Understanding the Relationship Between B chromosomes and Nondisjunction in Drosophila melanogaster

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Understanding the Relationship Between B chromosomes and Nondisjunction in

*Drosophila melanogaster*

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Abstract

B chromosomes are supernumerary, heterochromatic genetic elements that are found in hundreds of different plant and animal species. Recently, B chromosomes were discovered in a stock of *Drosophila melanogaster* and are carried at a high copy number of 10-12 B chromosomes per cell. B chromosomes are not known to carry any active genes, but when placed in a wild-type genetic background, they cause a significant increase in the frequency of chromosome 4 missegregation during meiosis. This project aimed to understand the relationship between a female’s B chromosome copy number and how often she passes on too many (or too few) copies of chromosome 4 to her progeny. To do this, females with B chromosomes were crossed to males that do not carry B chromosomes but do carry a specially marked copy of chromosome 4. This cross allows us to phenotypically identify instances where the parental female passed on a normal (one copy) or abnormal (zero or two copies) number of chromosome 4 to her progeny. To assess the B chromosome copy number in the parental female, I obtained karyotypes from metaphase chromosome spreads from her ovaries. I then plotted the frequency of chromosome 4 missegregation as a function of the number of B chromosomes the female carried. Our preliminary results indicate that there is not a distinct relationship between B chromosome copy number and nondisjunction frequency, and that any number of B chromosomes is enough to cause significant missegregation of chromosome 4 during female meiosis. Future studies will focus on the mechanism of how the B chromosomes promote aberrant chromosome 4 segregation, which will have major impacts on our understanding of how the presence of extra chromosomes during meiosis can lead to birth defects and infertility.
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Introduction

Within a eukaryotic organism, the genetic material that is essential for its growth and reproduction is organized into chromosomes (Man et al. 2021). Chromosomes reside in the nucleus of cells and have many unique features, such as telomeres and centromeres. Telomeres are repetitive sequences that protect the end of the chromosome (Giardini et al. 2014), and centromeres are the site of kinetochore assembly that enable chromosomes to be connected to the spindle and segregate properly during cell division (Verdaasdonk and Bloom 2011). These features allow the organism to replicate and develop properly. However, not all chromosomes work towards the goal of helping the organism survive.

In some species, additional, supernumerary chromosomes called ‘B’ chromosomes have been documented. The first documentation of these B chromosomes was in the plant insect Acanthocephal (Wilson 1907). Since then, B chromosomes have been documented in a wide range of species, including both plant and animal species (Palestis et al. 2004). The composition, origin, and structure of the B chromosomes can vary drastically from species to species. For several species, B chromosomes have been shown to originate from the essential chromosomes, such as in rye (Martis et al. 2012) and Drosophila melanogaster (Hanlon et al. 2018).

In the fruit fly Drosophila melanogaster, there are four sets of essential chromosomes. These chromosomes are the sex chromosome (X and Y chromosomes) and the three autosomes (chromosomes 2, 3, and 4). Recently, B chromosomes were discovered in a single laboratory stock (Bauerly et al. 2014). Their copy number within this stock ranges from 10 to 12 B chromosomes and they have centromeres (Bauerly et al. 2014).
al. 2014), telomeres, and no euchromatic protein-coding genic regions (Hanlon et al. 2018). The B chromosomes are highly heterochromatic and can be crossed into other genetic backgrounds, such as a wild type background (Bauerly et al. 2014). A particular feature of the Bs is that they are enriched with the AAGAT satellite repeat, which is also found on chromosome 4 (Hanlon et al. 2018). This satellite repeat indicates that the origin of the Bs is chromosome 4, most likely from a breakage of the geneless left arm (Hanlon et al. 2018).

