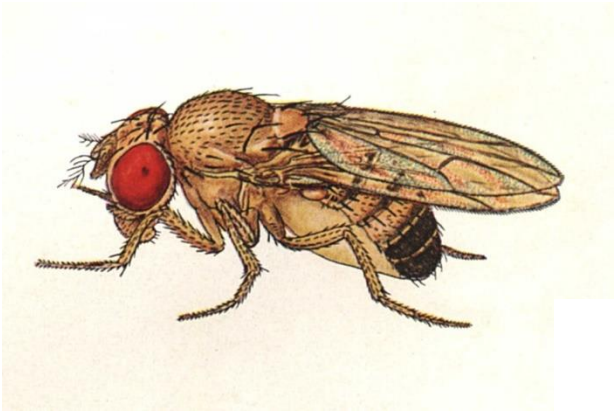
LOSS OF DIVERSITY DUE TO GENETIC DRIFT

STUDY OF THE EFFECT OF GENETIC DRIFT ON GENE FREQUENCIES EXTRAPOLATING A MODEL OF *DROSOPHILA MELANOGASTER* TO THE IBERIAN LYNX SITUATION

GENETICS

PSEUDONYM: DOÑANA



ABSTRACT

El lince Ibérico es una de las especies más amenazadas del planeta. El continuo declive de esta especie durante el siglo XX y los distintos cuellos de botella sufridos han incrementado y hecho más visibles las consecuencias directas de la deriva genética, que junto con la consanguinidad han erosionado la diversidad genética de esta especie llevándola al borde de la extinción. Este proyecto se centra en las causas y consecuencias de los efectos de la deriva genética sobre la erosión genética del lince Ibérico mediante la historia de la degradación de sus poblaciones. Asimismo, se analizan los efectos de la deriva genética sobre las frecuencias alélicas con un modelo experimental de *Drosophila melanogaster* a fin de extrapolar y comparar los resultados a la situación del lince Ibérico.

El proyecto concluye validando la hipótesis de que la deriva genética resulta en la pérdida de variabilidad genética, siendo sus efectos muy evidentes en poblaciones pequeñas, caracterizadas por una elevada consanguinidad. Por tanto, cuando el tamaño de una población disminuye, los efectos de la deriva genética y la consanguinidad aumentan, dando lugar a la pérdida de diversidad genética.

El linx ibèric és una de les espècies més amenaçades del planeta. El continu declivi d'aquesta espècie durant el segle XX i els diferents colls d'ampolla soferts han incrementat i fet més visibles les conseqüències directes de la deriva genètica, que juntament amb la consanguinitat han erosionat la diversitat genètica d'aquesta espècie, portant-la així a la vora de l'extinció . Aquest projecte està centrat en les causes i conseqüències dels efectes de la deriva genètica sobre l'erosió genètica del linx ibèric mitjançant la història de la degradació de les seves poblacions. També s'analitzen els efectes de la deriva genètica sobre les freqüències al·lèliques mitjançant un model experimental de *Drosophila melanogaster* per tal d'extrapolar i comparar els resultats a la situació del linx ibèric.

El projecte conclou validant la hipòtesi que la deriva genètica resulta en la pèrdua de variabilitat genètica, sent els seus efectes molt evidents en poblacions petites, caracteritzades per una elevada consanguinitat. Per tant, quan la mida d'una població disminueix, els efectes de la deriva genètica i la consanguinitat augmenten, donant lloc a la pèrdua de diversitat genètica.

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There has been a lot of work and effort put into the development of this research project. However, it would not have been possible, nor it would not have succeeded in the way it has without a series of people.

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1. INTRODUCTION

Gene frequencies describe the situation a species or population is at and they are determined by the four factors of evolution (natural selection, mutations, migrations, and genetic drift), which establish an equilibrium that allows the population to evolve. However, an unbalanced equilibrium between them endangers a species' situation, mainly in small populations.

Genetic drift is the random process that determines whether some alleles become more common or disappear, also defined as the random fluctuations in allele frequencies between populations over time. This evolutive force becomes more visible and has stronger effects in small populations, leading to loss of variation and damaging genetic variation, as this research will show.

The Iberian lynx is a clear example of how genetic drift and this unbalanced equilibrium are threatening its existence. This species was spread over the Iberian Peninsula, but in the end of the last century, it suddenly decreased in number, leaving only a hundred individuals distributed in two remnant populations in 2002. The sudden decline of this species made genetic drift generate high genetic damage and few chances for the species to survive.

This project consists of a study of the consequences of genetic drift on gene frequencies throughout an elaborated model with *Drosophila melanogaster* (fruit flies) that will attempt to extrapolate the results to the circumstances the Iberian lynx is undergoing.

1.1 PURPOSES

This research study aims to accomplish the following purposes:

1. Determine the effect of genetic drift on gene frequencies and small populations.
2. Determine the importance of population size and inbreeding in genetic variation.
3. Elaborate a model with *Drosophila melanogaster* to extrapolate the results to the Iberian lynx circumstances (taking its constraints into consideration) and reach a conclusion for the first two goals.
4. Obtain complete knowledge about the circumstances the Iberian lynx has been through and understand the causes of the genetic erosion and the importance genetic drift has in this situation.

5. Gain research skills (rewording, summarizing, reading comprehension, taking notes, writing, formulating a coherent hypothesis, laboratory skills, critical thinking, data analysis, and concluding), as well as provide a clear detailed study and information.

1.2 HYPOTHESIS

The hypotheses for this research, based on the first four aims, are that since genetic drift randomly establishes gene frequencies, the chance and probability of this process will lead to allele fixation depending on the equilibrium and overriding with the other evolutive forces, the strength of bottlenecks, population size, inbreeding rates, and the initial genetic variation. When genetic drift is not in equilibrium with the other evolutive forces (mutations, migrations, natural selection, and genetic drift), it will result in loss of diversity and genetic erosion.

The smaller a population is the more present inbreeding becomes and the more visible the consequences of genetic drift will be. Keeping this in mind, as population size decreases, inbreeding increases, and genetic variation is lost.

The model will demonstrate the two first hypotheses and will allow to extrapolate the results to the Iberian lynx situation (taking its constraints into consideration) since genetic drift will be the only present evolutive force.

2. THEORETICAL FRAMEWORK

2.1 THE *IBERIAN LYNX*

2.1.1 Characterization

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Carnivora

Family: Felidae

Genus:Lynx

Species: *L. pardinus*

Fig. 1. Iberian lynx. (Silwa, 2015)

The Iberian lynx is a graceful-looking feline known for its agility and sharp eyesight and hearing. It has long legs and a short tail with a black tassel at the end, which usually keeps upright shaking it in moments of danger or excitement. Its pointed ears end with a few stiff black hairs that favor its camouflage by decomposing the round silhouette of its head. It also has sideburns hanging from his cheeks that appear after a year of life and are longer in males than in females. Its fur varies from brown to greyish and presents black specks thin in some lynxes and thick in others.

Adult males weigh an average of 12.8 kg and females about 9.3 kg. They weigh roughly half as the Eurasian lynx. Both share the same ancestor: *Lynx issiodorensis*, and although their populations never overlapped much, the two lynxes were able to coexist until the late 19th century in the Pyrenees.

2.1.2 Habitat and behavior

The Iberian lynx lives in the Mediterranean forest and scrub, in very restricted areas of the Iberian Peninsula. In Spain, there are very few areas well preserved and isolated from human activity, while in Portugal they became extinct. This type of habitat provides shelter and open pastures to hunt rabbits, which make up 90% of their diet. The destruction of its habitat and the lack of rabbits belong to the main causes that brought the Iberian lynx to the edge of extinction.

They usually have a solitary existence after the first year of life and tend to frequent a small territory of about 26 square kilometers, far from agricultural or inhabited areas. However, last year it was observed that a female moved 300 kilometers and hunted in agricultural land, which is a hopeful sign of adaptation for this species (Bish, 2010).

2.1.3 Reproduction

The mating season begins between January and February and their dens are located in well-protected and hidden places. Litters are born between March and April and usually consist of one to four cubs. When they are two months old, they are able to accompany their mother on hunts and they become independent when they are seven or twelve months old (more or less when the female reenters breeding season).

Females can breed during their first winter, but it depends on demographic and environmental factors. In a dense population, their first reproduction depends on when a female obtains a territory, which occurs due to the death or expulsion of a resident. It is possible that a female will not reproduce until she is five years old, or until her mother dies and leaves her the territory (which creates a slow-growing population). They usually live about thirteen years, being fertile up to ten years.

2.1.4 Distribution

The Iberian lynx was anciently located in the Mediterranean part of the Iberian Peninsula and the south-west of France. Nevertheless, in the 20th century, a bottleneck made the species suddenly decrease in number and it was reduced to eight populations (Central Range, Montes de Toledo, Western Sierra Morena, Eastern Sierra Morena, Far-Eastern Sierra Morena, Vale do Saldo, Doñana, and Subbéticas) with a total of 2000 individuals in the south-west of the Iberian Peninsula (fig. 2). The isolation of these populations caused a lot of genetic damage, so the decline did not stop, and the Iberian lynx became one of the most endangered species in the world. In 2002, there were only 100 individuals left,

Fig. 2. Distribution of sampling across ancient and historical Iberian lynx ranges. Ancient range in light grey taken from Rodríguez and Delibes (2002). In Colour historical distribution according to country-wide surveys in the 1980s in Spain and 1989–1994 in Portugal, with populations delimited as in Rodríguez and Delibes (1992), except that we subdivided the largest Eastern Sierra Morena-Montes de Toledo population as suggested by genetic structure analyses. Points represent sampled localities, with outlined points corresponding to ancient samples and crosses representing contemporary samples; note that each point may represent several samples. Unsampled populations are shown in striped fill. (Godoy, Spatiotemporal Dynamics of Genetic Variation in the Iberian, 2017).

distributed in two populations in Doñana and Western Sierra Morena with few genetic variability and a high frequency of inbreeding (Godoy, Spatiotemporal Dynamics of Genetic Variation in the Iberian, 2017). However, thanks to conservation programs and the contribution of genetic techniques the Iberian lynx did not go extinct and the population rose to 550 individuals in 2017. There are five captivity stations and some members of the species have been reintroduced in new populations.

2.2 KEY ASPECTS

2.2.1 Evolution

Evolution is a process of transformation from a species to another thanks to the accumulation of variations that appeared among the descendants from one generation to another, generally after long periods of time.

The theory of evolution is the main synthetic theory in Biology that unifies the knowledge that different sciences provide, such as botany, zoology, paleontology, biogeography, or biochemistry, but mainly genetics. Evolution allows us to understand the development of life throughout history, and even predict how it will continue. Without this theory, biology would be a chaos of unconnected knowledge and a long chain of unanswered questions.

Above all, evolution essentially is a process of genetic change throughout time and population genetics is the biological specialty that supplies the theoretical principles of evolution (Barbadilla, 2010).

2.2.2 Population genetics

Population Genetics is the science that harbors the facts and processes explaining biological evolution, which essentially is the development of genetic changes over time (Barbadilla, 2010). The basis of this science is that evolutive changes on a small scale carry all necessary elements to explain evolution on a broad scale (macroevolution), so it would just be an extrapolation of the basic processes occurring in small populations. Almost every species includes one or more populations of breeding individuals forming a genetic exchange community called Mendelian population, the origin of evolution. In every population each individual leaves a different number of descendants, changing this way the allele frequencies every generation. Therefore, evolution is also an irreversible change of the proportions from different gene variants in populations. Thus, populations, not individuals, are the units of evolution (Barbadilla, 2010).

The evolutive forces, which are the agents changing allele frequencies, are mutations, migrations, genetic drift, and natural selection.

2.2.2.1 Mutations

A mutation is a stable and inheritable change in the genome, it alters DNA sequences and introduces new variants in a population. Most of these variants are most likely to be eliminated by natural selection and genetic drift; however, sometimes they are successful and become part of the gene pool. Given that mutations raise variation, a high mutation rate can increase adaptation since possessing more genetic variants is translated to more chances to have the appropriate variant to adapt to the environmental change; however, it also increases the chances for deleterious mutations to appear and make the individuals less adapted and more likely to go extinct (Barbadilla, 2010). For this reason, each species has a mutation rate modified by natural selection in order to face environmental changes. It is also necessary to mention that mutations do not present any kind of direction towards adaptation because they are completely random. Nonetheless, spontaneous mutation rates tend to be too low to modify allele frequencies in populations by themselves.

Mutations are classified as synonymous and non-synonymous. Synonymous mutations are the most common and do not generate changes in amino acids, so they are neutral. On the other hand, non-synonymous mutations generate changes in amino acids, which are usually deleterious for the individual or population (fig. 3 and 4). A mutation will be deleterious or beneficial depending on how it affects the individual: if this one becomes less adapted to the environment, the mutation is considered deleterious; otherwise, and beneficial when the individual becomes more adapted. (The mutation will be neutral when it does not affect the levels of adaptation).

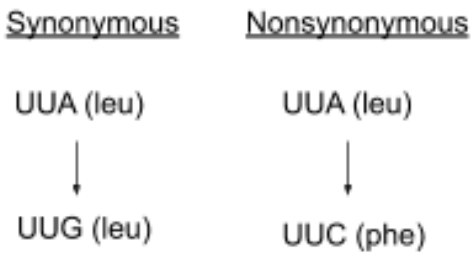

Fig. 3. Example of a synonymous mutation (does not affect the amino acid) and a nonsynonymous mutation (changes the amino acid) (own source).

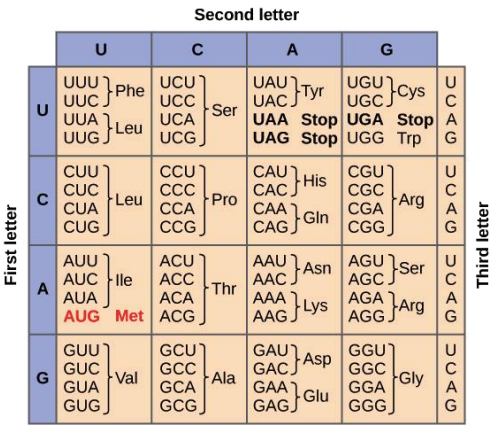

Fig. 4. This figure shows the genetic code for translating each nucleotide triplet in mRNA into an amino acid or a termination signal in a protein (OpenStax College, s.f.).

Dominant mutations have the same effect on big and small populations, each individual that presents a dominant allele will suffer its effects or the phenotype corresponding to that mutation. This way, it is much easier for selection to eliminate that mutation in both kinds of populations. On the other hand, recessive mutations will not be visible for selection when they are in a heterozygous individual; thus, selection will only be efficient on homozygous individuals (fig. 5). In this case, since large populations often present a higher frequency of heterozygosity it is more likely that a recessive mutation will be passed on generations.

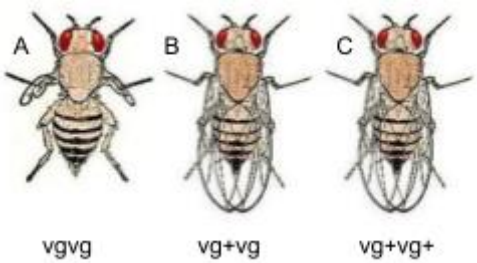

Fig. 5. Image of three *Drosophila melanogaster* individuals with different genotypes (vg (vestigial)<vg+ (wild)): recessive homozygous (A), heterozygous (B), dominant homozygous (C). (Skenderian, 2012)

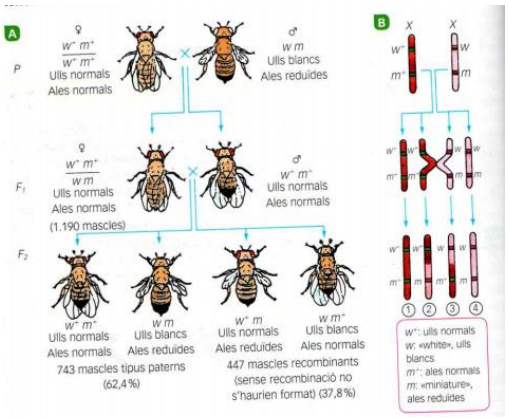

Fig. 6. Experiment that proved gene recombination. (A) Morgan proved that genes w+ and m+ are located in the X chromosome. Diagram of recombination in the formation of gametes of a female w+m+/wm. (Antonio Jimeno Fernández, 2016)

Although mutations increase genetic variability, it is necessary to mention that this genetic diversity mostly in prokaryotic organisms mostly relies on mutations; however, in eukaryotes, genetic diversity is mainly generated by recombination and a few mutations. Crossing-over or recombination is the exchange of fragments of chromatids between homologous chromosomes during meiosis; in other words, it is a process of gene recombination that allows gametes to have a new chromosome formed of random fractions from the two homologous chromosomes (fig. 6).

Linked-site genes of a chromosome tend to be clustered and inherited together. This tendency is called genetic linkage and it is detected when studying phenotypic proportions in the offspring since certain traits are presented together more frequently than expected by chance if they were undergoing an independent inheritance. This latter reason justifies why genetic linkage is also defined as the deviation from independent segregation compared to the proportions of a mating model.

When two linked-site genes are inherited separately it means a crossover has occurred during meiosis in the fragment of DNA between those two genes, so there has been a genetic recombination that made one gene belong to the original chromatid and the other to the chromatid of the homologous chromosome.

2.2.2.2 Migrations

Migrations are the genetic exchange between populations due to changes in the location of individuals, generating this way a variance in allele frequencies. In other words, they are the arrival of individuals from other populations (immigrants) or the exit of individuals from a population (emigrants).

The gene pool of a population varies due to the addition or subtraction of alleles with migrations. This variation is called genetic flow and its degree depends on the number of migrants regarding the number of residents and the different allele frequencies an allele presents in both populations.

2.2.2.3 Natural selection

Natural selection, also called “the survival of the fittest” (by Darwin), is the evolutive process that determines the reproduction and survival of certain gene variants from others and results in changes in allele frequencies which increase the average biological efficacy of the population. Thus, it results in the submission of phenotypic variation, differential reproduction or survival associated with variation, and inheritance of variation. If a population presents these conditions, there will be a change in genetic composition due to natural selection. In other words, natural selection is an accumulative process that allows to add improvements in each generation in order to obtain complex structures (Barbadilla, 2010).

Besides, natural selection increases the frequency of adaptations, which are characteristics in a population that have a positive direct effect on survival or the number of descendants of the individuals carrying them. However, there is not a better phenotype in the beginning, it depends on the environment that a species lives in so we can understand the cause of an adaptation.

Nevertheless, it is necessary to point out that natural selection is always beneficial to populations because it selects the best-adapted individuals (this is why it generates adaptations in populations). However, individuals and species might be negatively affected by this evolution force because it favors the survival of the fittest organisms. Consequently, if an individual is not fit enough or if a species does not present any adapted individuals, they will not leave descendants.

Depending on what genotype has more fitness, different types of natural selection with different effects on genetic diversity can be identified. Each of these types has different consequences on the loci they acted on, leaving patterns different from the neutral parts of the genome, which are shaped by demography (Nielsen, 2005):

- Positive selection, which increases the frequency of a beneficial allele.
- Negative or purifying selection tends to eliminate a deleterious allele. One of its effects on a gene undergoing this kind of selection is that it will present a low nucleotide diversity.

  Both types are considered directional selection since they continuously increase or decrease an allele's frequency, decreasing also the genetic diversity of that population. Directional selection also generates an excess of genetic differentiation compared with the neutral sites when selection favors different alleles or a failure in differentiation favoring the same alleles.

- Balancing selection tends to maintain the diversity favoring the coexistence of more than one allele. In this case, it is diversity itself and not just one allele, which gives the advantage to adapt better (Marmesat, 2020). The main consequence of this kind of selection is to cause a lot of diversity (mainly through heterozygous individuals) and a defect in differentiation. On the other hand, it also targets genes of which diversity is essential, such as MHC genes (the most important in the immune system), for the survival of an individual and reduces the effects of genetic drift. There are several mechanisms of balancing selection:

  - In loci, the advantage of heterozygotes occurs when biological efficacy is greater in individuals with heterozygote genotypes than any homozygous (Hedrick, 2012).
  - Selection depending on negative frequency, when the alleles with more biological efficacy are less frequent.
  - Sometimes, different populations or a population in different periods of time undergo different evolutive pressures and although directional selection is acting on them the pattern that all populations have been following is the maintaining of diversity (Marmesat, 2020).
  - If there is a multigenic family (several copies of the same gene) there is also haplotype balancing selection, which will favor the diversity of allelic lineages in each copy of the gene. In these situations, a new diversity can be generated due to interlocus recombination and genic conversion (Ohta, 1995)

- Disruptive selection, when the two extreme phenotypes are favored. For instance, in a population formed of individuals of three different sizes (small, medium, big), favoring the two extreme sizes since small individuals are not seen by their predator and big individuals are able to flee. As time goes by, the population spits in two populations (Antonio Jimeno Fernández, 2016).

