

Abstract:

Population genetics aims to explain how allele frequencies change over time and space. Genetic drift refers to the fluctuations in allele frequencies that are attributed to random sampling of gametes; it is most prevalent in small populations. *Drosophila melanogaster*, the fruit fly, is an insect species that has been extensively used as a model organism in genetic studies. The gene for eye color in fruit flies is sex-linked and is located on the X chromosome. Red eyed, or wild type, allele is dominant while white eye, or mutant, is recessive. The purpose of this study was to investigate the mechanisms of genetic drift and natural selection and their effects on eye color phenotype in populations of *Drosophila melanogaster* over three generations. To estimate allele frequencies for the red-eye allele, we used the *Hardy-Weinberg Equation*. We also conducted three simulations that modeled different amounts of selection on a novel allele that appears in a population. There were more fluctuations in frequency of the wild-type allele in the smaller populations than in the larger one. It was also observed that as the selection for the novel allele increased in strength, there was a faster evolutionary transition towards the advantageous trait.

Introduction: Population genetics is the study of genes in populations. It is a fascinating field of genetics that aims to measure genetic variation and explain how allele frequencies, within or between a species, change over time and space. Genetic drift refers to the fluctuations in allele frequencies that are attributed to chance and random sampling of gametes. It is an important force of evolution and its effects are most prevalent in smaller populations (Dobzhansky and Spassky 1961), usually ones below a certain threshold, or effective population size ; with fewer individuals to reproduce, the larger changes in allele frequency are more likely caused by chance rather than any other phenomenon.

Natural selection is another factor that drives evolution, in which variation among individuals is inherited and allows some individuals to be more successful at survival and reproduction than others. Unlike genetic drift which is random and non-directional, natural selection will tend to produce a directional change in allele frequencies from one generation to the next (Dobzhansky and Spassky 1961). The allele that consistently experiences greater success becomes more prevalent over time in that population. The most notable difference between the two factors is that while drift can behave differently in each population, the results of natural selection are consistent from population to population (Heil et al. 2012).

Genetic drift plays a vital role in many natural populations that are small as a result of habitat fragmentation, human interference, ecological constraints, climatic stresses, or natural disasters. By observing the collective action of these microevolutionary forces from within and across populations, we can understand their effects on genetic variation and perhaps study the consequential patterns of macroevolution, like speciation and extinction (Ragsdale 2019).

Drosophila melanogaster, the common fruit fly, is a widespread insect species that has been extensively used as a model organism in genetic studies. They develop and reproduce

rapidly and have a short life cycle, making them an ideal choice to study genetic variation and evolution over a short period of time. The gene for eye color in fruit flies is sex-linked and is located on the X chromosome. Red eyed, or wild type, allele is dominant while white eye, or mutant, allele is recessive (Reed and Reed 1949). Males have XY chromosomes and females have XX chromosomes, meaning the white eye allele must be present in males for the phenotype to be expressed (since there is only one X chromosome that dictates eye color), whereas both the X chromosomes must have the white eye allele in females in order for the phenotype to be expressed (since it is recessive and will not affect eye color in the presence of a dominant allele). As the red eye allele is dominant, we cannot distinguish between heterozygous females that carry one red and one white eye allele and homozygous females with two red eye alleles. Thus, the initial population of flies only contain homozygous (red or white-eyed) females.

The purpose of this study was to investigate the mechanisms of genetic drift and natural selection and their effects on eye color phenotype in populations of *Drosophila melanogaster* over three generations. Since the number of generations that could be included in the study were limited, a simulation using beans and dice was also conducted. The aim of the simulation was to study genetic drift and natural selection by manipulating the selection strengths of the alleles and observing the change in population over 10 rounds. We hypothesized that there would be more fluctuations in frequency of the wild-type allele in the smaller populations than in the larger one. The frequency of the red eye allele was expected to be higher in males than in females because (a) females tend to reproduce more with red-eyed males than white-eyed males (Schoonover 2020) and (b) the phenotype only depends on one X-chromosome. For the simulation, we also predicted that the allele with the stronger selection advantage would increase in frequency over time.

