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Lab Report: Natural Selection In Drosophila

Abstract:

The general goal of this lab is to set up a long-term natural selection experiment to demonstrate the evolutionary change of *Drosophila*. In order to investigate whether the evolutionary change occurred, genetic and allele frequencies needed to be estimated. Hardy-Weinberg equilibrium got tested by using chi-square test as well. The general hypothesis of this experiment is natural selection would occur in the *Drosophila* population overtimes, and the allele frequencies would be variant from one generation to the next as well. Overall, allele frequencies changed quite dramatically through the whole experiment, where the change occurred at the beginning of the experiment (from generation 2 to generation 3) was relatively more significant than the one occurred at the end of the experiment. The Hardy-Weinberg Equilibrium got rejected due to the large χ^2 value and associated small P value, which then further suggest that there was evolution occurred within the population. Lastly, Since the selection coefficient was estimated to against the white male alleles, and the value of selection coefficient in G1, G2, G4 and G5 were all greater than 0, indicating that the red-eyes males were favored during selection and further made the allele frequency of red-eyed males greater.

Introduction:

The general goal of this lab is to set up a long-term natural selection experiment to demonstrate the evolutionary change of *Drosophila*. The model organisms applied in this lab were *Drosophila*, which is one of the most critical model organisms in the field of biology. By tracking the changes in phenotypes of the *Drosophila* population, which further results in a change in the relative proportions of alleles in a population from one generation to the next, the evolutionary change will be essentially demonstrated during the experiment(Lab manual, 2018). In general, such experiment focuses on studying the distribution and change in frequency of alleles within the population, which is a significant aspect in the field of evolutionary biology. Factors like natural selection and genetic drift can be used to contribute the change in allele frequencies over time.

As a key mechanism of evolution, natural selection is the differential survival and reproduction of individuals due to differences in phenotype (Devin, 2006). Natural selection takes

places on an organism's phenotype, which determined by an organism's genotype and the environment in which the organism lives. When different organism in a population carries different versions of a gene for a particular trait, each of these versions is known as an allele (Douglas et al., 1996). Allele frequency refers to the relative frequency of an allele at a precise locus in a population (John H, 2004). Taking together, when natural selection results in a change in gene frequency of one generation to the next, evolution has occurred (lab manual, 2018). Even though some traits are driven by only one single gene, most traits are governed by the interactions of multiple genes, therefore, more than one allele must be present in population to allow the evolution to occur (lab manual, 2018). Further, the genetic composition of the population in the next generation can be created due to the different capacities of the carriers of alternate alleles. And this differential reproductive genotypes further results in a change in the relative proportions of alleles in a population from one generation to the next, which is 'the essence of evolutionary change.' (lab manual, 2018).

Other than natural selection, genetic drift is also an essential factor in contributing changes in allele frequencies over time. Compared with natural selection, the change in the frequency of an existing allele in the population is more random occurred in a genetic drift (Joanna,2011). Genetic drift happened in all populations of non-infinite size, but it usually brings most significant effects in small populations (Joanna, 2011). Sometime, several alleles including beneficial ones may be lost due to genetic drift, or rise to 100% frequency. Further, studies indicate that 'genetic drift can cause considerable effects when a population is strongly reduced in size due to the natural disaster (bottleneck effect) or when a small group splits off from the main population to found a colony (founder effect)' (Joanna, 2011).

Drosophila was the living model organism applied in this natural selection experiment to determine the evolutionary change. Two alleles for the X-linked gene controls the Drosophila eye color, and the two alleles are W (red eyes), and w (white eyes). One of the critical reason of using Drosophila as the model organism is because it's relatively more straightforward to distinguish white-eyed males from wild-type males. Since the gene is X-linked, the allele frequencies for both alleles (W and w) can be calculated directly (lab manual, 2018). Meanwhile, since

approximately 1/2 the red-eyed flies at the end of the experiment will have white alleles, by assuming Hardy-Weinberg equilibrium applies, the allele frequency of female flies can be calculated (lab manual, 2018).