2.2.2.3.1 Fitness

Depending on the growing population models fitness can adopt different definitions. According to Darwin, there is the absolute definition of fitness as the average number of descendants in a specific environment (Hedrick, Evidence for balancing selection at HLA, 1983). However, relative fitness is known as the ability of a species to be maintained in the following generation. On the one hand, in discrete and non-overlapping generations the total reproductive success reveals the selective advantage of each species in a certain generation. On the other hand, if the generations are continuous and overlapping, the data about descendancy will not be relevant unless a temporal pattern is known (Barbadilla, 2010).

Fitness as a consequence of natural selection does not refer to a physical magnitude, it just has a descriptive role that allows to predict a deviation pattern without providing any statements about the causes (Barbadilla, 2010). It gives the possible sources that act on the reproductive success of an individual (Sober, 1984). Moreover, there is a direct relationship between fitness and the environment, since this relationship determines the failure or success of a species or population (Kalisz, 1990).

This property that reflects the chances of reproductive success and/or survival associated with certain traits in a population, allows to distinguish between explanation and prediction in the theory of evolution (Sober, 1984). For this reason, it is not illogical that a group of individuals selected to favor a population ends up with a different variant fixed, especially in small populations. Likewise, two genetically identical individuals can also differ in the number of offspring. Fitness is a population attribute, not a particular one, of the different variants of a trait. However, the empirical estimation of absolute fitness requires the measurement of the total reproductive success of the different individuals (Barbadilla, 2010).

2.2.2.4 Allele frequencies

A population's allele frequency is the fraction of the copies of one gene that share a particular form, so it refers to how often a particular allele appears in a population and it is defined with the following equation:

$$Frequency\ of\ allele\ A\ = \frac{Number\ of\ copies\ of\ allele\ A\ in\ population}{Total\ number\ of\ A/a\ gene\ copies\ in\ population}$$

Random gene frequency changes are due to systematic factors (selection, mutation, and migration), which yield to an equilibrium point, and dispersive factors (chance fluctuations in finite populations and changes of systematic factors), which spread the gene frequency (Morton, 1955).

With background selection (loss of genetic diversity at a non-deleterious locus due to negative selection against linked deleterious alleles) if an allele frequency increases in one generation, it is likely to increase again in the next because recombination does not completely mix genes up every generation. Nevertheless, chance makes that what happens in one generation has no connection to what happens in the next; in other words, a different random fluctuation in allele frequencies is expected every generation in a population, and here is when genetic drift takes over an important role in evolution.

2.2.2.5 The Hardy-Weinberg law

According to the studies realized by Hardy and Weinberg in 1908, in a population formed of sexually reproducing individuals where each one of them mates randomly (panmictic population), and none of the evolutive forces are present, allele frequencies will be maintained in every generation. Therefore, the population will not evolve, which means that inheritance by itself does not cause evolution, since recombination randomly creates an almost infinite number of different combinations but does not change the allele's frequency. This equilibrium law has two main equations:

$$p + q = 1$$

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

The first equation takes allele frequencies into consideration and the second equation gives information about genotype frequencies. In both equations, p is the dominant allele frequency and q is the recessive allele frequency. Hence, p^2 is the genotype frequency for homozygous dominant individuals, $2pq$ for heterozygous individuals, and q^2 for homozygous recessive. This equation allows to predict the frequency individuals will present in a population under the

circumstances mentioned above, as well as to create a Punnett square and a graph that represent this law (figures 7 and 8).

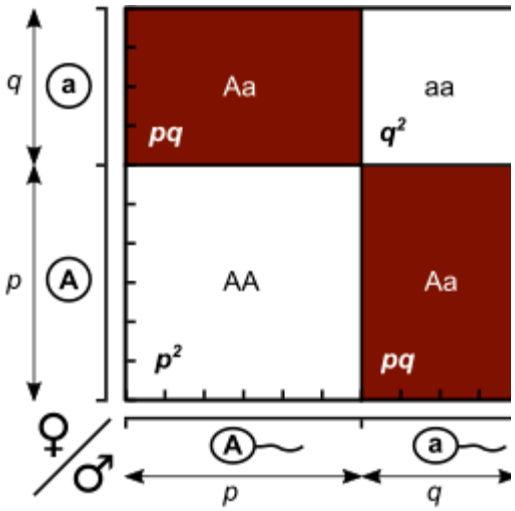

Fig. 7. H-W equilibrium Punnett square. Length of p, q corresponds to allele frequencies (here p = 0.6, q = 0.4). Then area of rectangle represents genotype frequencies (thus AA : Aa : aa = 0.36 : 0.48 : 0.16) (Wikipedia, 2009).

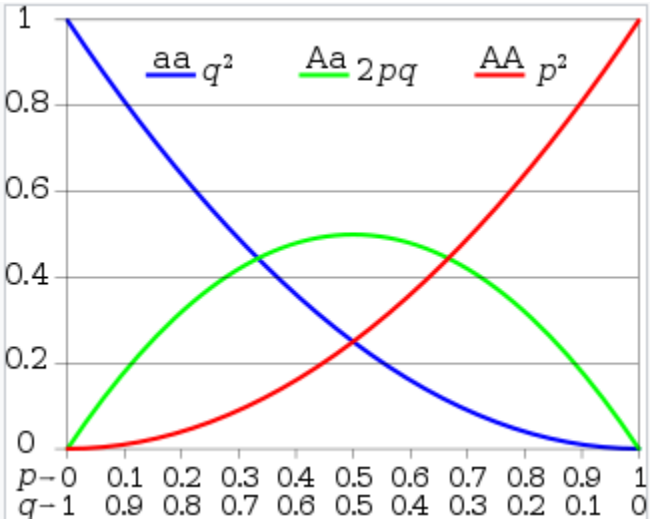

Fig. 8. Hardy–Weinberg proportions for two alleles: the horizontal axis shows the two allele frequencies p and q and the vertical axis shows the expected genotype frequencies. Each line shows one of the three possible genotypes (Wikipedia, 2009).

2.2.2.6 Genetic drift

Genetic drift is a mechanism of population divergence important in the evolution of living organisms and based on the random change (sampling error) in allele frequencies of a population over time. It is the random fluctuation of allele frequencies between generations that results from recombination in finite populations.

Genetic drift results in the loss of genetic variation by making gene variants disappear, counteracting this way the genetic diversity generated by mutations (Barbadilla, 2010). Besides, it can cause rare alleles to become much more frequent and even fixed. Therefore, genetic drift may result in the loss and fixation of some alleles (including beneficial ones). In other words, genetic drift tends to the fixation for one allele, implying a change in gene frequency and identical copies by descent. Fixation rate is inversely proportional to population size, so the smaller the population the larger the fixation rate. This is why genetic drift has a greater effect on small populations than in big ones.

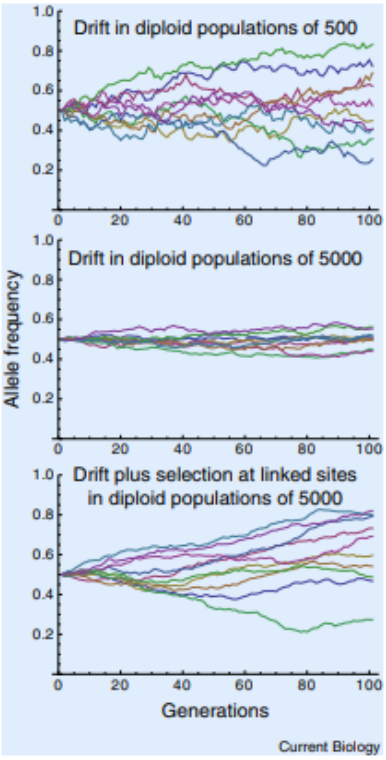

Figure 9. Drift and allele frequencies. Simulated allele frequencies in replicate populations. Drift happens faster in small populations (top panel) than larger ones (middle panel). Selection at linked sites (bottom panel) also increases the speed of change, but is not identical to a small population size (top panel).(Joanna Masel, 2011).

Summarizing, successive generations of genetic drift mostly cancel each other out, so that over the long term, an allele undergoing genetic drift has much less variation in its success than it would if it were linked to other genes under selection (Masel, 2011). Nevertheless, if an allele frequency hits zero, then the next generation of genetic drift cannot cancel that out. That allele stays extinct unless it appears again by mutation. Thus, genetic drift is important in determining whether a new mutation is lost, or whether it instead becomes common enough for selection to determine its fate. Therefore, the effect of genetic drift over a given time declines with increasing population size, and high-allele frequencies are only possible in a small population due to genetic drift. In other words, allelic fluctuations due to this evolutive force will be larger in small populations than in big ones since any change on few individuals would be more relevant than in a larger population size (fig. 9).

2.2.2.6.1 Bottlenecks

Bottlenecks are significant reductions in population size over a short period of time due to some random environmental event where genetic drift plays a major role. The survival chances of any member of the population are purely random and are not improved by any genetic advantage. Bottlenecks result in genetic erosion, fitness degradation, loss of adaptive potential, and radical changes in allele frequencies, completely independent of selection.

2.2.2.6.2 Founder effect

The founder effect occurs when a small group of members of a population forms a new colony by settling in another environment. This is how they create different gene, genotype and phenotype frequencies in the new population and reduce genetic variation. Thus, it is the random change in gene frequencies between a new colony and the parental population due to the little amount of founding individuals. In some cases, the founder effect leads to the formation of new species (Charles N. Rotimi, s.f.).

2.3 GENETIC DRIFT DAMAGING THE *IBERIAN LYNX* GENOME

2.3.1 Genetic variability

Genetic variability is the primary source of evolution. The gene pool of a population determines the ability of the population to evolve and adapt to environmental changes. This genetic variability is usually estimated by the gene and allelic diversity using identifiable regions of DNA known as genetic markers and ensures the survival of a species. Mutation generates new alleles and recombination randomly sorts alleles out and these alleles come to form part of the population's gene pool.

In order to have a better understanding of this concept, it is necessary to mention that if the repertoire of alleles of a species is sorted out in different sets corresponding to distinct populations, the levels of each population's diversity will be low even when the species diversity is high. Likewise, if the allele frequencies within a population are very biased, the levels of individual variation will be low even when the population allelic richness is high.

Genetic variability is measured as the average density between SNP (single nucleotide polymorphism) and the total nucleotides in the genome. The Iberian Lynx is one of the species with the lowest genetic variability ever seen, especially in Doñana, with a ratio of a hundred to a million (Godoy, Aplicación de la genética a la conservación del lince ibérico, 2018).

Genetic drift and the poor efficiency of natural selection, inbreeding, and bottlenecks are responsible for the loss of many alleles in these small populations and the accumulation and increase of deleterious alleles.

2.3.1.1 Natural selection vs genetic drift

Natural selection and genetic drift are in equilibrium, so in a big stable population, their effect is found at a certain rate that improves and maintains the evolution and fitness of the population with a certain genetic diversity. Since these forces are in equilibrium, when one of them has stronger effects, the other one becomes weaker. When a population is reduced, and isolated natural selection becomes less efficient. Because of this, recessive mutations in non-adapted individuals are more likely to not be eliminated and to increase in frequency due to genetic drift, which is the ongoing situation with the Iberian lynx. The populations of this species remained small and isolated long enough for genetic drift to damage its genetic diversity, which complicated the population to adapt to changes. Therefore, the Iberian lynx genetic erosion is in part due to the unbalanced forces of evolution, where genetic drift counteracts natural selection. This way, positive selection is not able to increase enough a beneficial allele's frequency, a higher number of deleterious alleles are not eliminated (purifying selection), and balancing selection has trouble in maintaining genetic diversity.

Those parts in the genome that codify proteins have too many variants that change the protein sequences and they probably are deleterious as a consequence of the poor efficiency of natural selection.

2.3.1.2 Inbreeding

Inbreeding is the mating between individuals in a population closely genetically related, which usually leads to genetic disorders. In small populations like the Iberian lynx's inbreeding is very common and dangerous because it causes an increase in homozygotes with recessive deleterious mutations. The high levels of inbreeding observed in the Iberian lynx made this species almost go extinct by making its immune system more vulnerable through fitness erosion, also known as inbreeding depression. This latter event has been accumulated in the form of heterozygosity-fitness correlations of sperm quality, reduced reproductive rates, increased nontraumatic mortality, and high rates of genetic diseases (Abascal, 2016). Thus, the increased inbreeding due to genetic drift led to inbreeding depression deteriorated the gene pool of the Iberian lynx populations and compromised its viability.

It is necessary to think about the fact that if you were to count the ancestors of an individual, you would be surprised by the large number you would obtain since it seems impossible that if you have two parents that had two parents, and so on, you will have over a trillion ancestors 40 generations ago. The only explanation for this fact is that some of those people were the same individuals, counted many times since they occur many times among your ancestors. In other words, inbreeding plays a role over generations, which means that your parents must have been related to each other. Therefore, any outbreeding finite species will be subject to the same argument (Felsenstein, 2019). Thus, inbreeding is a fact present in every species, but its rate will determine whether the survival of the species is compromised or not.

2.3.1.3 Bottlenecks

Poor genetic variability due to bottlenecks is not always an immediate consequence since chance could also favor the presence of fit individuals. However, even if the species could adapt to environmental changes at first, it would not survive in the future since the poor genetic variability would lead to inbreeding, deleterious mutations, and a low allele frequency.

Genetic drift damaged the Iberian lynx genetic variation mainly through bottlenecks, greatly reducing populations and leaving non-adapted individuals. The loss of variation left the surviving populations vulnerable to any new selection pressures or the ability to adapt to environmental changes since genetic variation is necessary in the population for natural selection to take place (Masel, 2011).

Moreover, most populations in every species carry recessive deleterious alleles or mutations, each of which is rare. When the Iberian lynx populations were bottlenecked a small number of those mutations became much more common. Then, inbreeding increased the frequency of

homozygotes (declining this way genetic variation) and rose the number of individuals with two copies of non-beneficial alleles, so the population became more vulnerable.

2.3.2 Demographic history

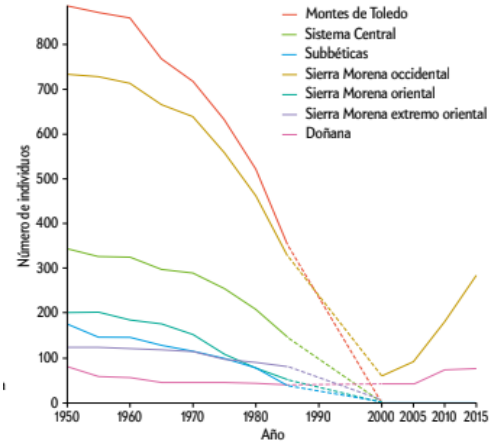

Fig. 10. Iberian lynx population sizes over time. Montes de Toledo and Western Sierra Morena were the biggest populations, but all of them except Doñana experienced a sharp decline until 2000, when they went extinct, aside from Western Sierra Morena, thanks to conservation programs. Doñana population also underwent a decline, but it always remained below 100 individuals (from 1950 until 2015) (Godoy, Aplicación de la genética a la conservación del lince ibérico, 2018).

First and foremost, it is necessary to briefly introduce an overall idea about the genetic situation extinct and remnant populations underwent. Central Range and Far-Eastern Sierra Morena populations did accumulate genetic erosion before their extinction by the late 20th century, while the central population of Montes de Toledo remained largely unaffected by genetic drift until its final extirpation due to human activity. However, we observe a striking persistence of the Doñana population despite a long history of small population size, isolation, and extreme genetic erosion, which according to recent studies may have affected reproduction and survival (Palomares, 2012) (fig. 10). The fact that Doñana is a nature reserve is the main cause of the persistence of the population, different from Western Sierra Morena, which survived because of its centric location, better habitat conditions, and less genetic erosion due to its size (big compared to the other populations). Therefore, the two remaining populations, Doñana and Sierra Morena, have a different background when they were reduced. On the one hand, Doñana's genetic variability decreased continuously along with its size due to genetic drift. On the other hand, Sierra Morena's population remained big without genetic drift deteriorating it until later on when a bottleneck made it almost disappear (fig. 11).

Population genetics theory predicts that small and isolated populations due to bottlenecks progressively lose genetic diversity and accumulate genetic load as a consequence of genetic drift, and this may be exposed through inbreeding, resulting in inbreeding depression (Hedrick, Conservation genetics: where are we now?, 2001). This way, they become less likely to adapt to environmental changes, as well as reproduce and survive. Besides, a decreasing population usually fragments and results in reduced gene flow. Finally, the above-mentioned processes operate independently in the resulting patches leading to increased genetic differentiation,

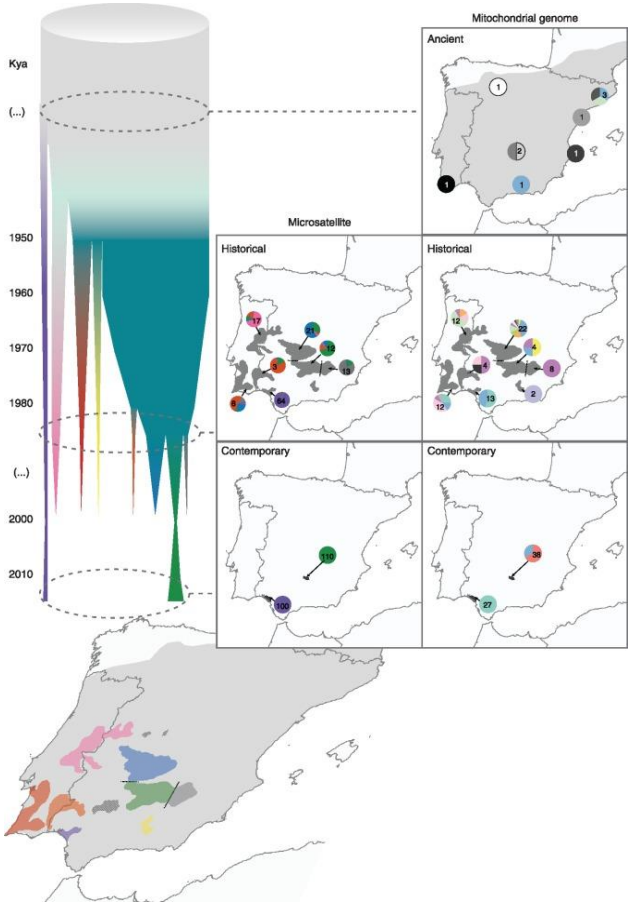

Fig. 11. Dynamics of population isolation and contraction, and genetic variation from ancient to contemporary times. The Iberian lynx population is represented by a cylinder projected on the distribution map, that becomes progressively fragmented into subpopulations which contract, become genetically differentiated and eventually go extinct. Maps represent the distribution of microsatellite (left) and mitogenomic variation (right) among ancient (top), historical (middle), and contemporary populations (bottom). Microsatellite pies represent the average coefficient of assignment to each of the clusters identified by STRUCTURE from historical (K = 6) and contemporary (K = 2) data sets. Mitogenome pies represent the distribution of mitochondrial genome haplotypes. Haplotypes observed only once are represented in shades of gray. Numbers within pies refer to sample size. (Godoy, Spatiotemporal Dynamics of Genetic Variation in the Iberian, 2017)

unrelated to local adaptation (Godoy, Spatiotemporal Dynamics of Genetic Variation in the Iberian, 2017). Nevertheless, low genetic diversity and high differentiation can also be the consequence of another demographic and evolutionary history which involves a different risk of extinction: a population with a history of small size and restricted gene flow which remains in equilibrium and presents a lower risk of inbreeding depression due to the action that selection has taken since the individuals are adapted to the local environment. In other terms, **historical genetic structure could be either the consequence of an ongoing fragmentation and decline process that started earlier than previously thought or the result of the species' natural demographic dynamics.** Thus, these two evolutionary histories need to be taken into consideration in order to assess the possible risks, inbreeding or outbreeding depression, and strategies to improve the current situation: to mix or not to mix the populations (which will be argued in the Conservation Genetics section).

Given these two circumstances, a study was made to determine what was the scenario belonging to the Iberian lynx. Finally, census sizes estimated for the historical populations over time revealed that populations varied widely in size and trend (Godoy, Spatiotemporal Dynamics of Genetic Variation in the Iberian, 2017). Montes de Toledo and Eastern Sierra Morena were still large by 1950 (above 750 individuals) but suddenly got reduced, while Far-E. Sierra Morena and Doñana remained always small (around 100 individuals). Finally, in

2000, the only two remnant populations (Eastern Sierra Morena and Doñana) reached 60 and 42 individuals.

