The Influence of Alcohol Dehydrogenase Activity Function on Ethanol Intoxication in Drosophila Melanogaster

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Abstract

Fermentation of fruit produces low concentrations of ethanol alcohol, animals which consume these ethanol-containing foods naturally evolved the alcohol dehydrogenase pathway to prevent alcohol intoxication. The fruit fly *Drosophila melanogaster*, which reacts to and metabolizes ethanol in a similar manner to humans. This makes Drosophila an excellent model to further understand the effects of alcohol metabolism and the genes that produce the enzymes capable of oxidizing alcohol. In this experiment the alcohol metabolism of *Drosophila melanogaster* will be investigated within three allelic variations of the alcohol dehydrogenase gene; Adh^{+}, Adh^{n1}, and Adh^{+}/Adh^{n1}. The alcohol dehydrogenase gene forms enzymes which function as dimers. *Drosophila* of the Adh^{+} variant form an active homodimer which oxidizes ethanol. The methyl-induced mutated Adh^{n1} variant forms an inactive homodimer. When two parental homodimers of the active and inactive form are crossed, the Adh^{+}/Adh^{n1} offspring produced will have both an active and inactive subunit forming a heterodimer. Studying the inheritance of methyl-induced genetic mutations such as Adh^{n1} offers insight into the epigenetics of enzyme activity.

To quantify *Drosophila*'s loss of postural and locomotive control upon exposure to concentrations of 0%, 5%, 15%, and 25% ethanol alcohol, specimens were placed in an inebriation chamber with vaporized ethanol and data was collected in five-minute intervals for thirty minutes. During this time the height of the *Drosophila* in the inebriation chambers decreased, more so as the concentration of alcohol increased. This was proved to be statistically significant through linear regression and an Anova test. Upon exposure to alcohol, *Drosophila* of the Adh^{n1} variant oxidized 0% of the ethanol and experienced the highest degree of ethanol intoxication, sedation, and fatalities. *Drosophila* of the Adh^{+} variant oxidized 96.72% of the ethanol and experienced the lowest degree of ethanol intoxication and sedation and had no fatalities. The Adh^{+}/Adh^{n1} offspring oxidized 87.35% of the ethanol and experienced an intermediate degree of intoxication, sedation, and fatalities. Additionally, the heterozygotes exhibited 89% of the ADH activity of the homodimer parent.

Introduction

Application and History

Drosophila melanogaster are an excellent model to examine the effects of alcohol since their entire genome has been sequenced. Like humans, *Drosophila* m. have alcohol dehydrogenase which functions as the pathway for the elimination of ethanol. This similarity allows the research collected from *Drosophila* m. to be applied to humans, which has the potential to offer further light as to why alcohol effects individuals so differently. This pathway evolved in *Drosophila* m. as a way to detoxify the ethanol that collects in the decaying fruit in which they feed and breed. When fruit decays it undergoes a process of fermentation[10] producing levels of 0.6-4.5% ethanol[8].

Alcohol Dehydrogenase Background in Drosophila melanogaster

Alcohol dehydrogenase is responsible for the production of the alcohol dehydrogenase polypeptides. Alcohol dehydrogenase polypeptides form enzymes composed of two subunits forming a dimer. These enzymes oxidize ethanol converting it into acetaldehyde which is then transformed into acetate by aldehyde dehydrogenase[5]. Without this pathway, ethanol accumulates in the blood and organs, in high enough levels this can be fatal. In *Drosophila m.* there two natural allelic variations[4] of alcohol dehydrogenase; Adh^F and Adh^S. Both variants of alcohol dehydrogenase form an active homodimer. That is a dimer with two identical subunits, both capable of oxidizing ethanol.

Methyl-Induced Mutations and Inheritance

An alcohol dehydrogenase-null mutation can be introduced to regions of the alcohol dehydrogenase gene through exposure to ethyl methanesulfonate[2], a mutagenic and teratogenic compound. In *Drosophila* of the Adh^{n1} mutation, a glycine-93 on the alcohol dehydrogenase locus is replaced with glutamic[1]. This changes the conformation of the resulting alcohol dehydrogenase polypeptide and forms an inactive homodimer, which is a dimer with two identical subunits, neither capable of oxidizing ethanol. The result for these *Drosophila* is a higher sensitivity to ethanol[6] which can present itself as an intoxication or death depending on the concentration of ethanol.

The result of crossing Adh^+ and Adh^{n1} *Drosophila* m. produces offspring whose alcohol dehydrogenase polypeptide forms a heterodimer. This heterodimer has one subunit in the active form and one in the inactive form. The association of the active and inactive subunit restores partial alcohol dehydrogenase activity. Consequently, the offspring have 50%[1] the alcohol dehydrogenase activity level of the active homodimer parent. In this experiment, Adh^+ and Adh^{n1} *Drosophila m.* are crossbred to produce a heterodimer F_1 generation. The ethanal sensitivity of the F_1 generation must be significantly different than the parental homodimers[6] to confirm that the offspring form heterodimers. Additionally, the predicted 50% alcohol dehydrogenase activity level will be compared to the actual outcome through a Chi-Square test.

	Adh^+ Dimer Subunits: active + active	**Adh^+** Dimer Subunits: active + active
Adh^{n1} Dimer Subunits: inactive + inactive	**Adh^+/Adh^{n1}** Dimer Subunits: active + inactive	**Adh^+/Adh^{n1}** Dimer Subunits: active + inactive
Adh^{n1} Dimer Subunits: inactive + inactive	**Adh^+/Adh^{n1}** Dimer Subunits: active + inactive	**Adh^+/Adh^{n1}** Dimer Subunits: active + inactive

Figure 1: Predicted outcome of crossing Adh^+ (forming active homodimer) and Adh^{n1} (forming inactive homodimer) *Drosophila* is 100% Adh^+/Adh^{n1} offspring (forming heterodimer).

Investigation

Ethanol intoxication and alcohol metabolism for *Drosophila* of Adh^{+}, Adh^{n1}, and Adh^{+}/Adh^{n1} allelic variants is measured for concentrations of 0%, 5%, 15%, and 25% ethanol alcohol. The construction of an inebriation chamber[8] is a method to quantify alcohol intoxication in *Drosophila* populations. A fourteen-centimeter glass tube was constructed with incremental markings every centimeter, and a cotton ball at the opening of the tube. To expose *Drosophila* to ethanol they are placed in the chamber and an ethanol solution is delivered into the cotton ball. The ethanol alcohol is vaporized, entering *Drosophila*'s bloodstream through inhalation, with blood alcohol levels rising throughout exposure. To ensure all individuals begin in the same location the inebriation chamber is shaken so all flies collect at the floor of the tube. The height of the *Drosophila* in the inebriation chamber is recorded in five-minute intervals for thirty minutes after exposure. Naturally *Drosophila* will climb upwards without difficulty, occupying the upper space of the inebriation chamber. Ethanol intoxication results in reduced movement, loss of postural control[6], inability to climb, and sedation. Thus, as ethanol concentrations increase within *Drosophila*, they will be unable to climb and will fall to lower heights of the chamber. By recording their height in the inebriation chamber[8], the degree of intoxication can be quantified.

Hypothesis: *Drosophila melanogaster* with the Adh^{n1} mutation experience a greater degree of alcohol intoxication and Adh^{n1}/Adh^{+} heterozygotes will experience intermediate alcohol intoxication when compared to the Adh^{+} variant.

Experimental Methods and Materials

Culture Preparation and Breeding

Drosophila melanogaster Adh^{+} and Adh^{n1} variants were obtained through Carolina Biological Supply Company.

