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Review

Applications of Microsatellites and Single Nucleotide Polymorphisms for the Genetic Characterization of Cattle and Small Ruminants: An Overview

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Abstract: The status of genetic diversity, adaptation to climate change or the identification of genes associated with traits of interest in livestock populations has been a major concern for scientists in the last decades. Biotechnology has evolved continuously, offering new tools and methodologies to analyse the genomes of livestock species. Biochemical markers or protein polymorphisms were the tools used for population studies many years ago, but over the last three decades the methodologies available to analyse livestock genomes have changed notably. The development of DNA molecular markers, especially microsatellites and Single Nucleotide Polymorphisms, opened new possibilities for a better understanding of livestock genomes, unthinkable until recently. However, Whole-Genome Sequencing technologies or genome editing techniques are changing the way to analyse or interact with the genomes, even before full advantage can be taken of all the possibilities open by the last group of molecular markers. The aim of this review is to summarize the opportunities available through livestock genome analysis in cattle and small ruminant populations, namely through the molecular markers most widely used over the last few years, including microsatellites and Single Nucleotide Polymorphisms.

Keywords: genomics; microsatellites; single nucleotide polymorphisms; cattle; small ruminants

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The current pattern of livestock genetic diversity is the result of a long history that began around 10,000 years ago, with the first steps of animal domestication. Since then, forces such as mutation, migration, natural and artificial selection and genetic drift have shaped the genetic characteristics of current livestock populations and explain, in addition to environmental factors, their enormous variety of phenotypes. Currently, groups of domesticated animals are characterized by some degree of phenotypic or genetic homogeneity, which has enabled them to be separated from other livestock populations, and they are currently called "breeds" [1]. The origin of livestock breeds has been dated to the late 18th or the early 19th century, and currently breeds are considered as the units of conservation. However, even 200 years after the recognition of the first livestock breeds, their definition is still being discussed, and it is much closer to a social construct than to a biological concept. In developed countries, breeds are generally well differentiated and easy to recognize, while in developing countries the differentiation it is not always so clear and breed differences are only gradual, mostly corresponding to a geographical separation.

The genetic erosion of livestock populations is a growing concern, such that in the last few years the number of breeds classified at risk has increased while the number of those classified as not at risk has decreased [2]. Furthermore, in the last decades two additional issues that compromise the viability of local breeds have emerged as a major concern: (1) the replacement of local breeds by high-performing cosmopolitan breeds or indiscriminate crossbreeding and (2) the number of breeds at unknown status has gone

up to 58%, and it mainly corresponds to local populations [2]. Those local breeds are an important source of genetic diversity, as reflected by the fact that they represent 88% of all the breeds recognized. The report *Status and trends of animal genetic resources–2020* [2] concludes that more than 25% of the livestock breeds currently recognized are considered to be at risk of extinction.

Furthermore, local breeds could play an important role in future humanity challenges, particularly those related with food supply to a continuously growing human population and the adaptation of livestock to climate changes [3]. In this context, several international plans of action have been developed in order to characterize and monitor animal genetic resources (AnGR) [4], as the in-depth knowledge of AnGR is crucial to guide future AnGR conservation plans and strategies, so that gaps of knowledge are a major concern to be solved.

Since the end of the 20th century, the development of biotechnology tools has had a major impact in establishing the foundations for the sustainable use and conservation of AnGR [2]. Currently, such molecular tools are crucial to develop global actions in order to preserve the genetic resources represented by livestock breeds. Among the major DNA markers commonly used for genetic diversity analysis [5], microsatellites and Single Nucleotide Polymorphisms (SNP) are currently the most frequently assessed. Over the last 15 years, microsatellites were the most common DNA markers used for genotyping livestock populations; however, in the last 5 years SNP markers have replaced microsatellites in genetic diversity studies [6].

The aim of this review is to give an overview regarding the use of microsatellites and SNP molecular markers for the genetic characterization and genetic diversity analysis of cattle and small ruminant breeds.

