Decreased Recombination Frequency in Lead Contaminated Drosophila melanogaster

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Abstract. Recombination frequency through testcross involving Drosophila melanogaster can be used as relevant data in assessing the effect of certain substances on organisms. This study aimed to analyze the effect of lead-contaminated culture media on the percentage of recombination events. D. melanogaster was selected as the model organism while crossing over was selected as the observed recombination event. Lead levels in each treatment were 0, 0.05, 0.075, and 0.1 grams. Crossing over data was collected by calculating the frequency of recombinant-type strains from testcross results involving wildtype strains and vestigial black double mutants. Successively, the recombinant frequencies in the 0-, 0.05-, 0.075-, and 0.1-gram lead groups were 32.40, 14.65, 0, and 0%. The results of the hypothesis test indicated that lead contamination had a significant effect on reducing the recombination frequency. Therefore, lead may negatively impact the molecular aspects that control recombination events. Because recombination is regulated by genes and involves various proteins, a decrease in recombination frequency indicates that lead has a negative impact on genes or proteins during gametogenesis.

1 Introduction

The genetic recombination event involves the exchange of DNA segments capable of creating new genetic variation within a population [1–3]. Under normal conditions, the frequency of recombination can depict the stability of molecular conditions in the gametogenesis process. However, changes in recombination frequency can be positioned as an indicator of the presence of changes at the molecular level in an organism [4–5]. Disturbances caused by mutagenic compounds or radiation can change recombination frequency [6–7]. The recombination mechanism involves several proteins [8] and various enzymes [9]. The presence of mutagens can damage genetic material involved in the recombination process or inhibit the expression of genes that produce important components involved during the recombination event. Therefore, an analysis of recombination frequency can provide...
implications for molecular damage induced by external factors or certain toxic compounds exposed to the organism under study.

One model organism often involved in toxicity research is the fruit fly (D. melanogaster) [10–12]. Various studies on compound toxicity have reported data on climbing ability [13–15], feeding behavior [16–19], flight behavior [20–21], sleep behavior [19], and even the development behavior of fruit flies [20–21]. Although this insect has been intensively involved in modeling organism responses to various toxic compounds, the utilization of recombination frequency data in toxicity studies has not been optimally explored. An example of recombination frequency that is easily collected from fruit flies is the crossover frequency that appears from the phenotype of offspring resulting from a testcross [1–22]. By evaluating changes in crossover frequency, researchers can identify compounds that have the potential to be mutagens and can be further investigated as toxic compounds.

Among the many toxic compounds that have contaminated the environment, lead (Pb) is a compound that is still often researched to this day [23–25]. The abundance of research investigating the effects of lead is driven by ongoing contamination in the environment [26–28] and its potential adverse effects on human health [23–29–30]. Regardless of the development of research findings related to lead toxicity, research related to organism responses to exposure to this compound is still essential to report because lead remains one of the toxic compounds that are still often found in various ecosystems and has a high exposure level in various regions [28–31]. Its potential to influence physiological [32], neurological [33], and developmental aspects [20–21] requires comprehensive investigation to uncover the complex mechanisms underlying its toxicity. In addition, the complex interactions between lead and other environmental factors, as well as its potential long-term consequences, demand ongoing exploration of established and emerging research paths. Therefore, ongoing research on lead that consistently reports the negative impacts of this compound can provide a basis for various parties to follow up on lead contamination in various regions.

In line with the information previously conveyed, research related to toxicity involving fruit flies has been reported many times. Some studies have reported the effects of lead on climbing behavior [34], social interaction [35], oxidative stress level [36], and even changes in fly morphology [37]. However, research reporting the effects of this substance on recombination frequency is still hard to find. Therefore, the aim of this study is to evaluate the effects of chronic and long-term lead exposure on crossover frequency in fruit flies. The findings of this study are expected to be able to reveal the relationship between lead exposure and crossover frequency. In addition, this research can also provide a basis for further research that also involves fruit fly crossover frequency data as an indicator of the toxicity of various other substances.

