Meiotic Crossovers Characterized by Haplotype-Specific Chromosome Painting in Maize

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Meiotic crossovers (COs) play a critical role in generating genetic variation and maintaining faithful segregation of homologous chromosomes during meiosis. We develop a haplotype-specific fluorescence in situ hybridization (FISH) technique that allows visualization of COs directly on metaphase chromosomes. Oligonucleotides (oligos) specific to chromosome 10 of maize inbreds B73 and Mo17, respectively, are synthesized and labeled as FISH probes. The parental and recombinant chromosome 10 in B73 x Mo17 F1hybrids and F2 progenies can be unambiguously identified by haplotype-specific FISH. Analysis of 58 F2 plants reveals COs located in regions very close to the centromere in recombinant inbred lines from an intermated B73 x Mo17 population, suggesting effective accumulation of COs in recombination-suppressed chromosomal regions through intermating and the potential to generate favorable allelic combinations of genes residing in these regions.

Probe design strategy

- Used the Chorus[1] software to generate single copy oligos (45 nt) from chromosome 10 of maize inbreds B73 and Mo17 for a total of 175,437 (B73) and 174,728 (Mo17) candidate probes.
- Selected probes present in one genome but absent from the other one and vice-versa (Present/Absent Variations - PAV), **6251** for B73 and **5506** for Mo17 (**PAV probe sets**).
- Selected probes with mismatches and/or indel located within the center part of probe:
  - **4353** sequences with 5 or more differences (**>5 SNPs probe set**)
  - **3894** sequences with 3 and 4 differences (**3-4 SNPs probe set**)
  - **6506** sequences with 2 differences (**2 SNPs probe set**)
  - **19,885** sequences with a single difference (**1 SNP probe set**)
- SNPs probes were designed as pairs, one probe specific to the B73 sequence and the other specific to the MO17 sequences

Experimental validation of probe sets

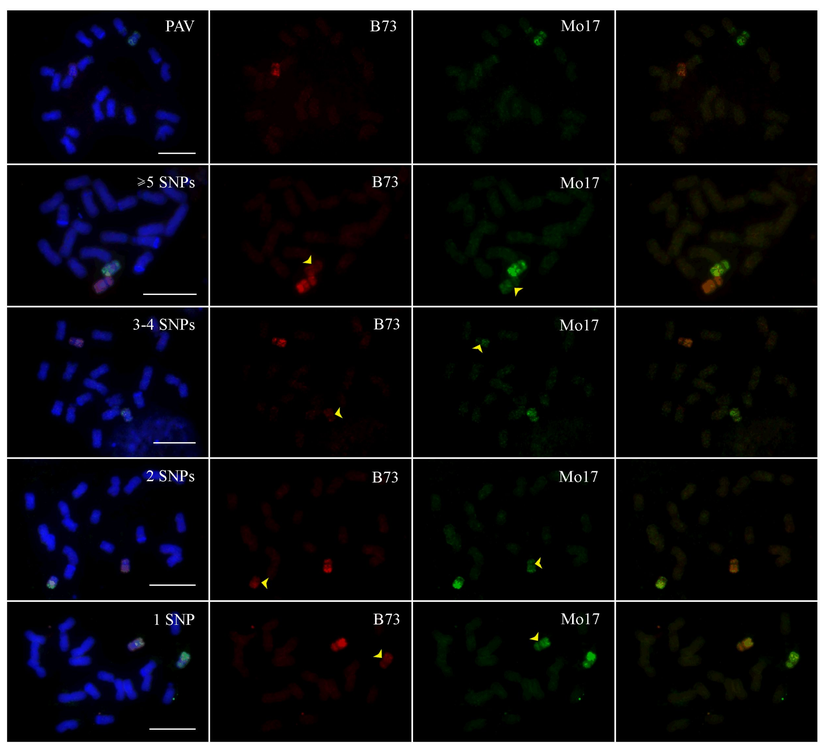
B73 probes labeled with biotin and detected with anti-biotin fluorescein.

Mo17 probes labeled with digoxigenin and detected with anti-digoxigenin rhodamine.

B73 and Mo17 probes were co-hybridized leading to competitive hybridization.

Yellow arrowheads indicate the cross-hybridization signals from B73-specific probes to Mo17 chromosome 10, and vice versa.

1 SNP probes can discriminate 2 copies.

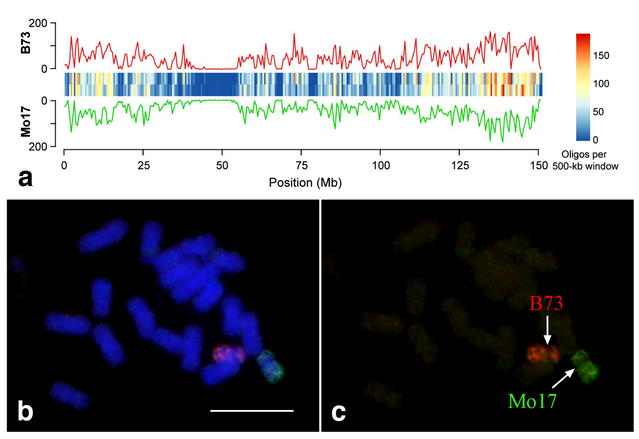


Final probe selection and chromosome coverage

Combined pools hapB (**14,498 probes**) and hapM (**13,753 probes**) of PAV, ≥5 SNPs, and 3–4 SNPs probe sets produce the best contrast of haplotype-specific FISH signals (Panel b, c).

