Synaptonemal Complex-Deficient Drosophila melanogaster Females Exhibit Rare DSB Repair Events, Recurrent Copy-Number Variation, and an Increased Rate of de Novo Transposable Element Movement

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ABSTRACT Genetic stability depends on the maintenance of a variety of chromosome structures and the precise repair of DNA breaks. During meiosis, programmed double-strand breaks (DSBs) made in prophase I are normally repaired as gene conversions or crossovers. DSBs can also be made by other mechanisms, such as the movement of transposable elements (TEs), which must also be resolved. Incorrect repair of these DNA lesions can lead to mutations, copy-number changes, translocations, and/or aneuploid gametes. In Drosophila melanogaster, as in most organisms, meiotic DSB repair occurs in the presence of a rapidly evolving multiprotein structure called the synaptonemal complex (SC). Here, whole-genome sequencing is used to investigate the fate of meiotic DSBs in D. melanogaster mutant females lacking functional SC, to assay for de novo CNV formation, and to examine the role of the SC in transposable element movement in flies. The data indicate that, in the absence of SC, copy-number variation still occurs and meiotic DSB repair by gene conversion occurs infrequently. Remarkably, an 856-kilobase de novo CNV was observed in two unrelated individuals of different genetic backgrounds and was identical to a CNV recovered in a previous wild-type study, suggesting that recurrent formation of large CNVs occurs in Drosophila. In addition, the rate of novel TE insertion was markedly higher than wild type in one of two SC mutants tested, suggesting that SC proteins may contribute to the regulation of TE movement and insertion in the genome. Overall, this study provides novel insight into the role that the SC plays in genome stability and provides clues as to why the sequence, but not structure, of SC proteins is rapidly evolving.

KEYWORDS meiosis whole-genome sequencing crossing over noncrossover gene conversion c(3)G corolla synaptonemal complex transposable element copy-number variation sister chromatid exchange

Programmed double-stranded DNA breaks (DSBs) made during prophase of meiosis I are a critical step in the formation of healthy gametes, yet they are potentially catastrophic events for cells. The meiotic break repair machinery must therefore accurately resolve DSBs as either crossovers (COs) or noncrossover gene conversions (NCOs). Because more DSBs are made than will be repaired as COs, the majority of DSBs are repaired as NCOs, which are nonreciprocal exchange events that result in the 3:1 segregation of alleles.

Crossing over is essential to ensure the proper segregation of homologous chromosomes during subsequent meiotic divisions. Crossovers occur within the context of a large, multiprotein structure called the synaptonemal complex (SC), which forms between homologous chromosomes. In most organisms, DSBs must be made before SC formation can occur, and functional SC is required for proper DSB repair (de Massy 2012; Zickler and Kleckner 2015). However, in Drosophila...
**melanogaster**, the SC is necessary for both robust DSB formation and DSB repair (Lake and Hawley 2012); in the absence of functional SC, DSBs are made at about 20–40% of the wild-type level (Mehrotra and McKim 2006; Collins et al. 2014).

The *Drosophila* SC protein C(3)G is functionally homologous to the transverse filament proteins SYCP-1 in mammals and ZIP1 in budding yeast (Page and Hawley 2001). While females heterozygous for a loss-of-function c(3)G allele appear to build normal SC, homozygous females do not build SC and are thus unable to resolve into COs those DSBs that do occur (Page and Hawley 2001). A previous study examining NCOGC events at a single locus in *Drosophila* recovered no events from *c(3)G* homozygous females but did not report the number of progeny scored (Carlson 1972), thus whether DSBs can be repaired as NCOGCs in females lacking functional SC is unknown. Like C(3)G, the *Drosophila* SC protein Corolla is also required for SC formation. Mutants in *corolla* exhibit phenotypes typical of *Drosophila* SC mutants, including a reduced number of DSBs (~40%) as assayed by γH2AV foci and increased levels of chromosome segregation defects (Collins et al. 2014). As with *c(3)G* homozygous females, it is not known how DSBs are repaired in *corolla* homozygotes.

While it is evident the SC plays a vital role in resolving DSBs into COs, its role in other meiotic processes is less obvious. For example, there is some evidence for a link between SC formation and the position of transposable elements (TEs), but the data are not definitive (Pearlman et al. 1992; Hernández-Hernández et al. 2008; Marcon et al. 2008; van der Heijden and Bortvin 2009). Transposable elements are mobile genetic elements active during different stages of gametogenesis. They can be divided into two classes: Class 1, or retrotransposons, replicate using a copy-and-paste method to insert copies of themselves into new locations in the genome, while Class 2, or DNA transposons, use a cut-and-paste method to move from one position in the genome to another. While the tripartite structure of the SC is highly conserved among many species, the genes that make up the SC are evolving rapidly at the sequence level, making their identification even within a species group challenging (Fraune et al. 2012; Hemmer and Blumenstiel 2016). The reason for the rapid sequence evolution of the SC is unknown, but it could be to counter the effects of TE movement during meiosis. In *Drosophila* female meiosis, the rate at which TE movement occurs and whether the SC has any role in facilitating or limiting TE movement remains unclear.

In the current study, whole-genome sequencing was used to investigate individual meiotic events in male offspring from females heterozygous or homozygous for a loss-of-function allele of *c(3)G*. While the number and distribution of CO and NCOGC events in individuals from females heterozygous for *c(3)G* was similar to wild type, in progeny arising from *c(3)G* homozygous mothers (which do not build SC), no COs and only one likely NCOGC event were recovered. The recovery of a single presumed NCOGC event suggests that while repair of DSBs via NCOGC may be possible in females lacking functional SC, it is not a common mechanism of DSB repair. Consistent with the high levels of chromosome missegregation observed in SC mutants, X0 males lacking a Y chromosome, males with 4th chromosome gain or loss, and flies that appeared phenotypically male were also recovered.