Upon arrival, carbon dioxide gas was administered to *Drosophila* for thirty seconds and then their cultures were poured onto a carbon dioxide air stone. This induced temporary unconsciousness which allowed for the sorting and sexing of *Drosophila*. The *Drosophila* were divided into six groups among six cultures. These cultures were created with 50mL Plastic Centrifuge Tubes, a large hole was cut into the lid of each tube and stuffed with cotton to allow for air circulation. Culture one contained 25 Adh^{n1} flies, culture two contained 25 Adh^{n1} flies, culture 3 contained 21 Adh^{+} flies, culture 4 contained 21 Adh^{+} flies, culture 5 contained male Adh^{+} flies and 5 female Adh^{n1} flies, culture 6 contained 5 female Adh^{+} flies and 5 male Adh^{n1} flies. All six cultures were kept at 25^{C}/65% humidity with 16/8-hour day/night light schedules, each culture contained a standard substrate[6] of 10% sucrose, 2% yeast, 3.3% cornmeal, and 1% agar[8]. The purpose of cultures 5 and 6 was to allow for the breeding of Adh^{+} and Adh^{n1} *Drosophila* to produce the Adh^{+}/Adh^{n1} F_1 generation. This breeding is ensured by housing opposite sexes of the two *Drosophila* variants together, this prevents breeding with members of

the same allelic variation. The six *Drosophila* cultures were left alone for fourteen days to allow for them to acclimate to their new environment and reproduce.

Experimental Material Preparation

Six inebriation chambers were built to measure the degree of ethanol intoxication and alcohol metabolism within the *Drosophila* populations. These chambers were constructed from a fourteen-centimeter glass tubes with incremental markings every centimeter. A solution of 0% ethanol alcohol was prepared in a 15mL Plastic Centrifuge Tube with 14mL of distilled water, measured with a PIPETMAN P1000. A solution of 5% ethanol alcohol was prepared in a 15mL Plastic Centrifuge Tube by diluting 1mL 70% ethanol alcohol (C_2H_5OH) in 13mL distilled water, measured with a PIPETMAN P1000. A solution of 15% ethanol alcohol (C_2H_5OH) was prepared in a 15mL Plastic Centrifuge Tube by diluting 3mL 70% ethanol alcohol in 11mL distilled water, measured with a PIPETMAN P1000. A solution of 25% ethanol alcohol (C_2H_5OH) was prepared in a 15mL Plastic Centrifuge Tube by diluting 5mL 70% ethanol alcohol in 9mL distilled water, measured with a PIPETMAN P1000.

Experimental Methods

Carbon dioxide gas was administered to the *Drosophila* for thirty seconds, introducing temporary unconsciousness. This allowed for Ten *Drosophila* from each of the six cultures to be transferred to an inebriation chamber. A cotton ball was inserted into the opening of the chamber to allow for air circulation but prevent any escapes. The *Drosophila* were allowed twenty minutes to regain consciousness and acclimate to the chamber before the experiments began.

To gather baseline measurements for the behavior and location of *Drosophila* in the six inebriation chambers a control was needed. 2mL of water (0% ethanol) was delivered to the cotton balls with a PIPETEMAN P1000. To ensure all individuals begin in the same location the inebriation chamber was shaken so all flies collected at the floor of the tube. The height of the ten *Drosophila* in each of the six inebriation chambers was recorded in five-minute intervals for thirty minutes after exposure. Additionally, any fatalities were recorded.

To replicate the concentration of alcohol naturally found in the food on which *Drosophila* feed, 2mL of 5% ethanol was delivered to the cotton balls with a PIPETEMAN P1000. To ensure all individuals begin in the same location the inebriation chamber was shaken so all flies collected at the floor of the tube. The height of the ten *Drosophila* in each of the six inebriation chambers was recorded in five-minute intervals for thirty minutes after exposure. Additionally, any fatalities were recorded.

Next, 2mL of 15% ethanol was delivered to the cotton balls with a PIPETEMAN P1000. To ensure all individuals begin in the same location the inebriation chamber was shaken so all flies collected at the floor of the tube. The height of the ten *Drosophila* in each of the six inebriation chambers was recorded in five-minute intervals for thirty minutes after exposure. Additionally, any fatalities were recorded.

Next, 2mL of 25% ethanol was delivered to the cotton balls with a PIPETEMAN P1000. To ensure all individuals begin in the same location the inebriation chamber was shaken so all flies collected at the floor of the tube. The height of the ten *Drosophila* in each of the six inebriation chambers was recorded in five-minute intervals for thirty minutes after exposure. Additionally, any fatalities were recorded.

Analysis of Ethanol Intoxication

The height in the inebriation chamber for the Adh^{+}, Adh^{n1}, and Adh^{+}/Adh^{n1} *Drosophila* variants at each ethanol concentration was averaged for each time interval. Additionally, the average overall height for the *Drosophila* variants at each ethanol concentration was calculated. This information was organized into a table to allow for analysis of the data.

To determine if the allelic variation and alcohol concentration statistically effected the average heights at each time interval a Two Factor Anova Test was performed at a 5% significance in Microsoft Excel.

Using Microsoft Excel scatterplot graphs were created to display the three variations of *Drosophila*'s average height in the inebriation chamber for each ethanol concentration. Similarly, a column graph was constructed using the average overall height for the *Drosophila* variants at each ethanol concentration. This allowed for the correlation between postural control and ethanol concentration to be visualized more clearly, as well as to generate r^2 values using linear regression. R^2 values explains what degree of change in one variable is the result of the other variable.

The average height of *Drosophila* in the inebriation chamber by itself is not enough for significant analysis. Therefore, increased ethanol sensitivity for each of the *Drosophila* variants at each ethanol concentration was calculated. This was calculated by dividing the average overall height of a specific *Drosophila* variant when exposed to water (0% ethanol alcohol) by the same variant's overall height at a concentration of ethanol alcohol. This method of analysis measured the change from the control experiment, thus taking into consideration the baseline behavior of the *Drosophila* populations in the inebriation chamber.

$$Increased\ Ethanol\ Sensitivity = \left(\frac{Mean\ Overall\ Height\ at\ Positive\ Control\ at\ a\ conc.}{Mean\ Overall\ Height\ at\ Ethanol\ Conc.}\right) x100\%$$

These calculations were organized into four graphs. Three which show the increased ethanol sensitivity at each concentration and a fourth which shows the compiled data. Through linear regression, r^2 values were calculated and added to the fourth graph.

To determine alcohol dehydrogenase activity, alcohol metabolism was determined using the previously calculated ethanol sensitivities. Since it is known that the Adh^{n1} variant forms an inactive homodimer and cannot oxidize alcohol, it can then be assumed that they experience the full effects of ethanol. Under this assumption, the alcohol metabolism of the Adh^{+} and Adh^{+}/Adh^{n1} *Drosophila* variants can be calculated by dividing their ethanol sensitivity at a

particular concentration by the sensitivity of the Adh^{n1} variant at the same ethanol concentration. This value is then multiplied by one hundred and then subtracted from one hundred.

$$Ethanol\ Alcohol\ Metabolism = 100 - \left(100 \cdot \frac{ethanol\ sensitivity\ of\ Adh^{+}\ or\ Adh^{+}/Adh^{n1} at\ a\ conc.}{ethanol\ sensitivity\ of\ Adh^{n1}\ at\ a\ conc.}\right)$$

The calculated alcohol metabolism for each *Drosophila* variant at the specific ethanol concentration was organized into a table. While analyzing data it became apparent that there was something wrong with the data collected during exposure to 5% ethanol alcohol, this is because it is an outlier and does not fit with any of the other results and logically does not make sense. Therefore, the average alcohol metabolism was calculated (excluding the data from the 5% ethanol alcohol experiment) for each *Drosophila* variant and organized into a table.

