Net Mineralization Rates Compared Between Tall Grass Prairie and Juniper-Invaded Site

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Abstract:

Nitrogen is one of the most essential elements for plant growth. However, plants must compete with microbes in the soil for acquisition of this nutrient. In soils with a low C:N ratio there is much more nitrogen available, thus these soils are considered more healthy. Soils with high C:N ratios have less nitrogen available for usage and are considered poor soils. The quality of soil can be measured by analyzing ammonia and nitrate levels to determine if nitrogen is readily available. With this in mind, we took samples from 2 sites, a native tall grass prairie which was predicted to have a low C:N ratio, and a juniper-invaded site which was predicated to have a high C:N ratio. We predicted the soil found in the prairie would have a much higher net mineralization rate than the soil found in the juniper area. After analyzing the soil collected from these 2 sites, it was determined there was no statistically significant difference in nitrogen levels between the two sites and thus our hypothesis was rejected.

Introduction:

Nitrogen is often times the most limiting factor for sustainable plant growth. Plants derive their nitrogen from the soil; however, they are at constant odds with microbes in the soil which also require nitrogen to live. If there is enough nitrogen in the soil for both plants and microbes to survive comfortably, there will usually be a low C:N ratio, which is indicative of high quality soil. On the other hand, a high C:N ratio is usually found in poorer quality soils in which plants have a more difficult time surviving. Soil organic matter (SOM) can be related to the C:N ratio in that the higher the SOM levels , the lower the C:N ratio. In other words, the healthier the soil, the more SOM, the

lower the C:N ratio because there is more nitrogen to go around relative to the levels of carbon. Anyone concerned with creating and maintaining a healthy soil environment should take into consideration the C:N ratio of that soil and the findings dealt with in this experiment.

The process of mineralization, which can best be understood via the "leaky pipe" model, is the decomposition of SOM to various states of nitrogen (NH_3, NO_2, etc). The leaky pipe model shows that SOM first breaks down to ammonia (NH_4^+) and then further decomposes into nitrate (NO_3^-). Along the way, NH_3, $N0_x$, N_20, and N_2 are released. For the purpose of this experiment, when searching for the net nitrogen mineralization rate (N-min) you can simply add the levels of ammonia in the soil to the levels of nitrate.

The main question we sought to answer in this lab was, do two separate sites differ in their N-min rates? This lab took place between two plots of land. One, a natural prairie which has never been cultivated has a high SOM level therefore a low C:N ratio. The second site, a juniper invaded section of land, has a low SOM level and should therefore have a high C:N ratio. Given this information, we hypothesized that the SOM from the native prairie will generate significantly different rates of N-min compared to the juniper-invaded site over a 3 week long incubation period. To further that hypothesis, we predicted that N-min rates will be higher in the prairie due to the higher SOM level and lower C:N ratio.

Methods:

Both the native tall grass prairie and juniper-invaded land are located in the Kansas Biological Reserve, 10 miles NE of Lawrence, KS. Our first 3 samples were

taken from the Rockefeller tall grass prairie, where we collected soil from the east side of the plot. Surrounding the prairie is a tract of mowed land, approximately 10m in width that functions as a barrier between the prairie and the surrounding vegetation. Prairie plant life, which consisted largely of tall grass and shrubs, varied from approximately 0.5m to 1m in height and was significantly higher towards the center of the land. The prairie is also burned every four years to remove encroaching vegetation and return nutrients to the soil. The soil in this location was very moist and fine compared to that of the juniper-invaded site

Our second set of 3 samples was taken from the juniper-invaded land, where we collected soil from the north side. The juniper-invaded land differs from the prairie in that it has been completely left alone for the past 50-60 years. At one point in time it was used for farming and agricultural purposes. When KU acquired the land it was decided it should be "let go" to determine what would happen to the land if it was left unaltered. Once maintenance on this land stopped, the junipers, which are often an indicator of poor soil quality since they can survive in soils of higher C:N ratios than many plants, began taking over. Because of this, the site was mainly composed of moderately tall junipers, shorts grasses, and weeds. The soil found at this location was very dry and clumpy compared to that of the prairie.