Methods: We first set up the initial populations (F0): a large population of 40 flies (20F, 20M) and two small populations of 8 flies each (4F, 4M). We placed the stock vials laterally on ice to temporarily immobilize the flies. We examined them under a microscope; the pattern of stripes on the abdomen helped sex the flies. The initial populations contained an equal number of flies with red and white-eyed phenotypes. As we could not distinguish between heterozygous and homozygous females, the initial population had only the latter. We transferred the flies to vials with a food medium and placed them on their side until the flies woke up. We incubated the flies at room temperature and tracked how each allele frequency changed over three generations. In week 3, we maintained a constant population size by randomly sampling 8 (and 40) flies - the progeny - to be parents for the next generation and recorded the frequency of the eye color phenotype. We repeated this in week 5, but without setting up a new generation. To estimate allele frequencies for the red-eye allele, we used the *Hardy-Weinberg Equation*. For males, the frequency, $p(m)$, was equal to the phenotypic frequency; for females, we used:

$p(f) = 1 - \sqrt{\text{frequency of white eyed females}}$. We also calculated the weighted average frequency using: $p(all) = (2p(f) + 1p(m))/3$.

For the simulation, we used beans to conduct three simulations that model different amounts of selection on an allele that appears in a population. The initial population was fixed for Bean A and Bean B was the novel allele (9A: 1B). The population was doubled at each round and the dice was used to determine the probability of an allele surviving to the next generation. We manipulated the probability of Bean B allele survival in each simulation: 50% chance in A, 66% in B, and 83% in C. Finally, we came up with a simulation D, where we added 9A and 1B to the population (to model a migration event) in round 6, randomly sampled to maintain carrying capacity, and observed the change in frequency of the alleles in the subsequent rounds.

Results: In *Small Population #1*, an increase in allele frequency in males in F1 from 0.50 to 0.63 was observed, while the frequency of females reduced significantly from 0.50 to 0.35 (Table. 1). In F2, the frequency of red-eyed females increased to around 0.70 whereas the frequency of red-eyed males remained steady at 0.63. The red-eye allele frequency for both males and females were greater than their values in the F0. The overall weighted frequency, $p(\text{all})$, of the wild-type allele decreased in F1 and increased in F2, with a final frequency of 0.68 (FIG. 1). *Small Population #2* shows a different trend. The red-eye allele frequency for both the male and female populations resist change in F1, only in females does the frequency drop slightly to 0.48 (FIG. 2). Interestingly, in F2, there is a drastic decrease in allele frequency in males, from 0.50 to 0.28, while the frequency in females remains relatively stable, only dropping to 0.43 (FIG. 2). The overall weighted frequency, $p(\text{all})$, of the wild-type allele showed a steady decline over time, with a final frequency of 0.38 (FIG. 2). The *Large Population* shows a more robust trend. In F1, wild type allele frequencies in both the male and female flies drop to 0.35 and 0.31 respectively (FIG. 3). There is a slight increase observed in F2 for frequencies in both males and females. The overall weighted frequency, $p(\text{all})$, of the wild-type allele decreased in F1 and slightly increased in F2, with a final frequency of 0.46 (FIG. 3).

The frequency of Bean A allele decreased in all three simulations over the 10 rounds, with steep declines in simulations B and C (FIG. 4). While the frequency in simulation A somewhat returned close to its initial value and the frequency in simulation B reached about 1/3 of its initial, the frequency in simulation C was driven to extinction in the last round. Simulation D had steady fluctuations in both Bean A and Bean B allele frequencies, similar to Simulation A, with the migration event causing a slight increase in Bean A in round 6. Nevertheless, the frequencies returned to their initial values by the last round (FIG. 5).

Discussion: In small population #1, the increase in red-eyed male frequency in the F1 generation (Table. 1) could be due to different fitness between the red eye and white eye allele, resulting in selection against the latter (Dobzhansky and Spassky 1961). In the F2 generation however, there was a drastic increase in red eye allele frequency in female progeny while that in males remained stable. This could be due to the elimination, or drastic reduction, of white-eyed males in the F1 generation; with few or no white-eyed males, the female flies would only produce offspring of the red-eyed males (Reed and Reed 1949). The X chromosome with the wild type allele from the male would inadvertently increase the frequency of red-eyed female progeny regardless of whether it mates with a white-eyed or red-eyed female - the allele for red eyes is dominant. According to the *Hardy-Weinberg Equilibrium*, expected frequency of red-eyed females is higher than the observed frequency of 0.38 (FIG. 1). It is unclear why, though it could be attributed to chance. The overall weighted frequency resulted in a higher red-eye allele frequency than the initial population. This could further suggest that the red eye allele has higher fitness in these conditions (Dobzhansky and Spassky 1961).