These data also provide information on what role, if any, the SC components C(3)G and Corolla play in facilitating or inhibiting TE movement during meiosis. In the current study of SC-defective mutants, novel TE insertions were curiously significantly elevated in *c(3)G* homozygotes but similar to wild type in *corolla* homozygotes. Shared and novel large-scale TE-mediated CNVs were also identified in progeny from all genotypes. Remarkably, one of these CNVs was observed in three unrelated individuals—two from this study and one from a separate study of individual meiotic events in wild type (Miller et al. 2016b)—suggesting that, similar to humans (Isara et al. 2009), recurrent CNVs may be a common occurrence in *Drosophila*. Overall, this work helps further our understanding of how meiotic cells cope with DNA breaks and maintain genetic stability.

**METHODS**

### Fly Stocks and husbandry

The loss-of-function allele *c(3)G* (Page and Hawley 2001) was crossed into stocks isogenic for either *w^{118}X* or Canton-S strain polymorphisms (Miller et al. 2012). Females homozygous for Canton-S X and 2nd chromosomes and heterozygous for the *c(3)G* loss-of-function allele were crossed to *w^{118}X* males to generate females heterozygous for Canton-S and *w^{118}X* strain polymorphisms. These heterozygous females were then crossed again to isogenic *w^{118}X* males and individual male progeny were isolated for sequencing (Figure S1). Females heterozygous for *w^{118}X* and Canton-S X and 2nd chromosomes and homozygous for *c(3)G* were crossed to isogenic *w^{118}X* males and individual male offspring were isolated for sequencing (Figure S1). Progeny from *corolla* homozygous females were generated by crossing virgin *corolla* females to sibling males and collecting both male and female progeny (Figure S1). All crosses were done using a single male and female, and females were allowed to lay eggs for 7 days before being removed from a vial. Male offspring used for sequencing were collected between days 12 and 15 post-fertilization. All flies were kept on standard cornmeal-molasses media and maintained at 25°C.

### DNA preparation and sequencing

For all flies, DNA was prepared from single adults using the Qiagen DNeasy Blood & Tissue Kit. All flies were starved for 4 hr before freezing at -80°C for at least 1 hr. One ng of DNA from each was fragmented to 250-bp fragments by adjusting the treatment time to 85 sec using a Covaris S220 sonicator (Covaris Inc.). Libraries were prepared using a Nextera DNA Sample Prep Kit and Biotrove NEXTflex DNA Barcodes. The resulting libraries were purified using Agencourt AMPure XP system (Beckman Coulter) then quantified using a Bioanalyzer (Agilent Technologies) and a Qubit Fluorometer (Life Technologies). Samples from *c(3)G* homozygous females were run on a HiSeq 2500 in rapid mode as either 100-bp paired-end or 125-bp paired-end samples using HiSeq Control Software 1.8.2 and Real-Time Analysis (RTA) version 1.17.21.3. Samples from *c(3)G* homozygous females and individual male progeny were isolated for sequencing (Figure S1). Progeny from *corolla* homozygous females were generated by crossing virgin *corolla* females to sibling males and collecting both male and female progeny (Figure S1). All crosses were done using a single male and female, and females were allowed to lay eggs for 7 days before being removed from a vial. Male offspring used for sequencing were collected between days 12 and 15 post-fertilization. All flies were kept on standard cornmeal-molasses media and maintained at 25°C.

### DNA alignment, SNP calling, and identification of CO and NCOGC events

Alignment to the *Drosophila* reference genome (dm6) was performed using bwa version 0.7.7-r441 using default parameters (Li and Durbin 2009). Single nucleotide and insertion or deletion polymorphisms were identified using SAMtools version 1.9 (Li et al. 2009). Candidate CO and NCOGC events were identified as described in Miller et al. (2016b).

### Depth-of-coverage calculations

Depth of coverage for each chromosome arm was calculated by summing the total read depth for each base position then dividing by the...
length of the entire chromosome arm. Because of the repetitive nature of the Y chromosome, analysis was limited to chrY:332,000–510,000 (Table S1).

**Validation of NCOGCs by PCR**

Nine candidate NCOGC events were identified in 93 males from c(3)G68 females and examined by PCR and Sanger sequencing. Phusion polymerase (NEB) was used according to the manufacturer’s instructions. Only one of the nine putative conversion events validated as real in male c3g-hom-6.4. All primers used can be found in Table S2.

**Calculation of expected NCOGC events**

The number of NCOGCs expected to be recovered from 93 individuals from c(3)G68 females if all DSBs on the X and 2nd chromosomes were repaired as NCOGCs was estimated by performing 100,000 trials of randomly distributing an estimated number of DSBs among the X and 2nd chromosomes using the SNP density of a w1118/Canton-S heterozygote. Given that DSBs in c(3)G68 females are made at 20% of the wild-type rate of 18–20 DSBs per meiosis (Mehrotra and McKim 2006), a per-arm number of DSBs was estimated as 0–2 per meiosis. Each break was randomly assigned to a chromosome arm, then to a random chromatid. A random chromatid was then selected to be recovered. NCOGC tract length was assumed to be a minimum of 250 bp and a maximum of 1000 bp (Miller et al. 2016b). An NCOGC was predicted to be recoverable if the tract involved at least one high-quality SNP that differentiated the w1118 and Canton-S genotypes. The estimate of the number of NCOGCs that should be recovered from individual offspring of c(3)G68/+ females was calculated by multiplying the wild-type per-arm NCOGC rate of 0.3 (Miller et al. 2016b) by 120, the number of arms studied.

**Identification of novel deletion polymorphisms**

Novel deletions were identified using two approaches. Deletions smaller than 30 bp were identified using SAMtools (Li et al. 2009). For each class of progeny (wild type, c(3)G68, c(3)G68/+), and corolla290) a custom script identified any deletion, regardless of quality score, from all vcf files that did not overlap repetitive regions as defined by Repeatmasker (Smit et al.). Novel deletions were those with quality scores over 200 (as determined by SAMtools) that did not fall within 100 bp of another deletion on a different offspring. Candidate novel deletions were validated visually using IGV (Thorvaldsdóttir et al. 2013). Data for both wild type and c(3)G68 were also analyzed using GATK HaplotypeCaller (McKenna et al. 2010), but no deletions not identified by SAMtools were found, thus the remainder of the analysis was completed with SAMtools. Larger deletions were identified using Pindel (Ye et al. 2009). For each class of progeny, Pindel was run using default settings with an average insert size of 200 bp. Output files for each class of progeny were analyzed as a group and candidate novel deletions were visually validated using IGV.

