Repeat associated small RNAs vary among parents and following hybridization in maize

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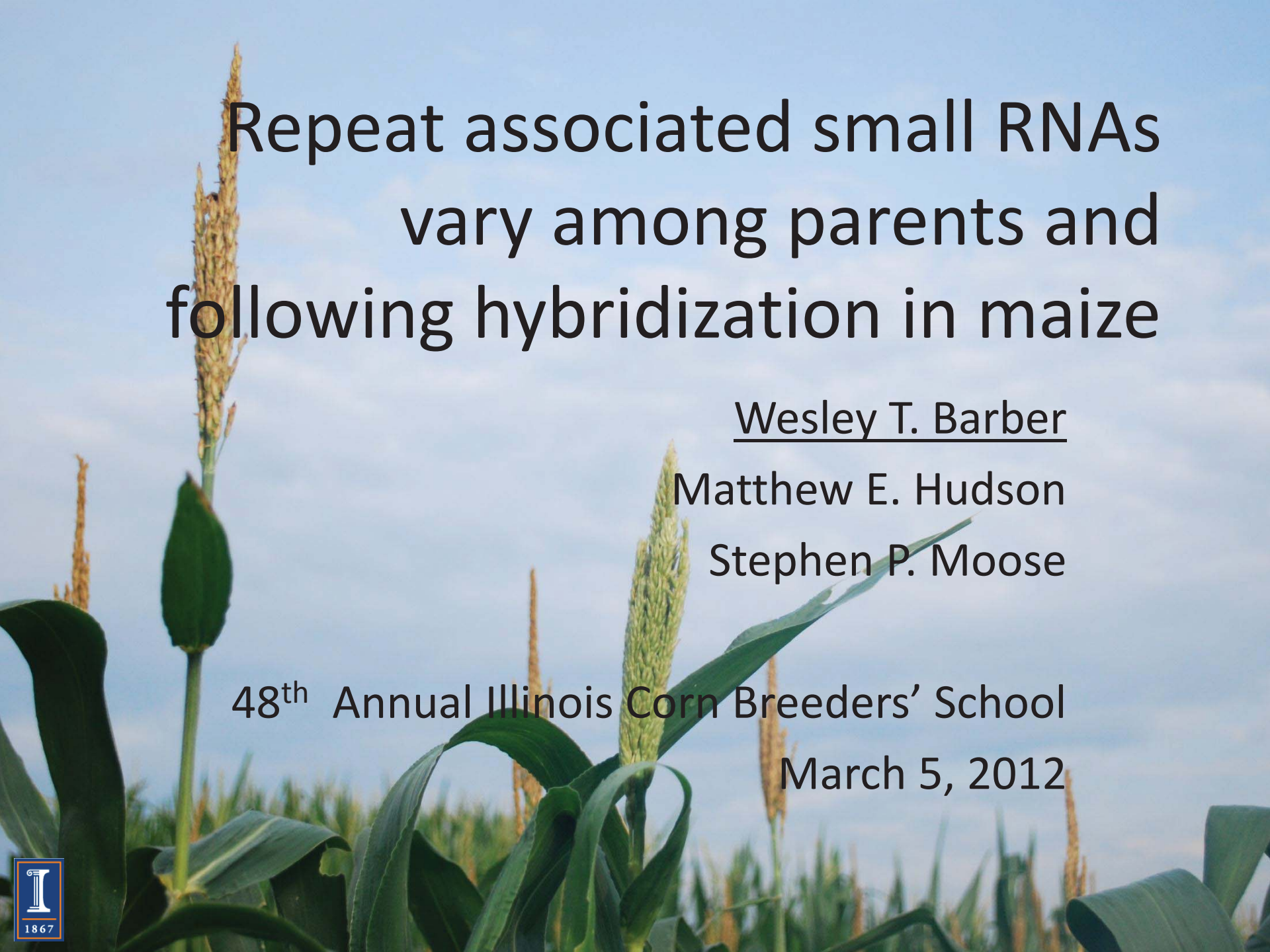
Abstract

Small RNAs (sRNAs) are hypothesized to contribute to hybrid vigor because they maintain genome integrity, contribute to genetic diversity, and control gene expression. We used Illumina sequencing to assess how sRNA populations vary between two maize inbred lines (B73, Mo17) and their hybrid. We sampled sRNAs from the seedling shoot apex and the developing ear, two rapidly growing tissues that program the greater growth of maize hybrids. We found that parental differences in siRNAs primarily originate from repeat regions. Although the maize genome contains greater number and complexity of repeats compared to *Arabidopsis* or rice, we confirmed that like these simpler plant genomes, 24-nt siRNAs whose abundance differs between maize parents also show a trend of downregulation following hybridization. Surprisingly, hybrid vigor is fully maintained when 24-nt siRNAs are globally reduced by mutation of the RNA-dependent RNA polymerase2 (RDR2) encoded by *modifier of paramutation1* (*mop1*). We also discovered that 21-22nt siRNAs derived from a number of distinct retrotransposon families differentially accumulate between B73 and Mo17 as well as their hybrid. We found that significant variation exists for 21-22-nt siRNA accumulation for these families among a larger set of diverse maize inbred lines. Thus, maize possesses a novel source of genetic variation for regulating both transposons and genes at a genomic scale, which may contribute to its high degree of observed heterosis.



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48th Annual Illinois Corn Breeders' School

March 5, 2012

Outline

- Heterosis/hybrid vigor
- sRNAs
  - 22-nt vs. 24-nt sRNAs
- Behavior of sRNAs following hybridization
- Variation in sRNAs between inbred lines
- What to learn from this talk
  - the maize genome provides a significant opportunity for inbred lines to vary for sRNAs
  - sRNAs are a source for genetic variation at the epigenetic and post-transcriptional levels

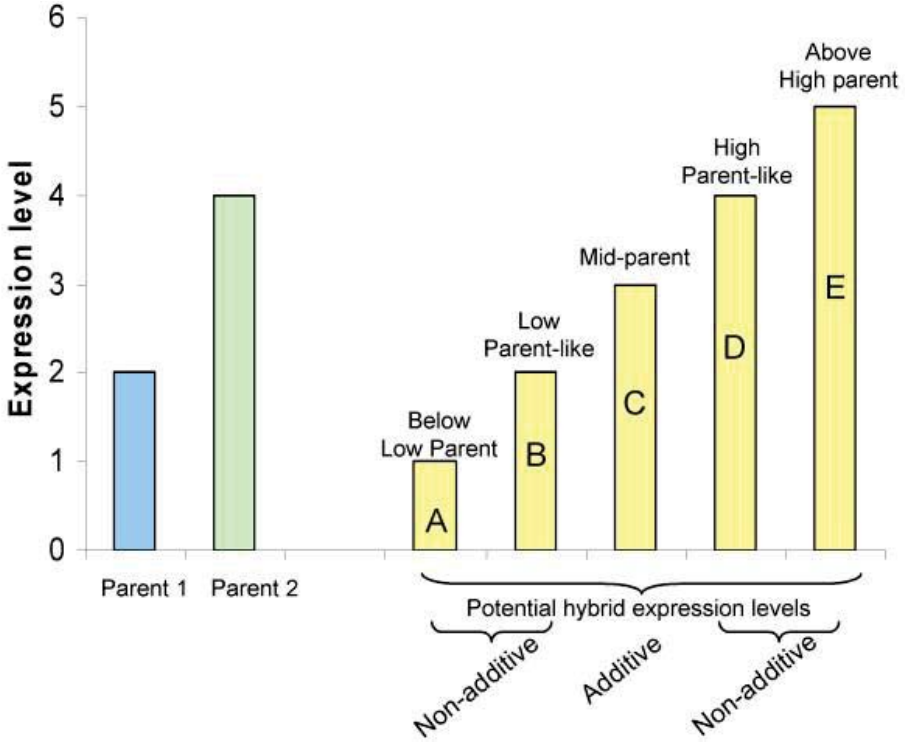
Hybrid vigor phenotype

- Offspring perform better than their parents
  - Greater cell proliferation in the hybrid

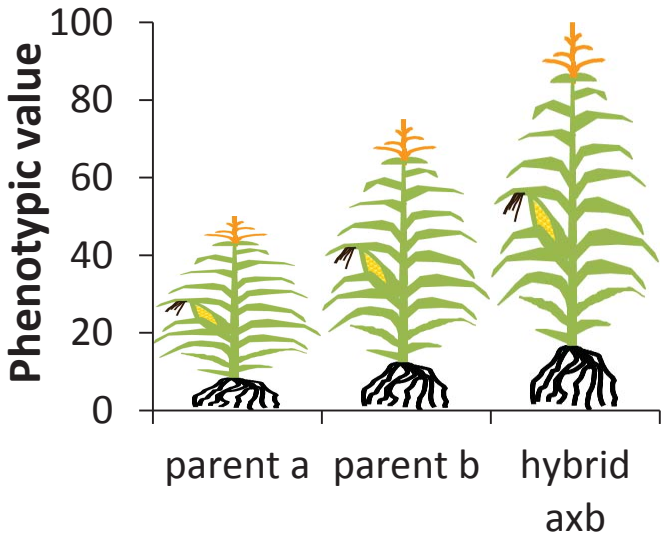
B73xMo17 (right) is taller than one of its parents, B73 (left).