Previous studies have shown that the presence of extrachromosomal elements that share repetitive sequences with the 4th chromosome can influence how the 4th chromosomes segregate during female meiosis (Hawley 1992). Meiosis is the process of cell division that produces gametes, or sex cells (Fellmeth and McKim 2022). The process of meiosis features two rounds of division (compared to only one round in mitosis) to produce four products that have only one copy of each chromosome (Figure 1A). Knowing that the Bs and 4th chromosome share many of the same satellite sequences, Bauerly et al. (2014) conducted a nondisjunction assay to understand if the presence of the B chromosomes can cause chromosome 4 to improperly segregate during female meiosis (Figure 1B). Nondisjunction of chromosome 4 during the first meiotic division results in two of the meiotic products receiving two copies of chromosome 4 and the other two products receiving zero copies (Figure 1B). In a wild-type background with B chromosomes (WT+B), chromosome 4 nondisjoined at a frequency of 27.1%, whereas without B chromosomes (WT), the frequency of nondisjunction was 0.3% (Bauerly et al. 2014).
Though it is clear that B chromosomes disrupt chromosome 4 segregation during meiosis, the mechanism of this disruption is not understood. My thesis work aims to explore the relationship between the B chromosomes and chromosome 4 during female meiosis by testing if the frequency of nondisjunction is dependent on B chromosome copy number. To begin, I repeated the experiments from Bauerly et al. to measure chromosome 4 nondisjunction in WT+B stocks. I found that nondisjunction of chromosome 4 is the highest when B chromosomes are first introduced into the wild-type background (fresh WT+B), and the nondisjunction frequency decreases as the stock has carried B chromosomes for multiple generations (aged WT+B). This result indicates that the stock carrying B chromosomes may adapt to their presence over time by somehow counteracting their detrimental effect on meiotic chromosome segregation.

Figure 1. Diagrams of female meiosis showing the movement of chromosome 4. (A) A normal female meiosis, in which the homologous chromosomes separate during the reductive round of division and the sister chromatids separate during the equational round of division. Here, each of the four gametes receives one copy of the 4th chromosome. (B) A female meiosis with a nondisjunction event occurring in meiosis I. The homologous chromosomes fail to separate during the reductive division, causing missegregation of the equational division. Two of the gametes have two copies of the 4th chromosome and two gametes have zero copies, respectively named diplo and nullo nondisjunction events.
Using a fresh WT+B stock, I proceeded to determine the relationship between B chromosome copy number and chromosome 4 nondisjunction. Individual females carrying B chromosomes were crossed to males without B chromosomes, and I determined the nondisjunction frequency of chromosome 4 for each cross. I then examined this nondisjunction as a function of B chromosome copy number in the parental female to determine if there is a relationship. I found that there appears to be no relationship between B chromosome copy number and nondisjunction frequency of chromosome 4, leading me to hypothesize that any number of B chromosomes in the female will lead to significant chromosome 4 missegregation during meiosis. Together, this work in the model B chromosome system in *D. melanogaster* has enabled us to better understand the influence of the nonessential B chromosomes on the segregation of the essential chromosomes during the complex cellular process of meiosis.
Materials and Methods

Growth conditions

All stocks were grown at 25°C in the Hanlon Lab incubator on the Bloomington formulation of Nutrifly cornmeal media, made with propionic acid and Tegosept, and supplemented with dry instant baker’s yeast. Fly sorting, collections, and crosses were done using CO₂ flowing from a pad.

Cross setup

Figure 2. Cross scheme depicting how the wild-type stock with B chromosomes (WT+B) was created. Flies in this stock carry on average 5-8 B chromosomes. For the experimental cross, the WT+B virgin females were used to conduct a nondisjunction assay. Flies that were used immediately in the experimental cross are labeled as “fresh”. Flies that were used after 10-15 generations are labeled as “aged.”

The crosses used to create the aged and fresh WT+B stocks (Figure 2) used ten virgin females and ten males in a single vial for each step. They were then placed in an incubator on day 0. After the final cross, the flies were transferred to a bottle to establish the stock.
For the experimental cross, I collected virgin WT+B females and aged them so that they would be 1-5 days old when the cross was set up. I collected males of unknown age from a stock carrying a compound fourth chromosome and the markers $ci$, $ey^R$, referred to as the D2 stock. Experimental crosses had one virgin WT+B female and three D2 males in a single vial, which were then placed in an incubator. On day 5, all vials were brooded into a second brood unless the female was dead, in which case the remaining parental males were discarded.