**Construction of synthetic genomes and sequencing reads**

To determine what percentage of small or large de novo deletion polymorphisms would be identified by SAMtools and Pindel, synthetic genomes were computationally modified with deletions of varying sizes then analyzed using the approach described above. Two classes of genomes were generated: 100 with deletions 1–10 bp long, and 100 with deletions 1–1,000 bp long. For each individual, two genomes were generated: one with an X and without a Y chromosome, and one with a Y and without an X. For each of these genomes, a single nucleotide was randomly changed approximately once every 500 nucleotides to a randomly selected A, G, C, or T. Next, for each genome with an X and without a Y chromosome 2–6 DSBs (approximately 20% of the 18–20 DSBs expected in wild-type (Mehrotra and McKim 2006)) were randomly placed on one of four haplotypes in a euchromatic location in the genome. Each of these DSBs was randomly determined to have a deletion between either 1–10 bp or 1–1,000 bp beginning at the site of the DSB. One haplotype of these four was then randomly chosen as the genome for the individual. For each individual, ART was used to generate synthetic reads for both genomes with a read depth of approximately 10x (Huang et al. 2012). FASTQ files were then combined into a single forward and a single reverse file—and thus represented data from an XY individual with 20x depth of coverage—that were then aligned to the D. melanogaster reference genome as above. SNPs, insertion/deletion polymorphisms, and larger deletions were identified as described above with SAMtools (Li et al. 2009) and Pindel (Ye et al. 2009). Deletions generated per individual genome can be found in Table S3.

**Identification of transposable element insertions**

To identify TE insertions, split and discordant read pairs were isolated from alignment files using SAMBLASTER (Faust and Hall 2014). BLAST (Altschul et al. 1997) was then used to annotate individual split or discordant reads using the D. melanogaster canonical TE set (Kaminker et al. 2002). Split and discordant clusters that contained more than five reads aligning to a specific TE family were considered candidate TE insertion sites. Novel insertions were detected by a custom script that compared insertions in one population or stock to related stocks or populations and were visually validated using IGV (Thorvaldsdóttir et al. 2013). Mosaic insertions on the X chromosome were identified visually using IGV.

**Identification of CNV events**

CNV events were identified as described in Miller et al. (2016b). Briefly, average depth of coverage for each individual chromosome arm was determined, then the log, depth of coverage for 5-kb nonoverlapping windows was calculated and plotted to reveal large regions of deletions or duplications.

**Data availability**

Illumina data generated for this project are available at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/). Data for individuals from c(3)G68 females can be found under project PRJNA565835, data for males from c(3)G68 heterozygous females are under project PRJNA565834, and data for males and females from corolla290 females are under project PRJNA565794. Wild-type data used in this study were obtained from project PRJNA307070 (Miller et al. 2016b). Original data underlying this manuscript can be accessed from the Stowers Original Data Repository at http://www.stowers.org/research/publications/libpb-1476. All code used in this project is available at GitHub (https://github.com/danrdanny/c3g-corolla-project/). Supplemental material available at figshare: https://doi.org/10.25387/g3.10006634.

**RESULTS**

**Analysis of individual meiotic events from c(3)G68 heterozygous and homozygous females**

While in many organisms DSBs are made in the absence of SC (de Massy 2012; Zickler and Kleckner 2015), Drosophila is unique in that SC is required for robust DSB formation (Lake...
and D. melanogaster females homozygous for loss-of-function alleles of SC genes make DSBs at a rate approximately 20–40% that of wild type (Mehrotra and McKim 2006; Collins et al. 2014), and it remains unclear how these DSBs are repaired. Studies using visual markers in Drosophila have shown that repair of DSBs by crossing over is substantially reduced or completely abolished in females unable to construct full-length SC, and it is not known if these DSBs can be repaired by other pathways, such as NCOGC or non-homologous end-joining (NHEJ) (Gowen 1933; Hall 1972; Page and Hawley 2001; Manheim and McKim 2003; Jeffress et al. 2007; Page et al. 2007; Collins et al. 2014).

To better understand this process, whole-genome sequencing was performed on individual male progeny from mothers heterozygous for wild-type Canton-S and \textit{w}^{118} X and 2\text{nd} chromosomes and either homozygous or heterozygous for the loss-of-function allele \textit{c(3)G}\textsuperscript{68} on chromosome 3. Male progeny from \textit{c(3)G}\textsuperscript{68} homozygous mothers (96 males from 10 females) represent the experimental group lacking SC and will hereafter be referred to as \textit{c(3)G} offspring, and male progeny from \textit{c(3)G}\textsuperscript{68} heterozygous mothers (40 males from two females) represent the control group with functional SC and will be referred to as \textit{c(3)G+/+} offspring (Figure S1).

While Drosophila males normally have an X and a Y chromosome, sex is determined by the ratio of X chromosomes to autosomes rather than the presence of a Y, thus X0 flies are male. This is seen when X chromosome missegregation (nondisjunction) leads to no maternal sex chromosome contribution, with a paternally inherited X. Triploid flies carrying three copies of each autosome and two X chromosomes may also be phenotypically male and are known as intersex males (Bridges et al. 1921). To assay for X and 4\text{th} chromosome nondisjunction and the presence of triploid flies, depth of coverage was calculated for each chromosome arm as a percentage of one of the autosomes (Table S1). Males carrying the expected number of X chromosomes should have X and Y chromosome depth of coverage half that of an autosome and 4\text{th} chromosome depth of coverage equal to an autosome. As expected, all 40 male offspring from the \textit{c(3)G+/+} control group were diploid with an X and a Y chromosome as well as two copies of the 4\text{th} chromosome. Meanwhile, among the offspring from the SC-deficient \textit{c(3)G} experimental group, 25 were found to be X0 males carrying an X chromosome with the \textit{w}^{118} haplotype and six were found to have three 4\text{th} chromosomes (Table S1, Figure S2). These 25 X chromosomes were most likely paternally inherited, since nondisjunction of the X or Y chromosome in wild-type Drosophila males has been shown to occur in fewer than 1 in 5,000 meioses (Boschi and Hawley 2012). It was noted an increase in the number of triploid individuals recovered from \textit{c(3)G} offspring (Figure S2).

