UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO:

Ecophysiological constraints of genome size in birds (Subclass Aves) and mammals (Class Mammalia)

Trabajo de integración curricular presentado como requisito para la obtención del título de Bióloga

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Dedicatoria

Con mucho cariño,

Quiero dedicar este logro principalmente a mi madre, Ofelia Sánchez, quien con su amor y esfuerzo me ha impulsado a seguir adelante siempre, por apoyarme, guiarme, aconsejarme y, sobre todo, por creer en mí, porque sin ella, nada de esto hubiera sido posible.

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Resumen

El genoma es el conjunto de genes presentes en un organismo. Este contiene la información necesaria para que cada individuo pueda crecer y desarrollarse. En organismos procariotas, la complejidad se correlaciona con el número de genes. Esto no pasa en eucariotas, donde el tamaño del genoma tiene una gran variación, incluso en individuos de la misma familia. Esta variación ha sido estudiada por la comunidad científica durante varios años, analizando y secuenciando genomas de diferentes especies, descubriendo de esta manera la "paradoja del valor C", la cual explica que la cantidad de ADN de un genoma haploide o valor C, no parece estar relacionado con la complejidad del organismo. En esta revisión bibliográfica, la variación del tamaño del genoma es brevemente descrita con un análisis enfocado en aves (subclase aves) y mamíferos (subclase mammalia), explicando los diferentes factores que podrían influir en el mismo, de acuerdo al ambiente en donde viven, tales como el clima, tasa de metabolismo basal (TMB) y la humedad. Además de comparar estos agentes para encontrar una relación genómica entre ellos, y de esta manera, tratar de predecir, el tamaño del conjunto de genes de mamíferos y aves, y las características fisiológicas que estos animales podrían presentar en un determinado ambiente. Adicionalmente, la paradoja del valor C está explicada desde cuando fue descubierta hasta los diferentes métodos usados para medir el tamaño del genoma, incluyendo la descripción del "ADN basura" y la estructura del genoma.

Palabras clave: ADN basura, tamaño del genoma, paradoja del valor C, mamíferos, aves.

Abstract

The genome is the set of genes that are present in an organism. It contains the genetic information needed by each individual to grow and develop. In prokaryotic organisms, the complexity is correlated to the number of genes. This does not occur in eukaryotes, where the genome size varies a lot, even between individuals from the same family. This variation has been studied by the scientific community during several years, analyzing and sequencing genomes from several species, finding out in this way, "the C value paradox", which explains that the amount of DNA in a haploid genome or the C value, does not seem to correspond to the complexity of an organism. In this bibliographic review, the genome size variation among species is briefly described with an analysis on focused on birds (Subclass Aves) and mammals (Subclass Mammalia), explaining the different factors that could affect its genome size based on the environment they live in, such as climate, basal metabolic rate (BMR) and humidity. Also, comparing these factors to find a genomic relationship between them and, in this way, try to predict the genome size of mammals and birds, and physiological characteristics that these animals might present in a determined environment. Additionally, the C value paradox is explained from when it was discovered until the different methods of measuring the genome size, including the description of "junk DNA" and genome structure.

Keywords: junk DNA, genome size, C value paradox, mammals, birds.

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Abbreviations

Deoxyribonucleic acid (DNA)

Base Pairs (bp)

Basal Metabolic Rate (BMR)

Picograms (pg)

Ribonucleic acid (RNA)

Flow Cytometry (FCM)

Feulgen Densitometry (FD)

Human Immunodeficiency Virus (HIV)

Ultraviolet Microscopy (UVM)

Long Interspersed Nuclear Element (LINE)

Short Interspersed Element (SINE)

Long Terminal Repeat (LTR)

Body Temperature (Tb)

Human Genome Project (HGP)

Transposable elements (TEs)

Section I: Introduction

The Earth is a very diverse planet. There are many organisms that inhabit this place, nevertheless, science still cannot tell the exact number of them. Generally, the best approximation to the total number of species on Earth, is presented by taxonomic experts, whose estimates range between 3 and 100 million species (1). Each of these organisms have different features that are determined by their genes, small segments of Deoxyribonucleic acid (DNA), which contains all the genetic information of living beings. Depending on the organism, the DNA can be found not only in the nucleus of the cell but also in cellular organelles such as mitochondria or plastids, present in eukaryotic cells (2).

Animals are eukaryotic individuals that are studied by zoology, a branch of biology that helps us to understand how they live, develop and survive in their environment. These are classified according to the alimentation, habitat, presence of spinal column, among other characteristics. According to the last one, animals are divided as vertebrates and invertebrates, in the first group, we have: amphibians, mammals, reptiles, fish and birds (3). In this research, we will focus on mammalian and bird classes, their genomes and metabolism.

Eukaryotic individuals are known to be more complex than prokaryotes, due to all the functions and systems they present, which allows them to have a superior efficiency in terms of structure and organization physiologically speaking. The complexity of an individual considers several features including body symmetry, number of body openings, body segmentation and the presence of a notochord (4). Since DNA is the material present in the genes, it would be logical to think that more complex organisms have more DNA, nevertheless, this is not true, because there are some animals that have a much larger genome size, even larger than humans, which are the most complex organisms on Earth. Then, genome size is not correlated to the complexity of an organism. This is described as the “C value paradox” (5).

In 1971, the geneticist C. A. Thomas Jr. described the “C value paradox”, a term used to explain that the size of the genome does not correlate to how complex an organism is

(6). The C-value is the amount of genetic information in a haploid nucleus measured in picograms (10^{-12} of a gram). The major part of modern genome size estimates are based on Feulgen densitometry (computerized image analysis), flow cytometry, among other methods (7).

The animal genome size could vary due to many factors, such as differences in noncoding DNA, transposable elements (sequences that change their position within a genome), microsatellites and other types of repetitive and intergenic deoxyribonucleic acid. This could create mutations or alter the genome size, which is known to have a direct impact on important physiological parameters such as cell size, cell cycle duration and developmental time (8) . Genome size also influences on the metabolic rate, due to the fact that larger cells need more energy to sustain essential functions. Although this is not totally known, there is a positive correlation between metabolic rate and body mass (9).

In amniotes such as reptiles (on a limited dataset), genome size has been related to metabolic rate; however, the studies on anamniotes were inconclusive. This relationship was demonstrated through the heart index, which is a unified indicator of metabolic rate in vertebrates. This is more general than the basal metabolic rate, which is measured by oxygen consumption. Heart design is more complex in vertebrates and so are the other organs. Then, it could be affirmed that exists a negative correlation between genome size and metabolic rate in all amniotes (10).

Also, in many crop plants, a positive relationship between genome size and latitude, has been observed, since genome size is generally larger in temperate species. In animals, most genome sizes are very small, less than 5 picograms (pg.), on the other hand, some invertebrates and amphibians have more than 10 picograms, which is considered a large genome and according to the Animal Genome Size Database, only the 8% of all animal species, have a genome greater than this quantity (11).

The study of this diversity of genomes is relevant nowadays. Genomics is the science that handles the analysis and mapping of animal genome activity. Its objective is the quantification and characterization of the genes in a living organism, which is relevant to determine and understand the function of the most complex systems, such as the brain. This is why eukaryotic genome size data is very important for comparative research. DNA sequencing is an essential technique to investigate the function of genes, since it provides the information that the cell needs to create RNA molecules and proteins (12).

1.1. Problem Statement

Diverse studies about genome demonstrated that there is a big variation in its size among many species. This variation is not related to the complexity of the organism, and scientists have demonstrated that approximately only 1,5% of the human genome is used to codify proteins, the rest is wrongly considered as "junk DNA", since it does not codify for any protein, which is described by the "C value paradox" or "C value enigma". Nevertheless, scientific studies have analyzed these DNA sequences, proving that is not "junk" because it is important for controlling gene activity (13).

Also, in endotherms, genome size has been associated to different factors, such as metabolic rate or body size. Smaller animals tend to have higher per-gram basal metabolic rates than large animals. The explanation behind this correlation is the nucleotypic effect, in which large nucleus and cells sizes affect cellular metabolism through surface area to volume ratios. Nevertheless, this description is not always true for metabolic rate and genome size, since it is conflicting among many scientific studies (9).

Therefore, there are still many doubts about the genome size variation among species and its relationship to different environmental or physiological factors. These factors are going to be discussed in this research work and their relationship to genome size, focusing only on endotherms such as birds and mammals.

1.2. Objectives

1.2.1. General Objective

To prepare a review document with pertinent information on the different studies and methods to measure the variables that influence animal genome size, focusing on the ecophysiological constraints of genome size in birds and mammals.

