LABORATORY 9. POPULATION GENETICS: GENE POOL AND ALLELE FREQUENCIES

LEARNING OUTCOMES

At the end of this lab practice, students should be able to:

- Perform a gel electrophoresis to determine different DNA fragment sizes.

- Describe the gene pool concept based on the analysis of a hypothetical *Drosophila* population.

-Estimate genotype and allele frequencies based on phenotypic data.

INTRODUCTION

Briefly, the study of the **amount** and **distribution** of genetic variation in populations is what we know as **population genetics**. This genetics' field also studies the different **evolutionary forces** that drive this variation (mutation, selection, genetic drift, etc.).

Imagine, for example, a *Drosophila* population where two alleles (A and a) from one gene are segregating through the different generations. It is possible that in an initial wild population you find three different genotypes "AA", "Aa" and "aa". However, the number of flies per each of these genotypes may change over time (generations) due several evolutionary forces. Then, the **gene pool concept** may be defined as the total amount of **alleles** and their **distribution** in a population at any **time**.

To better understand the gene pool concept, let's assume the following composition of an initial *Drosophila* wild population:

Genotype	Amount
AA	16
Aa	48
aa	36
Total	100

In this example, we can describe the gene pool of the population by simply analyzing the table: the total number of flies (N) is 100, the number of homozygous individuals is 74 (AA=38 and aa=36) and the number of heterozygous individuals is 26 (Aa). However, instead of using absolute counts of the different genotypes, a standardized option to describe the gene pool is to estimate **genotype frequencies**. In our case, we can do this by simply dividing each genotype's number by the total amount of flies:

Frequency of AA = 16/100 = 0.16

F(AA)=0.16

Frequency of Aa = 48/100 = 0.48

F(Aa)=0.48

Frequency of aa = 36/100 = 0.36

F(aa)=0.36

Now, the different genotypes are described as **frequencies** and hence, these values should add up to 1 (0.16+0.48+0.36=1). A reduced gene pool description of the *Drosophila* population can be proposed if we calculate the **allele frequencies**.

To estimate these values from **count numbers**, first, you must consider the total amount of **alleles** in the population. In our case the number of flies is N=100. As each individual is assumed to be **diploid**, then, the total amount of alleles will be 2N, that is, 200 alleles.

Now, let's start by estimating the frequency of the allele **"A"**, usually symbolized by the letter "*p*". By looking at the table, you can conclude that **16 individuals are "AA"**. Hence, the amount of "A" alleles is **16 X 2 = 32**.

Similarly, the table indicates that **48** individuals have **only one copy** of the allele "A" (heterozygous "Aa" individuals). Then, we can easily calculate the frequency of allele "A" as follows:

$$F(A) = p = \frac{(16X2) + 48}{200} = 0.4$$

A formula describing the calculation of **F(A)** would be:

$$F(A) = p = \frac{2AA + Aa}{2N}$$

Similarly, we can estimate the **frequency of allele "a"** in the population:

$$F(a) = q = \frac{(36X2) + 48}{200} = 0.6$$

A formula describing the calculation of **F(a)** would be:

$$F(a) = q = \frac{2aa + Aa}{2N}$$

Finally, we have a description of the gene pool of the observed *Drosophila* population based in only two numbers: **The allele frequencies *p* and *q***. Again, as these values are frequencies, their sum should add up to 1.

F(A)=*p*=0.4
F(a)=*q*=0.6
***p* + *q* = 1**

Under certain assumptions (i.e., equilibrium), we can make predictions of genotype frequencies in further generations, by combining these formulas as follow:

The expected frequency of the **homozygous dominant** genotype (AA) would be:

F(AA)= F(A) x F(A)
F(AA)= p x p
F(AA)= p^2

Similarly, the expected frequency of the **homozygous recessive** genotype (aa) would be:

F(aa)= F(a) x F(a)
F(aa)= q x q
F(aa)=q^2

The expected frequency of the **heterozygous** genotype (Aa) would be:

F(Aa)=2pq

And again, as these values are frequencies, their sum adds up to 1:

$p^2 + 2pq + q^2 = 1$

The Hardy-Weinberg principle

This principle states that in the absence of disturbing factors, if mating is random and the population is large, the genetic variation in a population will not change. That is, both genotype and allele frequencies will remain constant in a state known as **Hardy-Weinberg equilibrium**. This equilibrium can be disturbed by different evolutionary forces, including natural selection, mutation, non-random mating, gene flow, and genetic drift. This lab manual only mentions these fascinating topics; however, they are reviewed in detailed in further advance genetics courses (I.e., Evolutionary Processes, Plant Breeding, etc.).

MATERIALS (GEL ELECTROPHORESIS)

- PCR products from previous week.
- A gel chamber and power supply.
- Agarose, SB 1X buffer, SYBR-Safe dye, and DNA ladder.
- Pipettes and tips (10 – 20 ul)

PROCEDURE (GEL ELECTROPHORESIS)

1) Set-up the casting trays as illustrated in the video.

2) Dissolve the agarose in SB buffer (1% Agarose gel) by boiling in the microwave (20-45 seconds).

3) Cool down the agarose solution and add 1ul of the SYBR-Safe dye. Ask your TAs to perform this step. This dye will make your DNA fragments to fluoresce under UV light.

4) Pour the agarose gel on the tray and wait until it solidifies (around 10 minutes). Then, put the tray into the electrophoresis chamber.

5) Cover the gel with 1X electrophoresis buffer (SB). This salt-solution will allow the separation of the DNA fragments by maintaining a constant electric current through the gel.

6) Load 8 to 10 ul of the PCR product into the well. Place the safety cover. Check that the gel is properly oriented. Remember that DNA molecules will migrate towards the positive electrode (red) due the negative charge of the DNA (phosphate groups).

7) Connect the chamber cords to the power source. Set the voltage to 140 volts and the time to 30 minutes.

8) Run the electrophoresis. Ask one of your TAs for further instruction.

9) After completion of the electrophoresis, carefully remove the gel and casting tray. Observe the gel under UV-light and look at the different DNA fragments.

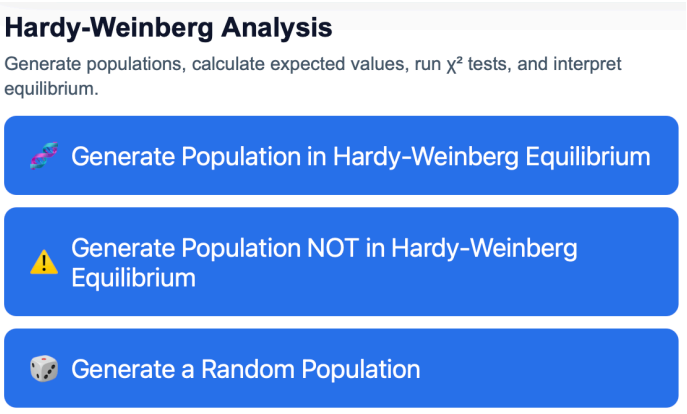
MATERIALS (POPULATION GENETICS SIMULATION)

- Desk or laptop computer. Small devices as cell phones or tablets **are not recommended**.
- Web browser (Chrome, Mozilla, Safari, etc.)
- Access to the **Genetics Virtual Lab** platform (www.ampossot.com/virtual_lab)
- Notebook and pen (or pencil)
- Calculator

PROCEDURE (POPULATION GENETICS SIMULATION)

1) **Launch the Genetics Virtual Lab**. Click on the Population **Genetics Generator button**.

2) You will use the following three apps in this lab:



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