1. Microsatellites

Microsatellites were described at the end of the 20th century as a new class of genetic marker, based on length variation of a short sequence of nucleotide motifs [7,8]. Microsatellites are also called Simple Sequence Repeats (SSR), Short Tandem Repeats (STR), Simple Sequence Length Polymorphisms (SSLP) or Variable Number of Tandem Repeats (VNTR). Their characteristics made them ideal to analyse livestock genomes: (1) they are codominant markers, so it is possible to distinguish heterozygotes from homozygotes; (2) they have high polymorphism and are very abundant in the eukaryote genomes; (3) it is feasible to amplify relatively small fragments by PCR (100–300), so it is possible to analyse them even from degraded samples; (4) sample preparation is easy, automatable and reproducible among laboratories; and finally (5) microsatellites are mainly located in non-coding regions of the genome, so they are considered as neutral markers [9].

The first articles describing the characteristics and applications of microsatellites in ruminant species were published in the early 1990s [10,11]. In the first stage of microsatellite application, the limiting factor was the development of a set of primers for PCR amplification, which needed a certain degree of genome knowledge that was scarce at the time in the majority of livestock species. However, the location and sequence of microsatellites are largely conserved among mammals and even more in closely related species [12], so microsatellites from cattle were often used for sheep and goat studies [13].

In the 1990s, the use of microsatellites in genetic diversity studies increased substantially due to their advantages relative to previous molecular marker tools [6]. The most common parameters estimated from microsatellite genotypes were: Wright's statistics parameters, observed and expected heterozygosity (H_O, H_E), population structure, genetic distance (Ds), effective/mean number of alleles (NEA, NAE) and Hardy–Weinberg equilibrium (Table 1). In addition, the use of microsatellites enabled the analysis of between-breed genetic diversity, which previously was not possible (e.g., genealogies) or not efficient (e.g., biochemical markers) with the information available at that moment [14]. In the late 20th century and very early 21st, most genetic diversity studies were focused on a small number of breeds or in particular geographic regions. In 2004, a survey among research

groups that involved countries from all over the world revealed the main characteristics of genetic diversity studies in livestock species [15]. Ruminants were the predominant livestock group analysed among the research groups that answered the survey, with cattle being the species more represented in such studies, followed by sheep and goats. The main reasons to include a breed in the study were (1) a long time of isolation, (2) unique phenotypic characteristics, (3) distribution in a particular environment and (4) economic importance. In this survey, half of the projects analysed included less than 10 breeds and only for 15% of the breeds the number of samples per breed was lower than 25. One of the major concerns in analyses with microsatellites was the possibility to combine genotypes from different studies, because the different PCR conditions used resulted in different allele size scoring of the same microsatellite marker. As a consequence, international efforts were developed to homogenize the use of microsatellite markers in livestock species, in order to facilitate the comparison among studies. The International Society of Animal Genetics (ISAG) was created as a FAO/ISAG consultant group on animal genetic diversity (1995) in order to recommend a list of microsatellites to be used in livestock species. In addition, a few years before, the FAO proposed the initiative called Measurement of Domestic Animal Diversity (MoDAD), intended to recommend technical guidelines in such studies and to promote genetic diversity studies in livestock species using DNA markers.

The intensive use of microsatellite markers for genetic characterization in the 1990s favoured a rapid increase in studies based on these new molecular markers, in both cattle and small ruminant species. In these studies, it became notorious that the analysis of allele frequency differences among populations was a powerful tool to analyse genetic relationships among them. For example, MacHugh et al. [16] analysed the genetic variation of 12 microsatellites in 6 European bovine breeds, assessing their usefulness in the study of population gene flow. In addition, the pattern of genetic variation among and within populations provided new insights for a better understanding of evolution, domestication and phylogeography in Bos taurus and Bos indicus populations. The first studies showed a clear genetic isolation of humpless (taurine) and humped (zebu) cattle, indicating a clear separate origin of domestication among them [17]. Further analysis supported two different domestication origins for taurine and indicine cattle, in the Fertile Crescent and Indus Valley, respectively. In addition, microsatellite markers were widely used since the end of the 20th century to analyse the genetic relationships and evolutionary history of bovine breeds located in close geographic areas or continents. Hanotte et al. [18], analysing 15 microsatellite markers in 50 African indigenous cattle breeds, suggested a single region of taurine expansion following an eastern route to the south of the African continent and two major entry points (the Horn and East Coast) of indicine genetic influence. More recently, the BioBovis consortium analysed 114 bovine breeds (including American, Iberian, British, continental European and African breeds) using autosomal and Y-chromosome microsatellites markers, in conjunction with mitochondrial DNA sequences, in order to elucidate the genetic background of Creole cattle breeds [19]. The results have shown that microsatellite markers still remain a powerful tool for analyses of breed genetic relationships and revealed that Creole cattle breeds occupy an intermediate position between African and European breeds, and some Creoles show a more distinctive Iberian signature than others. In addition, results confirmed the mixed ancestry of American Creole cattle and the role that African cattle have played in their development [19].