Microsoft Excel was also used to create a Colum graph displaying fatalities upon exposure to ethanol alcohol for the *Drosophila* allelic variants.

Analysis of Methyl-Induced Mutation Inheritance

A Chi-Square test was used to determine if *Drosophila* of the Adh^{+}/Adh^{n1} allelic variation had 50% the alcohol dehydrogenase pathway activity of the Adh^{+} variation. To perform this test, the data from the previously calculated ethanol alcohol metabolism was used. The expected values were calculated by multiplying the alcohol metabolism of the Adh^{+} variation at each ethanol concentration by .5 (the hypothesized activity of the heterodimer offspring). The actual values were simply the previously calculated alcohol metabolism of the Adh^{+}/Adh^{n1} variation at each ethanol concentration.

$$\chi^2 = \sum \frac{(o-e)^2}{e}$$

Additionally, to determine if the F_1 generation form heterodimers their ethanol sensitivity and alcohol metabolism must differ significantly from either parental homodimer. For this no further calculations are necessary.

Results

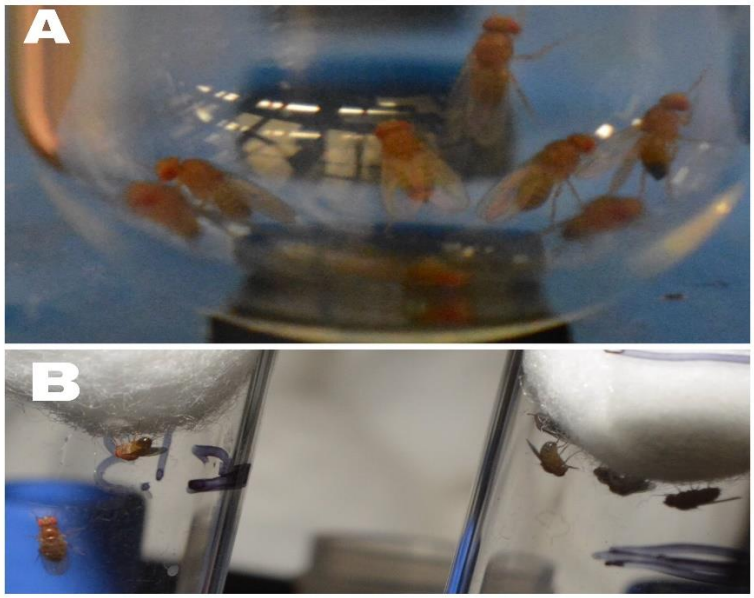


Image 1A: Drosophila collecting at the bottom of the inebriation chamber upon exposure to 25% ethanol alcohol. Displaying sedation and a loss of postural control.
Image 1B: Drosophila *collecting towards the top of the inebriation chamber in the absence of alcohol. Maintaining their postural control.*

		Time (In Minutes Since Exposure)							
		0	5	10	15	20	25	30	Average Height (in cm)
Height of Drosophila m. When Exposed to 0% Ethanol (in cm)	Adh(n1)	7.15	7.4	5.95	7.7	5.05	3.9	3.9	5.864
	Adh(+)	9.55	7.9	6.85	6.05	5.85	4.85	4.95	6.5714
	Adh(+)/Adh(n1)	10.55	12.2	9.175	8.8	9.45	11.55	9.4	10.161
Height of Drosophila m. When Exposed to 5% Ethanol (in cm)	Adh(n1)	3.65	1.3	0.65	0.65	0.65	0.65	0.65	1.1714
	Adh(+)	3.15	0.9	0.75	0.7	0.65	1.2	0.05	1.0571
	Adh(+)/Adh(n1)	10.15	9.45	8.75	8.65	7.3	6	5.4	7.9571
Height of Drosophila m. When Exposed to 15% Ethanol (in cm)	Adh(n1)	0	0.25	0.2	0.05	0	0	0	0.0714
	Adh(+)	4.1	2	1.15	1	1	0.05	0	1.3286
	Adh(+)/Adh(n1)	4.75	0.65	0	0	0	0.65	0.65	0.9571
Height of Drosophila m. When Exposed to 25% Ethanol (in cm)	Adh(n1)	0	0	0	0	0	0	0	0
	Adh(+)	0.1	0	0.1	0	0	0	0	0.0206
	Adh(+)/Adh(n1)	0.1	0	0	0	0	0	0	0.014

Figure i: Raw data for inebriation chamber experiments.

Ethanol Concentration	Genotype Group		
	Adh+	Adhn1	Adh+/Adhn1
Alcohol	9.55	7.15	10.55
	7.9	7.4	12.2
	6.85	5.95	9.175
	6.05	7.7	8.8
	5.85	5.05	9.45
	4.85	3.9	11.55
	4.95	3.9	9.4
5% Ethanol Alcohol	3.15	3.65	10.15
	0.9	1.3	9.45
	0.75	0.65	8.75
	0.7	0.65	8.65
	0.65	0.65	7.3
	1.2	0.65	6
	0.05	0.65	5.4
Alcohol	4.1	0	4.75
	2	0.25	0.65
	1.15	0.2	0
	1	0.05	0
	1	0	0
	0.05	0	0.65
	0	0	0.65
Alcohol	0.1	0	0.1
	0	0	0
	0.1	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	0

Anova: Two-Factor With Replication

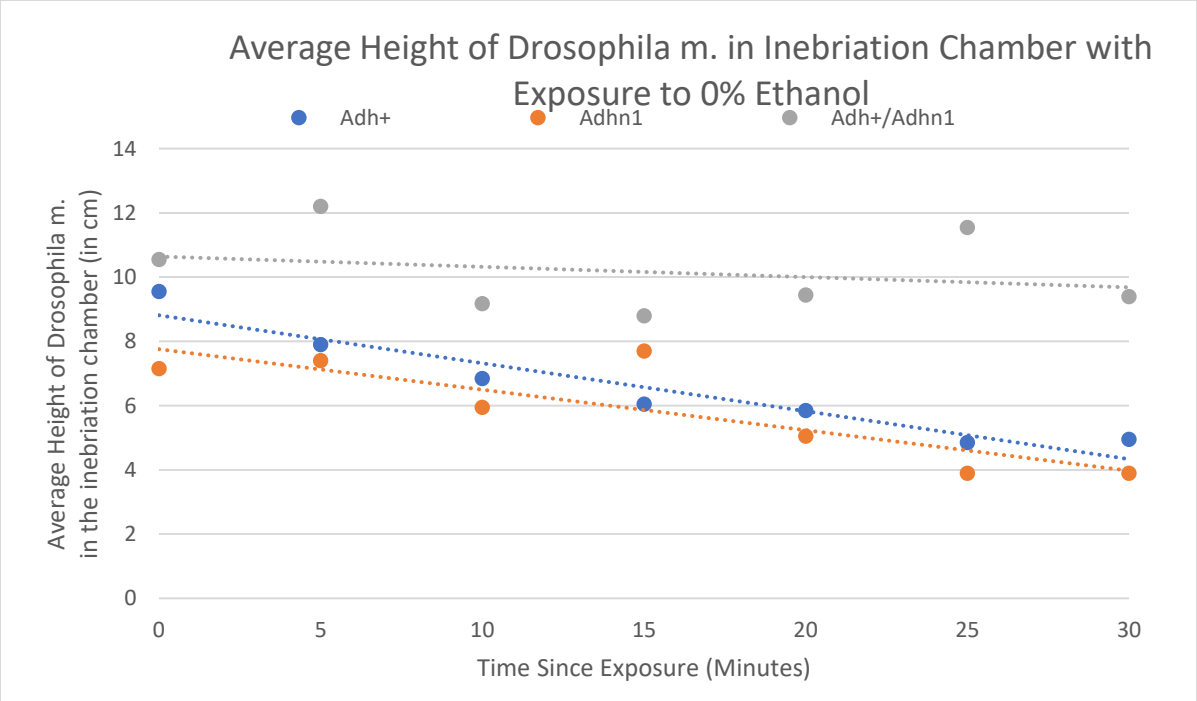
SUMMARY	Adh+	Adhn1	dh+/Adhn	Total
0% Ethanol				
Count	7	7	7	21
Sum	46	41.05	71.125	158.175
Average	6.57143	5.86429	10.160714	7.53214
Variance	2.84988	2.6281	1.6912202	5.8657
5% Ethanol				
Count	7	7	7	21
Sum	7.4	8.2	55.7	71.3
Average	1.05714	1.17143	7.9571429	3.39524
Variance	0.97119	1.25321	3.1578571	12.5427
15% Ethanol				
Count	7	7	7	21
Sum	9.3	0.5	6.7	16.5
Average	1.32857	0.07143	0.9571429	0.78571
Variance	1.96321	0.01155	2.9028571	1.75529
25% Ethanol				
Count	7	7	7	21
Sum	0.2	0	0.1	0.3
Average	0.02857	0	0.0142857	0.01429
Variance	0.00238	0	0.0014286	0.00129
Total				
Count	28	28	28	
Sum	62.9	49.75	133.625	
Average	2.24643	1.77679	4.7723214	
Variance	7.99591	6.86416	21.52321	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	724.406	3	241.46852	166.216	2.76293E-32	2.73181
Columns	145.357	2	72.678356	50.0284	2.39759E-14	3.12391
Interaction	153.346	6	25.557641	17.5927	1.99126E-12	2.2274
Within	104.597	72	1.4527406			
Total	1127.71	83				