Knowing about the action of recent genetic drift in local populations, the study reached a conclusion to favor the view of **an almost panmictic (random mating within a breeding population) ancestral population, which started to contract and fragment before the historical period** (Godoy, Spatiotemporal Dynamics of Genetic Variation in the Iberian, 2017). Although there can only be speculations about the causes of this historical decline, they may be related to an increase in anthropic pressures rather than natural causes, since it is known that humans are responsible for the massive extinction or reduction of some populations of certain species. Humans are drivers of global change, such as changes in ground or floors, use of energy, increase of population, etc. The 16th century was a period of intense human population growth in Iberia and across Europe in general and coincided with the extension of agriculture and the intensification of forest destruction, both of which may have initiated the decline of this Mediterranean area (Ellis, 2010). This continuous destruction of forest extension created patches in the natural habitat of the species and as a consequence, peripheral populations were the first ones to be isolated, while the central ones had more chances in staying connected with other natural patches, increasing the probability of survival since the effects of genetic drift were not so strong (Interview to María Lucena Pérez, own source, see section 3.1). Additionally, the loss of the main prey, the rabbit, had a great effect in the decline of the Iberian lynx since it is its primary source of nutrition. It is also relevant that in Montes de Toledo the direct persecution reflected the sudden disappearance of the population.

As mentioned, genetic drift started earlier and impacted to a greater extent the populations that became isolated sooner at the periphery, such as Doñana, and not central populations, which remained large and connected to each other, as well as to other more peripheral populations, until recently. During this process of decline, the Iberian lynx populations lost diversity and became genetically differentiated due to the random fluctuations in allele frequencies (genetic drift). Consequently, the bigger populations preserved genetic diversity and became a source of gene flow to other populations. Both ancient and historical bottlenecks have impacted genetic variation and are responsible for the Iberian lynx being apparently the mammal species with the lowest genome- and species-wide diversity today (al. A. e., 2016).

The fact that geographical isolation is in part responsible for the decline of the species and for driving genetic divergences between the populations in the Iberian lynx and the two possibilities, along with its consequences, that could explain the current situation in this species (mentioned in the first paragraph) lead to rely on the following statement: **in sexually**

reproducing species, geographic isolation can generate a barrier to gene flow that can result in genetic drift and/or local adaptation and differentiation (Futuyma, 1998).

Recapitulating, the Iberian lynx had already a low population size and underwent a sharp decline around the 16th–17th century, before the one in the 20th century that left two populations. However, the extremely low genetic diversity is also the result of intense genetic drift and fragmentation that occurred during the last decades and made inbreeding depression more dangerous. At the same time, the lower differentiation in historical and ancient times (revealed from the data of a study assessing the Iberian lynx genetic circumstances) suggested that the currently observed genetic differentiation between the two remnant populations is mainly the result of genetic drift in recent times and cannot be attributed to independent adaptive evolution over long periods, indicating low risks of outbreeding depression (al. F. e., 2011).

It is also relevant to mention that if a species has always presented a low genetic diversity it has no need to be a long-term viability threat since it survived lots of years like that. However, since the species is in a dynamic of loss in variation, it is dangerous to stay in this decline. The fact that a species disappears as a result of natural causes is not necessarily bad, but the bottlenecks and population decline in the Iberian lynx are a consequence of human activity; and here is when conservation genetic programs begin. A species can have low genetic variation and live millions of years, since all the species throughout the history of Earth have a cycle of birth and death, including humans. Thus, if a species had low diversity that was going to be maintained until its extinction it would not be possible nor make sense to try to increase it since it is an intrinsic characteristic of the species. The problem is that humans applied pressure on the species and made a particular case of low variation lose more (Interview to María Lucena Pérez, own source, see section 3.1).

In summary, the Iberian lynx has lost a relevant part of its already low genetic variation over time due to both recent and older demographic declines caused by anthropic pressures, and also the current high genetic differentiation between the remnant populations was the result of genetic drift during the last few centuries. Therefore, genetic variability has always been low but in the past, it was not as low as it is now; thus, now there is a long-term viability threat but before there was not. To conclude, the viability threat is determined by the amount of variation lost, not the original low variability.

2.3.2.1 Coalescent theory:

The coalescent theory has allowed scientists to build the demographic history of the Iberian lynx mentioned above. It consists of an allele of a population sharing a common ancestor with another allele of that population (Interview to María Lucena Pérez, own source, see section 3.1). For instance, let's create a fictitious situation where two siblings share an allele inherited from their mother and they both reproduce with another individual creating a family that goes on for generations. Then, it may be possible that by chance a descendant of each sibling shares that allele, that going back in time coalesce in the siblings' mother (the common ancestor). For this reason, a coalescent model is used to draw up the history of the individual alleles of a species, building a genetic demographic history, where it is possible to determine the level of inbreeding in a population and how a mutation has spread over generations (Interview to María Lucena Pérez, own source, see section 3.1).

The coalescent model in the Iberian lynx shows a temporal pattern consisting of the tendency of the oldest samples from each population to cluster together with other populations. For example, the oldest samples from Montes de Toledo and Eastern Sierra Morena belong to the same cluster (fig. 12). Thus, this explains how these two populations were one panmictic population, which were separated more recently, corresponding to the mentioned isolation dates (Godoy, Spatiotemporal Dynamics of Genetic Variation in the Iberian, 2017).

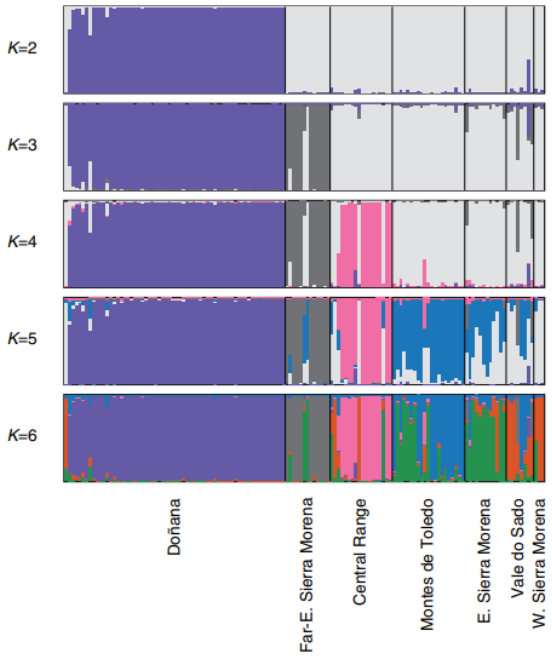

Fig. 12. Historical microsatellite variation. Samples were first subdivided into two clusters, separating almost all Doñana samples from the rest (K = 2). As K increases, other lynx populations are assigned to new clusters, becoming differentiated from the rest: Eastern Sierra Morena (K=3), Central Range (K=4), Montes de Toledo-Eastern Sierra Morena (K=5), and Western Sierra Morena-Vale do Sado (K=6). Older samples from Eastern Sierra Morena and Montes de Toledo (which remained large and interconnected until the second half of the 20th century) are both assigned to the green cluster, which means that they belonged to a single panmictic population that only recently became genetically differentiated. This genetic pool is probably the closest representation of the ancestral genetic variation of the species (Godoy, Spatiotemporal Dynamics of Genetic Variation in the Iberian, 2017).

2.3.2.2 Neutral theory of molecular evolution

Motoo Kimura created the neutral theory of molecular evolution in order to explain the patterns of genetic variation in species. It postulates that genetic drift is responsible for the great majority of evolutionary changes at the molecular level and it is also known as the “survival of the luckiest” (Kimura, 1989).

All evolutive forces produce changes in gene frequencies, however, their importance depends on the subject you take into consideration. This way, while natural selection is more relevant in functional sites of the genome, genetic drift has more importance in neutral sites (Barbadilla, 2010). According to this theory, natural selection is not the most important factor to explain DNA evolution, but mutation rate and genetic drift are. The hypothesis of this theory is that mutations suffered by individuals in a population tend to be neutral (their success in the population is determined by genetic drift) or deleterious (rapidly eliminated because of the few offspring they have) (fig. 13). Kimura claims that genetic variation in populations is a transitory state in a process of the random fluctuation of neutral alleles.

Due to its simplicity, intelligibility, and predictions about the pattern expected of molecular polymorphism and evolutive rate, the neutral theory of molecular evolution became the model with which to contrast selection. Both neutral and selective variation are molecularly abundant today (Barbadilla, 2010).

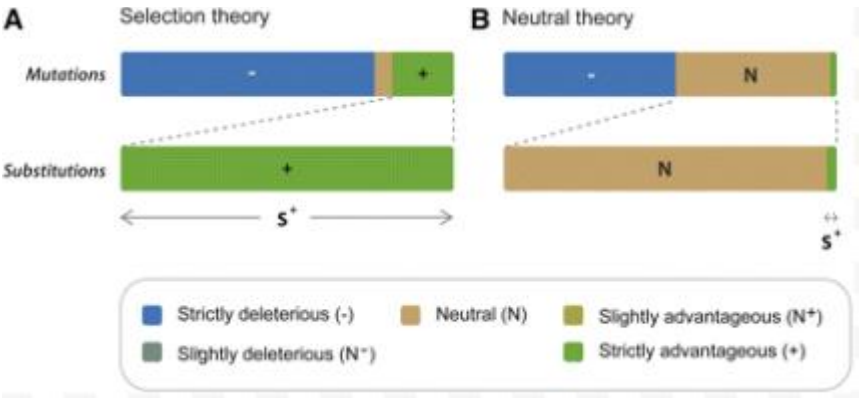

Fig. 13. (A) Darwin’s theory postulated the existence of deleterious (−) and advantageous (+) changes, but Darwin recognized the existence of neutral changes (Bernardi 2007). Deleterious mutations are immediately rejected by negative selection and neutral mutations are ignored. (B) The neutral theory (Kimura 1968, 1983) postulated the existence of a significant part of neutral mutations and a very small fraction of advantageous mutations. Neutral mutations are fixed by random drift and constitute the majority of substitutions. (Razeto-Barry, 2012).

2.3.2.2.1 Molecular clock

A random molecular clock is a consequence of the neutral theory of molecular evolution and it cannot be confused with a coalescent model. A molecular clock refers to the pace genetic variants are being substituted and the ratio it maintains to neutral mutation rate (not the common ancestor of individuals sharing an allele). As a result, when two populations or species split the number of genetic differences between them will be proportional to the time they have been apart. This way, the number of differences that exist between the DNA sequences of different species can be used as a molecular clock that organizes the dates those species split.

2.4 CONSERVATION GENETICS

Conservation genetics is an artificial solution to the loss of biodiversity caused by humans. The study of the genetic nature of populations and evolutive forces acting on them (natural selection, genetic drift, mutation, migration) are the pillars supporting conservation genetics (Benito, 2012).

Effective population size (Ne) quantifies the number of breeding individuals or variations in an individual's contribution to descendants, and it is the idealized population size if it were free of the cited causes for population drift (Benito, 2012). In natural populations, it is lower than the census of reproductive population size (N). Multiple Conservation genetic studies concluded that the minimum effective population size a population should have to be protected against genetic drift and the minimum below which population viability is compromised should be Ne = 500 individuals. However, considering that N should be ten times greater, N = 5,000, which compromises the survival of the Iberian lynx.

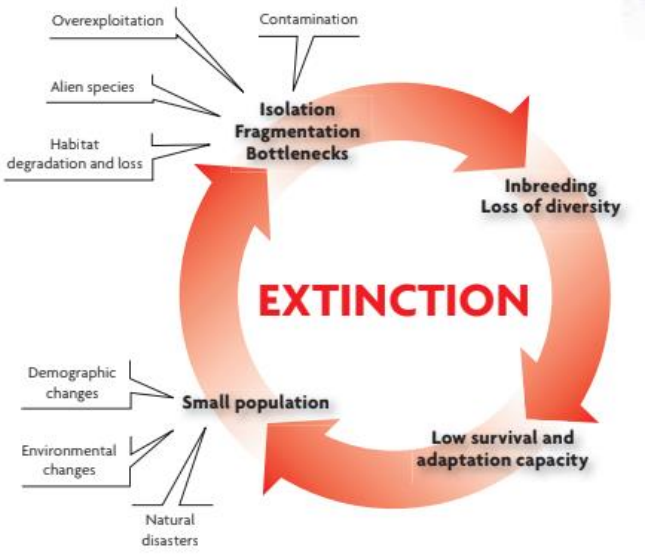

Fig. 14. Figure showing so-called «extinction vortices», which describe the mutual reinforcement between demographic, stochastic and genetic effects that promote extinction in small sized populations. (Rúa, 2013).

Extinction happens when an endangered species presents a compromised biological efficacy due to a combination of low genetic variability, inbreeding, deleterious mutations, and demographic, ecological, environmental, or stochastic factors. This phenomenon is known as vortex or extinction vortex (Rúa, 2013) (fig. 14).

In order to improve the genetic damage, there have been some successful Conservation Genetics strategies applied:

- In situ. It focuses on taking the necessary measures for population size to rise. Regarding genetic aspects, relocation (a certain number of individuals are moved to another population) of individuals in order to increase genetic diversity, decrease inbreeding, and cross genetically distant individuals to improve hybrid vigor (which can also have the opposite effect) are relevant.

- Ex-situ. It consists of keeping the population in captivity, when a limited number of individuals are taken from the wild and kept in centers. The main objectives are to avoid inbreeding, to maintain the highest possible genetic variation (so it can adapt to new environmental challenges), to make a genetically safe population, and to prevent the population from adapting to captivity.

When designing conservation measures and strategies for endangered species it is not only essential to know the genetic situation of the populations and the relationship between them, but also to consider and assess the facts which generated those patterns (Rúa, 2013). For instance, both remnant populations in the Iberian lynx present low diversity and high differentiation; however, the genetic management to improve this situation can be really different depending on the cause of this pattern. On one hand, if this is the result of a demographic contraction caused by a random fluctuation in allele frequencies, together with an aleatory loss of variants and an accumulation of deleterious mutations generated by genetic drift; crossing populations would help to reduce inbreeding and increase diversity since the hybrid individuals would result less homozygous and there would be enough variants to ease adaptation. On the other hand, if this genetic situation was due to an accumulation of differences over a long period of isolation, the populations would have developed certain traits of local adaptation, which would be lost if the populations were mixed, causing outbreeding depression (loss of fitness due to the crossing of populations too genetically different).

The current genetic patterns were shaped by genetic drift and not natural selection, which supports the genetic management and measures applied in the Genetic Conservation program mixing both remnant populations through captivity centers and reintroductions, which has only had positive results. Thus, low-genetic diversity together with higher risks of inbreeding than outbreeding depression support the ongoing admixture of the two genetic pools both in captivity (Rúa, 2013) and in the wild through translocations (Simon et al. 2012).

As mentioned before, genetics is a very important aspect which is helping the species to survive. In order to have detailed information about its genetic variability, two types of studies

have been realized: genetic and genomic. Genetic studies analyzed 36 microsatellite markers (STR, short tandem repeats) which are DNA sequences from 1 to 6 base pairs located randomly in the genome between genes. They do not codify proteins and their purpose is to identify individuals and assign their progenitors, thus, they give an overall information about inbreeding in a population. Besides, they allow us to quantify the genetic variability and compare it with older populations. In genomic studies, genome sequences and SNP (single nucleotide polymorphisms) markers which are locations in the genome where a nucleotide changes, are analyzed. Thanks to having a genome to compare with the species, we can know if the changes in a protein sequence are deleterious or not. 1500 SNP representing the Iberian Lynx's genetics have been chosen, from the 1.5 million identified. Genomic studies are cheaper and more precise than genetic studies, and they have led to the discovery of the demographic and evolutive history and identified more informative genetic markers for the populations of the species. The recent genetic erosion of Iberian lynx has been severe and has affected both microsatellite and mitogenomic diversity, which has led to loss of diversity through time within populations, an increasing differentiation between populations, and extinction of genetically differentiated populations at the edges of the historical distribution

3. EXPERIMENTAL STUDY

The experimental study of this research consists of an interview that provided information for the theoretical framework and an experiment that used *Drosophila melanogaster* as a model. Given the circumstances that the Iberian lynx is undergoing and the consequences of genetic drift in small populations, this experiment attempts to demonstrate the effects of genetic drift in allele frequencies in small populations. The theoretical framework is based on the ongoing situation in the Iberian lynx; however, since it would not be possible to experiment with this endangered species, the experiment was executed with a model of *Drosophila melanogaster*, also known as fruit flies, and aims to establish a relationship between the Iberian lynx loss of variability and the results of the model. This species is a very useful common species to experiment with in genetics, mainly because of their simple maintaining, low cost, and considerable amount of mutants and offspring.

3.1 INTERVIEW TO MARÍA LUCENA PÉREZ

During the research of information to develop the theoretical framework, an interview (appendix 1) was realized with María Lucena Pérez on the twelfth of August of 2020 in order to acquire a better understanding of the genetic erosion and the several bottlenecks the Iberian lynx has been through, genetic diversity, mutations, and genetic and genomic studies.

María Lucena Pérez is a doctoral student in Doñana's Biological Station-Spanish National Research Council (Spanish: Estación Biológica de Doñana - Consejo Superior de Investigaciones Científicas, EBD-CSIC), where she studies variations in the Iberian lynx genome and the role of natural selection in maintaining genetic diversity.

María Lucena Pérez explained that bottlenecks causing massive extinctions are mainly due to human activity. The decline of the Iberian lynx population started with the absence of rabbits (its main prey) and was followed by the change of soil that destroyed the forest extension creating patches in the natural habitat of the Iberian lynx and isolating populations. For this reason, peripheral populations were more likely to be extinct first. The two remnant populations survived because Doñana is a protected area and Sierra Morena is a central population that had a larger number of individuals, so genetic drift had a weaker effect on this population. Although Montes de Toledo had the same characterization as Sierra Morena it disappeared since the members of the population were hunted.

The dynamics of loss of variation and not the fact that the species has always presented a low genetic diversity (since it survived a long period of time with this intrinsic characteristic)

became a threat for the population. Every species has a date of birth and death, but making it disappear due to anthropic pressures and not natural causes forces conservation genetic programs to take place. Therefore, the genetic variability of the Iberian lynx has always been low, but it had never experienced such a sharp decline, so now there is a long term viability threat that did not exist before.

Furthermore, information about mutations was provided, beginning with an explanation of synonymous (neutral) and non-synonymous (produce changes in amino acids, mostly deleterious) mutations. Then she explained that it is possible to know what amino acid is affected by a mutation and how, but not how the new structure of the protein interferes with other proteins nor the phenotypic effect on the individual; concluding that artificial selection and genomic edition exist, but it is very difficult to identify where to operate and how, so mixing populations and improving the habitat of a species is more significant when it comes to improving its long-term viability. Currently, GWAS studies are trying to establish relationships between phenotypes and genotypes. Nevertheless, most living organisms belong to multigene families, so different variants cause an effect or another. Moreover, the fact that two variables are related to each other does not mean that one is the cause of the other. In other words, correlation does not imply causality. Nowadays, correlations are established first, but it is quite difficult to find the cause.

Since large populations often present a higher frequency of heterozygosity it is more likely that a recessive mutation will be passed on generations because selection cannot eliminate it (considering it is deleterious). However, in small populations, recessive mutations become visible for selection to eliminate (inbreeding generates more homozygous individuals), but depending on how small the population is and the circumstances it is undergoing, genetic drift overrides selection and make it more difficult to completely eliminate that mutation.

Iberian lynx researches are based on genetic and genomic studies. María Lucena Pérez ended the interview by explaining the main difference between them. Genetics works on specific sites and genomics in the total variability, which means a qualitative and quantitative improvement since it allows us to access functional sites; whereas microsatellites do not provide information about functional sites, so they are neutral (Non-Selectively Constrained). Genomics is more detailed than genetic studies; however, sometimes it is more efficient to use genetics since it provides enough tools to solve the initial problem without having to develop the entire genome. For instance, it would only be necessary to use microsatellites when analyzing the effects of genetic drift (since it affects neutral sites), as long as it is not being compared with natural selection.

Finally, it was mentioned that genetic drift causes genetic erosion since not only decreases diversity but also leads to the fixation of deleterious mutations because it overrides purifying selection. Nonetheless, although it is very visible in neutral sites, selection cancels it out in functional sites because there are alleles that strongly need to maintain diversity, such as MHC (immune system genes).

3.2 DROSOPHILA MELANOGASTER

3.2.1 Characterization

Drosophila melanogaster is a type of fly that belongs to the order of Diptera in the family Drosophilae (fig. 15). It is also known as fruit fly since it is a diurnal species that feeds and reproduces on fruit or other decaying plants. Like other insects, it has six legs, and its body is divided into three parts: head, thorax, and abdomen. It presents an articulated external skeleton that provides protection to the inner parts of the body.

