An investigation of the fitness and strength of selection on the white-eye mutation of *Drosophila melanogaster* in two population sizes under light and dark treatments over three generations

Image Source: http://diogenesii.files.wordpress.com/2010/07/lewis-16.gif

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ABSTRACT

Drosophila melanogaster, the common fruit fly, is known to have an observable phenotype of white eyes that is caused by the *white* X-linked recessive mutation. In this experiment, finite small and large populations of *Drosophila* were established and observed from the parental (P) generation to F_3 generation under light and dark conditions. Using *Drosophila*, two of the four major evolutionary forces, the natural selection acting on the *white* mutation and genetic drift, were examined. Two assumptions were made: immigration was closed and mutation was negligible in this experiment. All parental populations had the maximum heterozygosity value of 0.5 at the *white* locus. The results indicated that in both small and large populations, declines in heterozygosity and the frequency of *white* allele were observed under the light and dark treatments after three complete generations. In addition, the variance of the frequency of white allele increased over the generations. The declines in both heterozygosity values and frequency of *white* allele may be explained by the directional effect of selection that reduced genetic variation and acted against the white mutation. Genetic drift may have affected the fluctuations of allele frequencies due to random sampling errors. This experiment indicated that evolution occurs through changes in gene frequencies driven by factors such as selection and genetic drift.

INTRODUCTION

Evolution occurs through the process of changes in inherited phenotypes of a population of organisms from one generation to the next. Though changes between generations are relatively minor, differences accumulate with each subsequent generation and can, over time, cause substantial changes in the organisms. Inherited traits come from the genes that are passed on to offspring during reproduction. Mutations in genes can produce new or altered traits in individuals, resulting in the appearance of heritable differences between organisms. In species that reproduce sexually, new combinations of genes are produced by genetic recombination, which can increase the variation in traits between organisms. Evolution occurs when these heritable differences become more common or rare in a population, through four major forces: selection, gene flow, mutation and genetic drift.

Drosophila melanogaster is a dipteran insect known as the common fruit fly. It has a holometabolous lifecyle and a complete metamorphosis from the larva to adult stage. *Drosophila* has a relatively short generation time and lifecycle. It is inexpensive to culture and the sexes are easily distinguished in the adults. These advantages confirmed *Drosophila* as an excellent model species to investigate the evolution of various population sizes and treatments (Chippindale *et al.* 2008). In addition, the *Drosophila* system has been extensively studied by researchers, and the conditions of evolution for the populations can be replicated and manipulated to examine a particular aspect of the evolutionary forces.

In this experiment, two assumptions were considered. Gene flow was completely closed and mutation was negligible. Populations were assumed to be influenced and

acted on by only two major forces, the non-random natural selection and random genetic drift. For any given diallelic system at a locus, the frequencies of the two alleles will depend on the strength of selection on an allele in different population sizes, and the chance for drift via random sampling errors. While evolution by natural selection acts on fitness that is heritable, genetic drift has no impact on fitness of the organisms. However, both are equally important in regards to producing evolutionary changes over the generations.

The focus of the experiment was on the recessive and X-linked white mutation allele that produces observable white eyes in *Drosophila*. This particular trait is always expressed in males if they carry only one copy of the white allele. Like other mutants, the white mutation creates a fitness cost. It causes effective blindness and slower development in *Drosophila.* However, the white-eyed flies are more vigorous. The experimental populations were set up with an equal allele frequencies at the diallelic locus ($p=0.5$, $q=0.5$) at the parental stage and a maximum heterozygosity value of 0.5. For each subsequent generation, the progenies are counted to assess the viability of the white allele on fitness under two selective conditions for two different population sizes, small and large. In the light, natural selection against the white-eyed phenotype is likely to be stronger, while in the dark, selection tends to be weaker. Over the generations, the white allele frequency and the overall heterozygosity would likely to decrease in both the light and dark treatments.

In this study, small and large populations of *Drosophila melanogaster* were established to measure fitness under various conditions. The purpose of this study was to examine the changes in the gene frequency of the white allele in *Drosophila* over three

complete generations and the two evolutionary forces, selection and genetic drift, in different sized populations under light and dark treatments.