Figure ii: Two Factor Anova Test at 5% significance level for Drosophila height in the inebriation chamber with exposure to concentrations of 5%, 15%, and 25% ethanol alcohol. Displaying that for factor 1 (ethanol alcohol concentration) the F-calculated value is 166.2, the F-critical value is 2.7, and the P-value is 2.7E-32. For factor 2 (Drosophila genotype) the F-calculated value is 50.0, the F-critical value is 3.1, and the P-value is 2.4E-14. For interaction of ethanol concentration and Drosophila genotype the F-calculated value is 17.6, the F-critical value is 2.2, and the P-value is 2.0E-12.

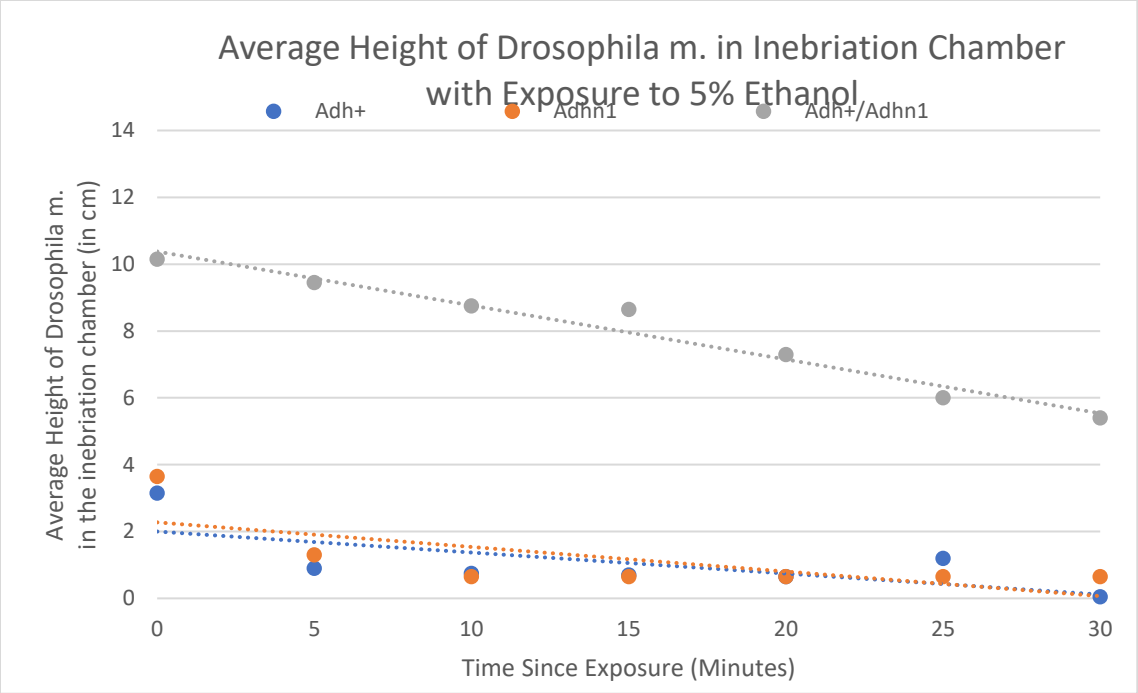
Height of Drosophila in Inebriation Chambers



Graph 1: Average Height of Drosophila m. in Inebriation Chamber with Exposure to 0% Ethanol. Scatter plot displaying the average height of the Drosophila in the inebriation chamber when exposed to a concentration of 0% ethanol alcohol over the

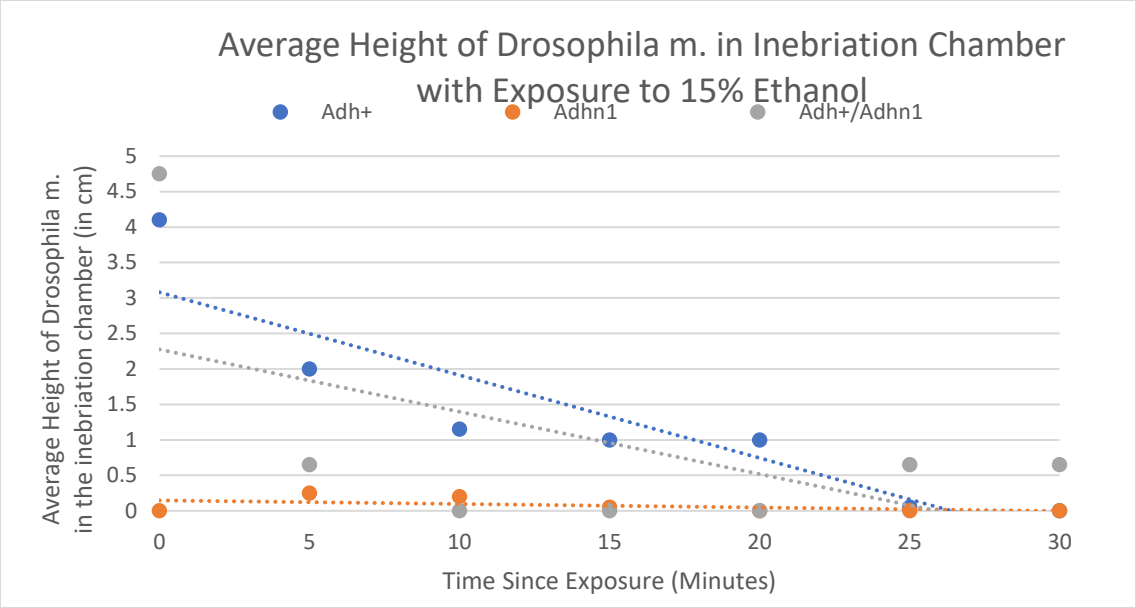
span of 30 minutes. This data provides a baseline, which is used to determine the normal behavior of Drosophila in normal conditions.

