The Winthrop McNair Research Bulletin

Volume 5 *The Winthrop McNair Research Bulletin Volume*

Article 10

2019

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Cover Page Footnote

This research was supported by the Ronald E. McNair Post-Baccalaureate Achievement Program and SC INBRE grants from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health.

Locating Mutagen-sensitivity Gene *mus109* in the *Drosophila melanogaster* Genome Using Deficiency Mapping

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ABSTRACT

The complex processes involved in repairing damaged DNA are still being elucidated. Some genes that are known to have roles in the DNA repair process have been identified, such as the mutagen-sensitivity genes, or *mus* genes, in *Drosophila melanogaster*. However, the precise genomic location of some *mus* genes is still unknown, including *mus109*. It is known that mutations in *mus109* cause chromosomal aberrations resulting in larval death, and previous research has mapped *mus109* to a region of the X chromosome consisting of over 520,000 nucleotides and 41 genes. Therefore, this study aimed to locate *mus109* using deficiency mapping. The $mus109^{D2}$ male flies were crossed to four deficiencies covering the 8F10-9B1 region of the genome producing four possible classes of offspring. Brood 1 larvae and Brood 2 larvae were treated with H_2O and 0.05% MMS, respectively. Offspring were scored for sex and eye phenotype, and this data was used in complementation analysis to narrow the probable genomic location of *mus109* to 12% of the original.

INTRODUCTION

In one day, a mammalian cell's DNA can be damaged with ~10,000 single-strand breaks and 10-50 double-strand breaks (Vilenchik and Knudson, 2003 qtd. in Madabhushi et al. 2014), as well as other damages such as lesions (Madabhushi, et al. 2014). An inability to repair DNA has been linked to various human diseases and disorders. For example, studies have shown that defective DNA repair has a role in neurodegeneration (Madabhushi et al. 2014), and the inability to fix DNA damage caused by UV radiation results in xeroderma pigmentosum, a disease resulting in an elevated risk of skin cancer (Driscoll 2012). Therefore, the study of DNA repair will not only aid us in understanding the human body and how it works, but it will also assist us in treating the many diseases and disorders caused by defective DNA repair like xeroderma pigmentosum and neurodegeneration.

An organism commonly used for the study of DNA repair is the fruit fly *Drosophila melanogaster*. The use of *D. melanogaster* as a model to study genetics has many advantages in comparison to other models. For example, it is more cost effective than using other organisms, and the flies do not need significant storage space. Additionally, flies produce many generations within a relatively short time span because they have generations every 10 days. Finally, because they only possess four chromosomes and have a fully sequenced genome, the genetic analysis of this organism is simplified (Hales et al. 2015). Forward genetic screens have identified 58 "*mus*" (mutagen-sensitive) genes within the *D. melanogaster* genome, 14 of which have been mapped molecularly (Sekelsky 2017). The unmapped *mus* gene that will be the focus of this research is *mus109*. It is an essential gene, and homozygous mutants are sensitive to several mutagenic agents. Mutations in this gene commonly cause chromosomal lesions in the junctions between heterochromatin and euchromatin, resulting in cell death in the larva (Baker et al. 1982). There are three publicly-available recessive alleles of *mus109*: $mus109^{ts}$ (Baker et al. 1982), $mus109^{D1}$ (Smith 1976), and $mus109^{D2}$(Mason et al. 1981).

This study aims to map the *mus109* gene within the genome of *D. melanogaster* using a form of complementation analysis called deficiency mapping. In deficiency mapping, female flies with deletions in the X chromosome are crossed with male flies carrying an allele of *mus109*. If a deletion fails to complement the *mus109* allele, then it can be concluded *mus109* is within the deleted region.

MATERIALS & METHODS

Fly Stocks

Five stocks of *Drosophila melanogaster* were ordered from the Bloomington Drosophila Stock Center (Indiana University). One fly stock contained a known allele of *mus109* (*sn*^{3} *mus109*^{D2}/*C(1)Dx, y*^{1} *f*^{1}) and the remaining four stocks contained deficiencies covering the 8F10-9B1 region of the genome, referred to as Df(A), Df(B), Df(C), and Df(D) in this work. Flies were kept on standard corn syrup/soy media (Bloomington recipe) in a 25°C incubator on a 12-hour dark;12-hour light cycle.

Deficiency Mapping

Deficiency-mapping was conducted using the mutagen-sensitivity protocol reviewed in Sekelsky (2017). Briefly, virgin female flies from each Df stock were identified based on both time (less than 8 hours from eclosion) and physical appearance, and were collected for up to 4 days. Then five 1-4 day old virgin females were crossed with five *sn*^{3} *mus109*^{D2} males per vial for a total of 10 vials for each Df (Brood 1). The day of crossing was designated as Day 0. On Day 3, the flies were flipped into new vials, producing Brood 2, and the following day each vial of Brood 1 was mock-treated with 250 µl of autoclaved water. On Day 5 of the overall experiment (Day 2 of Brood 2), the parent flies were cleared from the Brood 2 vials. On Day 6, Brood 2 was treated with 250 µl of 0.05% MMS per vial. On Day 18 for each Brood, all offspring were frozen. This process was repeated for each Df to obtain technical replicates. Due to viability issues, *Df(C)* was the exception to this protocol: four replicates with fewer vials were used in lieu of two replicates of 10 vials each (number of vials used ranged from 5-10 vials per replicate).

