



Review

Molecular Genetics in the Conservation of Zoogenetic Resources

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ABSTRACT

Background: Advances in molecular biology techniques, such as PCR testing, and the use of polymorphic sequences of mitochondrial DNA in the d-Loop region, have been frequently used to describe maternal lines in animals, especially in birds. **Aim:** To conduct a review of laboratory techniques applied to molecular genetics, and their importance in the preservation of zoogenetic resources. **Development:** Molecular markers, especially microsatellites, became more useful and profitable, as protocols and technology were optimized; genetic methods provide reliable information that helps in the preservation of zoogenetic resources, particularly when the most traditional methods are inadequate. **Conclusions:** The utilization of molecular techniques offers objective measurements of diversity among and inside races, which permits to study their genetic relations, and to provide evidence of unique genetic traits or genetic isolation phenomena from the past.

Key words: birds, microsatellites, sequencing, zoogenetic resources (*Source: DeCS*)

INTRODUCTION

One of the most alarming global problems today is the extinction of species and breeds due to several reasons. One of the most significant has a natural character, as it derived from the loss or reduction of resistance to some diseases, as well as the incapacity to adapt to certain types of climate. It occurs thanks to the loss of genetic resources caused by intensive production, genetic selection, foreign species introduced to new habitats, and the costs of cryopreservation of genetic material and genetic analysis, which create difficulties for preservation and related studies (Zinovieva *et al.*, 2019; Roh *et al.*, 2020).

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Genetic variability at the molecular level of individuals in a population can be studied and determined thanks to the advances in molecular biology techniques, such as the discovery of polymerase chain reaction (PCR), the utilization of microsatellite molecular markers and mitochondrial DNA (mtDNA). It is used to genetically breed and conserve these populations (Toalombo, 2020). The techniques enable researchers to identify individuals in a breed or species, determine genetic and parent kinship among individuals of some particular species, evaluate susceptibility to genetic diseases or establish qualitative characteristics (Aranguren-Méndez, Rincón-Carruyo, and Bravo, 2017).

In recent years, scientists have used genetic markers in order to know genetic structures. Polymorphic structures of mitochondrial DNA in the d-Loop region are used to discover maternal lines in animals, mainly birds (Meydan, Pish Jang, Yilduz Ali, and Weigend, 2016). DNA inheritance is not transmitted in a Mendelian form, but exclusively by the mother, which is of great help in the construction of a phylogenetic tree, besides its elevated rate of mutation, which is favorable to identify the origin and distribution of the population (Malomane *et al.*, 2019).

Some of these populations have unique traits in specific environments, and suffer a genetic dilution or extinction that leaves them without the possibility of morphological or genetic characterization. Therefore, it is important to evaluate every animal coming from an ever increasing number of agroecological regions and production systems (Dhorne-Pollet, Barrey, and Pollet, 2020).

Considering the previous elements, the purpose of this research is to conduct a review of the laboratory techniques applied in molecular biology, and their importance in the conservation of zoogenetic resources.

DEVELOPMENT

Genetic resources

The United Nations Food and Agriculture Organization (FAO) has stated that the diversity of domestic animals is an important component of global biodiversity. More than forty species of domestic animals and plants help compensate for the needs of food worldwide, as they supply meat, fiber, milk, eggs, furs, and manure. More than 8 000 breeds constitute the animal genetic resources (AGR) of high importance for nutrition and agriculture (Miller *et al.*, 2018).

The extinction of different species, both wild and domesticated have caused the loss of biodiversity, so it is necessary to take preventive steps. Human nutrition relies on approximately thirty plant species and fourteen animal species (birds and mammals), regardless of aquaculture resources. Estimates show that 30% of mammal breeds and domesticated birds are threatened (Miller *et al.*, 2018). The implementation of plans to conserve genetic diversity is necessary to

comply with sustainable management of AGR for handling and conservation of animal genetic resources, in order to contribute to food safety and sovereignty.