Drosophila stayed in the upper levels of the inebriation chamber, but elevation decreased over the interval of thirty minutes. At this concentration there were no fatalities within any *Drosophila* population.



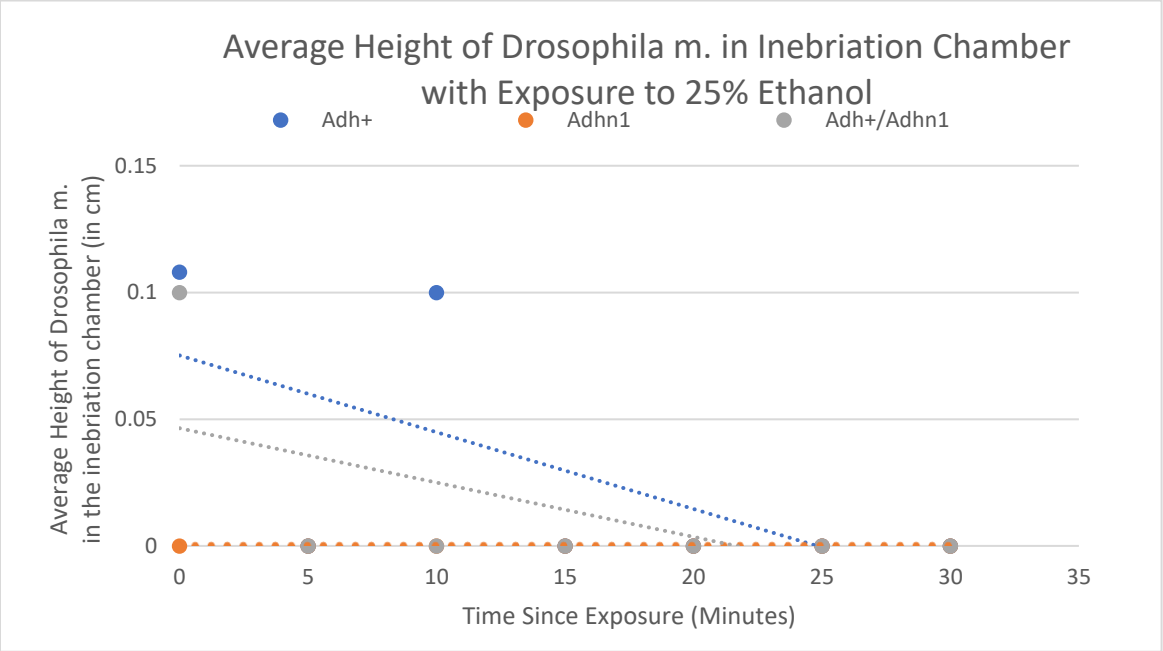
Graph 2: Average Height of Drosophila m. in Inebriation Chamber with Exposure to 5% Ethanol. Scatter plot displaying the average height of the Drosophila in the inebriation chamber when exposed to a concentration of 5% ethanol alcohol over the span of 30 minutes.

Upon exposure to a concentration of 5% ethanol alcohol, the elevation of the *Drosophila* decreased over the interval of thirty minutes. At this concentration there were no fatalities within any *Drosophila* population.



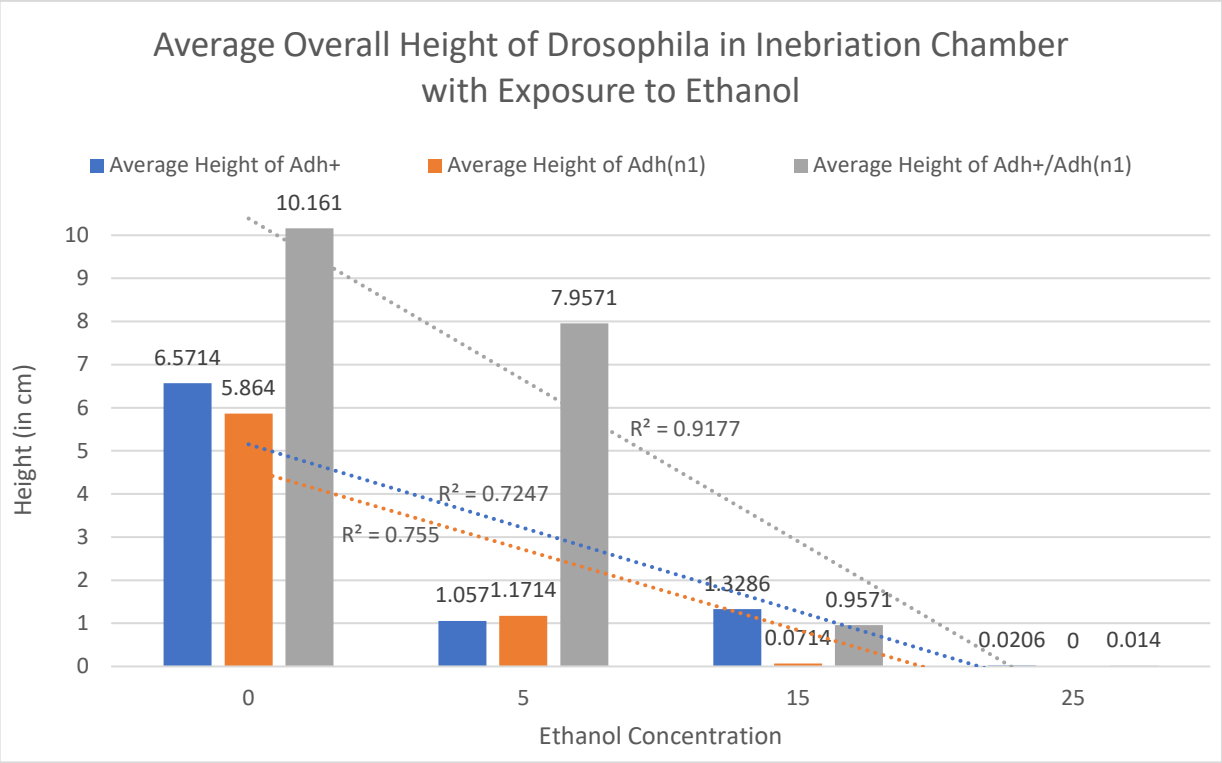
Graph 3: Average Height of Drosophila m. in Inebriation Chamber with Exposure to 15% Ethanol. Scatter plot displaying the average height of the Drosophila in the inebriation chamber when exposed to a concentration of 15% ethanol alcohol over the span of 30 minutes.

Upon exposure to a concentration of 15% ethanol alcohol, the elevation of the *Drosophila* decreased rapidly. At this concentration there were no fatalities within any *Drosophila* population.



Graph 4: Average Height of Drosophila m. in Inebriation Chamber with Exposure to 25% Ethanol. Scatter plot displaying the average height of the Drosophila in the inebriation chamber when exposed to a concentration of 25% ethanol alcohol over the span of 30 minutes.