In the first decade of the 21st century, microsatellites were the dominant markers in livestock genetic diversity studies (81%), while the use of other molecular markers such as mitochondrial DNA and SNP arrays was less relevant [6]. In addition, various large-scale international projects were set up to analyse genetic diversity of ruminant breeds and their genetic relationships. The ECONOGENE project analysed 3401 samples from 57 sheep and 47 goat populations across Europe, the Middle East and Egypt using 30 microsatellites recommended by the FAO, with an attempt to maximize overlapping with other projects (for an overview of the project see [20]). The results of the ECONOGENE project demonstrated the power of microsatellite markers to investigate within-breed

genetic diversity and between-breed genetic relationships. Sheep breeds located near the domestication centre and southeast regions harbour higher levels of genetic diversity [21]. On the other hand, a geographical differentiation among breeds was suggested by PCA and STRUCTURE results, indicating a genetic cline from the Middle Eastern and south-eastern European breeds towards north-western and western European breeds. In addition, the phenotypic characteristics also showed a geographical clustering, where fat-tailed sheep that are well adapted to harsh and dry environmental conditions formed an isolated cluster from the remaining Middle East and south-eastern European sheep breeds [22]. The results for goat breeds indicated four clear genetic clusters: eastern Mediterranean, central Mediterranean, western Mediterranean and central/northern European. As for sheep breeds, the eastern Mediterranean cluster was the most variable, but the north-central Europe cluster evidenced the highest degree of breed differentiation [23]. Therefore, in both small ruminant species a clear southeast to northwest gradient pattern was observed.

Table 1. Number and percentage of publications reporting different types of diversity parameters, estimated based on 68 studies and software used. Table from Olschewsky et al., 2021 [6].

Parameter	n	%	Software
Wright's F-statistics	61	90	Arlequin, Cervus, FSTAT, GDA, GenAlEx, Genepop, Genetix, HP-Rare, MolKin, POPGENE, Populations, SAS
Observed Heterozygosity	58	85	Arlequin, Cervus, GenAlEx, Genetix, FSTAT, Microsatellite, Toolkit, MolKin, PHYLIP, POPGENE
Expected Heterozygosity	58	85	Arlequin, Cervus, FSTAT, GenAlEx, Genetix, Microsatellite, Toolkit, MolKin, POPGENE, PHYLIP
Population structure/Admixture	51	75	BAPS, CLUMPP, Distruct, Genetix, Leadmix, Structure
Genetic distances	49	72	Arlequin, Dispan, Genetix, MolKin, Phase, PHYLIP, POPGENE, Populations
Effective/mean number of alleles	48	71	Arlequin, FSTAT, GenAlEx, Genetix, Microsatellite Toolkit, MolKin, POPGENE
Hardy–Weinberg equilibrium test	48	71	Arlequin, Cervus, GenAlEx, Genepop, POPGENE, SAS
Neighbor-joining-/phylogenetic tree	37	54	Dispan, Mega, PHYLIP, r, SplitsTree
Allele frequencies	36	53	Cervus, FSTAT, GenAlEx, Genetix, Genepop, Microsatellite Toolkit, MolKin, Populations
Allelic richness	28	41	FSTAT, GenAlEx, HP-RARE, POPGENE
Polymorphic information content	23	34	Cervus, Excel, Microsatellite Toolkit, MolKin
Analysis of molecular variance	16	24	Arlequin, GenAlEx
Principal component analysis	15	22	Fortran, GenAlEx, MVSP, r, SAS, SPSS, XLSTAT
Private alleles	12	18	FSTAT, GenAlEx, GDA, HP-RARE, Microsatellite Toolkit
Populations Linkage disequilibrium	10	15	Genepop, SAS
Null alleles	8	12	Cervus, FreeNA, Micro-Checker
Genetic relationships/coancestry	8	12	Admixture, Genetix, MolKin, r
Gene diversity	5	7	FSTAT, Genetix, Microsatellite Toolkit
Proportion of shared alleles	5	7	Microstat
Effective population size	4	6	Cervus, GenAlEx, POPGENE
Multidimensional scaling	4	6	r, DARwin, GenAlEx
Allelic diversity per locus	3	4	Microsatellite Toolkit, MolKin
Multiple co-inertia analysis	2	3	r
Percentage of polymorphic loci	1	1	POPGENE

