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Application of advanced molecular marker technique for improvement of animal: A critical review

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Abstract
The present study was conducted in collaboration with Department of Animal Genetics and Breeding, OUAT and WBUAFS to review on new class of advanced molecular marker techniques derived from combination of earlier basic molecular marker for the genetic improvement of livestock from December, 2016 to June, 2017. In the protein coding region of mtDNA, the cytochrome oxidase I gene has recently gained more attention in developing DNA barcodes for species identification and biodiversity analysis. Among, nuclear marker techniques, RAPD and AFLP are being applied to cDNA based templates to study patterns of gene expression through advanced technique like ESTs and SSCP. While RFPL and microsatellites techniques are used for detecting marker-QTL linkages that accomplished by the use of MAS, genetic fingerprinting and mixture models based on segregation analysis principle. The present study objective was to review the utility of advanced molecular marker technology for selection and genetic improvement of animals.

Keywords: DNA Barcoding, ESTs, mtDNA, QTL, MAS, SSCP

1. Introduction
During last century animal breeders had exploited the application of methods based on population genetics and statistics allowed the development of animals with a high productive efficiency. These systems are based on simplified models of genic action that assume a large number of or genes with small individual effects in the expression of the phenotype (polygenes) and emphasizes the average genic effects (additive effects) over their interactions [1]. Traditionally, the genetic improvement of livestock breeds has been based on phenotypic selection. The past century was characterized by the development of quantitative theory and methodology towards the accurate selection and prediction of genetic response [1]. The basis is predicting the breeding values of the animals using phenotypic and genealogical information. Properties of the predictions are equivalent to the levels of correlated random effects of a mixed linear model or *best linear unbiased predictors* (BLUP) which is based to a large extent on the work of Henderson [2]. Important advances to some of the economically important characters in several species of livestock has been achieved based on phenotypic performance, however, several limitations of these methods of improvement based on population genetics alone are becoming evident with time. Their efficiency decreases when the characteristics are difficult to measure or have a low heritability. Later, phenotype based genetic markers for Drosophila led to the establishment of the theory of genetic linkage [3]. This application used external animal characteristics as a marker that called morphological markers (i.e. udder shape, coat color, body shape, skin structure, and anatomical characteristics) [4]. These markers depend on visual observation and measurement to identify, classify, and characterize the genetic evolution of different species or populations. The conclusions reached through applying morphological markers are often not completely accurate when they used for the evaluation of farm animal genetic because these markers based on subjective, judgments, and descriptions. Another type of markers represent by using of cytological markers that were included several criteria such as chromosome karyotypes, bandings, repeats, translocations, deletions, and inversions to investigate the genetic resources of animals [5]. The chromosome mutations lead to genetic variation [6]. These mutations were used as markers to identify a certain location of the gene on a specific chromosome.

In the domestic animals, cytological markers allow to investigate their genetic diversity by comparing chromosome number and structure between domesticated animals and their wild ancestors [7]. Cytological markers still widely used in elucidating the origin and classification of species [8] because of its good properties; rapid, economic, and straightforward technique. The third type of markers is biochemical markers such as blood type and isozymes. These markers represent biochemical traits that could be analyzed by protein electrophoresis. The differences in the amino acid composition of isozymes and soluble proteins were used to investigate the genetic variation within species and phylogenetic relationships between species [9]. The application of these markers was limited because the proteins and isozymes are not genetic materials. They are products of gene expression, so they could affect by environmental factors [10]. Yang *et al*. [5] mentioned that the molecular markers are the most reliable markers available based on the nucleotide sequence mutations within the individual's genome. Molecular markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell are not confounded by the environment, pleiotropic and epistatic effects. Several types of molecular markers, including mitochondrial DNA (mtDNA) and nuclear DNA markers, are available but none of them can be regarded as optimal for all applications [11]. For molecular analysis, these markers are first amplified by PCR using conserved primers and the amplicons are sequenced. Sequencing data are then aligned and compared using appropriate bioinformatics tools. Substantial advances have been made over the past decades through the application of molecular genetics in the identification of loci and chromosomal regions that contain loci that affect traits of importance in livestock production [12]. This has enabled opportunities to enhance genetic improvement programs in livestock by direct selection on genes or genomic regions that affect economic traits through marker-assisted selection and gene introgression [13]. Molecular methods play an important role in estimating the genetic diversity among individuals by comparing the genotypes at a number of polymorphic loci [14]. So, the objective of the present review was to integrate the utility of advanced molecular marker technology, pedigree data and phenotypic information through the markers and polygenic inheritance for selection, genetic development of animal and their various applications in characterizing animal genetic resources.

