

From Mice to Maize: a multiparental population for fine mapping in *Zea mays*

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Rationale

Advanced crossing designs originally developed in mice (Churchill et al., 2004) showed a superior power for complex traits mapping. We transferred the multiparental approach to maize to create a multi-purpose tool for the genetic and molecular dissection of complex traits once the maize genome would have been sequenced. The advantages in maize as compared to mice were i) less generations needed (7 vs. 25), ii) easier RILs storage in form of seeds, and iii) a much cheaper cost.



Building the 8-way RILs population

Eight maize inbred lines (A632, B73, B96, F7, H99, HP301, Mo17 and W153R) were chosen as parents to maximize genetic and phenotypical variation. CML91 was also added to the founding lines as a backup parent. Parental lines were crossed following an half-diallele scheme (Figure 1). The 28 so obtained 2-ways hybrids were intercrossed according to a "disjoint diallel" scheme (Figure 2), in which only crosses between entries with no parents in common were allowed. Finally, the 210 so obtained 4-ways hybrids were bulked in 70 pools, each composed by the three 4-ways hybrids bearing the same alleles in all possible parent-of-origin cis combinations, and 35 8-ways hybrids were produced by crossing pairs of complementary 4-ways hybrids in a second disjoint diallel (Figure 3). Both 4-ways and 8-ways hybrids were produced by collecting and pooling pollen from at least 5 individuals for each cross and used to fertilize at least 3 ears. The resulting seeds were weighted and mixed, maintaining a balanced contribution from each of the fertilized ears. The inbreeding stage was initiated from the 35 pools of 8-ways hybrids by random weighted selection of 5,000 founders individuals. After 5 selfing generation in the summer 2011 > 1300 8W-RILF6 were obtained by single seed descent. For about 1,000 F6 lines enough seed was produced to set up phenotypic trials.

Inbred line	Group	Subgroup	x	A	B	C	D	E	F	G	H
A632	SS	B14A	►	A							
B73	SS	B73	►	B							
B96	TS	Suwani	►	C							
F7	Mixed	-	►	D							
H99	NSS	NSS-mixed	►	E							
HP301	Popcorn	-	►	F							
Mo17	NSS	CO109xMo17	►	G							
W153R	NSS	NSS-mixed	►	H							