1.2.2. Specific Objectives

i. To analyze the animal genome size, focusing on mammals and birds principally.
ii. To describe bird (Subclass Aves) and mammals (Class Mammalia) genome size, based on the currently biological activity studies.
iii. To describe the different factors that have an influence on genome size.
iv. To report general details about the methods employed to analyze and compare the constraints of the genome size variation.

Section II: Methodology

The kind of study to be developed is a narrative bibliographic review. The purpose of this research is the bibliographic search for articles or reviews that contain information of relevance to our topic; such as definitions, descriptions, characteristics or factors that have an influence on animal genome size. The search strings employed for the research have been the following: genome structure, mammalian genome size, bird genome size, genome size variation, C value paradox, junk DNA, transposable elements (in that order specifically). There were no time restrictions for the selection of the papers and documents, due to the fact that, some of the concepts described in this investigation, have remain constant through the years since their discovery, such as genome structure or DNA. Also, there were no restrictions in the type of study that was carried out as a methodology for the documents to be analyzed, such as experimental, non-experimental, descriptive, among others. More than one hundred papers were found typing the search strings mentioned before, nevertheless, only 84 were selected. In order to select the information for this investigation project, the only conditions were: that the documents were related to bird or mammalian genome principally, and that these were found in trusted and verified pages and databases such as the ones mentioned below.

In this paper, three processes are going to be observed: bibliographic search, analysis of the information found, selection, and synthesis.

2.1. Bibliographic search and databases used

PubMed: National Center for Biotechnology Information (NCBI) is responsible for this investigation work. This free search engine allows the access to MEDLINE database of references and abstracts of life sciences and biomedical topics.

Google Scholar: this Google database provides us articles and investigations with a solid scientific base. This information is very reliable since it comes from university publishers, universities, professional associations, and other academic organizations.

Animal Genome Size Database: this is a catalogue of published genome sizes measurements carried out in vertebrate and invertebrate animals. This investigation was based on this database, since it contains the C values (haploid genome size) from many species of mammals and birds of interest for our study.

2.2. Analysis and synthesis of information

This investigation was developed with the collection of scientific papers found, each of them was reviewed and analyzed, selecting only the relevant information.

Inclusion/exclusion criteria:

- Time Restriction: No time restrictions.
- Type of Study: Unrestricted.
- Language restriction: Documents were filtered with English as the main language and Spanish as a second language.
- Keywords were included in the study title, summary and text.

Section III: Results

3.1. Genome: structure and organization

A genome could be defined as the compilation of all the genetic information from each organism. Nevertheless, it is relevant to point out that genome is not totally similar even between individuals from the same family (table 1). For instance, there is a variance of 0,1% in humans. Two people from the same sex are enough to understand the contrast in the skin color, eyes and hair type, also, size and sensibility or resistance to any illness

or virus; attitude, aptitude, physiological and metabolic differences or response to environmental factors such as temperature, hypoxia, nutrition, drugs. The genome of mammals is different by approximately 3 billion nucleotide bases and 20 to 25 thousand genes. It is calculated that at least one million of them determine the distribution of possible genetic markers in all regions of the genome (14).

Family	**Species**	**Common name**	**Genome size (picograms)**
Felidae	*Acinonyx jubatus*	Cheetah	2,56
	Felis catus	Domestic cat	3,1
	Felis concolor	Cougar	3,05
	Felis lynx	Lynx	2,92
	Felis silvestris	Wildcat	3
	Panthera leo	African lion	2,95
	Panthera tigris	Tiger	2,71
	Panthera unica	Snow leopard	3,54
	Neofelis nebulosa	Clouded leopard	2,77

Table 1: *Genome size variation even in individuals from the same family.*

Until 1944, the genetic base material was still unknown. Oswald Avery, Colin McLeod and Maclyn McCarty used bacterial extracts in order to determine what was present in the nucleus and if it could be purified. This was subsequently identified as deoxyribonucleic acid (DNA). They could demonstrate that DNA is the material which allows the genetic characteristics to be transferred from one bacterial strain to another. In this case, it involved the transfer of *S. pneumoniae* strains that form small colonies with rough surfaces (strain R) to strains with large colonies and smooth surfaces (strain S). R strains that do not provoke pneumonia can transform into pathogenic S strains when cultured with bacterial extracts of S. The fact that nucleic acids were more than just a component of the cell nucleus astonished the scientific environment of that time (15).

DNA structure was not revealed until 1953, year in which James Watson from United States and Francis Crick from England reported their surprising work on the structure of the double helix. For these investigations, they were awarded with the Nobel Prize in medicine and physiology in 1962, together with Maurice Wilkins. DNA is a macromolecule constituted by deoxyribonucleotides, and each one of these is composed by a phosphate group, a pentose (deoxyribose in this case) and a nitrogenous base (16).

3.2. Some methods to measure the animal genome size

Animal genomes have a big difference in size, there is over 3000-fold variation between the smallest and the largest one. Nevertheless, it is very difficult even nowadays, trying to explain this big variation, due to the lack of knowledge about the genome sizes of many species. This information could be very important to be aware of some strange situations that happen to the genome such as duplication and polyploidy. It could also provide us very valuable information about the evolution of the genome, which allow us to develop next generation genomic sequencing projects (17).

It has been proposed that genome size is associated with a myriad of organismal aspects, such as egg size, characteristic paternal features, cell size, metabolic rate and body size (18). Species with larger genome sizes have been associated with lower metabolic rates, constraining them to more stable habitats that allow them to have a slower development. On the other hand, species with smaller genomes could be widely distributed in numerous habitats (19).

Several methods had been utilized to measure nuclear DNA content and even though that they were very vague, these techniques were enough to prove that it was constant among conspecific individuals and to realize the big variation in genome sizes between organisms of different species. Some of these methods employed bulk biochemical DNA extractions. The first determinations of genome size were made through colorimetry assays on DNA that was previously extracted from a determined number of cells. However this method did not totally work due to the difficulties at the moment of the identification of subpopulations of cells with different amounts of genetic material (20). André Boivin and Colette Vendrely carried out the first measurements of nuclear genetic material in 1948, and two years later, in 1950 Hewson Swift developed the concept of C-value. Flow cytometry methods were utilized to analyze the various types of DNA amounts present in the studied cells (21).

The conventional and reliable methods for determination of genome size such as Feulgen densitometry, Feulgen image analysis densitometry and flow cytometry, are mainly restricted to organisms that can be raised in a lab or simply found in nature and easily transported to a lab, due to the fact that, these techniques require that the tissues have in great measure safe cells, therefore the tissues must be live, frozen or adequately

fixed (17). There are several manners to measure the genome size of a species, some more accurate or complicated than others. According to the animal genome size database, to achieve this measurement, we could use densitometry methods, biochemical analysis, ultraviolet microscopy, among other several techniques. The results obtained could vary slightly (even in individuals from the same species), depending on the method or the type of cells to be analyzed (table 2). A few of them will be described below:

Common name	**Scientific name**	**Genome size (picograms)**	**Technique**	**Cell type**
Domestic cattle	*Bos taurus*	3,15	UVM	Sperm
		3,6	FCM	Leukocytes
Sheep	*Ovis aries*	2,34	UVM	Sperm
		3,3	FD	Leukocytes, liver
Domestic pig	*Sus scrofa domesticus*	3	FCM	Leukocytes
		3,15	FD	Sperm
Domestic dog	*Canis familiaris*	2,85	FCM	Leukocytes
		3,43	FD	Liver
Domestic cat	*Felis catus*	2,86	FD	Lung (culture)
		3,1	FCM	Leukocytes

Table 2: *Genome size variation according to the method and cell type utilized.*

3.2.1. Feulgen densitometry (FD)

Genome size measurements were not done with densitometric methods until 1930s. These techniques were first applied for nucleic acid quantifications. Feulgen densitometry was occupied to analyze the correlation between DNA content and nuclear surface in blood smears of the species of interest. The normal process used to be done with chicken (22).

This method is very utilized to measure genome size, due to the fact that the results obtained are very accurate. Nevertheless, there could be some mistakes if the process is not correctly done, for instance, the hydrolysis time could vary according to the species that is being analyzed (23). The fuchsin used for this method are normally four: Pararosiline, Rosaniline, Magenta II and New Fuchsin, that are normally utilized as

chemically pure dyes for this technique. The Feulgen method selectively stains DNA and under determined conditions can be used for photometric analysis of genetic material content. It relies on the premise that the amount of stain bound is directly proportional to the amount of DNA present and the quantity of stain to be used is related to the amount of light it absorbs. Feulgen densitometry has only few issues that complicate the quantification of the stained molecules attached to the genetic material, such as the fact that it is not possible to measure absorbance directly, since it is not-emitted light and it can only be measured using the transmittance, which is the amount of light that passes through the object (24).