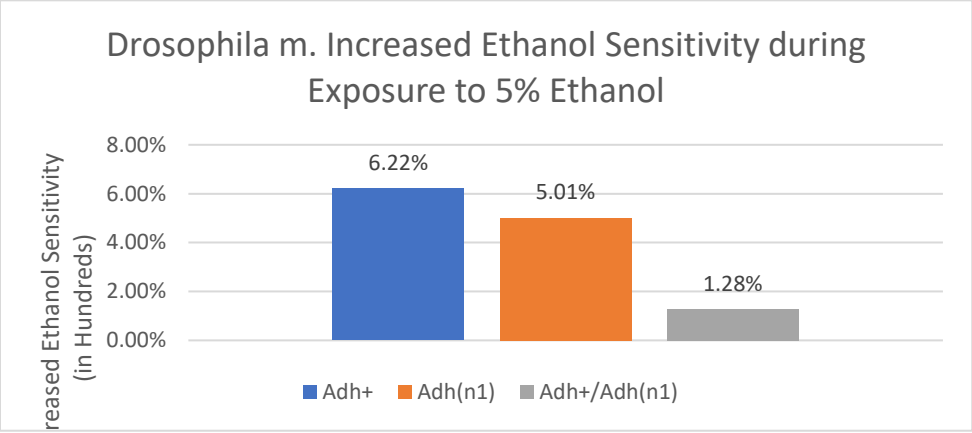
Upon exposure to a concentration of 25% ethanol alcohol, the elevation of the *Drosophila* decreased rapidly. At this concentration there were fatalities within the Adh^{n1} and Adh^{+} /Adh^{n1} populations.



Graph 5: Average Overall Height of Drosophila *in Inebriation Chamber with Exposure to Ethanol. Column graph displaying the average overall height of the Drosophila in the inebriation chamber when exposed to concentrations of 0%, 5%, 15%, and 25% ethanol alcohol over the span of 30 minutes.*

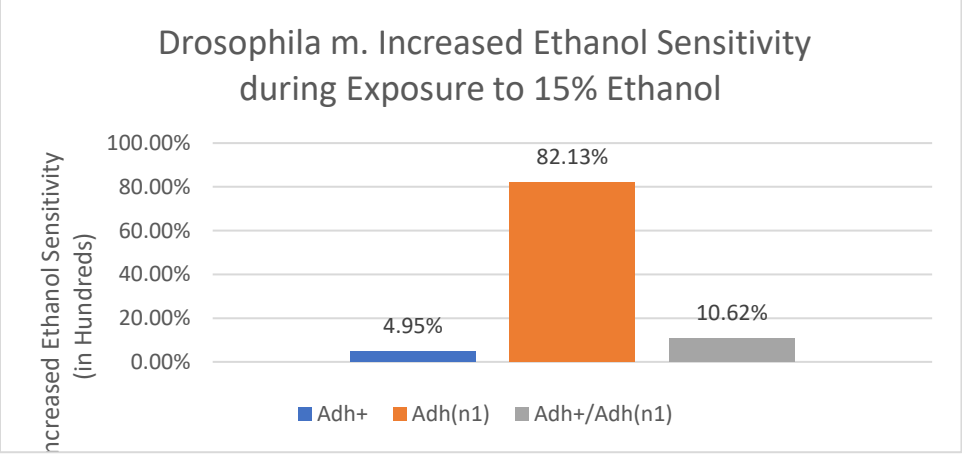
Upon exposure to a concentration of ethanol alcohol, the elevation of the *Drosophila* decreased rapidly. Significant differences emerge upon exposure to concentrations at and above 15% ethanol alcohol. The Adh^{n1} population experienced the most extreme decrease in elevation. The Adh^{+} population experienced the least extreme decrease in elevation. The Adh^{+} /Adh^{n1} population experienced a decrease in elevation which was intermediate of the homodimer parents. The R^2 values were calculated through linear regression and explain what degree of change in one variable is the result of the other variable. For example, the Adh^{n1} population has a r^2 value of 0.755, this means that 75.50% of the change in average height can be explained by the change in ethanol concentration.

Ethanol Sensitivity and Fatalities



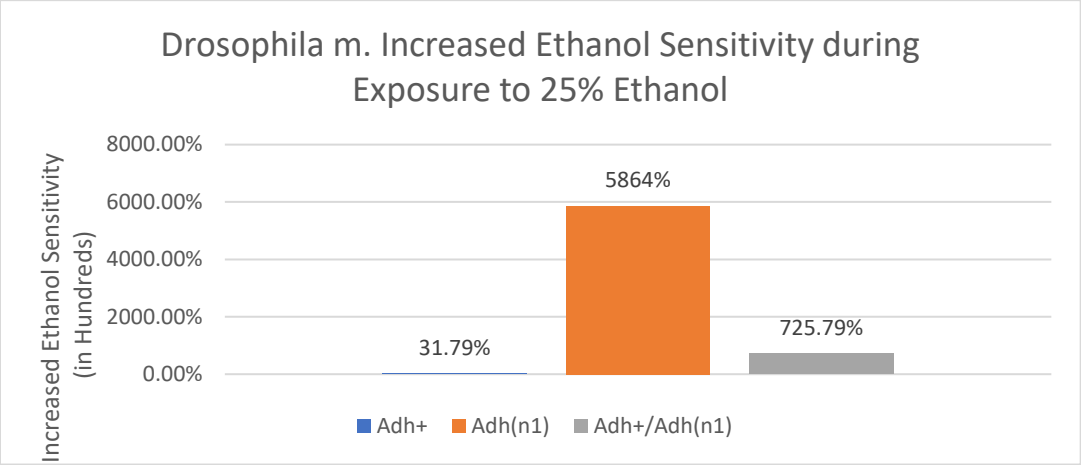
Graph 6: Drosophila m. Increased Ethanol Sensitivity during Exposure to 5% Ethanol. Column graph displaying the percent increase in ethanal sensitivity when Drosophila are exposed to a concentration of 5% ethanol alcohol. Showing that the Adh^{+} genotype is the most sensitive, the Adh^{+} /Adh^{n1} genotype is the least sensitive, and that the Adh^{n1} genotype is intermediately sensitive to ethanol at this concentration.

Increased ethanol sensitivity measures the change in height between the *Drosophila*'s exposure to the control (water) and exposure to ethanol alcohol. At the concentration of 5% ethanol alcohol, the Adh^{+} population was the most sensitive to ethanol, with a 622% increase in sensitivity. The Adh^{n1} population was intermediately sensitive, experiencing a 501% increase in sensitivity. The Adh^{+}/Adh^{n1} population was the least sensitive, experiencing a 128% increase in sensitivity.