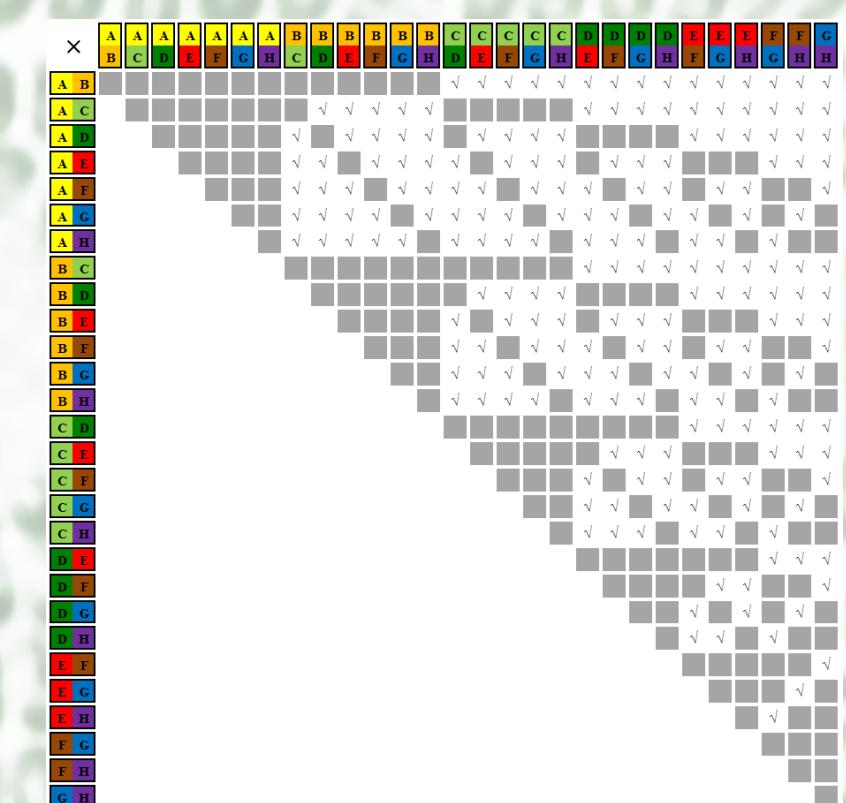


Figure 1. Two-way crossing scheme. The eight founder inbred lines are indicated by letters (A to H). Check marks indicate allowed crosses (no shared parents); gray squares indicate prohibited crosses. Letters A to H in rows and columns headers indicate the combination of founder lines in each of the crossed 2-way hybrids.

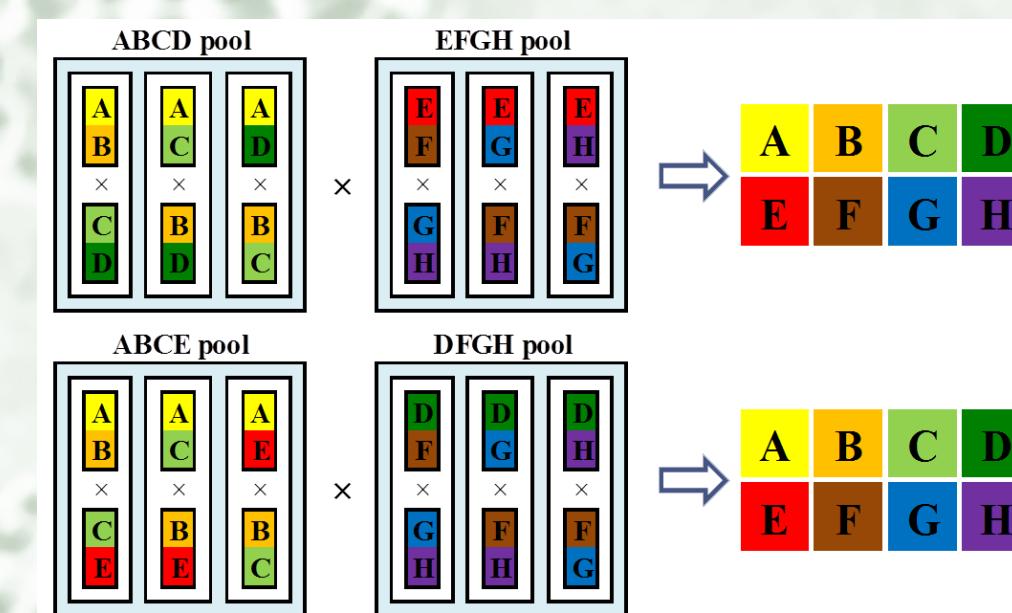


Figure 2. Four-way crossing scheme (first disjoint diallel). Check marks indicate allowed crosses (no shared parents); gray squares indicate prohibited crosses. Letters A to H in rows and columns headers indicate the combination of founder lines in each of the crossed 2-way hybrids.

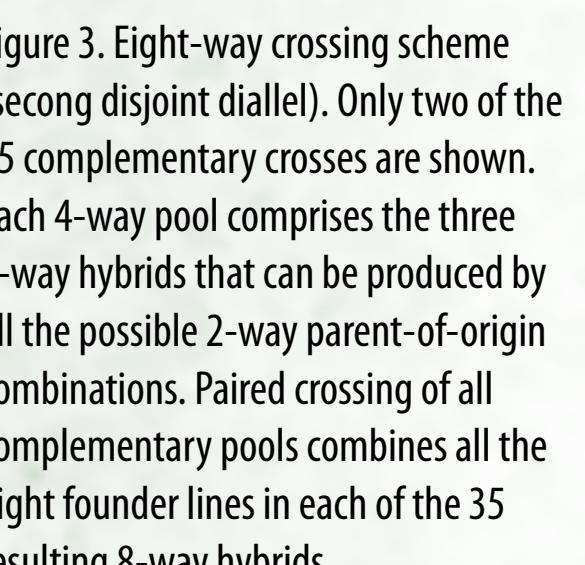


Figure 3. Eight-way crossing scheme (second disjoint diallel). Only two of the 35 complementary crosses are shown. Each 4-way pool comprises the three 4-way hybrids that can be produced by all the possible 2-way parent-of-origin combinations. Paired crossing of all complementary pools combines all the eight founder lines in each of the 35 resulting 8-way hybrids.