In small population #2, the red-eye allele frequency remains fairly stable for both males and females (FIG. 2) in F1; however in F2, a stark decrease in allele frequency in males is observed along with a slight decrease of that in females. Looking through the lens of sexual selection, an increase in white-eyed females in F1 could be the cause of a decrease in red-eyed males in F2 (Schoonover 2020). A higher number of white-eyed females would increase the probability of producing a white-eyed *male* offspring, regardless of which fly the female mates with. White allele is associated with poor eyesight and is detrimental for feeding and reproduction ; however, the increase in this allele frequency implies that the deterministic effect of genetic drift is highly variable, since it can increase the alleles which may have little to no

survival value (Heil et al. 2012). On the other hand, this could merely be due to the short life cycle of *Drosophila melanogaster*, limiting the flies' survival and fertility till before week 5. There is a slight decrease in overall weighted frequency, $p(\text{all})$, as it reached 0.38 (FIG. 2), a value below the initial population, surprisingly indicating an increase in fitness for the white eye allele in both male and female populations. Both small populations, albeit having the same initial population and environment, showed different trends. Adaptations in independent populations could occur via parallel changes in gene expression or fluctuating enrichment of pre-existing alleles (Dobzhansky and Spassky 1961). Replicate experiments can give disparate results, with the selection rates being unpredictable. This suggests that even with similar populations, genetic drift in small populations is merely a consequence of chance (Reed and Reed 1949).

In the large population, a sharp decrease in red eye allele frequencies was observed in F1 in both males and females with a gradual increase in F2 (FIG. 3). While the adaptive genotype may be present in an organism's DNA, relative fitness is not solely defined by its success in one environment. Rather, the level of fitness remains context-dependent on the variable conditions applying selective pressures (Heil et al. 2012). The allele frequency at F2 somewhat reverted back to the initial population, with the overall weighted frequency, $p(\text{all})$, at 0.48. Over the three generations observed in *D. melanogaster*, each generations' mean allele frequency changed, showing that each generation was evolving. A change in allele frequencies occurred in both population sizes, but the more drastic fluctuations occurred in the small populations, which is consistent with our expectations for genetic drift and the first hypothesis. However, fluctuations between allele frequencies between males and females was slightly different than predicted, perhaps due to the presence of strong selection in female flies leading to lesser red-eyed males (Reed and Reed 1949).

In simulation A (no selection), there was a gradual, slightly decreasing frequency of the Bean A allele following the introduction of the novel allele Bean B. As the selection for the novel allele increased in strength, the evolutionary transition towards the advantageous trait can be observed in simulations B and C. Simulation C emerged with the most drastic decrease in Bean A frequency, even resulting in extinction at round 10, as there was an 83% chance of being selected against. The selection of the Bean B allele reduced the frequency of Bean A, supporting the initial hypothesis. This is associated with selective sweeps, the spread of an advantageous mutation over multiple generations (Ragsdale 2019). Simulation D posed the same selection probability for Bean B as simulation A (no selection), except it consisted of a migration event occurring at round 6 - the addition of the initial population. We hypothesized an increase in Bean A frequency and a decrease in Bean B following the migration; however, the effect, although present, was negligible as the populations returned to their initial states by round 10. Even though there were changes in allele frequencies during the experiment and the simulation, the genetic diversity in the population remained stable if looked at over a longer period of time.

This study supported the hypotheses in the investigation of genetic drift; however, it has some limitations. The laboratory populations of flies, although ideal, may be already adapted to the controlled environment (Ragsdale 2019). The absence of natural stimuli like interactions with other species may diminish the generalizability. One extension to the study involves the implementation of digital organisms - computer programs capable of self-replication, mutation, and competition within a virtual environment, which could enhance the study's precision (Ragsdale 2019). Incorporating comparative studies with other fly species could offer valuable insights into the generalizability of the observed trends as well, fostering understanding of the vast evolutionary processes driven by genetic drift.