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CO and NCOGC events were then identified on the X and 2\text{nd} chromosomes in both \textit{c(3)G} and \textit{c(3)G+/+} male offspring through changes in polymorphisms in each fly. (CO and NCOGC events were not analyzed on the 3\text{rd} because \textit{c(3)G} lies on this chromosome, nor were they analyzed on the 25 paternally inherited X chromosomes carried by X0 \textit{c(3)G} offspring.) A total of 41 single COs and 7 double COs were identified in \textit{c(3)G+/+} offspring (Figure 1A, Table S4), with a frequency of exchange similar to previous observations in wild type for all three arms (Figure 1B). A total of 32 NCOGCs were also identified (Figure 1C, Table S5), not significantly different than the 36 expected to be recovered based on wild-type rates (Miller et al. 2016b). Previous work has shown rates of crossing over similar to wild type for the \textit{c(3)G}\textsuperscript{68} allele when heterozygous (Hall 1977), but higher rates of crossing-over for the \textit{c(3)G}\textsuperscript{17} as a heterozygote (Hinton 1966). While the \textit{c(3)G}\textsuperscript{68} allele is a known point mutation, the \textit{c(3)G}\textsuperscript{17} allele (also historically known as \textit{c(3)G}1) is a TE insertion that disrupts the function of the gene (Page and Hawley 2001), and the reason for the difference in exchange between these two alleles is not clear.

Among 93 \textit{c(3)G} homozygous offspring, no CO events were recovered on the X or 2\text{nd} chromosomes, but a single NCOGC event in male \textit{c3g-hom-6.4} was identified and validated by PCR and Sanger sequencing. This event occurred on a chromosome with the Canton-S haplotype, which could have occurred only in the heterozygous \textit{w}^{118}/Canton-S mother and thus was not contributed by the isogenic \textit{w}^{118} father. This NCOGC was minimally defined by a 4-bp deletion on the 5’ side (2R:23,350,969–23,350,972, release 6 coordinates) and a single polymorphism on the 3’ side (2R:23,351,148) (Figure 1E, Table S5). Because it was defined by these two closely located polymorphisms that created two changes identical to the other haplotype used in this study, it is unlikely the event was the result of \textit{de novo} somatic mutation. Moreover, the average depth of coverage within the 1-kb interval surrounding the two polymorphisms was 54x, similar to the average depth of coverage for chromosome 2R for this individual, making it unlikely that this NCOGC was due to a deletion or duplication of this interval. Additionally, the minimum and maximum possible widths of this NCOGC are 180 bp and 2,507 bp, respectively, well within ranges observed in wild type (Hilliker et al. 1994; Miller et al. 2016b). Because homologous chromosomes pair prior to meiotic onset, this NCOGC could be the result of DSB repair in a pre-meiotic cell (Bosco 2012; Joyce et al. 2012). Unfortunately, there are no reliable estimates of the rate at which pre-meiotic NCOGC occurs, making the likelihood difficult to assess.

Females homozygous for \textit{c(3)G} loss-of-function alleles make DSBs at ~20% of the level of wild type (Mehrotra and McKim 2006). To estimate the number of NCOGCs that should have been recovered in the \textit{c(3)G} dataset if DSB repair as NCOGCs occurred frequently, a simulation was performed. This model randomly distributed DSBs among 68 X and 93 2\text{nd} chromosome arms as if they occurred at a rate 20% that of wild type. The model estimated that 37–62 NCOGCs should have been recovered if all DSBs that occurred were repaired as NCOGCs (since COs do not occur in \textit{c(3)G}\textsuperscript{68} homozygotes). The recovery of a single candidate NCOGC event is significantly less than the 37–62 expected NCOGCs (P = 0.001, Fisher’s exact), indicating that like COs, full-length SC is crucial for the repair of DSBs into NCOGCs. Indeed, using these data, the rate of NCOGC in an SC-deficient female can be estimated as approximately 1x10^{-10} per bp per meiosis, markedly lower than the wild-type rate of 1.9x10^{-8} NCOGCs per bp per meiosis (Hilliker et al. 1994; Miller et al. 2016b). This raises the obvious question, which will be considered next: if they are rarely repaired as COs or NCOGCs, what is the fate of DSBs that occur in SC-deficient flies?
Figure 1 CO and NCOGc events recovered in this study (details are in Table S4 and Table S5). (A) Individual NCOGc, single crossover (SCO), and double crossover (DCO) events recovered per chromosome arm in c(3)G68/+ females. No DCOs were recovered on 2R in c(3)G68/+, and no COs of any type were recovered in c(3)G68 homozygotes. Gray shading indicates heterochromatic regions; black circles represent centromeres.

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Most DSBs in SC-deficient females are repaired by an error-free process

In addition to meiotic CO or NCOGC, other potential mechanisms exist for repair of meiotic DSBs. One such mechanism, NHEJ, is often described as an error-prone process resulting in deletions (Bétrémier et al. 2014), but data suggest the canonical NHEJ pathway is a higher-fidelity system than previously believed (Kabotyanski et al. 1998; Feldmann et al. 2000). Alternatively, single-strand annealing (SSA), alternative end-joining (alt-EJ), and microhomology-mediated end-joining (MMEJ) are pathways that may result in small deletions that could be detected as novel deletion polymorphisms in whole-genome sequencing data (Wang et al. 2003, 2005; Guirouillé-Barbat et al. 2007; Rass et al. 2009). Finally, repair of DSBs using the sister chromatid as a template may occur and leave little or no evidence detectable by whole-genome sequencing.

Gene conversion with the sister chromatid has been shown to be a significant repair pathway in both S. cerevisiae (Goldfarb and Lichten 2010) and mammalian cells (Johnson and Jasin 2000), thus it is reasonable to assume it may be active during Drosophila female meiosis as well. Indeed, ring chromosome assays have shown a decrease in the recovery of ring chromosomes in the absence of c(3)G, suggesting breaks in c(3)G homozygous females can be repaired by intersister recombination (see Discussion for a description of the ring chromosome assay) (Sandler 1965). Furthermore, in both FM7/+; c(3)G68 and FM7/+ females with functional SC, the recovery of Bar revertants through unequal exchange between sister chromatids supports the hypothesis that sister chromatid exchange does occur in flies (Hall 1977; Miller et al. 2016a).