Phenotyping

A total of 576 8-way RILs F6 were chosen as core population for phenotyping and genotyping, as the ones with the largest seed stock available. Phenotyping (Figure 4) was carried out in two fields with a complete random block design with two replicas. Each RIL was phenotyped for plant height, ear height, yield and flowering time. Each of the 35 original pools was represented.



Figure 4. An example of the phenotypic variability deriving from just one of the 35 families.

Genotyping (work in progress...)

The same pool of 576 8-way RILs are being genotyped to assess the power provided by such population in complex traits mapping. The genotyping technology to be employed was chosen after comparing the performances of genotyping-by-sequencing (Elshire et al., 2011) and Illumina Infinium maize 50K SNP assay (Ganal et al., 2011) in genotyping parental lines. Infinium assay showed higher reliability and consistency of results, and yielded more than 40k SNPs polymorphic in at least one parent (Fig. 5). The Illumina platform was employed to genotype test F1s among parental lines (Fig. 6) plus an initial batch of 184 8-way RILs F6. Preliminary results are encouraging, as show an unprecedented potential for complex traits mapping in maize. Recombination blocks were severely reduced in length, as shown by the steep LD decrease (Fig. 7). Kinship among RILs is low and uniform (Fig. 8), resulting in highly differentiated lines with low genetic structure (Fig. 9).

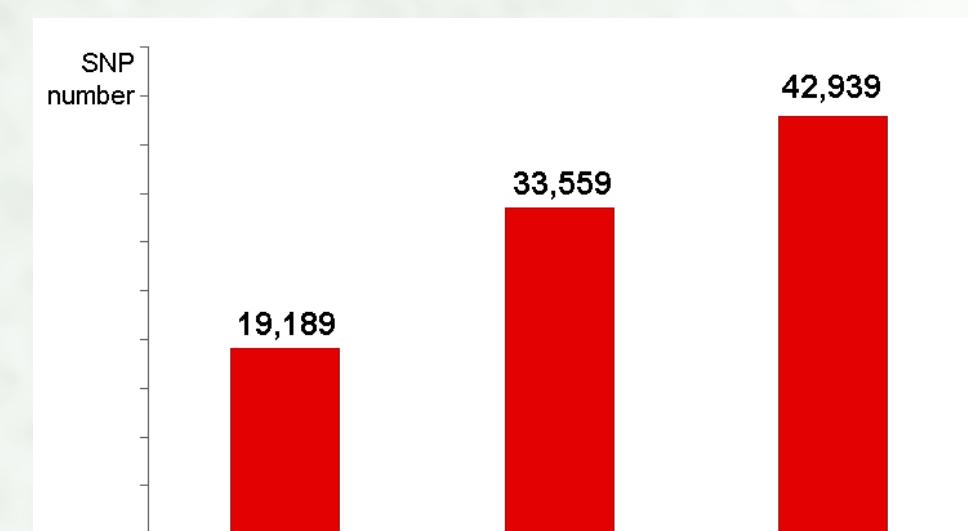


Figure 5. Mapped and polymorphic SNPs generated with Illumina Infinium assay, with 2, 4 or 8 parental populations. The comparison shows how the 8 parents approach more than doubled the variability in RILs population when compared with standard breeding approaches.