In addition to genetic diversity studies, microsatellites have also become the most useful and widely accepted tool for parentage verification. Identification of pedigree errors is a prerequisite for successful breeding programs, because it is essential to link performances to the correct families, in order to estimate the breeding values of the animals, even if we do not have their own data but only those for relatives. Pedigree errors affect negatively the genetic gain of breeding programs and bias the estimation of genetic parameters, breeding values and selection response [24]. It has been estimated that a level of 10% in pedigree inaccuracies can result in up to 18% reduction in the genetic trend of breeding values, 3% loss in selection response and 13% effect on the inbreeding coefficient [25]. Even though the number of microsatellites in livestock genomes is high, not all of them are informative for parentage verification. The usefulness of a microsatellite for such purposes is determined by the Polymorphism Information Content (PIC), which evaluates the informative value of a marker and depends on the number and frequency of the alleles and their deviation from Hardy–Weinberg equilibrium. In order to homogenize and facilitate this activity, the ISAG proposed a set of well-tested microsatellites in the major livestock species (12 for cattle and 13 for small ruminant species) (www.isag.us (accessed on 19 August 2022)). Parentage verification based on microsatellite molecular markers has been used since the end of the 20th century [26–29], i.e., for more than three decades.

The wide genome distribution of microsatellites favoured the construction of more detailed linkage maps in cattle and small ruminants [30–39] and, as a consequence, the development of studies with the aim to identify Quantitative Trait Loci (QTL) or single genes affecting economically important traits. The first QTLs identified in sheep were related to wool, milk and parasite resistance traits [31]. In addition, various single gene traits associated with fertility (Booroola and Inverdale), meat (callipyge) or horns were identified [40–44]. In 2006 the goat genetic map contained nearly half the number of markers reported in the sheep map, with 622 and 1392 markers for each species, respectively [44]. However, many QTLs were identified in the goat genome associated with milk [45], conformation traits [46], birth weight [47] or cashmere traits [48]. In cattle breeds the number of QTL was higher than that in small ruminant breeds. In the AnimalQTLdb web page (https://www.animalgenome.org/cgi-bin/QTLdb/index (accessed on 19 August 2022)) there are 122 entries with the word microsatellite in the publication, while in sheep the number is 38.

2. Single Nucleotide Polymorphisms

In a strict molecular sense, Single Nucleotide Polymorphisms (SNPs) are single base changes in a DNA sequence; however, indels (insertions and deletions) are also considered as SNPs. The maximum number of alleles in an SNP are four, corresponding to each of the four nucleotides, but SNPs are usually biallelic. There are two main reasons for this: (1) the low frequency of single substitutions in the nuclear genomes (between 1×10^{-9} and 5×10^{-9} per year per neutral position in mammal genomes), so the probability that a single base has two different mutations is low, and (2) a clear bias towards transitions over transversions [49]. The main characteristics of SNPs are their high frequency in the genomes, with approximately one SNP every 1000 bases [50], their location in coding and non-coding regions, thousands or even several hundred thousand can be genotyped in a single reaction and, in comparison with microsatellites, their mutational mechanism is better understood [51]. Since 2015, SNPs have been the predominant molecular markers in analyses of local farm animal genetic diversity [6] and there are commercial high-throughput arrays for the majority of livestock species [14]. Two major commercial SNP arrays are available for livestock species, (i) Illumina's Infinium iSelect Microarray or BeadChip, based on single nucleotide extension or allele-specific primer extension [52], and (ii) Affymetrix's GeneChip or AxiomArray, based on molecular inversion probe hybridization [53].