References:

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Figures and Tables:

	Small Population #1			Small Population #2			Large Population		
	p(m)	p(f)	p(all)	p(m)	p(f)	p(all)	p(m)	p(f)	p(all)
F0	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
F1	0.63	0.35	0.44	0.50	0.48	0.49	0.35	0.31	0.32
F2	0.63	0.70	0.68	0.28	0.43	0.38	0.39	0.49	0.46

Table. 1. Final frequencies of the wild type (red) allele in male and female flies as well as its overall weighted frequency in 2 small populations (N=8) and a large population (N=40) over 3 generations.

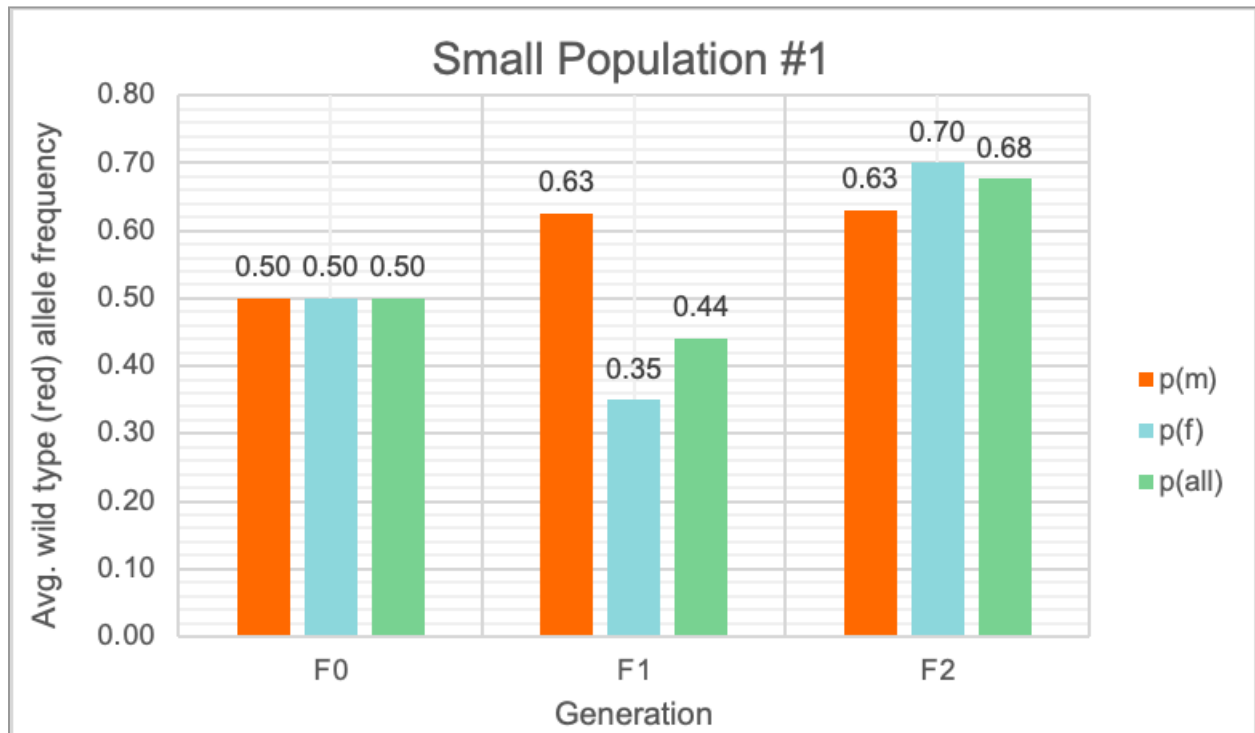


FIG. 1. Average wild type (red) allele frequency in male and female flies as well as its overall weighted frequency in small population #1 (N=8) over 3 generations.

