additivity. Dominance/epistasis may play an important role.
- In temperate germplasm, barrenness is the trait most responsive to density. Selection based upon this trait explained grain yield per unit area in inbreds.
- Density interacts strongly with reciprocal differences, at least during the first growing stages. Higher inter-plant competition reduced the effect of differences in plant vigor due to kernel size.

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