2. Molecular and Quantitative Genetics

The most economically important traits in livestock are continuous distributed and found genetically in the form of quantitative trait loci. So, to exploit the genetic variation among such traits two types of models can be adopted on the basis of quantitative genetics, the infinitesimal model and on the basis of molecular genetics, the finite loci model [15]. The infinitesimal model describes that the quantitative traits are determined by an infinite number of unlinked and additive loci each with an infinitesimally small effect [16]. This model forms the root for the discovery of breeding value estimation theory to determine the genetic merit of an individual for selection [2]. The finite loci model is the concept applicable to genome postulate that a finite amount of genetically inherited material exist that guide the trait [17]. The inherited genetic materials are confined at a particular location in a chromosome called loci. The effect of these loci on quantitative traits could be classified to few genes with large effect and many genes of small effect [3]. To get familiarize with molecular markers first approach is to finding the candidate gene for the trait. A large number of candidate genes may be affecting a particular, so many genes must be sequence and a large sample of animals is needed. Usually, a gene responsible for certain trait could undergo mutation causing variation in that trait by changing the DNA sequences [18]. If the mutation occurred in non-coding DNA further increases the amount of sequencing required. So, when the mutation associated with the phenotypic variation for certain trait that occurs in another gene is considered as a non-candidate gene [19]. Till now, many types of molecular markers have been discovered to detect the variation among individual and population in gene level. These markers can be classified into three groups; protein variants (allozymes), DNA sequence polymorphism, DNA repeat variation [20]. In 1980s, with the invention of Polymerase chain reaction (PCR) many different types of DNA based molecular markers had been discovered e.g. Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Single-Strand Conformation Polymorphism (SSCP) and Microsatellite DNA. All of these DNA-based markers contain specific advantage and disadvantages accordingly they used in the evaluation of genetic diversity of farm animals [9]. With development in computer science, innovative strategies such as whole genome SNP chips and DNA Barcoding have been discovered in which statistical analysis is applied to solve the molecular biological problems through advanced mathematical algorithms. At present scenario, advanced DNA molecular marker techniques are widely used in the fields of species identification, phylogenetics and genetic structural analysis to observe the diversity of the population [21, 22].

3. Molecular marker technique based on genetic sample

3.1 Mitochondrial ribosomal RNA marker

Animal mitochondria contain two ribosomal RNA (rRNA) genes i.e., 12s rDNA and 16s rDNA. Typically, mitochondrial 12s rDNA is highly conserved, so it is being used to understand the genetic diversity of higher categorical levels like phylum [23]. On the other hand, the 16s rDNA is applied for analyzing the genetic diversity at middle categorical levels such as in families or genera [24]. For molecular analysis, these markers are first amplified by PCR using conserved primers and the amplicons are sequenced. Sequencing data are then aligned and compared using appropriate bioinformatics tools. Alvarez *et al*. [23] found that a specific haplotypes of 12S rRNA gene is responsible to study the effects of geographical isolation on genetic divergence of endangered spur thighed tortoise *(Testudo graeca*). Using 12S rRNA, a 394–nucleotide fragment of gene sequence was analyzed to examine the genetic variation in *Testudo graeca* using 158 tortoise specimens belonging to the four different sub-species [25]. Lei *et al*. [26] testified the mitochondrial rRNA genes of Chinese antelopes and determined that average sequence divergence values for 16S and 12S rRNA genes were 9.9 percent and 6.3 percent respectively. But, the 12S rRNA fragment of *Testudo graeca* was found to be less variable than the D–loop fragment due to the inherently slower evolutionary rate of rRNA genes than the variable parts of the D–loop [27]. Similar types of findings was found on endangered Pecoran lower sequence diversity in 16S rRNA gene has lower sequence

diversity than cytochrome b gene both between and within species. However, the 16S rRNA gene carries larger number of species–specific mutation sites as compared to cytochrome b gene [28].

3.2 Mitochondrial Protein coding gene marker

Due to their faster evolutionary rates compared to ribosomal RNA genes, the mitochondrial protein–coding genes are regarded as powerful markers for genetic diversity analysis at lower categorical levels, including families, genera and species. Animal mitochondria contain 13 protein–coding genes; however, one of the most extensively used protein coding genes of the mitochondrial genome for molecular analysis is cytochrome b (cyt b). Zhang and Jiang [21] established that mitochondrial cyt b sequences have been used to understand the genetic diversity for better conservation management of Tibetan gazelle (*Procapra picticaudata*), a threatened species on the Qinghai–Tibet Plateau of China. Partial cyt b based molecular analysis of genetic distances has revealed that there is considerable genetic divergence between the Korean goral and the Chinese goral, but virtually none between Korean and Russian gorals [29]. The Korean gorals possessed two haplotypes with only one nucleotide difference between them. While the Japanese serows (Japanese goat-antelope) showed slightly higher sequence diversity with five haplotypes [29]. Another important mitochondrial protein coding gene, NADH dehydrogenase subunit 5 (318 bp), has been used for phylogenetic analysis of multiple individuals of different species from Felidae family [30]. Mitochondrial cytochrome oxidase I (COI) gene has recently gained more attention in developing DNA barcodes for species identification and biodiversity analysis discussed below under suitable heading [22, 31].

3.3 Mitochondrial D-loop marker

Mitochondrial DNA (mtDNA) is an extra-chromosomal genome in the cell mitochondria that resides outside of the nucleus [32], and is inherited from mother with no paternal contribution [33]. This has been attributed to a faster mutation rate in mtDNA that may result from a lack of repair mechanisms during replication [34]. Mitochondrial DNA contains a non–coding region termed the control region (CR or D–loop) due to its role in replication and transcription of mtDNA. The D–loop segment exhibits a comparatively higher level of variation than protein–coding sequences such as the cytochrome b gene due to reduced functional constraints and relaxed selection pressure [35]. The length of the D–loop is approximately 1 kb and it can easily be amplified by PCR prior to sequencing to determine the molecular diversity. Due to higher evolutionary rates of mtDNA relative to the nuclear genome [36], this marker is preferred in constructing phylogenies and inferring evolutionary history, and is therefore, ideal for within- and between-species comparisons [32]. Hu *et al*. [37] have studied the genetic diversity and population structure in 40 samples of the Chinese water deer (*Hydropotes inermis inermis*) by analyzing the 403 bp fragment of mitochondrial D–loop and detected 18 different haplotypes. They concluded that the samples having the haplotype diversity of 0.923 and nucleotide diversity of 1.318, whereas no obvious phylogenetic structure among haplotypes was found for samples of different origin. Onuma *et al*. [38] analyzied the sequence of the D-loop region of the sun bear has been used to measure molecular diversity and to identify conservation units for better management of the species. Wu *et al*. [39] have sequenced a portion of mitochondrial CR (424 bp) to assess the population structure and gene flow among the populations of black muntjac (*Muntiacus crinifrons*) using 47 samples collected from three large populations. It has been suggested that the coexistence of distinct haplotypes in a specific population was induced by historical population expansion after fragmentation and that the current genetic differentiation should be attributed to the reduction of female–mediated gene flow due to recent habitat fragmentation and subsequent loss [39]. A comparison between various types of mitochondrial DNA type is given in Table 1.