The main parameters estimated with SNP data are similar to those with microsatellites (Table 2), but the software is different, due to the different characteristics of the two molecular markers. Furthermore, numerous studies have compared both molecular markers in

genetic diversity analysis [51,54–56]. The genetic diversity parameters with higher correlation among both markers were expected heterozygosity (He), F_{IS} and allelic richness (Ar), while observed heterozygosity (Ho) showed the lowest correlations [51,57–60]. In addition, the higher variance in the genetic metrics with microsatellite data, due to their higher polymorphism, results in more frequent non-significant differences between populations when this type of marker is used. In contrast, significant differences are often achieved with SNP markers due to the higher number of markers used [61]. Several studies have shown that all the parameters related to genetic differentiation have high correlations when estimated either with SNPs or microsatellites [59,60,62,63]. In general, both markers are useful for analysing the pattern of genetic structuring of livestock populations and show consistent patterns. However, microsatellite results are much noisier than those from SNP markers that can detect tenuous genetic relationships with higher precision in confidence intervals [55]. In summary, the three main advantages of SNPs are: (1) the confidence intervals of genetic diversity parameters are much smaller, so the distinction between populations is improved, (2) the higher number of markers used, in spite of being less informative, increases the power to detect substructure in the populations analysed and (3) SNP markers allow addressing new questions that cannot be answered with microsatellites [56]. Thus, high-throughput genotyping technologies and high-density SNP panels have made possible new or more detailed analyses than those performed with conventional molecular markers, such as microsatellites.

Table 2. Number and percentage of publications reporting different type of diversity parameters, estimated with microsatellites based on 68 studies and software used. Table from Olschewsky et al., 2021 [6].

Parameter	n	%	Software
Population structure/Admixture	35	85	Admixture, fastSTRUCTURE, Python, Structure, TreeMix
Wright's F-statistics	32	78	Arlequin, Genepop, Golden Helix SNP Variation Suite, Powermarker, Plink, r, VCFtools
Neighbor net/ neighbor-joining-/max. likelihood tree	28	68	Arlequin, hapFLK, Mega, PHYLIP, r, RAxML, SplitsTree, TreeMix
FROH/other inbreeding coefficients than FIS	28	68	Arlequin, Haploview, Plink, r
Principal component analysis	26	63	Eigensoft, Eigenstrat, GCTA, Golden Helix SNP variation Suite, Plink, r
Linkage disequilibrium	26	63	Haploview, Plink, r, SNeP
Expected heterozygosity	26	63	Arlequin, Plink, r
Observed heterozygosity	23	56	Arlequin, Plink, r
Effective population size	21	51	NeESTIMATOR, Plink, r, SNeP
Genetic distances	20	49	Arlequin, hapFLK, Genepop, PHYLIP, Plink, Power marker, r
Multidimensional scaling	15	37	Haploview, Plink, r
Relationship/coancestry	11	27	Admixture, GCTA, Haploview, Plink, r
Allelic richness	10	24	Adze, r
Analysis of molecular variance	7	17	Arlequin
Proportion of polymorphic markers/loci	6	15	Plink, r
Allele frequency	5	12	Plink, Golden Helix SNP variation Suite
Hardy–Weinberg equilibrium test	4	10	Plink
Proportion of shared alleles	3	7	Plink

In Saravana et al. [64] can be found a more detailed description of the most popular software used in livestock genome diversity studies.

2.1. Traceability and Breed Assignment

The high number of SNPs that can be analysed simultaneously using SNP arrays allows the assignment of individuals to breeds or populations and the estimation of breed proportions in the genetic make-up of an individual, predicted with high levels of confidence. Doods et al. [65] assigned genotyped samples to four New Zealand sheep breeds correctly with high prediction accuracy. Lewis et al. [66] concluded that 250 to 500 selected SNPs from the Bovine HapMap are enough to accurately assign 19 cattle breeds. Later on, a set of 108 and 110 SNPs was enough to correctly assign 21 sheep breeds from Italy and Slovenia [67]. In addition, Wilkinson et al. [68] showed that the minimum number of SNPs for a correct breed assignment was 60. The importance of individual breed origin in conservation genetics, breeding programs or authentication of livestock products have favoured continuous analysis for the development of SNP sets, enabling the achievement of such objectives in cattle, sheep and goat breeds. [69–75].