The steps to follow for this method are only two. First the fixed material must be treated with 1N HCl in a water bath or oven at 60°C during approximately 10 minutes. Next, the material should be rapidly put into Schiff reagent at room temperature for at least 30 minutes until the tissue stains deep purple. The Schiff reagent is mainly used to detect aldehydes and the staining of biologic tissues, for instance, if it is used in human skin, this will stain normally due to that skin contains aldehydes functional groups in the saccharides that are present (25). Afterwards, the material should be squashed in acetocarmine or aceto orcein, another type of dye. It is very recommended to analyze the material on the same day, if it is not possible, it could be kept at 4°C for several days. (26).

3.2.2. Flow Cytometry (FCM)

FCM is a technique that uses a diversity of fluorescent reagents, such as fluorescently conjugated antibodies, DNA binding dyes, viability dyes, ion indicator dyes and fluorescent expression proteins; and it also needs lasers as light sources in order to generate scattered and fluorescent light signals that are read by detectors like photodiodes or photomultiplier tubes. This method is very useful nowadays, since it is applied in many disciplines such as immunology, molecular biology, virology, among other important topics. For instance, it could be occupied for common clinical laboratory tests, review of CD4 cell count in HIV (Human Immunodeficiency Virus) patients or the identification of inflammatory cells active in diseases like lupus or psoriasis. (27).

This technique measures the different properties of a cell in a flow system. The cells or nuclei that is analyzed should be in suspension and free of clumps. This process can be performed on a wide diversity of tissues such as peripheral blood, skin biopsies, etc. It is carried out in a buffered salt based solution, where the suspended single cells or particles past single or several lasers, that are used to study the visible light dispersion or fluorescence parameters. This allows to obtain information on several cellular processes, such as expression of surface markers, intracellular cytokine and signaling proteins (28).

Flow cytometry could be also used to analyze or distinguish chromosomes on the basis of their DNA content, in this case, it is called Flow karyotyping. Nevertheless, this method has few disadvantages, such as that it could produce extensive quantities of information, turning the analysis and studies of the results, very complicated and tedious (29).

3.2.3. Ultraviolet Microscopy (UVM)

This useful method combined with computational techniques, can generate maps of nucleic acid or protein mass. This is carried out through the perfect image resolution, obtaining an increasing contrast enhancing. The resolution of the microscope depends mainly on the wavelength of the light source, for instance, the short wavelength of ultraviolet light (180 to 400 nanometers) helps to have more resolution of the image that is being analyzed than the diffraction limit of optic microscopes that use normal white light, which has around 400-700 nanometers (30).

UV techniques have been improved through the years, due to that it can provide valuable information using accurate experimental methods. For instance, for the analysis of lignin present in plants, UVM provides an image of this polymer in a particular area of woody tissue, which is very helpful to determine the microscopic distribution of the cell wall (31).

3.3. Genome Size Variation

The term “genome” was proposed by H. Winkler in 1920. Scientists define it as the total amount of genetic material or ADN present in an organism, that codify for the necessary proteins to allow the development of life. Various analysis of genome from different individuals, indicate that in less complex organisms such as prokaryotes (Archaea and Bacteria), genome size is directly related to the number of genes. This does not happen with more complex organisms like eukaryotes, since there is no correlation between those two factors. Complex eukaryotic organisms do not have a greater genome size. This is clearly observed in the case of salamanders, which genome is approximately ten times bigger than that of human, and obviously, these animals are not more complex than humans (32).

The analysis of genome size is based on the classification of life on the earth, the phylogenetic studies about gen 16S rDNA, which is very conserved at an evaluative scale and codifies for the small subunit of ribosomal RNA, allows us to analyze the relationship between different living organisms, establishing that cell life is divided in three main domains: bacteria, archaea and eukaryotes. Each of them has a particular genome size in determined groups (33).

Bacteria and archaea domains have a genome size that varies between 0,58 Mb (1Mb= one million base pairs (bp), until 10 Mb or more in several species of cyanobacteria. Generally, the major part of genome sizes is lower than 5Mb, nevertheless, in the case of bacteria, it is within 50 Mb. Therefore, in both domains, there is a relationship between genome size and the number of genes. Then, the size of a prokaryotic gen is practically uniform and the genetic density is constant in bacteria and archaea. Being that, prokaryote organisms that have greater genome size, have more genes and consequently, are more complex (34).

In eukaryotes, genome size is defined as C value, which is the amount of DNA per haploid genome, and it is known to be constant or characteristic in a determined species. It is evident that eukaryotes have larger genome sizes than prokaryotes, with some exceptions, this is why the range of sizes is very wide. However, these organisms are characterized for having a big quantity of DNA. Nevertheless, eukaryotes do not have a clear relationship between the genome size and the complexity of an organism, as it was

mentioned before, this could be clearly noticed if we compare unicellular protists, like amoebas, which have a C value that varies from 23,5 Mb to 686.000 Mb. On the other hand, humans have 3.400 Mb. Then, amoeba has approximately 200 times more DNA than humans, nevertheless, this organism clearly is not more complex than a human being (35)(36).

There are more species such as amphibians, fish, or even plants that have larger genomes than mammals, however, if apparently similar organisms are compared according to its complexity, the results are that they have wide differences in their C values. This lack of correspondence between the amount of genetic material and codifying genes is called “C value paradox”, for the reason that, it is not possible to determine whether a species is going to possess lees DNA than the required for their vital functions (37).

Previous investigations have demonstrated that the eukaryotic genome is composed of a big amount of repetitive DNA and no coding sequences. Also, introns, which are eliminated in the maturation process of RNA. These sequences are not part of the protein synthesis process. Both repetitive and no coding DNA are considered as “junk DNA”, which is what influences the most in the C value variation among eukaryotes and could explain the paradox about genome size (38).

The sequencing of genomes allows us to determine more accurately the number of genes that are present in those sequences, and even more specific mechanisms that are used by several domains, such as expansion of proteome, which is applied by eukaryotes to obtain more than one protein from one DNA sequence. Besides, the presence of large introns in mammalian genome, could hinder the information that cannot be obtained only with the DNA sequencing. There is still a long investigation ahead until we discover the number of proteins that an organism is capable of synthesize. Nevertheless, it is true that more complex organisms have more genic functions (39).

3.3.1. Factors that influence in genome size

An important aspect about genome is that there are several factors that could alter its size, for instance, mutational mechanisms that could occur in a big scale, such as

duplication of genome, or in a small scale like loss or gain of nucleotides. Being that, these alterations could be transmitted to the progeny, if it is the case that the mutations appear in the gametes. The chromosomic mechanisms could also cause drastic variations with only one mutation, since they may affect a whole chromosome or part of it. These variations could emerge not only in animals, but also in plants. Notwithstanding, mutations have a beneficial effect in genome evolution (40).

There are more factors that could affect genome size such as transposable elements, which duplicate themselves and the copies get inserted in the DNA sequence. This induces fast increments in size or act as controllers of proliferation, since they selfishly emerge in the genome instead of other sequences. Spontaneous deletions or insertions of a few nucleotides are also, very influent in the genome size in long term. When these insertions emerge in no codifying regions or pseudogenes, the sequence will deactivate and disappear from the genome. Also, microsatellites and minisatellites are other mechanisms that have an influence on genome size, these regions possess a quantity of repeated nucleotides that vary a lot among a species, due to the recombination or mistakes during replication of DNA that cause the increase or decrease on the number of repetitions in the sequences (41)(42).

All of these alterations and mutations in genome size, appear in a periodic time among species, including humans. According to the advantages or disadvantages of these alterations for the organisms, natural selection will keep them or eliminate them (43).

3.3.2. Junk DNA and the C value paradox

During the 1800s and early 1900s, cattle tissue was one of the most used sources of DNA for investigation. Several kilograms of cow pancreas were dissolved in hundreds of liters of dilute sulfuric acid to obtain a feasible quantity of genetic material. In 1948, André Boivin and two of his students started an investigation about DNA content compared in several cattle tissues, including pancreas, thymus, liver and kidney and also liver from pig and guinea pig. The analysis exposed that the nuclei from all of these tissues had approximately the same amount of DNA, which is roughly twice as the quantity present at sexual cells, such as sperm, from the same families. Then, knowing that the genome size constancy is the role of genetic material within species, the logical

conclusion was that this quantity is equivalent to the number of genes, however, this hypothesis started to be cleared out when it was analyzed in different species (44).

