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Testing the Allelic Strength of Drosophila melanogaster mus109 Alleles

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Testing the Allelic Strength of *Drosophila melanogaster mus109* Alleles

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Abstract

Drosophila melanogaster, commonly referred to as fruit flies, possess a group of genes that when mutated can cause sensitivity to DNA damaging agents. These mutagen sensitive (*mus*) genes are likely involved in DNA repair, and one of these genes, *mus109*, was the focus of this study. To perform the experiment, complementation crosses were set up between the three *mus109* alleles: $mus109^{lS}$, $mus109^{D1}$ and $mus109^{D2}$. The wild-type *DGRP-59* was used as the control. For each cross, twenty vials were scored. Brood one contained ten vials that were mock treated with distilled water, and brood two contained ten vials that were treated with the alkylating agent methyl methanesulfonate (MMS). The relative survival was calculated as the ratio of mutants to non-mutants in brood 2, normalized to brood 1. An ANOVA analysis indicated that there was not a significant difference in survival rate between the various *mus109* allelic combinations. However, ANOVA analysis indicated that the relative survival value for all *mus109* alleles was significantly different from wild-type ($p<0.0001$).

Introduction

- Human cells are constantly being exposed to DNA damaging agents throughout their lifetime[4].
- DNA repair is an essential process that corrects damage from exposure to mutagens.
- In DNA repair mutants, DNA damage promotes increased cell death[6].
- *Drosophila melanogaster* is a model organism in genetics due to their similarities to humans relating to their disease-causing genes[5].
- A mutation in the *mus109* gene correlates to a greater sensitivity and decreased DNA repair capacity when in the presence of DNA damaging agents[1,2,3].
- The comparative strength between *mus109* alleles is unknown, but research confirmed that $mus109^{lS}$, $mus109^{D1}$ and $mus109^{D2}$ are sensitive to methyl methanesulfonate (MMS)[1,2,3].
- The objective of this study was to test allelic strength between the three *mus109* alleles: $mus109^{lS}$, $mus109^{D1}$ and $mus109^{D2}$ to compare their phenotypes with their molecular information.

Methods

- 4 fly stocks were acquired from Bloomington Drosophila Stock Center. Three fly stocks contained the known alleles of *mus109*: $mus109^{lS}$, $mus109^{D1}$ and $mus109^{D2}$, and one fly stock *DGRP-59* contained wild-type flies to use as the control.
- Virgin females from each allele were crossed to males carrying the same allele or remaining allele types (Table 1).
- A balancer chromosome, *FM7*, *Bar* was crossed with each mutant allele to create flies heterozygous for a balancer chromosome and the desired mutation (Figure 1).
- Each of the six fly crosses contained 10 vials of brood 1, mock treated with distilled water, and 10 vials of brood 2, treated with 250µL of 0.05% methyl methanesulfonate (MMS) (Figure 2).
- The relative survival was calculated as the ratio of mutants to non-mutants in brood 2, normalized to brood 1 (Table 2).

1. $\frac{mus109^{D1}}{Bar} x \frac{mus109^{D1}}{Y}$	2. $\frac{mus109^{D1}}{Bar} x \frac{mus109^{D2}}{Y}$
3. $\frac{mus109^{lS}}{Bar} x \frac{mus109^{D1}}{Y}$	4. $\frac{mus109^{lS}}{Bar} x \frac{mus109^{D2}}{Y}$
5. $\frac{mus109^{D2}}{Bar} x \frac{mus109^{D2}}{Y}$	6. $\frac{DGRP-59}{Bar} x \frac{DGRP-59}{Y}$

Table 1. Mating scheme for complementation analysis. "*Bar*" represents *FM7* balancer chromosome.

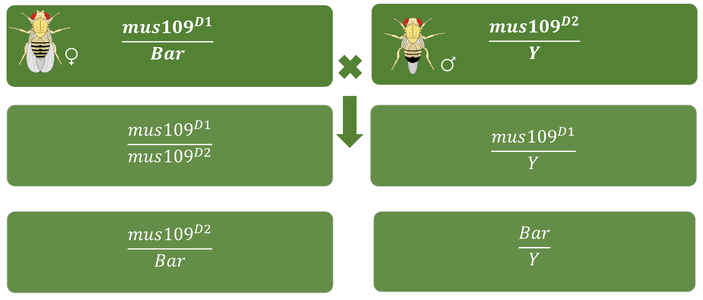

Figure 1. Complementation cross offspring. Heterozygous female fly $mus109^{D1}/Bar$ crossed to $mus109^{D2}$ male yields four possible offspring: $mus109^{D1}/mus109^{D2}$ female with wild-type eyes; $mus109^{D1}$ male with wild-type eyes; $mus109^{D2}/Bar$ with Bar eyes; and Bar male with Bar eyes.
https://www.netclipart.com/isee/xxJxh_male-and-female-fruit-flies/

