Natural Selection in *Drosophila melanogaster*

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Abstract

Studying whether or not a population is in Hardy-Weinberg equilibrium is an incredibly useful and popular tool in population genetics. By comparing the frequency of alleles across generations of offspring, it can reveal the evolutionary forces that are acting on the population as well as what allele is being favored. In this experiment, a population of *Drosophila melanogaster* that started with equal allele frequencies of the dominant wild-type red eye allele and the recessive white eye allele, was observed over the course of 10 weeks to determine if natural selection was acting on the population and if so, which trait was being favored. When reviewing the data collected, it became apparent that the population was not in Hardy-Weinberg equilibrium because the allele frequencies were changing from generation to generation. Additional calculations of the selection coefficient in the population further revealed that the red-eyed wild-type allele was greatly favored in this controlled population.

Introduction

In this experiment, the presence and effects of natural selection are observed in a population of *Drosophila melanogaster*. For the purpose of this study, one trait - the X-linked eye color trait – was monitored in the population over the course of five generations, starting with a parental generation that contained the eye coloration determining allele in equal frequencies. The alleles that were focused on were the dominant wild-type allele that causes a fly to have red eyes, and the recessive allele that in hemizygous males and homozygous recessive females results in white eyes. These flies were kept in a sealed population cage to prevent the loss or introduction of individuals and were under near constant conditions throughout the experiment in order to remove the potential effects of as many variables as possible. Through this experiment, the composition of each generation that exhibited either the red-eyed allele or the white-eyed allele was tracked so that statistical tests could be performed, proving quantitatively that natural selection is occurring in this population. One of the key principles of this experiment is the Hardy-Weinberg principle which states that a large population lacking the effects of natural selection, mutation, random mating, and migration, will maintain the same allele frequencies from one generation to the next (Wigginton, 2005). Furthermore, without these evolutionary pressures acting on the population the genotypic frequencies observed will be a function of the population’s allele frequencies, making it very easy to compute from one to the other (Wigginton, 2005). The ease of this computation has made it an incredibly useful, quick and popular means of determining if the genotypes observed in a population conform to those expected under Hardy-Weinberg equilibrium (Wigginton, 2005).

One of the benefits of testing if a population is in Hardy-Weinberg equilibrium is that it can help reveal what evolutionary forces are currently influencing the population. Natural selection is the process that results when one phenotype increases the fitness of an individual in a population and consequently increases the frequency of that allele when that individual produces more offspring than individuals that lack that trait. It is usually examined retrospectively in populations, like with this *D. melanogaster* experiment, and can lead to speciation over a considerable amount of time (Via, 2009). In addition to the effects of natural selection on this experiment, it is also expected that genetic drift will play a large role in affecting the allele frequencies across generations. Unlike natural selection, which is purposeful in the advancement of certain alleles because those alleles are beneficial in a way that increases fitness, genetic drift is the result of random events that may increase an individual’s ability to pass on their traits to their offspring. This occurs regardless of the advantages certain traits may offer and can result in
fluctuations in allele frequency across generations, potentially eliminating alleles all together if
the population becomes too small or a large scale event happens (Masel, 2011). Genetic drift
may be entirely luck driven but it can have a huge impact on a population under the right
conditions. By examining population trends using the Hardy-Weinberg equilibrium equation, the
mechanism of evolution for eye color in Drosophila melanogaster, whether it is natural
selection, genetic drift or both in unison, can be determined based on the changes seen in the
allele frequencies across the four generations of offspring.

Materials and Methods

Maintaining and Transferring Flies

The population of Drosophila melanogaster studied in this experiment were kept within a
shoebox sized clear plastic container. To prevent the escape of the flies through any crevices, all
of the edges, including around the food bottles, were sealed using plumber’s putty. Four food
bottles were inserted into the bottom of these cages and secured using the plumber’s putty. All
food bottles used throughout the experiment were prepared using 40mL of fly food and 40mL of
fly water. The prepared fly food was allowed to sit until the water was fully absorbed before they
were secured to the bottom of the fly cages. An additional hole cut into the bottom of the plastic
cage was used to add the initial flies to the population cage. This was sealed with a foam plug.