At the prairie, we began our sample collecting on the afternoon of August 29th, 2006 at approximately 2:30PM. We started with each group 5m away from each other forming a straight line approximately 5m in from the mowed section. Using a soil probe with a width of 2.5cm, we took a soil sample that was 5cm deep. Prior to the collection of this and all other soil samples, we removed the litter layer from the top of the soil. After

moving another 5m towards the center of the prairie, we repeated this procedure and collected another soil sample. Finally, we moved an additional 5m towards the center, collected our final sample, and placed all 3 samples into a double zip-loc bag for storage.

A similar procedure was followed at the juniper-invaded site, and each group again started 5m away from the closest group. Starting from the north edge of the site, we again collected a 5cm deep sample of soil. Moving 5m in we took our second sample, and moving an additional 5m we collected our third and final sample. When groups were confronted with a juniper tree, we angled our straight-line path in order to collect soil that was as far away from the tree roots as possible. Finally, we combined all 3 samples into another double zip-loc bag.

At approximately 4:00PM we returned from the field and set out to begin analysis of our samples. We took 3g of soil from each site and placed it into 2 separate 50mL centrifuge tubes. We labeled those tubes as PR (prairie) and JI (juniper-invaded) and called them T_1 to be set aside for incubation in the dark at 23^{o}C. Those soil samples would incubate for 21 days and then be analyzed. Next, we again took 3g of soil from each site and placed it into 2 separate 50mL centrifuge tubes. These tubes were also labeled as PR and JI, the only difference being these were our T_0 samples which would be analyzed today. We then added 15mL of 2M KCl to each of the T_0 tubes and shook vigorously for 30 minutes. Once we completed shaking the samples, we decanted the extractant (KCl with inorganic N extracted in it) into filter paper held by a funnel, which funneled the extractant into vials labeled "PR T_0" and "JI T_0."

After the 21-day incubation was complete for the T_1 samples, they were handled in a similar manner. We again added 15mL of 2M KCl to the centrifuge tubes.

However, this time we shook the tubes for 15 minutes and then allowed them to sit for 2 hours. After that process was complete, we again decanted the extractant into filter paper held by a funnel, which funneled the extractant into vials that were this time labeled as "PR T_1" and "JI T_1."

Following the decantation of all 4 samples, ammonia and nitrate levels were analyzed using a Lachat autoanalyzer. This raw data was in the form of ppm (parts per million) and needed to be converted to μgN/g soil/day (micrograms of nitrogen per grams of soil per day). To do this, we multiplied by 0.15L (amount of KCl) and then by 1000 (to convert to micrograms) and then divided by 2.49g of soil (3g of soil minus the 17% moisture content) and by 21 days. Once that conversion was completed we found the difference in levels of ammonia, nitrate, and the sum of the two (N-min rate), between T_1 and T_0. After that, we found the mean of the differences between the 2 locations and calculated the standard deviation with the help of Microsoft Excel and Cathy Collins. Using these means and deviations we created bar graphs for visual analysis as well as performing a two-tailed t-test to determine if our results were significant.

Results:

Figure 1a

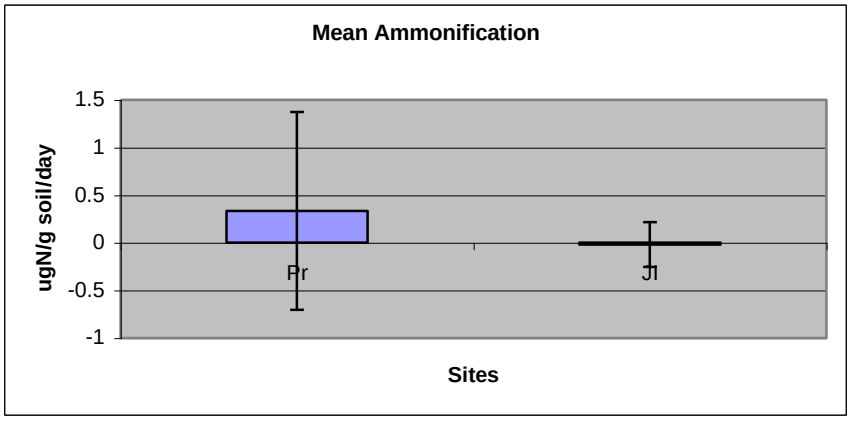

Figure 1b

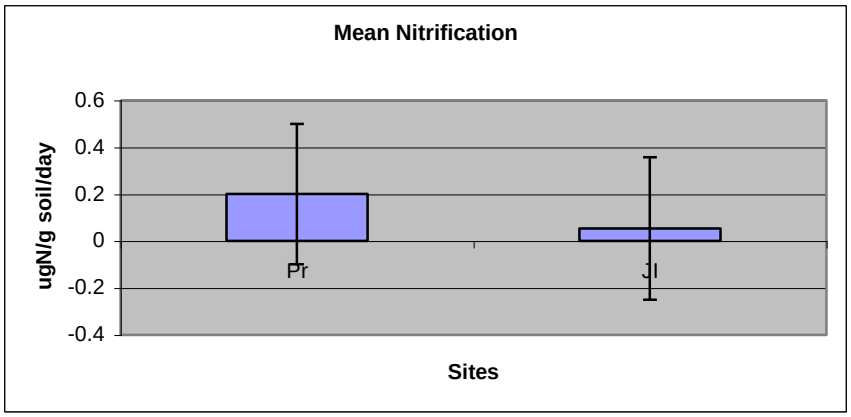

Figure 1c

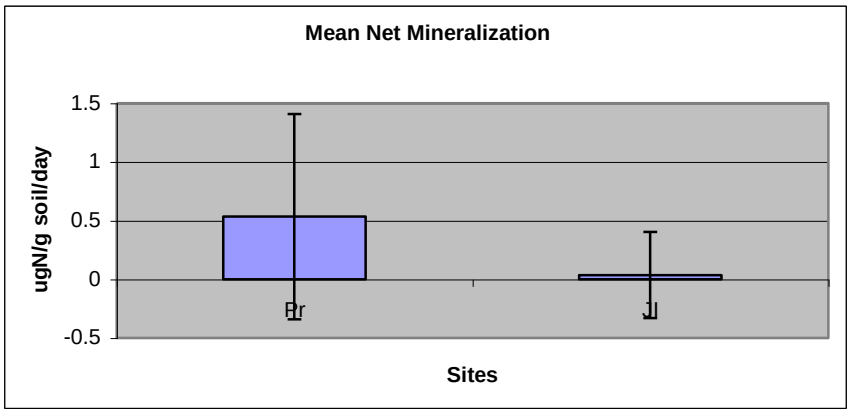

Figure 1. Graphical representation of the means of the differences with standard deviation between T_1 and T_0 of our two sites for (a) Ammonification, (b) Nitrification, and (c) Net Mineralization.

Table 1a

	PR NH4	*JI NH4*
Mean	0.33284157	-0.017621506
Variance	1.077459517	0.055224747
df	7	
t Stat	0.871238093	
P(T<=t) two-tail	0.412504836	
t Critical two-tail	2.364624251	

Table 1b

	PR NO3	*JI NO3*
Mean	0.200434391	0.053766085
Variance	0.089356434	0.09263132
df	12	
t Stat	0.909628822	
P(T<=t) two-tail	0.380925722	
t Critical two-tail	2.178812827	

Table 1c

	PR SUM	*JI SUM*
Mean	0.533275961	0.036144578
Variance	0.764291628	0.134212532
df	8	
t Stat	1.387586781	
P(T<=t) two-tail	0.202688764	
t Critical two-tail	2.306004133	

Table 1. Calculated using a "two-sample assuming unequal variances" t-Test. Showing results for (a) Ammonia statistics, (b) Nitrate statistics, and (c) Net mineralization statistics.