**Nondisjunction assay**

To assess the frequency of nondisjunction within the progeny of our experimental cross, I scored the progeny. Scoring is done by surveying the progeny by phenotype assessment and recording the amount of each type of progeny (Figure 3). Progeny that received one copy of chromosome 4 from the parental female will look phenotypically wild type. Progeny that received two copies of chromosome 4 from the parental female are phenotypically $sv^{spa-pol}$, which is an identifiable mutation of eye texture. Progeny that received zero copies of chromosome 4 from the parental female are phenotypically $ci$, $ey^R$, which are two identifiable mutations of wing vein interruption and small eyes respectively.

Scoring was done on or around days 10, 14, and 18, but never after day 18 as the F2 progeny will begin to eclose after that point. To calculate nondisjunction frequency, the data from scoring was added to an Excel spreadsheet using an established formula that calculates chromosome 4 nondisjunction by doubling the number of nondisjunctional progeny. The number is doubled to account for the inviable progeny that received either
four 4th chromosome copies or zero 4th chromosome copies. The total number is then divided by the number of flies scored to calculate the frequency of 4th chromosome nondisjunction.

**Figure 3.** Punnett square showing both expected and exceptional progeny after a cross with the D2 stock that carries a compound 4 chromosome. In the green boxes is the expected progeny, which will be phenotypically wild type. In the light grey boxes are the other expected progeny, which are minute flies that often die or are very sick because they only have one copy of the 4th chromosome. The blue box shows diplo nondisjunction, in which the parental female passed down both of her 4th chromosomes. These flies phenotypically look svspa-pol. The pink box shows nullo nondisjunction, in which the parental female passed down no 4th chromosomes. These flies inherit the compound 4th chromosome from the parental male and phenotypically look ci, eyR. The dark grey boxes show flies that received either four copies of the 4th chromosome or zero copies and are thus inviable. Figure made with BioRender.
Ovary mitotic preps

To conduct an ovary mitotic prep, flies were anesthetized with CO$_2$ until they were just asleep. The female parental fly was separated out and the male parental flies were discarded. The female fly was moved to a well slide with 50 μL 0.7% sodium chloride (NaCl). The fly was held ventral-slide down and the second to last tergite toward the posterior was pulled to create an opening for the ovaries to come out. If it was not large enough, more of the cuticle was pulled to create sufficient space. The ovaries were then further pulled on to remove them from the carcass and transferred to the other well which is also filled with 50 μL 0.7% NaCl. In the second well, a dissection needle was used to carefully sever the tips of the ovaries, which contain the ovarioles, and no eggs past stage 6 were included. After the germarium tips are carefully cut, they were moved to a hypotonic solution of 50 μL drop of 0.5% sodium citrate. After 5 minutes, the tips were moved into an acetic acid paraformaldehyde (AAP) solution and allowed to fix for 4 minutes. As the 4 minutes came to an end, a siliconized 22mm diameter round coverslip was prepared with one 3 μL drop of 45% acetic acid to the center. Then the ovary tips were placed onto the coverslip and gently teased apart. The slide was added and squashed using body weight. The slide was then put into a hand clamp and squished for two minutes. The pressure was applied perpendicularly in order to avoid lateral pressure and coverslip movement. After the two minutes, the slide was quick-frozen in liquid nitrogen for at least five minutes before removal. Using a razor blade, the coverslip was removed by popping it. The slide was then added to -20°C 70% ethanol for 10 minutes, then moved to -20°C 100% ethanol for another 10 minutes to dehydrate the sample. Once removed from the ethanol, the slide leaned against a rack, sample-side down to air-dry.
**Quick fluorescence in situ hybridization (FISH)**