Probes are not uniformly distributed on chromosome 10 (Panel a), with low coverage between 42–54 Mb (around centromere) and near ends.

These regions showed weak or undetectable FISH signals (Panel c).



Crossover detection

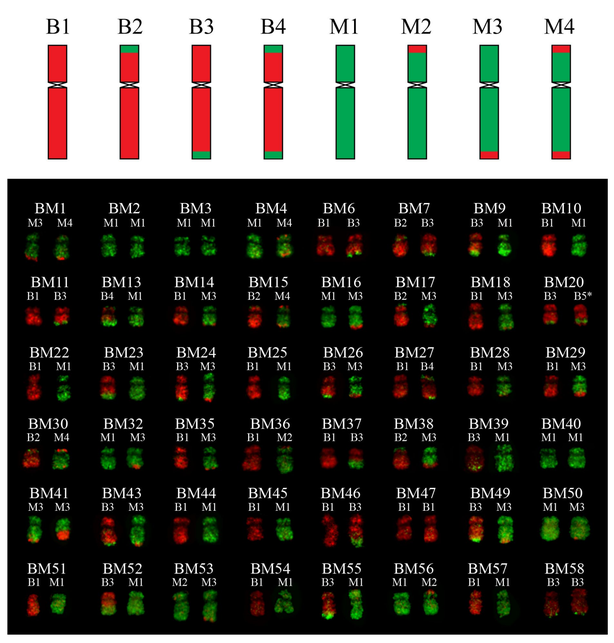
Produced F2 plants by pollinating sibling B73 × Mo17 hybrid plants.

Performed oligo-FISH experiments on somatic metaphase chromosomes from 58 F2 plants (BM1-BM58; 116 copies of chr 10).

Chromosomes can be cataloged as 8 different types based on the positions of chromosomal exchanges (top).

Identified at least one unambiguous B73-Mo17 chromosomal exchange on 50 (43%) of the 116 chromosomes, including 6 chromosomes with an exchange on both arms.

Three or more crossovers per chromosome were never identified in this analysis.



Correlation with sequencing data

An intermated B73 × Mo17 recombinant inbred line (IBMRIL) population was developed by randomly intermating plants for four generations following the F2 generation.

Genotyping data was obtained by genomic sequencing of 10 IMBRILs.

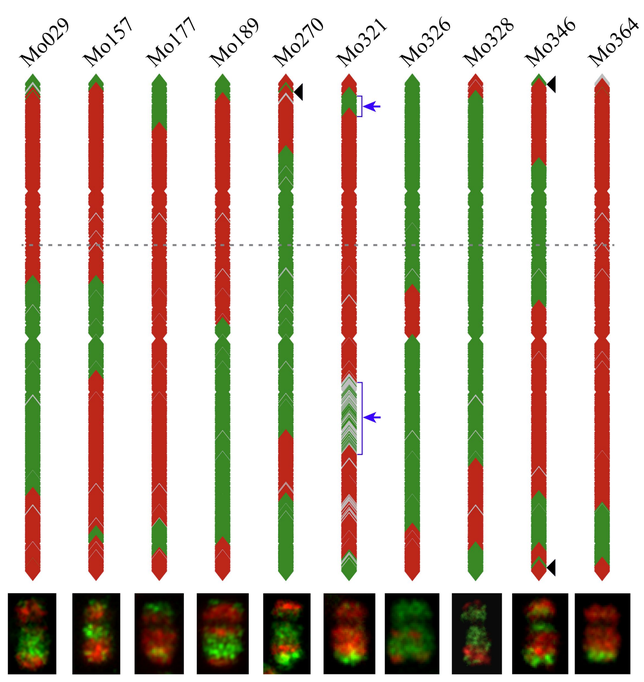
Genotyping data illustrated by location of markers from B73 (red) and Mo17 (green), respectively (top panel). Gray indicates missing data. A dashed line marks the putative position of the centromere.

FISH hybridization with pools HapB (red) and HapM Green) (bottom panel).

Regions indicated by blue arrows on Mo321 were not consistently visualized by oligo-FISH.

Black arrowheads on Mo270 and Mo346 point to small regions that were not identified by oligo-FISH.

Crossovers close to the centromere of chromosome 10 were detected in several IBMRILs.



Potential of haplotype-specific chromosome painting

- While PAV and multi-SNPs probes give better contrast, probes with only one SNP can still discriminate between homologous chromosomes
- Meiotic crossovers between homologous chromosomes can be visualized
- Chromosomal breakpoints from historical or multiple crossovers can be mapped
- Specific chromosomes derived from a single genotype can be tracked
- Extent of somatic recombination could potentially be examined
- True homologous chromosome pairing could potentially be distinguished from pairing of homeologous chromosomes with minor structural variation in polyploid species

REFERENCES

1. Chorus is available from https://github.com/forrestzhang/Chorus

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