Fall 12-1-2012

Changes in Meiotic Crossover Distribution and Frequency In Response to Chromosome Structural Variants

Christiana Wang
Case Western Reserve University

Nicole Crown
Case Western Reserve University, knc38@case.edu

CHANGES IN MEIOTIC CROSSOVER DISTRIBUTION AND FREQUENCY IN RESPONSE TO CHROMOSOME STRUCTURAL VARIANTS

Christiana Wang[1], Nicole Crown[1]
[1]Department of Biology, Case Western Reserve University

Abstract

Meiosis is an important mechanism that generates genetic variation for sexually reproducing organisms through recombination. In order for an organism to successfully propagate its genetic material from one generation to the next, its genome must be properly delivered as gametes. Meiotic crossing over of DNA ensures proper homolog segregation by repairing programmed double-stranded breaks. Meiotic defects caused by chromosome structural variants are detrimental to reproduction. During the interchromosomal effect, heterozygous inversions suppress crossing over between affected chromosomes while increasing crossing over between normal chromosome pairs. These defects in chromosomal dynamics trigger the pachytene checkpoint, leading to a delay in prophase progression. It has been suggested that this delay in prophase causes the interchromosomal effect on recombination. However, whether the interchromosomal effect on crossing over is caused directly by defects in chromosome dynamics or indirectly by the delay in prophase remains unclear. We are distinguishing between these two hypotheses by investigating the distributions and frequencies of crossovers in Drosophila mutants when prophase is extended by utilizing maelstrom mutants that trigger the pachytene checkpoint independently of chromosome defects. We are analyzing the changes in crossover distribution and frequencies in these mutants using recessive markers on unaffected chromosome. We are also collecting confocal imaging data of the mutants' germarium to visualize the effects of maelstrom mutations on meiosis. Our data will provide insights into the mechanisms of the interchromosomal effect and reveal whether or not the interchromosomal effect is directly mediated by a delay in pachytene or, alternatively, mediated by disrupted crossover control mechanisms.

CHANGES IN MEIOTIC CROSSOVER DISTRIBUTION AND FREQUENCY IN RESPONSE TO CHROMOSOME STRUCTURAL VARIANTS

Christiana Wang[1], Nicole Crown[1]
[1]Department of Biology, Case Western Reserve University

WHY CHROMOSOME STRUCTURAL VARIANTS?

- Consequences of chromosome structural variants when they prevent the exchange of genetic material
- Cancer – large numbers of chromosome rearrangements
- Normal cell – rearrangements cause problems in reproduction
- Meiosis and infertility using *Drosophila melanogaster* – fruit flies

CHANGES IN MEIOTIC CROSSOVER DISTRIBUTION AND FREQUENCY IN RESPONSE TO CHROMOSOME STRUCTURAL VARIANTS

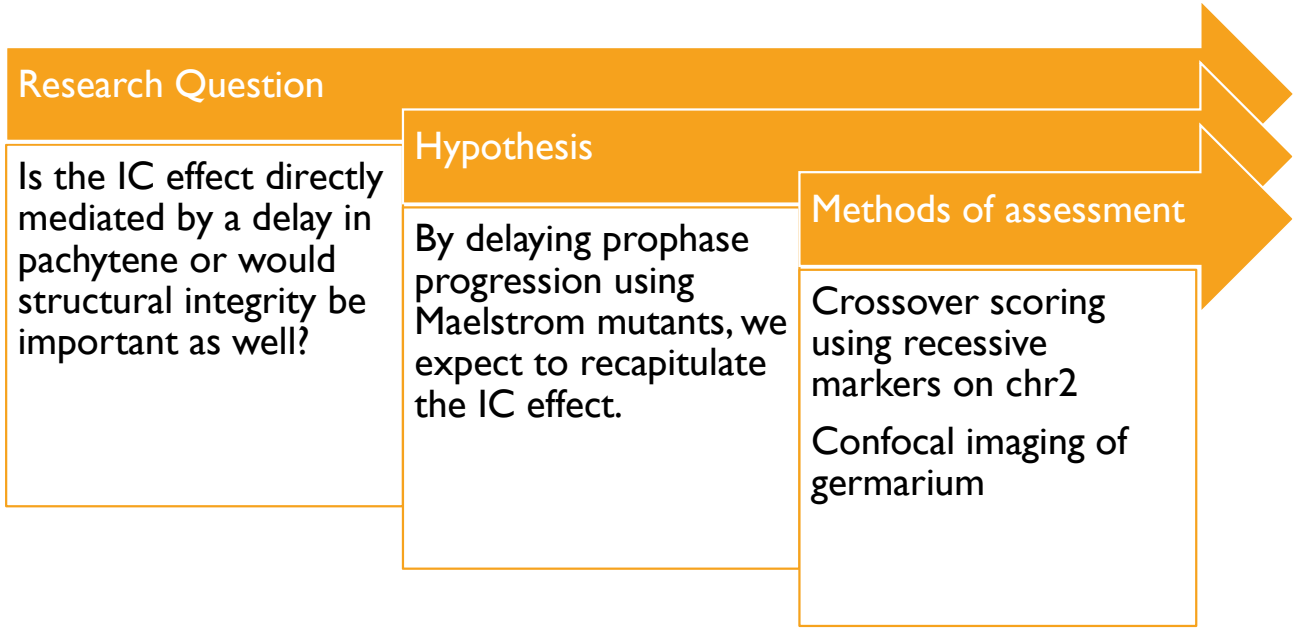
Christiana Wang[1], Nicole Crown[1]
[1]Department of Biology, Case Western Reserve University