In 1971, C.A. Thomas described the “C value paradox”, which explains that even similar species can have very different genome sizes. The letter “C” in the term “C value”, means constant or characteristic value of haploid DNA content per nucleus, measured in picograms (1 picogram is roughly 1 gigabase). There are several species from the same genus that differ a lot in their haploid genome sizes. Many hypotheses have been proposed to explain this paradox, nevertheless, the most accepted, was proposed by Susumu Ohno in 1970, Ph.D., a scientist at the City of Hope, National Medical Center in Duarte. It says that genomes carry some fraction of genetic material that has no adaptive advantage for the organism, Ohno called this “junk DNA”. This term appeared when he published his famous paper titled “So much junk DNA in our genome”, and its meaning was extended to all non-coding sequences, which do not code for proteins. However, it is not correct to call all the noncoding genes as “junk”, due to the fact that, there are genes that are regulated by these fractions of genetic material. "The earth is strewn with fossil remains of extinct species; is it a wonder that our genome too is filled with the remains of extinct genes?" wrote Ohno (6)(45).

It has been established by several scientists that only a small fraction of the human genome is composed of protein coding sequences and that some non-coding genes have main biological purposes. The term “junk DNA” is used nowadays, to mention any DNA sequence that does not have an important function in development, physiology, or some other organism-level dominion. Nevertheless, it is not totally certain that this type of genetic material exists in the genome, due to the fact that it is not adequate to argue that most or all noncoding DNA sequences are “useless junk” (46).

In 1964, the German biologist Friedrich Vogel did an approximate calculation about how many genes has a human genome, or if it is only formed by genes. Vogel affirmed that human genome must have an awful lot of them, 6.7 million genes. Nevertheless, there was no confirmation that our cells produce 6.7 million proteins or anything close to that number. Because of that, Vogel assumed that the genome was mainly integrated of essential noncoding DNA, that probably function as switches, turning genes on and off. However, several scientists disagreed with this idea (mathematically speaking), due to the reason that, for instance, each baby that is born,

has roughly 100 new mutations; if every sequence of genome were important, then a large number of those mutations would cause remarkable birth defects, thus the species would become extinct, because of the number of these defects multiplied over the course of generations (47).

Then, due to this paradox, Crick, scientist who discovered the three dimensional structure of DNA with Watson in 1953, and other investigators deduced that the genome is not exaggeratedly packed with coding genes, on the contrary, the genome was conformed principally of noncoding DNA, around 98,5% of the total human genome (figure 1), and most of it was considered as junk, since it apparently has no function (48).

Scientists have found that some of these repeats present in the genome, are no more than "genetic parasites", short DNA sequences left behind over the years by retroviruses or other pathogens that unintentionally copy themselves as a sequence of Cytosine, Guanine, Adenine, and Thymine. They also found that all of the regulatory elements (promoters and enhancers) needed for gene transcription are located in the non-coding DNA (49).

Then, the term "junk DNA" refers to pseudogenes, which are useless copies of protein coding genes. These are considered as mobile elements and studies have investigated the dependence of pseudogenes on other sequences such as long interspersed nuclear elements or LINEs, in order to move across the genome (50).

In 1980, it was possible the recognition and isolation of the first human genes by positional cloning, that allowed scientists the sequencing of the total human genome, which was the largest collaborative scientific project in the world. The main discovery of those who worked in the Human Genome Project (HGP), was that only 1.5% of the genome encodes approximately 21,000 distinct protein coding genes (figure 1). Then, what does the rest of the DNA do? Many of the noncoding sequences are repeated transposable elements, around 45% of the total genome, that make possible genomic rearrangements, and are important for evolution (51). Transposable elements (TEs) are mobile genetic units that present a wide variety in their structure and transposition mechanisms. Most of the plant and eukaryotic genomes are dominated by TEs and their movement and accumulation have a major role in driving genome evolution, since they facilitate genomic rearrangements (52).

These elements were first discovered by Barbara McClintock in the 1940s during her studies of maize: she noticed that the patterns of color changed in several breeding crosses. She analyzed her results and concluded that the genes were regulated by some type of "controlling elements" that could go from one place to another on the chromosomes. The changes in color were due to their reactions on pigmentation genes. For this investigation, she received the Nobel Prize in Physiology or Medicine in 1983. Her principal conclusion was that these elements have a major influence on the development of the organisms (53).

Transposable elements are generally divided in two groups according to the type of mobilization: they can be DNA transposons and retrotransposons. The first group move through a "cut and paste" method, the sequence shifts to another position in the genome. On the other hand, retrotransposons move using a "copy and paste" mechanism since it doubles the element into a different genomic location through an RNA intermediate, for this reason, retrotransposons become greater in number than DNA transposons (54).

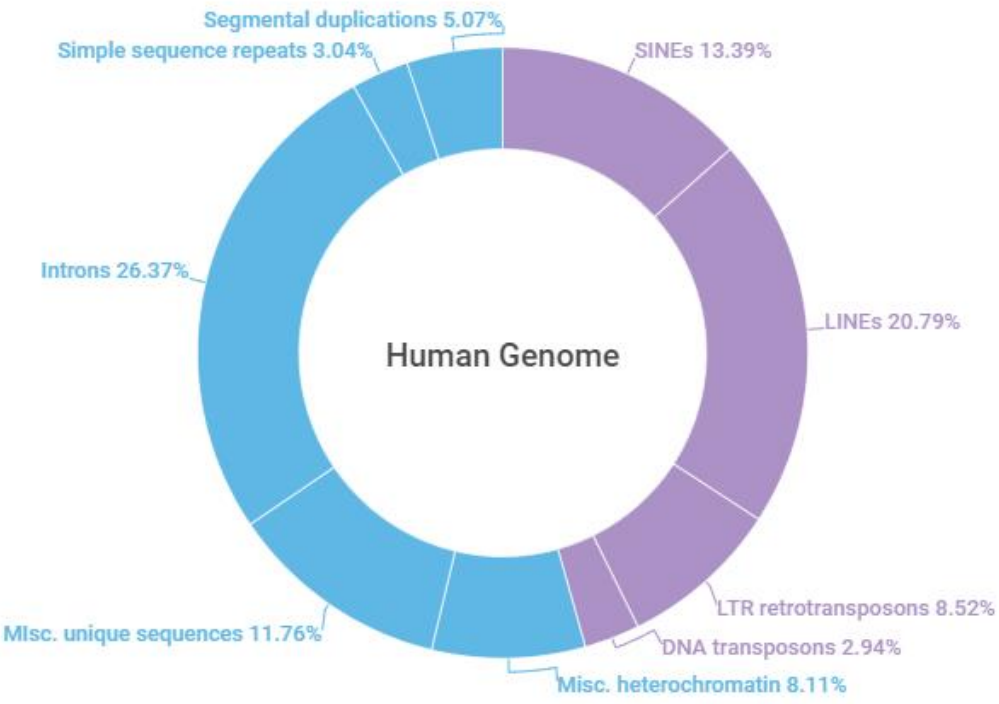

***Figure 1**: Components of Human Genome. Only approximately 1.5% are protein coding sequences (light blue sections). Own elaboration. Data from the International Human Genome Sequencing Consortium.*

The human genome is packed with these tandemly recurrent DNA sequences or retrotransposons, which can be divided into: long terminal repeat (LTR) and non LTR. Both contain genes for the inverse transcriptase or retrotranscriptase (the enzyme that catalyzes the inverse transcription). The first group is similar to retroviruses in structure and mechanism, LTR retrotransposons have more than 5 kilobases long. On the other hand, non LTR do not have long terminal repeats, as their name explains it. These are divided in: long interspersed element (LINE) and short interspersed element (SINE), which conforms the 20% and 13% of the genome, respectively (figure 1). Around 45% of primate genomes are made up by transposable elements (TEs) and at least a million Alu repeats (type of transposon), also 25% is conformed of shorter tandem repeats such as satellites, minisatellites and microsatellites (55)(56).

Alu elements are fragments of DNA of 300 approximately, that could be found in several places at primate genome. Their name proceeds from the Alu endonuclease, which is used to analyze these parts of genetic material. These elements act on the genome on a large number of manners, causing gene conversion, insertion mutations, recombination between elements and alteration in gene expression. Alu elements are very useful for the analysis of human population genetics and primate comparative genomics due to the fact that they are neutral genetic markers of identical offspring with known ancestral states (57).