Genetic principles of hybrid vigor

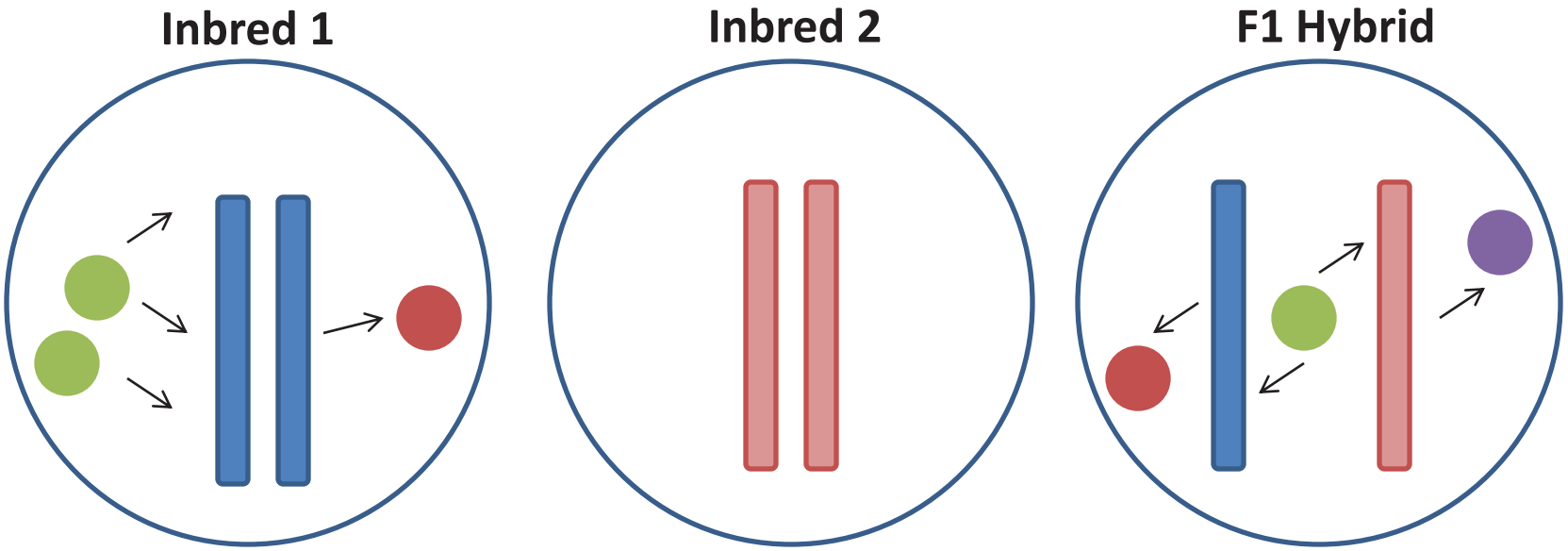
- Multiple mechanisms
- Genetic variation between parents
  - Variation in regulatory genes = large effects?
- Altered genetic states in hybrids
  - Additivity is an altered state
- Genomics uncovers more ways for how parents may vary