In order to calculate the allele frequency, Hardy-Weinberg equilibrium was assumed applied in this lab. The Hardy-Weinberg equilibrium demonstrates that allele and genotype frequencies in a population will endure constant from generation to generation in the absence of other evolutionary influences such as mate choice, genetic drift, and mutation (Crow, 1999). Thus, under this condition, the frequency of the male white-eye allele can be calculated by using the number of white-eyed males divided by the total number of males. Further, since Hardy-Weinberg equilibrium indicates that allele frequencies sum to 1, the frequency of the male red-eyed alleles can be estimated by using one minus the frequency of the male white-eyed allele (lab manual, 2018). Selection coefficient is another term applied in this lab. In population genetics, selection coefficient is a measure of differences in fitness, which determine the change in genotype frequencies attributable to selection (John H, 2004).

In this lab, the sex of the fly was determined by using the dissecting microscope or just by the naked eye. Overall, the population and phenotype of five generations of *Drosophila* were collected and analyzed. The general hypothesis of this lab is the *Drosophila* would undergo natural selection over generations, and the allele frequencies would change from one generation to the next as well.

Materials and Methods:

First of all, the fly culture vials were created by mixing 40mL of 'fly food' and 40mL of 'fly water.' The fly culture vials provided flies with sufficient nutrients to development. In order to make the culture clean, the culture vials had to be changed out regularly.

Secondly, during the process of collecting and analyzing the phenotype of flies, carbon dioxide gas was used to anesthetize the flies. Turned the gas valves of CO₂ gas on and checked the flow rate to make sure the flow rate was appropriate for flies transferring. Held the inverted vial in one hand and CO₂ blunted needle in the other, then pushed the needle into the side of the

vial. The blunted needle was removed after all flies were fell to the low point. Then removed the cotton and poured flies onto the fly pad for examination.

At the beginning of the semester, 10 wild-type females, 10 white-eye females, 10 wild-type males, and 10 white-eye males were placed in the fly culture vials. The parents were removed after a week, and the only offspring were left in the culture. Two weeks after, the old food bottles contained larvae and pupa were removed, and new food bottles came in. The old food bottles were sealed with foam plugs and saved for the next week. After a week, the G1 adult in the old bottles was anesthetized and transferred to a fly pad. The population and phenotypes of G1 were analyzed and recorded. Similarly, new food bottles were rotated out, and flies in the bottles were counted every two weeks, and total five counts were performed through the experiment. After collecting all data of five generations, the allele and genotypic frequency were estimated.

In order to estimate phenotypic frequencies of female flies in five generations, the number of wild-type female flies and the number of white-eye female flies were needed.

For example, there were 48 red-eyed females and 16 white-eyed females in generation 1.

Thus, the genotypic frequency of the red-eyed female in generation one is

$$p^2 = 48 / (48 + 16) = 0.75$$

The genotypic frequency of the white-eyed female in generation one is

$$q^2 = 16 / (48 + 16) = 0.25$$

According to Hardy Weinberg Equilibrium, the allele frequency of white-eyed female in generation one will be

$$q = \sqrt{q^2} = 0.5$$

During the process of calculating male *Drosophila* allele frequency, there's not necessary to take the square root of the male genotypic frequency as male *Drosophila* only have one copy of the gene.

For example, there were 61 red-eyed males and 7 white-eyed males in generation 1.

Thus, the allele frequency of red-eyed males in generation one is

$$p_m = 61 / (7 + 61) = 0.897$$

The white allele in males in generation one is

$$q_m = 7 / (7 + 61) = 0.103$$

At the same time, to test Hardy-Weinberg Equilibrium, population of male flies and their allele frequencies were needed.

For example, there were 61 red-eyed males and 7 white-eyed males in generation 1, and the allele frequencies of generation 0 is 0.5 (for both red and white eyed).

Thus, the expected number of male flies assuming HWE can be calculated as

$$\text{Red} = 0.5 * (61 + 7) = 34$$

$$\text{White} = 0.5 * (61 + 7) = 34$$

Then, the chi-square test can be performed and associated P value can be further investigated.

In order to calculate the selection coefficient (s) against the white (w) allele, equation

$$S_m = (q_f - q'_m) / (1 - (q_m * q_f)) \text{ will be used.}$$

where: q_f = 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

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

For example, there were 48 red-eyed females, 16 white-eyed females, 61 red-eyed males and 7 white-eyed males in generation 1.