Conservation of zoogenetic resources

In Latin America and the Caribbean (LAC), approximately 20% of existing classified breeds are considered endangered. The total percent of birds in danger of extinction has gone drastically from 5% in 1995, to 45% in 1999. These figures are appalling, so a great deal of efforts are required to promote the defense of endangered genetic resources. This seemingly very serious situation should be better understood (Macrì *et al.*, 2019).

Concerns over the conservation of genetic variability began in wild animals, first linked to species conservation, and later to maintenance of genetic diversity in every species. This concern paved the way for zoos, natural protected areas, and the implementation of conservationist policies, such as bans, reservations, protected species, etc. It was known that up until the past century this process was not socialized or spread to the domestic animal world, since concerns over the dangers of genetic variations of domestic species were not considered at the level of breed, and within breeds (Malomane *et al.*, 2019).

Neglect of protection measures causes the extinction of many wild species, and brings about a progressive process of homogenization of domestic species. Thus leading to the loss of breeds, which might have a special genetic aptitude for some purposes, but for diverse reasons they begin to lose importance. The conservation of genetic resources of farm animals refers to all human activities, including strategies, plans, policies, and measures adopted to guarantee the maintenance of the diversity of such resources, in order to contribute to the food and agricultural production and productivity now and in the future. Besides, the ultimate goal of conservation is people's well-being; hence, the value assigned should not be exclusively limited to their economic value in the market or their genetic singularity, but also to the social and cultural values (Wiśniewska *et al.*, 2019).

In the 1980s, the interest aroused in relation to the conservation of domestic animal species, which includes genetic resources and ecosystems, was evidenced during Technical Advisory Committee on Animal Genetic Resources, Conservation, and Husbandry, organized by FAO, in Rome. The final document laid the foundations and recommendations in terms of the genetic resources of every country, resulting in the Animal Genetic Resources Information Bulletin, in 1983. The outcome of that cooperation was the Data Bank for Animal Genetic Resources (Malomane *et al.*, 2019).

The diversity of domestic animals is the set of ecosystems, species, and genetic varieties that exist in a country. Although every country is entitled to manage their own genetic resources, these should be considered an asset of humanity. The main causes for the loss of biodiversity is the accelerated pace of the growth of human population, an increase in the consumption of

natural resources, and unplanned exploitation. Consequently, there is new concern for acquisition of new better knowledge about zoogenetic resources, particularly about native domestic animal species to every region, since their adaptability and high productivity pose new challenges to ecological and sustainable livestock raising (Wiśniewska *et al.*, 2019).

In Latin America and the Caribbean, native breeds evolve in different agroecological areas under traditional management practices, but because of the perception that they are little productive, they are being replaced slowly by exotic genotypes. This is probably a wrong idea to hold in limited sanitary and feeding conditions, and extreme climates (Malomane *et al.*, 2019).

Unfortunately, the studies that compare native breeds to exotic ones just consider few production variables, not the whole production cycle of animals, such as survival, reproductive rate, longevity, and more specifically, maintenance costs in adverse production conditions, considering that adaptation of zoogenetic resources to certain agroclimatic, management, and market conditions are the basis for the development of sustainable production systems where other factors intervene. They are particularly important in adverse environments with large environmental variations throughout the year, like most production systems in tropical or arid regions of Latin America and the Caribbean. Among the factors that determine animal adaptation are tolerance to heat are the ability to consume low quality forages, and the potential to survive in the presence of pests and diseases (Perezgrovas-Garza, 2016).

The United Nations Organizations for Food and Agriculture has raised a red flag on the extinction of at least one breed of domestic animals every month, which has occurred uninterruptedly since 2002. This situation will become even more dramatic in the short-term because of the effects of environmental modifications and the emergence of new diseases; the dilution of local breeds as a result of crossings has called the attention as well, as stated by FAO, which has shown concern over genetic losses that hinder their study and evaluation of their real potential (Macrì *et al.*, 2019).