METHODS AND MATERIALS

In the parental generation, eight virgin heterozygous (+/w) females were added into each of the 20 vials containing eight wildtype and eight white-eyed males. A small amount of yeast was placed at the bottom of the vials to stimulate oviposition. Randomly, ten vials were selected to be light-proofed using construction papers, while the other ten vials are light vials. When all the vials were prepared and positioned in the incubator. After 48 hours, the eggs for F1 generation have been laid and the adult flies were discarded. The eggs were allowed to develop into adults. To start the F2 generation, the vials were gently tapped down and F1 adult flies were transferred to empty vials. The adults were then given a light dose of FlyNap. Upon the FlyNap, the flies scored and recorded to compile the F1 generation data. To set up the F2 generation, eight females were randomly removed from each population of the five light and five dark vials and placed in fresh vials. This composed the F2 small populations. For large population, all the flies were mixed together and collected randomly eight females into each of five light vials and five dark vials. The same protocol was followed to propagate twelve populations for F3 generation. The phenotypes were recorded at each generation.

RESULTS

The allele frequencies changed in both the dark and light treatments. In small populations of light treatment, the *white* frequency (q) decreased from 0.5 in the parental

generation to 0.23 in F3, whereas in the dark, *q* increased from 0.5 to 0.533. In large populations, white frequency decreased from 0.5 to 0 and 0.5 to 0.271 in the light and dark conditions respectively. Thus, the *white* allele frequencies overall changed more rapidly in the light than in the dark over the three generations in two population sizes. The *q* increased in dark while decreased in light for small populations, and the *q* decreased in both light and dark treatments in large populations. Figure 1 and Table 1 describe and quantify these results. The variances did change and increased over the generations in both light and dark conditions. There was a significant difference in the mean variances of *q* between the light and dark treatment over three generations (two-tailed, paired t-test, p=0.062911). The variances in the mean *white* allele frequency was significantly greater in the light treatment (one-tailed, paired t-test, p=0.001587) and greater in the dark treatment (one-tailed, paired t-test, p=0.000256) than in the parental generation. The mean difference between the two treatments was calculated to be 0.0686 ± 0.01159. Compared to the group data, the overall section data indicated a decline in *q* over three generations in the small and large populations under both light and dark treatments, whereas the group data showed the same trend but an increase in *q* for small populations in dark treatment. Both the group and section data indicated increases in variance of the white allele frequency in light and dark treatments across all populations. The observed differences could be expected from random practical sampling errors and the impact of this on small populations. Other theoretical reasons to expect these differences could be natural selection and possibly an increased fitness for the *white* allele due to a higher physical aggressiveness of *Drosophila* in the dark. In light and

large population, the *q* approached 0 at F3 generation and this fixation could be expected from high selection magnitude and genetic drift forces.

The white allele disappeared more rapidly in the light treatment of both small and large populations compared to the dark treatment. Figure 2 and Table 4 describes and quantifies the results. The final mean *q* for the light condition was 0.252 compared to 0.399 in the dark condition for the small populations and 0.265 compared to 0.362 in large populations over the three generations. In addition, it was found that the final mean *q* was significantly greater in the dark than in the light treatments of large populations (one-tailed, paired t-test, p=0.068181), while the final mean *q* was not significantly greater in the dark than in the light for small populations (one-tailed, paired t-test, p=0.024351). A one-tailed test was used because of an *a priori* expectation. Differences were expected that the final mean *q* in both populations were greater in the light than dark treatment, but this difference was not observed in the small populations. This might be explained by possibly sampling errors and genetic drift that can have a stronger impact on small populations.

The variance in the mean frequency of *white* allele increased over time for all sized populations and in both the light and dark treatments (Figure 3). The mean variances in q progressively increased in the small populations under light and dark treatments. The mean variance of q in the dark and large population increased until F2 and then decreased in F3, but the mean variance increased over the generations (Table 3). In addition, the light and large population indicated a fluctuation in the change of mean variance of q over the three generations. An increase in the mean variance was observed during F1 and then followed by a decrease in F2 and an increase in F3. It was found that there were

significant differences between the light and dark treatments in the final mean variances of q for the small populations (two-tailed, paired t-test, p=0.437069) and for the large populations (two-tailed, paired t-test, p=0.057871). Furthermore, it was found that the final mean variances at F3 in the light (one-tailed, paired t-test, p=0.001434) and dark treatment (one-tailed, paired t-test, p=0.001879) were significantly greater or equal to the parental variance. Over time, the mean variances in q increased and this pattern was observed in the light and dark treatments of large and small populations.