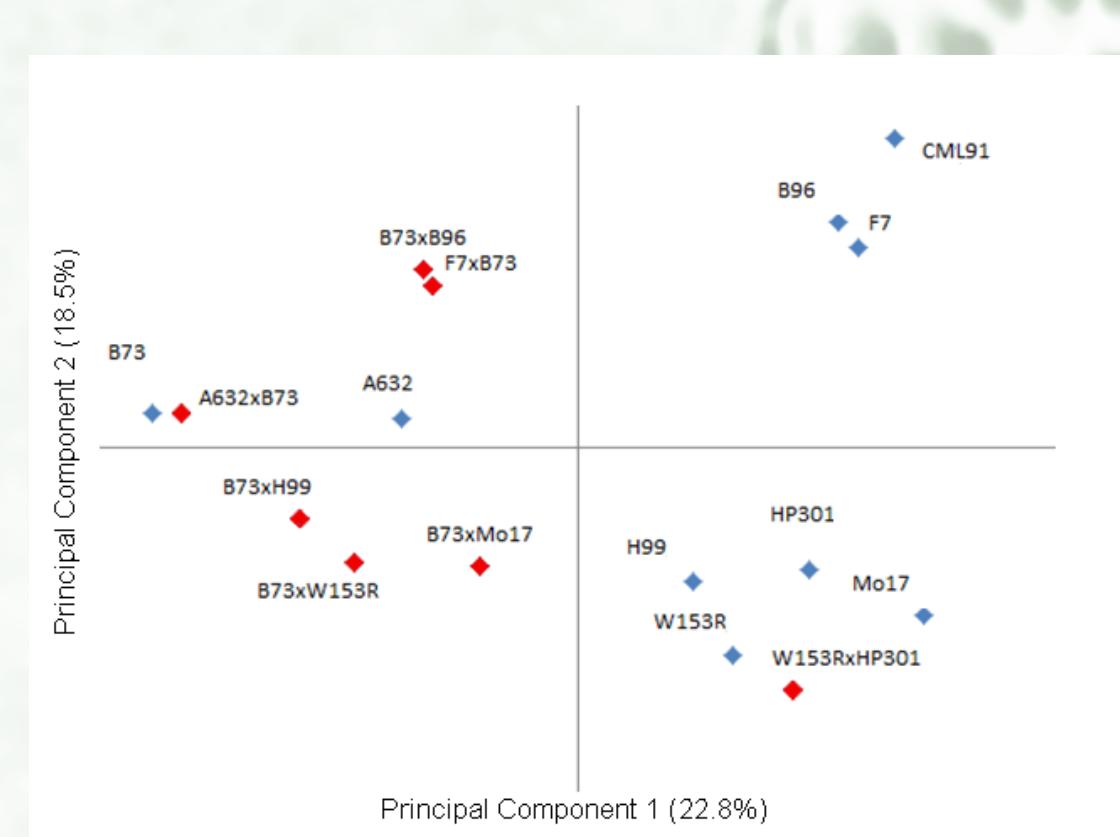


Figure 6. PCA space representing the genetic relations among parental lines (blue diamonds) and a set of test F1 (red diamonds). Calculations based on the set of 42,939 mapped polymorphic SNPs obtained through Illumina Infinium assay. Note how parents are sorted following their breeding group (Figure 1). F1s are consistently placed in between the corresponding parents, confirming assay reliability.