Although mtDNA loci can exhibit large numbers of alleles per loci, the limited number of markers available on the mtDNA molecule positions its PIC values higher than those for allozymes but lower than highly variable nuclear markers such as RAPDs, microsatellites, AFLPs, and SNPs. Due to its non-Mendelian mode of inheritance, the mtDNA molecule must be considered a single locus in genetic investigations [20]. In addition, because mtDNA is maternally inherited, the phylogenies and population structures derived from mtDNA data may not reflect those of the nuclear genome due to gender-biased migration [33] or introgression [40]. The drawbacks of mtDNA analyses include hybridization, introgression, and incomplete lineage sorting. Moreover, mtDNA is of little use in investigating the recent loss of genetic variation and any individual-level events such as identity, individual dispersal, and mating systems [41]. In addition, mtDNA markers are subject to the same problems that exist for other DNA-based markers, such as back mutation (sites that have already undergone substitution are returned to their original state), parallel substitution (mutations occur at the same site in independent lineages), and rate heterogeneity or mutational hot spots (large differences in the rate at which some sites undergo mutation when compared to other sites in the same region).

Table 1: Comparison between various Mitochondrial DNA markers.

Type of Molecular Marker	Characteristics
mtDNA	Inherited from the mother (maternal lineage); rare exceptions do exist
	Degrades slower than nuclear DNA. It can be used in degraded or old samples
	Evolves about 10–fold faster than nuclear DNA
12s rDNA	Highly conserved; used for high–category levels: phyla and subphyla
16S rDNA	Usually used in mid–category differentiation such as families
Protein–coding genes	Conserved; used in low–categories such as families, genera and species
D-loop region	Hypervariate; used for identification of species and sub–species

4. Advances in molecular marker technique

The technical advancements and genome based discoveries has lead to the enhancement of molecular marker techniques. These advanced molecular marker techniques are an amalgamation of the advantageous characteristics of several basic techniques as well as incorporation of modifications in the methodology to increase the sensitivity and resolution to detect genetic discontinuity and distinctiveness.

4.1 DNA Barcode markers

Parallel with the development of innovative practical computer technologies, a new technology has emerged in biological field for automatic scanning and identification of a population. Biological taxonomists referred this technique as DNA barcode. DNA barcoding has become a novel tool for accurate identification of various taxa and unrecognized new species in various classes of animal kingdom. It has become the efficient method to improve the way the world relates to wild biodiversity [42]. Moreover, the introduction of DNA barcoding has highlighted the expanding use of the cytochrome oxidase I (COI) as a genetic marker for species identification as a means to quantify global biodiversity [31]. A DNA barcode is a Short DNA sequence of 600–800 bp segments selected from a standardized region of the genome. Then the barcode is used for identifying the species by applying the integrated innovative bioinformatics concept with population genetics (Fig. 1). Tautz *et al*. [43] was the first biological taxonomist who pertain the DNA sequences in systematic biological taxonomy, called it as DNA taxonomy. After that, Hebert *et al.* [22] proposed the concept of DNA Barcoding by suggesting the COI as the only sequence in mtDNA gene for animal DNA barcode analysis. DNA Barcoding has a high accuracy of 97.9% [44], and provides a new, quick, and convenient identification strategy for animal genetic diversity [45]. This approach like other molecular markers have some disadvantages that the genome fragments are very difficult to obtain and are relatively conserve with minimal variations. In some species the COI cannot be identified because of the low evolution rates of COI sequences in due course of period. Witt *et al*. [46] proposed that determining genus *Hyalella* of amphipod crustaceans is very difficult taxonomically by DNA barcoding technique. Moreover, COI is an mtDNA sequence of maternal origin, which could bias species diversity [42]. The above disadvantages can be minimized by using one or more nuclear gene barcodes together to make a standardized analysis for animal genetic resource. However, still many biological taxonomists apply this technique for species classification and identifying new one.