After visually analyzing our results using Figure 1, it appears as if there are significant differences between the prairie and juniper-invaded sites. The prairie rates of mineralization are higher on all three graphs compared to the juniper site (Figure 1). However, once the standard deviation error bars are taken into account, these apparent differences become much less significant. In all three graphs the error bars overlap thus meaning the differences between the sites could have just as easily been equal to each other and not shown the differences we see above. The only pattern that is similar

between all three graphs is that the maximum standard deviation for the JI sites was lower than that of the PR sites. Therefore, when looking solely at the graphs, it would appear as if our results were not significant primarily due to the large standard deviation.

The data from our t-tests supports this interpretation. Our t-test had an α=0.05 meaning that p-values greater than 0.05 would be determined as not significant. Table 1a shows no significant statistical difference between our ammonification rates at the two sites (T_{crit} = 2.365, P_{val} = 0.413). Table 1b also shows no significant statistical difference between our nitrification rates at the two sites (T_{crit} =2.178, P_{val} = 0.381). Finally, table 1c shows no significant statistical difference between our net mineralization rates at the two sites (T_{crit} = 2.306, P_{val} = 0.203). All three of these P values are greater than 0.05 meaning that statistically all of our results were not significant.

Discussion:

Overall, our statistical and graphical evidence did not support our predictions or hypothesis. Our P values for nitrification, ammonification, and net mineralization were all greater than 0.05 (Table 1). However, one fact that can be taken into consideration is that the variation within the prairie site was higher than the variation in the juniper site. A high variation is more indicative of a heterogeneous environment, i.e. more variation equals more differences in soil composition. On the other hand, a low variation, as seen in the juniper site, is more likely to be seen in a homogenous environment. This being said, it could be inferred that the juniper site is more homogenous because the soil is nutrient poor throughout the entire site. Contrarily, the prairie site is more heterogeneous because while it may have some nutrient poor segments, it also has nutrient rich segments, which would account for the large degree of variation.

Our findings do not mean there is no discernable difference between the prairie and the juniper sites, merely that our methods were unable to find those differences. For future experiments, taking larger sample sizes, taking samples from closer to the center of the prairie, and having more sample groups, could help find data which would prove to be more significant.

One factor that could have affected our data was the different methods of preparation our soil samples went through. The soil we analyzed for T_0 was shaken for 30 minutes and then decanted whereas the soil for T_1 was shaken for 15 minutes and then let sit for 2 hours. These different techniques could have affected the yield determined by the Lachat autoanalyzer, which in turn would have affected the means of the differences we later calculated.

However, the conclusion that must be drawn based solely on our data is that the hypothesis of finding a statistically significant difference in net mineralization between the prairie and juniper site, must be rejected. Our data, both graphical and analytical, does not support this hypothesis. Only future experiments using the changes suggested above could determine if there is in fact a significant difference between these two sites.

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September 30, 2004

Jeff Cole – Tuesday AM

Gene Mapping in Fruit Flies

Abstract

Drosophila melanogaster are easy to raise and breed and thus have been used for genetic analysis by scientists for years. Our goal for part A of this experiment was to separate *Drosophila* by sex based on observable traits. For part B, we were to analyze 1000 *Drosophila* and record the occurrence of certain traits. We were successfully able to separate the flies in part A by sexual features. All 1000 flies were analyzed and our results were recorded for part B. We also determined the order of that the order of genes was *y+ cv+ sn+* and the distances between the genes was 13.275MU from *y+* to *cv+* and 7.788MU from *cv+* to *sn+*. These results were found by interpreting the data obtained from part B of this experiment. Finally we were able to determine that the genes were sex linked based on a breeding between a wild type female and a mutant male.