Eukaryotic species present a big difference in the amount of TEs in their genomes, these variations contribute to modify them, for this reason, there is a wide diversity among eukaryotic genome sizes and even between closely related species (52).

Genome size varies enormously among species, at least 7000 fold among animals. These variations are not related to the organism complexity or the number of protein coding genes as it has been mentioned before; for instance, the human genome is much smaller than that of a lungfish (figure 2), even though humans are the most complex creatures in the planet (physiologically speaking). Nevertheless, organisms that have a very large genome are not scarce, for example, from more than 200 salamander genomes that have been sequenced thus far, all are around 4 to 35 times larger than human genome. According to the Animal genome size database, the largest genome among animals is found in the marbled lungfish (*Protopterus aethiopicus*) with a measurement of 132.83 picograms; while the smallest genome belongs to a plant parasite nematode (*Pratylenchus*

coffeae), that has a genome of only 0.02 picograms of size (table 3). These wide variations observed in genome sizes also happen in closely related species with the same biological characteristics and equal ploidy level (46)(38).

Common name	**Species**	**Genome size (picograms)**
Plant-parasitic nematode	*Pratylenchus coffeae*	0,02
Human	*Homo sapiens*	3,5
Salamander	*Salamandra salamandra*	40,11
Marbled lungfish	*Protopterus aethiopicus*	132,83

***Table 3**: Comparison between different genome sizes (the biggest, human, salamander and the smallest genome size).*

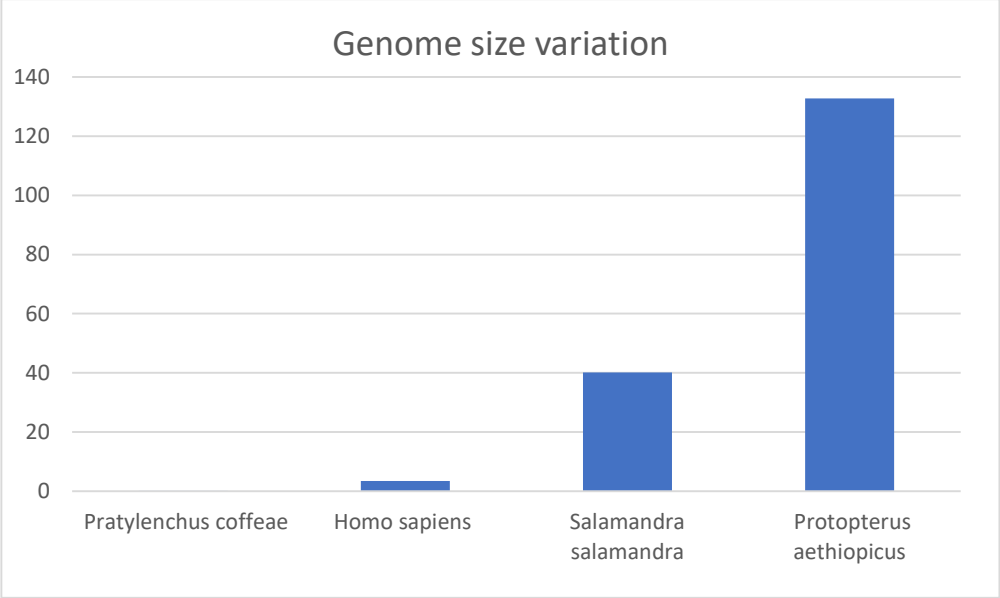

***Figure 2**: Graphic representation of the information presented in the table 3. Own elaboration. Data from the Animal Genome size database.*

3.3.3. A modern view of the C value paradox: The C value enigma.

A paradox is not an accurate term to describe this "puzzle" regarding to the genome size, since there is no paradoxical aspect about the discrepancy between C value and gene number and the term "paradox" is used to describe something seemingly absurd or opposed to common sense. However, this "paradox" has been figured out, since Dover

(1990) pointed out that there was no longer any paradox, to be addressed because several molecular mechanisms can explain the sources of noncoding genes, which conforms the major part of the genes, for instance, the human genome comprises less than 1,5% protein-coding regions, being the rest 98,5%, non-coding DNA (58).

Genome size evolution is much more accurately referred as “C value enigma”, a term that was endorsed at the second plant genome size discussion and workshop at the Royal Botanic Gardens in the UK, and several scientists began to adopt this manner to call it. Also, the term paradox brings a lack of understanding of one of the most basic features in eukaryotic genomes, the noncoding DNA. Besides, this term has made the investigators to seek for a simple one dimensional solution to what really is a multi-faceted puzzle (59).

The C value enigma is a complex puzzle which consists of several component questions, surrounding the extensive variation in the nuclear genome size among eukaryotic species. These questions are: What types of non-coding DNA are found in different eukaryotic genomes, and in what proportions? Where does this non-coding DNA come from? and how is it spread and/or lost from genomes over time? What effects or functions, does this non-coding DNA have for chromosomes, nuclei, cells, and organisms? And why do some species exhibit remarkably streamlined chromosomes, while others possess massive amounts of non-coding DNA? However, even a conclusive answer to one of this questions does not solve the whole puzzle. It requires several approaches to be applied and integrated (50).

3.3.4. Animal Genome Size Database.

Measuring genome size is clearly important to understand its evolution. Several studies use whole genome sequencing analysis, however these methods have some disadvantages, despite all the investigations that have been done around this topic, there are only few examples of complete genome assemblies. These tend to be shorter than the genomes they represent, due to the fact that these concepts are not the same. Sequenced data can give information about genome size, nevertheless, it is not always precise or accurate, and according to the method that is being used, the results could vary in each case, underestimating features or lacking some type of information about genome (60).

Most of the questions related to the C value enigma, require large amounts of genome size data that is obtained from a wide taxonomic sampling. Botanists have compiled data from plant C values, focusing on angiosperms, since 1970, and it was available online since 1997. In 2001, the Animal Genome Size Database was launched online and nowadays, it contains nearly 4000 species, including roughly 2500 vertebrates and 1300 invertebrates. Even though, the animal and plant databases are maintained separately, both offer the free distribution of all available genome size data and require the development of standardized methods to make sure that the measurements obtained are more accurate and reliable (61).

This database is very visited by several researchers in just one day, for instance, in 2004, it was receiving about 100-150 hits per day on its various pages and it has been used in almost 100 countries. Unfortunately, these data are not available for other groups such as fungi or protists, although currently, it would be very useful for many investigations, such as this one, which was based on the Animal Genome Size Database information, in order to compare and analyze it accurately (20).

3.3.5. Bird Genome Size: Why is it smaller?

Eukaryotic genome sizes are shaped by not only intrinsic but extrinsic factors, such as transposable elements proliferation, genomic deletions and duplications, or also genetic drift and selection. It has been observed that birds have a small amount of DNA when compared to other terrestrial vertebrates. For instance, approximately 200 species of birds present 1-2 pg in mass. This presumed constraint for the bird genome size diversity is explained by the fact that birds need small cells and high surface areas for gas exchange, therefore their cells have smaller genomes; in order to maintain the oxygenation of blood and release of carbon dioxide produced in cellular respiration, this mechanism is very necessary to balance the acid-base corporal state, thermoregulation and vocalization. These adaptations allow birds to cover the metabolic demands that are needed for flight (62).

The concept that flight ability is related to the genome size in birds is supported by the fact that flightless birds have larger genomes. On the other hand, birds that are expert flyers have smaller genomes (figure 3). The variability in the nuclear genome size

has been a reason for investigation and discussion during several decades. Comparative patterns are analyzed to identify what is the main force that shapes the genome size diversity. As an example of genome reduction related to the flying capacity, we could analyze bats (even though they are not birds). It is an evidence that flight constraints the genome size due to its metabolic demands. For this reason, this animal has substantially less DNA than other mammals. Birds possess even less genetic material than do their closest living relatives, the reptiles. This characteristic is an adaptation that was achieved through the years by natural selection (63).

Then, avian genomes are of interest for its rapid metabolic rate that constraints the genome size, and it is associated with flight ability. Also, heart and muscle size, are both thought to be promotors of flight. Nevertheless, genome size is directed by the expansion or contraction of introns and repetitive sequences, such as transposable elements (TEs). Therefore, avian genomes are smaller for the reason that they have smaller introns and also, small insertions and deletions in repetitive sequences. However, there are three different mechanisms to obtain a genome size estimation: the assembly of sequenced genomes, those that are analyzed through k-mer method, which bases on the number of substrings contained within the sequence), and those obtained from the Animal Genome Size Database, which is mainly based on flow cytometry measurements of cellular DNA content (64).