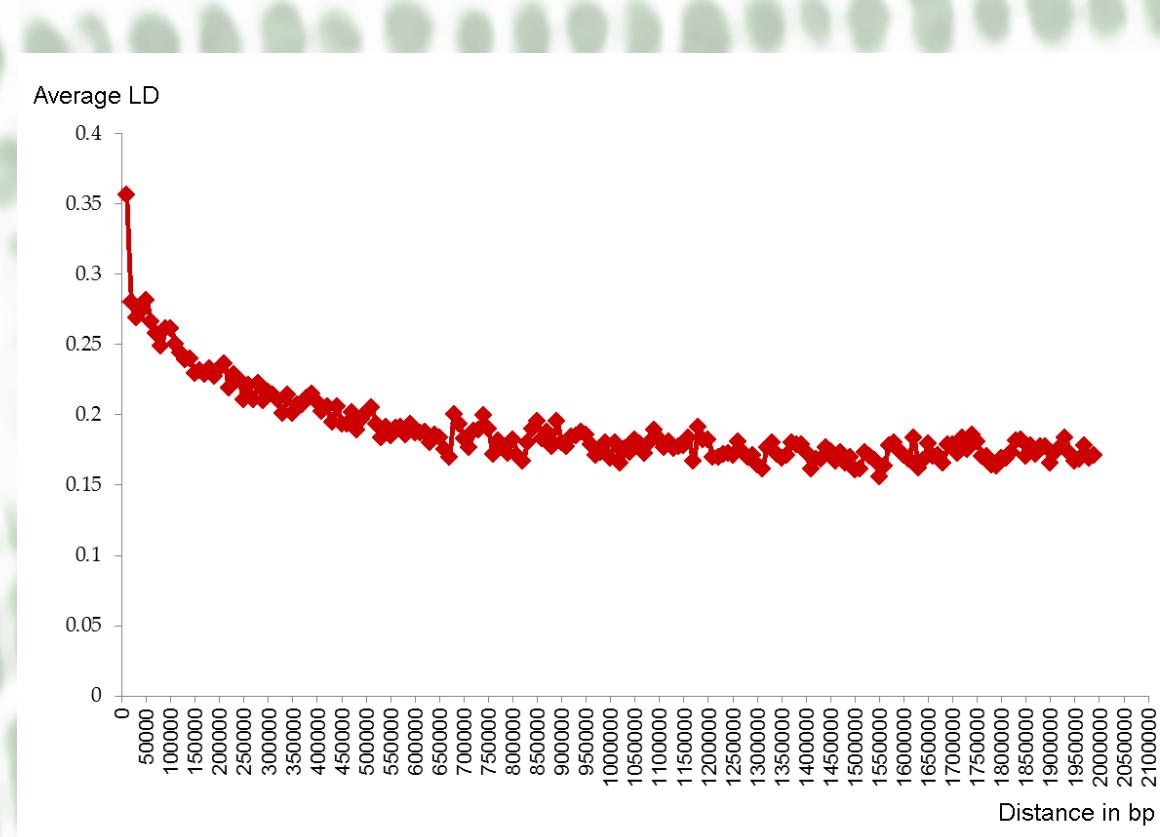


Figure 7. Linkage disequilibrium as a function of physical distance between markers, calculated as a mean value each 10 kb interval in all chromosomes upon 184 RILs genotypes. The asymptotic curve prevents from calculating precise flex points, yet the steepest drop is advisable before 100 kb. This indicates that, on average, haplotypes should be very small.