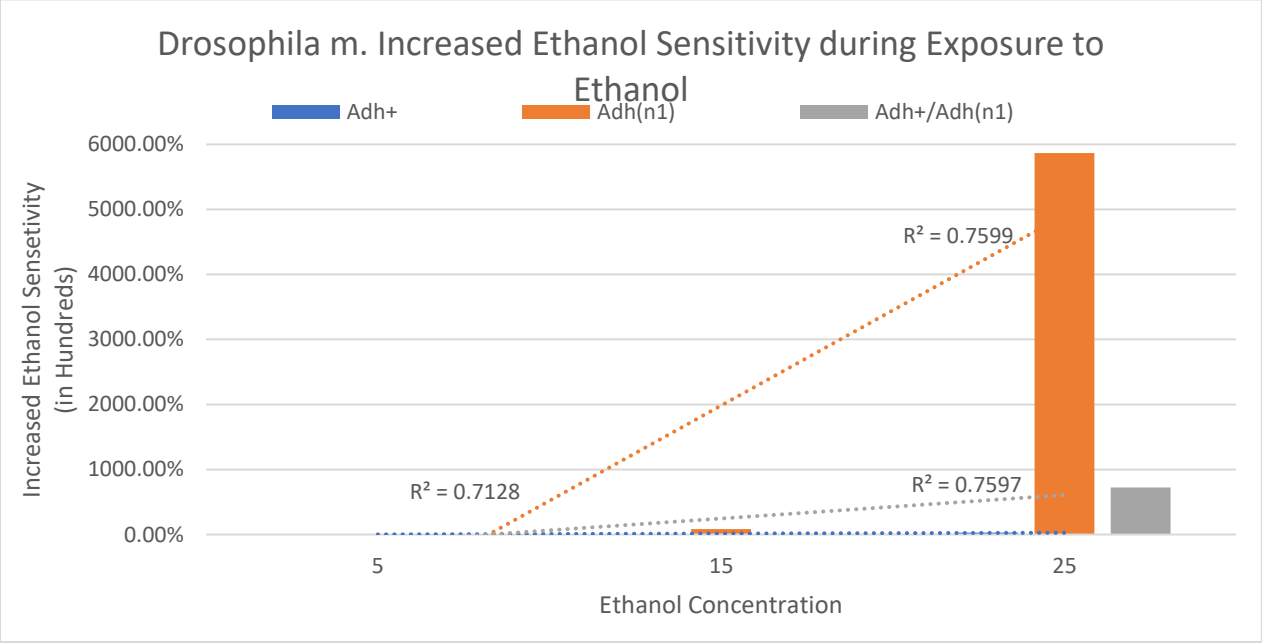
Graph 7: Drosophila m. Increased Ethanol Sensitivity during Exposure to 15% Ethanol. Column graph displaying the percent increase in ethanal sensitivity when Drosophila are exposed to a concentration of 15% ethanol alcohol. Showing that the Adh^{n1} genotype is the most sensitive, the Adh^{+} genotype is the least sensitive, and that the Adh^{+} /Adh^{n1} genotype is intermediately sensitive to ethanol at this concentration.

Increased ethanol sensitivity measures the change in height between the *Drosophila*'s exposure to the control (water) and exposure to ethanol alcohol. At the concentration of 15% ethanol alcohol, the Adh^{n1} population was impacted the greatest, experiencing an 8213% increase in ethanol sensitivity. The Adh^{+} population was the least affected experiencing a 495% increase in ethanol sensitivity. The Adh^{+}/Adh^{n1} population was intermediately affected, experiencing a 1062% increase in ethanol sensitivity.



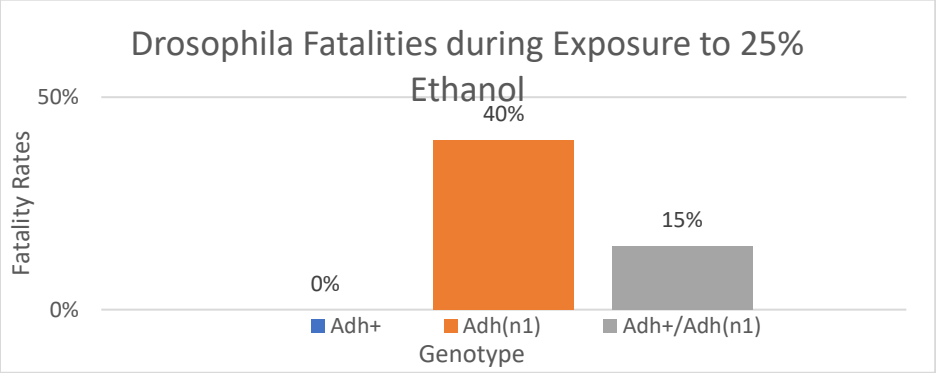
Graph 8: Drosophila m. Increased Ethanol Sensitivity during Exposure to 25% Ethanol. Column graph displaying the percent increase in ethanal sensitivity when Drosophila are exposed to a concentration of 25% ethanol alcohol. Showing that the Adh^{n1} genotype is the most sensitive, the Adh^{+} genotype is the least sensitive, and that the Adh^{+} /Adh^{n1} genotype is intermediately sensitive to ethanol at this concentration.

Increased ethanol sensitivity measures the change in height between the *Drosophila*'s exposure to the control (water) and exposure to ethanol alcohol. At the concentration of 25% ethanol alcohol, the Adh^{n1} population was impacted the greatest, experiencing a 586400% increase in ethanol sensitivity. The Adh^{+} population was the least affected experiencing a 3179% increase in ethanol sensitivity. The Adh^{+}/Adh^{n1} population was intermediately affected, experiencing a 72579% increase in ethanol sensitivity.



Graph 9: Drosophila m. Increased Ethanol Sensitivity during Exposure to Ethanol. Column graph displaying the percent increase in ethanal sensitivity when Drosophila are exposed to concentrations of 0%, 5%, 15%, and 25% ethanol alcohol. Showing that the Adh^{n1} genotype is the most sensitive, the Adh^{+} genotype is the least sensitive, and that the Adh^{+} /Adh^{n1} genotype is intermediately sensitive to ethanol alcohol.

Increased ethanol sensitivity measures the change in height between the *Drosophila*'s exposure to the control (water) and exposure to ethanol alcohol. Upon exposure to varying concentrations of ethanol alcohol, the Adh^{n1} population was the most affected, the Adh^{+} population was the least affected, and the Adh^{+} /Adh^{n1} population was intermediately affected. The R^2 values were calculated through linear regression and explain what degree of change in one variable is the result of the other variable. For example, the Adh^{n1} population has a r^2 value of 0.7599, this means that 75.99% of the change in ethanol sensitivity can be explained by the change in ethanol concentration.



Graph 10: Drosophila Fatalities during Exposure to 25% Ethanol. Column graph displaying the fatality rate when Drosophila are exposed to a concentration of 25% ethanal alcohol. Showing that the Adh^{n1} genotype experiences the highest fatality rate, Adh^{+} genotype experiences the lowest fatality rate, and that the Adh^{+} /Adh^{n1} genotype experiences intermediate fatality rates.

Upon exposure to a concentration of 25% ethanol alcohol there were several recorded fatalities. Eight of the twenty Adh^{n1} *Drosophila* died, experiencing a fatality rate of 40%. Three of the twenty Adh^{+}/Adh^{n1} *Drosophila* died, experiencing a fatality rate of 15%. No members of the Adh^{+} population died, and thus experienced a fatality rate of 0%.

Alcohol Metabolism and Inheritance of Methyl-Induced Mutations

Ethanol Detoxification by Drosophila Genotype			
Ethanol Alcohol Concentration	**Adh+**	**Adh^(n1)**	**Adh+/Adh^(n1)**
5%	-24.15%	0%	25.54%
15%	93.97%	0%	87.07%
25%	99.46%	0%	87.62%

Figure ii: Ethanol Detoxification by Drosophila Genotype. Displaying the amount of ethanol alcohol metabolized and detoxified via the alcohol dehydrogenase pathway upon exposure to concentrations of 5%, 15%, and 25% ethanol alcohol. Showing that the Adh^{n1} genotype detoxifies no ethanol alcohol, the Adh^{+} genotype detoxifies almost all ethanol alcohol, and that the Adh^{+} /Adh^{n1} genotype detoxifies the majority of ethanol alcohol.

To determine alcohol dehydrogenase activity, alcohol metabolism was determined using the previously calculated ethanol sensitivities.

	Adh+	Adh^(n1)	Adh+/Adh^(n1)
Alcohol Metabolism	96.72%	0%	87.35%

Figure iiiv: Alcohol Metabolism averages for the Adh^{n1}, Adh^{+}, and Adh^{+} /Adh^{n1} Drosophila genotypes. Alcohol detoxification at a concentration of 5% ethanol alcohol was excluded from these calculations due to outliers in data.