The heterozygosity values decreased over three generations for both light and dark treatments in the small and large populations. Table 4 quantifies the heterozygosity results. The decline in heterozygosity was more rapid in the light than in the dark for both large and small populations (Figure 4). The heterozyosity values decreased from 0.5 to 0.377 in small populations and 0.5 to 0.389 in large populations under light conditions. In dark treatments, heterozygosity was reduced from 0.5 to 0.480 and 0.5 to 0.462 in small and large populations respectively. It was found that the F3 heterozygosity was significantly less than the parental or initial heterozygosity of 0.5 in the light treatment (one-tailed, paired t-test, p=0.00445) and in the dark treatment (one-tailed, paired t-test, p=0.002441). The pattern was found to be the same in the light and dark treatments. Moreover, the heterozygosity at F3 was found to be significantly greater in the small population under dark than light treatment (one-tailed, paired t-test, p=0.142552), and greater in the large population under dark than light treatment (one-tailed, paired t-test, p=0.249207). A one-tailed t-test was used, because of *a priori* expectation of an increase or decrease in the heterozygosity over time in both the light and dark treatments. One-

tailed test was supplemented to determine the direction of heterozygosity change over three generations in both treatments.

Wright-Fisher

The Wright-Fisher expectations for the variance in the F3 generation for each of the four treatments are shown in Table 5. Compared to the class' mean variance values, variances in all four treatments were larger than the expected Wright-Fisher variances. From the comparative analysis of difference in variances between the four treatments, there was a 51.3% increase in observed variance compared to the expected variance in small population under light treatment and a 140.9% increase under the dark treatment. In the large population, the percent increase in variance under light treatment was 267.9% and 264.3% under dark treatment. The increase in variance is observed to be higher in the large populations than in the small populations comparatively. Thus, the class' mean observed variances did compare favourably for small populations in both dark and light conditions than in the large populations under light and dark.

DISCUSSION

In the present study, the mean frequencies of the mutant *white* allele in *Drosophila* were observed to have decreased more rapidly in the light than in the dark treatment of both the small and large populations over the three generations. The mean variances over time increased across all populations and treatments. Heterozygosity was found to have decreased from the parental maximum heterozygosity of 0.5 in large and small populations under the light and dark environment. The heterozygosity values observed in the dark treatment were higher than those observed in the light treatment across two

population sizes. These observations could be explained by the physiological differences between the mutant and wildtype flies in fitness and survival and departures from random mating, in addition to the two evolutionary forces, the non-random natural selection and the random genetic drift.

The change in allele frequency of the white-eyed allele could be due to selection on the relative fitness of the two phenotypes of Drosophila. The mean white frequency declined more rapidly in the light treatment than in the dark for large and small populations. According to Geer and Green (1962), a direct relationship was found between the density of eye pigmentation and successful mating: the greater the mating success, the greater the density of eye pigmentation. This indicated a substantial physiological advantages and increased fitness for the wildtype compared to mutants. An increased and heritable fitness of the wildtype red-eye allele would be favoured by natural selection in the light, while the white-eye allele would be strongly unfavoured and selected against in the light setting. Thus a more rapid decline in white frequency can be expected. However, this mating advantage was not shown to be suffered by the mutant flies when matings were allowed in total darkness (Connolly *et al.* 1969). A difference in visual ability or visual discrimination rather than vigor or other factors might account for the advantage of wildtype male in mating under the light environment. Therefore, a greater decline in the *white* allele frequency might be explained by the differences in fitness due to physiology of the mutant white eyes.

In addition, non-random matings might provide explanations for the declines in heterozygosity values. Mating was likely to be more random in the dark regardless of the eye colours of the competing males than in the dark environment (Geer and Green 1962).