At the beginning of the experiment 48 total flies were added to the cage as the parental
generation. These flies included 12 red eyed males, 12 white eyed males, 12 homozygous red
eyed females and 12 homozygous white eyed females. The flies were carefully anesthetized
using carbon dioxide, to maintain their fertility, and transferred to the natural selection cage. The
flies were then allowed to mate freely with each other for the next two weeks. Every two weeks
the food bottles were removed from the cage, sealed, and then replaced with fresh food bottles.
The sealed bottles, containing the offspring in the larvae and pupae stages, were put aside for
another week before the flies inside were sexed and phenotyped for the eye color trait. This
process was repeated until four generations of offspring were raised.

Determining Phenotypes and Genotypes

Flies were counted and phenotyped the week following the one during which the food
bottles were replaced. These flies, contained within the old food bottles, were first anesthetized
using carbon dioxide and then further anesthetized using an ether tap to prevent them from
escaping while they were being counted. Flies were sorted into groups by gender based on the
appearance of the fly’s thorax. Male flies were identified as having darker, curved abdomens,
while female flies had plumper, stripped abdomen. The number of each gender that had white
eyes or red eyes as their phenotype was then recorded. Genotypes for the male flies were easily
determined because the gene for eye color is an X linked trait. Since males only have one X
chromosomes, their displayed phenotype is the same as their genotype. The only genotype that
could be determined with complete certainty for the female flies were those that displayed the
recessive white eyed phenotype. Since this trait is recessive, female flies must contain two copies
of the recessive allele in order to display that phenotype.

Data Analysis

Several techniques were used when conducting the data analysis. First, the dominant
allele frequencies were calculated for the female flies using the Hardy-Weinberg equations: \( p + q = 1 \) and \( p^2 + 2pq + q^2 = 1 \). Where \( p \) is equal to the allele frequency of the dominant allele
for red eyes (\( w^+ \)), and \( q \) is equal to the allele frequency for the recessive allele for white eyes
(\( w \)), in the first equation. The second equation uses the allele frequencies to calculate the
assumed genotypic frequencies in the female flies. Since the trait that controls eye color is X-linked, males can only have one allele determining their genotype and phenotype. For this reason, calculating the allele frequency and genotypic frequency for males was done by simply determining the ratio of one trait to the other out of the total number of male flies. Using the allele frequencies from the male flies for each generation, a chi-squared test was performed to determine if there were significant changes in allele frequency from one generation to the next. Additionally, the selection coefficient, a measurement of the reproductive fitness of the flies containing the white allele, was used to prove whether or not selection was occurring on the white allele and in which direction it was pushing it.

Results

Using the allele frequencies, the abundance of the recessive white trait and the dominant red trait can be compared across the different generations. Both the male and female flies started the experiment with an equal distribution of the two alleles being studied, as seen under the Parental G0 column. The allele frequencies were allowed to diverge naturally over the span of four generations. In the male flies a vast change in allele frequency can be observed from the parental generation to the final F4 generation, where the dominant wildtype allele is more frequent. This trend holds with the female flies across the 5 generations but to a smaller magnitude. The wild type allele is more frequent in the female flies but the difference between the wildtype and recessive allele is smaller than the difference seen in the males.