2 Method

2.1 Model organism preparation and experimental design

Two strains of D. melanogaster were used in this study: the N strain (wildtype) and the double mutant bvg strain (black body vestigial wings). The D. melanogaster used in this study were obtained from the Genetics Laboratory at Malang State University. The flies were cultured in 200 ml cylindrical glass bottles using the standard medium used by Fauzi et al [38]. There were four groups of flies, and each group was cultured in a medium containing lead with treatment levels of 0, 0.05, 0.075, and 0.1 grams. Each treatment consisted of five crosses.
2.2 Testcross procedures

The testcross procedure was used to obtain the frequency of crossover events in this study. Wildtype females were crossed with bvg males as the first parental generation. The heterozygous N strain females resulting from this cross were then crossed with homozygous bvg strain males. Subsequently, the F2 single mutant b strain (black body) and single mutant vg strain (vestigial wings) offspring were recorded as recombinant type offspring and their percentage was calculated from the total F2 produced.

2.3 Data analysis

The recombinant frequency data in this study was based on the crossover values in each treatment. Crossover values were obtained by calculating the frequency of recombinant type offspring obtained in F2. This frequency was obtained by dividing the number of recombinant offspring by the total number of F2 produced and then multiplying by 100%.

3 Results and Discussion

The observed cross-over frequencies exhibited a distinct pattern within the experimental groups, as summarized in Table 1. The cross-over frequency in the 0-gram treatment group was notably prominent, accounting for approximately 32.40%. Intriguingly, a discernible decline was observed as the dosage increased to 0.05 grams, with the cross-over frequency diminishing to 14.65%. Strikingly, the concentrations of 0.075 grams and 0.1 grams yielded parallel outcomes, both registering a complete absence of cross-over events, implying a substantial impact on the recombination process. These results underscore the intricate relationship between lead exposure and genetic recombination dynamics, suggesting a dose-dependent effect on the recombination frequency.

Furthermore, the ANOVA results, as presented in Table 2, provided evidence regarding the significant impact of the treatments on the observed reduction in cross-over frequencies (F=17.461, p<0.001). This finding underscores the susceptibility of genetic recombination to lead exposure, substantiating the notion that the variations in lead dosages exert discernible effects on the genetic exchange processes.

The mechanism of genetic recombination in D. melanogaster involves an intricate process of crossing over between homologous chromosomes during meiosis [1]. This fundamental process contributes to genetic diversity by exchanging genetic material between chromatids [39]. During prophase I of meiosis, homologous chromosomes align closely, and molecular interactions between proteins facilitate the formation of crossover points, or chiasmata [22]. This physical linkage leads to the exchange of genetic material, which can include alleles for different traits. The subsequent stages of meiosis involve the separation of homologous chromosomes and chromatids, resulting in the formation of gametes with unique combinations of alleles [39]. The intricacy of crossover events is underpinned by a sophisticated network of molecular factors, among which enzymes and proteins play pivotal roles [40].

There are several other genes that are involved in crossing over events, such as mei-9, mei-41, and mei-W68. The product of mei-9 is involved in the meiotic recombination pathway which, if mutated, will disrupt its function during meiotic division [41]. Based on FlyBase [42], together with Ercc1 products, mei-9 products will interact with mus312 products to facilitate meiotic crossover. Along with mei-9, damage to mei-41 has also been reported to disrupt the meiotic crossover pattern in Drosophila [9]. Furthermore, DSB repair involves a protein kinase coded for by the mei-41 gene which plays an important role in the crossing over process [9]. On the other hand, the protein encoded by the mei-W68 gene is
involved in the formation of double strand breaks [43]. Apart from these genes, crossing over also requires structural proteins known as synaptonemal complex (SC) and cohesin complex [40]. SC is a protein complex involved in aligning homologous chromosome segments [44]. Similar to SC, the cohesin complex is also an important component in crossing over events in Drosophila [45–46]. In line with these various proteins, topoisomerase, and nuclease enzymes are also important components when crossing over occurs.

Table 1. Recapitulating the F2 strain results from a testcross between the wild type of strain (N) and the vestigial wing black body double mutant (bvg).