Meiosis Crossovers and the Interchromosomal Effect

- Meiosis – specialized cell cycle that generates haploid cells
- Crossovers – programmed repairs of double stranded breaks to ensure proper homolog segregation
- The Interchromosomal (IC) effect - suppression of COs on one chromosome will lead to the increase of COs on the other chromosomes, observed with flies with heavily inverted chromosome
- Possible causes of the IC effect:
  - Simply triggering the pachytene checkpoint leads to a delayed prophase which allows more time for CO events, thus resulting in the IC effect (McKim and Joyce, 2011)
  - Disrupting in CO controls that is caused by chromosome structural variants leading to pachytene checkpoint activation, resulting in the IC effect

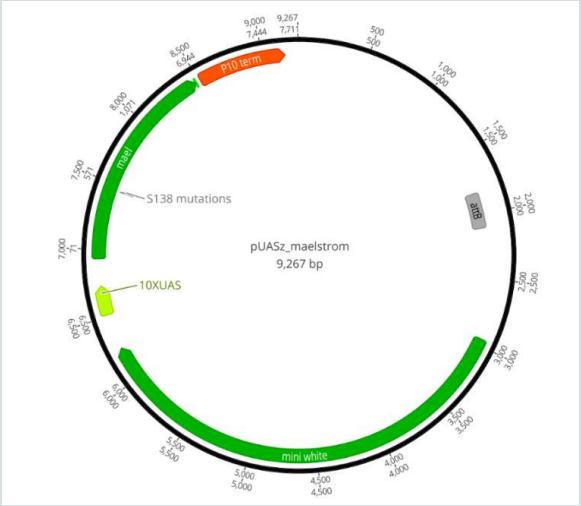
CHANGES IN MEIOTIC CROSSOVER DISTRIBUTION AND FREQUENCY IN RESPONSE TO CHROMOSOME STRUCTURAL VARIANTS

Christiana Wang[1], Nicole Crown[1]
[1]Department of Biology, Case Western Reserve University



CHANGES IN MEIOTIC CROSSOVER DISTRIBUTION AND FREQUENCY IN RESPONSE TO CHROMOSOME STRUCTURAL VARIANTS

Christiana Wang[1], Nicole Crown[1]
[1]Department of Biology, Case Western Reserve University



Final transgene construct of *mael* incorporated in pUASz vector. pUASz contains an attB site for *phiC31* integration into the genome, a mini-white gene to screen successful transformants in adult flies, a 10XUAS promoter to be driven by a Gal4 promoter, and a P10 terminator sequence for regulation translation. The *mael* gene and the location of the mutations is also shown.

Infusion reaction of *mael* transgenes with pUASz, followed by transformation and mini/midi-prep clean up

→ DNA injection into yw stock embryos using phiC31 by Best Gene, Inc.

→ Generated recombinant flies with a single copy of the transgene in a null background ($mael^{M391}$) with recessive markers on chr2

↓

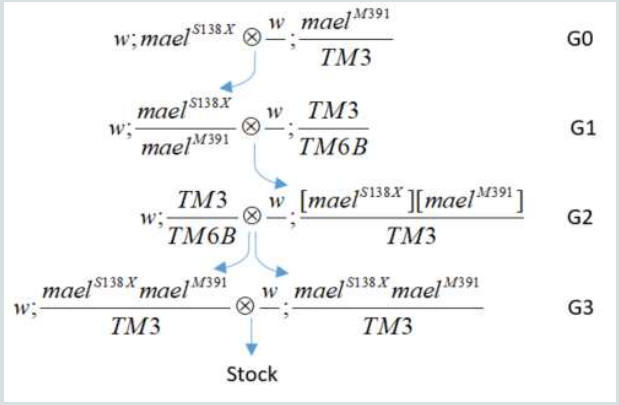
Generated nanos::UAS driver stock carrying the deficiency allele (Df(3L)79e)

→ Score COs between driver stock and recombinant stock

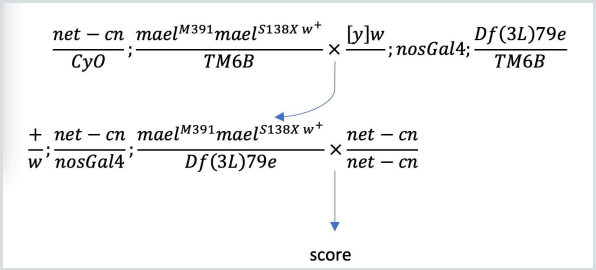
→ Collect and fix ovaries for confocal imaging to determine germarium progression