SNPs have advantages over microsatellites as they have (1) lower mutation rate, (2) more reproducibility among laboratories, (3) suitability for standardization and (4) high potential for automation [74]. Their application in parentage verification has been widely used since the development of SNP high-throughput genotyping techniques. The number of SNPs used for parentage verification must be higher than for microsatellites, due to their lower polymorphic content. Different panels of SNPs for parentage testing have been analysed in cattle and small ruminant populations [25,76–80], and various factors can affect the number of SNPs necessary for parentage verification. Therefore, it is not easy to answer the question of how many markers are needed to obtain the probability that we consider adequate (often close to, but not necessarily 100%) to report that the parentage is likely correct. The ISAG recommends for cattle populations a core panel of 100 SNPs, and an additional one of 100 SNPs, for a total of 200 SNPs that has a probability of exclusion of 0.999 and 0.9999999, respectively. However, for sheep and goat populations there is currently no ISAG recommendation regarding an SNP panel for parentage testing. Another important question to be answered is the acceptable number of genotype mismatches among a true parent–offspring relationship; the ISAG suggests 1% as the maximum value, but there is not an international consensus on this. In spite of SNPs having many advantages over microsatellites for parentage verification, and that there are many studies that have successfully developed SNP panels for parentage testing in different livestock breeds, there is not a common SNP panel recommended for cattle or small ruminant breeds, beyond the panel suggested by the ISAG for cattle populations that is currently under study. This is one possible reason, along with the need to re-genotype samples, new laboratory requirements or higher cost, which so far has not favoured the transition from microsatellites to SNPs for parentage verification.

2.2. Linkage Disequilibrium (LD)

LD, which could be defined as the non-random association between loci, is a key parameter to study the evolution of populations and to fine mapping Quantitative Trait Loci (QTL) [81]. The LD concept was introduced in 1917 [82], and posteriorly in 1970 was developed the methodology for their quantification [83]. In addition to mapping association studies that are based in LD among markers, LD is an indicator of the genetic forces that have influenced the genetic diversity of current populations. In terms of population demography, LD analysis allows the estimation of effective population size (Ne). In populations with lower Ne, the effect of genetic drift is higher and originates LD among alleles at a rate inversely proportional to Ne [84]. In spite of LD theory having been introduced at the beginning of the 20th century [82], the development of high-throughput genotyping technologies and dense SNP arrays have improved the analysis of LD patterns in livestock populations considerably [85–90], among others in cattle, sheep and goat breeds.

LD analysis in cattle populations has revealed that at short distances the r2 values are smaller than at longer ones in indicine breeds when compared with taurine cattle, so it is assumed that the census of their ancestral populations was larger than that from which taurine populations were domesticated [91]. In addition, two or three bottlenecks were achieved in cattle populations, associated with domestication, breed formation and selection for milk or meat in some breeds, in contrast to other populations such as humans, which has expanded exponentially in the same period. Furthermore, LD levels in taurine breeds are higher than those in indicine populations, as several studies have shown [92–94]. In sheep breeds LD analyses have revealed a different scenario than in cattle populations, suggesting a common ancestral group for all domesticated populations, as a consequence of a high degree of LD phase conservation between sheep breeds [95]. More recent studies showed that the LD decay was slower in sheep breeds than that in dogs, pigs, goats and cattle [96], and a higher level of LD was found in European sheep populations than in Asian ones [97]. The analysis of more than 80 goat breeds has revealed that modern goat populations are descendants of bezoar-like ancestors that followed different dispersal routes along the east–west Afro-Eurasia, and this has favoured a clear genetic differentiation among Asian and European and African populations. As expected, as the distance from the domestication centre increases, the genetic diversity of the population declines, and the LD tends to increase [98].

2.3. Inbreeding

The high-density SNP arrays allow the identification of genome segments of homozygosity, called Runs of Homozygosity (ROH), which expand throughout the genome. ROH are the consequence of the inheritance of two copies of an ancestral haplotype from the same ancestor. The length and frequency of ROH depends on the demographic history of populations, so ROH analysis can answer properly questions related to population history, structure and demographic events. For example, shorter and less frequent ROH are expected in admixed populations compared to in bottlenecked ones [99]. Several software packages are suitable to estimate ROH, and probably PLINK [100] is one of the most used. Whatever the software used, an important concern in ROH estimation is the parameters used to assess ROH, because they can vary substantially, making difficult the comparison between studies [101–103].