In other words, more discrimination towards the male flies was probably present in the light than dark. This could explain that the declines in heterozygosity and in the mean *white* allele frequency over the generations were smaller in the dark than in the light environment. In fact, deviation from non-random mating, such as assortative mating, can have dramatic effects on the heterozygosity of subsequent generation in reducing the heterozygosity value by half per generation (Chippindale *et al.* 2008). Although assortative mating itself does not cause changes in allele frequencies, but when coupled with non-random natural selection, allele frequencies could rapidly alter. Consequently, th observed pattern of declines in heterozygosity across all treatments and population might be due to the directional selection from assortative mating.

Although physiological differences in phenotype contribute to the relative fitness and mating success in males, genetic drift can produce dramatic random effects on evolution through chance events known as sampling errors. Among the nonselective mechanisms of evolution, genetic drifts in finite populations are absolutely random and are simply blind differential reproductive success that does not lead to adaptations (Freeman and Herron 2007). A similar study on Drosophila conducted by Peter Buri (1956) revealed and confirmed that alleles did become fixed or completely lost while the heterozygosity declined under genetic drift. The fixation of one allele was observed in this experiment under the group data. The *white* allele frequency was entirely lost and the wildtype allele frequency reached 1 in the large population of light treatment at F3. This observation could be an outlier because it is unlikely to observe fixation in a diallelic locus for a large population size. However, although genetic drift is more rapid in small populations and slower in large populations, given sufficient time, genetic drift can

produce dramatic alterations in allele frequencies in large-sized populations (Freeman and Herron 2007)

The mean variances were observed to have increased in both the large and small populations under the light and dark environment. Over time, the changes in variance in a particular allele frequency depend on the population size, the number of generations elapsed and the initial allele frequencies (Chippindale *et al.* 2008). According to the Wright-Fisher model, as the effective population size (N) increases, the variance changes slowly in finite populations and it would not change at all in infinite population (Chippindale *et al.* 2008). This prediction was observed in the results where the final mean variances in the small populations were greater than that of the large populations in both treatments. The changes in variances over the generations were expected due to genetic drift. The differences between the expected and observed mean variances might be due to sampling errors, but they were considerably close, especially in small populations.

In this experiment, non-random and random evolutionary forces were examined in *Drosophila* under different population sizes and various treatments over three generations. The heterozygosity and the mean *white* allele frequency both decreased over the generations in all populations and treatments, and were observed to be more rapid in the light treatment. The final mean variances were indicated to have increased from the parental generation. These observations could be explained by the dynamic impact of evolutionary forces such as genetic drift and natural selection and departure from non-random mating. Future researches are needed to provide further insight into other factors that might have decreased the survival of the white allele in the light environment.

APPENDIX

Table 1: The mean white allele frequencies over three generations in small and large populations under light and dark treatments.

	Light small	Dark small		Light large	Dark large
P	0.5	0.5	P	0.5	0.5
F1	0.272	0.472	F1	0.137	0.398
F2	0.23	0.533	F2	0.242	0.248
F3	0.428	0.416	F3	0	0.271

Table 2: The variance in the mean white allele frequency of small populations under light and dark treatments. (Group data)

	Light small	Dark small
P	0	0
F1	0.0931	0.0372
F2	0.113	0.0344
F3	0.107	0.0357

Table 3: The allele frequencies over three generations. No means and variances were obtained for larger populations because N=1. *q* represents the frequency of the white allele and *p* represents the frequency of the wildtype. (Group data)

Light Treatment	**Generation**	**Mean *p*All**	**VAR*p***	**Mean *q*All**	**VAR*q***	**Mean Sum of adults**
Light, small	F1	0.572	0.0931	0.428	0.0931	63.6
Light, small	F2	0.728	0.113	0.272	0.113	46.4
Light, small	F3	0.770	0.107	0.230	0.107	61.8
Dark, small	F1	0.584	0.0372	0.416	0.0372	63.8
Dark, small	F2	0.545	0.0344	0.472	0.0344	134
Dark, small	F3	0.467	0.0357	0.533	0.0357	34.4
Light, large	F1	0.863	-	0.137	-	298
Light, large	F2	0.758	-	0.242	-	878
Light, large	F3	1	-	0	-	289
Dark, large	F1	0.602	-	0.398	-	392
Dark, large	F2	0.752	-	0.248	-	609
Dark, large	F3	0.729	-	0.271	-	670