CHANGES IN MEIOTIC CROSSOVER DISTRIBUTION AND FREQUENCY IN RESPONSE TO CHROMOSOME STRUCTURAL VARIANTS

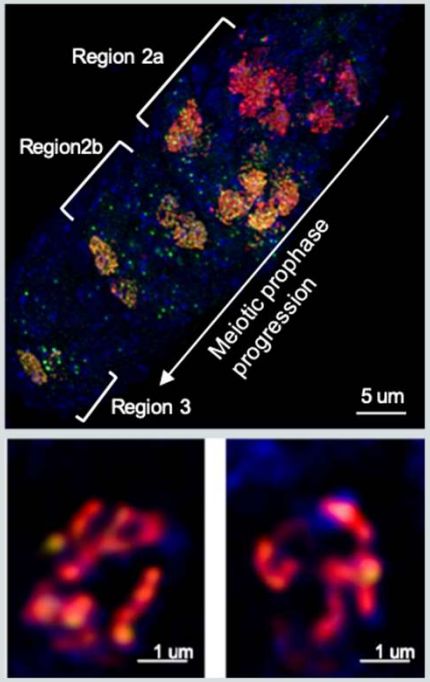
Christiana Wang[1], Nicole Crown[1]
[1]Department of Biology, Case Western Reserve University



Screening cross scheme for selecting recombinant flies with w+ marker. PCR is used to select for M391 presence.



Scoring cross scheme with nanos::Gal4 driver and a single copy of maelstrom carrying recessive markers on chr2.



Sample confocal imaging. Top: 63X deconvolved maximum projection image of prophase progression in a wildtype germarium. Multiple 16-cell cysts enter meiosis and proceed through the germarium in an assembly-line fashion. The synaptonemal complex is pseudocolored red, Vilya in green. Vilya initially appears as foci in late region 2a and becomes threadlike as it moves through region 2b and region 3. Bottom: 63X with 4X zoom, deconvolved single z-section of two different meiotic nuclei showing that individual synaptonemal complexes (red) with Vilya foci (yellow) can be resolved. Adapted from Pek *et al.* 2012.

CHANGES IN MEIOTIC CROSSOVER DISTRIBUTION AND FREQUENCY IN RESPONSE TO CHROMOSOME STRUCTURAL VARIANTS

Christiana Wang[1], Nicole Crown[1]
[1]Department of Biology, Case Western Reserve University

CURRENT PROGRESS AND FUTURE DIRECTIONS

- Resolve sterility observed in the recombinant scoring cross and collect CO distribution and frequency data
- Collect CO distribution and frequency of controlled cross – yw stock – using net-cn markers
- Collect ovaries for confocal imaging to confirm the typical two-oocyte phenotype observed in pachytene delays
- Analyze and interpret CO data

RELATIONSHIP TO ADDITIONAL PROJECT EXTENDED FROM SOURCE SUMMER RESEARCH

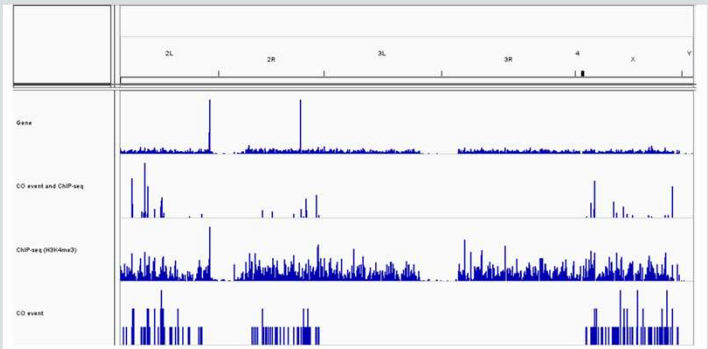


Figure 2: IGV visualization of the interaction between the CO dataset and the ChIP-seq result of H3K4me3, along with the original CO dataset and the combined ChIP-seq result. All chromosomes of the *Drosophila* genome are shown. Blue peaks correspond to signal intensity, either for CO frequency or ChIP seq signal. **Gene**: number of genes in that region of the chromosome. **CO event and ChIP-seq**: the number of overlapping signals between H3K4me3 signals and CO events. **ChIP-seq (H3K4me3)**: signal intensity of the ChIP-seq calls from MACS2. **CO event**: number of CO events in that genomic location.

- Summer project – CO distribution and epigenetic characteristics
- Extended project - Assess downstream effects on fertility when CO is allowed on a structural variant using whole genome sequencing and bioinformatic analysis

CHANGES IN MEIOTIC CROSSOVER DISTRIBUTION AND FREQUENCY IN RESPONSE TO CHROMOSOME STRUCTURAL VARIANTS

Christiana Wang[1], Nicole Crown[1]
[1]Department of Biology, Case Western Reserve University

ACKNOWLEDGMENT

- We thank Nigel Muhammad and Haosheng Li from the Crown lab for help in general lab duties. We thank Toshie Kai from Osaka University for the generous donation of the original mael transgenes.
- KNC is supported by NIH grant R00GM118826 and start up funds from Case Western Reserve University. Christiana Wang is supported by SOURCE summer research funding provided by Case Alumni Association.