To determine if DSB repair in SC-deficient females occurs by an error-prone process, novel deletion polymorphisms were identified in the three previously described classes of progeny (wild type, c(3)G68/+, and c(3)G0) plus an additional class unable to repair DSBs by crossing over. Females carrying loss-of-function mutations of the SC gene corolla are unable to construct full-length SC, and thus have a high rate of nondisjunction, yet still make DSBs at a rate approximately 40% of wild-type (Collins et al. 2014), similar to the phenotype observed in c(3)G loss-of-function mutations. 50 individual males and females from three females homozygous for a nonsense mutation in the SC protein corolla (corolla29) were sequenced. Of these 50 individuals, 11 were the result of X chromosome nondisjunction, with 3 X0 males and 8 XX female; 9 were triplo-2; 2 were nondisjunctional for both the X and 4th chromosomes; and no X or 4th chromosome mosaics were observed (Table S1). The genetic background of the 2nd and 3rd chromosomes of females homozygous for corolla was not controlled, thus candidate CO and NCOGC events could not be identified, but previous studies have shown a nearly complete absence of exchange in corolla homozygous females (Collins et al. 2014).

In all four classes of progeny (wild type, c(3)G68/+, c(3)G68, and corolla29), de novo deletions were searched for using two different approaches (see Methods). First, vcf files generated by SAMtools (Li et al. 2009) were analyzed for deletion polymorphisms (these are generally less than 20 bp), and second, larger deletions were identified using Pindel (Ye et al. 2009). Separately, the output of GATK HaploType caller (McKenna et al. 2010) was compared to SAMtools and was found to produce similar results, thus only data from SAMtools was analyzed. Both approaches identified a similar number of de novo deletions per fly in all four classes of progeny. Specifically, SAMtools identified 11 deletions 1-11 bp in size in previously published data of 196 wild-type males, a single 21-bp deletion in 40 c(3)G/+ offspring, 8 deletions ranging from 1-14 bp from 93 c(3)G offspring, and one 3-bp deletion from 50 corolla individuals (Table S6). Pindel identified only one large novel deletion among all genotypes, a complex 17-bp deletion in a c(3)G homozygous male.

Because DSBs are made at different rates in wild type and SC-deficient mutants, comparing the absolute number of deletions among progeny is not informative. A more accurate measure of deletion frequency is deletions per DSB. While approximately 18–20 DSBs are made during wild-type meiosis, only 4–8 are made in the SC mutants studied here (Mehrotra and McKim 2006; Collins et al. 2014). Although the number of DSBs made in c(3)G68 heterozygous females is unknown, it is assumed to be similar to wild type because the landscape of CO and NCOGC events is similar to wild type. Using these estimates, the rate of deletions per DSB per meiosis (assuming only 25% of DSBs are recovered in the offspring) can be calculated as approximately 1% for wild-type and c(3)G/+ females, 3% for corolla females, and 10% for c(3)G females. The rate for c(3)G females is significantly higher than wild type (P = 0.008, Student’s 2-tailed T-test) but the rate for corolla females is not (P = 0.6, Student’s 2-tailed T-test).

Thus, while there is a subtle but significant increase in the rate of de novo deletions recovered in offspring from c(3)G mothers, this does not sufficiently explain what happens to the majority of DSBs made during meiosis. It does suggest that the majority of DSBs are repaired by a non-error-prone process between the homolog or with the sister chromatid. Of course, secondary alignment and/or analysis errors may make de novo deletions difficult to detect.

To test whether the analysis approach was robust enough to detect both large and small deletions, 200 D. melanogaster genomes with novel random single nucleotide and deletion polymorphisms were generated computationally. Two different classes of genomes were created, 100 with deletions 1–10 bp in size, and 100 with deletions 1–1,000 bp in size (Table S3). Synthetic reads were generated based on these genomes and aligned and analyzed using the same steps as the experimental samples. A total of 713 synthetic deletions were generated, with 339 1–10 bp deletions and 374 1–1,000 bp deletions. SAMtools identified 86% of 1–10 bp deletions on the X, 2nd, and 3rd chromosomes. Pindel recovered 57% of synthetic 1–1,000 bp deletions (213 of the 374) with the highest fraction of deletions recovered on chromosome 2L (72%) and the fewest on chromosome 2R (44%) (Table S7). These models indicate that had deletions occurred at a higher rate, the additional small and large deletion polymorphisms
created by error-prone repair mechanisms should have been detected in offspring from the c(3)G or corolla females.

**De novo transposable element insertions are more frequent in c(3)G68 homozygous females**

While the SC is essential for repair of DSBs as CO and NCOGCs, it is unknown if the SC regulates other molecular events such as the movement of TEs. Absence of the yeast c(3)G homolog Zip1 has been shown to result in a decreased insertion rate of the retrotransposon Ty1, suggesting a role for the SC in TE movement in some organisms (Dakshinamurthy et al. 2010). The rate at which TE movement occurs during Drosophila meiosis is unknown, therefore to determine the baseline transposition rate, previously published data were used to identify 44 novel TE insertions from the X, 2nd and 3rd chromosomes from 196 wild-type individuals (Miller et al. 2016b) (Figure 2, Table S8). In this dataset, a single novel insertion on the euchromatic TE insertion can be found in Table S8. Seven of the 44 insertions occurred close enough to a polytene chromosome insertions + (93 offspring × 4 autosomes), 64 novel transposition events were identified—12 on the X chromosome, 10 on 2L, 15 on 2R, 13 on 3L, and 14 on 3R (Figure 2, Table S8). Analysis of sibling X chromosome haplotypes confirmed that all 12 maternally inherited X chromosome insertions were de novo. Considering the 440 maternally inherited chromosomes together, the per arm per meiosis rate of novel transposition insertion events in c(3)G offspring was 0.58 insertions per arm per meiosis for the X, 2nd, and 3rd chromosomes, which is significantly higher than wild type (t-test) and was also significantly different from that observed for maternally inherited chromosomes (P = 0.58, Student’s T-test) and was also significantly higher than wild type (P = 0.002, Chi-square test). There was not a significant difference in the per-arm per meiosis insertion rates of 0.71, 0.54, and 0.58 for the maternally inherited X, 2nd, and 3rd chromosomes, respectively (P = 0.36, one-way ANOVA). The 25 paternally inherited X chromosomes were analyzed separately and 4 de novo TE insertions were observed resulting in a de novo transposition rate of 0.64 insertions per arm per meiosis. This rate was not statistically different from that observed for maternally inherited chromosomes (P = 0.58, Student’s T-test) and was also significantly higher than wild type (P = 0.002, Chi-square test).

