A whole-genome approach to understanding meiotic recombination and crossover patterning

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Background

During meiosis, correct placement of crossovers (crossover patterning) ensures correct segregation of homologs. Crossovers are derived from homologous recombination, a pathway that repairs double-strand breaks using a homologous template.

Homologous recombination pathways can be traced via distinguishable tracts of heteroduplex DNA (hDNA), DNA in which the strands are derived from two different parental chromosomes.

Although it is normally obscured by corrections in mismatches between strands, we can resolve heteroduplex structure in recombinants of a test locus in mutants of *Drosophila melanogaster* deficient in short-patch and canonical mismatch repair. Previous analysis of heteroduplex DNA at this locus has revealed that heteroduplex DNA patterns not anticipated by the classical model of homologous recombination frequently occur.

Further analysis of heteroduplex DNA in both wild-type and mutants affecting crossover control is warranted to continue refining the homologous recombination model. However, analysis of heteroduplex DNA at this test locus is tedious, low-yield, and subject to bias due to disparities in SNP densities throughout the locus. **These complications warrant development of new tools to continue analyzing meiotic events.**

Aims

Resolve heteroduplex structure genome-wide by taking advantage of genetic tools in *Drosophila*, and use this data to inform our current molecular model of homologous recombination.

Simulate recombination between homologous chromosomes computationally to inform whole-genome sequencing analysis.

Introduction

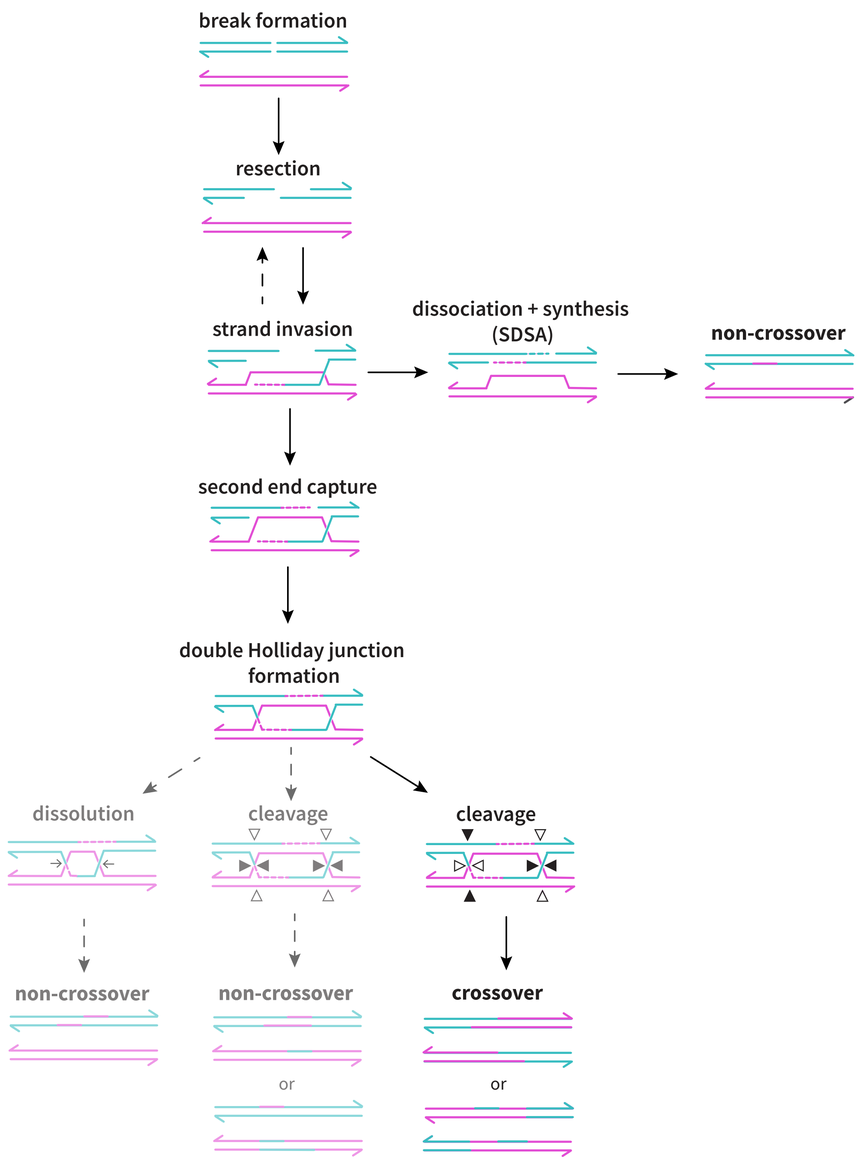

Figure 1. A classical model of meiotic recombination. Double-strand breaks are processed by resection. They then invade a homologous template (D-loop formation). After synthesis, the D-loop can dissociate and the break can repaired via synthesis-dependent strand annealing (SDSA), yielding a non-crossover. Otherwise, the intermediate can undergo second-end capture and double Holliday junction (dHJ) formation. The dHJ can be resolved via dissolution (non-crossover-forming), cleavage of two strands (non-crossover-forming), or cleavage of all four strands (crossover-forming). Formation of non-crossovers via dHJs (faded) is not predicted to occur during meiotic recombination. Dashed lines indicate events that have not been proven to occur during meiotic recombination in *Drosophila*.