Stupar et al. 2008



Non-additive possibilities from genes behaving additively



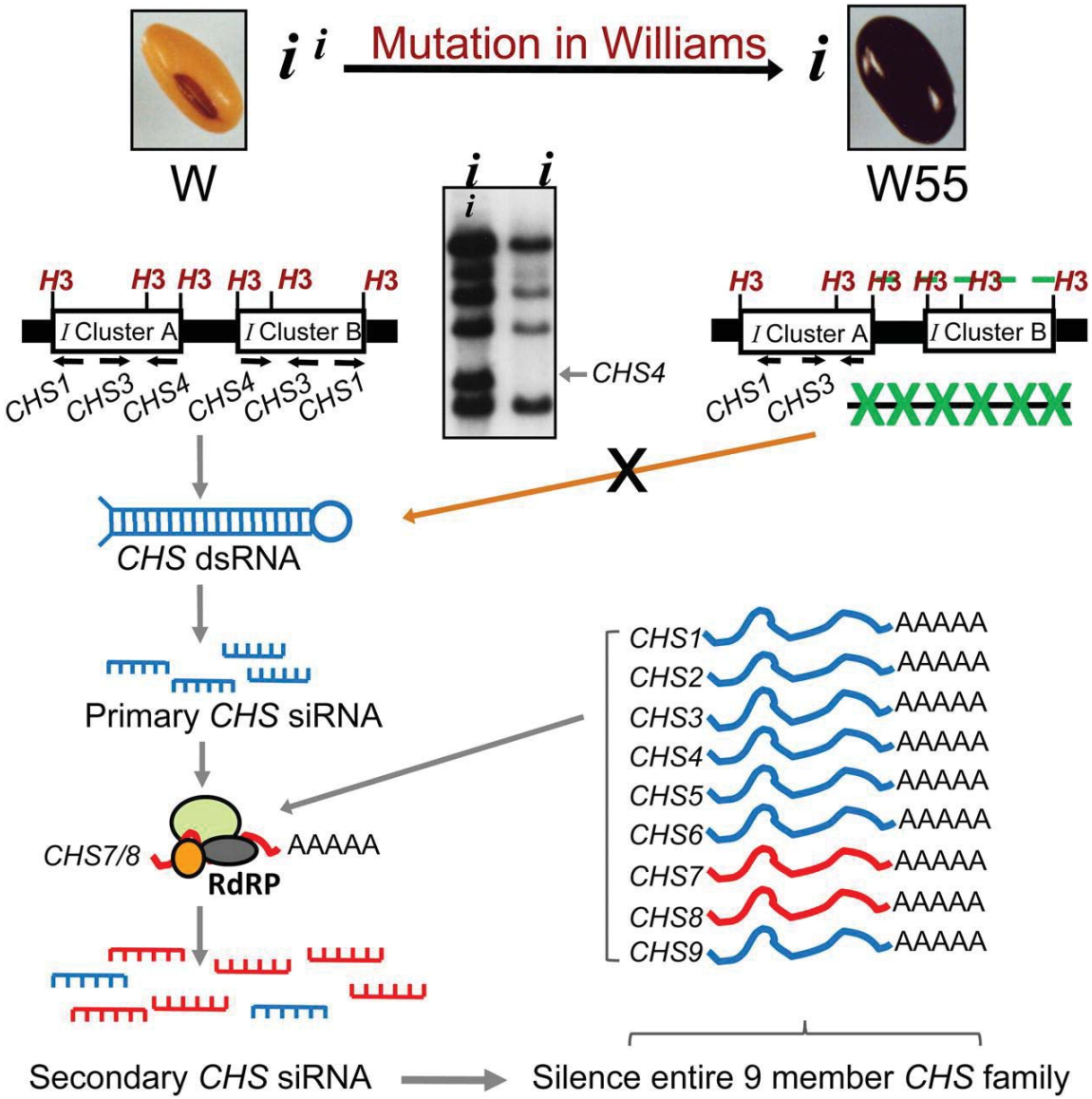
= regulatory gene

= proteins

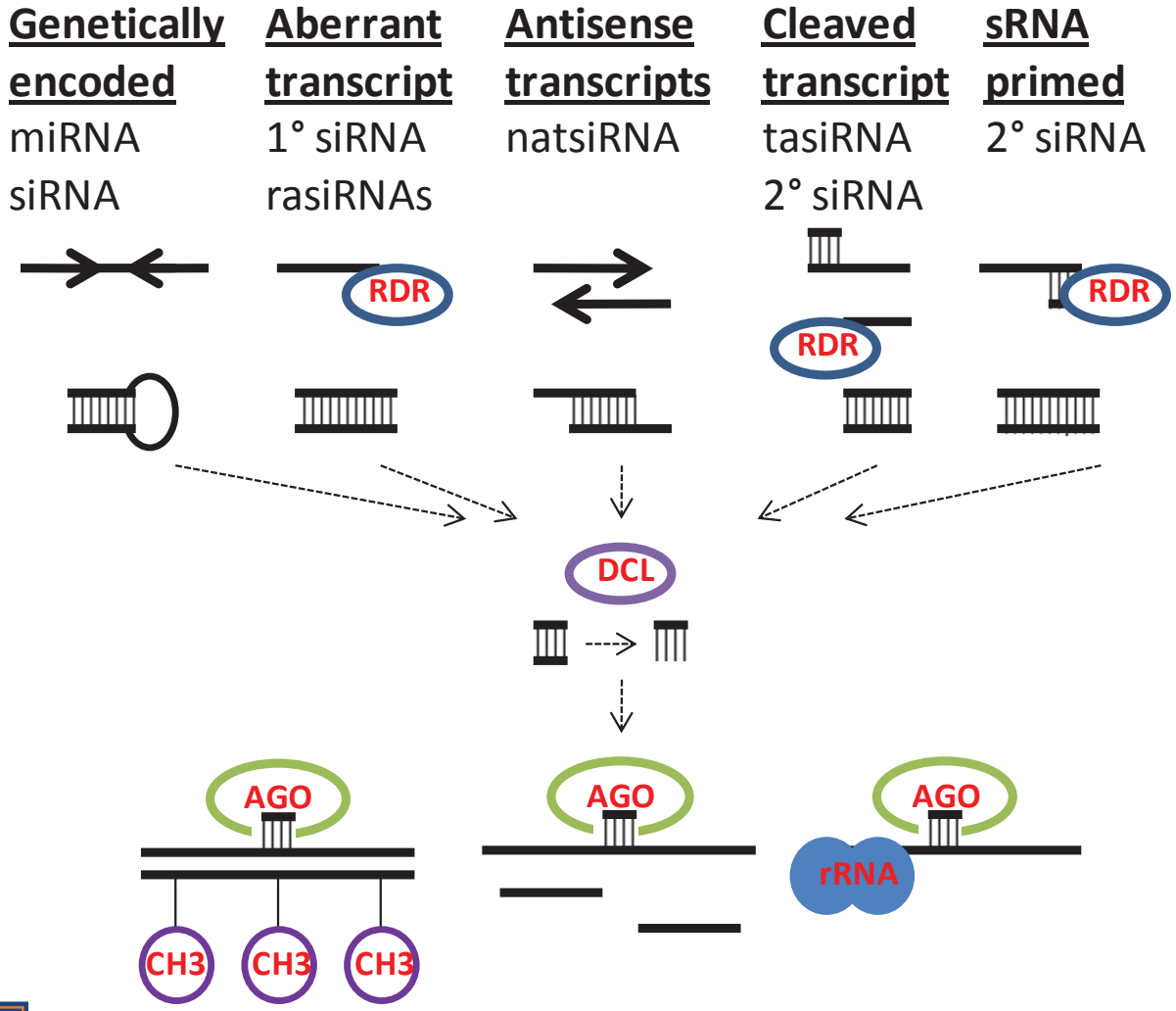
Possibility for something new in hybrid (non-additivity) even from factors behaving additively

sRNAs regulate crop phenotypes

- Soybean seed coat color
  - Tuteja et al. 2009
- Flowering time, biomass in maize
  - Lauter et al. 2005



sRNAs are major regulators of the genome

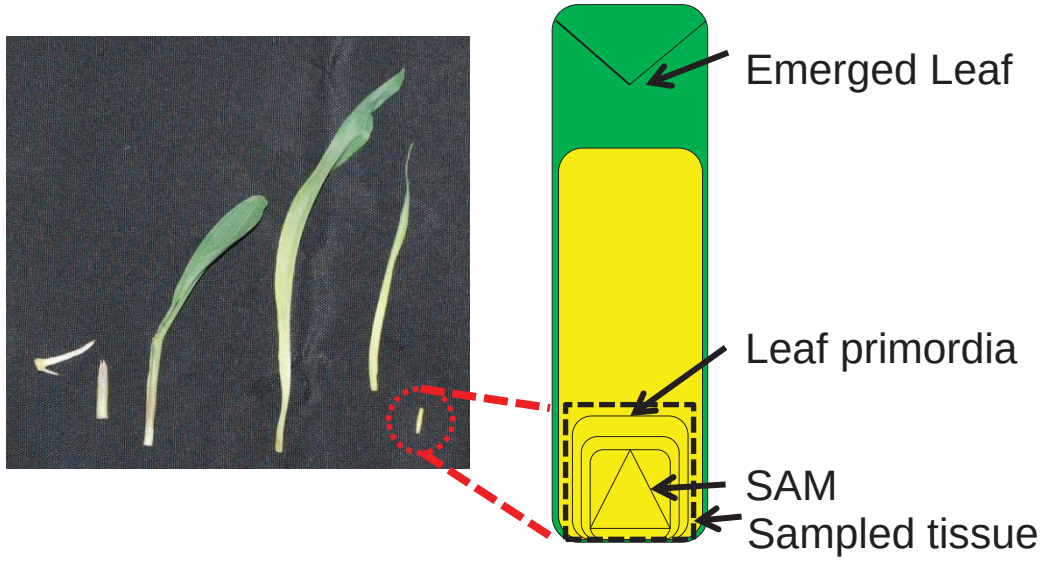


- Small interfering RNAs (siRNAs) regulate genes and repeats
  - RDR enzymes
  - Maintain genome integrity
- 21/22-nt siRNAs
  - post-transcriptional
- 24-nt siRNAs
  - transcriptional

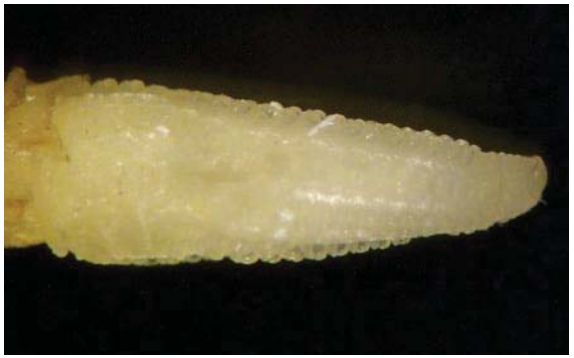
How do sRNAs behave following hybridization in maize?

- sRNA sequencing from B73, Mo17, and their hybrids
  - Compared abundances (reads per million) of individual sRNAs and blocks of sRNAs between the genotypes
- sampled actively growing tissues that program greater growth of hybrids

Shoot apex (tips) at 11 DAS



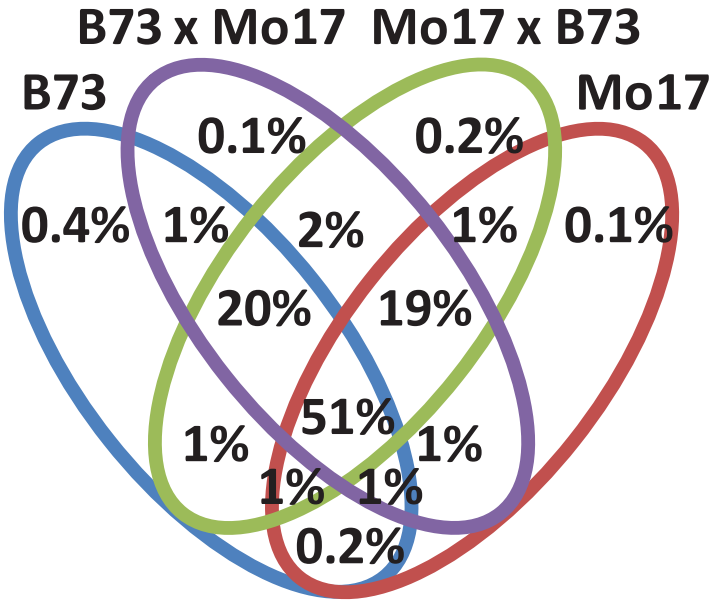
Ear at V12 growth stage



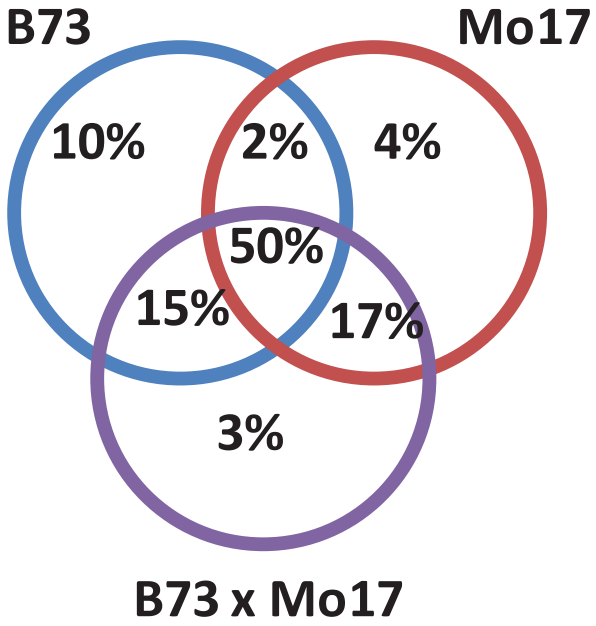
Differences between parents and hybrids result from B73 and Mo17 passing on divergent siRNA populations