First, the sample was marked around the bounds and coverslip boundary on the back of the slide using an ethanol-resistant marker. A humidity chamber was created by placing wetted paper towels into a large, black gem case and placing a flat tip rack piece of plastic on top to avoid direct contact between the slide and the paper towel. The chamber was placed in the 30°C benchtop incubator to warm up while the hybridization buffer is made. The buffer was created by using the components listed below, with this protocol creating a 1x solution meant for 1 slide. This buffer was made by combining 10 μL of Formamide (ultrapure), 0.9 μL of UltraPure water, 0.1 μL of FISH probe, and 10 μL of Salty DS. Each slide had 21 μL of the hybridization buffer added to it. A 22mm x 22mm non-siliconized coverslip was then placed on top and all bubbles are gently pushed out. The slides were placed on a 95°C heat block, covered to protect them from the light, and allowed to denature for 5 minutes. After this, the slides were added to the pre-warmed humidity chamber and incubated for at least an hour at 30°C. After the hour, the slides were checked to make sure they have not dried out underneath the coverslip. The slides were dunked into a shallow amount of 0.1x SSC and the coverslip was removed under the SSC. The slides were then loaded into a Coplin jar or slide rack that was filled with 0.1x SSC. The jar was then put on an orbital shake for 5 minutes at room temperature and in the dark. After the 5 minutes, the 0.1x SSC in the jar was discarded and fresh 0.1x SSC was added for another 5-minute shake. This process was then repeated again for a final 5-minute wash. The slides were then removed from the 0.1x SSC and using a house airline, as much liquid as possible was carefully removed. The
slides were then mounted with 5 μL Vectashield+DAPI under a 22mm x 22mm non-siliconized coverslip. The edges of the coverslip were then sealed with clear nail polish and allowed to dry completely before any imaging occurs. Slides were either imaged within a week or stored 4°C for up to two weeks.

*DeltaVision Ultra Imaging*

To image using DeltaVision Ultra Imaging Microscope, the slide was dabbed with a drop of Cargille microscopy oil (ND = 1.512), and then placed face-down into slide holder. The 100x objective was used and moved into position. The stage was also moved to the center of the sample over the objective. The Z-touchdown position “Tissue squash” was selected and the sample was brought into focus manually using the fine focus knob. Once in focus the x-y position was moved to the left-most side of the sample. The entire boundary of the sample was searched for at least 12 metaphase chromosome spreads. If necessary, I searched within the sample. Each spread was marked with a point. The marked metaphase spreads were imaged automatically by visiting the marked points. The Contrast Autofocus on DAPI (DNA) channel was performed, then wavelength-then-z images in the desired channels from least-to-most energy (red > green > blue). A 0.45-micron offset was used for the green channel. All images and imaging logs can be found in the folder for this project (HanlonLab_DVElite -> AAP -> AAP1).

*Data availability*

The data for the creation of a wild type stock with B chromosomes can be found in lab notebook AAP1-1A. The data for the results presented in this thesis can be found in lab notebooks AAP1-1E & AAP1-1F (aged WT+B) and AAP1-1G & AAP1-1H (fresh
WT+B). Additional nondisjunction data was generated in collaboration with Shell Chen and can be found in lab notebook SLC1-1D and SLC1-1E.
Results

A stock carrying B chromosomes for several generations does not exhibit nondisjunction at the levels observed for stocks with freshly introduced B chromosomes.

In order to further assess B chromosomes and 4\textsuperscript{th} chromosome nondisjunction without any other mutations in the background, a stock with a wild-type background and B chromosomes had to be created. I established a wild-type stock with B chromosomes (WT+B) by crossing the original B chromosome stock to a wild type stock without B chromosomes (Figure 2). After its establishment, I kept this WT+B stock under normal growth conditions until it was needed for nondisjunction assays (see Materials and Methods).