Table 4: The allele frequencies over three generations from pooled data. (Section data)

Treatment	**Generation**	**Mean pAll**	**VAR(p)**	**Mean qALL**	**VAR(q)**	**H**

Light Small	F1	0.639046	0.01587	0.360954	0.01587	0.46133
Light Small	F2	0.73182	0.033047	0.26818	0.033047	0.392519
Light Small	F3	0.748212	0.03443	0.251788	0.03443	0.376782
Light Large	F1	0.615233	0.012099	0.384767	0.012099	0.473443
Light Large	F2	0.72723	0.007063	0.27277	0.007063	0.396733
Light Large	F3	0.735382	0.017142	0.264618	0.017142	0.38919
Dark Small	F1	0.588813	0.018861	0.411187	0.018861	0.484225
Dark Small	F2	0.596147	0.035657	0.403853	0.035657	0.481512
Dark Small	F3	0.600745	0.054798	0.399255	0.054798	0.479701
Dark Large	F1	0.578119	0.008262	0.421881	0.008262	0.487794
Dark Large	F2	0.628215	0.021636	0.371785	0.021636	0.467122
Dark Large	F3	0.637243	0.016968	0.362757	0.016968	0.462329

Table 5: Comparison between the Wright-Fisher expectations for the variance vs. the observed mean variance in the F3 generation for each of the four treatments.

Treatments	**Wright-Fisher expected variance**	**Observed mean variance**	**Difference (observed – expected)**	**Percent Increase (Difference/Expected) X 100%**
Light small	0.0227471	0.03443	0.01168	51.347%
Dark small	0.0227471	0.054798	0.03205	140.897%
Light large	0.004658	0.017142	0.01248	267.926%
Dark large	0.004658	0.016968	0.01231	264.277%

(A) Light Treatment

(B) Dark Treatment:

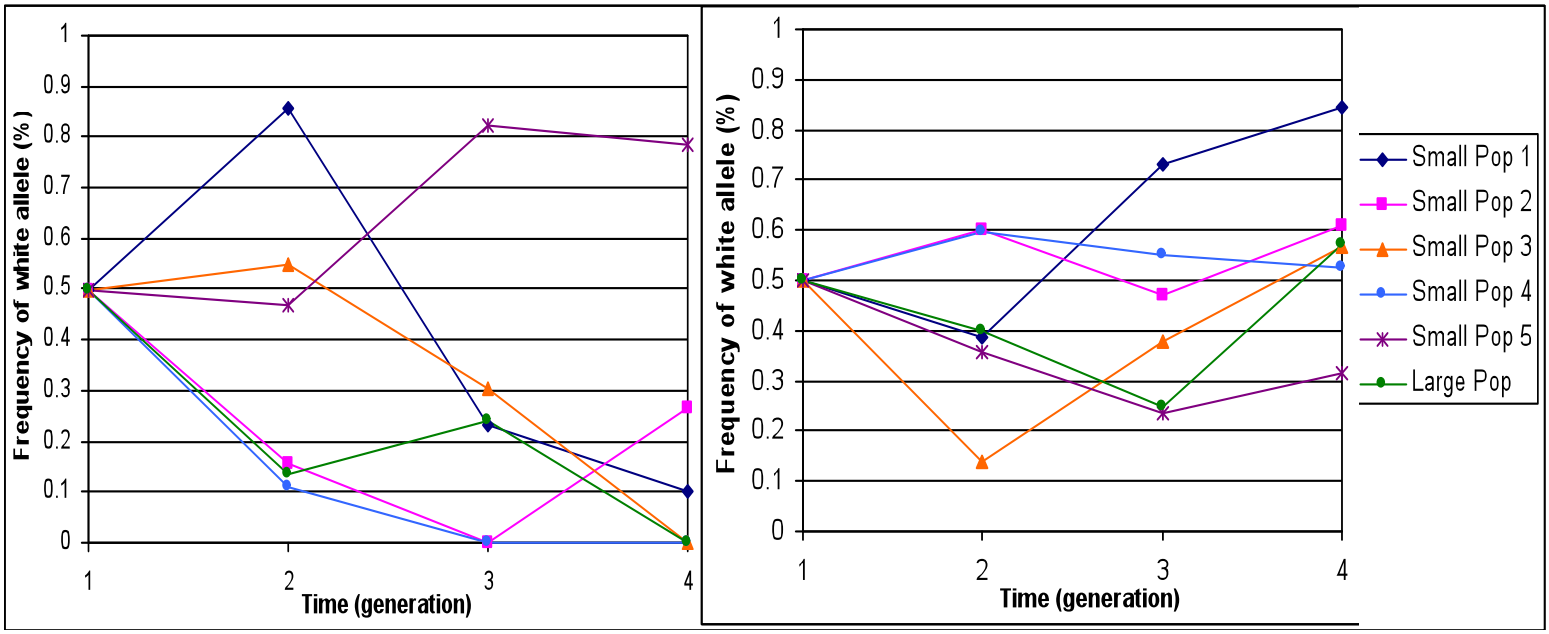

Figure 1: (a) The change in allele frequency of the white allele in *Drosophila melanogaster* from the P generation to the F_3 adults for each of the six different experimental populations in the light treatment. (b) The change in allele frequency of the white allele in *Drosophila melanogaster* from the P generation to the F_3 adults for each of the six different experimental populations in the dark treatment.

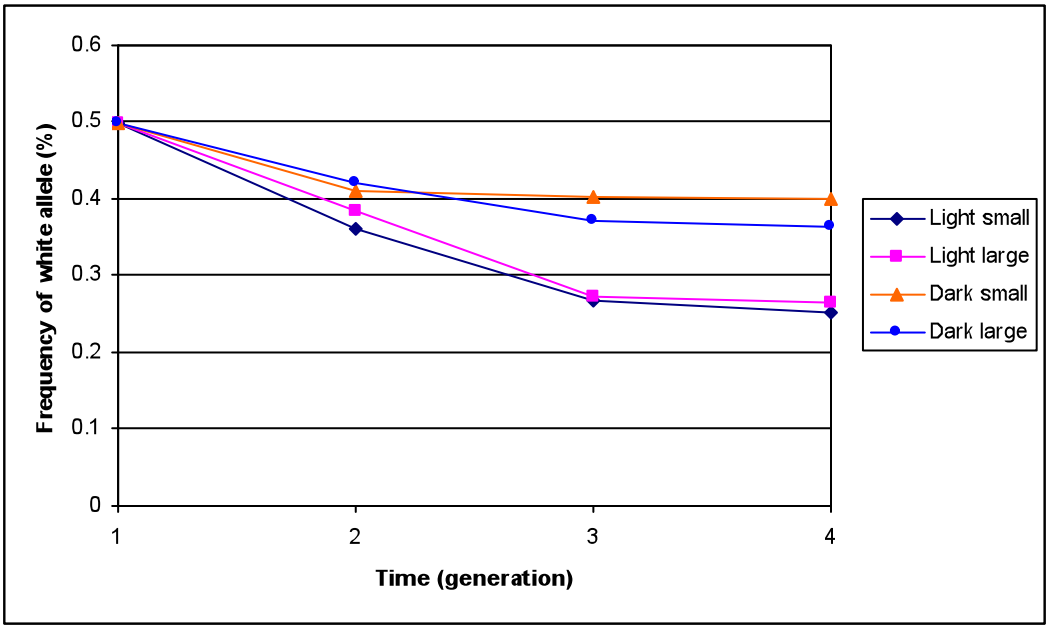

Figure 2: The average change in allele frequency of white allele from the P generation to the F_3 adults of *Drosophila melanogaster* in the light and dark treatments of two different population sizes, small and large.