% of 21-24-nt siRNAs

Shoot apex



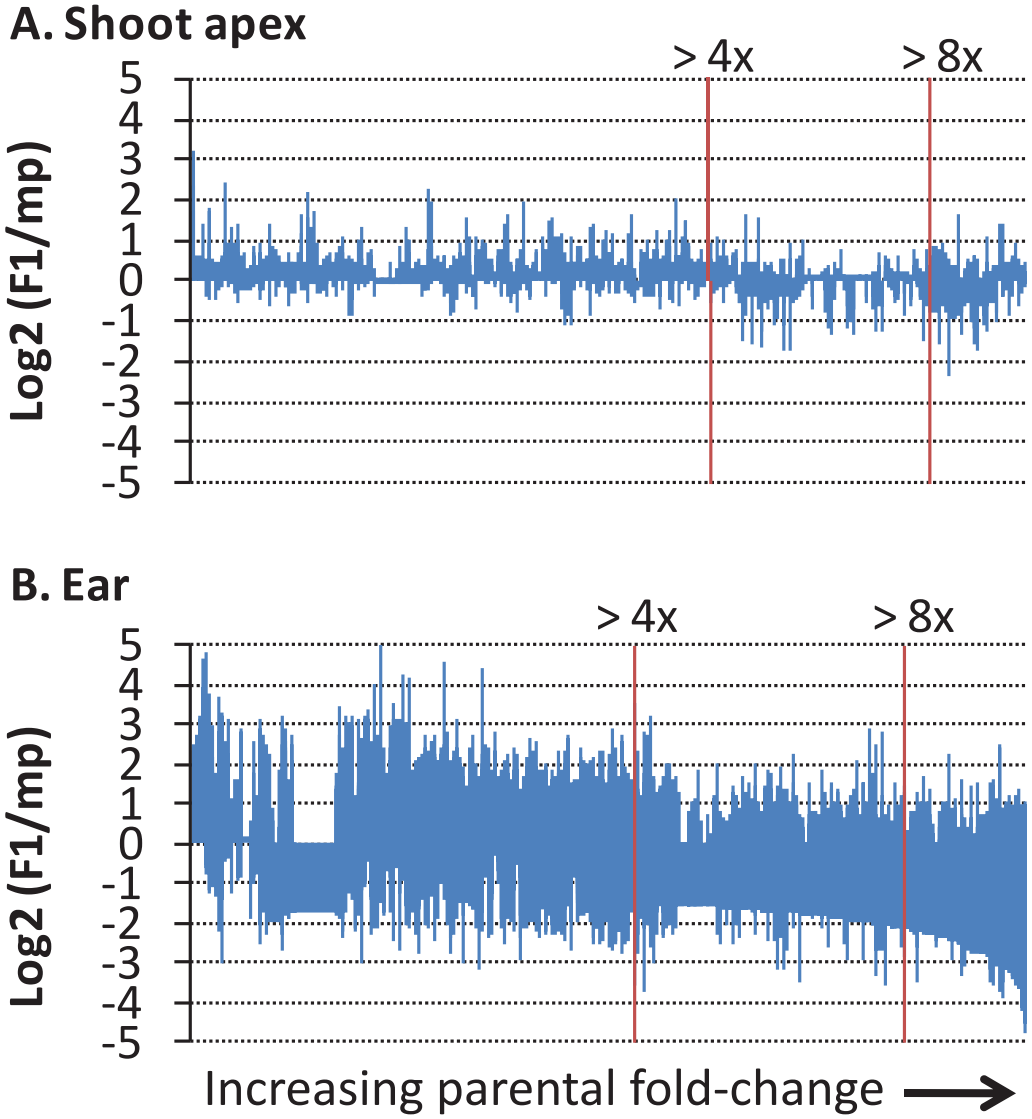
Ear



Genotypes

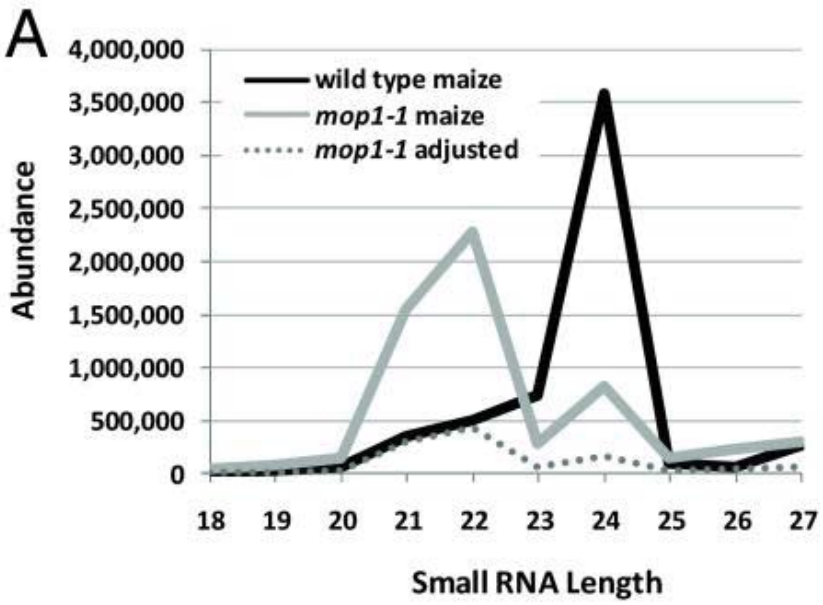
- B73
- Mo17
- B73 x Mo17
- Mo17 x B73

Parental differences in 21-24-nt siRNAs are inherited at levels below mid-parent in the ear



- 24-nt siRNAs reduced in *Arabidopsis*, rice, wheat hybrids/polyploids
  - Connected to epigenetic and gene expression changes (Groszman et al. 2011)
- Transgressive phenotypes of tomato RILs are influenced by siRNAs (Shivaprasad et al. 2011)

MOP1 (*RDR2*) provides unique system to test importance of 24-nt siRNAs to hybrid vigor