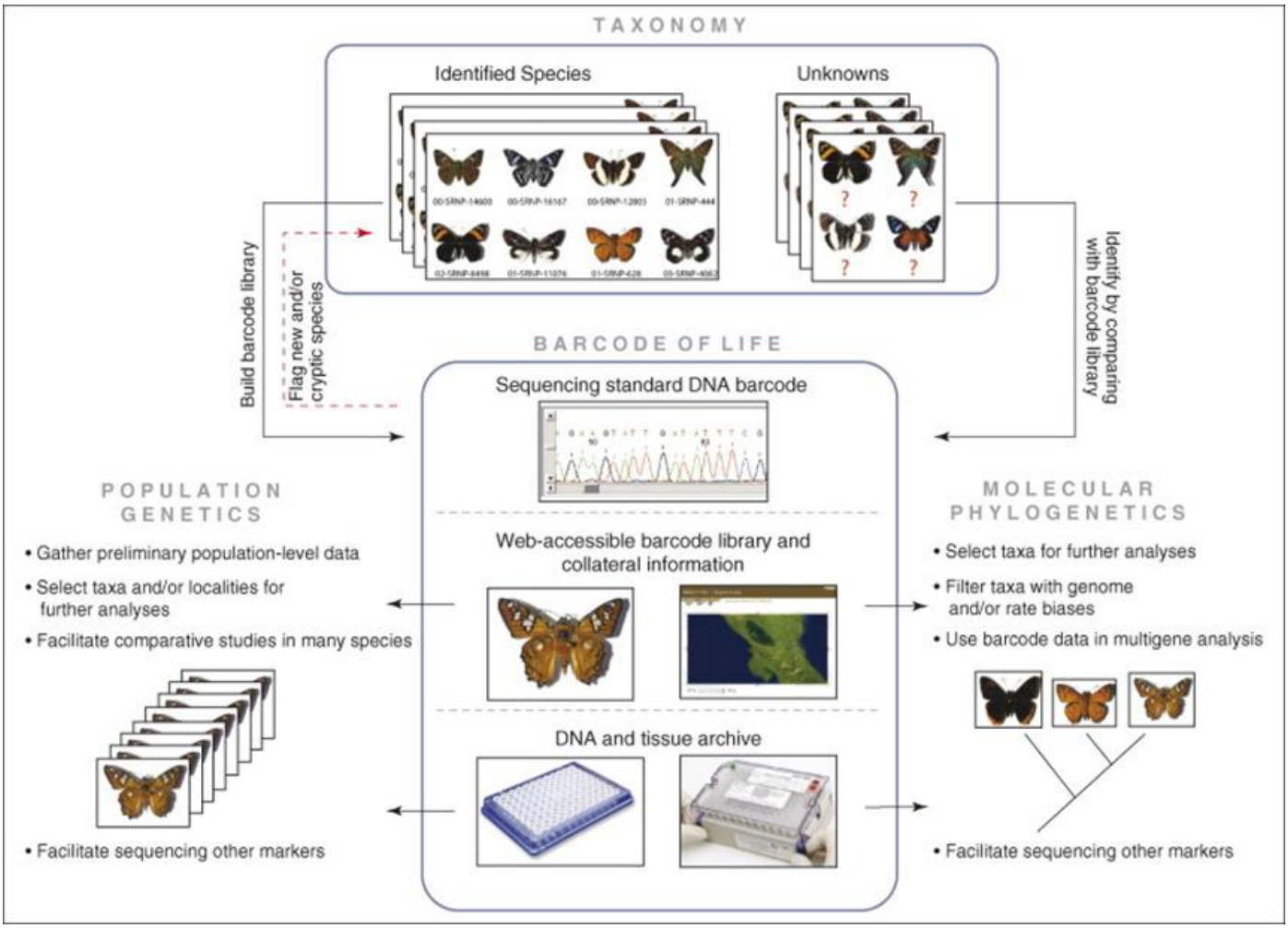

Fig 1: Application of DNA Barcoding technique for new species identification.

4.2 Expressed sequence tags (ESTs)

Adams *et al*. [47] discovered that Expressed sequence tags (ESTs) are single-pass sequence obtained from random sequencing of cDNA clones. It is an efficient way to identify genes and analyze their expression by means of expression profiling [48]. ESTs are useful for the development of cDNA microarrays that allow the geneticist to determine the analysis of differentially expressed genes in a systematic manner for genome mapping [49, 50]. It offers a rapid and valuable first look at genes expressed in specific tissue types, under specific physiological conditions, or during specific developmental stages [51]. The steps for analyzing the gene expression by ESTs technique of GPRS gene is given in the Fig. 2. Cox *et al*., [52] studied cattle and swine genome mapping and explained that ESTs are most useful for linkage mapping and physical mapping in animal genomics where radiation hybrid panels are available for mapping non-polymorphic DNA marker. Typically, a radiation panel is consist of hybrid cells

lines, with each hybrid cell containing small fragments of irradiated chromosomes of the species of interest. Characterization of the chromosomal break points within many hybrid cell lines would allow linkage and physical mapping of markers and genes in the specific mammalian genome [53, 54].

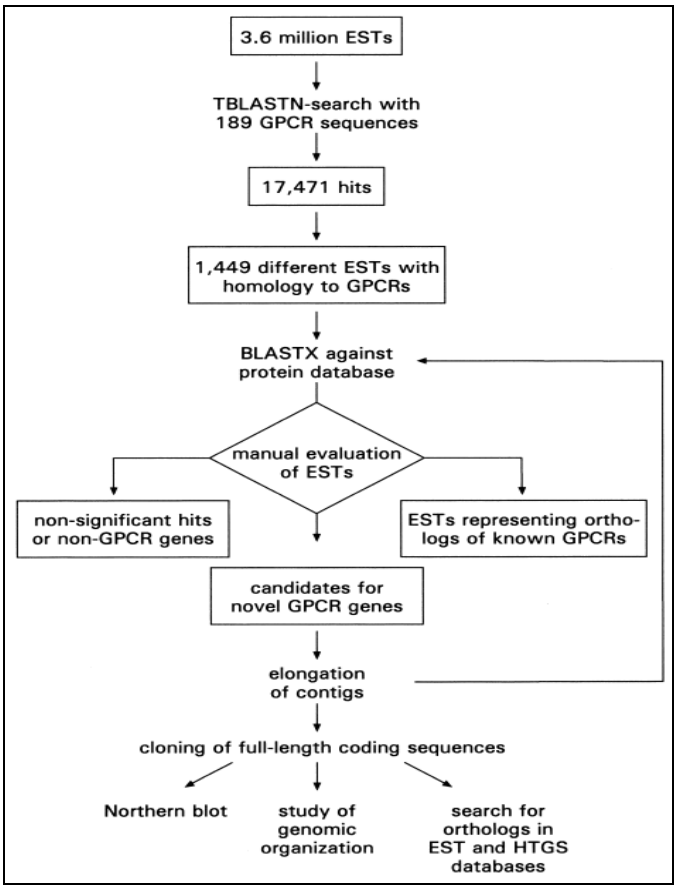

Fig 2: Steps for analyzing the expression of GPCR gene by ESTs.