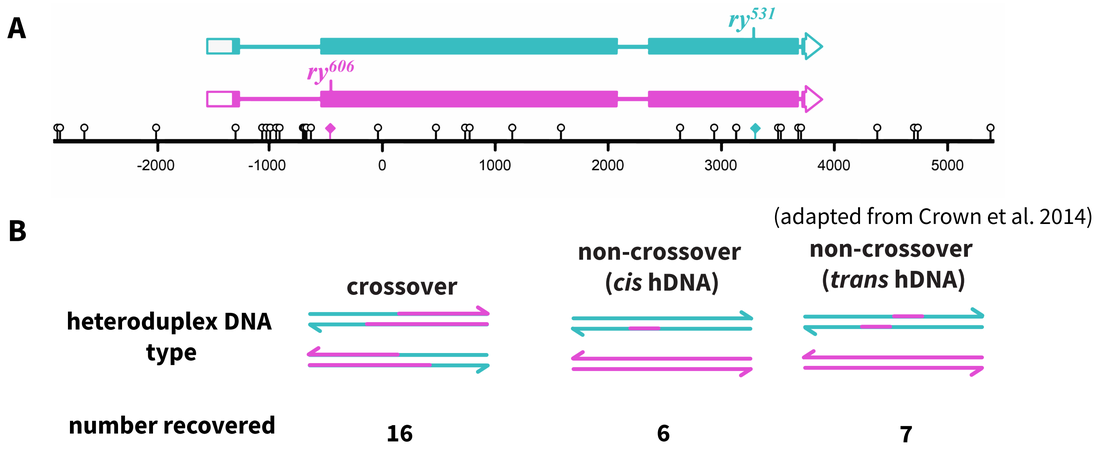

Figure 2. Unexpected hetereoduplex structures are observed in the absence of mismatch corrections. (A) Recombinants of the rosy test locus can be selectively isolated and heteroduplex can be characterized via PCR for SNPs along the locus. (B) Types of heteroduplex that were isolated from the assay. Only one crossover heteroduplex pattern (out of two possible patterns) was found. Non-crossovers exhibited both the *cis* heteroduplex pattern (indicative of SDSA) and *trans* heteroduplex pattern.

Approach

How can we identify heteroduplex DNA at the genome scale?

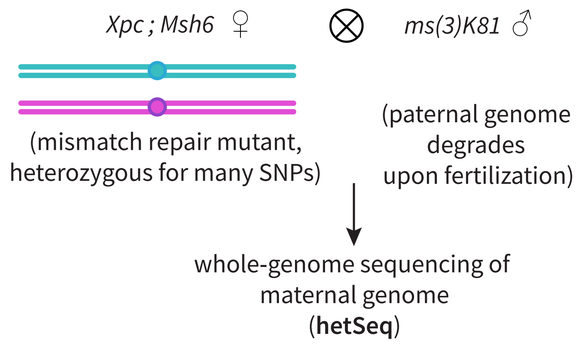

Figure 3. hetSeq ennables recovery of heteroduplex DNA genome-wide. Mismatch repair-defective females heterozygous for many SNPs genome-wide are mated to males with a sperm telomere protection defect that causes the paternal genome to rapidly degrade upon fertilization. Approximately 20% of offspring survive to gastrulation, enabling sequencing via Oxford Nanopore.

How can we simulate recombination computationally?

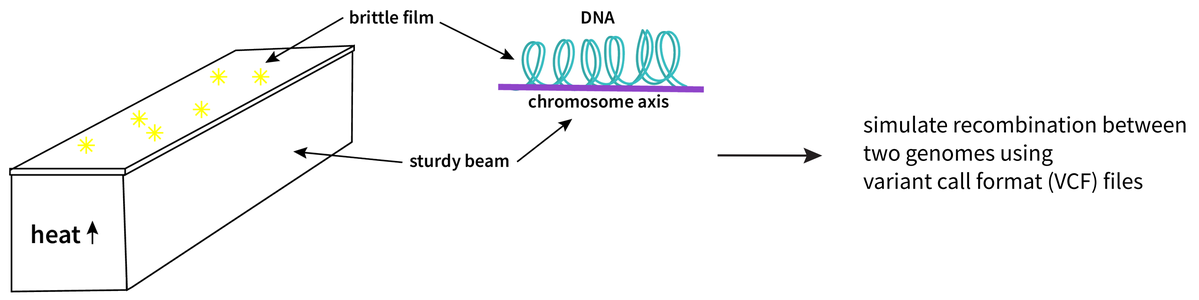

Figure 4. Simulation of recombination between two genomes. The beam-film model can be used to simulate crossover interference, which can then be applied to real genomes to determine how many recombination events are traceable in meiotic products via SNP differences.