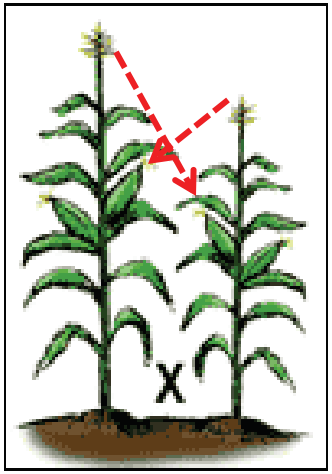


Nobuta et al. 2008

Parent 1 X **Parent 2**

B73- wild type × Mo17- wild type

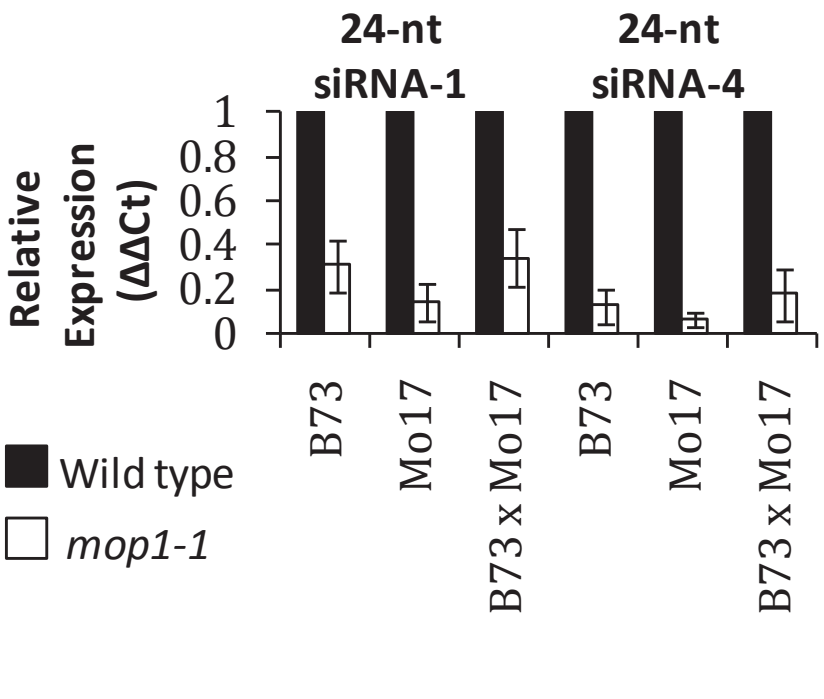
B73- *mop1-1* × Mo17- *mop1-1*

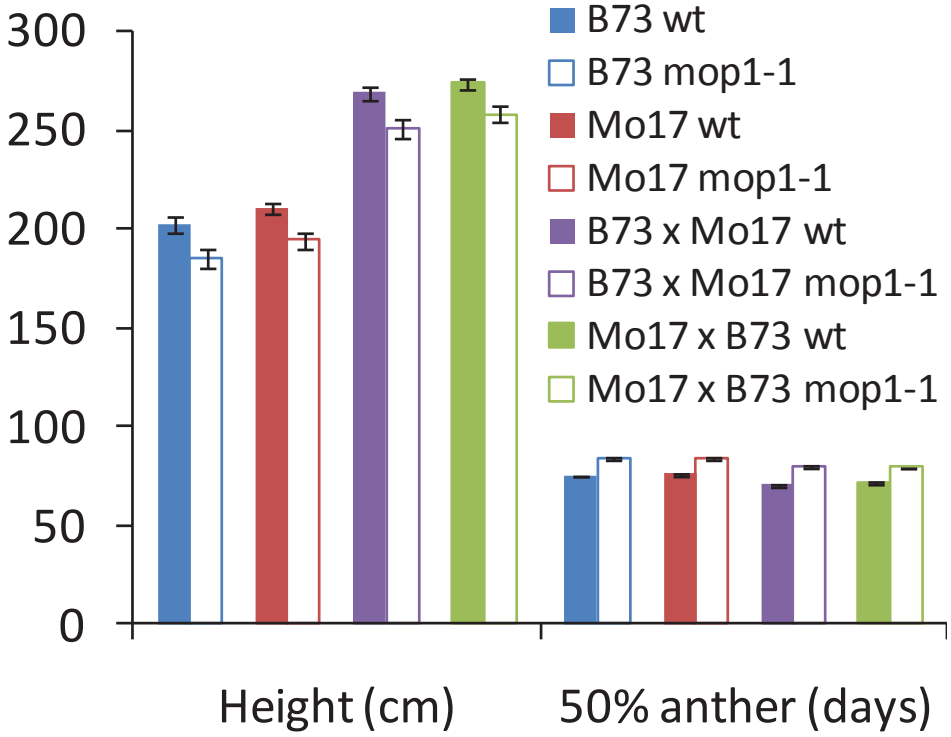


Reciprocal hybrids

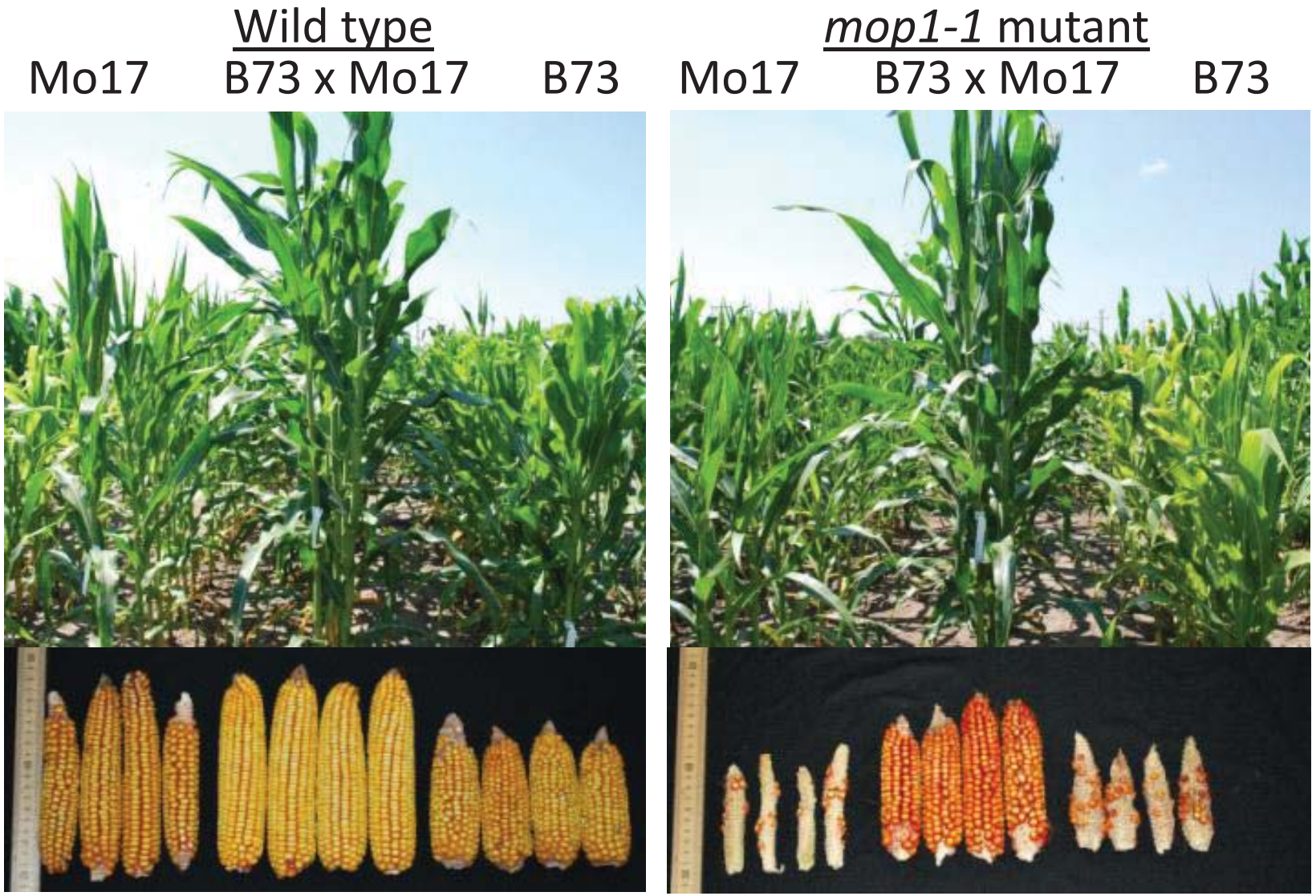
Do the mutant hybrids still show as much heterosis as wild type hybrids?

Loss of *MOP1* reduces 24-nt siRNAs and height and delays flowering for each genotype

