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Genetics Lab Report #3: Natural Selection in Drosophila

April 19, 2018

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**ABSTRACT**

In this Drosophila melanogaster flies are used to study evolution, natural selection, genetic drift, and X-linked inheritance. By tracking the phenotypes, in this case wild-type eye color (red) and mutant eye color (white), within a fly population across five generations, it was possible to look for evidence of evolution, either by natural selection or genetic drift. Over the course of several months, the changes in eye color among the population were recorded and later analyzed by determining the changes in allele frequencies. This experiment was done by taking a sample population from the population cage every two weeks, for twelve weeks. Each sample of flies was then categorized by sex and eye color. This occurred for five generations. Once all the data was gathered, allelic frequencies were calculated and further analyzed by using Chi-Square, Hardy-Weinberg Equilibrium formula, and selection coefficient formula. The data shows that some type of evolution occurred within the population, however, it is hard to explain by which means because there was a lot of experimental error. However, the data was not following the expectations of the Hardy-Weinberg Equilibrium, which states that allele frequencies will remain constant so long as evolutionary influences occur within the population. Table 1 shows that allele frequencies in wild-type males and white-eye males are inverse. As the wild-type frequency increases, the mutant one decreases, which demonstrates that natural selection is taking place. However, Chi-Square values and sm values were inconsistent because of experimental error, so it does not fully support the idea that natural selection was the cause of evolution.

Yet, even though the means of evolution, whether natural selection or genetic drift, cannot be determined because of the inconsistent data, it can still be stated that evolution did occur. The allelic frequencies and decreasing number of white eye flies are proof that evolution occurred. Therefore, the hypothesis that evolution will occur is accepted. However, the idea that evolution will occur because of natural selection is rejected, if it were according to the data obtained. Experimental error could be decreased by counting all of the flies. The fact that in one of the generations there were more than 800 flies that were not counted could have drastically changed the data, results, and conclusion.

**INTRODUCTION**

This experiment deals with population genetics, which studies genetic differences within and between populations; in other words, it is the study of the dissemination and alteration in frequency of alleles within populations, and it plays a key role in the field of evolutionary biology. Natural selection and genetic drift are important processes of evolution and will serve as evidence of evolution in this experiment. Natural selection is the process by which organisms who have traits that allow them to adjust better to specific environmental pressures, as predators, changes in climate, competition for food or mates, will survive and reproduce more than others of their kind, therefore, guaranteeing the continuation of those advantageous traits in later generations. However, in order for natural selection to occur, there has to be a heritable variation between individuals within a population and this variation has to let certain individuals to be more prosperous at surviving and reproducing than others. Genetic drift is a disparity in the comparative frequency of unlike genotypes in a small population, owing the disappearance of particular genes as individuals die or do not reproduce. Although these concepts may seem similar, genetic drift is random while natural selection yields a directional change in allele frequencies that is visible from generation to generation. In other words, the products of natural selection are consistent from population to population, while genetic drift can have different results in each population. Contrary to natural selection, genetic drift is non-directional because it results from random sampling of alleles in each different generation; its results can typically be appreciated in small populations.

For this lab, the examined population is *Drosophila melanogaster* (fruit fly). This organism was chosen because it can be easily cultured, they are low maintenance, and the time it takes for a complete new generation to be produced is two weeks. For this experiment and with this particular population, an X-linked gene that determines eye color will be examined. X-linked recessive inheritance is a type of inheritance where a mutation in a gene on the X chromosome causes the phenotype to be expressed in heterozygous males (because they only have 1 X gene) and in homozygous females (because they have 2 X genes). The 2 alleles for the X-linked gene controlling eye color are W, which is wild-type eye color (red) and w, which is the mutant (white). In males, the frequency of the white-eye allele is the number of white-eyed males divided by the total number of males. Then, to calculate the frequency of the red-eyed allele the frequency of the white-eyed alleles is subtracted to 1 because all allele frequencies sum to 1. Therefore, if a male doesn’t have white eyes it must have red eyes. To prove evolution is occurring within the population Hardy-Weinberg Equilibrium principles will be used. The Hardy-Weinberg Equilibrium model states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences.