Importance of native zoogenetic resources

The main livestock species from the Iberian Peninsula have evolved for more than 500 years in diverse and adverse environments, thus suggesting that native animals have genes that help them adapt at different frequencies from exotic breeds. Rearing domestic animals is fundamental for nutrition and agriculture; a high percent of the value of the global agricultural sector is provided by them, about two billion people depend, at least partially, on rearing animals for their support. The production of meat, milk, and eggs will need to double in the next 20 years to meet the nutritional needs of the world population (Dancause Vilar, Steffy, and Lum, 2011).

Animal production is also critical, since it refers to foods and derivatives. The manure used as fertilizer and fuel, drafting animals, and the utilization of fiber, leather, and fur as clothing (Ayala and Carbone, 2020). The demographic statistics indicate that by 2050, the human population of

the world will reach 9 600 billion, one of the most stressing challenges of present time (World Economic Forum, GAP Report®, 2017). Consequently, there is greater pressure on natural resources, since food production will have to increase using the same or less farm area, due to the new challenges posed by society, such as animal well-being, safety of foods, and the reduction of pollutants. Among the expected effects of climatic change are the appearance of pests and diseases, shortages or abundance of water, quantity and quality of forages, heat stress, and the reduction of biodiversity (Haile, 2020), all of which should be considered for conservation and sustainable use of zoogenetic resources.

Conservation genetics

Genetic resources

Genetic diversity (GD), is understood as the genetic ability to vary the capacity of populations to evolve in terms of their response to environmental changes and changes in the objectives of selection. Genetic variability is the basis of their evolution (Morales-González *et al.*, 2020).

GD is one of the three levels of biological diversity acknowledged by the International Union for the Conservation of Nature (IUCN), as worthy recipients of conservation (Meydan, Jang, Yıldız, and Weigend, 2016). Conservation genetics is a discipline that focuses on the preservation of genetic diversity, which is tied to segmentation, reduction in size, and all the factors that can affect it (Kress, García-Robledo, Uriarte, and Erickson, 2015). Moreover, it is considered an applied science that uses molecular tools to help solve problems of species conservation and management, an interdisciplinary specialty in which scientists apply genetic studies together with traditional ecological field work, and other techniques to explore molecular variation, population limits, and evolutionary relations, in order to protect biodiversity, and identify unique populations.

For many centuries, animal breeds were selected according to their morphological features and appearance. Most of these species of animals have a large genetic variability as a result of constant selection activities (Macrì *et al.*, 2019).

The main methods used to study the genetics of populations are nuclear microsatellites and mitochondrial DNA (mtDNA) markers used to evaluate genetic diversity, mating systems, kinship, and genetically different populations, to report on management decisions. Next generation DNA and RNA sequencing, environmental DNA, and epigenetics are beginning to be applied to the evolution, physiology, and ecology (Dancause *et al.*, 2011).

PCR technique

In the 1980s, the Polymerase Chain Reaction (PCR) technique was developed as a method that amplifies specific DNA segments at usable concentrations (Lawrence *et al.*, 2019), which led to several research studies linked to the variation of the DNA sequence used both in humans and

animals. In recent years, several molecular markers were developed and enhanced, such as single nucleotide polymorphism (SNP). SNPs are single base changes in a DNA sequence (Liu, 2014). They offer different sequencing alternatives (alleles) in individuals from one or several given populations; however, the allele frequencies should be greater than 1% in the population to be considered as SNP. SNP (Larsen and Matocq, 2019) microsatellites (Lawrence *et al.*, 2019), are designed to study characterization and genetic diversity topics of domestic animal populations and breeds; this development is in concert with the implementation of easy to use statistical packages that offer greater automation of sequencing, and other tools associated to genetic analysis (Lv, Edwards, Zhou, and Xu, 2019).

The complete genome of birds was sequenced (Silva *et al.*, 2020) using SNP markers (Li *et al.*, 2013; Xu *et al.*, 2020). It was determined that there are only 11 variable sites in the D-loop region, five of which occur on sites 217 and 261 (Sweeney *et al.*, 2020), which is the optimum region for polymerase chain reaction (PCR) amplification.