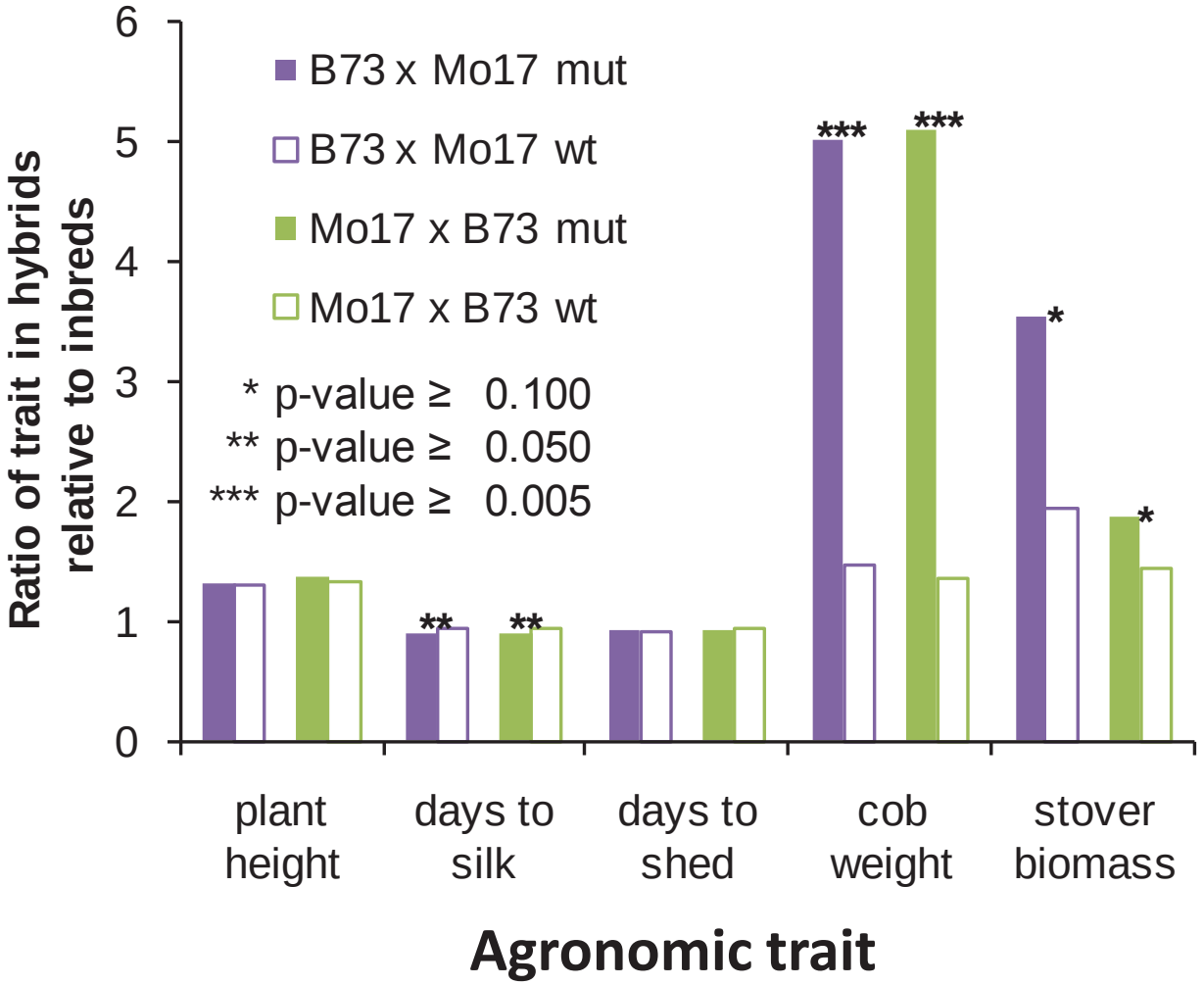




Loss of *MOP1* does not suppress hybrid vigor for B73xMo17



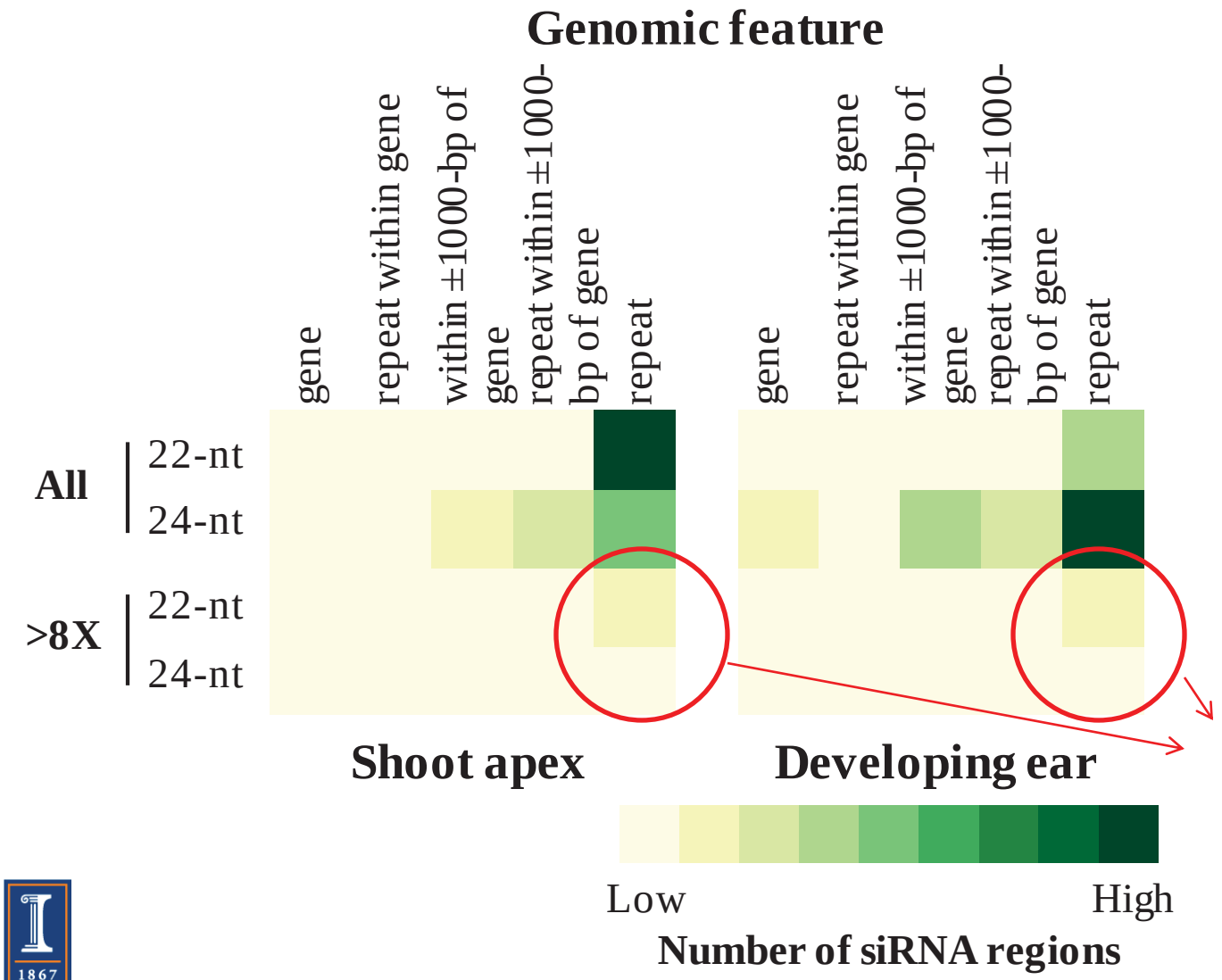
MOP1 mutant hybrids show the same degree of heterosis as wild type hybrids



Conclusions on how sRNAs vary following hybridization

- Hybridization doesn't cause global changes to sRNA populations or core components of sRNA biogenesis
- *MOP1* result questions the functional significance of 24-nt siRNAs to hybrid vigor
  - 24-nt siRNAs may play a bigger role in other processes (inbreeding?)

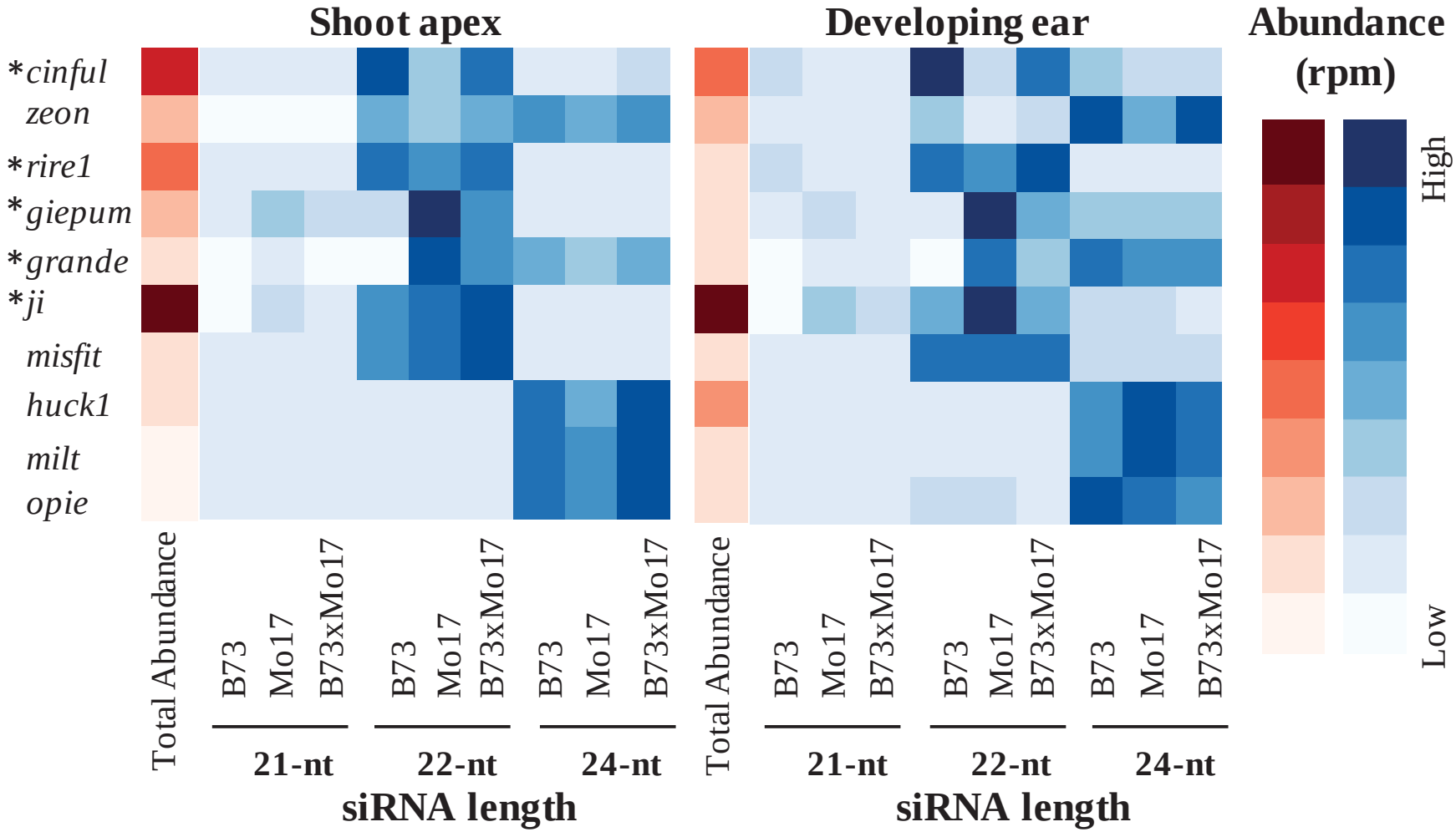
Differences in 21-24-nt siRNAs between B73 and Mo17 primarily originate from repeat regions