<table>
<thead>
<tr>
<th>Generation -&gt;</th>
<th>Parental G0</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red (w+)</td>
<td>0.5</td>
<td>0.563</td>
<td>0.72</td>
<td>0.859</td>
<td>0.753</td>
</tr>
<tr>
<td>White (w)</td>
<td>0.5</td>
<td>0.437</td>
<td>0.28</td>
<td>0.141</td>
<td>0.247</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red (w+)</td>
<td>0.5</td>
<td>0.525</td>
<td>0.808</td>
<td>0.635</td>
<td>0.668</td>
</tr>
<tr>
<td>White (w)</td>
<td>0.5</td>
<td>0.475</td>
<td>0.192</td>
<td>0.365</td>
<td>0.332</td>
</tr>
</tbody>
</table>

Further analysis of the divergence in allele frequency amongst the male flies from the experiment is shown below in Figure 1. The third generation of offspring showed the greatest difference between the two alleles, as well as the greatest change from the initial allele frequencies for the male flies.

Figure 1.
The greatest Chi-squared value for all the flies in a single generation was calculated to be 15.396 for the F4 generation. The F1 generation had the smallest Chi-squared value out of all the generations. The P-values used to determine statistical significance for each Chi-squared value are also displayed below in Table 2. All of the P-values, except for the first generation of offspring, were less than 0.05, the standard used to determine statistical significance.

Table 2.

<table>
<thead>
<tr>
<th>Generational Chi-Squared and P Values for Male Drosophila</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generation</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>Chi-Squared</td>
</tr>
<tr>
<td>P-Value</td>
</tr>
</tbody>
</table>

Selection Coefficients against the white allele were calculated for each generation and displayed in Table 3. The smallest selection coefficient was observed in the F3 generation, with a value of 0.054. The greatest selection coefficient was that of the F2 generation (0.246), almost triple the next highest number of 0.089.

Table 3.

<table>
<thead>
<tr>
<th>Selection Coefficient (s) Against the White Allele (w) in Male Flies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generation</td>
</tr>
<tr>
<td>q_m</td>
</tr>
<tr>
<td>q'_m</td>
</tr>
</tbody>
</table>
Discussion
This experiment showed that the population was not in Hardy-Weinberg equilibrium, contrary to expectations. This conclusion can be drawn based on several of the calculations performed. For starters, if the allele frequencies are compared from generation to generation for both the male and female flies, it can be clearly seen that they differ for each generation. If the population was in Hardy-Weinberg equilibrium, there would be no change in allele frequency. The Chi-squared tests performed further supports this idea. All but one of the four generations of offspring had a calculated Chi-squared value greater than 3.84, the Chi-squared critical value needed to be considered significant when the p-value is 0.05. This allows the null hypothesis, that the generation is in Hardy-Weinberg equilibrium, to be rejected, indicating that an outside force is acting on the population that is causing the allele frequencies to change. The variables that could have affected the population could have been any combination of the Hardy-Weinberg conditions: natural selection, random mating, small population size, or mutation.

The most rapid changes in allele frequency were observed within the first two generations of offspring. The natural selection pressures acting on this population caused the rapid change in allele frequency. These pressures are quantified in this study as the selection coefficient against the white recessive allele. Throughout the experiment, the selection coefficient varied greatly, though it always remained in favor of the dominant red eyed allele. The selection coefficient varied as much as it did because of the effects of genetic drift. Though the red eyed allele was being favored, through events of random chance flies containing the white allele could have had greater reproductive success, affecting the allele frequencies in the following generation. Without the effects of genetic drift, the selection coefficient could have been expected to remain consistent until the least favored allele was no longer present in the population, at which point the selection coefficient for the more favorable allele would become zero.

The fluctuations in the selection coefficient shows the influence that genetic drift played on the population. From the allele frequencies alone, it can be concluded that this population is not in Hardy-Weinberg equilibrium; however with analysis like the selection coefficient, explanations as to why these population trends existed. The change in allele frequency can most likely be attributed to genetic drift and natural selection, especially since many other variables like food availability and cage condition was kept as consistent as possible.

| qf | 0.5 | 0.475 | 0.192 | 0.332 | - |
| Selection Coefficient (s) | 0.084 | 0.246 | 0.054 | 0.089 | 0.11825 |

Works Cited