Taxonomic rank
Kingdom: Animalia
Phylum: Arthropoda
Class: Insect
Order: Diptera
Family: Drosophilidae
Genus: Drosophila
Species: *Drosophila melanogaster*

Fig. 15. Taxonomic rank of *Drosophila melanogaster. (*own source*)*

Wild flies present a brownish-yellowish body, which varies in mutants, and dark rings across the abdomen, different in males than in females (see sex identification section). It also has stiff sense hairs over its body.

Thorax is also divided into three parts: prothorax, mesothorax, and metathorax, with two legs in each one. Red eyes are characteristic of wild flies since it changes color in mutants.

Diptera have one pair of fore wings on the mesothorax and a pair of halteres (reduced back wings) on the metathorax that stabilize the flight. There are different mutants with different fore wing sizes and shapes, but in wild flies, they are light, flat, and almost colorless; as well they have a rounded edge, and they are extended beyond the abdomen. Moreover, they have a set of veins that give them stiffness (necessary for flight) and they are divided into cells (fig. 16).

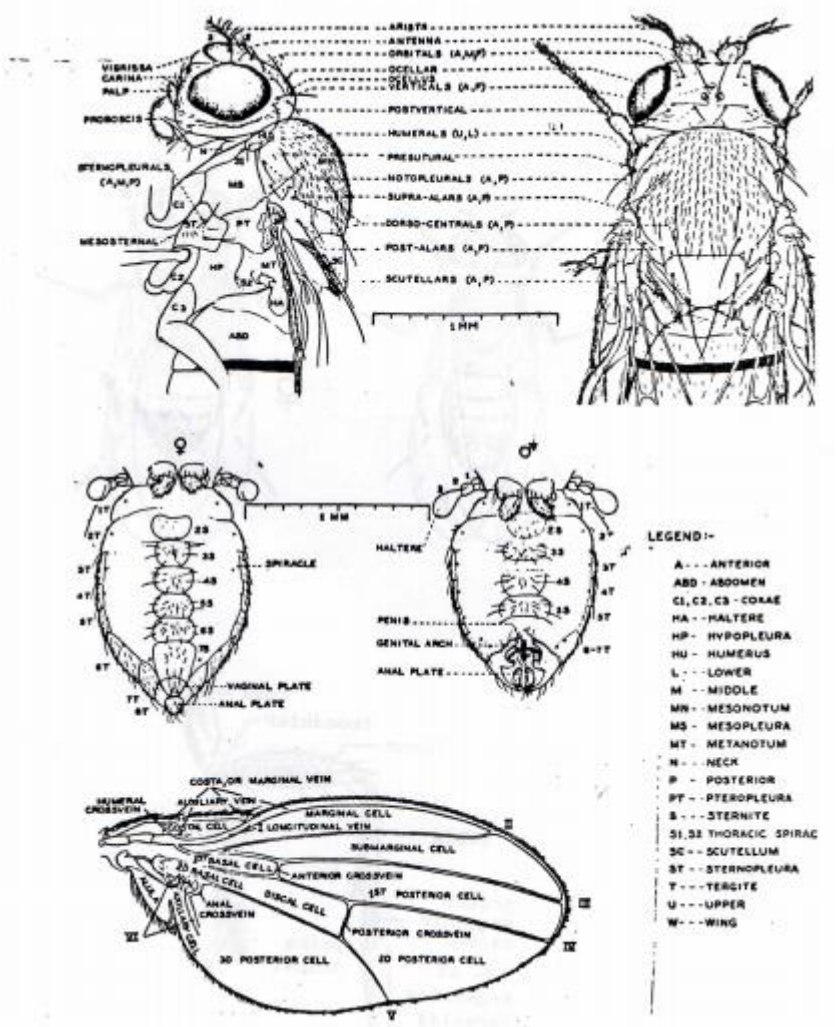

Fig. 16. Morphologic aspects of *Drosophila melanogaster*. (L. Lindsley and E.H. Grell).

3.2.2 Biological cycle

Drosophila melanogaster are insects whose development consists of four stages: embryo, larva, pupa, and adult (table 1). The duration of this life cycle depends on the temperature the flies are living with. At 20ºC the cycle lasts about 15 days and at 25ºC 9 days. Under this latter temperature, each stage has a different duration (fig. 17).

The egg's fecundation is placed in the uterus and once ejected the embryo is covered by two membranes that allow gas exchanges with the atmosphere. The eggs are laid in the media and present two antennae that allow them to float.

Larvae are very active and eat the media creating galleries and cavities. They become pupae when they are mature enough metamorphosis occurs, which makes the larva progressively get harder and darker. When the imago is formed it breaks the cover and emerges. At first, the adults have their wings folded and no pigmentation. They remain virgin the six first hours, however, on the tenth day there usually is a distinguished decline in reproduction. Adults life

depends on the temperature and the available food, but under apt conditions, they can last a few weeks.

horas	días	estadio
0	0	huevo
0-22	0-1	embrión
22	1	larva (estadio 1)
47	2	primera muda (estadio 2)
70	3	segunda muda (estadio 3)
118	5	formación del "puparium"
122	5	muda prepupal (estadio 4)
130	5,5	pupa: formación de la cabeza, alas y patas
167	7	pigmentación de los ojos
214	9	emergencia del adulto con las alas plegadas
215	9	alas desplegadas

Table 1. *D. melanogaster* development (Experimental genetics guide, UAB).

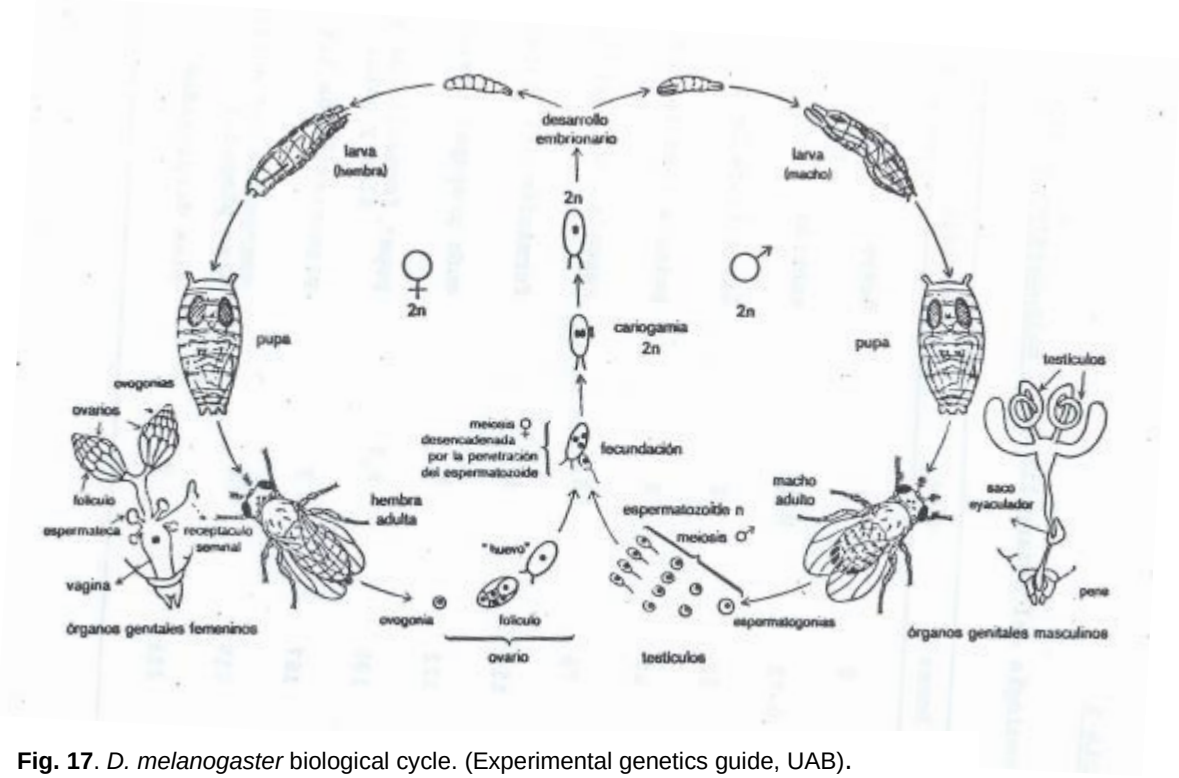

Fig. 17. *D. melanogaster* biological cycle. (Experimental genetics guide, UAB).

3.2.3 Sex identification

When it comes to experimenting with this species, it is necessary to know the different characteristics males and females present.

During the larval phase, the males' testicles are larger than the female's ovaries. This difference is easy to appreciate during the third larval stage through a binocular.

Through the mature pupa's cover, the sex combs become visible (only in males) in the first tarsus pair. In order to observe this trait, it is necessary to examine the pupae's ventral face.

The adult flies have clearer sex differences. The pigmentation in the dorsal face of the distal part of the abdomen is continuous, generating this way a dark stain. Females are bigger than males, and the abdomen tip is more pointed than in males. Sex combs are still visible in males (only through a binocular). Moreover, females present a pale vaginal plate, besides the anal plate, and males a brown genital arch, aside from the anal plate, too.

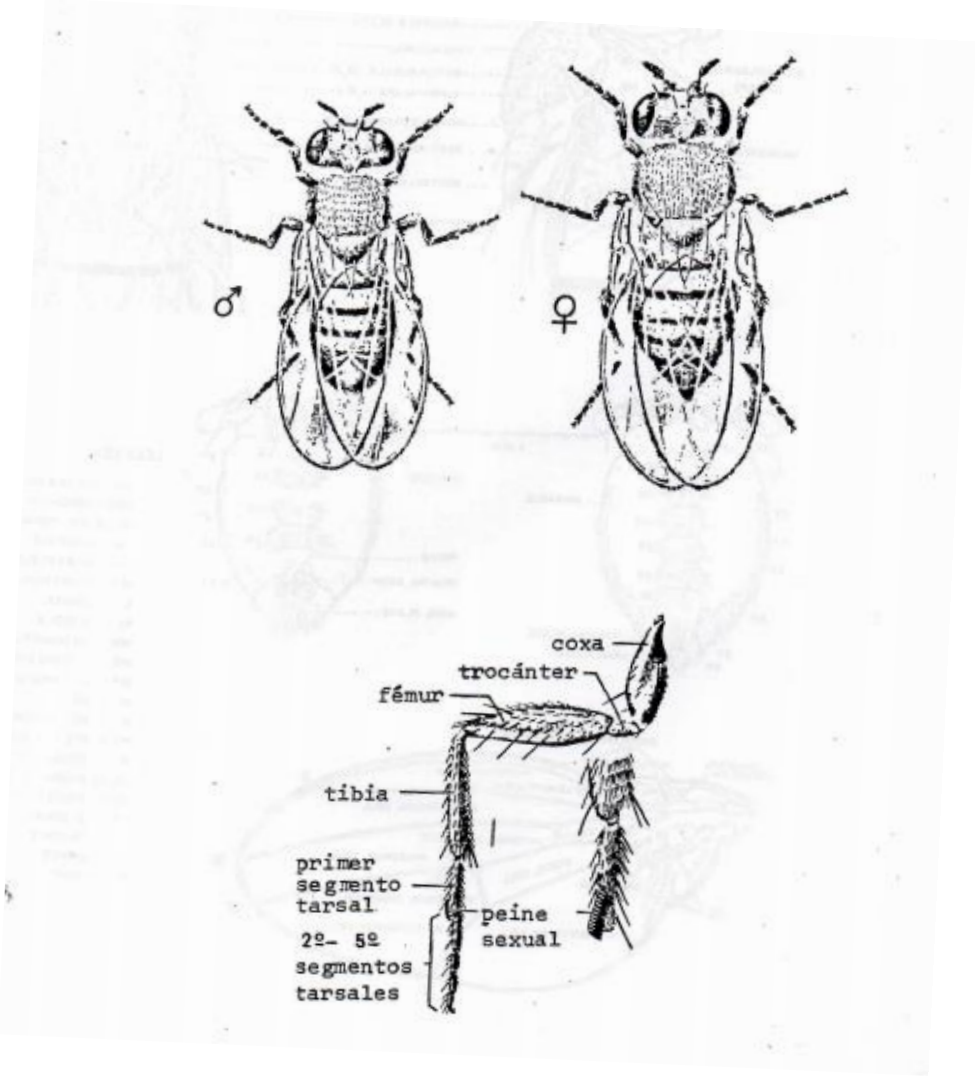

Fig. 18. Male and female of *D. melanogaster*. Sex comb of a male. (Experimental genetics guide, UAB).

3.2.4 Manipulation

In order to elaborate a correct experiment with *Drosophila melanogaster*, it is necessary to have some knowledge on how to manipulate these insects in the laboratory.

Drosophila melanogaster populations are kept in vials covered using a piece of cotton as a lid. They usually are under 23ºC-25ºC and fed with a certain culture medium (see appendix).

When crossing individuals and creating populations or observing and classifying individuals from a population it is necessary the use of ether in order to numb them. Realizing this procedure requires a vial with the same or a similar diameter as the population vial. Firstly, the culture vial will be gently hit a few times, so the flies fall at the bottom and do not escape, and the cotton lid will be removed. It is recommendable to have a shock-absorbing surface, such as cork, when gently hitting the vials in order to not damage them and prevent the culture

medium from falling. Secondly, the top of both vials will be joined (the empty one at the bottom and the population vial on top, so it is upside down) and the flies will fall in the new vial (softly hitting the vials if necessary). Finally, a wet (with ether) piece of cotton covered with a thin gauze will be used as a lid. Few seconds later the flies will be numbed and ready to be observed through the binocular and manipulated with a thin brush. It is important to mention that as soon as the flies are anesthetized, they need to be transferred to a petri dish to be observed, since ether can kill them if they remain longer under its effects. These insects last approximately seven minutes numbed, thus, it is advisable not to work with more than thirty at a time, since they could escape if they wake up in the petri dish. If this latter scenario occurred, the insects would need to be etherized again.

Once the flies are classified a ready to form a new population they cannot be directly put in the new vial since they can get stuck in the medium vial, so different solutions could be used:

- Put the vial horizontally so the flies are not in contact with the culture medium and wait until they awake to put the vial vertically (fig. 20).

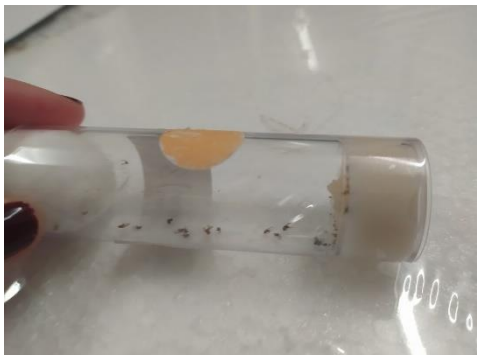

Fig. 20. (own source)

- Leave the numbed flies in the petri dish or a piece of paper and place the vial upside down on the flies, thus, when awake they will be inside the vial and, since they tend to go up, there will be no flies left on the petri dish or the paper and the vial will be ready to flip and cover (fig. 21). The experiment in this project was made with this option since it is easy to notice when the flies are awake. The other options were not considered since it takes a while to elaborate the cones and transfer the flies with the brush to the vial or cone. Therefore, if the flies are left on a surface and covered with the vial, they will be entering the vial themselves when they wake up and in the meantime the experiment can continue. Thus, while waiting for a population to awake, other populations or flies can be manipulated.

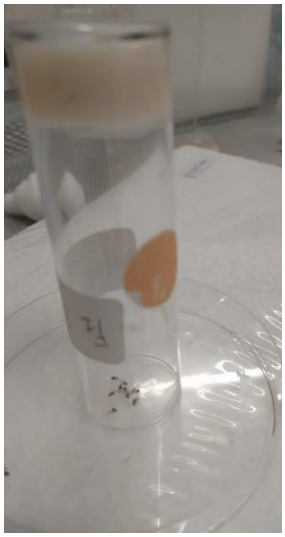

Fig. 21. (own source)

3.2.5 Obtention of virgin females

An essential aspect to take into consideration when experimenting with *Drosophila melanogaster* is having virgin females since they first need to be assigned to a population, so they will only mate with the individuals of the new population and the genetic results will be valid and reliable. Females can be separated from males when they are pupae or adults, however, it is easier to do it when they are adults since pupae are easier to damage and hard to take off the vial.

When adults emerge, since the parents will have been removed previously during pupae stage to avoid inbreeding, it takes between six and eight hours for them to be fertile, so this is the interval of time to separate males from females. The adults will be manipulated as explained above. If there are enough individuals to form the wanted population, put the number of males and females desired together in the new vial. Otherwise, if there is a lack of individuals to form a population, place the obtained females in a vial and the males in another, and wait until more flies are born.

The first days or hours there usually are more females emerging because their life cycle ends first, so the abundance of females in the beginning is normal.

3.2.6 Genetics

Drosophila melanogaster owns four pairs of chromosomes (2n=8), the first one belongs to sexual chromosomes (XX in females and XY in males) and the rest are autosomes (II, III, and IV) (fig. 22). Therefore, females have four pairs of homologous chromosomes and males three (the Y chromosome barely has genes). As a result, we can distinguish four ligament groups: X, II, III, and IV. The last one is smaller, so it has less genes. Its genome was sequenced in 2000, so the 13600 genes of this species were classified and organized through gene mapping.

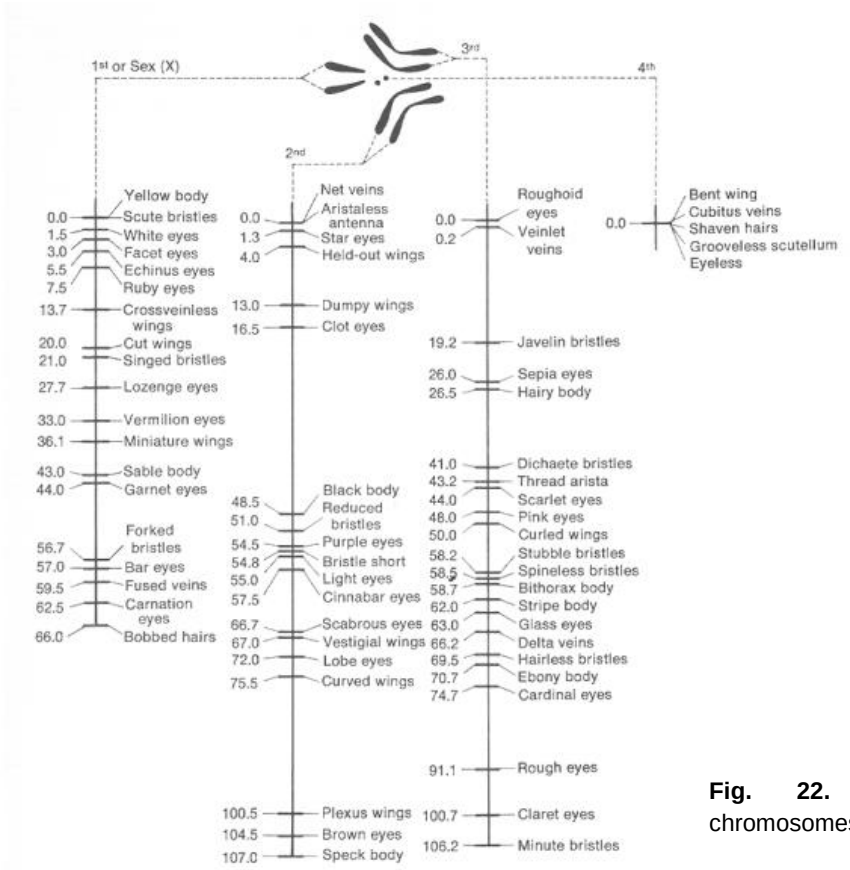

Fig. 22. *Drosophila melanogaster* chromosomes (Madl, 1997).

3.2.6.1 Mutations

Drosophila melanogaster has plenty of mutations that are used in different experiments (table 2). This project will study the effects of genetic drift on gene frequencies through the vestigial mutation (vg), located in chromosome 2 and locus 67. The phenotype of this recessive mutation is expressed as reduced wings that stop the insects from flying. This mutation will not affect the fitness of the population because the flies will be kept in an environment where their wings are not necessary, so natural selection will not be able to act on this trait.

	Wild-type (+)	Mutant Type
Eye	red, oval in shape, and many faceted	white, black, apricot, scarlet red, pink, or brown; changes in shape and number of facets
Wing	smooth edges, uniform venation, extend beyond the abdomen	changes in size and shape; absence of specific veins; changes in position in which wings are held when at rest
Bristle	fairly long and smooth (note distribution on head and thorax)	shortened, thickened, forked, or deformed (note changes in pattern of distribution)
Body Color	basically gray, with pattern of light and dark areas	black (in varying degrees), yellow (in doubtful cases color can often be determined clearly on wing veins and legs)

Table 2. Different mutations used in *Drosophila melanogaster* experiments.