Repeats = DNA transposons, MITEs, helitrons, retrotransposons

Majority of genomic intervals annotated as high copy retrotransposon families

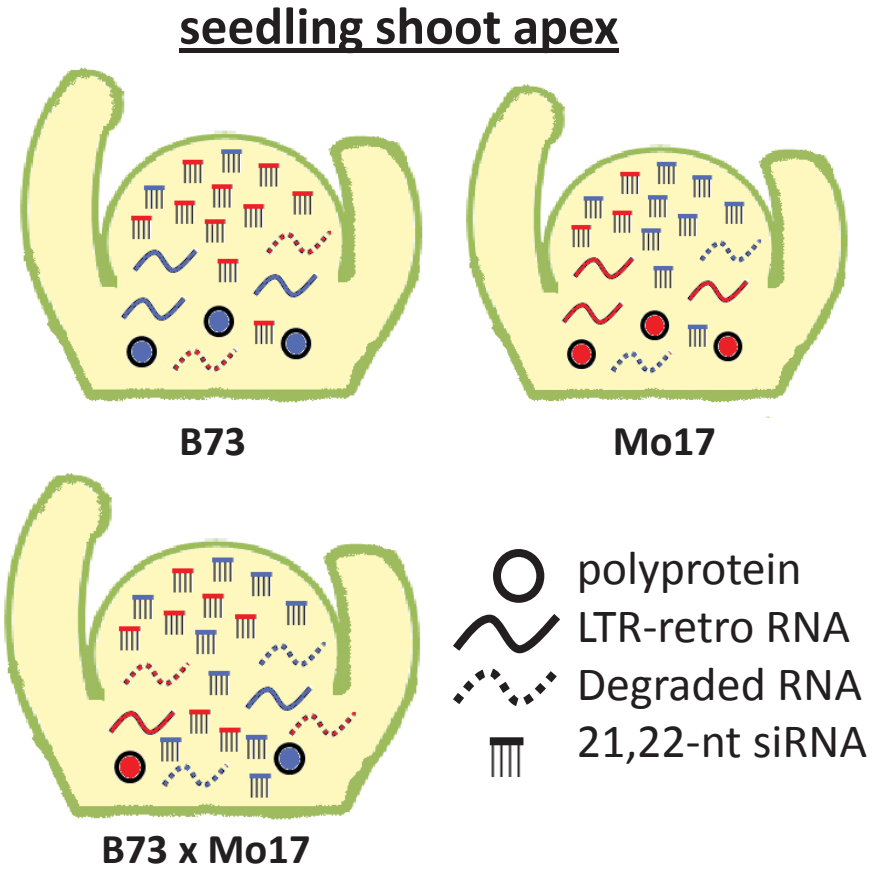
Parental differences in retrotransposon siRNA activity are driven by 21/22-nt siRNAs, not 24-nt siRNAs



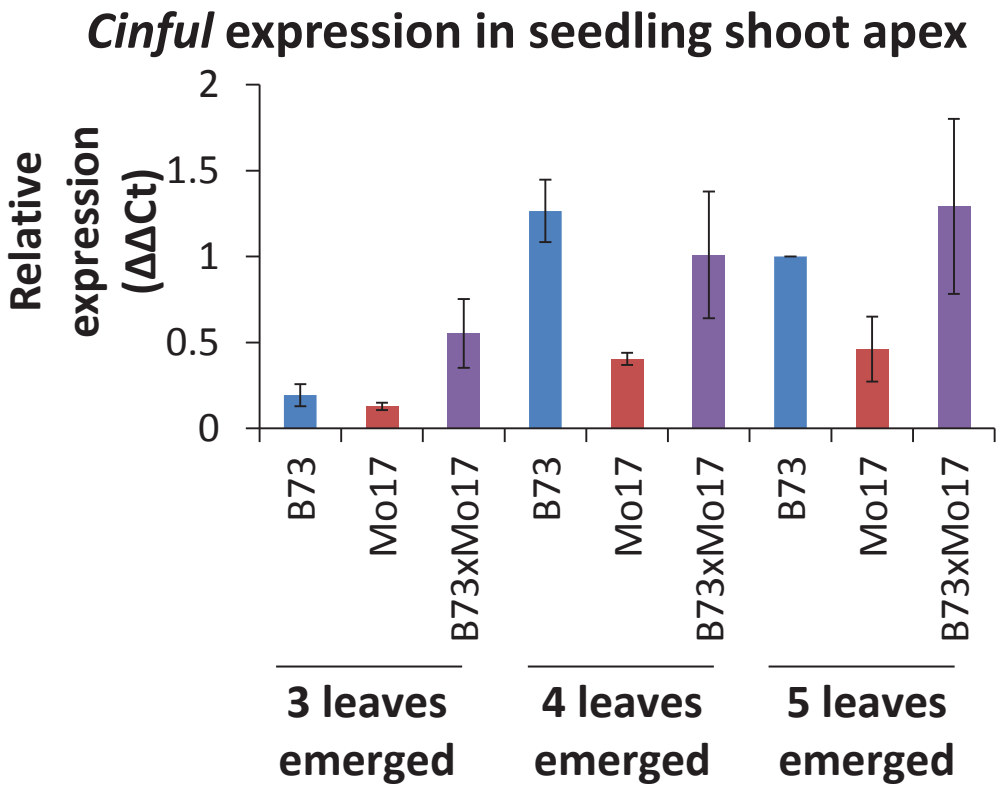
* Indicates that for the length of siRNA investigated, B73 and Mo17 were found to significantly differ in their abundance of siRNAs in the same direction in both tissues (χ^2 test, Bonferroni corrected p-value ≤ 0.01).

Retrotransposon derived siRNAs and cellular proliferation

- Retrotransposon derived siRNAs in human stem cells may regulate DNA repair, cell cycle control and chromatin
  - (Wang et al. 2011)
- Active retrotransposons in pollen cells are dealt with at post-transcriptional level
  - Slotkin et al. 2009



Combining divergent 21/22-nt rasiRNA *cinful* populations in the hybrid doesn't lead to greater silencing



- What are retrotransposon derived siRNAs doing in proliferating tissues?
  - Regulate themselves?
  - Regulate genes? (Ohtsu et al. 2007)
  - Recent evidence for gene regulation found in *Arabidopsis* (McCue et al. 2011)

Current work: How do rasiRNAs vary among a diverse set of inbred lines in maize?

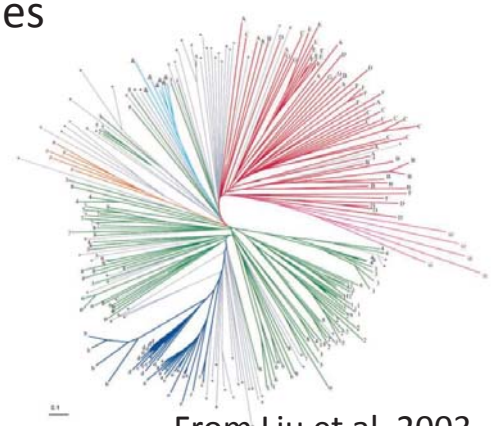
- Characterizing 21/22-nt rasiRNA across diverse maize germplasm combined with genetic tests of hybrid performance
- Determining if genes are targeted by rasiRNAs
- Investigating inheritance/stability of rasiRNAs