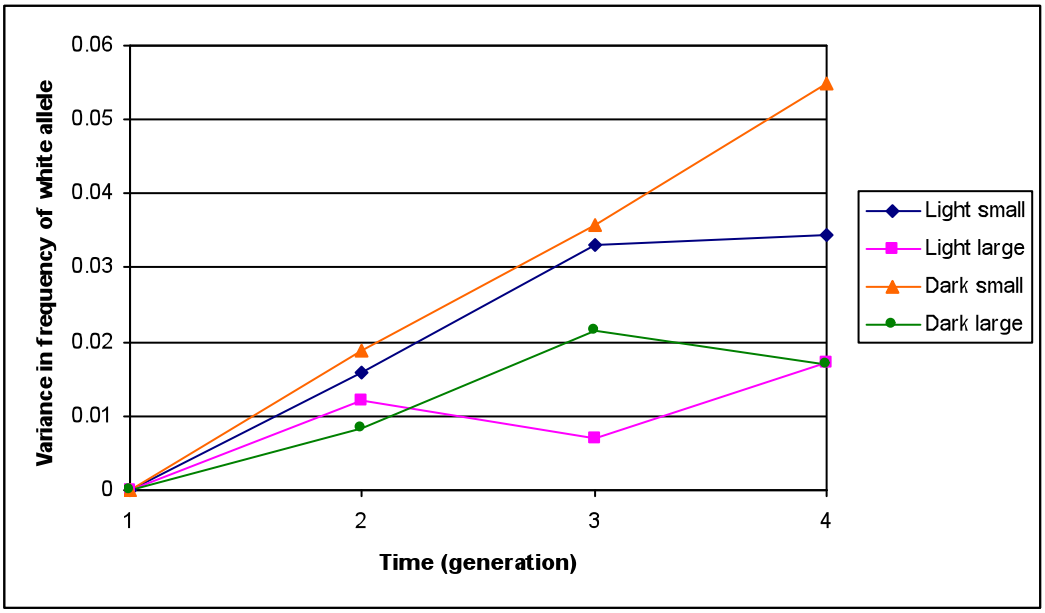

Figure 3: The variance in allele frequency of white allele from the P generation to the F_3 adults of *Drosophila melanogaster* in the light and dark treatments of two different population sizes, small and large.

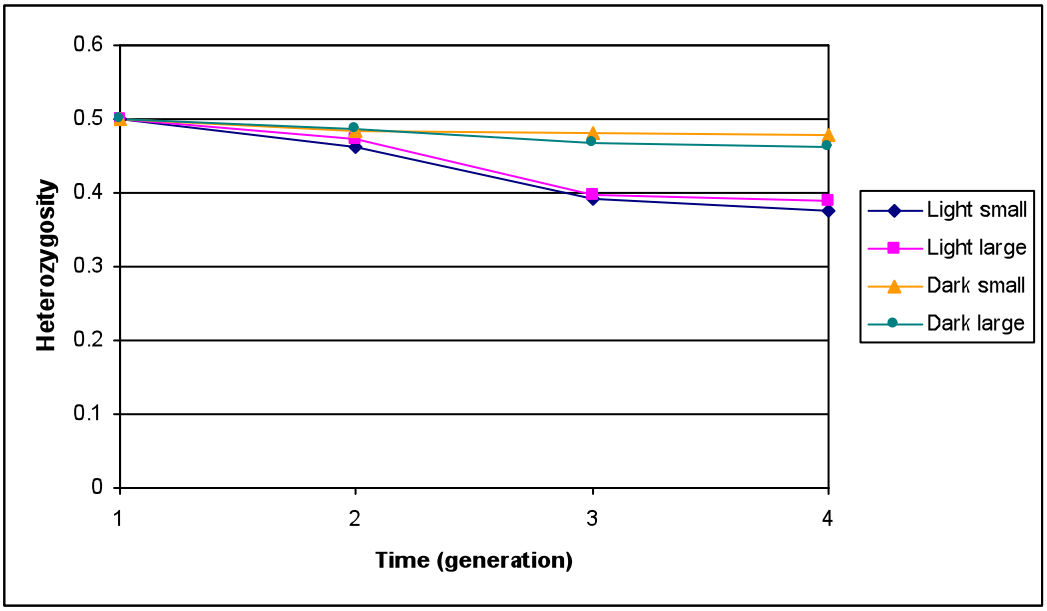

Figure 4: Figure 3: The heterozygosity value from the P generation to the F_3 adults of *Drosophila melanogaster* in the light and dark treatments of two different population sizes, small and large.

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