Molecular markers

Molecular markers are defined localization DNA sequences in the genome (specific loci), polymorphic sequences insensitive to the environment and known inheritance, which can be of two types: type I markers, which are related to codifying sequences of the genome; and type II markers, which are found in anonymous or non-codifying regions (Chauhan and Rajiv, 2010).

Importance of molecular markers

The most important molecular markers used in research today are microsatellites and single nucleotide polymorphisms (SNP).

Molecular markers, especially microsatellites, became more useful, cost-effective, and generalized, as the protocols and technology were improved, thus the genetic techniques offer reliable information, particularly when the traditional methods prove to be inadequate to demonstrate hypotheses. It is necessary to mention that genetic data will be more valuable when they are combined with complementary information, such as behavior, demography, space, etc. (Razgour *et al.*, 2019).

Microsatellites

DNA microsatellites are molecular markers located in non-codifying DNA regions made between 10 and 30-fold tandem-repeats of 1-6 nucleotide sequences (Meydan, Jang, Yıldız, and Weigend, 2016) produced in the genome of most eukaryotes (Rodríguez-Osorio, 2019), like polydinucleotide repetitions (for instance, ACACAC ...), which are inherited in a codominant manner.

The number of repetitions varies among alleles; there are thousands of microsatellite loci in all the genomes (Islam *et al.*, 2020); they work as a genetic tool to determine the diversity and characterization from broader to more specific forms in individuals, besides establishing kinship (Kinney *et al.*, 2019), the genetic structure of populations (Meydan, Jang, Yıldız, and Weigend, 2016), and comparison between species (Jangtarwan *et al.*, 2019), the basis of most genetic maps, which are useful to characterize the diversity within livestock populations and breeds (Islam *et al.*, 2020).

The variation between microsatellite alleles is evaluated by measuring length differences of the repeated sequence, which result from the addition or removal of repeated units. The overall high mutation rate in the microsatellite loci results in high polymorphic levels, which make these molecular markers ideal for the study of the genetic structure of the population, the genetic relation, patterns of genetic flow, genetic drift, and the size of the population. Estimations of heterozygosity in the loci of microsatellites are 10-fold higher than heterozygosity detected by protein electrophoresis in large mammal species (Wainwright, Glenk, Akaichi, and Moran, 2019)..

The different alleles are named according to the nomenclature determined and agreed internationally by the International Society of Animal Genetics (ISAG) (ISAG, 2012). Genotyping provides the genetic formula of every animal analyzed.

Based on the type of sequence repeat or tandem, according to Meydan, Jang, Yıldız, and Weigend (2016) they are classified into:

- Perfect microsatellites: the repeated sequences are not interrupted by any base that does not belong to them. For instance, GAGAGAGAGAGA.
- Imperfect microsatellites: there are bases between repeated sequences that do not coincide with them, TATATATGTTATATATA.
- Interrupted microsatellites: a tiny sequence that does not correspond to repetitions is observed, CGCGCGCGTAGCCG.
- Compound microsatellites: these contain distinctive adjacent sequences, CGCGCGCGTATATATATATA.

Microsatellites have three main uses in domestication studies:

1. Quantifying genetic variation inside and between livestock populations and breeds (Razgour *et al.*, 2019).
2. They help learn the genetic mix between livestock populations (Islam *et al.*, 2020).
3. The data can be used to assign individuals to similar genetic groups in the population, breed or levels of species (Yang *et al.*, 2020).

Microsatellite applications

- ***Individual identification and paternity tests***

The method consists in comparing the genotype and/or phenotype of the progeny to that of their parents, since one of the alleles from an individual comes from the father, and the other from the mother. This combination offers 97% likelihood of detecting or assigning one of the two incorrect progenitors, and approximately 100% likelihood of crossing between individuals from other breeds (Roques *et al.*, 2019).

- ***Genetic maps and comparative genomics***

This is a useful tool that helps identify genes with traits of interest; it looks into associations among several alleles in any marker, separates individuals with interest traits into populations to identify regions of the genome where the gene responsible for that trait is more likely to be located (Sargin *et al.*, 2019).