The purpose of this lab is to observe evolution on living organisms by keeping track of the phenotypes of this specific population, *Drosophila melanogaster*. The evidence of evolution will be in the form of natural selection or genetic drift. If natural selection takes place, then throughout the 5 generations there will only be males with white eyes and the number of males with white eyes will decrease overtime.

**MATERIALS & METHODS**

To set up the experiment, the population cage and food had to be assembled. First, 30 mL of prepared flakes (food for the flies) and 30 mL of water were added to large bottles. This was the main source of nutrients for larvae’s development; however, these cultures had to be changed regularly because it produced yeast. Then, carbon monoxide gas was added to a bottle given by the TA, which had male and female flies. Once anesthetized, the flies were sorted out based on sex and eye color. The bottles of food were attached to a plastic box and the flies were added to the plastic box; the box contained 10 wild-type males (red eyes), 10 mutant males (white eyes), 10 wild-type females (red eyes), and 10 mutant females (white eyes). The box was then put in a cabinet and left there for 2 weeks.

After the two weeks passed, the old food bottles, containing larvae and pupae, were replaced with new food bottles. The old food bottles were sealed with foam plug and saved for the following week, where a full count of the G1 generation was done. To count the G1 generation, the flies in your food bottles were anesthetized and transferred to a fly pad. There, the sex and eye color of each fly was determined. This data is used to estimate the allele frequencies in the first generation. For the rest of the experiment, new food vials were placed in the population cages and population cage counts took place every two weeks for a total of 5 counts in the semester. Once all the data was gathered, specific calculations had to be done in order to analyze it.

In order to find allele frequency in males, you need to add both groups of males together (sum of white eyed males + red eyed males) and then divide the number of wild-type males by the total of males, to get the wild-type allele frequency. The same can be done to find white eyed male frequency. To find the mutant female allele frequency, the Hardy-Weinberg equation is used: p2 + 2 pq + q2 where p represents the wild-type (dominant, red) trait and q represents the mutant (recessive, white) trait. The first step is the same as in males, add up both female groups (wild-type and white eyes) and then take the number of white eyed females and divide it by the total of female flies; the answer yielded is the q2 so the square root of the difference needs to be taken. The result is the q, which is the allele frequency of white-eye females. To find the allele frequency of wild-type females, you subtract 1-q that was found. These calculations were done to find all of the allele frequencies. A Chi-Square test was then done to test the Hardy-Weinberg equilibrium for each male generation. Lastly, the estimation of the selection coefficient against the w allele in males was calculated by using the following equation: sm = (qf – q’ m)/1− (qm x qf ); for which qf is the frequency of the white allele in females in the previous generation (using HWE), q'm is the frequency of the white allele in males in the current generation, qm is the frequency of the white allele in males in the previous generation.

**RESULTS**

**Table 1: Allelic Frequencies of Males & Females Over 5 Generations**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Allele Frequencies |  | G0 | G1 | G2 | G3 | G4 | G5 |
| Males | pm | 0.5 | .44 | .83 | .99 | 1 | .  .98 |
|  | qm | 0.5 | .55 | .16 | .004 | 0 | .01 |
| Female | q^2 | .05 | .11 | .02 | 0 | 0 | 0 |
|  | qf | 0.5 | .33 | .16 | 0 | 0 | 0 |
|  | pf = (1 - q) | 0.5 | .67 | .84 | 0 | 0 | 0 |

The table above shows the variations in red-eyed and white- eyed allele frequencies of the five generations. The values represent the allelic frequencies for red-eyed flies (p) and white eyed (q) flies in the population. The trend shown in males is that the wild-type allele’s frequency becomes larger while the white-eye decreases. On the other hand, the female’s allelic frequency could not be calculated for generation’s 3-5 because there were no white-eyed females in these populations.

**Figure 1: Plot of Male Allelic Frequencies Over 5 Generations**

This plot shows the allelic frequencies ‘ data in Table 1. As stated before, there is an inverse relationship between the male’s wild-type and white eye frequencies: as one grows, the other decreases.