Genetic Material

36 inbred lines

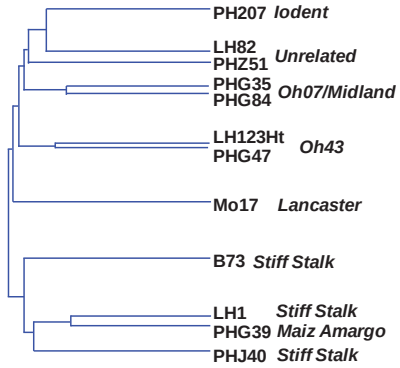
B73, Mo17

22 NAM founders



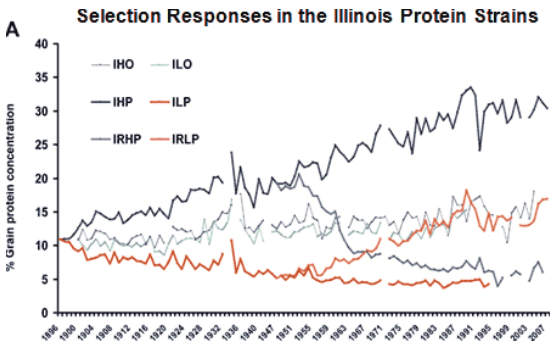
From Liu et al. 2003

10 PVP lines

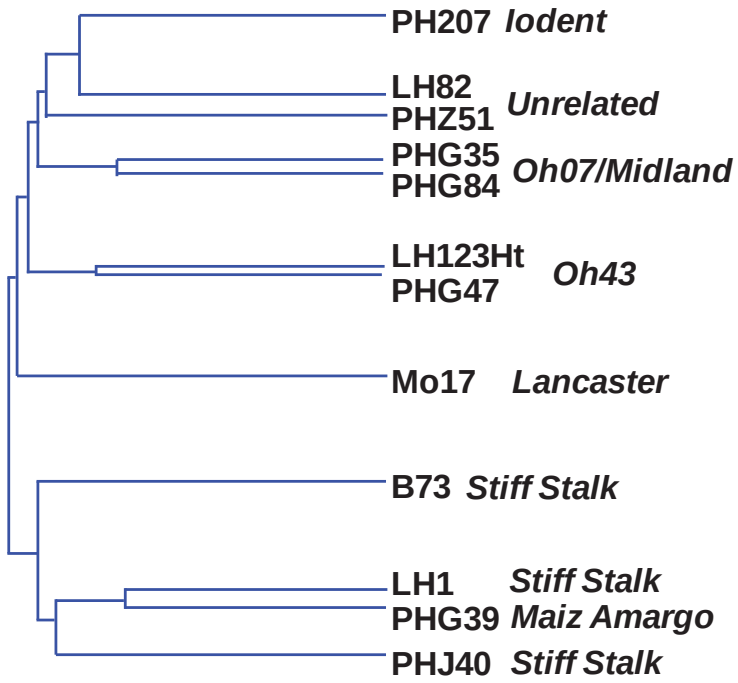


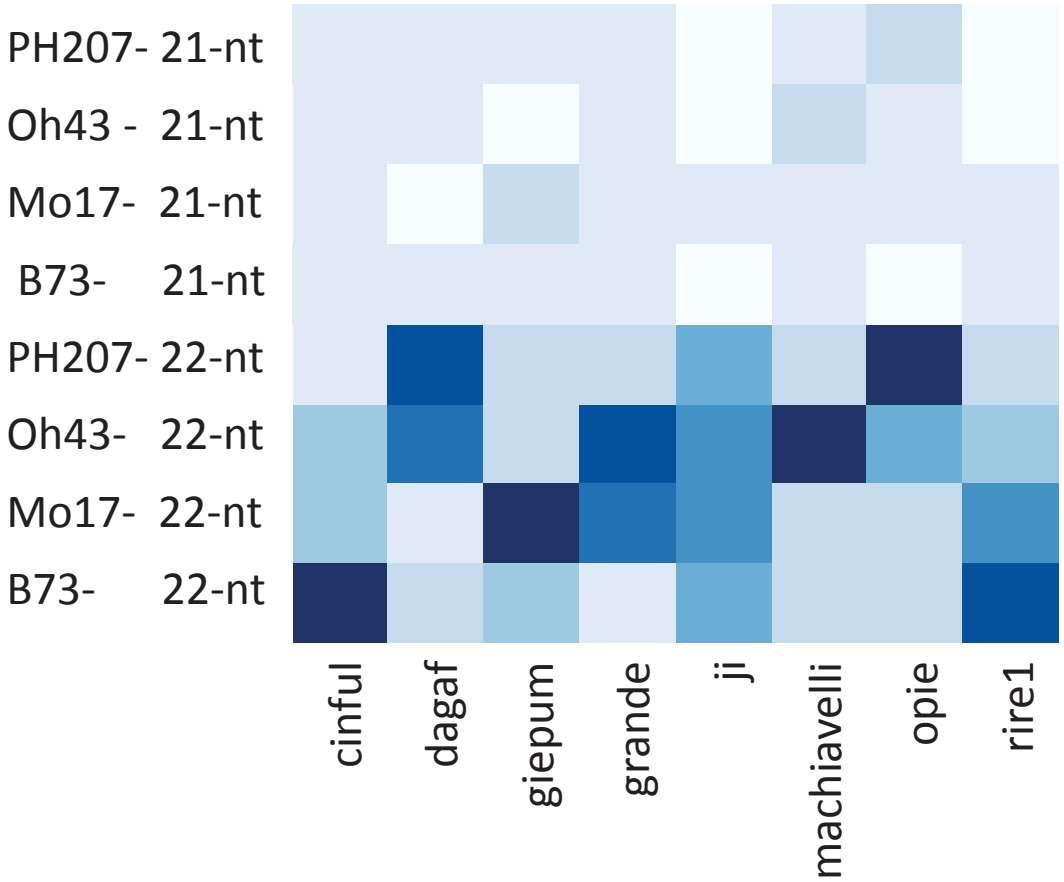
IHP1

ILP1



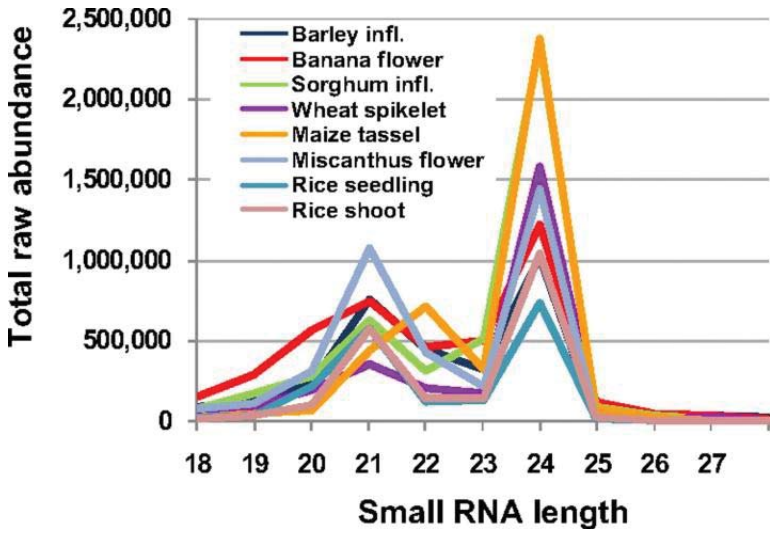
Are heterotic subgroups defined by specific patterns of 21/22-nt rasiRNA activities?



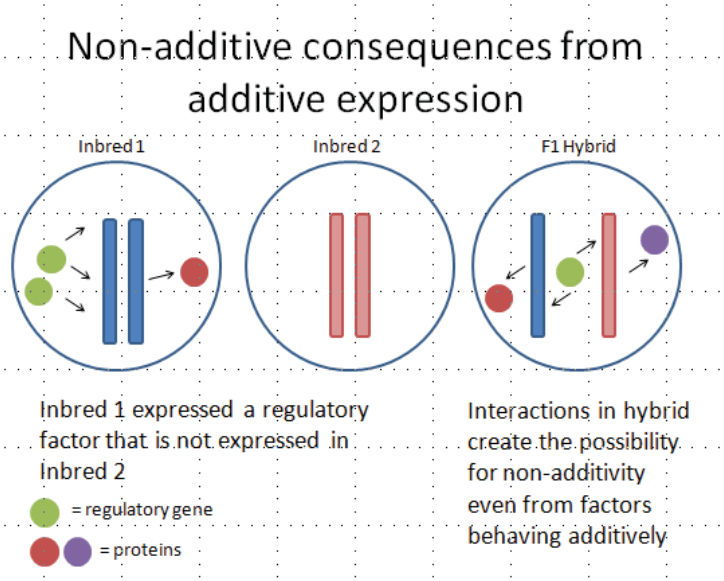


Conclusions for parental variation in siRNAs

- Studies in *Arabidopsis*, rice, and wheat did not find significant variation for 21/22-nt siRNAs derived from retrotransposons
- Heterosis cannot be compared across genera because the relative degree of genetic differences varies (East, 1936)
  - Related to the high degree of heterosis observed in the species?



Nobuta et al. 2008



Take home messages

- The maize genome provides a significant opportunity for inbred lines to vary for sRNAs
  - 21/22-nt rasiRNAs from retrotransposons may allow inbred lines to differ in their post-transcriptional regulation of repeat elements and genes at a genomic scale
- sRNAs are a source for genetic variation at the epigenetic and post-transcriptional levels
  - Could this genomics information be useful to guide breeding strategies?

Acknowledgements

- National Science Foundation
  - Heterosis Challenge Grant
- Pioneer Hi-Bred
  - Bob Seifert Honorary Plant Breeding Fellowship
- Moose Lab
  - Qing Li
  - Wei Zhang
- Hudson Lab
  - Kranthi Varala
  - Wendy Win
  - Ying Li
- Jane Dorweiler
- EBI/Miscanthus
  - Magdy Albady
  - Chris Austin