Introduction

Drosophila melanogaster have been used as a tool of geneticists for decades. These flies are easy to raise, breed, and analyze for a large number of traits which makes them a favorite choice in this field. Ever since Morgan's[1] *Sex Linked Inheritance in Drosophila* essay, describing the possibility that genes could be sex linked, students have been able to replicate his experiments on their own, gaining valuable insight into the

discipline of genetics. In modern times, with the development of computers and software which were never available to Morgan and his associates, students are able to carry out the breeding of huge quantities of *Drosophila* all with the click of a button; making observation and analysis that much easier.

The basic theory behind crossing over, which plays a large part in this lab, is that during the pachytene stage of prophase I in meiosis, an exchange occurs between nonsister chromatids. As the nonsister chromatids are joined at the synaptonemal complex they are able to come in contact with each other which can result in an exchange known as crossing over. Crossing over involves cutting, at the same position, one chromatid from each chromatid pair. The subsequent reshuffling and splicing of the cut regions produces two recombinant chromatids of the original size. Recombination simply refers to the fact that the chromatids end up with genetic material that does not identically match what the cell started with at the beginning of meiosis.[2]

If a trait is sex linked it means it resides on a sex chromosome, in this case X or Y, as opposed to residing on an autosomal chromosome, which are the same regardless of sex. Tests can be done to determine if a trait is sex linked by breeding a wild type female with a mutant male and comparing those results to a breeding of a mutant female with a wild type male. If the traits of interest are sex linked, there will be a difference between the two breedings since, in this case, females have two X chromosomes and males have an X and a Y chromosome. If the traits of interest are not sex linked, the two breedings will result in exactly the same progeny.

Methods – Part A

For part A our goal was to learn the proper technique for handling and analyzing certain characteristics in *Drosophila.* Once we were comfortable finding these characteristics, our purpose was to separate the flies by sex. We started by obtaining a vial of stock flies and a bottle of fly nap to anesthetize the flies. When anesthetizing the flies we had to make certain the fly vial was on its side so the flies would not drown in their food when they become unconscious.

Once the flies had passed out we placed them on an index card (to increase visibility) under the dissection microscope. We visually analyzed such characteristics as wings, body color, body size, bristles, genitalia, sex comb, and abdomen shape. Next, we separated the flies by sex to complete this portion of the experiment.

Methods – Part B

For part B our goal was to breed and analyze 1000 *Drosophila* using the supplied computer software. The traits to be analyzed for this portion of the experiment were body color (yellow or wild type) wings (crossveinless or wild type) and bristles (singed or wild type). We began by using the software to generate a homozygous wild type male and female and a homozygous mutant male and female. For part 1 we bred the mutant female with the wild type male. We then took one female from this cross (a heterozygous female) and bred it with the original mutant male, thus performing a backcross. From this backcross we analyzed 1000 of the offspring for the above-mentioned traits.

Finally we checked for sex linkage by performing a reciprocal cross with a wild type female and a mutant male. After analyzing the F1 generation we were able to reach our conclusion as to the possibility of sex linkage in the traits in question.

Results – Part A

The flies were successfully anesthetized. The characteristics of body color, wing venation pattern, bristle type, genitalia, eye color, and abdomen size were identified and the flies were separated based on sex.

Results – Part B

y = Yellow body color / ***y+*** = Wild type body color, a grayish yellow

cv = Wings are crossveinless / ***cv+*** = Wild type wings, have crossveins

sn = Bristles are singed / ***sn+*** = Wild type bristles, not singed

Table 1:

Phenotype	**Genotype**	**Number**
Wild Type	*y+ cv+ sn+*	5896
Singed, Yellow, no veins	*y cv sn*	6068
Yellow	*y cv+ sn+*	889
Singed, Crossveinless	*y+ cv sn*	976
Singed	*y+ cv+ sn*	518
Yellow, Crossveinless	*y cv sn+*	524
Crossveinless	*y+ cv sn+*	60
Yellow, Singed	*y cv+ sn*	66
Total		**14997**