Thus, in this case, the q_f = frequency of the white allele in females in G0 generation = 0.5

q'_m = frequency of the white allele in males in G1 generation = $7 / (61 + 7) = 0.1$

q_m = frequency of the white allele in males in G0 generation = 0.5

$S_m = (0.5 - 0.1) / (1 - 0.5 * 0.5) = 0.53$ (In this case, S_m indicates the selection coefficient against white allele from generation 0 to 1).

Results:

After the experiment, the number of flies and their correlated eye color were observed and collected. Table 1 shows the number of red-eyed females, red-eyed males, white-eyed females, and white-eyed males *Drosophila* for five generations. In general, the populations of

flies underwent a dramatic change through five generations. Generation 1 had the least flies observed while most flies were collected in generation 4. In generation 3, there were no white-eyed females observed. In generation 2, most white-eyed females were collected. Meanwhile, generation 4 had the most white-eyed males collected while generation 1 had the least white-eyed males observed.

Table 1. The population of Red-Eyed Females, White-Eyed Females, Red-eyed Males, and White-eyed males *Drosophila* for Five Generations

	Red-eyed females	White-eyed females	Red-eyed males	White-eyed males
G0	10	10	10	10
G1	48	16	61	7
G2	62	28	51	29
G3	837	0	708	9
G4	711	1	775	57
G5	173	4	112	34

Table 1: table 1 shows the population of red-eyed females, white-eyed females, red-eyed males, and white-eyed males flies for five generations. From the table, one can tell that the population of flies underwent a dramatic change through five generations. There were no white-eyed females observed in generation 3. In generation 3, there were no white-eyed females observed. In generation 2, most white-eyed females were collected. Meanwhile, generation 4 had the most white-eyed males collected while generation 1 had the least white-eyed males observed.

In order to further investigate the evolutionary changes occurred during the experiment, allele frequencies for each of the 5 generations for both sexes were calculated by using the data collected in table 1. Table 2 exhibits the changes in red-eyed and white-eyed allele frequencies of five generations. In table 2, p^2 represents the genotypic frequency of red females flies; p_f represents the red allele frequency in females; q^2 indicates the frequency of white-eyed females; q_f represents the frequency of the white allele in females; p^m indicates the allele frequency of red-eyed males in the current generation, and q^m represents the frequency of the white allele in males in the current generation. According to the results, in males, white-eyed allele frequencies are relatively lower than red-eyed allele frequencies. In generation 3, the genotypic and allele frequency of white-eyed females are 0. In order to estimate overall allele frequency changes in

males overtime more obviously, figure 1 shows the plot of the allele frequency changes of males over time. According to the scheme, one can tell that a rapid shift in allele frequencies occurred from generation one to generation three, and the change from generation three to generation four were less significant.

Table 2.Changes of Genotypic and Allele Frequencies of Males and Females Drosophila over Five Generations

	q ² (genotypic frequency of white-eyed females)	qf (allele frequency of white-eyed females)	pf (allele frequency of red-eyed females) =1- qf	p'm (allele frequency of red-eyes males in the current generation)	q'm (allele frequency of white-eyes males in the current generation)
G0	0.25	0.5	0.5	0.5	0.5
G1	0.25	0.5	0.5	0.897	0.103
G2	0.311	0.56	0.44	0.637	0.363
G3	0	0	1	0.987	0.013
G4	0.001	0.316	0.684	0.931	0.069
G5	0.023	0.152	0.848	0.767	0.233

Table 2: Table 2 shows the changes in red-eyed and white-eyed allele frequencies of five generations. According to the table, in males, white-eyed allele frequencies are relatively lower than red-eyed allele frequencies. In generation 3, the genotypic and allele frequency of white-eyed females are 0. Figure 1. Plot of Allele Frequency Change in Males Over Time