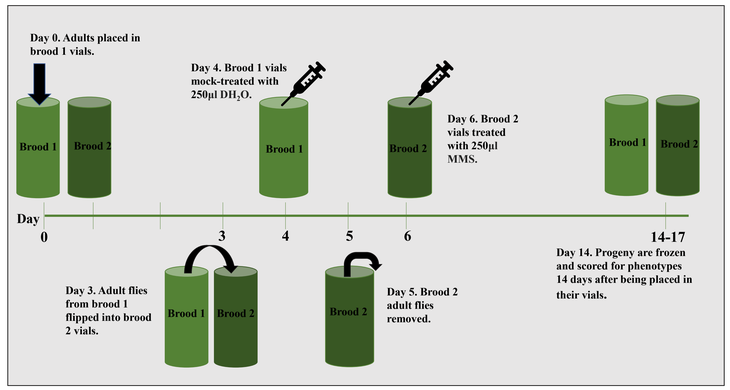

Figure 2. Mutagen sensitivity assay. On day 0, adult flies were crossed to begin Brood 1. On day 3, adult flies from Brood 1 were flipped into Brood 2 vials. On day 4, Brood 1 vials were mock treated with 250µl DH_2O. On day 5 , brood 2 flies were removed from their vials. On day 6, Brood 2 vials were treated with 250µl of 0.05% MMS. Brood 1 progeny were frozen and subsequently scored on day 14. Brood 2 progeny were frozen and subsequently scored on day 17. Figure adapted from literature[6].

Results

- All *mus109* alleles displayed sensitivity to MMS (Figure 3 and Table 2).
- An ANOVA indicated that the relative survival values for all *mus109* genotypes were statistically different from *DGRP-59* ($p<0.0001$) and not statistically different from each other.

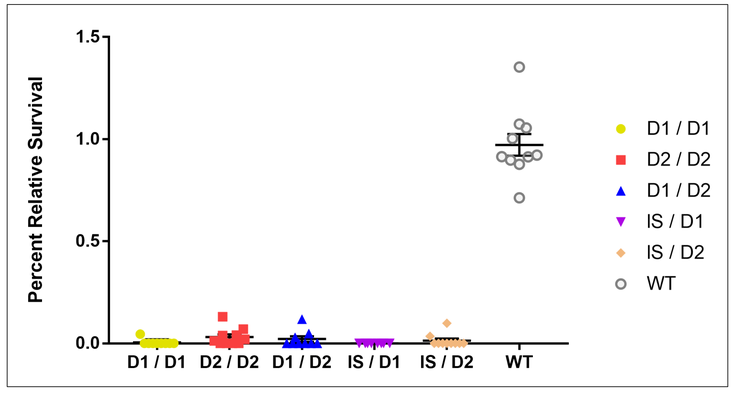

Figure 3. Relative survival of each *mus109* genotypic combination when treated with 250µL of 0.05% methyl methanesulfonate (MMS). Each point represents one vial. The horizontal line represents the mean, and the vertical bars represent the standard deviation. A relative survival value of 1 indicates 100% survival.

	D1 / D1	D2 / D2	D1 / D2	IS / D1	IS / D2
Percent Relative Survival ± std. dev.	0.5 ± 1.5%	3.1 ± 4.2%	2.2 ± 4.0%	0%	1.3 ± 3.2%
N	1242	1501	1494	654	1106

Table 2. Total number of flies scored for each allelic combination and their corresponding relative survival.

Discussion

- All mutations experienced sensitivity, but $mus109^{lS}$ displayed the greatest allelic strength as hypothesized, because it is a null allele (Figure 3 and Table 2).
- Of the remaining alleles, $mus109^{D1}$ exhibited greater allelic strength (lower percent relative survival) compared to $mus109^{D2}$ (Figure 3 and Table 2).

Acknowledgements

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