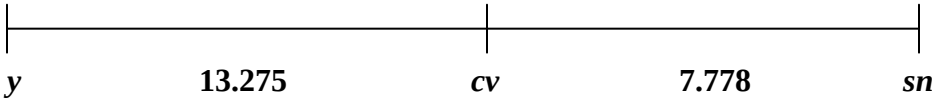
Table 2:

Parental Types	*y+ cv+ sn+ / y cv sn*
Single Recombinants	*y cv+ sn+ / y+ cv sn*
	y+ cv+ sn / y cv sn+
Double Recombinants	*y+ cv sn+ / y cv+ sn*

Table 3:

Genotype	**Number**	**Percentage**	**Map Units**
y cv+ sn+ / y+ cv sn	1991	13.275%	13.275 MU
y+ cv+ sn / y cv sn+	1168	7.788%	7.788 MU
y+ cv sn+ / y cv+ sn	122	.813%	N/A

Figure 1:



Sex Linkage Test

After breeding a wild type female with a mutant male, all F1 progeny were wild type regardless of sex.

Interference

Expected number of DCO = 0.13275 x 0.07788 = 0.01034 = **1.034%**

Observed number of DCO = **0.813%**

Coefficient of coincidence = Observed/Expected = 0.813/1.034 = **0.7863**

Interference = 1 – Coefficient of coincidence = **0.2137**

Discussion

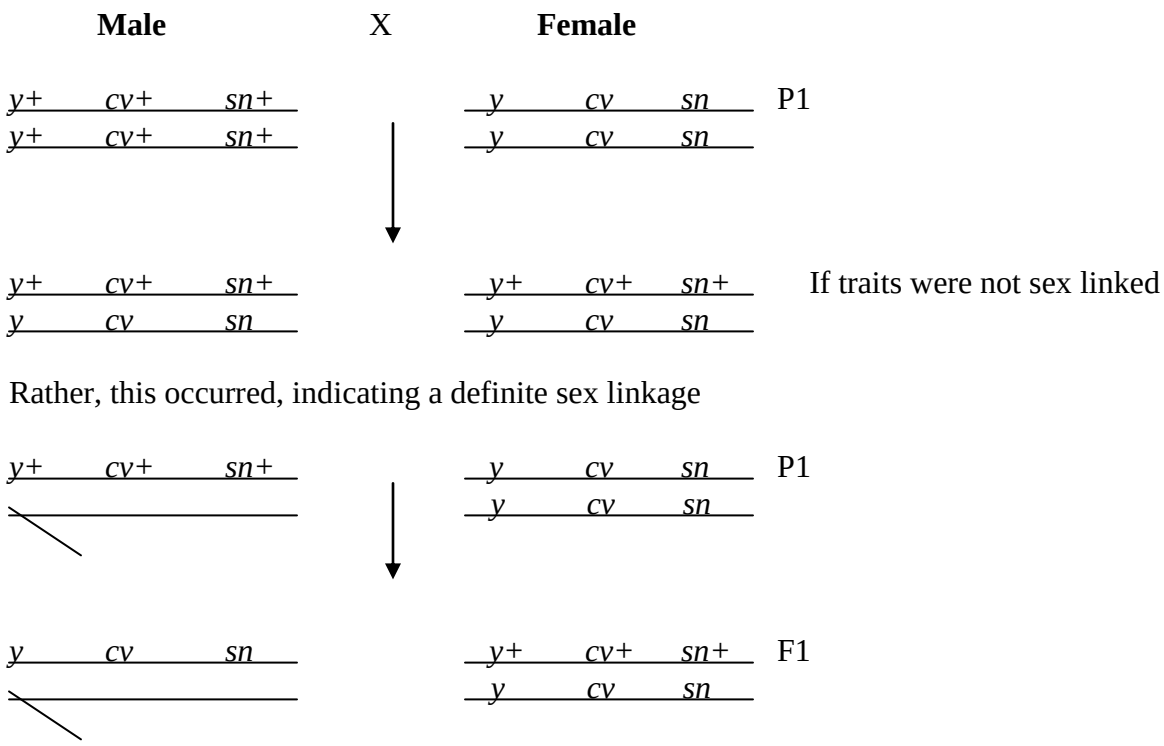
For part A, the results mean that it is possible to sort *Drosophila* by sex, based on observable traits that differ between the two genders. It is also possible to find genotypic differences between specific *Drosophila* based solely on phenotypic observations.