The alcohol metabolism, which is the activity of the alcohol dehydrogenase polypeptide for the Adh^{+}, Adh^{n1}, and Adh^{+} /Adh^{n1} *Drosophila* were calculated as 96.72%, 0%, and 87.35% respectively.

Ethanol Alcohol Concentration	Expected Detoxification e	Observed Detoxification o	e-o	(e-o)^2	[(e-o)^2]/e
5%	-12.075	25.54	-13.465	181.306	-15.015
15%	46.985	87.06928	-40.084	1606.727	32.1965
25%	49.7285	87.6229	-37.8944	1435.986	28.8765
					$\sum$ 48.058

Figure iv: Chi-square calculations for the expected vs observed alcohol metabolism for Drosophila of the Adh^{+} /Adh^{n1} genotype. The $\chi^2 calc = 48.06$, at a 5% significance level for two degrees of freedom the $\chi^2 crit = 5.99$.

The Chi-Square test was performed using the data from the alcohol metabolism calculations. Where expected detoxification is half the alcohol metabolism of the Adh^{+} population, and observed detoxification is the alcohol metabolism of the Adh^{+} /Adh^{n1} population. At two degrees of freedom at a 5% significance level that the $\chi^2 crit = 5.99$, it was then calculated that $\chi^2 calc = 48.06$.

Discussion

The data collected from exposing *Drosophila* to 0% ethanol alcohol in the inebriation chambers provided information that explains the baseline behavior and motor ability in the absence of alcohol. The change in position of *Drosophila* in the inebriation chamber between exposure to water (0% ethanol) and a concentration of ethanol is measured as increased ethanol sensitivity.

Upon analysis of the 5% ethanol alcohol experiment it becomes apparent that something went wrong. The average height of the Adh^{+}/Adh^{n1} *Drosophila* is significantly higher than either of the homodimers across all time intervals. This indicates experimental error, because the heterodimer offspring lack the capability to oxidize more ethanol than the active homodimer parent. Additionally, at this concentration the Adh^{+} *Drosophila* have the greatest increase in ethanol sensitivity. This is impossible because they have the greatest capability to oxidize ethanol. There are two possible explanations for this aberrant data. The first being the more obvious, human error. It is possible that the ethanol concentration was not diluted correctly or that the solution was contaminated by an unknown chemical. The second explanation involves

the breeding behavior of *Drosophila*. *Drosophila* prefer to breed in ethanol-containing medias with a concentration below 10% ethanol.[6] Additionally, multiple studies have reported that low concentrations of ethanol lowers inhibition[7] and increased sexual arousal in *Drosophila*, often inducing courtship behaviors. Therefore, it is possible that the ethanol concentration triggered this response, resulting in the populations gravitating to the bottom of the chamber to reproduce.

However, a clear pattern emerges when *Drosophila* are exposed higher concentrations of ethanol alcohol. At concentrations of 15% and 25% ethanol the Adh^{n1} population were located at the lowest average heights, experienced the highest increase in ethanol sensitivity, and experienced the greatest number of fatalities. In contrast, the Adh^{+} population were located at the greatest average heights, experienced the least increase in ethanol sensitivity, and experienced no fatalities. The Adh^{+}/Adh^{n1} population were located at intermediate heights, experienced an intermediate increase in ethanol sensitivity, and experienced an intermediate number of fatalities.

Across the experiments all three variations of *Drosophila* experienced a degree of intoxication and consequently their height in the chamber decreased due to a loss of postural control and sedation[6]. With an r value of 0.86243 there is strong positive correlation between the concentration of ethanol alcohol and its effects on the postural control of *Drosophila*. This means that as the concentration of ethanol alcohol increases so does its deliberating effects. R^2 was calculated to be the value of 0.7448. Therefore, 74.48% of the change in ethanol sensitivities can be explained by the variation of ethanol concentrations. However, this degree of intoxication differed across the variants. To determine the statistical significance of these differences a two factor Anova test was performed at a 5% significance level. Using the average height at five-minute intervals for each allelic variation of *Drosophila* with exposure to concentrations of 5%, 15%, and 25% ethanol alcohol. Because the F-calculated value for the factor's ethanol alcohol concentration and *Drosophila* genotype are larger than their F-critical values ($166.2 > 2.7$ and $50.0 > 3.1$ respectively), it can be concluded that the factors have a significant effect on the height of the *Drosophila* in the inebriation chamber. Additionally, the F-calculated value for interaction is larger than the F-critical value ($17.6 > 2.2$). Therefore, it can conclude that ethanol alcohol concentration and *Drosophila* genotype had a combined effect on the population's height in the inebriation chamber. This is further defended because the P-value is less than the 5% significance level ($0.05 > 2.0E-12$).

Overall, *Drosophila* of the Adh^{+} allelic variation experienced the lowest degree of alcohol intoxication. Which is the result of their functional alcohol dehydrogenase polypeptides which form active homodimers capable of oxidizing 96.72% of ethanol. Whereas, *Drosophila* of the Adh^{n1} allelic variation experienced the highest degree of alcohol intoxication. Which is the result of their null alcohol dehydrogenase polypeptides, which form inactive homodimers, incapable of oxidizing ethanol. This proved to be fatal at the concentration of 25% ethanol alcohol.

Additionally, *Drosophila* of the Adh^{+}/Adh^{n1} allelic variation experienced an intermediate degree of alcohol intoxication. Which is the result of their alcohol dehydrogenase polypeptides, which form a heterodimer composed of an active and inactive subunit. While it was predicted that they would have 50% the alcohol dehydrogenase activity of the Adh^{+} allelic variation, this was tested through a Chi-Square test. For two degrees of freedom at a 5% significance level that the $\chi^2 crit = 5.99$, it was then calculated that $\chi^2 calc = 48.06$. When $\chi^2 crit < \chi^2 calc$, the prediction is rejected. Because, $5.99 < 48.06$, it can be concluded that the Adh^{+}/Adh^{n1} heterozygotes did not have 50% of the alcohol dehydrogenase activity of the Adh^{+} parent. Rather, the results of the experiments show that the heterodimer offspring can oxidize 87.35% of ethanol, which means they have 89% the Adh activity of the homodimer parent. This indicates that upon the association of the active and inactive subunits to form a heterodimer, methyl-induced alterations to the conformation of the alcohol dehydrogenase polypeptide were corrected, in turn partially restoring enzyme activity.

With all results and calculations considered it can be concluded that *Drosophila melanogaster* with the Adh^{n1} mutation experienced the greatest degree of alcohol intoxication, and that the heterozygotes experienced an intermediate degree of alcohol intoxication. Therefore, the hypothesis is accepted. This conclusion can be supported by the multitudes of publications that study alcohol dehydrogenase in *Drosophila*. Research has shown that the functional activity of alcohol dehydrogenase directly influences preference, sensitivity and tolerance to ethanol[6]. Additionally, the same study showed that homozygote Adh^{n1} *Drosophila* had a significantly lower ethanol tolerance than either homozygote Adh^{+} or heterozygote Adh^{+}/ Adh^{n1} *Drosophila*[6]. A similar study[1] examined the activity of the alcohol dehydrogenase enzyme in eight methyl-induced mutated Adh^{n} *Drosophila.* They found that when the mutated flies were bred to homozygote Adh^{+} *Drosophila*, the heterozygote offspring regained partial or complete enzyme activity[1]. To improve the experiment the 5% ethanol alcohol concentration test should be repeated, other concentrations of alcohol should be examined and a higher quantity of *Drosophila* should be used in the determinations. Additionally, to prove that *Drosophila* are indeed homozygotes or heterozygotes the DNA of the Adh fragment can be isolated and amplified through PCR.

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