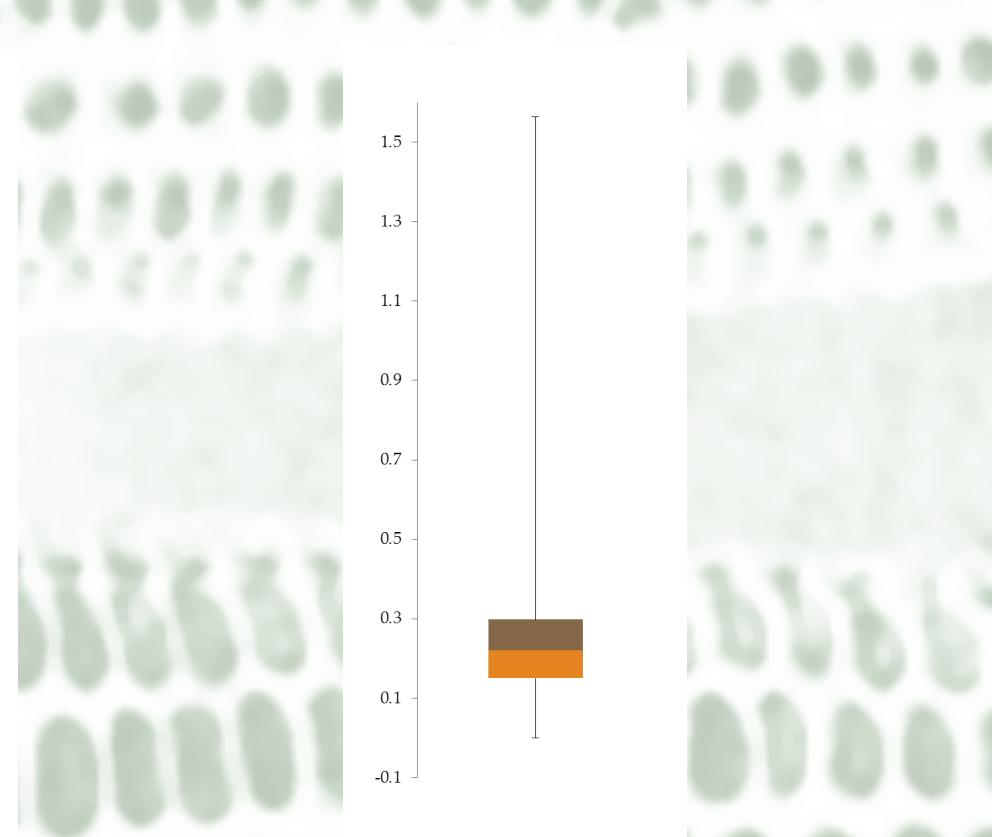


Figure 8. Boxplot showing the low (0.23 in average) and uniform distribution of kinship values among 184 RILs.

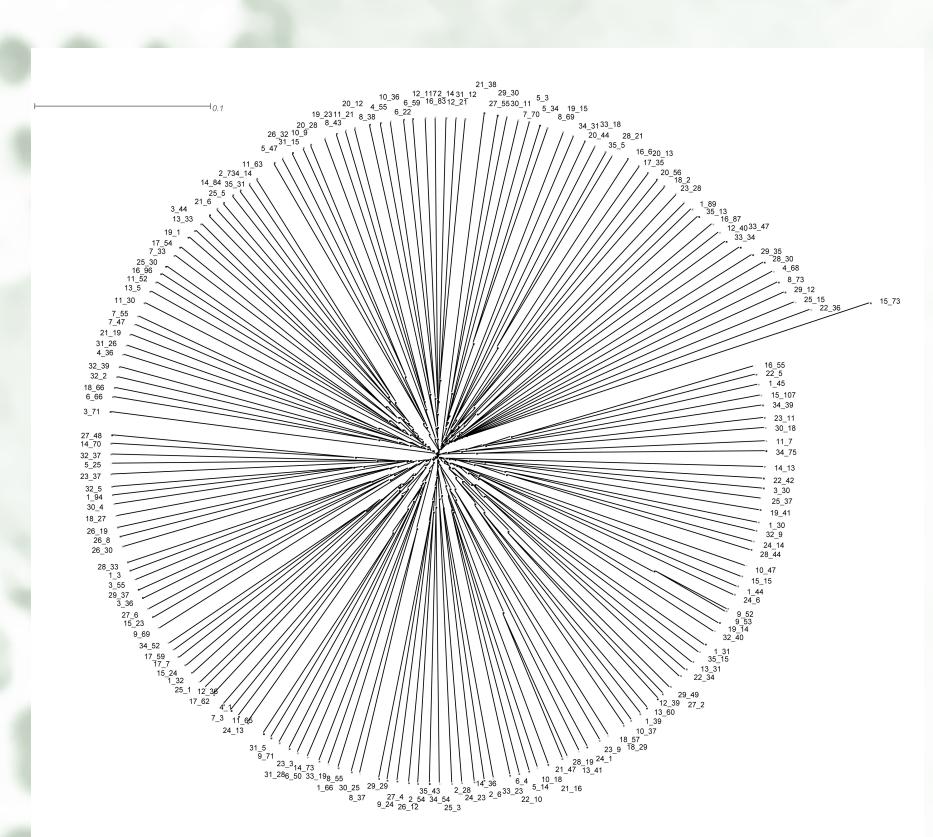


Figure 9. UPGMA phylogeny of 184 RILs. The 35 founding 8-way populations are mixed throughout. The long terminal branches connecting individual RILs to very deep nodes suggest high recombination rates and extremely low population structure.

References

- Elshire, Robert J., et al. "A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species." *PLoS One* 6.5 (2011): e19379.
- Churchill, Gary A., et al. "The Collaborative Cross, a community resource for the genetic analysis of complex traits." *Nature genetics* 36.11 (2004): 1133-1137.
- Ganal, Martin W., et al. "A large maize (*Zea mays* L.) SNP genotyping array: development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome." *PLoS One* 6.12 (2011): e28334.