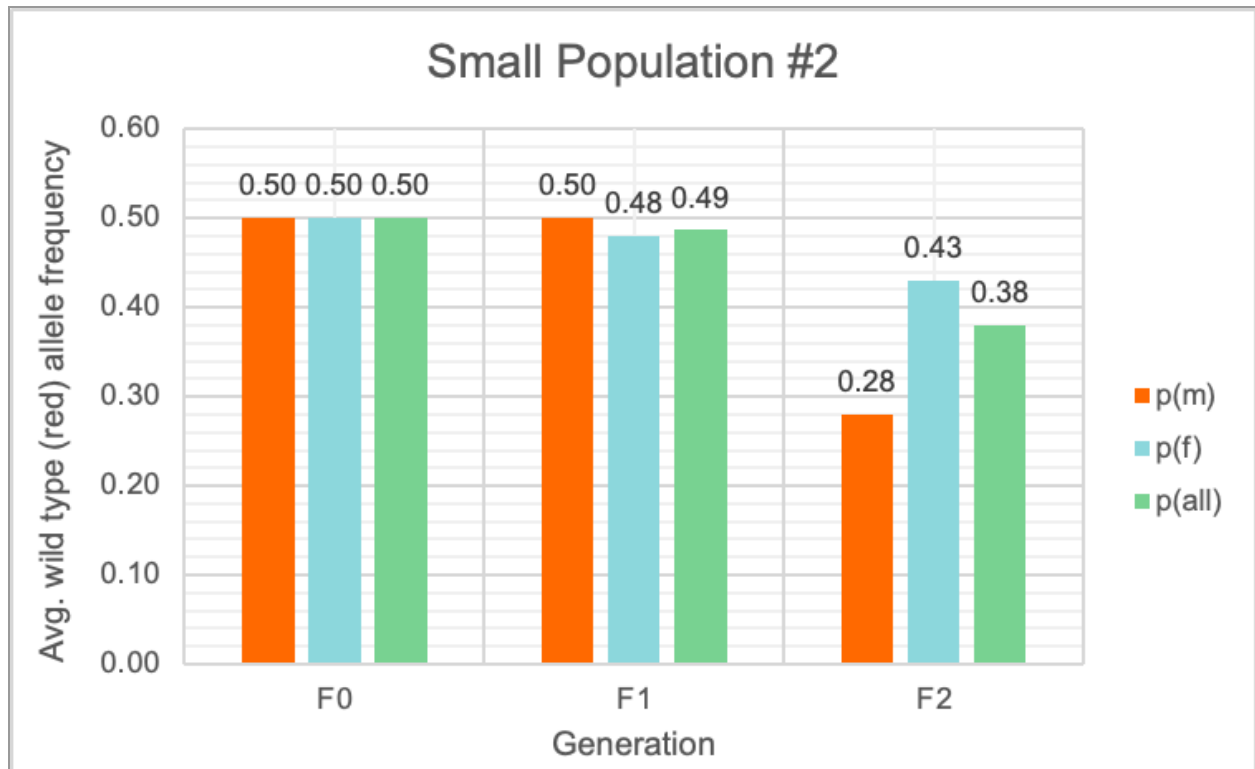


FIG. 2. Average wild type (red) allele frequency in male and female flies as well as its overall weighted frequency in small population #2 (N=8) over 3 generations.