For part B the fact that the *y+ cv+ sn+ / y cv sn* genotypes showed up the most indicates they are the parental types. The *y cv+ sn+ / y+ cv sn* genotypes were the next most common indicating they are the single recombinants that are the furthest apart. The *y+ cv+ sn / y cv sn+* genotypes were the third most common indicating they are the single recombinants that are closest together. Finally the *y+ cv sn+ / y cv+ sn* genotypes appeared the least indicating they are the double recombinants.

On the double recombinant genotypes, *y+ cv sn+ / y cv+ sn*, because the "*cv*" gene is the "odd one out," it is the gene that is in the middle. Using this knowledge, and the percent of recombination for the other genotypes, the chromosome can be mapped out as seen in the results section.

As to sex linkage, when a mutant female was bred with a wild type male, all the female progeny were heterozygous and thus wild type and all the male progeny were mutants. When a wild type female was bred with a mutant male, all the progeny were wild type, regardless of sex. These results indicate a definite sex linkage. If the traits were not sex linked, the first breeding, between the mutant female and wild type male, would have resulted in all wild type progeny; however this did not occur (shown below).

Figure 2:



Rather, this occurred, indicating a definite sex linkage

As to interference, the fact that there was an interference of 0.2137 indicates that some factor was prohibiting all double crossovers from occurring. It could just be that we did not analyze enough progeny or it could be that something in the chromatid was preventing double crossovers from occurring all the time. Either way, the fact that there was interference proves something was impeding the double crossovers.

Conclusion

It is possible to separate *Drosophila* by sex based on phenotypic characteristics. When mapping the genes of *y* (body color), *cv* (wing veins), and *sn* (bristle type), the *cv* gene is located in the middle. Also, the distance between *y* and *cv* is 13.275 MU and the distance between *cv* and *sn* is 7.778 MU. Double recombinations occur with a frequency of 0.813%. These traits are all sex linked in that they appear only on the female *x*

chromosome and not on the male *y* chromosome. Finally, there was an interference of 0.2137 indicating some factor was prohibiting 100% of the double crossovers from occurring.

Table 1 shows the final results for all *Drosophila* breeding, thus allowing us to calculate all other factors needed for this experiment. Table 2 shows which genotypes are parental, which are single recombinants, and which are double recombinants; presenting a summarized view of the analyzed results of Table 1. Table 3 displays the transition from genotype to number of occurrences, to percent of occurrences, to distance in map units between genes. Figure 1 shows the results of Table 3 in graphic form to create a clear picture of the chromosome of interest. Finally, Figure 2 shows a pictorial explanation as to why the target genes in the *Drosophila* have to be sex linked and cannot be autosomal.

The goals for this experiment, to be able to analyze *Drosophila* based on physical characteristics, to determine the map of this specific part of the *Drosophila* chromosome of three traits, and to determine the percent of recombination for these traits, were all met and are discussed above in full.

This experiment could be taken further in the future by mapping out more *Drosophila* genes and traits. It could also be taken further by using the experience gained here in the analysis of other organisms in order to create genetic maps of their chromosomes.

Literature Cited

1. Morgan, T.H., Sex Limited Inheritance in Drosophila. *Science* Magazine, Vol. 32, 1910, p 120-122.

2. Hartwell, Leland H., et al. Genetics, From Genes to Genomes. 2nd edition. McGraw-Hill Publishers, New York, NY. p 56-57, 2004.