The first studies analysing ROH segments were published In 2010 for cattle populations, 2014 for goats and 2015 for sheep [104,105]. Later on, several studies clearly showed that ROH pattern varies among breeds, as a consequence of the differences in their demographic history. Ferencakovic et al. [106] indicated that cattle breeds with historic admixture showed short ROH lengths, while close populations without migration events showed larger segments [107]. Furthermore, ROH analysis has become a standard approach to analyse inbreeding in livestock populations [108]. F_{ROH} (ratio of the total length of ROH in an individual in the selected ROH length to the total length of the genome of the animal or the total length of the genome covered by SNPs) is usually calculated as the length of all the ROH segments of an individual divided by the total length of the autosomal genome covered by the SNPs included in the analysis. Longer or shorter ROH lengths are correlated with recent and ancestral inbreeding, respectively, so that FROH estimations could be used to assess recent or past inbreeding events. For example, ROH lengths of 10, 5 and 2.5 Mb are correlated with inbreeding events occurring nearly 5, 10 and 20 generations ago, respectively. Furthermore, ROH longer than 16 Mb were formed less than three generations ago, and those smaller than 8 Mb probably more than eight generations ago [109,110]. In addition, as SNP arrays cover all the genome, it is possible to estimate F_{ROH} per chromosome, providing a more detailed inbreeding estimation and revealing the consequences of specific demographic or selection processes in the populations. Inbreeding estimation from ROH has clear advantages to that from pedigree records: (1) it can be estimated in individuals without pedigree information, (2) it is not a probability measure, but rather a direct measure from genotype information, (3) it can predict more precisely the

autozygosity of the genome, (4) it can predict autozygosity from common ancestors more than 50 generations ago and (5) it can be estimated across the genome, for example, per chromosome [14].

Several studies have analysed the correlation among inbreeding coefficients from SNP, pedigree and even microsatellite data. The results are very variable and varied from correlations around 0.3 to higher than 0.8, but in general the correlations are low [54,111,112]. Inaccuracies of pedigree records, the parameters used to estimate ROH, and the different structures of the population can explain the discrepancies among both inbreeding estimators.

In genomic regions under positive selection the number of ROH is higher, and such "genomic hotspots" have been used to identify genetic variants associated with inbreeding depression and Quantitative Trait Loci related with reproductive, growth, dairy and disease resistance traits in cattle, sheep and goat populations [113–119]. Such regions are called ROH islands, first referred to in 2010 by Nothnagel, and defined as highly inbred genomic regions [120,121]. In addition to positive selection, they are also the consequence of demographic events (bottlenecks, for example), repressed recombination or even artefacts due to SNP gaps or Copy Number Variants (CNV) [97,101,122]. Recently, an online repository of ROH islands from eight animal species (cat, cattle, dog, goat, horse, pig, sheep and water buffalo) was [108] created and made available via OSF (https://doi.org/10.17605/OSF.IO/XJTKV (accessed on 19 August 2022)).

2.4. Ascertainment Bias (AB)

Initially, SNP arrays were available only for species with reference genomes. The comparison of whole genome sequences of samples from different breeds allowed the development of commercial arrays that are used in genetic studies and many other applications. However, as few samples and breeds are used in SNP array development, an ascertainment bias can occur when larger or different breeds are genotyped. There are two main reasons that explain the ascertainment bias, (1) Minimum Allele Frequency (MAF) and (2) sub-population bias. As SNPs with intermediate frequencies are preferentially selected, those loci with low frequencies are under-represented in SNP commercial arrays. As a consequence, genetic parameters can be biased [123,124]. When the samples used for SNP discovering are not representative of the breed or a small number of breeds are used, the genetic parameters estimated in genetically distant populations can be underestimated and the heterozygosity of close breeds can be overestimated [125,126]. Linkage Disequilibrium pruning or the use of ancestral SNPs are two options to mitigate the AB of SNP commercial arrays [127].

3. Conclusions

This manuscript aimed at reviewing the most common DNA molecular markers used in genetic diversity analysis. Microsatellites and SNPs have contributed considerably to a better use and conservation of animal genetic resources. However, biotechnology evolves continuously, and new tools arise all the time, opening new ways to understand the complexity of the eukaryote genomes. Whole-Genome Sequencing or genome editing methodologies have the potential to create a major change in livestock population studies. The new sequencing technologies have reduced considerably the cost for sequencing genomes, so currently WGS procedures are more affordable than years ago, and probably in the near future the genome of all domesticated species and likely their relative wilds will be sequenced. CRISPR (clustered regularly interspaced short palindromic repeats) technology has become the genome editing methodology of choice due to its ease of use [128,129]. Such technologies allow us to study the genomes with an unthinkable depth compared to years ago and we can also interact with them.

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