3.3 EXPERIMENTAL DESIGN

3.3.1 Introduction

This experiment will use *Drosophila melanogaster* populations in order to study the effect of genetic drift (together with population size and inbreeding) on gene frequencies by generating bottlenecks in every generation of each population. It attempts to elaborate a model with populations that will only be undergoing one evolutive force: genetic drift, in order to assure that the observed results are the effects of this evolutive force. Hence, this experiment studies the frequency of a neutral allele (vestigial wings) in finite populations that will be completely isolated; so, migrations and natural selection cannot take place and it is not likely for a mutation to appear because there will not be any environmental changes nor enough generations for it to occur.

3.3.2 Problem to solve

This experiment aims to find the answer to the following question:

Is genetic drift present? If it is, how does it affect gene frequencies and what is the influence of population size and inbreeding? If it is not, how should the experiment be like so that genetic drift is present and how does the absence of this evolutive force affect gene frequencies?

3.3.3 Hypothesis

The results will not match the Hardy-Weinberg law, which indicates that every population will be undergoing the effects of genetic drift. This evolutive force (in the form of consecutive bottlenecks) together with the small population size and the increase of inbreeding in each generation will lead to loss of variation and to the fixation of the vestigial (vg) allele in some populations. For this reason, the smallest populations with the highest initial allele frequency will tend to be fixed first.

3.3.4 Variables

The independent variables in this experiment will be population sizes and parental population genotype frequency. The dependent variable will be the gene frequencies resulting from the bottlenecks in each filial generation. Control variables will be temperature (23-25ºC), culture medium prepared, habitat provided (vials with the same diameter only containing food), and to maintain the bottlenecks based on chance.

3.3.5 Materials

- 20 vials (being used during the experiment) and extra vials (recommendable to be able to handle unexpected situations).
- Binocular magnifier.
- Brush.
- Petri dish.
- Culture medium (see appendix 3 for recipe).

3.3.6 Procedure

The experiment will consist of two starting *Drosophila melanogaster* populations, all wild (dominant homozygotes) and all mutants (vestigial recessive mutation), which will be reproduced concurrently until there are enough individuals to start the experiment, which is 80 individuals (40 males and 40 females) in each population.

1. Begin with two populations in two different vials with a gene frequency 1:1 (50%-50%) in a population and 4:1 (75% wild - 25% vestigial) in the other. Thus, the two parental population will consist of the following individuals:
    - 20 vg+vg+ ♂, 20 vg+vg+ ♀, 20 vgvg ♂, and 20 vgvg ♀.
    - 30 vg+vg+ ♂, 30 vg+vg+ ♀, 10 vgvg ♂, and 10 vgvg ♀.
2. Calculate the H-W equation.
3. When the larvae are visible, move the parents from each population to another vial and keep them until the experiment is over in order to observe the frequencies in the resulting population.
4. When the adults emerge (9-10 days since the eggs were laid if the room temperature is 25ºC) put each population in a different vial (one at a time) and use ether to numb them so they can be manipulated.
5. Now, the offspring of the two populations will result in twelve populations (six from each parental population) undergoing a bottleneck. Each new F1 population will consist of a certain number of individuals randomly chosen. From each initial population, there will be two F1 populations (total of four) made up of eight individuals, four males and four females; an eight individual F1 population (total of two) with randomly selected sex; two F1 populations (total of four) with a census population size of sixteen, eight males and eight females; and, finally, an F1 population (total of two) consisting of sixteen individuals randomly selecting the sex, (see fig. 23).

6. Then, when the larvae are present, the parents from each F1 population will be removed from the vial and released, leaving the offspring until they are born.
7. Realize steps 4, 5, and 6 in order to get an F2, then F3, F4 … until Fn, when fixation is reached, and the experiment is over.

* It is necessary to mention that bottlenecks can create a situation where a generation is only formed of members of one sex (only in the populations where sex is randomly selected), so they will die without leaving descendants or having an allele fixed.

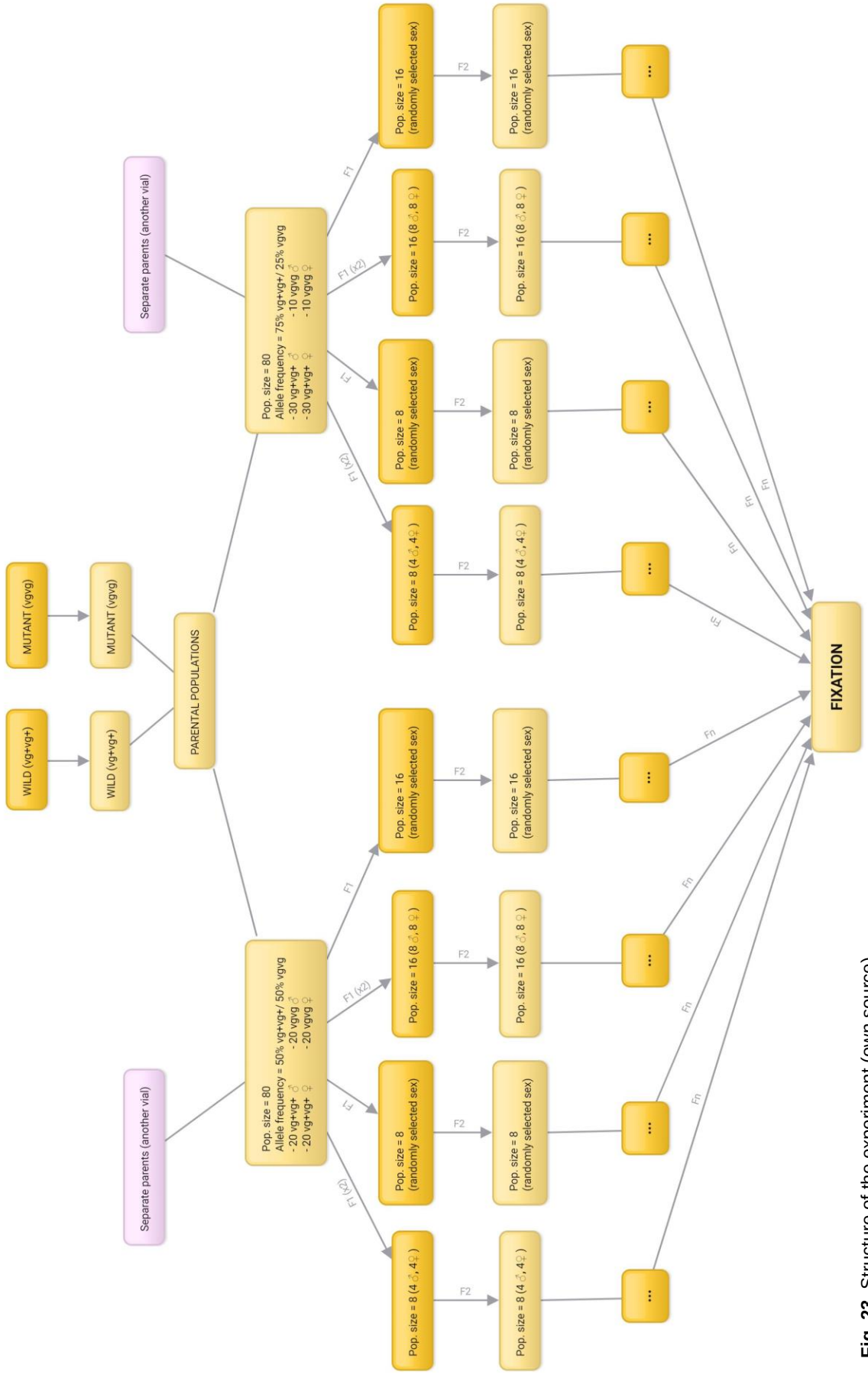

Fig. 23. Structure of the experiment (own source).

3.3.7 Method applied

The organization of the vials and information consisted of a certain pattern. Each vial was color-tagged depending on its paternal population gene frequency: yellow for 1:1 and orange for 3:1. Mutant and dominant homozygous vials had a blue tag. Moreover, they also had a label that identified the vial and prevented confusion. There were four different types of labels (filial generations followed the same pattern) (fig.24 and 25).

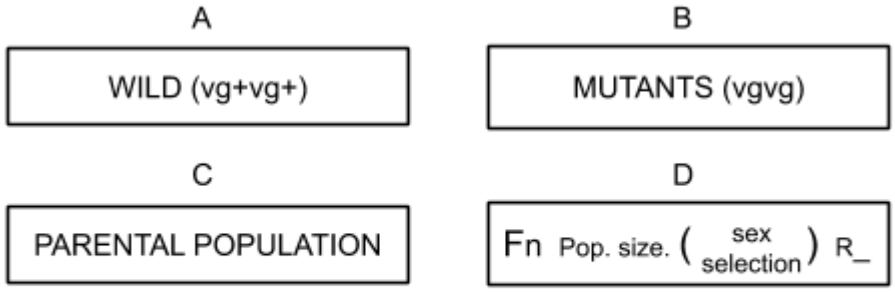

Fig. 24. (A) Label for dominant homozygous vial. (B) Label for vestigial vial. (C) Label for parental population vial. (D) Label for filial generation vials, where *n* is the generation, *pop. size*. is the number of individuals forming that population, *sex selection* indicates whether the sex of the bottlenecked individuals is chosen by chance or not, and R_ refers to the replicas.(own source).

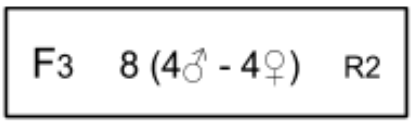

Fig. 25. Example of filial generation label: second replica of the third generation of a population formed of eight bottlenecked individuals where half of them are males and the other half females. (own source).

The results of each filial generation were written in a table following the same pattern. Each table is titled with the parental population gene frequency (1:1 or 4:1), the population size of the bottlenecks (4 or 16), the kind of sex selection applied, and the number of replica (if there are any) (table 3).

Parental population gene frequency			**Pop. size (sex selection)**			**R_**					
F1						F4					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male			Male			Male			Male		
Female			Female			Female			Female		
F2						F5					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male			Male			Male			Male		
Female			Female			Female			Female		

F3						F6					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male			Male			Male			Male		
Female			Female			Female			Female		

Table 3.

This experiment was planned to be made in summer since it is easier to reach the optimum temperature (23-25ºC). However, CESIRE was not able to provide *Drosophila melanogaster* due to COVID-19. In the end, the experiment started on October seventh of 2020 with two wild vials of homozygous individuals (vg+_) and two vestigial vials (vgvg) (fig. 26). Wild flies were supposed to be homozygous (vg+vg+), but when the offspring were observed, one of the wild vials contained flies that presented an unknown mutation, later identified as another form of vestigial mutation (fig. 27). Thus, that vial was not used in the experiment.

Fig. 26. Vestigial flies (own source).

Fig. 27. Vestigial flies (own source).

Furthermore, the flies obtained to form the parental generation were born within 20 days (twice as much as the *Drosophila melanogaster* biological cycle) because the experiment was made during fall and winter, so the temperature needed to be higher. This experiment was made in a residence (so an incubator or other technology advanced extra tools were not available), and in order to reach the needed temperature, the vials were put under the sun during warm daylight hour (i.e. midday), by the fire or in a pot with hot water (fig. 28) during cold afternoons and evenings, by an oil heater, or in a carton box during the night. The temperature in the carton box was raised by putting foam core boards (since it is an isolating material) on the inner sides, top and bottom, blankets (to keep the heat) and battery charged hand warmers (they emit heat). Later on, an old yogurt maker replaced the hand warmers, since it reached

the optimum temperature and maintained it for a longer period of time (fig. 29). Moreover, it does not need to be charged since it works by plugging it in and the blankets did not have to be used anymore.

Fig. 28. Vials in a pot with hot water with plastic lids on the sides to stabilize them (own source).

Fig. 29. Carton box with yogurt maker and foam core boards on the sides. A thermometer is used to check the temperature (own source).

This experiment could only reach seven generations before its deadline day; for this reason, there were some populations that were not fixed, since it was chance what determined gene frequencies. Other populations presented 100% of wild individuals in the sixth generation (F6), so instead of letting it reproduce and obtain a seventh generation a backcrossing was made in order to find out whether the vestigial allele had been lost or not. A backcrossing is a process that takes into consideration Mendelian genetics and consists of crossing a dominant individual with a recessive individual and analyzing the offspring in order to identify the genotype of the dominant individual. The backcrossings in this experiment consisted of separating wild males and females of the F6 in different vials (so they would not mate between them) and putting vestigial individuals of the opposite sex (i.e., wild males with vestigial females). Parents were moved to another vial when the first pupae were visible and then the offspring were analyzed. Thus, if there was any vestigial individual, it would mean some F6 individuals were heterozygous. On the other side, if all the offspring were wild, it would most likely mean that F6 individuals were homozygous for the dominant allele.

3.4 RESULTS AND DISCUSSION

3.4.1 Hardy-Weinberg calculation

The Hardy-Weinberg calculation will use the data of the frequency of parental populations and the results will be compared with the obtained results of the experiment. Then a Chi-squared calculation will be developed in order to make this comparison. As the theoretical framework explained, there are two equations for the Hardy-Weinberg equilibrium:

$p + q = 1$

$\mathrm{p}^2 + 2\mathrm{pq} + \mathrm{q}^2 = 1$

Firstly, it is necessary to make sure that the established frequencies in each parental population match the equations:

Parental population 1:1:

$0.5 + 0.5 = 1$

$0.5^2 + 2 \cdot 0.5 \cdot 0.5 + 0.5^2 = 1$

Parental population 4:1:

$0.75 + 0.25 = 1$

$0.75^2 + 2 \cdot 0.75 \cdot 0.25 + 0.25^2 = 1$

Now, we can find the genotype and phenotype proportions in Hardy-Weinberg equilibrium.

Parental population 1:1:

$\mathrm{p}^2 = 0.5^2 = 0.25$

$\mathrm{q}^2 = 0.5^2 = 0.25$

$2pq = 2 \cdot 0.5 \cdot 0.5 = 0.5$

Thus, the genotype frequency in Hardy-Weinberg equilibrium for the following generations will be 1:2:1.

Parental population 4:1:

$p^2 = 0.75^2 = 0.5625$

$q^2 = 0.25^2 = 0.0625$

$2pq = 2 \cdot 0.75 \cdot 0.25 = 0.375$

Thus, the genotype frequency in Hardy-Weinberg equilibrium for the following generations will be 9:1:6.

Calculating the phenotype ratio only requires adding p^2 + 2pq for the dominant allele (vg+) and using the value of $q^2$for the recessive allele (vg).

Parental population 1:1:

$p^2 + 2pq = 0.25 + 0.5 = 0.75$

$q^2 = 0.5^2 = 0.25$

Thus, the phenotype frequency in Hardy-Weinberg equilibrium for the following generations will be 3:1.

Parental population 4:1:

$p^2 + 2pq = 0.5625 + 0.375 = 0.9375$

Thus, the phenotype frequency in Hardy-Weinberg equilibrium for the following generations will be 15:1.

3.4.2 Filial generations results

The obtained results in this experiment demonstrate the effects of genetic drift on gene frequencies and the importance of population size and inbreeding. The random fluctuations over generations through bottlenecks and the continuous increase of homozygous individuals in each generation proves the presence and effects of genetic drift and inbreeding.

Bottlenecked populations with eight individuals were fixed before the ones with sixteen individuals, which all were either fixed in the seventh generation or not fixed. We can also observe the significance of population size in population 1:1 8 (random selected sex) and 1:1 16 (8♂ - 8♀) R1. Although the second one had a 7:9 phenotype frequency in the F1, the first one was fixed (for the vg allele) a generation earlier starting with an F1 of a 3:1 ratio (table 4).

Population size ↓	Parental population gene frequency			
	1:1		4:1	
	Generation of fixation	F1 phenotype frequency	Generation of fixation	F1 phenotype frequency
8 (4♂ - 4♀) R1	F4 (vg)	5:3	F6 (vg+)	7:1
8 (4♂ - 4♀) R2	Not fixed (8:0)	5:3	F5 (vg)	5:3
8 (random selected sex)	F6 (vg)	3:1	Extinction (only females left in F3)	8:0
16 (8♂ - 8♀) R1	F7 (vg)	7:9	Not fixed (1:1)	3:1
16 (8♂ - 8♀) R2	Not fixed (16:0)	15:1	Not fixed (16:0)	15:1
16 (random selected sex)	Not fixed (1:3)	3:1	Not fixed (5:3)	13:3

Table 4.

Table 4 also shows how the Hardy-Weinberg law since the populations were not infinite due to the continuous bottlenecks they experienced and the presence of genetic drift. None of the final frequencies of the bottlenecked populations matched the Hardy-Weinberg law (except 4:1 16 (8♂ - 8♀) R2), which adds another prove about the Hardy-Weinberg equilibrium not being reached. It is very likely that the last frequency of population 4:1 16 (8♂ - 8♀) R2 matched the Hardy-Weinberg law because of a coincidence, since every population experienced changes in allele frequencies in every generation.

Sharp increases in the number of vestigial offspring are evidence of inbreeding effects. In F1 generations it is not possible to know to what extent the individuals are related to each other because they come from populations of 80 individuals where it is likely that some individuals will share a common parent but not which one or to what extent. Thus, inbreeding is more visible in small populations, so they were fixed first because a smaller number of individuals means higher inbreeding (there are higher chances of having close related individuals after every bottleneck). Random selected populations had the highest inbreeding rate since the difference between the number of male and female individuals results in offspring sharing the same parent (detailed explanation in the results of the populations where this occurred). Nevertheless, it is difficult to say to what extent inbreeding affects non-random selected sex populations, since there were some agents that also favored the persistance of vestigial individuals, such as the easy manipulation with vestigial flies and the impossibility for them to escape. The reliability and extent of inbreeding in these populations will be argued in the discussion section. The following results do not mention the presence of these agents because they aim to justify and find the presence of genetic drift and inbreeding with the data, but they

are taken into consideration for the next section, so the reliability of genetic drift and inbreeding will be discussed.

Parental populations allele frequency was also significant to determine the allele frequency of F1 populations and compare them. As table 4 shows, 1:1 F1 populations had a higher number of vestigial populations than 4:1 F1 populations (which explains why populations having the 1:1 parental population reached a faster fixation), except 4:1 8 (4♂ - 4♀) R2, 4:1 16 (8♂ - 8♀) R1, and 4:1 16 (8♂ - 8♀) R2; which had the same F1 phenotype frequency as some 1:1 F1 populations.

Population 1:1 8 (4♂ - 4♀) R1 started with a 5:3 phenotype frequency and had the vestigial allele fixed in the F4. However, some of the offspring in the second generation flew away when some spontaneous breeze made the cotton lid fall, so the F3 was mainly formed of vestigial individuals, which favored the fixation of this allele.

F1 of population 1:1 8 (4♂ - 4♀) R2 presented a 5:3 ratio (same as population 1:1 8 (4♂ - 4♀) R1) but was not fixed. In fact, the wild allele was close to being fixed, since F5 and F6 were 100% wild. However, not all the members of the population were homozygous since a backcrossing made showed one vestigial descendant. Thus, the vg allele was still present in this population but genetic drift and inbreeding increased the frequency of dominant homozygous (vg+vg+) individuals because the vestigial allele was in decline.

Population 1:1 8 (random selected sex) started with a 3:1 phenotype and had the vestigial allele fixed in the sixth generation with five females and three males. Since it was a random selected sex population, there were generations where there was a significant difference in number between sexes, such as the F2, which only had two vestigial males and six wild females. This means that all the wild offspring from that generation were heterozygous, and the vestigial resulted from the crossing of vestigial males and heterozygous females. Moreover, this situation increases the inbreeding rate since all the offspring have a 50% chance of sharing the same father.

Population 1:1 16 (8♂ - 8♀) R1 started with a 7:9 phenotype proportion and lost the dominant allele (vg+) in the F7 bottleneck. This population had a high number of vestigial offspring from the beginning due to its F1 phenotype frequency favoring the existence of vestigial individuals. Each bottleneck accumulated vestigial individuals in every generation until F6 generation only left eight wild individuals (and 24 vestigial) that were not selected (due to genetic drift) for the following generation.

Population 1:1 16 (8♂ - 8♀) R2 was not fixed although the F6 had a phenotype frequency of 16:0. It was not likely to have the vestigial allele fixed since it had a 15:1 F1 phenotype frequency. Thus, the number of vestigial flies not significant enough to not be eliminated with the bottleneck. Generation F6 only presented wild flies, but it was likely that the dominant allele (vg+) had not been fixed yet because the presence of a vestigial male among the F5 individuals increased the chances of having heterozygous flies when mating with a wild female. Despite of it, a backcrossing was made to assure this prediction, which was proved when observing the vestigial offspring.