I first wanted to reproduce the nondisjunction frequency that was previously observed in WT+B females (Bauerly et al. 2014). To do this, I crossed WT+B virgin females to males from the D2 stock (Figure 3). With this experimental cross, I can observe instances where 4\textsuperscript{th} chromosome nondisjunction has occurred through phenotypic assessment. The expected progeny, which have inherited one 4\textsuperscript{th} chromosome from their parental female and the compound 4\textsuperscript{th} chromosome from the parental male, will be wild type. The exceptional progeny will exhibit either sy\textsuperscript{spa-pol} (diplo nondisjunction) or ci, ey\textsuperscript{R} (nullo nondisjunction). Through scoring the progeny, I can determine the exact number of nondisjunction events and calculate an average frequency of nondisjunction for each vial and an overall average for the entire trial.

In each of two trials, I found that the nondisjunction frequency was consistently low, resulting in an average nondisjunction frequency of 13.34% (Table 1). This frequency is lower than the expected 27.1% (Bauerly et al. 2014), leading me to question
if the parameters of my assay were different from the previous study. After a careful comparison of the experimental methods, I concluded that the primary difference between my assay and the one conducted in Bauerly et al. was the age of the WT+B stock: I had used females from my WT+B stock I established 10-15 generations prior, whereas the previous study used WT+B females from a stock that had been made fresh. This discrepancy led me to hypothesize that the age of the WT+B stock influences the frequency of nondisjunction of chromosome 4.

To test this hypothesis, I re-created the WT+B stock and used it in the experimental nondisjunction assay immediately rather than allowing it to propagate. I will herein refer to it as the “fresh” WT+B stock to distinguish it from the previous one, herein referred to as “aged” WT+B. When the fresh WT+B stock was used, the frequency of 4th chromosome nondisjunction was found to be 22.34% (Table 1). This was much higher than the aged WT+B stock and much closer to the findings reported by Bauerly et al. (2014). Regardless, both aged WT+B and fresh WT+B stocks had more nondisjunction than a wild type (WT) stock without B chromosomes. In total, I found 1.3% 4th chromosome nondisjunction in WT (Table 1), which is slightly higher than the nondisjunction frequency of 0.3% reported by Bauerly et al. (2014).

Additionally, I found that the ratio of diplo to nullo nondisjunction was not conserved between fresh and aged WT+B stocks. The diplo nondisjunction to nullo nondisjunction ratio for the aged WT+B stock was 6.3 to 1. In contrast, the diplo-to-nullo ratio for the fresh WT+B stock was 2.7 to 1. The ratio for the fresh stock is much closer to the 2.2 to 1 ratio reported by Bauerly et al. (2014). The ratio discrepancy between fresh and aged WT+B additionally suggests that the age of the stock plays a role in the
No distinct relationship was identified between B chromosome copy number and frequency of 4th chromosome nondisjunction

The presence of B chromosomes is known to cause 4th chromosome nondisjunction during female meiosis in wild type backgrounds at a rate of 27.1% (Bauerly et al. 2014). However, at present it is not understood if there is any relationship between B chromosome copy number and the frequency of nondisjunction produced.

If the frequency of nondisjunction increased as the B chromosome copy number increases, one possible explanation for this positive correlation is that the B chromosomes may be overcrowding the meiotic spindle and promoting the missegregation of chromosome 4. Another possibility is that nondisjunction is high when there are fewer Bs, but as the number of Bs increase, the nondisjunction frequency

Table 1. 4th Chromosome nondisjunction values in wild type, “fresh” WT+B, and “aged” WT+B