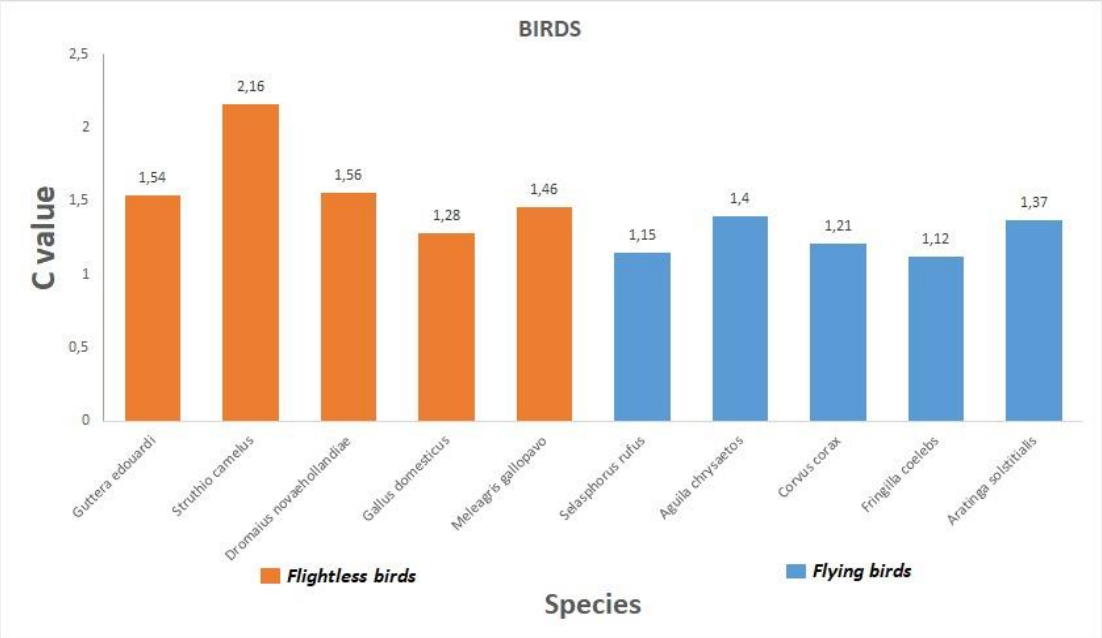

Figure 3*: Genome size variation in birds. Flightless and Flying birds. Own elaboration. Data from the Animal Genome size database.*

Subsequently, reduced DNA content would be expected in small, vigorous birds such as, for instance, hummingbirds of the family Trochilidae, which most species measure 7.5-13 cm in length. Despite of that, several investigations have demonstrated that bird genome size have the shortest scale of interspecific diversity of any vertebrate class. Even though their small genome size, modern bird species are ecologically diverse (65).

3.4. Mammals and Birds

Mammals are part of a very numerous species, almost 5000 divided into 26 orders. These animals have different shapes and sizes, one of the principal features they have is the presence of mammary glands in order to nourish their offspring. Also these organisms have a very unique skin, due to the fact that it is composed of two layers, the outer nonvascular epidermal layer and the inner layer of the corium, that allows them to protect themselves from fungus and bacteria, thanks to its surface that is covered by lipids and organic salts. However, all mammals have hair during some time of their development and others have hair all their lives. This hair is made of keratin and allows them to regulate their temperature according to their needs, it also works as a sensory organ, due to the specialized hairs called vibrissae (66)(67).

These creatures are divided in three major groups, monotremes (Prototheria), marsupials (Metatheria) and placental mammals (Eutheria), these three groups have different characteristics, however, they are all united by the feature of embryonic development. The main differences between them, is the type of reproduction, for instance, monotremes deliver eggs and incubate them during 10 to 11 days. All mammals are viviparous except for monotremes. On the other hand, the other groups, marsupials and placental mammals have various features, such as a posterior urogenital sinus: two vaginae and the urethra in marsupials, they also have a marsupium, a small sac located in the lower abdomen in which the offspring is fed and kept after birth, due to that the marsupial newborns are very little developed. Finally, eutherians or placental mammals, in which group, humans are included. Even though, mammals are a very successful species, these animals are much less diverse than invertebrates, since they are more capable to resist extreme ecological conditions than mammals. Nevertheless, these

animals have adapted through physiological and behavioral characteristics to reproduce and survive in their ecosystem (68)(69).

Mammalian C values average is about 3.5 pg (figure 4), almost exactly as human genome size. However, marsupials tend to have mean genome sizes (~4.0 pg) larger than most placentals (~3.4 pg). Bats (order Chiroptera) have the lower size only around 2.5pg. Monotremes have small genome sizes too, for instance the duck-billed platypus at 3.06 pg and the short nosed echidna at 2,89 pg. On the other hand, birds with nearly 10,000 species, are very diverse, nevertheless, their genomes have not been studied totally, since they are the least well studied group of vertebrates. Avian genome sizes are highly constrained, with an average of around 1.4 pg (42)(44).

Biologists express about mammals and birds as homeotherms, however, these animals usually have variations in their body temperature that could be regional or temporal, which are favorable for their genotypes that function over a wide range of temperatures. This is very important because the performance of any organism depends on its body temperature (70).

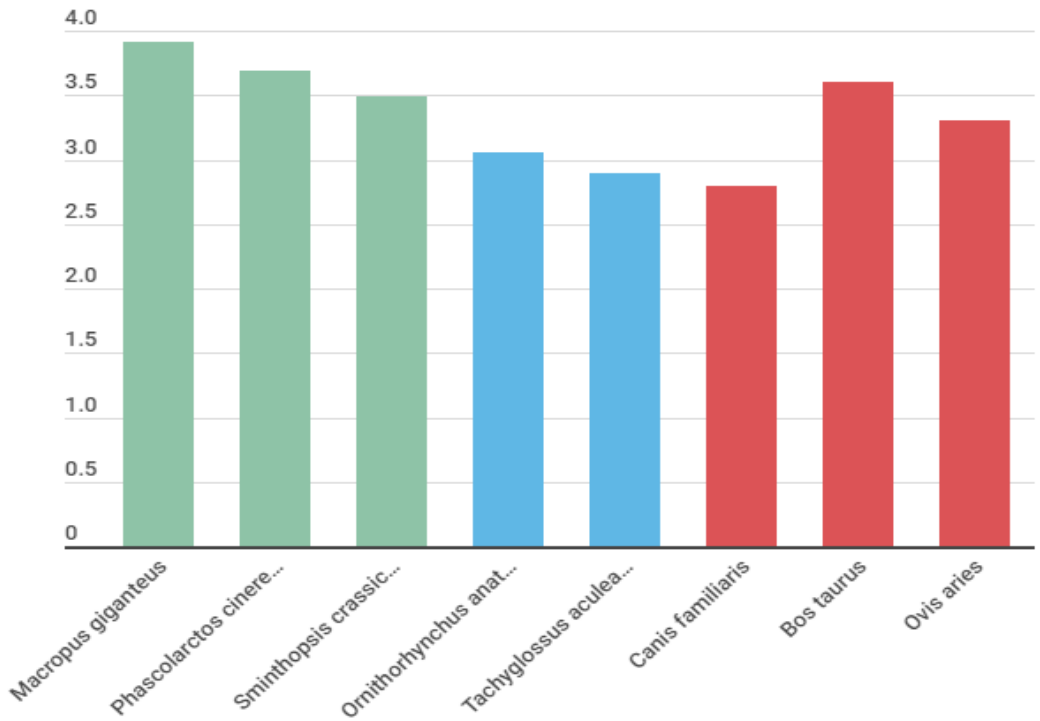

Figure 4: *Genome size variation among mammals. Marsupials (green), Monotremes (Light blue) and placentals (red). Own elaboration. Data from the Animal genome size database.*

3.4.1. Metabolic rate and thermoregulation

Metabolic rate is not the same in all animals, it principally depends on body size. For instance, among endotherms, smaller animals tend to have higher per gram basal metabolic rates than larger animals. This fact has been analyzed in many biological systems, such as interspecies observations of mammals and birds or analysis in the same species through the years with the variation in size, just as happens with mice. These animals have a BMR per kilogram (BMR/kg) nearly thirteen times higher than elephants. In other words, if we observe the metabolic rate of the entire organism, a bigger animal will have a higher metabolic rate, because it has more metabolizing tissue than a smaller one. Nevertheless, if we observe the per mass metabolic rate, the situation changes, for example, a gram of mouse tissue is much faster than a gram of elephant tissue at the metabolism process (71).