4.3 Single strand conformation polymorphism (SSCP)

Orita *et al*. [55] discovered that single strand conformation polymorphism is the mobility shift analysis of single-stranded DNA sequences on neutral polyacrylamide gel electrophoresis, to detect polymorphisms produced by differential folding of single-stranded DNA due to restrained differences in sequence often a single base pair (Fig. 3). In the absence of a complementary strand, the single strand experiences intra strand base pairing, resulting in loops and folds, that gives it a unique 3D structure which can be considerably altered due to single base change resulting in differential mobility [55]. The SSCP analysis proves to be a powerful tool for assessing the complexity of PCR products as the two DNA strands from the same PCR product often run separately on SSCP gels [56]. Thereby, providing the opportunities to keep count a polymorphism by resolving internal sequence polymorphisms in some PCR products from identical places in the two parental genomes. The PCR-based SSCP analysis is a rapid, simple and sensitive technique for detection of various mutations, including single nucleotide substitutions, insertions and deletions, in PCR-amplified DNA fragments [57]. Thus, it was a powerful technique for gene analysis particularly for detection of point mutations. However, unlike RFLP analysis, SSCP analysis can detect DNA polymorphisms and mutations at multiple places in DNA fragments [58]. The SSCP gels have been used to increase throughput and reliability of scoring during mapping by PCR fingerprinting in animal. Fluorescence-based PCR-SSCP (F-SSCP) is an adapted version of SSCP analysis involving amplification of the target sequence using fluorescent primers [59]. The major disadvantage of the technique is that the development of SSCP markers is labor intensive and costly and cannot be automated.

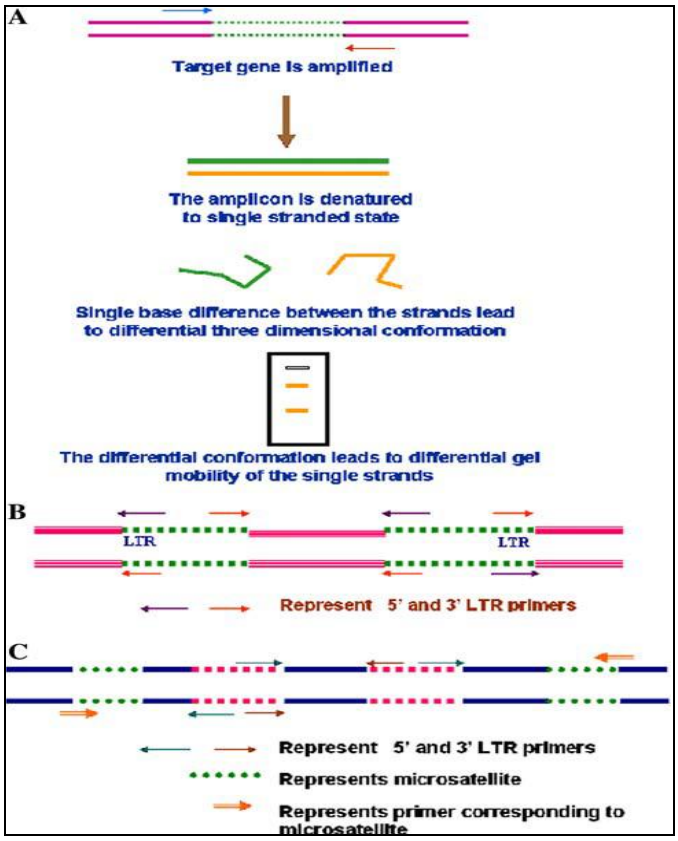

Fig 3: (A) SSCP analysis is based on the mobility shift of the single stranded DNA which is due to nucleotide changes (For SSCP analysis, the amplified target sequence is denatured and analysed on a native polyacrylamide gel). (B) IRAP markers are generated by the proximity of two LTRs using outward-facing primers annealing to LTR target sequences. (C) REMAP technique relies on amplification using one outward-facing LTR primer and a second primer from a microsatellite.

4.4 RNA-based molecular markers

Biological responses and developmental programming are regulated by the precise control of genetic expression. To obtain the detail information about the genetic expression the scientist started to concentrate on the differential patterns of gene expression at RNA level. PCR-based marker techniques, such as, cDNA AFLP and RAP-PCR are used for differential RNA study by selective amplification of cDNAs. cDNA-SSCP analysis of RT-PCR products was used to evaluate the expression status presence and relative quantity of highly similar homologous gene pairs from a polyploid genome. Replicate tests showed that cDNA-SSCP reliably separates duplicated transcripts with 99% sequence identity [60]. This technique has been used to gain remarkable insight into the global frequency of silencing in synthetic and natural polyploids.

4.5 RNA fingerprinting by arbitrarily primed PCR (RAPPCR)

Welsh *et al.* [61] illustrated that the RAP-PCR technique involves the fingerprinting of RNA populations using arbitrarily selected primer at low inflexibility for first and second strand cDNA synthesis followed by PCR

amplification of cDNA population [62]. The method required very minute quantity of total RNA usually in nanograms and remains unaffected by low levels of genomic DNA contamination. Differential PCR fingerprintings are detected for RNAs from the same tissue isolated from different individuals or for RNAs from different tissues from the same individual was expressed by Welsh *et al*. [61]. The individual-specific differences revealed are due to sequence polymorphisms which is useful for genetic mapping of genes. The tissue-specific differences are useful for studying differential gene expression. A novel RNA fingerprinting technique is cDNA-AFLP developed to display differentially expressed genes [63]. The methodology includes digestion of cDNAs by two restriction enzymes followed by ligation of oligonucleotide adapters and PCR amplification using primers complementary to the adapter sequences with additional selective nucleotides at the 30 end [64]. The cDNA-AFLP technique is a more stringent and reproducible than RAP-PCR [65]. In contrast to hybridization-based techniques, such as cDNA microarrays, cDNA-AFLP can distinguish between highly homologous genes from individual gene families. There is no requirement of any preexisting sequence information in cDNA-AFLP as it is required in case of ESTs, thus it is valuable as a tool for the identification of novel process-related genes [66, 67]. Recently, the major of area of research in cDNA-AFLP is applied for identification of stress-regulated genes [68].