To help delineate whether the increase in de novo transposition events is a general property of SC-deficient females or specific to c(3)G68 homozygous females, novel TE insertions were identified in offspring from the previously described corolla mutant females. Using the same approach as above, 12 de novo transposon insertions were identified on the X, 2nd, and 3rd chromosomes of 50 individual offspring (Figure 2, Table S8). Of the 12 novel insertions identified, none occurred on the X in a male with a paternally inherited X chromosome, and one occurred on the X chromosome of an XXY female carrying two maternally inherited X chromosomes. The distribution of events was similar to that observed in wild type. These 12 events were recovered.
from all five chromosome arms, giving a rate of 0.19 insertions per arm per meiosis in corolla mutants, similar to the rate of 0.18 observed in both wild type and c(3)G\textsuperscript{68}/+ heterozygous females but significantly less than the observed de novo TE insertion rate in c(3)G\textsuperscript{68} homozygous females. This suggests that the increase in de novo TE insertions may not be a general property of SC-deficient mutants, but may be specific to c(3)G\textsuperscript{68} homozygotes.

Previous work has shown that the de novo movement of TEs in somatic cells contributes to cellular diversity in Drosophila (Eickbush and Eickbush 2011; Treiber and Waddell 2017). While relatively low depth of coverage and difficulty with phasing due to limited SNP density make identifying post-meiotic somatic TE insertions on the autosomes challenging, it is easier to identify mosaic insertions on the single X chromosome carried by males. None of the 6 de novo X chromosome insertions among males from wild type, c(3)G\textsuperscript{+}, and corolla\textsuperscript{females} appeared to be somatic events. In c(3)G\textsuperscript{G} offspring, however, two of the 16 de novo X chromosome insertions identified were likely somatic events (males c3g-hom-7.14 and c3g-hom-9.7, Table S8). Both of these insertions were Doc elements, similar to somatic events identified by Treiber and Waddell (2017), and are present in approximately 50% of reads, suggesting they happened after the first but before the second mitotic division. It is likely that additional somatic insertions occurred in later mitotic divisions that are not detectable in this dataset given the depth of coverage in this experiment and challenges in identifying these events using short reads.

**De novo copy-number variation occurs in the absence of full-length SC**

Copy-number variation is a significant source of genetic variability within populations (Kaminker et al. 2002; Lee and Langley 2010). CNVs may be beneficial or deleterious to an individual and may involve a large or small number of genes. Previous studies in *D. melanogaster* have revealed surprisingly high rates of de novo CNV both in single offspring and shared among siblings (Watanabe et al. 2009; Cardoso-Moreira and Long 2010; Cardoso-Moreira et al. 2012; Miller et al. 2016b). Large CNVs have been found to frequently form between sister chromatids and are flanked by transposons, suggesting that TEs may play a key role in de novo CNV formation (Miller et al. 2016b). Smaller CNVs that average 50–100 bp have also been identified in Drosophila, and these are associated with replication timing, but not with TEs (Cardoso-Moreira et al. 2011). It is not known whether functional SC is critical for CNV formation either between sisters or homologs.

Here, large CNVs were identified by plotting depth of coverage for all chromosome arms for all individuals from c(3)G\textsuperscript{68}/+, c(3)G\textsuperscript{68}, or corolla\textsuperscript{females} and revealed 5 total events. No de novo CNV events were observed in offspring from c(3)G\textsuperscript{68}/+ females, 4 events were recovered in offspring from c(3)G\textsuperscript{68} females, and 1 event was seen in an offspring of a corolla\textsuperscript{females} (Figure 3, Table S9). Among the 4 events recovered from the c(3)G\textsuperscript{68} females, one was shared among multiple siblings—a 223-kb deletion of chromosome 2R involving 27 genes, which was recovered from 14 male offspring from females #4, 5, and 6 (Figure 3A). That this event was observed in individuals from multiple crosses of individual males and females makes it likely that the deletion occurred at least two generations prior and did not have a significant impact on the reproductive fitness of those individuals carrying it.

The remaining CNVs recovered were only observed in single individuals and, based on haplotype analysis, were likely de novo events. One event, a complex de novo CNV involving both a deletion and a duplication was identified at the white (w) locus on chromosome X in one male from a c(3)G\textsuperscript{68} homozygous female (Figure 3B). This event was mediated by unequal crossing over between roo elements. Previous work describing ectopic recombination in *D. melanogaster* focused on unequal exchange between roo elements at the w locus, similar to the event observed here (Goldberg et al. 1983).

Two de novo CNVs (one duplication and one deletion) occurred at the exact same position on chromosome 2R in two individuals. The duplication was recovered from the offspring of a c(3)G\textsuperscript{68} female, while the deletion was recovered from the offspring of a corolla\textsuperscript{females} (Figure 3C,D). This 856-kb event included 107 genes and is flanked on both sides by a hobo element. Remarkably, a CNV with the exact same breakpoints was identified in a previous study of individuals from wild-type females (Miller et al. 2016b). All three of these CNVs were de novo events validated using the haplotypes of the siblings that did not carry the CNV. All three crosses were between individual males and females and multiple genetic backgrounds are involved (w\textsuperscript{111H}, Canton-S, and the undefined corolla\textsuperscript{background}), thus these CNVs are not variants segregating at low frequency in the population and are recurrent de novo events.