There are many factors that have an influence on the animal metabolic rate, such as temperature, mass and phylogenic affinities (72). Mammalian thermoregulation is essential to ensure the functioning of their organisms through the homeostasis, in which are implicated central, endocrine and metabolic functions (73). According to the source of corporal temperature regulation, organisms are classified as endotherms or ectotherms. Endothermic animals need more energy than other ectotherms of the same size, due to the fact that they have a higher metabolic rate in order to generate heat and conserve their body temperature (74).

Every organism needs energy to sustain their life, in order to do that, they break molecules into simpler ones. The energy obtained is utilized to transport ions, pump blood, move the body and several other important functions such as produce sufficient internal heat to maintain their body temperature, in the case of endotherms. Ectotherms, on the contrary, cannot produce sufficient internal heat to increase their body temperature above the environment. In older literature, the term endotherm was interchangeable with homeotherm, which is incorrect, because these two terms do not mean the same. Homeotherms are organisms that maintain constant their internal body temperature, usually within a narrow range of temperatures. On the other hand, endotherms regulate their own internal body temperature through metabolic processes. Then, an organism

could be considered as homeotherm not because it is thermoregulating, but due to the constant temperature of the environment. Poikilotherms, on the contrary, have a temperature that varies substantially, generally due to environmental temperature; while ectotherms rely only on the external environment. For instance, poikilothermic animals include various species of fish, amphibians and reptiles, also birds and mammals that lower their metabolism and body temperature, which is needed to survive extreme environmental temperature changes as a part of hibernation or torpor. Some ectotherms can also be called homeotherms due to the stable ambient temperatures that maintains their internal temperature constant, for example, some species of tropical fish (75).

Endotherms have also insulation to help them keep their temperature, such as fur, feathers or fat. Mammals and birds have been considered to be endotherms (in their majority). They are different from other endotherms due to the fact that these animals can maintain high body temperature at rest. Mammals and birds need a high basal metabolism to elevate their body temperature above environmental temperature, even if the animal is resting. This happens because of the higher mitochondrial volume, greater membrane surface per tissue volume and higher aerobic enzyme activity that endotherms have in comparison to ectotherms. Such characteristics are caused by the cellular membrane of endotherms, that is "leakier" than those of ectotherms, since it allows greater ion fluxes. This leakiness has been analyzed to be responsible for the higher basal or standard metabolic rate in endotherms. Nevertheless, these endotherms that have high food needs, do not always have the adequate ecological conditions to survive, such as climate or food, this is why they diminish their energy consumption for a while. This mechanism is called torpor and is applied by endotherm and ectotherm organisms (76)(77). This research paper focuses on endotherms principally, since it is about mammal and bird genomes.

Various are the factors that influence on the Basal Metabolic Rate (BMR) in mammals, such as body mass, food habits, climate, habitat, substrate, a restriction to islands or highlands, torpor and reproduction. The most influential is body mass, since it affects the energy expenditure, due to the heat loss, which is proportional to the surface area of the animal. Small animals tend to lose heat faster than bigger ones. This happens following the surface to volume ratio and how it changes according to size. For instance, a small cell has a larger surface compared to its volume, than a bigger one. Therefore, a small animal will have a larger surface to exchange heat with the environment, than bigger animals. This is why smaller animals need more energy and a higher BMR to maintain

constant their internal body temperature (in an environment lower than their corporal temperature). BMR determines how much food an animal needs to ingest to avoid losing corporal mass. In order to measure it, the sympathetic nervous system cannot be stimulated, this requires complete rest (78).

The benefits of thermoregulation rely on the spatial and temporal conditions, food consumption affects its advantages; for instance, the food shortage diminishes the maximal rate of energy gain. Notwithstanding, when food is not enough to ensure growth, thermoregulation could use up energy stores. Therefore, organisms that consume less food should thermoregulate less adequately. If the cost of thermoregulation rises, selection favor genotypes that depress their metabolism and temperature, such as they do during torpor or hibernation (79).

The factors that influence thermoregulation in birds affect the maternal brooding. Birds commonly brood their offspring during a determined time. This enhances survival and development of the species, even though it consumes the energy that could be spent in other activities. Endothermic physiologies present adaptations within the constraints imposed by genetics (63).

3.4.2. Torpor

The International Union of Physiological Sciences defines torpor as "A state of inactivity and reduced responsiveness to stimuli"(80). It is known as a period of time where mammals and birds reduce their energy expenditure to reach a hypometabolic state in order to survive stochastic ecological conditions. This physical state could be seasonal such as hibernation, estivation, among others, also it could be non-seasonal like nocturnal hypothermia or daily torpor. During this period of time, animals could save from 10% to 88% of energy. Endotherms are able to return to their normal temperature by shivering or non-shivering thermogenesis, which is the process of heat production in organisms (81).

Generally, mammal temperature varies approximately from 35° to 38°C in spite of the climate fluctuation of the environment. Nevertheless, according to the ambient temperature (Ta), these animals go through "torpor" or "hibernation", which are the terms used to define the dormancy at lower environmental temperatures (82).

Torpor and hibernation are not the same concepts. The main difference that has been established between them is the duration of each process. Torpor can last several hours (less than 24), while hibernation lasts several days or even months. These mechanisms could be induced at any time of the year in some species, as a response to exposure to low environmental temperature or low food availability. Hibernators start this process after a period of fat accumulation, prior to the cold of winter. The decrease in body temperature causes a diminishing in metabolic rate, food consumption, and activity, which leads to the reduction of fecal and urinary water loss. This is why several species use torpor during the dry season, such as drought. In this case, the correct term is "estivation", which is used to describe the dormancy or lethargy that occurs as a response to very high environmental temperatures or water shortage, particularly in environments where food and water availability are unpredictable. (83).

This fluctuation in the body temperature (Tb), causes a reduction in the metabolic rate. This depends on the pattern of lethargy, for instance, during hibernation, Tb goes down and heat production is very low, due to the fact that this lasts for one or two weeks, with short periods of normal temperature. Nevertheless, daily torpors last for less than a day and therefore, Tb and Basal Metabolic Rate (BMR) in this organisms, are maintained at higher levels than hibernators. The decrease of BMR during torpor is essential for the reduction of energy consumption. This reduction on the metabolism rate occurs due to the pattern of torpor, body mass and body temperature during lethargy (84).

Section IV: Discussion

4.1. Analysis of genome sizes in endothermic animals

The metabolic rate of endothermy is affected by body size due to the heat loss to the environment, which is proportional to the surface area of the animal. Also, the energy expenditure is influenced by climate, food and habits. Body size, which is measured by body weight is essential in the determination of energy expenditure in endotherms (78).

For this section, two factors will be compared and analyzed, C value (obtained from Animal Genome Size Database) and Basal Metabolic Rate (BMR) in birds and mammals, obtained from Brian McNab's investigation papers.

4.1.1. Mammals

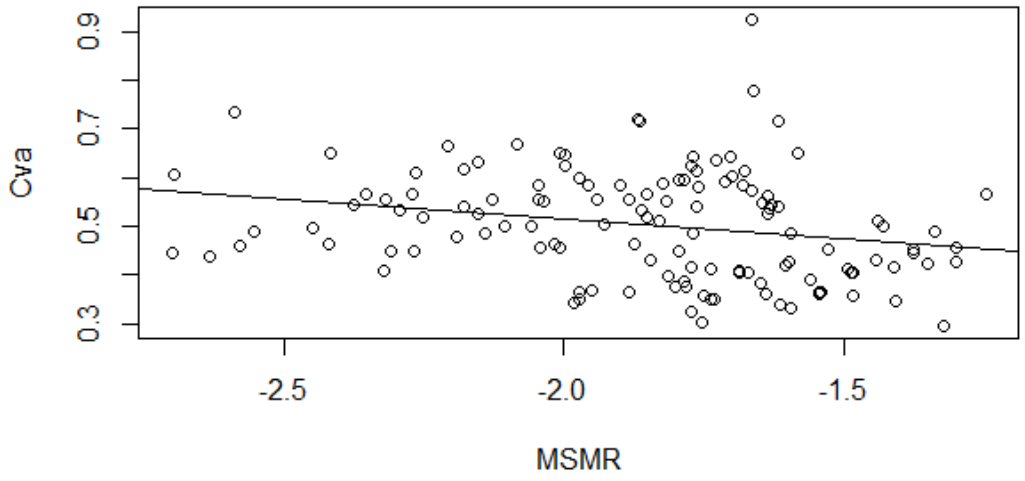

***Figure 5**: Logarithmic regression made on R program, between C value and BMR on mammals. Own elaboration. Data from the Animal genome size database.*

Genome size has been thought to have an influence on metabolic rate in several groups of animals. The nucleotypic effect explains that large nucleus and cells sizes have an effect on metabolism, described by the surface area to volume ratio. Nevertheless, the relationship between these factors, has not totally been explained, since there are many blank spaces in the knowledge about this topic. The correlation between genome size and metabolic rate has only been studied in tetrapods vertebrates and it varies a lot according to the organismal biology. The sizes of cells could influence on aerobic metabolism due to the oxygen consumption, which is proportional to cell surface, and thus, the capacity of efficiency on providing oxygen to the determined tissues, just as happens with erythrocytes, which have a larger surface and no nucleus, in order to absorb more oxygen that will be later transported to the whole organism. Smaller cells usually divide faster and have a higher metabolic rate (85).