4.6 Marker Assisted Selection (MAS)

To improve the performance of animals, breeders have to tackle the situation of selecting the best individual. From time being it has been accomplished either by pedigree data analysis or by phenotypic data estimation by Best Linear Unbiased Prediction (BLUP) that ultimately responsible to calculate estimated breeding values (EBVs). But with the development of DNA markers techniques a new approach named Marker assisted selection (MAS) has been discovered. MAS can be based on DNA in linkage equilibrium with a quantitative trait locus (QTL) by the phenomenon LE-MAS with the help of LE Marker. LE refers to the genotype frequencies at one locus which is independent of genotype frequencies at the second locus. Andersson [12] described LE markers stand for the loci that are in population-wide linkage equilibrium with the functional mutation in outbred populations. The LE markers can be readily detected on a genome-wide basis by using breed crosses or analysis of large half-sib families within the breed. Such genome scans require only sparse marker maps (15 to 50 cM spacing, depending on marker information and genotyping costs; to detect most QTL of moderate to large effects. Similarly, MAS can be based on molecular markers (LD marker) in linkage disequilibrium with a QTL by the phenomenon LD-MAS [69]. LD refers to the non-random association of alleles between two loci-, or based on selection of the actual mutation causing the QTL effect (Gene-MAS). LD markers: loci that are in population-wide linkage disequilibrium with the functional mutation [70]. The LD markers are by necessity close to the functional mutation for sufficient population-wide LD between the marker and QTL to exist within 1 to 5 cM, depending on the extent of LD, which depends on population structure and history. The LD markers can be identified using candidate genes or fine-mapping approaches [12]. Third type of marker loci i.e., the direct markers (i.e., polymorphisms that code for the functional mutations) are the most difficult to detect because causality is difficult to prove and, as a result, a limited number of examples are available, except for single-gene traits [12]. Whereas direct markers and, to a lesser degree, LD markers, allow for selection on genotype across the population because of the consistent association between genotype and phenotype, use of LE markers must allow for different linkage phases between markers and QTL from family to family. Thus, the ease and ability to use markers in selection is opposite to their ease of detection and increases from direct markers to LD markers and LE markers [70]. In what follows, selection on these three types of markers will be referred to as gene-assisted selection (GAS), LD markers-assisted selection (LD-MAS), and LE marker-assisted selection (LE-MAS). All three types of MAS are being used in the livestock industries [70]. Evaluation of the success of marker-assisted selection in breeding programs for different types of markers is given in Table 2.

Table 2: Evaluation of the success of marker-assisted selection in breeding programs for different types of markers.

Level of evaluation	Direct marker	Linkage disequilibrium marker	Linkage equilibrium marker
Frequency of marker locus	Direct population estimate	Direct population estimate	Within-family assessment
Frequency of target locus	Direct population estimate	Population-wide marker-trait association	Within-family marker-trait association
Phenotypic effect of target locus	Population-wide marker-trait association	Population-wide marker-trait association	Within-family marker-trait association
Genetic merit of population	Line comparison	Line comparison	Line comparison

Pongpisantham [71] established that the application of markers could increase the genetic response to selection for growth rate in a population of chickens up to 15% compared to that of family based selection. Similar type of result was also obtained by Ruane and Colleau [72] revealed that by the adaptation of MAS in the selection response for milk production in cattle nucleus that used multiple ovulation and embryo transfer (MOET) in the first six generations of selection was increased by 6 to 15%. So, significant increases to the selection response are possible in several species using MAS [73]. The estimated range of increase in selection response combined parameters with maximum responses was 2 to 38% and with minimum responses was –0.7 to 22.4% as analyzed by Clarke [74]. However, Ruane and Colleau [73] illustrated that possible increase in selection response of only 0.2 to 1% in six generations using a single marker by applying in cattle ONBS method of selection. The variation arises because of differences in the assumed sizes of the QTL effects, population structures, allelic frequencies, environmental variances, residual polygenic variances, of generations of selection, and selection procedures and most important factor recombination rates between the marker and the QTL and type of marker (single or haplotype). An unusual result was determined by Meuwissen and Goddard [69] that possible increases upto 64% in the genetic response to selection of different characteristics in the first five generations by MAS while the QTL explains only 33% of the genetic variation in the base population. Meuwissen and

Goddard [69] concluded the following result from their study by the use of Marker assisted selection (MAS):

1. MAS only can increase the rate of genetic gain in the long term, when there is a continuous advantage of new identified QTL (obviously, this has a biological limit determined by the maximum proportion of the genetic variance that can be explained by the segregation of QTL).
2. The extra genetic gain due to the MAS decreases very quickly with the number of generations of selection for a same QTL. The rate of identification of new QTL is difficult to predict.
3. The gain due to MAS for a certain QTL is higher when the characteristic is measured after the selection, as it happens with the fertility and carcass characteristics.