<table>
<thead>
<tr>
<th>Lead Concentration</th>
<th>Strains</th>
<th>Total Flies</th>
<th>Crossover Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 gram</td>
<td>N</td>
<td>56</td>
<td>32.40%</td>
</tr>
<tr>
<td></td>
<td>bvg</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vg</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>0.05 gram</td>
<td>N</td>
<td>61</td>
<td>14.65%</td>
</tr>
<tr>
<td></td>
<td>bvg</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vg</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.075 gram</td>
<td>N</td>
<td>69</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>bvg</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vg</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.1 gram</td>
<td>N</td>
<td>23</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>bvg</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vg</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Summary of ANOVA test results on crossover frequency data.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean of square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>2122.8</td>
<td>3</td>
<td>707.6</td>
<td>17.461</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Within groups</td>
<td>648.4</td>
<td>16</td>
<td>40.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2771.2</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the findings and analysis results reported in this study, lead is identified as a substance that can reduce the crossover frequency of fruit flies. Several mechanisms potentially cause these findings. One possible mechanism is the emergence of disturbances in enzymes or proteins involved in controlling crossovers. Lead may interfere with several enzymes or proteins, such as mei-41 product or SC involved in the crossover process. If these proteins are disturbed, then the exchange of both DNA segments will also be disturbed [9–47].

In addition to the direct impact on the molecular mechanisms of crossover events, the effects of lead on chromosome structure and DNA integrity can also contribute to a decrease in crossover frequency. Exposure to lead has been linked to DNA damage and alterations in chromosome structure [48–49] that may inhibit homologous processes and affect the formation of recombination foci. Various types of DNA damage, such as double-strand breaks and single-strand breaks, can be caused by lead exposure. If these breaks are not efficiently repaired, they can disrupt the normal processes of crossover. Furthermore, alterations in chromosome structure due to lead exposure, such as chromosome fragments or inversions, can interfere with the alignment of homologous sequences during crossover.

In line with the focus of this study, crossover frequency data is crucial to investigate. The occurrence of crossovers, which is part of the recombination phenomenon [50], is an essential mechanism in generating organism diversity [1]. This diversity not only enhances the
adaptive potential of the species in response to evolving environments but also introduces a dimension that could influence their susceptibility to environmental stressors [51], such as toxic substance exposure. Considering the role of recombination as the basis for diversity within a population, this research finding also indicates the role of toxic compounds in influencing diversity. By delving into the intricacies of these molecular mechanisms, insights are gained into how Drosophila's genetic landscape responds to external pressures, thereby potentially unraveling the complex interplay between genetic recombination and environmental factors in shaping the species' overall resilience.

Furthermore, a decrease in crossover frequency potentially has implications for the evolutionary trajectory of a species. Crossover events are a primary source of genetic variation [1], which is the raw material for evolution. In line with this statement, a decrease in crossover frequency will suppress the number of new genetic combinations, potentially reducing the population's ability to cope with changing environmental conditions [52]. The reason is that genetic variation within a population is one of the bulwarks for the population to avoid extinction due to environmental changes. In addition, toxic compounds like lead, which was studied in this research, can also influence the direction of evolution by exerting selective pressure on certain traits. If the presence of these toxic compounds reduces the fitness of certain variants, then those individuals will be less capable of reproducing and have a chance to pass on their genetic profile to the next generation. Over time, this could lead to changes in the genetic composition of the population. Therefore, studies and understanding related to the effects of toxic compounds on changes in crossover frequency include important efforts that are not only limited to the scope of short-term health risks but also for predicting long-term evolutionary outcomes.

Overall, this study provides new insights into the potential use of crossover frequency as an indicator of toxic effects on fruit flies. The results of the study show that a decrease in crossover frequency occurs due to exposure to toxic substances in culture media. These findings indicate that crossover frequency is sensitive to toxic substance exposure and can therefore be used as a useful variable in assessing the toxic effects of a compound. The implications of these findings create new potential in toxicological research using fruit flies’ crossover frequency as a data to understand the impact of toxic compounds at the genetic level. By continuing to understand the interplay between environmental factors and genetic reactions, future studies can strengthen the scientific basis needed to protect human and environmental health from the impacts of toxic substances.

4 Conclusion

In this study, *D. melanogaster* was used as a model organism to investigate the effects of lead exposure on crossover frequency. The lead levels in the various treatments were 0, 0.05, 0.075, and 0.1 grams. Crossover frequency was determined by a cross test between wildtype and double mutant black vestigial strains, with recombinant frequencies of 32.40%, 14.65%, 0%, and 0%, respectively. The results of the hypothesis test indicate that lead exposure has a significant influence on reducing crossover frequency.

These findings reveal the potential use of crossover frequency data as a tool for further toxicological research. This data can be a sensitive indicator in assessing the impact of toxic compounds at the genetic level and provide an important contribution to understanding the relationship between the environment and genetic reactions. Thus, this study opens up opportunities for further development in the field of toxic risk assessment and a deeper understanding of environmental impacts on organisms.
References