Population 1:1 16 (random selected sex) had a 3:1 ratio for the F1 and showed a continuous increase of vestigial individuals over time, although it did not have enough time to reach fixation since F7 had a 1:3 ratio. The difference in number between males and females in each generation could also increase inbreeding but it was not as significant as in other random selected sex populations. The F5 generation only had one wild male (and five vestigial males), which increased the number of vestigial and heterozygous individuals; thus, the frequency of the vestigial allele.

Population 4:1 8 (4♂ - 4♀) R1 started with a 7:1 phenotype and the vestigial phenotype disappeared in F4 and the following bottlenecks. A few F4 offspring were vestigial but excluded from the following bottlenecked population. The sixth generation was also wild so instead of letting it reproduce and obtain a seventh generation a backcrossing was made in order to find out whether the vestigial allele had been lost or not. Both wild male and wild female backcrossings (with vestigial flies of the opposite sex, as explained before) resulted in all wild offspring. Thus, it is very likely that the continuous bottlenecks fixed the dominant allele (vg+).

Although its parental population had a 4:1 phenotype frequency, the F1 bottlenecked generation of the population 4:1 8 (4♂ - 4♀) R2 had the same ratio, 5:3, as 1:1 8 (4♂ - 4♀) R1 and 1:1 8 (4♂ - 4♀) R2. This population (4:1 8 (4♂ - 4♀) R2) had the vestigial (vg) allele fixed within five generations, which suggests the presence of high inbreeding leading to homozygous recessive individuals.

Population 4:1 8 (random selected sex) was not fixed nor able to reproduce further than three generations because it went extinct. It started with an 8:0 phenotype frequency with some heterozygous individuals (there were vestigial offspring), but the F3 bottlenecked population was only formed of females, so this generation (with a 7:1 phenotype frequency) could not leave any descendants. Furthermore, as in other random selected sex bottlenecks, there was

a higher probability of inbreeding since generations did not have the same number of males and females, specially F1 with two males and six females.

The first bottleneck in the population 4:1 16 (8♂ - 8♀) R1 left the F1 with a 3:1 phenotype frequency. Within seven generations none of the alleles were fixed, but vestigial individuals increased in frequency in every generation until F7, with a 1:1 proportion. This data suggests a high inbreeding rate since the number of vestigial offspring was quite significant. Furthermore, the fact that F3 had ten vestigial offspring but only one vestigial individual among the parentals indicates the presence of several heterozygous individuals. This latter aspect together with inbreeding led to the increase of the vestigial allele.

Population 4:1 16 (8♂ - 8♀) R2 was not fixed either. Its F1 generation had a 15:1 ratio (like population 1:1 16 (8♂ - 8♀) R2) and there were few vestigial individuals, which stayed in low numbers in every generation. The seventh generation had no vestigial individuals (16:0), but it was not possible to make a backcrossing due to lack of time. However, it is likely that the vestigial allele was not extinct because some of the offspring in every generation were vestigial and having at least one vestigial individual in a bottlenecked population (except F3 and F7) increases the probability of heterozygous offspring.

Population 4:1 16 (random selected sex) had a starting phenotype frequency of 13:3 and reached a 5:3 ratio in F7. This data provides evidence of inbreeding since homozygous recessive individuals progressively increased in frequency. Generations F3 (with only three males) and F5 (with only three females) led to a following generation (F4 and F6) with high inbreeding because of most individuals of the same sex. Moreover, the F5 generation only left 19 descendants, so the bottleneck forming the F6 population only excluded three individuals, which assured the persistence of vestigial individuals.

As the latter table showed, having more females than males does not affect as much as if the situation were the opposite. The number of offspring is not as low as when there are more males than females because females are the ones who lay eggs and a decline of this kind of individuals means less eggs will be laid. On the other hand, if there are less males than females the number of offspring does not necessarily lead to a sharp decline because they can reproduce with more than one female. However, this scenario highly increases inbreeding due to the number of offspring sharing the same father.

3.5 DISCUSSION

The results confirm the hypothesis since the data of each population does not match the Hardy-Weinberg law because phenotype frequencies (and probably allele frequencies but it could not be tested) randomly changed over generations, which indicates that every population underwent the effects of genetic drift. This evolutive force was present because no mutations appeared, migrations could not occur since the flies were kept in vials and natural selection was absent since the experiment worked with neutral alleles and the habitat provided to the flies only had culture medium (the flies were completely isolated so there were no predators nor any interactions with other species). Genetic drift (in the form of consecutive bottlenecks) together with the small population size and the increase of inbreeding in each generation led to loss of variation in almost every population and to the fixation of the vestigial (vg) allele in four populations and the wild allele (vg+_) in one. For this reason, the smallest populations with the highest initial allele frequency tended to be fixed first.

The hypothesis did not take into consideration the fixation or increase in frequency of the vg+ allele. The results let us argue that genetic drift leads to loss of variation and the fixation of alleles together with inbreeding. However, not only it increases the frequency of homozygous recessive individuals, but it can also increase the frequency of homozygous dominant, as the results showed in population 1:1 8 (4♂ - 4♀) R2 and in the fixation of the vg+ allele in population 4:1 8 (4♂ - 4♀) R1. This might indicate the lack of heterozygous individuals in these populations, although we cannot know for sure.

The results of the evolution of gene frequencies in populations 1:1 8 (4♂ - 4♀) R1, 1:1 8 (4♂ - 4♀) R2, and 4:1 8 (4♂ - 4♀) R2 represent clear evidence of how genetic drift randomly changes gene frequencies and produces different results. The parental population of 4:1 8 (4♂ - 4♀) R2 was in a 4:1 ratio but the first bottleneck created an F1 with a 5:3 ratio so populations 4:1 8 (4♂ - 4♀) R2 and 1:1 8 (4♂ - 4♀) R1 were fixed with a difference of only one generation although they had very different parental population frequencies (4:1 and 1:1). Then, population 1:1 8 (4♂ - 4♀) R2 ended with no vestigial individuals. This explains how bottlenecks never represent the gene pool or gene frequencies of the parental population.

The theoretical framework together with the cited studies in this research have shown that genetic drift leads to loss of variation and affects to a greater extent small populations, as well as inbreeding is higher in small populations and contributes in loss of diversity by raising the frequency of homozygous individuals. The results of this experiment showed how the smallest populations had a faster loss of genetic variation and justified how inbreeding was present and to what extent. The only explanation for these results in genetic terms (since there were no

other evolutive forces and the experiment showed how bottlenecks consisted of a random selection of individuals) is that random fluctuations over generations (genetic drift) favored an allele or the other due to inbreeding increasing the frequency of homozygous individuals, which is the same situation the Iberian lynx has undergone.

Furthermore, populations with a fixed allele and not fixed populations with a significant change in frequency not only suggest a high inbreeding rate but prove that this event leads to homozygosity since a fixed allele requires the 100% of the individuals to be homozygous.

Nonetheless, it should be mentioned that vestigial flies had more chances to be randomly chosen in each generation than wild flies since they were easy to manipulate because wild flies sometimes escaped, and their wings were very fragile and easy to get damaged. Moreover, some wild flies died when they were born because their wings were stuck in the culture medium, which became sticky and less solid when larvae ate it. Thus, this agent favored vestigial individuals to persist over generations.

Another possibility to observe is that during the experiment flies always tended to stay near the cotton lid when they were not eating. Wild flies reached that location flying, but vestigial flies had to walk up the walls of the vial, which could get hard because larvae had been moving in that area and spreading the sticky culture medium. For this reason, there is a chance that some vestigial flies reproduced between them before reaching the cotton (where they would randomly reproduce with vestigial or wild flies) since they were closer to each other. However, this situation cannot be proved, it is just a speculation.

Although these last two paragraphs generate doubts about the results of inbreeding, the presence of this agent has been justified previously. There is no doubt that random selected sex populations had a high inbreeding rate (although there was no specific experiment on testing it made), but the presence of inbreeding loses reliability with what the last two paragraphs exposed. However, the fact that this experiment worked with small populations and the information provided in the theoretical framework, allows us to state that in non-random selected sex populations there was an inbreeding rate higher than in usual populations but the fixation for the vestigial allele and the loss of diversity was not only due to genetic drift and inbreeding, but also due to these environmental factors.

Populations where the sex was randomly selected were a closer model to the Iberian lynx populations due to the random mating and the higher inbreeding. One of them (4:1 8 (random selected sex)) allows us to explain an extreme effect of bottlenecks: extinction. The population did not go extinct because of genetic drift causing genetic erosion (Iberian lynx situation), but because of genetic drift eliminating the males of a population.

The results obtained in this experiment allow us to extrapolate them to the Iberian lynx circumstances but taking some constraints into consideration. First of all, the environmental agents favoring recessive homozygous individuals needs to be taken into consideration when reaching a conclusion for this experiment. Secondly, this investigation studied the effects of genetic drift by itself on neutral allele frequencies (which are harmless), whereas in the Iberian lynx populations the other evolutive forces are present and the loss of variation caused by genetic drift and inbreeding has affected negatively on the health of the population. Besides, this experiment was focused on one locus, so the effects of genetic drift in the Iberian lynx populations are much larger. Nevertheless, this experiment is able to extrapolate the results to the Iberian lynx circumstances because both *Drosophila melanogaster* populations and Iberian lynx populations were under the effects of genetic drift and inbreeding and experienced loss of variability.

Hence, an improvement for this experiment should be using a mutation that enhances the random selection of individuals, such as eye color. Moreover, doing this study with more populations would have increased the trustworthiness of the results, but it was not possible since this experiment was made during the school year and in a residence.

All these observations and results about genetic drift not only yield to conclusion about this study, but also to other research questions or studies that could be done about genetic drift, such as how the number of generations between bottlenecks affects genetic erosion, or a study about the equilibrium between natural selection and genetic drift, observing when and how it becomes unbalanced and its consequences.

3.6 EXPERIMENT CONCLUSION

This experiment has elaborated a *Drosophila melanogaster* model that has allowed to identify the effects of genetic drift on gene frequencies and the presence of inbreeding over generations. It has been observed how each bottlenecked population experienced random fluctuations over generations with the tendency to raise the number of homozygous individuals due to three agents: genetic drift, inbreeding, and environmental factors.

Moreover, although there were environmental agents acting against them, this experiment concludes that it proved the presence of genetic drift and inbreeding (producing a higher number of homozygous individuals). It is also necessary to claim that the allele frequencies of the parental populations influenced the time of fixation of the bottlenecked populations, but it was genetic drift, inbreeding, and the mentioned environmental factors that determined the course of gene frequencies in each bottlenecked population. Furthermore, this study agrees with the theoretical framework in stating that the smaller a population is the more visible

genetic drift and inbreeding will become, which may lead to extinction by eliminating the members of one sex.

Finally, this experimental study reaches the ultimate conclusion that genetic drift leads to loss of variation and the inbreeding rate between the bottlenecked individuals influences on the time of fixation, establishing this way a direct relationship between the loss of diversity of the *Drosophila melanogaster* bottlenecked populations and the Iberian lynx populations, with the main difference of this latter species experiencing an actual damage on viability. Thus, this model has succeeded in extrapolating its results to the Iberian lynx circumstances taking into consideration the consequences in the viability of each species: genetic erosion threatening the Iberian lynx existence and *Drosophila melanogaster* losing a neutral allele. However, it can be stated that genetic drift affects small populations to a greater extent and together with inbreeding they deteriorate the genetic diversity of a species.

4. CONCLUSIONS

Given the conclusions of the experimental study, we can proceed to conclude this project.

This research study accomplished the purposes set in the introduction. It has determined the effect of genetic drift on gene frequencies and small populations; shown the importance of population size and inbreeding in genetic variation; elaborated a model with *Drosophila melanogaste*r and extrapolated the results to the Iberian lynx circumstances (taking its constraints into consideration); obtained complete knowledge about the circumstances the Iberian lynx has been through and understood the causes of the genetic erosion and the importance genetic drift has in this situation; and finally, it has allowed me to gain research skills as well as to provide a clear detailed study and information. For this reason, it can show solid conclusions.

As the theoretical framework has shown, the Iberian lynx has undergone several bottlenecks throughout its demographic history and its genetic diversity was so low that the species would have gone extinct if conservation genetic programs had not been applied. This species is characterized by a low genetic diversity, which is not dangerous, but it lost so much variation that became one of the most endangered species in the world. The bottlenecks the Iberian lynx suffered are evidence of an unbalanced equilibrium between evolutive forces. Several studies and a coalescent model have provided information about the high inbreeding in the populations and how compromised the long-term viability of this species is. Thus, all the information included in the theoretical framework allows us to understand the seriousness of unbalanced evolutive forces, genetic drift in this situation, and conclude that the main consequence of this evolutive force overriding natural selection is loss of diversity and genetic erosion. This project has also shown to what extent genetic drift affects gene frequencies: extinction. Both the experimental study and theoretical framework have proved extinction is a consequence of genetic drift, so random fluctuations in small inbred populations represent a threat for a species.

However, it is significant to outline that the effects of genetic drift that this research has shown are due to anthropic pressures. Humans have demonstrated the ability to save a species from extinction and improving its genetic damage situation through several research studies and conservation programs. In spite of it, the circumstances the Iberian lynx has been through are due to human activity and the advanced technology and knowledge of genetics has allowed us to be conscious about this situation and save this species. But it is important to realize the genetic damage we have caused to this species, which started its decline with a change in its environment. The Iberian lynx has not been the first species to reach extinction vortex due to

anthropic pressures and will not be the last. Therefore, besides solving the problems we have caused, we should also take into consideration the problems we could cause and search for measures to solve them. We could avoid having to save a species by improving the environment we all share.

In conclusion, this research project accepts its initial hypothesis since it has been observed through the theoretical framework and experimental study that genetic drift randomly establishes gene frequencies and when it is not found in equilibrium with the other evolutive forces (mutations, migrations, natural selection, and genetic drift), it results in loss of diversity. Furthermore, the smaller a population is the more present inbreeding becomes and the more visible the consequences of genetic drift are. Therefore, as population size decreases, inbreeding increases and genetic variation is lost.

5. REFERENCES

Abascal, F. C. (2016). Extreme genomic erosion after recurrent demographic bottlenecks in the highly endangered Iberian lynx. *Genome Biology*.

al., A. e. (2016). Extreme genomic erosion after recurrent demographic bottlenecks in the highly endangered Iberian lynx. *Genome Biology*.

al., E. e. (2010). Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography*.

al., F. e. (2011). Predicting the Probability of Outbreeding Depression. *Conservation Biology*.

Antonio Jimeno Fernández, M. B. (2016). *Biologia.* Santillana.

Barbadilla, A. (2010). *Genética de Poblaciones*. Obtenido de http://bioinformatica.uab.es/base/base3.asp?sitio=geneticapoblaciones&anar=presentacio

Benito, C. a. (2012). Genética, conceptos esenciales.

Bish, P. O. (2010). *National Geographic*. Obtenido de https://www.nationalgeographic.com.es/mundo-ng/grandes-reportajes/el-lince-iberico_2307/1

Charles N. Rotimi, P. (s.f.). *Nationa Human Genome Research Institute*. Obtenido de https://www.genome.gov/genetics-glossary/Founder-Effect

Ellis, E. C. (2010). Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography*.

Felsenstein, J. (2019). *Theoretical Evolutionary Genetics.*

Futuyma. (1998). Wherefore and Whither the Naturalist?

Godoy, J. A. (2017). Spatiotemporal Dynamics of Genetic Variation in the Iberian.

Godoy, J. A. (2018). Aplicación de la genética a la conservación del lince ibérico. *Investigación y ciencia*.

Hedrick & Murray, 1., & Endler, 1. (s.f.).

Hedrick. (1983). Evidence for balancing selection at HLA. *Genetics*.

Hedrick. (2001). Conservation genetics: where are we now? *Science Direct*.

Hedrick. (2012). What is the evidence for heterozygote advantage selection? *Science Direct*.

Kalisz, M. J. (1990). The causes of natural selection. *Evolution International Journal of Organic Evolution*.

Kimura. (1989). The neutral theory of molecular evolution and the world view of the neutralists.

Madl, P. (1997). Drosophila melanogaster protocol.

Marmesat, E. (2020). *Dinámica de la variación genética en poblaciones en declive; variación neutral y funcional en el lince ibérico.*

Masel, J. (2011). Genetic drift. *Cell Press*.

Mathematics. (2016). Obtenido de https://math.stackexchange.com/questions/2009660/basic-question-about-using-the-chi-square-table

Morton, J. F. (1955). Measurement of Gene Frequency Drift in Small Populations. *Society for the Study of Evolution*, 202-214.

Murray, H. &. (1983).

Murray, H. &., & Endler. (1983, 1986).

Nielsen. (2005). Annual Reviews. *Molecular Signatures of Natural Selection*, 197-218.

Ohta, T. (1995). Gene conversion vs point mutation in generating variability at the antigen recognition site of major histocompatibility complex loci. *Springer Link*.

OpenStax College, B. (s.f.). *The genetic code*. Obtenido de https://openstax.org/books/biology/pages/15-1-the-genetic-code

Pablo Razeto-Barry, J. D. (2012). The Nearly Neutral and Selection Theories of Molecular Evolution Under the Fisher Geometrical Framework: Substitution Rate, Population Size, and Complexity.

Palomares. (2012). Possible Extinction Vortex for a Population of Iberian Lynx on the Verge of Extirpation. *Conservation Biology*.

Razeto-Barry, P. (2012). The Nearly Neutral and Selection Theories of Molecular Evolution Under the Fisher Geometrical Framework: Substitution Rate, Population Size, and Complexity. *Genetics*.

Rúa, A. C. (2013). Genética de la conservación: la aplicación de los conceptos de la evolución a la conservación de la diversidad biológica.

Silwa, D. A. (2015). *ISEC*. Obtenido de https://wildcatconservation.org/wild-cats/eurasia/iberian-lynx/

Skenderian. (2012). Obtenido de https://skenderianscience.weebly.com/drosophila-melanogaster.html

Sober. (1984). Force and disposition in evolutionary theory. *Cambridge University Press*.

Wikipedia. (2009). Obtenido de https://en.wikipedia.org/wiki/Hardy%E2%80%93Weinberg_principle

6. APPENDICES

6.1 APPENDIX 1. GLOSSARY

Adaptation: in evolution, any inheritable trait of the phenotype of an individual that increases its probability of survival and reproduction in the environment in which it lives.

Allele: one of the different forms of a gene that exists at a single locus.

Artificial selection: breeding of consecutive generations through human selection of certain parental phenotypes or genotypes in each new generation.

Chromatid: each of the two copies, both joined together, which result from the division of the chromosome.

Diploid: A cell that contains two copies of each type of chromosome (except sex chrom. compare haploid).

DNA polymorphism: natural variation in a DNA sequence in a certain part of the genome.

Dominant allele: allele expressed in the phenotype even when the individual is heterozygous. Thus, if A is dominant over an individual with the genotype AA and Aa will present the same phenotype.

Endogamy: mating or union between individuals within a group or subgroup instead of doing it randomly in a population.

Filial generation: F1, F2, etc. In mendelian genetics the 1st, 2nd, etc. Generation in the line at descent.

Fixed allele: allele for which all members of the population under study are homozygous, so there is no other allele for that locus in that population.

Functional genomics: the study of the patterns of transcribed gene and protein expression and the molecular interactions of the whole genome.

Gamete: mature haploid male or female reproductive cell which is able to unite with another of the opposite sex in order to form a diploid zygote (egg and sperm in mammals).

Gene equilibrium: normal phenotype requiring a gene proportion of 1:1 in the genome.

Gene pool: stock of different genes in an interbreeding population.

Gene: fundamental functional physical unit of inheritance, which passes information on from one generation to the next. It is a portion of DNA formed of a region that is transcribed and a regulative sequence that makes transcription possible.

Genetic marker: allele used as an experimental probe to analyze an organism, tissue, cell, nucleus, chromosome, or gene.

Genetic polymorphism: genetic differences that occur naturally between members of a population.

Genome: complete set of genes or genetic material present in a cell or organism.

Genotype: allelic composition of a cell, referred to the genome, to a certain gene, or to a group of genes. In diploid individuals, half of the genes are inherited from the father and the other half from the mother.

Heterozygous: having two different alleles in each homologous chromosome of a particular gene.

Homozygous: having identical alleles in each homologous chromosome of a particular gene.

Hybrid: (1) Heterozygous. (2) The offspring of two individuals with different genotypes.