Some studies have shown possible economic advantages of the use of the MAS in dairy cattle [72, 75, 76] when the benefits are evaluated for the industry as a whole (Fig. 4). In most of the developed countries, it is being applying with progeny testing or multiple ovulation and embryo transfer (MOET) for nuclei improvement purpose.

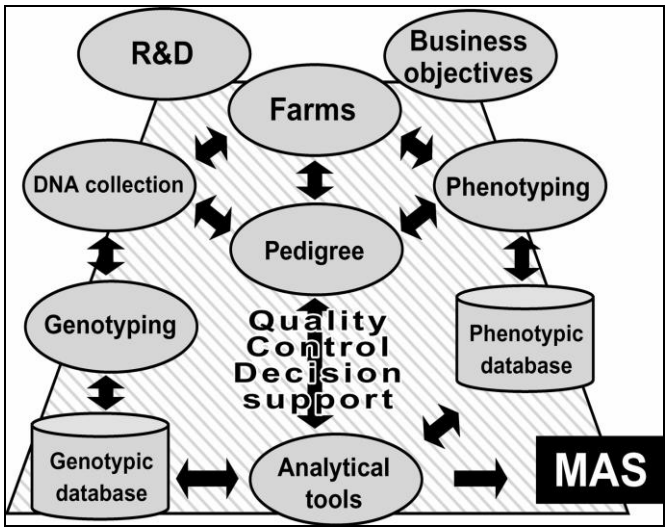

Fig 4: Components of an integrated system for the use of molecular genetic information in breeding programs for marker-assisted selection (MAS).

4.7 Marker Introgression Selection

The introgression involves the induction of a major gene in from a donor population into the recipient population by means of backcrosses assisted by molecular markers. The introgression programme is divided into three steps as shown in Fig. 5.

1. induction of the desired trait by crossing donor and recipient population,
2. subsequent backcrossing of the crossberd progenies with the recipient population, and
3. intercrossing between final crossberd progenies to estabilish a new population.

The repeated backcrossing aims to remove the doner line chromosomal segments unliked to the introgressed gene in order to recover the recurrent parental genome by 50 percent. Therefore, about eight or more generations of backcrossing are required to recover more than 99 percentage of the recipient genome [77]. A continuous character with additive genetic effects additives it is not advantageous to use single genetic marker information, in comparison with the use of only phenotypic information [78]. The genetic markers can be used in introgression of gene from one line or breed to other line or breeds, this phenomenon is known as Marker Assisted Introgression (MAI) [78]. Use of a single or two flanking markers linked to the allele for its introgression, result in substantial decrease in its allelic frequency after several generation of backcrossing; however when two flanking markers were used for bracketing the allele, the decline is relatively slow [77]. Nevertheless, it seems feasible that using a dense map that involves many chromosomal regions and with more than one allele of interest, the time for fixation of the major genes can be reduced [78]. This could be done through the selection for recurrent parent like characteristic traits in each or several backcross generations accelerate the recovery of the characteristic traits encoded by genes either linked or unlinked to the introgressed genes [77]. But the efficiency of such breeding strategy is doubtful for the selection of low heritable traits.

Hospital *et al*. [79] explained the advantage of using markers to monitor gene introgression and the predicted gain in time expected from such selection is about two-backcross generation. A density of 2-3 markers per 100 cM seems to be a optimal choice as with the increase in density leads to detriment. Use of distant markers around the introgressed gene is more effective than proximal markers, especially in early generations and when selection intensities are not very high. Groen and Smith [78] explained a simulation study on the use of genetic markers to increase the efficiency of introgression through selection for the background genotype. They experimented that selection using markers has small advantages over phenotypic selection for at least first three backcross generation. Selection was made in two stages: first of all the animals were selected carrying the allele to be introgressed and among these animals, those with the best phenotype or with least number of markers from the recipient line were then again selected. Hospital *et al*. [79] observed that markers were efficient in introgression backcross programmes for simultaneously introgression of an allele and selecting for the desired genomic background. Using a marker spacing of 10-20 cM gave an advantage of one or two backcross generations selection relative to random or phenotypic selection.

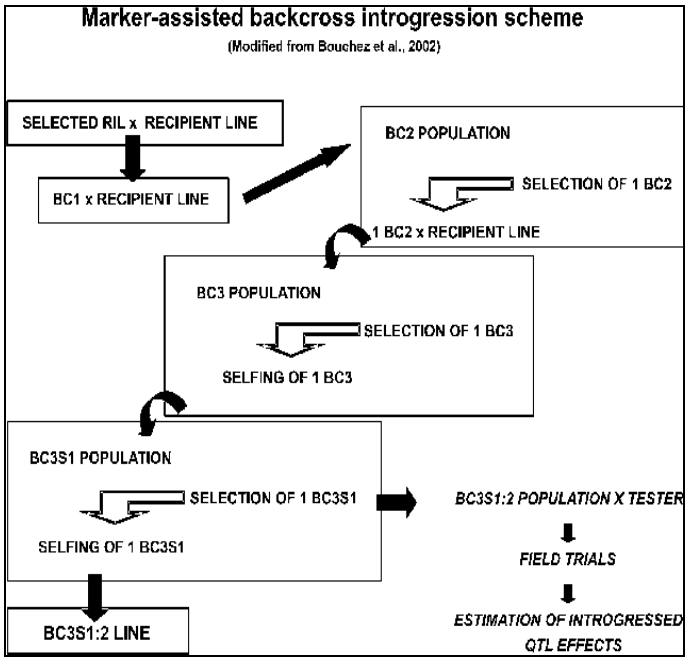

Fig 5: Steps of Marker Introgression Selection Scheme.