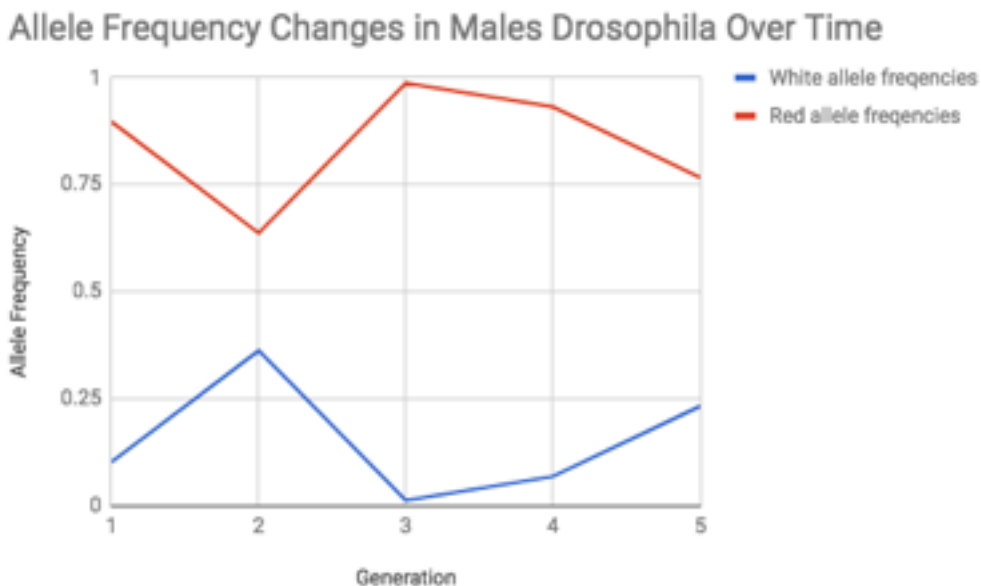


Figure 1. Figure one is the plot of allele frequency change in males overtime, which provides a more explicit estimation of the change in allele frequency. In general, a rapid change of allele frequencies occurred from generation one to generation three, and the change from generation three to generation four were less significant.

Allele frequencies were calculated and collected in table 2, and the allele frequencies of male flies were used to test for Hardy-Weinberg Equilibrium in each of the 5 generations. Chi-square test was applied to test Hardy-Weinberg Equilibrium as well. Table 3 shows the chi-square value and P values for each generation. According to the results, one can tell that the chi-square value under the assumption of Hardy-Weinberg Equilibrium were all relatively significant, and their associated p-value was extremely small.

Table 3. Chi-Square Value and Associated P Value of Five generations

	χ^2	P Value
G1	42.88	Less than 0.0001
G2	58.3	Less than 0.0001
G3	380.84	Less than 0.0001
G4	199.78	Less than 0.0001
G5	60.77	Less than 0.0001

Table 3: Table 3 shows the chi-square value and their associated P of five generations. According to the table, one can tell that the chi-square value is all relatively big, and the associated p-value is extremely small.

Lastly, assuming that selection, not drift, is the only evolutionary force performing in this experiment, the values of selection coefficient in males against the white allele over all five generations can be calculated. Table 4 indicates the value of selection coefficient against the white allele in males of five generations. According to the results, one can tell that the value of selection coefficient was positive ($s > 0$) under most of the generations (G1, G2, G3, G5). However, the value of selection coefficient performed negatively in generation 4.

Table 4: Value of Selection Coefficient Against the White Allele in Male Flies of Five Generations

	qf	q'm	qm	Selection Coefficient (s)
G1	0.5	0.103	0.5	0.529
G2	0.5	0.363	0.103	0.144
G3	0.56	0.013	0.363	0.687

G4	0	0.069	0.013	-0.013
G5	0.316	0.233	0.069	0.085

Table 4: Table 4 shows the values of selection coefficient against the white allele in males of five generations. According to the results, one can tell that the value of selection coefficient was positive ($s > 0$) under most of the generations (G1, G2, G3, G5). However, the value of selection coefficient performed negatively in generation 4.

Discussion:

Evolution refers to change in the heritable characteristics of biological populations over successive generations (Hall, 2008). Evolutionary processes play a crucial role in the field of biology as it gives rise to biodiversity at almost every level of biological organization, including the levels of species, and molecules (Hall, 2008). Thus, by performing a long-term natural selection experiment, this lab provides an opportunity to establish a further understanding of evolutionary change of living organisms. This lab focuses explicitly on studying the distribution and change in frequency of alleles within the population by using *Drosophila* as the model organism. The overall hypothesis of this experiment is over generations, natural selection or genetic drift would occur in the *Drosophila* population. Another prediction is the allele frequencies would variant from one generation to the next.