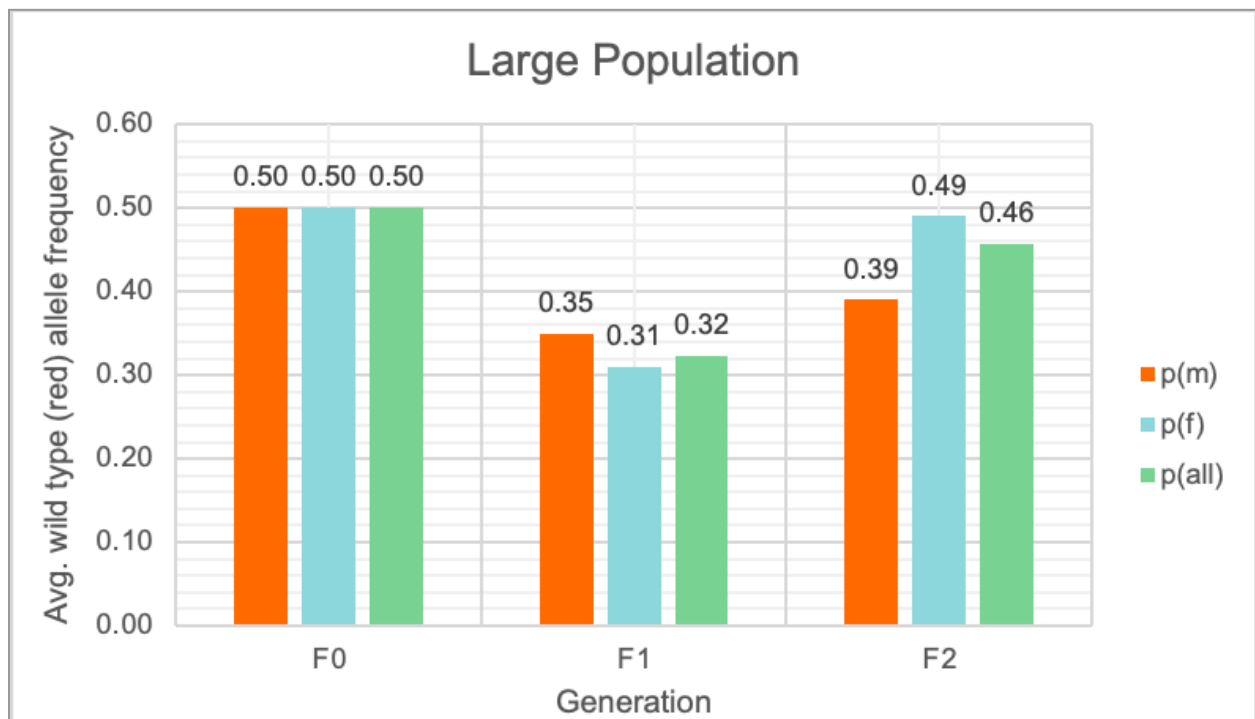


FIG. 3. Average wild type (red) allele frequency in male and female flies as well as its overall weighted frequency in the large population (N=40) over 3 generations.