Predictions

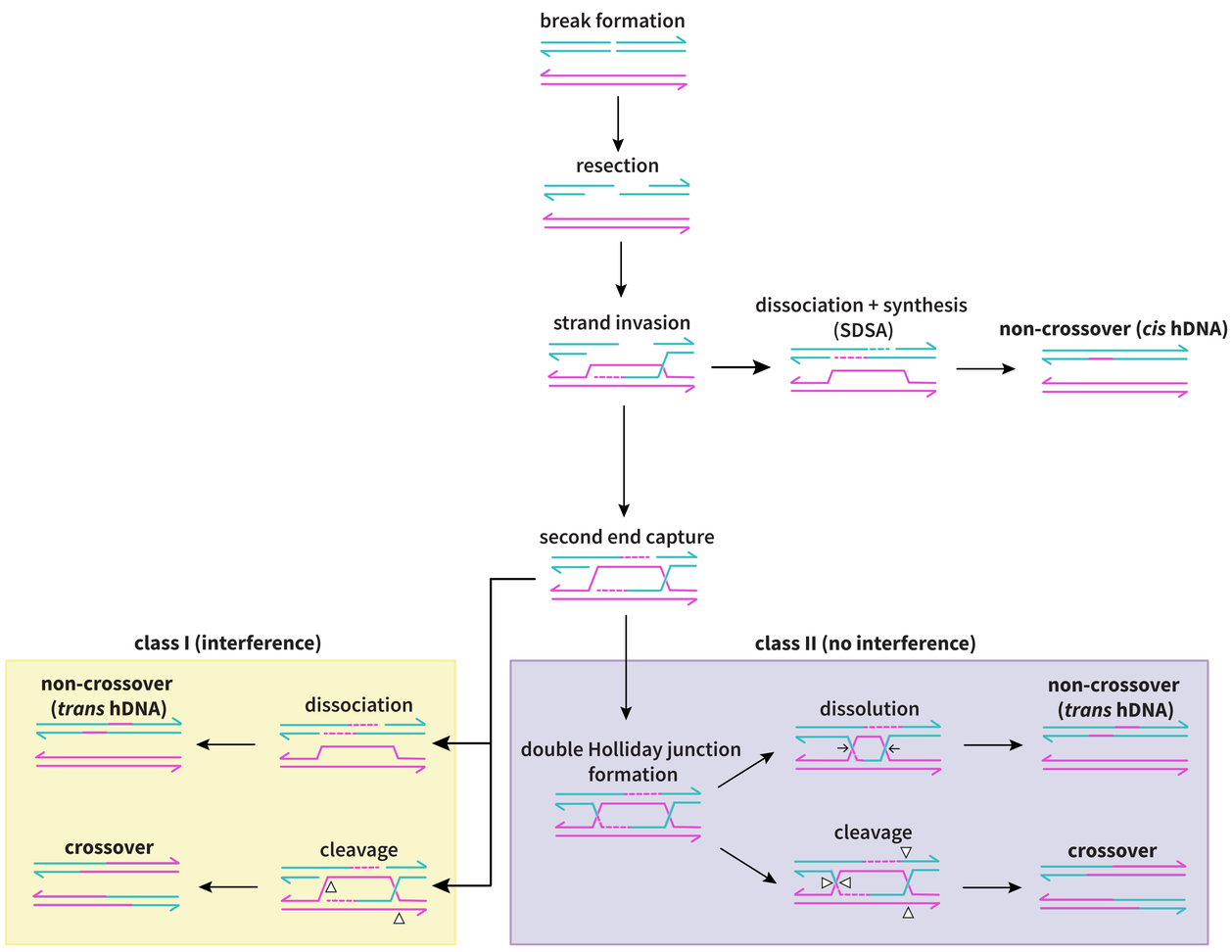

Figure 5. A predicted model of homologous recombination in *Drosophila.* SDSA yields non-crossovers with *cis* hDNA, while second-end capture can give rise to crossovers and *trans* non-crossovers via two pathways: class I (cleavage/dissociation without dHJ formation) or class II (dHJ formation followed by cleavage or dissolution).

Future Directions

Use hetSeq to determine how heteroduplex structure and crossover patterning are affected in meiotic mutants (*Blm*).

Assess regional differences in heteroduplex DNA within the genome to determine whether *cis* and *trans*-hDNA are localized to distinct regions of the chromosome arms (and whether this distribution is affected in crossover control mutants).

References

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Acknowledgements

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