Scoring and Analysis

Frozen offspring were scored and data recorded based upon the sex and eye shape phenotype. The four possible phenotypic outcomes of the crosses were: male with wild-type shaped eyes, female with wild-type shaped eyes, male with Bar eyes, and female with Bar eyes (**Figure 1)**. Relative survival was then calculated as the ratio of mutant (non-Bar): control (Bar) flies in the MMS-treated vials, normalized to the same ratio in the mock-treated vials. Deficiencies showing sensitivity to MMS (less than 10% relative survival) were presumed to uncover the *mus109* locus (Laurencon et. al 2004).

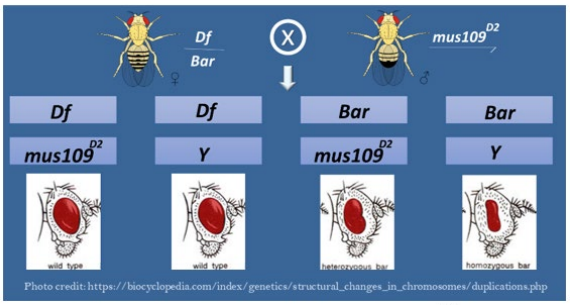

Figure 1: Female deficiency *(Df) / Bar* flies crossed to *mus109*^{D2} male flies produce four possible classes of offspring: *Df / mus109*^{D2} females with wild-type eyes; *Df / Y* males with wild-type eyes; Bar / *mus109*^{D2} females with Bar eyes; and *Bar / Y* males with Bar eyes.

RESULTS

Vial observations show differences between Brood 1 and Brood 2

The larvae and pupae were observed on Day 21 for both Broods 1 and 2. The vials that were mock-treated with water had pupal cases that were light in color and there were larvae visible at the bottom of the vials (Figure 2A). However, stark differences were seen when observing the vials of Brood 2. These vials, which were treated with MMS, had dark pupal cases indicative of organismal death and very few larvae (Figure 2B). These differences were observed for every deficiency analyzed.

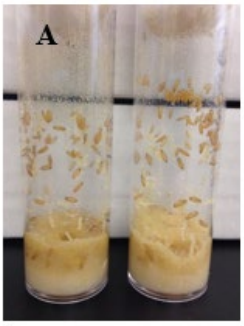

Figure 2A: Vials of deficiency B Brood 1 (H_2O).

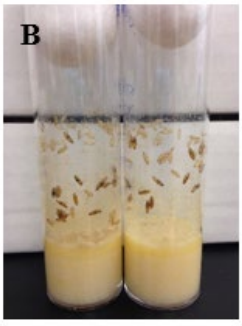

Figure 2B: Vials of deficiency B Brood 2 (MMS).

Figure 2A & 2B: These vials represent what was typically observed for all of the vials of each deficiency.

Relative Survival Percentages

After scoring the offspring from all deficiencies, the relative survival percentage was calculated for each. In all cases, relative survival was 0% (Table 1).

Table 1: Relative survival percentage

Deficiency	Brood 1 (n)	Brood 2 (n)	Relative Survival
A	1,673	1,111	0%
B	1,497	978	0%
C	832	430	0%
D	1,057	693	0%

DISCUSSION

There is little known about the mutagen-sensitivity gene *mus109* including its precise location in the *Drosophila melanogaster* genome. However, the gene was previously mapped to a region on the X chromosome that contained over 520,000 nucleotides and 41 genes (Mason et. al 1981). Thus, this study continued to narrow the location of *mus109* using a mutagen-sensitivity assay (Sekelsky 2017) paired with deficiency mapping.

Female flies with a deficiency were crossed with a male fly that had the $mus109^{D2}$allele. Brood 1 was mock-treated with water and Brood 2 was treated with MMS. The flies were then scored to determine whether there was a difference in survival for mutant and non-mutant flies by calculating the relative survival percentage. All relative survival percentages were calculated to be 0%, which indicated that the flies were sensitive to the DNA damaging agent MMS (Laurencon et. al 2004). These percentages suggest that *mus109* was within each deficiency and therefore, was in a region where all the deficiencies overlapped. This narrowed region consisted of just over 62,000 nucleotides and 9 genes (Gramates et al. 2017*)*. The genes included previously studied genes and currently unstudied "CG" genes. Future work will seek to identify a candidate *mus109* gene by using computational methods to learn more about the function of all nine genes. Then the candidate gene will be sequenced for all alleles of *mus109 (*$mus109^{ls}$*,* $mus109^{D1}$*,* and $mus109^{D2}$*)* and a rescue construct will be constructed.

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