Inbreeding rate: the probability of having homozygous individuals in the offspring that results from the zygote receiving two copies of the same allele present in an ancestor.

Locus: position of a chromosome where each gene is placed.

Microsatellite marker: DNA sequences from 1 to 6 base pairs located randomly in the genome between genes. They do not codify proteins and their purpose is to identify individuals and assign their progenitors.

Molecular marker: DNA heterozygosity site, not necessarily associated with a phenotypic variation, that is used as a label for a particular chromosomal locus.

Mutant allele: an allele that differs from the allele present in the wild type.

Neutral evolution: non-adaptive evolutive changes that occur due to genetic drift.

Neutral mutation: a mutation that does not affect the survival and reproductive rate of the organism that has it.

Neutral variation: variation in DNA sequences that is not under selection.

Nucleotide: molecule composed of a nucleobase, a sugar, and a phosphate group; it is the basic structural element of nucleic acids.

Parental generation: In mendelian genetics the individuals that give rise to the 1st filial generation F1.

Phenotype: (1) Effects of a trait (or group of traits) in a certain individual. (2) Visible effects of a genotype.

Polymorphism: presence in a population (or between populations) of several phenotypic forms associated with different alleles of a gene or with different homologs of a chromosome.

Recessive allele: allele whose phenotypic effect is not expressed in heterozygous individuals.

Single nucleotide polymorphism (SNP): natural variation in a single pair of nucleotides in a certain situation of the genome of two or more individuals.

Speciation: formation of new species by separating, from an ancestral species, two or more new species incapable of exchanging genes between them.

Species: a group of organisms that are able to exchange genes between them and that are reproductively isolated from members of other groups.

Viability: probability of survival and development of a fertilized egg in an adult individual.

Wild Type: The genotype or phenotype that is found in nature or in the standard laboratory stock for a given organism.

6.2 APPENDIX 2. INTERVIEW TO MARÍA LUCENA PÉREZ

¿A qué es debido que Doñana y poblaciones periféricas fueron contrayéndose progresivamente de modo que la deriva genética tuvo más efecto y en Sierra Morena y Montes de Toledo se mantuvo grande y conectada para después desaparecer rápidamente? ¿Cuáles son las principales causas de los cuellos de botella y el daño genético sufrido?

Algunos cuellos de botella o procesos de deriva genética son debido a causas naturales. Sin embargo, generalmente el evento que estamos viendo a día de hoy de extinción masiva de muchas poblaciones o disminución de su tamaño en cualquier especie tienen origen antrópico. Los humanos somos conductores de cambio global, como cambios de suelo, consumo de energía, aumento de la población, etc. En el caso del lince Ibérico ha tenido

mucha importancia el cambio de suelo, ya que ha generado una destrucción continua de una extensión de bosque creando parches en el hábitat natural de esta especie. Por esta razón, las poblaciones periféricas fueron las primeras en quedarse aisladas, mientras que las centrales tuvieron más posibilidades de mantenerse conectadas con otros parches naturales, aumentando la probabilidad de supervivencia. En el caso de Montes de Toledo, la persecución directa del animal quedó reflejada en la desaparición súbita de la población. Asimismo, la desaparición de la presa principal, el conejo, también repercutió de gran manera en el declive de la especie.

La población de Doñana lleva mucho tiempo aislada en comparación a las otras poblaciones, pero biológicamente poco tiempo. Si este aislamiento es debido a la deriva, se debería mezclar con otras poblaciones, pero si es debido a la selección natural no, porque es lo que determina las adaptaciones locales.

En los estudios genómicos, ¿cómo determináis si un alelo o mutación es deletérea?

Las mutaciones se clasifican en sinónimas y no sinónimas. Las sinónimas no producen cambios en aminoácidos, de modo que son neutrales; por otro lado, las no sinónimas producen cambios en aminoácidos, que mayoritariamente generan efectos deletéreos para el individuo o población. No obstante, si la mutación aumenta el fitness del individuo será beneficiosa, hecho que generalmente no ocurre. Por tanto, se determina si una mutación no sinónima es beneficiosa o deletérea dependiendo de los cambios y adaptaciones producidos en el individuo.

Cuando hay una mutación deletérea, ¿se puede identificar su efecto? Si es así, ¿se podría modificar artificialmente esa mutación para evitar sus efectos y fijación? Es decir, ¿se pueden identificar y controlar mutaciones deletéreas?

Se puede saber a qué aminoácido afecta y cómo cambia, e incluso hay algunos modelos matemáticos que se aproximan a cómo repercute en la estructura de la proteína. Sin embargo, no es posible identificar cómo esa nueva estructura de la proteína interfiere con otras proteínas o cómo la mutación afecta fenotípicamente al individuo.

Para determinar el efecto de ciertas mutaciones se comparan los fenotipos de distintos individuos para determinar el gen común que presentan y qué alelos repercuten sobre ese cambio. Actualmente, los estudios GWAS intentan relacionar el fenotipo con el genotipo.

Sin embargo, la mayoría de seres vivos pertenecen a familias multigénicas, y suelen ser distintas variantes las que causan un efecto concreto u otro. Por ejemplo, las enfermedades genéticas, en las que no solo un gen es el responsable del efecto que tienen sobre el

individuo, sino que hay distintas variantes que combinadas la originan. No obstante, correlación no implica causalidad, es decir, que dos variables estén relacionadas no significa que una sea causa de la otra. A día de hoy, primero se establecen correlaciones, pero llegar a la causalidad requiere otro paso al que difícilmente se puede llegar.

Por tanto, la selección artificial ya existe y la edición genómica es posible, lo difícil es identificar lo que hay que cambiar y con qué cambiarlo, de modo que es más eficiente y relevante mezclar poblaciones y mejorar el hábitat para conseguir una viabilidad a largo plazo de la especie.

¿Hay alguna mutación dominante o todas son recesivas? ¿Qué consecuencias hay para cada tipo? ¿Cuál es la relación entre la homocigosidad de mutaciones recesivas y la deriva genética? ¿Qué consecuencias tiene y cuál es la gravedad de la situación?

Dominant mutations have the same effect on big and small populations, each individual that presents a dominant allele will suffer its effects or the phenotype corresponding to that mutation. This way, it is much easier for selection to eliminate that mutation in both kinds of populations. On the other hand, recessive mutations will not be visible for selection when they are in a heterozygous individual; thus, selection will only be efficient on homozygous individuals. In this case, since large populations often present a higher frequency of heterozygosity it is more likely that a recessive mutation will be passed on generations. However, in small populations inbreeding generates more homozygous individuals so a recessive mutation will express its effect. This way, selection can eliminate that mutation. Nevertheless, we have to take into consideration the size of the population and the effect of genetic drift, since the smaller a population is the more efficient genetic drift becomes, and since it is in equilibrium with natural selection this latter evolutionary force loses efficiency. Therefore, in small populations, recessive mutations become visible for selection to eliminate, but the circumstances might make genetic drift can counteract selection and make it more difficult to completely eliminate that mutation.

¿Los otros linces también tienen poca variabilidad genética o solo el Ibérico?

Sí, sobre todo el boreal, es decir, la baja variabilidad es una característica intrínseca de la especie.

¿Por qué el lince Ibérico sólo sobrevivió en el sur de España? También, los linces americanos no presentan ningún daño genético y el lince boreal sólo ha desaparecido en Europa Occidental, ¿es este lugar un mal hábitat para el animal?

En Doñana sobrevivió ya que era una reserva natural, y el resto está relacionado con la actividad humana, la densidad original de linces y el azar.

En cuanto a Europa Occidental, las principales causas son la gran presión antrópica que sufría en comparación a Asia y el hecho de ser zonas periféricas, ya que es el primer lugar donde una especie empieza a desaparecer.

Si la diversidad genética siempre ha sido baja, ¿cómo es posible que no sea una amenaza para la supervivencia a largo plazo de la especie? ¿No habría entonces más deriva genética, cuellos de botella, reducción de población, y por tanto mayor probabilidad de llegar a vórtice de extinción otra vez? En otras palabras, si su historia está caracterizada por cuellos de botella y declive de la población, ¿no es bastante probable que vuelvan a haber cuellos de botella y vuelva a acabar en la misma situación en la que está ahora?

Si una especie siempre ha presentado baja diversidad no tiene por qué ser una amenaza ya que ha sobrevivido muchos años así. Sin embargo, como ya la especie venía de una dinámica de pérdida de diversidad es especialmente peligroso que siga perdiendo diversidad. El hecho que una especie desaparezca por causas naturales no tiene por qué ser malo, pero los cuellos de botella y pérdidas de población en el lince ibérico han estado provocados por actividad humana, entonces ahí es donde entra en juego la genética de conservación. De por sí una especie puede tener baja diversidad y vivir millones de años, todas las especies a lo largo de la historia de la tierra tienen ciclo de nacimiento y muerte, incluida la humana. Entonces, si una especie tiene baja diversidad y se iba a mantener en baja diversidad hasta que desapareciera no puedes pretender que esa especie presenta mayor diversidad ya que su baja diversidad es una característica intrínseca. El problema es que nosotros hemos ejercido una presión sobre esa especie y algo que ya de por sí era un caso de diversidad particularmente baja provocamos que pierda más. Por tanto, la variabilidad genética del lince ibérico siempre ha sido baja, pero nunca había perdido tanto, de modo que ahora hay una amenaza en la viabilidad de la especie a largo plazo pero antes no la había.

¿Pero el hecho de que una especie presente poca diversidad determina que su vida va a ser más corta, osea que esa especie se va a extinguir antes de las que presentan mucha diversidad?

Que a mí me conste no, no lo sé.

El que una especie se extinga antes o no depende de un montón de cosas, por ejemplo, el ciclo de vida de las especies marinas suele ser más largo porque tienen un medio más homogéneo y estable.

Cabe mencionar, por eso, que en un escenario de cambio global, como que las especies se van a tener que enfrentar a nuevas situaciones, es bueno que la especie cuente con cierta diversidad para responder a esos cambios, por eso es más conveniente que haya individuos heterocigotos.

Si los estudios genómicos son más precisos, detallados y baratos que los genéticos, ¿por qué se siguen usando los dos? ¿Qué tienen los microsatélites que los marcadores SNP no tienen?

Ahora son más precisos, detallados y baratos que los genéticos, antes no. Además, primero se necesita desarrollar el genoma, que es más costoso. La genómica es más detallada, pero a veces, depende de la situación a la que te enfrentes, es más eficaz utilizar genética. Es decir, depende del problema planteado. Hay veces que no necesitas hacer un genoma completo porque ya tienes herramientas genéticas suficientemente buenas y poderosas para contestar a la pregunta. Entonces lo barato es hacer genética y es igualmente eficaz.

En la genética se miran cosas detalladas y en la genómica casi todo. Para hacer genómica se necesita una primera inversión muy grande y en genética no. Además, no todo el mundo puede hacer genómica porque la formación para estudiar la genómica es muy difícil.

La genómica se debería incluir en la genética, pero se refiere a que la genética se basa en sitios concretos y la genómica en la variabilidad total. La genómica tiene una mejora a nivel cuantitativo y cualitativo. La genómica siempre te va a contestar todo, pero hay veces que con la respuesta de la genética ya es suficiente.

En los microsatélites la variación es no funcional, es decir, los microsatélites son neutrales (Non-Selectively Constrained). En cambio, con la genómica se accede a toda la parte que sí es funcional, hecho que supone un salto cualitativo muy importante. Por eso, para ver el efecto de la deriva simplemente te vale con usar microsatélites (ya que la deriva afecta a zonas neutrales), siempre y cuando no se esté comparando con cualquier tipo de selección.

A pesar de que la deriva en zonas neutrales se ve de una manera muy evidente, en zonas funcionales la selección la contrarresta, ya que hay alelos que deben mantener diversidad, como los MHC (alelos de inmunidad). Es muy beneficioso que el sistema inmunitario tenga mucha diversidad de este modo la selección contrarresta la deriva (hecho que ocurre dependiendo la fuerza de la selección y del alelo sobre el que actúa).

La deriva causa erosión genética porque además de perder diversidad también fija mutaciones deletéreas a causa de que la selección purificadora no tiene tanta fuerza para eliminarlas.

6.3 APPENDIX 3. CULTURE MEDIUM RECEIPE

Recipe provided by the Department of Genetics of the Department of Biology of Barcelona University.

	Nº of vials		
	10	24	
Agar	5.6	13.5	grams
Sugar	1	2.5	tablespoon
Water	375	900	cc
Wheat flour	91	218.3	grams
Water	250	500	cc
Nipagin	0.9	2.2	grams
Ethyl alcohol	10.3	24.7	cc

Preparation of the culture medium (example for 10 vials):

1. Put the 5.6 g of agar and 1 tablespoon of sugar in 375 cc of water. Put everything in a pot and boil it in order to avoid the formation of lumps.
2. When the mixture boils add 91 g of wheat flour dissolved in 250 cc of water and cook, stirring for 10 - 15 minutes minutes.

3. Remove the mixture from the heat and add 0.9 g of nipagin dissolved in 10.3 cc of ethyl alcohol.
4. Put the resulting “porridge” in the vials before it hardens.
5. Once the culture medium is cold and set (about 24 hours after resting in the vials) dry the moisture that may be using a little cellulose paper.
6. Once dry, insert a paper folded in a zigzag pattern of about 3 cm height for the larvae to climb and become pupae (optional).
7. Add some crumbled yeast (to feed the adult flies).
8. The final result of the "porridge" depends on many factors such as the type of utensils used, the amount of water, the regulation of the stove... You can try different ways to make the "porridge" (put or extract water to vary its viscosity, cook it over a softer or stronger fire...) until getting the most optimal result.

6.4 APPENDIX 4. EXPERIMENT DATA TABLES

Parental population 1:1					
Individuals	vg+_	vgvg	Offspring obtained for the F1	vg+_	vgvg
Male	20	20	Male	39	18
Female	20	20	Female	41	15

Parental population 4:1					
Individuals	vg+_	vgvg	Offspring obtained for the F1	vg+_	vgvg
Male	30	10	Male	47	6
Female	30	10	Female	42	16

1:1 8 (4♂ - 4♀) R1											
F1						F3					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	2	2	Male	5	2	Male	2	2	Male	3	6
Female	3	1	Female	4	4	Female	0	4	Female	4	5
F2						F4					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	3	1	Male	7	2	Male	0	4	Male	-	-
Female	1	3	Female	5	4	Female	0	4	Female	-	-

1:1 8 (4♂ - 4♀) R2											
F1						F5					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	2	2	Male	6	3	Male	4	0	Male	9	0
Female	3	1	Female	5	4	Female	4	0	Female	8	1
F2						F6					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	3	1	Male	8	3	Male	4	0	Male	-	-
Female	2	2	Female	7	3	Female	4	0	Female	-	-
F3						Backcrossing vg+_ ♂					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	4	0	Male	8	0	Male	4	0	Male	9	1
Female	3	1	Female	9	2	Female	0	4	Female	9	0
F4						Backcrossing vg+_ ♀					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	4	0	Male	9	0	Male	0	4	Male	8	0
Female	3	1	Female	7	2	Female	4	0	Female	7	0

1:1 8 (random selected sex)											
F1						F4					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	2	1	Male	2	3	Male	2	2	Male	2	5
Female	4	1	Female	7	0	Female	1	3	Female	4	5
F2						F5					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	0	2	Male	3	2	Male	0	4	Male	2	8
Female	6	0	Female	4	3	Female	1	3	Female	1	6
F3						F6					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	2	1	Male	4	2	Male	0	3	Male	-	-
Female	3	2	Female	2	5	Female	0	5	Female	-	-

1:1 16 (8♂ - 8♀) R1											
F1						F5					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	3	5	Male	9	7	Male	0	8	Male	3	12
Female	4	4	Female	8	10	Female	2	6	Female	5	9
F2						F6					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	4	4	Male	7	10	Male	0	8	Male	4	11
Female	3	5	Female	9	9	Female	2	6	Female	4	13
F3						F7					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	2	6	Male	8	9	Male	0	8	Male	-	-
Female	3	5	Female	7	11	Female	0	8	Female	-	-
F4						F8					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	3	5	Male	4	11	Male	-	-	Male	-	-
Female	2	6	Female	6	9	Female	-	-	Female	-	-

1:1 16 (8♂ - 8♀) R2											
F1						F5					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	8	0	Male	12	3	Male	7	1	Male	15	4
Female	7	1	Female	14	0	Female	8	0	Female	15	1
F2						F6 - backcrossing					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	7	1	Male	15	2	Male	8	0	Male	-	-
Female	8	0	Female	10	4	Female	8	0	Female	-	-
F3						Backcrossing vg+_ ♂					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	7	1	Male	13	3	Male	8	0	Male	18	0
Female	6	2	Female	12	4	Female	0	8	Female	15	3

F4						Backcrossing vg+_ ♀					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	7	1	Male	16	3	Male	0	8	Male	15	2
Female	7	1	Female	14	4	Female	8	0	Female	17	0

1:1 16 (random selected sex)											
F1						F5					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	4	2	Male	12	4	Male	1	5	Male	6	9
Female	8	2	Female	13	4	Female	5	5	Female	4	10
F2						F6					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	3	2	Male	9	3	Male	3	6	Male	5	10
Female	10	1	Female	8	5	Female	1	5	Female	8	7
F3						F7					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	8	1	Male	9	6	Male	2	7	Male	-	-
Female	4	3	Female	14	3	Female	2	5	Female	-	-
F4						F8					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	5	4	Male	5	9	Male	-	-	Male	-	-
Female	6	1	Female	11	7	Female	-	-	Female	-	-

4:1 8 (4♂ - 4♀) R1											
F1						F5					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	4	0	Male	7	4	Male	4	0	Male	11	0
Female	3	1	Female	9	3	Female	4	0	Female	13	0
F2						F6 - backcrossing					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	2	2	Male	7	4	Male	4	0	Male	-	-
Female	3	1	Female	10	0	Female	4	0	Female	-	-

F3						Backcrossing vg+_ ♂					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	3	1	Male	12	1	Male	4	0	Male	12	0
Female	4	0	Female	9	2	Female	0	4	Female	11	0
F4						Backcrossing vg+_ ♀					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	4	0	Male	13	0	Male	0	4	Male	10	0
Female	4	0	Female	9	3	Female	4	0	Female	9	0

4:1 8 (4♂ - 4♀) R2											
F1						F4					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	3	1	Male	7	2	Male	1	3	Male	5	7
Female	2	2	Female	8	3	Female	1	3	Female	3	5
F2						F5					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	4	0	Male	7	4	Male	0	4	Male	-	-
Female	2	2	Female	6	4	Female	0	4	Female	-	-
F3						F6					
Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Bottlenecked individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	2	2	Male	3	6	Male	-	-	Male	-	-
Female	1	3	Female	7	4	Female	-	-	Female	-	-

4:1 8 (random selected sex)					
F1					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	2	0	Male	4	1
Female	6	0	Female	6	0
F2					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	3	0	Male	4	2
Female	5	0	Female	8	1

F3					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	0	0	Male	-	-
Female	7	1	Female	-	-

4:1 16 (8♂ - 8♀) R1											
F1						F5					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	7	1	Male	13	4	Male	6	2	Male	13	4
Female	5	3	Female	12	3	Female	4	4	Female	9	9
F2						F6					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	7	1	Male	13	2	Male	7	1	Male	11	7
Female	7	1	Female	9	4	Female	3	5	Female	10	9
F3						F7					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	8	0	Male	11	6	Male	5	3	Male	-	-
Female	6	2	Female	11	4	Female	3	5	Female	-	-
F4						F8					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	6	2	Male	12	7	Male	-	-	Male	-	-
Female	6	2	Female	9	6	Female	-	-	Female	-	-

4:1 16 (8♂ - 8♀) R2											
F1						F5					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	8	0	Male	14	5	Male	6	2	Male	14	3
Female	7	1	Female	16	2	Female	7	1	Female	18	1
F2						F6					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	7	1	Male	18	2	Male	7	1	Male	14	2
Female	7	1	Female	15	3	Female	8	0	Female	17	0

F3						F7					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	8	0	Male	14	2	Male	8	0	Male	-	-
Female	8	0	Female	16	3	Female	8	0	Female	-	-
F4						F8					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	7	1	Male	15	5	Male	-	-	Male	-	-
Female	8	0	Female	17	2	Female	-	-	Female	-	-

4:1 16 (random selected sex)											
F1						F5					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	4	1	Male	15	2	Male	9	4	Male	7	3
Female	9	2	Female	11	4	Female	2	1	Female	5	4
F2						F6					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	8	1	Male	8	3	Male	7	2	Male	13	4
Female	5	2	Female	13	4	Female	4	3	Female	10	6
F3						F7					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	2	1	Male	11	4	Male	6	1	Male	-	-
Female	9	3	Female	13	4	Female	4	5	Female	-	-
F4						F8					
Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg	Individuals	vg+_	vgvg	Offspring obtained	vg+_	vgvg
Male	5	2	Male	12	6	Male	-	-	Male	-	-
Female	6	3	Female	11	2	Female	-	-	Female	-	-

6.5 APPENDIX 5. EXPERIMENT JOURNAL

10/2/2020

CESIRE prepared the vials.