<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th>Wild type (cumulative)</th>
<th>Fresh WT+B</th>
<th>Aged WT+B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4</td>
<td>616</td>
<td>276</td>
</tr>
<tr>
<td>(chr 4 Nondisjunction</td>
<td>44</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Adjusted Total</td>
<td></td>
<td>621</td>
<td>355</td>
</tr>
<tr>
<td>% diplo-4</td>
<td>1.30%</td>
<td>16.29%</td>
<td>11.52%</td>
</tr>
<tr>
<td>% nullo-4</td>
<td>0%</td>
<td>6.04%</td>
<td>1.82%</td>
</tr>
<tr>
<td>Total chromosome 4 Nondisjunction</td>
<td>1.30%</td>
<td>22.34%</td>
<td>13.34%</td>
</tr>
</tbody>
</table>
decreases. This negative relationship could indicate that when present in low copy numbers, the Bs are more likely to disrupt chromosome 4 pairing, but as the number of B chromosomes increase, they prefer to pair with each other and chromosome 4 pairing with the Bs becomes less frequent. If no correlation between B chromosome copy number and nondisjunction is observed, then it may indicate that the B chromosomes – at any copy number – interfere with the chromosome 4 heterochromatic pairing that normally ensures its proper segregation.

To determine if the number of B chromosomes correlates with the nondisjunction of chromosome 4, I conducted multiple nondisjunction assays using virgin females from the fresh WT+B stock and males from a stock with a marked, compound 4th chromosome. I used both genetics through fly crossing and advanced cytology to assess this question. The progeny of the crosses were assessed for nondisjunction by identifying phenotypic markers (Figure 3). Using this method, the data was collected and analyzed to determine nondisjunction frequency for each parental female and her progeny. Next, ovary mitotic preps (OMPs) were conducted on the parental female to determine B chromosome copy number, described in detail in Materials and Methods. Then, I determined each parental female’s B chromosome copy number and compared it to her frequency of chromosome 4 nondisjunction.

When the frequency of nondisjunction was plotted for each B chromosome copy number, it appears that there is no direct correlation between B chromosome copy count in the parental female and the amount of nondisjunction in her progeny (Figure 4). Rather, it seems that B chromosomes in any copy number are enough to cause high (> 15%) nondisjunction. From this portion of the project, I conclude that the number of B
chromosomes does not seem to directly influence the levels of chromosome 4 nondisjunction. This finding suggests that the presence of B chromosomes may be interfering with the 4th chromosome missegregation regardless of copy number, perhaps through heterochromatic pairing or another mechanism independent of the number of Bs.

**Figure 4.** Relationship between B chromosome copy number and 4th chromosome nondisjunction frequency. B chromosome copy number was obtained by conducting ovary mitotic preps (OMPs) on the parental females (see Materials and Methods). The data shown above was gathered in collaboration with Shell Chen.
**Discussion and Future Directions**

Through our experiments, I have been able to better understand B chromosomes in *Drosophila melanogaster* and specifically the role B chromosomes play in causing chromosome 4 nondisjunction. Through the data I have produced, I can confirm results from previous studies, but also begin to explore the mechanisms that are causing 4th chromosome nondisjunction. Below, I present potential mechanisms that explain my findings and an overview of future directions for this project to be pursued by our laboratory.

An important question that emerged from our initial experiment was understanding if the age of the WT+B stock influenced the frequency of 4th chromosome nondisjunction that occurred when the stock was used. The first trials of this project, named Trials E and F, used a WT+B stock that was aged 4-5 months, which is equates to approximately 10-15 generations. In this aged stock, the average frequency of nondisjunction when used in an experiment was 13.34%. In comparison, the average frequency of nondisjunction when a fresh stock was used was 24.34%. This difference is important for follow-up experiments as it alters how they will be conducted. It is clear that in order to measure the true effect of the B chromosomes within an experiment, they must be recently incorporated into a wild type stock. Additionally, it is interesting that there is such a large reduction of nondisjunction of chromosome 4 in aged WT+B stocks. My results indicate that over time, the stock is able to acclimate to the presence of the B chromosomes and reduce their detrimental effect on chromosome stability.