The results obtained in figure 5, show a slight negative relation between basal metabolic rate (BMR) and genome size in mammals, that needs to be clarified with the addition of more data to achieve a better result. It is still essential to sequence several genome sizes that still unknown nowadays, from many species that have not been totally studied yet. This could allow a complete analysis of genome and find how it is affected or how it affects many other factors that it is thought to be correlated. This is of vital importance to understand evolution and all of its effects in the development of biodiversity.

4.1.2. Birds

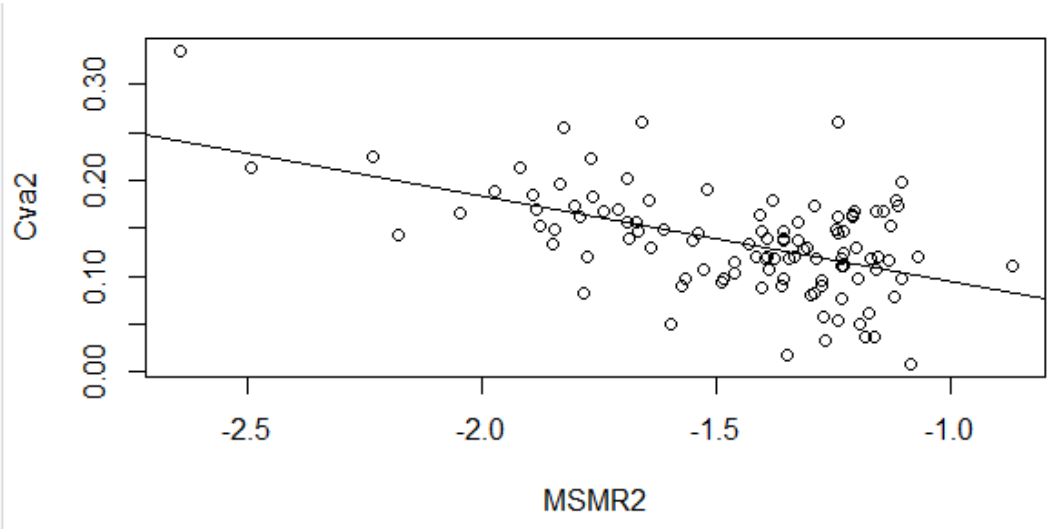

***Figure 6**: Logarithmic regression made on R program, between C value and BMR on birds. Own elaboration. Data from the Animal genome size database.*

In birds, it has been proved that C value is smaller than in other animals. This is either a result or a necessary condition for flight, due to the fact that flightless birds have larger genome sizes. Also, these migrating species have a higher metabolic rate in order to get rid of any extra weight that would be unnecessary to travel long distances. Given the very high metabolic demands involved, it has been argued that powered flight proposes solid constraints on cell and genome sizes. On this basis, a progressive reduction in genome size may have occurred during or after the evolution of flight (44).

In figure 6, we observe a slight negative relation between avian BMR and genome sizes values in birds, just as it was observed in figure 5 in mammals. Nevertheless, it is not well defined, since the values are scattered and there is no linear correlation between those variables presented in the graphic. Then, it could be affirmed that there is no relation between these two factors in birds. However, it is necessary to add and compare more data to affirm that. In birds the values are a bit different due to the higher metabolism some individual present, principally the flyer experts or migratory species.

BMR in birds may contribute and play a role in controlling the rate of insertions and deletions (indels). Since higher BMRs are associated with faster cell cycles and therefore, in more replication-dependent mutations. Also, it has been exhibited that birds have a higher rate of indels, even though they have low variation genome size. Then, reduced DNA content would be expected in a small, vigorous birds such as hummingbirds. Even though birds have a small and consistent genome size, modern individuals are ecologically diverse (9).

4.2. Comparison of Genome Size Variation Studies (Mammals and Birds)

Genome size has changed over the evolutionary time, by the addition or subtraction of different types of sequences through various processes. It has thought to be correlated with many features, such as size of the nucleus, size of the cell, rate of metabolism, and also, the rate of development. Accumulation of noncoding DNA and therefore, genome size or C value, increases the costs of cell division, due to the fact that the genome size becomes larger. Nevertheless, these costs may not be high enough to balance mutation pressure for “junk DNA” accumulation (42).

We call genome at the set of all genes from a cell of a determined species. The structure and function state of the genome is very dynamic. Individual development of organisms and their existence in a variable environment requires constant and ordered switching the expression status of the gene groups, often associated with genomic DNA rearrangements, caused by mutations and other processes. also, genome size is related to the size and number of introns and a number of other genomic properties associated to both, protein coding and non-coding regions (86).

Many of these relationships had been suspected to exist and even studied in few comparison papers. Nevertheless, it has been not enough analyzed to understand this enigma. For instance, some studies done in eukaryotes, affirm that there is no relation between genome size and the number of coding genes, therefore, the complexity of the organism. However, some other studies confirm that genome size and gene number, in fact, are positively correlated across a broad arrays of eukaryotes, in a much smaller and more taxonomically limited sample of genomes. It is worth say that the authors did not take phylogeny into account (32).

There are many external factors that influence on animal genome such as climate temperature and all the variables that are related to it. This influences the lifestyle of many animals, forcing them to adapt and develop several evolutionary features through time in order to survive. This had allowed them to resist extreme conditions. For instance, animals that go under a torpor, hibernation or estivation state. Torpor in endotherms is characterized by a periodic lowering of the set point for body temperature regulation with a corresponding reduction in metabolism for energy and water conservation in the organism, observed principally in mammals and birds. These processes affect the metabolic rate of animals and therefore, its genome size. In endothermic vertebrates, physiological demands have constrained its genome size evolution, by favoring smaller cells to facilitate the faster gas exchange. Significant negative correlations between oxygen consumption and genome size have been reported in birds and mammals (11)(78).

Nevertheless, there are many other factors that influence on genome size, such as chromosomal mutations, insertions or deletions, transposable elements, replication and duplications. These processes have been proved to be related to the C value of the organisms, named like that to reflect the relative constancy in genome size, observed among individuals of a given species. Scientists were surprised to discover that genome size did not correlate with apparent organismal complexity, but there was a positive correlation between erythrocyte size and genome size noted in vertebrates. Thus, it is of vital importance to analyze completely the genome sizes of every species and the characteristics that relate to it, in order to have a better comprehension of the evolution and its effects on the life on earth (45).

Section V

5.1. Conclusions

Currently, the study of the genome is essential to analyze and prevent several illnesses, due to the fact that it contains all the genetic information about each organism. For instance, in humans, which have approximately 30,000 genes, around 6,000 are linked to pathologies or illnesses. Then, the study of genome allows us to predict if an individual is going to be susceptible or resistant to determined affections, and therefore, take the most accurate preventive actions. There are still many gaps in the knowledge of all genes functions and characteristics. This is why, there has been special interest and analysis in all the non-coding genes, wrongly called “junk DNA”, which in humans, conform around the 98,5% of the genome. The determination of complete genome sequences provides us with an opportunity to describe and analyze evolution at the comprehensive level of genomes.

Various are the factors that influence on genome size, as it was studied in this investigation. Factors such as chromosomal mutations, insertions or deletions, transposable elements, replication and duplications; have been demonstrated to affect C value. It is not totally known which factors influence genome size, even nowadays. Many animal genomes remain poorly studied. This is particularly true of invertebrates, which means that the majority of genome size diversity continues unknown.

In general, organisms with bigger genomes tend to have more coding genes, therefore, larger introns and more transposable elements than organisms with smaller genomes. There are some species such as salamanders, which have genomes around thirty times larger than the human genome. However, these species are obviously less complex than humans, concluding with this data, that the complexity of an organism is not related to the genome size.

This is why, it is fundamental to study and analyze deeply, the functions of the whole genome, since each part of our DNA carries out a determined activity, important to our development.

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