The final CNV observed, a 4.2-Mb duplication on chromosome 2L not flanked by a TE or low-complexity sequence, was recovered in a single male from a c(3)G\textsuperscript{68} female (Figure 3E). Analysis of read pairs revealed that this was a tandem duplication, with reads mapping to the proximal end of the duplication linked to reads mapping to the distal end of the duplication. The log\textsubscript{2} depth-of-coverage ratio for this interval is 1.25, 0.25 higher than expected for a diploid and less than the log\textsubscript{2} depth-of-coverage ratio of 1.5 that would be seen in an autosomal duplication occurring before the first mitotic division. Thus, this duplication is present in half the cells in the individual sequenced and likely occurred during the first mitotic division, possibly as a consequence of a re-replication event that was then repaired by recombination between the duplicated segments (Green et al. 2010). It is notable that the fly was able to tolerate such a large duplication, involving 513 genes, present in half of all cells. Although the possibility that there was selection against cells carrying the large duplication cannot be excluded, a log\textsubscript{2} depth-of-coverage ratio of 1.25 does strongly suggest there was limited selection against those cells with the duplication. If selection was acting strongly on these cells the log\textsubscript{2} ratio would fall below 1.25 and perhaps become undetectable.

**DISCUSSION**

In this study, whole-genome sequencing was used to evaluate individual offspring from females either heterozygous or homozygous for loss-of-function alleles of two different SC components, c(3)G\textsuperscript{G} and corolla. Loss-of-function variants in either gene prevent the construction of full-length SC and reduce the number of meiotic DSBs to approximately 20% of wild type in c(3)G\textsuperscript{G} and 40% of wild type in corolla (Mehrotra and McKim 2006; Collins et al. 2014). Previous studies have shown that these DSBs are rarely repaired as COs (Gowen 1933; Hall 1972; Collins et al. 2014), therefore how DSBs are repaired in the absence of SC remains an open question.

While evaluating the results of this study, several possible confounding factors should be kept in mind. First, as not all experiments were done at the same time, subtle differences in food, humidity, or temperature could affect these results. Next, the number of females from which offspring were collected from was not balanced. For example, while 96 individuals were taken from 10 c(3)G\textsuperscript{G} females, 40 males were taken from 2 c(3)G\textsuperscript{G} females. This could result in subtle female-specific effects being amplified in the results. Finally, offspring for sequencing were collected between days 12 and 15 post-fertilization and flies which would have eclosed after day 15 were not collected. Previous work has
shown that maternal age affects CO events (Hunter et al. 2016), and it is not unreasonable to assume that age may have an impact on other factors such as the formation of CNVs and movement of TEs.

CO and NCOGC events were identified in males from c(3)G68/+ females and compared to wild type (Figure 1). In agreement with previous studies of crossing over in c(3)G68/+ (Hall 1972), the frequency and distribution of events identified in this study appears similar to wild type, although the small sample size may mask subtle differences in the heterozygous genotype. Next, among 93 males from c(3)G68 homozygous females, no CO events and a single NCOGC event were identified. Recovering no CO events was not unexpected based on prior studies of c(3)G homozygous females (Gowen 1933; Hall 1972). The single

Figure 3 Copy-number variants recovered in this study (details are in Table S9). Gray shading indicates heterochromatic regions and © represents the centromere. (A) 223-kb deletion shared among 14 males from 3 different females that likely occurred at least two generations prior. (B) A complex 27-kb duplication and 13-kb deletion at the white locus that was recovered in a single offspring and is likely to be de novo based on sibling haplotypes lacking the rearrangement. (C, D) An 856-kb duplication identified in a single male from a c(3)G68 female has identical start and end coordinates as an 856-kb deletion recovered in a single male from a corolla129 female and is identical to an 856-kb duplication recovered in a single male from a wild-type female from a prior study (Miller et al. 2016b). (E) A large 4.17-Mb duplication observed in a single individual that is likely mosaic based on its lower log2 ratio of 0.25.
NCOGC recovered was defined by two polymorphisms and validated using PCR and Sanger sequencing. While this event is likely real and not an artifact of sequencing or alignment, it is not possible to definitively determine whether this event occurred during meiosis or was instead a pre-meiotic conversion event.

To determine the fate of DSBs made in females unable to construct full-length SC, novel deletion polymorphisms were identified in wild type, c(3)G864+, c(3)G68, and corolla129 offspring. Comparing the absolute number of deletions between genotypes is not accurate as DSBs in SC mutants are made at a fraction of the wild-type rate, thus a better comparator is deletions per DSB. When analyzed in this way, approximately 10% of DSBs made in individuals from c(3)G86 homozygous females resulted in a novel deletion—significantly more than the 1% observed in the wild-type and c(3)G86+ datasets—while only 3% of DSBs in corolla homozygous females were recovered as deletions, an observation not different from wild type.

The increased frequency of de novo deletions in offspring from c(3)G86 homozygous females does not explain the fate of the remaining 90% of DSBs made in these flies. Sandler (1965) used a ring chromosome assay in a c(3)G background to determine whether these DSBs are repaired by sister chromatid exchange. In that assay, females carrying one ring X chromosome and one normal rod X chromosome are constructed. The absence of any exchange yields an equal number of progeny with a ring or a rod chromosome. If exchange occurs between homologs (between the ring and the rod chromosome), single CO events will result in anacentric and dicentric chromosomes that will not segregate properly, and because both chromosomes will be equally affected, an equal but decreased number of ring and rod chromosomes will be recovered. In the absence of homologous recombination but in the presence of sister chromatid recombination, rod chromosomes will be transmitted at a normal level, since sister chromatid exchange events on a rod chromosome will not affect their structure or segregation. Single sister chromatid exchange events on a ring chromosome will result in a large dicentric ring that will not segregate properly, thus the number of progeny inheriting a ring will be lower than the number of rod chromosomes recovered.

Sandler found that in the c(3)G mutant background, rings are recovered less frequently than the homologous rod X chromosome, demonstrating that sister chromatid exchange is occurring in the absence of C(3)G (Sandler 1965). Separately, Hall (1977) was able to recover Bar revertants from FM7+/c(3)G86 females which, in the absence of homologous recombination, occur through unequal exchange between sister chromatids (Hall 1977). Thus, taken together, it is most likely that the majority of DSBs in SC-deficient flies are repaired either by a high-fidelity repair process such as canonical NHEJ or by sister chromatid repair.