Here, I have observed the B chromosomes not being able to maintain their ability to cause rampant nondisjunction at a steady rate. Rather, the Bs can cause high levels of

20
nondisjunction when they have recently been introduced to the stock and this level tapers off the longer they have been incorporated in the stock. One theory that may explain this finding is that because the B chromosomes are causing nondisjunction and increasing the amount of exceptional progeny, there is a selective pressure for B chromosome tolerance. Flies that harbor B chromosomes but have a lower rate of chromosome 4 nondisjunction may produce more progeny than flies with B chromosomes and a high frequency of chromosome 4 nondisjunction since the latter would have more inviable progeny. After several generations under this selection, the overall population of flies may have a reduced frequency of chromosome 4 nondisjunction. It is uncertain which mechanism or mutation carries out this selective pressure but ultimately, this tolerance leads to B chromosomes causing lower frequencies of nondisjunction.

The primary goal of this project was to understand how B chromosome copy number affects the frequency of 4th chromosome nondisjunction. Through my experiments, I conclude that there is no linear relationship between copy number and nondisjunction frequency (Figure 4). Rather, the data indicate that any number of B chromosomes is enough to cause significant nondisjunction. With this in mind, I can attempt to further understand what the B chromosomes are doing during female meiosis to cause such rampant missegregation. One possible theory is that the B chromosomes interfere with the chromosome 4 heterochromatic pairing that normally ensures its proper segregation. To further investigate this, we could look at the heterochromatic threads of the 4th chromosome. Heterochromatic threads have been shown to play a role in ensuring that achiasmate chromosomes, such as the 4th chromosome, segregate properly (Hughes et al. 2009). If chromosome 4 cannot properly form heterochromatic threads between
them, then their segregation during meiosis is disrupted. Chromosome 4 may be pairing with the B chromosomes instead of its homolog, which may result in its missegregation. Staining and visualizing the heterochromatic threads could offer insight into the movement of the 4th chromosome, and live imaging could perhaps reveal if the Bs are interfering with the heterochromatic threads and thus causing nondisjunction.

Another important future direction for the B chromosome copy number and frequency of 4th nondisjunction project is supplementing the data with additional trials. The data I gathered to assess the relationship between copy number and 4th nondisjunction frequency is highly concentrated from 5-7 B chromosomes but lacks more points for other B chromosome copy numbers. To address the lack of thorough representation of lower (1-4) and higher (8-12) B chromosome copy numbers, additional experimental trials need to be conducted. The creation of a WT+B stock with both lower and higher numbers of B chromosomes is possible and would be done by altering the WT+B cross scheme (Figure 2).

To create a lower copy number WT+B, I would switch the sexes of the first cross as males pass on fewer B chromosomes than females (Hanlon and Hawley 2023). To create the higher copy number WT+B, I would start the cross scheme with a pre-existing WT+B male rather than a WT male with no B chromosomes. The WT+B male should already carry between 5-8 B chromosomes and will contribute half of those throughout steps of the cross scheme, resulting in a final stock that has a higher number of B chromosomes. Overall, conducting additional trials with the lower and higher B chromosome copy number stocks is an essential future direction for this project to fully understand the relationship between the Bs and chromosome 4 nondisjunction.
Through this project, I have been able to gain a broader understanding on how B chromosomes influence segregation of the 4\textsuperscript{th} chromosome in \textit{Drosophila melanogaster}. With the findings of my project and the future directions for the study, I hope to have aided the current understanding of how supernumerary non-essential chromosomes can impact an organism and specifically how non-essential chromosomes can influence meiosis. Humans are known to have small supernumerary marker chromosomes (sSMCs) (Liehr \textit{et al.} 2004), and sSMCs have been shown to play a role in causing both genetic syndromes (Jafari-Ghahfarokhi \textit{et al.} 2015) and infertility (Armanet \textit{et al.} 2015). Through a greater understanding of the B chromosomes’ influence on missegregation and the mechanism they use to cause missegregation, we can hopefully gain further insight into how sSMCs may be operating. This would allow for further knowledge on the cause of genetic syndromes and infertility in humans and could influence their diagnosis and treatment.
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