**Table 2: Tests of Hardy-Weinberg equilibrium in each of the 5 male generations**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Generation | Observed | Expected | (O-E)2 - E | X2 value | P value |
| G1 red eye | 17 | 19 | .21 | .42 | .5169 |
| G1 white eye | 21 | 19 | .21 |
| G2 red eye | 84 | 44.4 | 35 | 61.7 | < 0.0001 |
| G2 white eye | 17 | 55.5 | 26.7 |
| G3 red eye | 482 | 401.7 | 16 | 89.4 | < 0.0001 |
| G3 white eye | 2 | 77.4 | 73.4 |
| G4 red eye | 92 | 91.08 | .009 | .377 | . 5392 |
| G4 white eye | 0 | .368 | .368 |
| G5 red eye | 99 | 101 | . 039 | .039 | .8434 |
| G5 white eye | 2 | 0 | undefined (can’t be divided by 0) |

Table 2 shows a Chi-Squared Test for all five male generations assuming the Hardy-Weinberg Principle. The calculations above show the number of observed red-eyed verse white- eyed flies in each generation of the population, compared to the number of flies expected based on the allelic frequencies calculated in Table 1, which were used to find the χ2 and P values. The allelic frequencies from prior generations were used as the expected allele frequency for the generation following it.

**Table 3: Numerical values of the selection coefficient against the w allele in males**

|  |  |
| --- | --- |
| Generation | sm |
| G1 | -.5025 |
| G2 | -.0115 |
| G3 | .0128 |
| G4 | 0 |
| G5 | -.01 |
| Average | -.1022 |

Table 3 shows the values of selection coefficient in males against the white-eye color allele over the 5 generations. Moreover, it shows the average over all the generations. The values may not be completely correct due to the fact that the female’s generation 3-5 did not have any white eyed flies. The equation used was the following:

Equation 1: sm = (qf – q’ m)/1− (qm x qf )

qf = frequency of the white allele in females in the previous generation (using HWE) q'm = frequency of the white allele in males in the current generation

qm = frequency of the white allele in males in the previous generation

**DISCUSSION**

From the results above, it can be determined that evolution did occur; however, the data is not in accordance with the expectations of Hardy-Weinberg equilibrium because there were no white-eyed females from generations 3-5 and no white-eyed males in generation 4. This could have been an error in the experimental procedure because we did not count all the flies, for example, in one generation we only counted 255 and there were approx. 1,000; therefore, approximately 800 flies were not counted and there could have been white-eyed males and females in those. There is a big chance that this could have occurred because if there were no white-eye males in generation 4 and there were 2 in generation 5, then in those 800 flies, there could have been at least 2 white eyed flies, 1 male and 1 female because they had to mate. Figure one demonstrates these allelic frequencies and an inverse relationship can be seen. As red-eye males’ frequencies increases, white-eye males’ frequency decreases. Allele frequencies changed more rapidly towards the end of the experiment because the mutant allele was already fading off as it was passed through more generations and the sample size became smaller.

The data found in Table 2 showed the χ2 and P for all generations, some the values were significant and some weren’t but this is due to the experimental error talked before. Therefore, HWE can’t be completely accepted or rejected. Moreover, for generation 5, the χ2 value had to be calculated solely with the data from the red eye males because the (O-E)2/E could not be calculated because it was 2/0 and it can’t be divided.

The selection coefficient was very inconsistent throughout the experiment; therefore, it is difficult to assume that the red-eyed male flies were naturally selected in comparison to white-eyed male flies, even if they were. This inconsistency is because there were 0 white-eyed females in the last 3 generations and 0 white-eyed males in the fourth generation. The data with experimental errors is needed for the equation to find the selection coefficient and it could be a reason for why there was no consistency in these values. Not all of the sm values are greater than 0; therefore, if it were based solely on the data obtained, it cannot be implied that selection favored red-eyed males, even if it does. Genetic drift was not an important factor, mostly because there was great experimental error that can be used to account for the discrepancies in the data and results. For further experiments, in order to get accurate results, there needs to be a limit for fly reproduction so all flies can be counted and accounted; this would greatly reduce the experimental error that occurred in this experiment.

**REFERENCES**

http://www.dictionary.com/browse/natural-selection