- ***Studies of population genetics***

These studies help estimate levels of variability, genetic relations within populations, evaluations of genetic diversity and kinship in populations of domestic animals in danger of extinction. It can also be used in the study of phylogeny (Tabatabaei, Fard, Hashemi, and Dianatpour, 2020). It studies the genetic distances between populations or individuals and population structure.

Factors that intervene and diminish marker sensitivity

Mutation: They are variations of genetic material that contain from simple substitutions of a single nucleotide to deletions or insertions of one or more nucleotides. Generally, mutations in animals are not observable in the phenotype, since they take place in non-codifying regions (silent mutations). Codifying regions or sequences expose a low rate of mutation, which is reflected in the reduced variability within species and the high level of conservation of these regions within species (Nomura *et al.*, 2018).

Null alleles: These alleles cannot be amplified by PCR, due to a mutation at the hybridization point of the primer. One of the alleles does not amplify, and therefore, the individual is described as homozygote to the other allele. It is hard to detect when it has a low frequency in the population, and when there is not reliable genealogical information available. Another way to detect the presence of null alleles would be based on the calculation of the deficit of heterozygotes for Hardy Weinberg equilibrium (Larsen and Matocq, 2019).

Homoplasia: It is the fact that the same trait present in two species does not always derive into the same ancestral trait. Two alleles are homoplasic when they have an identical status, though it may not be acquired by descendancy (Miller *et al.*, 2018); it is also manifested when they are the

same size (bp), but not because they are identical. They are regarded as equal because they are the same size, though there are evident intrinsic differences as to their structure, presence of insertions and/or deletions, base changes or variations in the flanking region.

These factors increase the likelihood of error in genetic studies. They are patterns of mutation, null alleles, and homoplasia.

As a molecular technique

The United Nations Organization for Food and Agriculture (FAO), in collaboration with the International Society of Animal Genetics (ISAG), designed guidelines for analysis of molecular genetics by implementing domestic animal diversity measurements (DADM), which included microsatellite markers for nine common livestock species (Miller *et al.*, 2018).

Microsatellite use to conserve zoogenetic resources

The safety trends in intensive meat and egg production from hens is one of the main factors considered in the selection of breeds to create highly productive lines, and therefore, introduce them internationally, following a reduction of genetic diversity. Current avian production, which includes fowls, commercial, and experimental lines, is changing in terms of extensive rearing that ensures organoleptic, native, and animal well-being characteristics applied to ecological livestock raising adapted to particular farming areas (Francesch, 2011).

Agroecological adaptability would be one of the causes that strengthens the importance of utilizing zoogenetic resources in every location, since they are a source of genetic variability. At this point, molecular markers, such as microsatellites, are recommended as a tool to fulfill that goal, since they can show the differences of allele frequencies between populations. Their application in birds can be cited from Bariloche (2018) and Vargas (2020), and the conservation strategies designed by FAO.

Generally, studies begin with a genetic characterization that includes data collection of the size and the structure of the population, geographical distribution, production systems hosting the breeds, faneroptical characteristics (physical characteristics, levels of yielding, and any other characteristic that becomes important in production), historical evolution of crossbreds, and selection (Francesch, 2011).

It can also be done through biochemical polymorphisms and molecular markers, especially microsatellites to analyze genetic variability within or between populations. Besides, to obtain mean values of kinship, paternity tests, to identify heterozygote individuals, to quantify relationships between populations, and detect introgressions (Mathema, Dondorp, and Imwong, 2019).

It is important to highlight that the genetic information achieved is,

- Standardized breeds
- Experimental lines that become commercial
- Native birds from every region

Molecular markers help provide a definition of the identity of the breed, and assign individuals to their original population. All the results offer thorough information on the genomic variability and the structure of the population of domestic birds. The downside is that performance cannot be predicted, and that monitoring genetic resources of fowls is not always a priority for some countries.

Mitochondrial DNA

Mitochondrias are intracellular organelles located in the cell cytoplasm. They are tiny (16-20 kb) covalent closed circular molecules made of 37 genes (13 messenger RNA, 2 ribosomal RNA, 22 transference RNA), and a region known as control (1 Kb) or D-loop, which controls replication and transcription in the molecule, with an exceptionally high rate of substitution and polymorphisms in many taxons (Serna-Lagunes *et al.*, 2015).