First of all, according to Figure 1, the allele frequencies changed quite dramatically through the whole experiment. The most rapid changes were observed at the beginning and the end of the experiment. However, the change of allele frequencies at the beginning of the experiment was slightly more significant compared with the end of the experiment. Especially the change occurred from generation 2 to generation 3, according to table 2, the allele frequency of white-eyed males was declined from 0.363 to 0.013 ($\Delta q = 0.013 - 0.363 = -0.35$). Another relatively rapid change occurred at the end of the experiment (from generation 4 to generation 5), and the change of allele frequency was $0.233 - 0.069 = 0.164$. By comparing the absolute value of the change of allele frequencies during this two-stage ($|-0.35| > |0.164|$), one can tell that the change occurred at the beginning of the experiment (from generation 2 to generation 3) was relatively more significant than the one happened at the end of the experiment. This is likely due to the fact that the initial population size was relatively small, which further triggered the most rapid population sizes change during the first few generations. According to table 1, the

number of flies in generation 3 was significantly increased from 80 to 717. However, the number of white-eyed males were dropped from 29 to 9, which further suggest that white-eyed alleles were being against in the population.

Secondly, Chi-square test was applied to test Hardy-Weinberg Equilibrium as the null hypothesis in the experiment as well. The Hardy-Weinberg equilibrium demonstrates that allele and genotype frequencies in a population will endure constant from generation to generation in the absence of other evolutionary influences such as mate choice, genetic drift, and mutation (Crow, 1999). According to table 3, where the χ^2 were extremely large in almost all five generations, and χ^2 associated P values were all appeared small ($p < 0.0001$ in all five generations). This results found were not accordance with the expectation of Hardy-Weinberg equilibrium; thus, Hardy-Weinberg Equilibrium got rejected as a null hypothesis. Since the Hardy-Weinberg Equilibrium got rejected which then further suggest that there was evolution occurred within the population and caused all these significant deviations.

According to table 4, one can tell that the value of selection coefficient underwent the significant change from generation to generation. At the beginning of the experiment, the value of selection coefficient decreased from generation 1 (0.529) to generation 2 (0.144); from generation 2 to generation 3 (0.687), there was a rapid increase of the value of selection coefficient. However, the value of selection coefficient significantly decreased to a negative value in generation 4 (-0.013). At the end of the experiment, it became positive again (0.085). Since the selection coefficient was estimated to against the white male alleles, and the value of selection coefficient in G1, G2, G4, and G5 were all greater than 0, indicating that the red-eyed males were favored during selection and further made the allele frequency of red-eyed males greater. Also, when the selection coefficient is strong, natural selection will get rid of recessive allele quicker. The strongest selection coefficient occurred in generation 3 (0.687), and there were no white-eyed females and relatively least number of white-eyed males presented. In generation 4, the negative value of selection coefficient means the selection starts again, which may explain why the white allele got less against in the last two generations. At the same time, sampling error could be another reason triggered the non-consistency of selection coefficient over time. In general, all

these results suggest the increasing allele frequencies toward red-eyed flies over white-eyed flies was due to natural selection.

In this experiment, there was a significant chance that genetic drift played a substantial role in changing allele frequencies within populations. First of all, studies demonstrate genetic drift usually brings most significant effects in small populations (Joanna, 2011). A finite number of flies were contributed in this lab, especially in the first and last generations, even though the allele frequencies were relatively less selected/directed when the smaller sample size had been applied in the beginning and end of the experiment compared to bigger sample sizes collected in G3 and G4. Further, genetic drift can cause considerable effects when a population is sharply reduced in size due to the natural disaster or when a small group splits off from the primary population (Joanna, 2011). During the experiment, larvae and pupae left in the fly culture could be counted as a small group split off from the primary population, further confirmed that the change over genotypic and allele frequencies over time might be caused by genetic drift.

Lastly, sampling error could be another potential causation of changes in allele frequencies. During the process of transferring flies out of fly culture, the mortality of flies could be increased, which further influenced the population size to some degree. The sex and the phenotype could be incorrectly assigned during the experiment, which might cause the inaccuracy of the results as well.

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