These results also help clarify the role the SC plays in determining which chromosome is used for DSB repair. During mitosis, an intersister bias exists as the sister chromatid is preferred for repair. Alternatively, there is interhomolog bias during meiosis, as the homolog is preferred for DSB repair. How the cell determines which chromatid to use for repair is an area of active research. In S. cerevisiae, DMC1 (the E. coli RecA homolog) promotes interhomolog DSB repair and Dmc1-deficient yeast only undergo intersister DSB repair during meiosis (Bishop et al. 1992; Schwacha and Kleckner 1997). Both D. melanogaster and C. elegans lack an ortholog of DMC1, thus it is unclear how interhomolog bias is established during meiosis. One hypothesis is that these species no longer need DMC1 because the SC is built prior to the initiation of recombination. The data presented here, along with prior studies showing that sister chromatid repair occurs in the absence of SC, support the hypothesis that the SC plays a critical role in establishing interhomolog bias in Drosophila and that in the absence of SC, repair from the sister chromatid is possible.

Whole-genome sequencing of individual meiotic products allowed for the characterization of other meiotic events as well, such as whether the absence of full-length SC affects the movement of TEs. The observed rate of novel TE insertions in this study was significantly higher in offspring from c(3)G86 females when compared to the other three classes of progeny studied (wild type, c(3)G86+, and corolla129). Surprisingly, the elevated rate in maternally derived c(3)G86 chromosomes was similar to the rate from paternally derived X chromosomes, which came from males with two wild-type copies of c(3)G. The increased rate of de novo transposition in an SC mutant may provide new clues as to other roles that SC components might play in facilitating or preventing the movement of TEs.

This increased rate of novel TE insertion in c(3)G86 females could be explained by a model in which C(3)G prevents mobilized TEs from inserting into genomic DNA. In the absence of C(3)G, a greater number of TEs may be available to insert into nuclear DNA. A higher number of active TEs may also explain why the rate of TE insertions was similar on X chromosomes derived from wild-type males, but this would require that TE insertions occur post-fertilization.

Alternatively, the increased rate of TE insertion in c(3)G females could be explained by differences in genetic background leading to an increased rate of transposition (Kidwell et al. 1977). Previous studies have reported “bursts” of TE insertions from a specific TE class and attributed the observation to differences in genetic background (Pasynkova and Nuzhdin 1993; Page et al. 2007; Guerreiro 2011). It is worth noting that 20 of the novel insertions identified in the homozygous c(3)G86 dataset were doc elements, which seems to support the idea that different genetic backgrounds might exhibit different rates of transposition. Although, the genetic background in these experiments was somewhat controlled as females both heterozygous and homozygous for c(3)G86 were heterozygous for the same w1118 and Canton-S X and 2nd chromosomes, which were from the same stocks used in the wild-type experiment (Miller et al. 2016b). These two stocks differed in that females heterozygous for c(3)G86 carried one copy of a w1118 3rd chromosome, while those homozygous for c(3)G86 did not. Although the fact that these chromosomes have been used in previous experiments might reduce the contribution of genetic background to the elevated TE insertion rate, it may not completely eliminate it. The background of corolla mutations was not controlled. A unifying explanation may be that c(3)G itself plays a previously unappreciated role in the prevention of TE movement and that this is separate from the role, if any, played by fully functional SC.

Analysis of reads spanning de novo TE insertion sites revealed that two of the 16 total de novo TE insertions identified on the X chromosome were mosaic. Approximately 50% of reads at the TE insertion site spanned the junction and did not contain TE sequence, suggesting that the insertion occurred after the first, but before the second mitotic cell division. Both of the likely mosaic insertions were doc elements, with one occurring on a maternally inherited X chromosome and the other on a paternally inherited X. Removing both of the insertions does not significantly alter the overall rate of TE insertions per arm per meiosis calculated using the observed data, but does decrease the rate of insertions observed on the paternally inherited X chromosome from 0.64 to 0.48, suggesting a de novo insertion on the paternal X chromosome would be recovered approximately 1 in every 8 meioses instead of 1 in every 6.

The increased rate of TE movement in c(3)G homozygotes suggests that SC components may play a role in mediating TE movement. While the tripartite structure of the SC is widely conserved across species,
the amino acid sequence of the components themselves are not (Handel and Schimenti 2010; Fraune et al. 2012, 2013). Indeed, the rate of the amino acid change of SC components makes it challenging to find homologs of the transverse filament protein ε(3)G and other previously identified D. melanogaster SC components even in other species of Drosophila (Hammer and Blumenstiel 2016). At a larger evolutionary scale, there is little sequence homology among lateral element, transverse filament, or central element components of C. elegans, D. melanogaster, and M. musculus, suggesting the rapid rate of evolution is not limited to the Drosophila species group (Fraune et al. 2016). This is unlike components of the recombination machinery, which associate with the SC during meiosis and are highly conserved at the amino acid level (Zalesky et al. 1999; Villeneuve and Hillers 2001). Thus, it is reasonable to posit that the rapid rate of evolution observed for SC components across species and organisms may in part be a response to the ongoing evolution of TEs.

The recovery of large TE-mediated CNVs in females unable to construct SC demonstrates that these types of CNVs can occur independent of normal meiotic synopsis and DSB formation, perhaps depending only on the presence of a chromosome axis. Of course, it is also possible the events recovered here, including the events identified in the wild-type dataset, occurred during mitosis. While this study only sought to identify large CNVs, it is likely that many smaller CNVs occurred which were not identified. Indeed, previous studies in Drosophila using both microarray and whole-genome sequencing have shown that both small and large CNVs are segregating in wild populations (Emerson et al. 2008; Cridland and Thornton 2010; Cardoso-Moreira et al. 2011, 2012), however these studies did not evaluate the frequency of TE-mediated events because they were done using microarrays or filtered reads that partially mapped to TE sequences.

That two different TE-mediated CNV events (a deletion and a duplication) in two different genetic backgrounds were recovered at the exact same coordinates as a duplication observed in wild-type individuals was surprising and suggests that the rate of CNV formation and the ability to tolerate a large CNV may not be uniform across the genome. As would be expected in mutants with defective homologous recombination and meiotic drive are properties of wild-type Drosophila melanogaster male meiosis. Genetics 172: 305–316.

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