Mitochondrial DNA (mtDNA) has a series of specificities in relation to nuclear DNA: their genes have no introns, the two DNA strands are known as light (L) and heavy (H), one of them is rich in purines, and the other is in pyrimidines; it is exclusively passed from the mother, without recombination. Vertebrate double-stranded closed buckle mtDNA can self-replicate, with matrilinear inheritance (Yan and Chen, 2020).

The application of molecular genetics contributes to the estimation of the isolation degree between a population or group of population, providing a more direct access to knowing the legacy and inheritable intraspecific differentiation (Meydan, Jang, Yıldız, and Weigend, 2016). Some of these features are quick evolution, a simple molecular structure, high rate of nucleotide substitution, a matrilinear clonal inheritance (inheritance exclusive from the mother), absence of recombination, no tissue specific, and a high correspondence in mammals, and most other vertebrates (Sargin *et al.*, 2020).

MtDNA is one of the most commonly used markers for domestication studies and phylogenetic relations. Thanks to their characteristics and specific functions, it is conserved enough to allow for identification of the ancestral population that gives rise to the target population and distribution of domestic animals, it is variable, and it is structured in geographical areas, allowing for an approximate location of the domestication site, evolving quickly at a constant rate (Raza *et al.*, 2019). One of the applications is to know the history of animal domestication of livestock (Colli *et al.*, 2015), dogs, horses, and pigs (Elad *et al.*, 2020). Moreover, it has a preponderant role in intraspecific genetic studies of species with management and conservation priorities (Sargin *et al.*, 2020).

MtDNA studies in birds are based on sequences of the control region (partial), (CR; D-loop: nucleotide position 1–1232; NC_007235 (Nishibori, Shimogiri, Hayashi, and Yasue, 2005).

The mitochondrial genome is different in every bird breed, whose sequences have been completed recently. Chang and Larracunte, 2019 were the first to clone and sequence the mitochondrial genome of Leghorn hens (16 775 bp). Bao *et al.*, 2008, sequenced mitochondrial DNA (mtDNA) from Shouguang and Silky birds, consisting in 16 784 bp and 16 785 bp long, respectively, in addition to the genome of Tibetan birds, which varies between 16 784 bp and 16 786 bp. Yan *et al.*, 2020, noted that the total length of the mitochondrial genome of Daweishan Mini birds was 16 785 bp; Xie *et al.*, 2016, sequenced the mitochondrial genome of Cenxi birds (16 786 bp long) (Avadanei, Giusca, Negura, and Caruntu, 2018).

Mitochondrial DNA and the D-loop region in domestic chickens have a higher rate of evolution in comparison to the genomic DNA; it is less difficult to obtain. The geographical origins of several local populations of birds are evaluated and identified (Meydan, Jang, Yıldız, and Weigend, 2016; Malomane *et al.*, 2019; Islam, Osman, and Nishibori, 2019), which led to two hypotheses. The first hypothesis was related to the monophyletic origin in which one subspecies of red jungle fowls (JF) (*G. g. Gallus*) was the main progenitor of all the domestic birds, whose domestication event may have taken place in southeast Asia (Meydan, Jang, Yıldız, and Weigend, 2016). The second hypothesis would be the multiple origin in which birds could be domesticated from multiple geographical regions from south and southeast Asia, which favors polyphyletic origins (Islam, Osman, and Nishibori, 2019).

CONCLUSIONS

The utilization of molecular techniques provides objective measurements of diversity between and within breeds, allowing for the study of genetic relationships among them, and also showing unique genetic traits of genetic isolation phenomena in the past.

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AUTHOR CONTRIBUTION

Author participation was as follows: Conception and design of research: AVG, GEGV; analysis and interpretation of data: AVG, GEGV; redaction of the manuscript: AVG, GEGV.

CONFLICT OF INTERESTS

The authors declare the existence of no conflicts of interests.