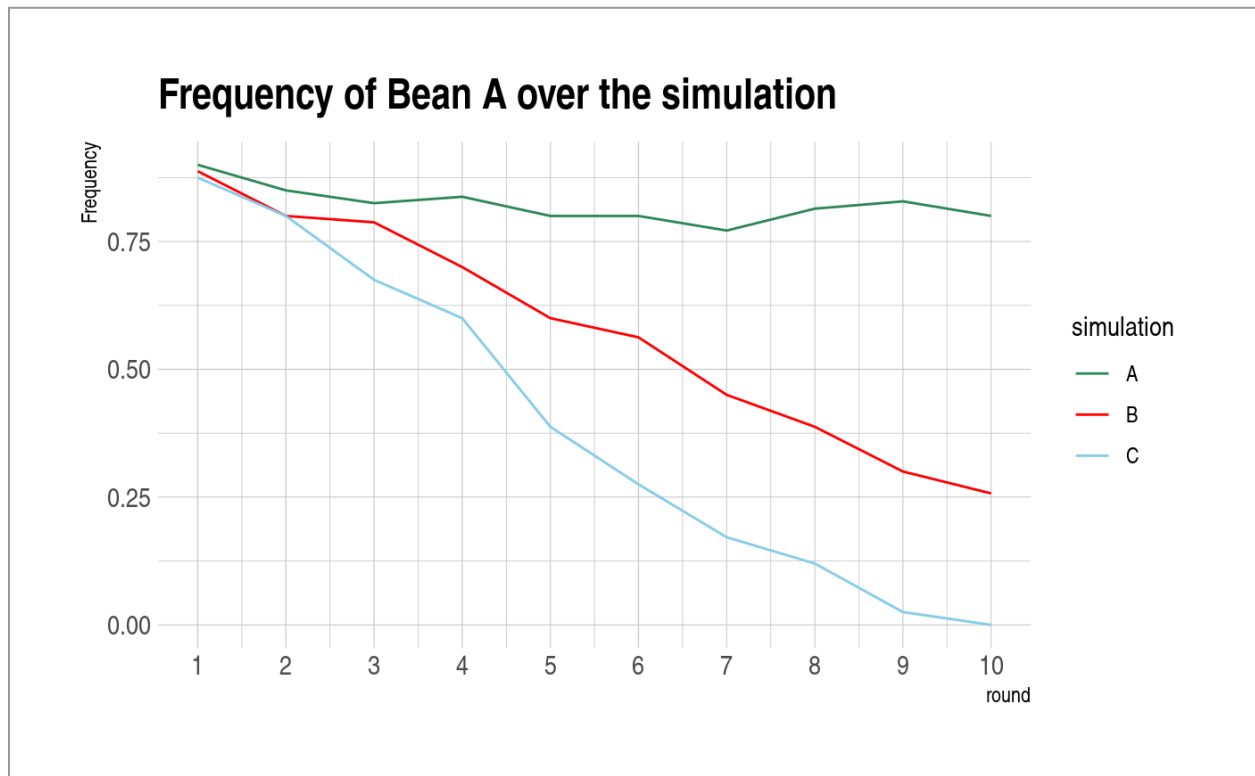


FIG. 4. Average Bean A allele frequency in simulations A(no selection), B(weak selection), and C(strong selection) over 10 rounds/generations; initial population was fixed for Bean A allele.

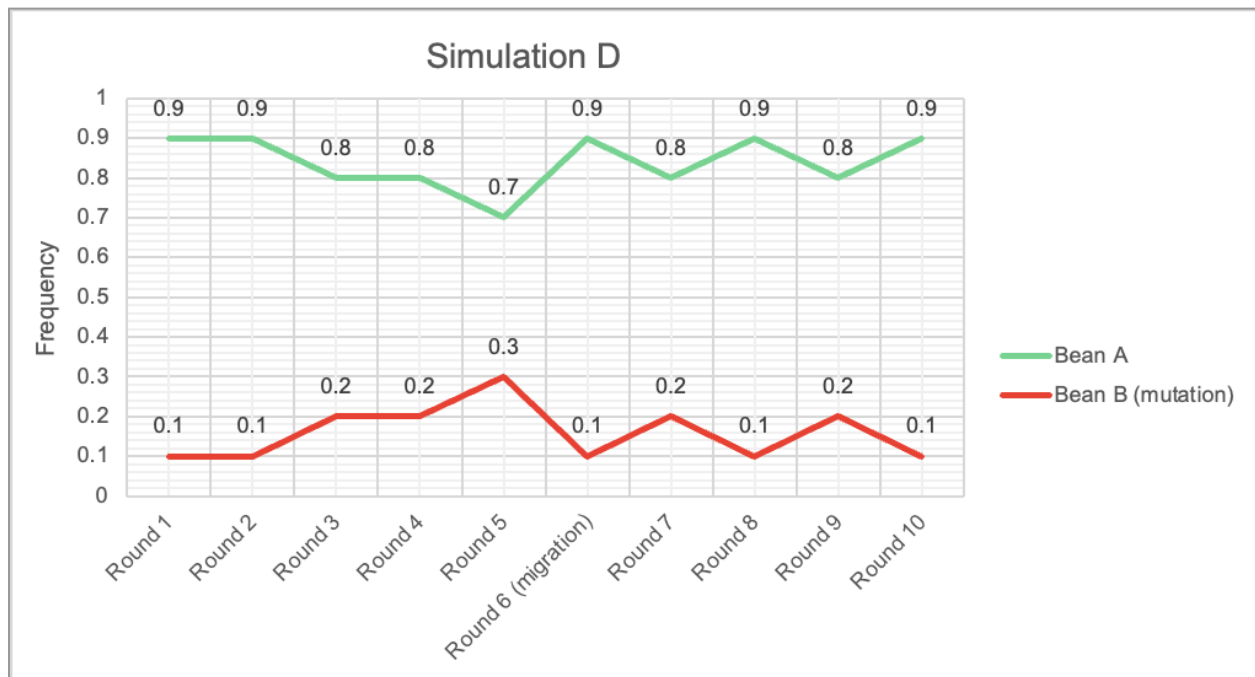


FIG. 5. Average Bean A and Bean B allele frequency in simulation D over 10 rounds/generations; initial population was fixed for the Bean A allele and was reintroduced in round 6.