10/7/2020

Collection of vials.

10/19/2020

Adult flies are born. Manipulation after lunchtime and dinner time. Males and females are separated in different vials to keep the females virgin.

10/20/2020

Formation of parental population 1:1. 20 vg+vg+ ♂, 20 vg+vg+ ♀, 20 vgvg ♂, and 20 vgvg ♀.

Separation of other flies in order to reach enough for the other parental population.

10/21/2020

Formation of parental population 4:1.

11/1/2020

Methods applied to give heat and shorten the biological cycle are working. F1 population (of parental population 1:1) is born during the morning, afternoon, and night. Bottlenecks forming the F1 in each population:

1:1 8 (4♂ - 4♀) R1	vg+_	vgvg
Male	2	2
Female	3	1

1:1 8 (4♂ - 4♀) R2	vg+_	vgvg
Male	2	2
Female	3	1

1:1 8 (random selected sex)	vg+_	vgvg
Male	2	1
Female	4	1

1:1 16 (8♂ - 8♀) R1	vg+_	vgvg
Male	3	5
Female	4	4

1:1 16 (8♂ - 8♀) R2	vg+_	vgvg
Male	8	0
Female	7	1

1:1 16 (random selected sex)	vg+_	vgvg
Male	4	2
Female	8	2

11/2/2020

F1 population (of parental population 4:1) is born during the morning, afternoon, and night. The ones born in the morning are not used since it is not possible to know if they are virgins. Bottlenecks forming the F1 in each population:

4:1 8 (4♂ - 4♀) R1	vg+_	vgvg
Male	4	0
Female	3	1

4:1 8 (4♂ - 4♀) R2	vg+_	vgvg
Male	3	1
Female	2	2

4:1 8 (random selected sex)	vg+_	vgvg
Male	2	0
Female	6	0

4:1 16 (8♂ - 8♀) R1	vg+_	vgvg
Male	7	1
Female	5	3

4:1 16 (8♂ - 8♀) R2	vg+_	vgvg
Male	8	0
Female	7	1

4:1 16 (random selected sex)	vg+_	vgvg
Male	4	1
Female	9	2

11/12/2020

F2 populations are born (parental population 1:1).

1:1 8 (4♂ - 4♀) R1

Total offspring: 5 vg+_ ♂, 4 vg+_♀, 5 vgvg ♂, 3 vgvg ♀.

Bottleneck: 3 vg+_ ♂, 1 vg+_♀, 1 vgvg ♂, 3 vgvg ♀.

1:1 8 (4♂ - 4♀) R2

Total offspring: 6 vg+_ ♂, 5 vg+_♀, 3 vgvg ♂, 4 vgvg ♀.

Bottleneck: 3 vg+_ ♂, 2 vg+_♀, 1 vgvg ♂, 2 vgvg ♀.

1:1 8 (random selected sex)

Total offspring: 2 vg+_ ♂, 7 vg+_♀, 3 vgvg ♂, 0 vgvg ♀.

Bottleneck: 0 vg+_ ♂, 6 vg+_♀, 2 vgvg ♂, 0 vgvg ♀. → Creates high inbreeding.

1:1 16 (8♂ - 8♀) R1

Total offspring: 9 vg+_ ♂, 8 vg+_♀, 7 vgvg ♂, 10 vgvg ♀.

Bottleneck: 4 vg+_ ♂, 3 vg+_♀, 4 vgvg ♂, 5 vgvg ♀.

1:1 16 (8♂ - 8♀) R2

Total offspring: 12 vg+_ ♂, 14 vg+_♀, 3 vgvg ♂, 0 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 8 vg+_♀, 1 vgvg ♂, 0 vgvg ♀.

1:1 16 (random selected sex)

Total offspring: 12 vg+_ ♂, 13 vg+_♀, 4 vgvg ♂, 4 vgvg ♀.

Bottleneck: 3 vg+_ ♂, 10 vg+_♀, 2 vgvg ♂, 1 vgvg ♀.

11/13/2020

F2 populations are born (parental population 4:1).

4:1 8 (4♂ - 4♀) R1

Total offspring: 7 vg+_ ♂, 9 vg+_♀, 4 vgvg ♂, 3 vgvg ♀.

Bottleneck: 2 vg+_ ♂, 3 vg+_♀, 2 vgvg ♂, 1 vgvg ♀.

4:1 8 (4♂ - 4♀) R2

Total offspring: 7 vg+_ ♂, 8 vg+_♀, 2 vgvg ♂, 3 vgvg ♀.

Bottleneck: 4 vg+_ ♂, 2 vg+_♀, 0 vgvg ♂, 2 vgvg ♀.

4:1 8 (random selected sex)

Total offspring: 4 vg+_ ♂, 6 vg+_♀, 1 vgvg ♂, 0 vgvg ♀.

Bottleneck: 3 vg+_ ♂, 5 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

4:1 16 (8♂ - 8♀) R1

Total offspring: 13 vg+_ ♂, 12 vg+_♀, 4 vgvg ♂, 3 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 7 vg+_♀, 1 vgvg ♂, 1 vgvg ♀.

4:1 16 (8♂ - 8♀) R2

Total offspring: 14 vg+_ ♂, 16 vg+_♀, 5 vgvg ♂, 2 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 7 vg+_♀, 1 vgvg ♂, 1 vgvg ♀.

4:1 16 (random selected sex)

Total offspring: 15 vg+_ ♂, 11 vg+_♀, 2 vgvg ♂, 4 vgvg ♀.

Bottleneck: 8 vg+_ ♂, 5 vg+_♀, 1 vgvg ♂, 2 vgvg ♀.

11/23/2020

F3 populations are born (parental population 1:1).

1:1 8 (4♂ - 4♀) R1

Total offspring: 7 vg+_ ♂, 5 vg+_♀, 2 vgvg ♂, 4 vgvg ♀. → some wild offspring flew away.

Bottleneck: 2 vg+_ ♂, 0 vg+_♀, 2 vgvg ♂, 4 vgvg ♀.

1:1 8 (4♂ - 4♀) R2

Total offspring: 8 vg+_ ♂, 7 vg+_♀, 3 vgvg ♂, 3 vgvg ♀.

Bottleneck: 4 vg+_ ♂, 3 vg+_♀, 0 vgvg ♂, 1 vgvg ♀.

1:1 8 (random selected sex)

Total offspring: 3 vg+_ ♂, 4 vg+_♀, 2 vgvg ♂, 3 vgvg ♀.

Bottleneck: 2 vg+_ ♂, 3 vg+_♀, 1 vgvg ♂, 2 vgvg ♀.

1:1 16 (8♂ - 8♀) R1

Total offspring: 7 vg+_ ♂, 9 vg+_♀, 10 vgvg ♂, 9 vgvg ♀.

Bottleneck: 2 vg+_ ♂, 3 vg+_♀, 6 vgvg ♂, 5 vgvg ♀.

1:1 16 (8♂ - 8♀) R2

Total offspring: 15 vg+_ ♂, 10 vg+_♀, 2 vgvg ♂, 4 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 6 vg+_♀, 1 vgvg ♂, 2 vgvg ♀.

1:1 16 (random selected sex)

Total offspring: 9 vg+_ ♂, 8 vg+_♀, 3 vgvg ♂, 5 vgvg ♀.

Bottleneck: 8 vg+_ ♂, 4 vg+_♀, 1 vgvg ♂, 3 vgvg ♀.

11/24/2020

F3 populations are born (parental population 4:1).

4:1 8 (4♂ - 4♀) R1

Total offspring: 7 vg+_ ♂, 10 vg+_♀, 4 vgvg ♂, 0 vgvg ♀.

Bottleneck: 3 vg+_ ♂, 4 vg+_♀, 1 vgvg ♂, 0 vgvg ♀.

4:1 8 (4♂ - 4♀) R2

Total offspring: 7 vg+_ ♂, 6 vg+_♀, 4 vgvg ♂, 4 vgvg ♀.

Bottleneck: 2 vg+_ ♂, 1 vg+_♀, 2 vgvg ♂, 3 vgvg ♀.

4:1 8 (random selected sex)

Total offspring: 4 vg+_ ♂, 8 vg+_♀, 2 vgvg ♂, 1 vgvg ♀.

Bottleneck: 0 vg+_ ♂, 7 vg+_♀, 0 vgvg ♂, 1 vgvg ♀. → Extinction. No males.

4:1 16 (8♂ - 8♀) R1

Total offspring: 13 vg+_ ♂, 9 vg+_♀, 2 vgvg ♂, 4 vgvg ♀.

Bottleneck: 8 vg+_ ♂, 6 vg+_♀, 0 vgvg ♂, 2 vgvg ♀.

4:1 16 (8♂ - 8♀) R2

Total offspring: 18 vg+_ ♂, 15 vg+_♀, 2 vgvg ♂, 3 vgvg ♀.

Bottleneck: 8 vg+_ ♂, 8 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

4:1 16 (random selected sex)

Total offspring: 8 vg+_ ♂, 13 vg+_♀, 3 vgvg ♂, 4 vgvg ♀.

Bottleneck: 2 vg+_ ♂, 9 vg+_♀, 1 vgvg ♂, 3 vgvg ♀.

12/4/2020

F4 populations are born (parental population 1:1).

1:1 8 (4♂ - 4♀) R1

Total offspring: 3 vg+_ ♂, 4 vg+_♀, 7 vgvg ♂, 5 vgvg ♀.

Bottleneck: 0 vg+_ ♂, 0 vg+_♀, 4 vgvg ♂, 4 vgvg ♀. → Fixation.

1:1 8 (4♂ - 4♀) R2

Total offspring: 8 vg+_ ♂, 9 vg+_♀, 2 vgvg ♂, 2 vgvg ♀.

Bottleneck: 4 vg+_ ♂, 3 vg+_♀, 0 vgvg ♂, 1 vgvg ♀.

1:1 8 (random selected sex)

Total offspring: 4 vg+_ ♂, 2 vg+_♀, 2 vgvg ♂, 5 vgvg ♀.

Bottleneck: 2 vg+_ ♂, 1 vg+_♀, 2 vgvg ♂, 3 vgvg ♀.

1:1 16 (8♂ - 8♀) R1

Total offspring: 8 vg+_ ♂, 7 vg+_♀, 9 vgvg ♂, 11 vgvg ♀.

Bottleneck: 3 vg+_ ♂, 2 vg+_♀, 5 vgvg ♂, 6 vgvg ♀.

1:1 16 (8♂ - 8♀) R2

Total offspring: 13 vg+_ ♂, 12 vg+_♀, 3 vgvg ♂, 4 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 7 vg+_♀, 1 vgvg ♂, 1 vgvg ♀.

1:1 16 (random selected sex)

Total offspring: 9 vg+_ ♂, 14 vg+_♀, 6 vgvg ♂, 3 vgvg ♀.

Bottleneck: 5 vg+_ ♂, 6 vg+_♀, 4 vgvg ♂, 1 vgvg ♀.

12/5/2020

F4 populations are born (parental population 4:1).

4:1 8 (4♂ - 4♀) R1

Total offspring: 12 vg+_ ♂, 9 vg+_♀, 1 vgvg ♂, 2 vgvg ♀.

Bottleneck: 4 vg+_ ♂, 4 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

4:1 8 (4♂ - 4♀) R2

Total offspring: 3 vg+_ ♂, 7 vg+_♀, 6 vgvg ♂, 4 vgvg ♀.

Bottleneck: 1 vg+_ ♂, 1 vg+_♀, 3 vgvg ♂, 3 vgvg ♀.

4:1 16 (8♂ - 8♀) R1

Total offspring: 11 vg+_ ♂, 11 vg+_♀, 6 vgvg ♂, 4 vgvg ♀.

Bottleneck: 6 vg+_ ♂, 6 vg+_♀, 2 vgvg ♂, 2 vgvg ♀.

4:1 16 (8♂ - 8♀) R2

Total offspring: 14 vg+_ ♂, 16 vg+_♀, 2 vgvg ♂, 3 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 8 vg+_♀, 1 vgvg ♂, 0 vgvg ♀.

4:1 16 (random selected sex)

Total offspring: 11 vg+_ ♂, 13 vg+_♀, 4 vgvg ♂, 4 vgvg ♀.

Bottleneck: 5 vg+_ ♂, 6 vg+_♀, 2 vgvg ♂, 3 vgvg ♀.

12/15/2020

F5 populations are born (parental population 1:1).

1:1 8 (4♂ - 4♀) R2

Total offspring: 9 vg+_ ♂, 7 vg+_♀, 0 vgvg ♂, 2 vgvg ♀.

Bottleneck: 4 vg+_ ♂, 4 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

1:1 8 (random selected sex)

Total offspring: 2 vg+_ ♂, 4 vg+_♀, 5 vgvg ♂, 5 vgvg ♀.

Bottleneck: 0 vg+_ ♂, 1 vg+_♀, 4 vgvg ♂, 3 vgvg ♀.

1:1 16 (8♂ - 8♀) R1

Total offspring: 4 vg+_ ♂, 6 vg+_♀, 11 vgvg ♂, 9 vgvg ♀.

Bottleneck: 0 vg+_ ♂, 2 vg+_♀, 8 vgvg ♂, 6 vgvg ♀.

1:1 16 (8♂ - 8♀) R2

Total offspring: 16 vg+_ ♂, 14 vg+_♀, 3 vgvg ♂, 4 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 8 vg+_♀, 1 vgvg ♂, 0 vgvg ♀.

1:1 16 (random selected sex)

Total offspring: 5 vg+_ ♂, 11 vg+_♀, 9 vgvg ♂, 7 vgvg ♀.

Bottleneck: 1 vg+_ ♂, 5 vg+_♀, 5 vgvg ♂, 5 vgvg ♀.

12/16/2020

F5 populations are born (parental population 4:1).

4:1 8 (4♂ - 4♀) R1

Total offspring: 13 vg+_ ♂, 9 vg+_♀, 0 vgvg ♂, 3 vgvg ♀.

Bottleneck: 4 vg+_ ♂, 4 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

4:1 8 (4♂ - 4♀) R2

Total offspring: 5 vg+_ ♂, 3 vg+_♀, 7 vgvg ♂, 5 vgvg ♀.

Bottleneck: 0 vg+_ ♂, 0 vg+_♀, 4 vgvg ♂, 4 vgvg ♀. → Fixation.

4:1 16 (8♂ - 8♀) R1

Total offspring: 12 vg+_ ♂, 9 vg+_♀, 7 vgvg ♂, 6 vgvg ♀.

Bottleneck: 6 vg+_ ♂, 4 vg+_♀, 2 vgvg ♂, 4 vgvg ♀.

4:1 16 (8♂ - 8♀) R2

Total offspring: 15 vg+_ ♂, 17 vg+_♀, 5 vgvg ♂, 2 vgvg ♀.

Bottleneck: 6 vg+_ ♂, 7 vg+_♀, 2 vgvg ♂, 1 vgvg ♀.

4:1 16 (random selected sex)

Total offspring: 12 vg+_ ♂, 11 vg+_♀, 6 vgvg ♂, 2 vgvg ♀.

Bottleneck: 9 vg+_ ♂, 2 vg+_♀, 4 vgvg ♂, 1 vgvg ♀.

12/26/2020

F6 populations are born (parental population 1:1).

1:1 8 (4♂ - 4♀) R2

Total offspring: 9 vg+_ ♂, 8 vg+_♀, 0 vgvg ♂, 1 vgvg ♀.

Bottleneck: 4 vg+_ ♂, 4 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

1:1 8 (random selected sex)

Total offspring: 2 vg+_ ♂, 1 vg+_♀, 8 vgvg ♂, 6 vgvg ♀.

Bottleneck: 0 vg+_ ♂, 0 vg+_♀, 3 vgvg ♂, 5 vgvg ♀.

1:1 16 (8♂ - 8♀) R1

Total offspring: 3 vg+_ ♂, 5 vg+_♀, 12 vgvg ♂, 9 vgvg ♀.

Bottleneck: 0 vg+_ ♂, 2 vg+_♀, 8 vgvg ♂, 6 vgvg ♀.

1:1 16 (8♂ - 8♀) R2

Total offspring: 15 vg+_ ♂, 15 vg+_♀, 4 vgvg ♂, 1 vgvg ♀

Bottleneck: 8 vg+_ ♂, 8 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

1:1 16 (random selected sex)

Total offspring: 6 vg+_ ♂, 4 vg+_♀, 9 vgvg ♂, 10 vgvg ♀.

Bottleneck: 3 vg+_ ♂, 1 vg+_♀, 6 vgvg ♂, 5 vgvg ♀.

12/27/2020

F6 populations are born (parental population 4:1).

4:1 8 (4♂ - 4♀) R1

Total offspring: 11 vg+_ ♂, 13 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

Bottleneck: 4 vg+_ ♂, 4 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

4:1 16 (8♂ - 8♀) R1

Total offspring: 13 vg+_ ♂, 9 vg+_♀, 4 vgvg ♂, 9 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 3 vg+_♀, 1 vgvg ♂, 5 vgvg ♀.

4:1 16 (8♂ - 8♀) R2

Total offspring: 14 vg+_ ♂, 18 vg+_♀, 3 vgvg ♂, 1 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 8 vg+_♀, 1 vgvg ♂, 0 vgvg ♀.

4:1 16 (random selected sex)

Total offspring: 7 vg+_ ♂, 5 vg+_♀, 3 vgvg ♂, 4 vgvg ♀.

Bottleneck: 7 vg+_ ♂, 4 vg+_♀, 2 vgvg ♂, 3 vgvg ♀.

1/6/2021

F7 populations are born (parental population 1:1) + results of backcrossings in other populations.

1:1 8 (4♂ - 4♀) R2

Backcrossing vg+_ ♂ x vgvg ♀: 9 vg+_ ♂, 9 vg+_♀, 1 vgvg ♂, 0 vgvg ♀.

Backcrossing vg+_ ♀ x vgvg ♂ : 8 vg+_ ♂, 7 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

Not fixed.

1:1 16 (8♂ - 8♀) R1

Total offspring: 4 vg+_ ♂, 4 vg+_♀, 11 vgvg ♂, 13 vgvg ♀.

Bottleneck: 0 vg+_ ♂, 0 vg+_♀, 8 vgvg ♂, 8 vgvg ♀.

→ Fixation.

1:1 16 (8♂ - 8♀) R2

Backcrossing vg+_ ♂ x vgvg ♀: 18 vg+_ ♂, 15 vg+_♀, 0 vgvg ♂, 3 vgvg ♀.

Backcrossing vg+_ ♀ x vgvg ♂ : 15 vg+_ ♂, 17 vg+_♀, 2 vgvg ♂, 0 vgvg ♀.

→ Not fixed.

1:1 16 (random selected sex)

Total offspring: 5 vg+_ ♂, 8 vg+_♀, 10 vgvg ♂, 7 vgvg ♀.

Bottleneck: 2 vg+_ ♂, 2 vg+_♀, 7 vgvg ♂, 5 vgvg ♀.

1/7/2021

F7 populations are born (parental population 4:1) + results of backcrossings in other populations.

4:1 8 (4♂ - 4♀) R1

Backcrossing vg+_ ♂ x vgvg ♀: 12 vg+_ ♂, 11 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

Backcrossing vg+_ ♀ x vgvg ♂ : 10 vg+_ ♂, 9 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

→ Fixation.

4:1 16 (8♂ - 8♀) R1

Total offspring: 11 vg+_ ♂, 10 vg+_♀, 7 vgvg ♂, 9 vgvg ♀.

Bottleneck: 5 vg+_ ♂, 3 vg+_♀, 3 vgvg ♂, 5 vgvg ♀.

→ Not fixed.

4:1 16 (8♂ - 8♀) R2

Total offspring: 14 vg+_ ♂, 17 vg+_♀, 2 vgvg ♂, 0 vgvg ♀.

Bottleneck: 8 vg+_ ♂, 8 vg+_♀, 0 vgvg ♂, 0 vgvg ♀.

→ We cannot know if it is fixed without a backcrossing.

4:1 16 (random selected sex)

Total offspring: 13 vg+_ ♂, 10 vg+_♀, 4 vgvg ♂, 6 vgvg ♀.

Bottleneck: 6 vg+_ ♂, 4 vg+_♀, 1 vgvg ♂, 5 vgvg ♀.

→ We cannot know if it is fixed without a backcrossing.