5. Application of Molecular marker technique

DNA markers have a potential application over a relatively broad field in animal breeding and genetics. The technique has direct practical application for livestock breeders, for example for parentage verification, individual identification and identification of certain genetic disorders. A very exciting and fast developing application of genetic markers is in the mapping of the various animal genomes. Conservationists also use various genetic markers in evolutionary and genetic biodiversity studies.

5.1 Genome mapping

In 1990, Human Genome Project was established to map the estimated 30,000 genes in the human genome in a targeted period of 15 years, it is an example of first innovative approach towards Genome mapping in world. This project had far reaching implications for genetic research including the quest to map genomes of other species [80]. After that, many projects were initialed to map the genomes of different species and the maps for different species are comparatively analyzed among themselves [81]. For most of the domesticated species such as cattle, pigs, sheep and equine, genetic maps are under construction. Ten laboratories are involved in the mapping of the bovine genome, while six laboratories are contributing to the mapping of the sheep genome. Comparative mapping of the human genome and those of various farm animal species were also carried out [82, 83]. Comparative mapping between pig chromosome 4 and human chromosome 1 based on QTL association for fatness and growth was demonstrated by Berg *et al.* [84]. Comparative mapping has several potential advantages including the identification of conserved regions between species, important contributions to the search for QTL and the provision of valuable information for gene expression studies [84]. A total of 154 autosomal shared segments have been identified between the chicken genome and the human genome, which is very helpful in studying the human genetic and diseases [85]. Approximately, 105 conserved segments between the human and bovine genomes have been reported by Band *et al.* [86]. Livestock genome mapping is a complex and time-consuming effort but, once completed, holds promise for finding functional genes, Quantitative Trait Loci (QTL) and genes associated with disease resistance which would beneficial for both human and animal improvement.

5.2 Quantitative Trait Loci (QTL)

In farm animals, most of the genetic traits of economic importance are the result of quantitative variation is mostly locate at a loci that so-called QTL [87]. By means of statistical analysis, genetic and phenotypic data are combined and it is possible to find the most likely location on the chromosome responsible for a specific trait by QTL mapping as shown in Fig. 6 [88]. In order to identify a QTL for a specific trait, many animals have to be genotyped for a large number of markers on different chromosomes [89]. In QTL mapping only a single candidate locus was mapped instead of the whole genome scan in the search for a QTL [1]. The identification and confirmation of QTL is complex, time-consuming and also rather costly, but promises lucrative commercial returns. QTL must be confirmed and tested in a target populations where the animals can be genotyped and genetic and phenotypic information be combined to predict genetic merit [90]. It is typically carried out by the phenomenon of Marker Assisted Selection (MAS). MAS are applicable for the traits where major genes are involved or with traits of low heritability [90]. The calpastatin gene associated with beef tenderness has initially been identified as a candidate gene for MAS [91]. But later on Casas *et al.* [92] discovered that QTL affecting both growth and carcass composition in cattle associated with myostatin. A diagnostic test for a major gene for marbling in beef cattle was also developed as geneSTA for measuring the characteristics of beef is commercially available in the USA, Australia and southern Africa. Likewise, in dairy cattle the quantitative trait loci responsible for milk production and protein and fat content have received considerable attention. Initially, milk yield and protein quantity were linked to chromosomes 14 and 21 as studied by Vaiman [93] respectively. Further work on this concept, it was discovered that five possible regions were associated with milk and protein yield, fat and protein percentage and milk yield, fat and protein yield, fat yield and protein percentage on chromosomes 1, 6, 9, 10 and 20, respectively [93]. Potential QTL for fat percentage and yield and protein percentage were recently reported for chromosomes 3 and 6 respectively [89]. Conformational type traits, associated with dairy form and milk yield have been found on chromosome 27 [87, 94]. Van Kaam *et al.* [95] tested 368 markers, scanning the whole chicken genome in their search for QTL affecting body weight in the chicken and identified chromosome 1 as the most likely position. Further work on mapping QTL for growth and fatness on chickens is underway [96]. The HMGI-C gene has also been identified as a candidate gene for the adw locus or autosomal dwarf gene in the chicken, which is of importance in the broiler breeding stock programmes [97].

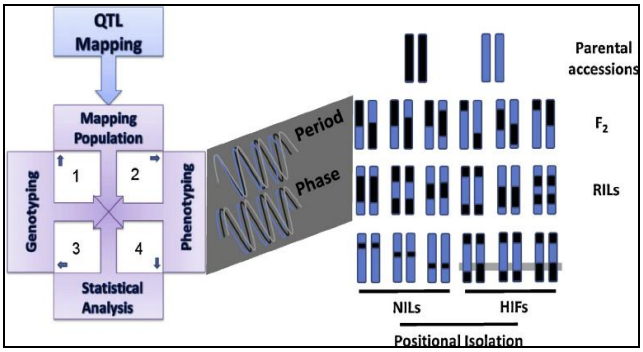

Fig 6: Schematic diagram of a QTL mapping.

5.3 Biodiversity studies

It is to be anticipated that selection for a long duration of time leads to inbreeding, thereby increases the homozygosity within the existing population which are prone to various type of deformities and disease condition [98]. Also, to improve the performance of livestock, crossbreeding systems has been opt that may lead to the loss of genetic variation within breeds [99] but the breed itself may become extinct. For this reason, the scientific community identified the need for the conservation of livestock resources. During 1992 the Food and Agricultural Organization (FAO) launched a programme for the Global Management of Farm Animal Genetic Resources, with the main objective being to identify new breed, conservation activities and create an awareness of possible losses of genetic resources on an international basis [100, 101]. A global programme was launched for all the livestock species for genetic characterization using DNA markers [101]. Genetic markers such as DFP's, RAPD and microsatellites have been used in studying genetic variability in different livestocks like cattle [102], sheep [103], goats [104], chickens [105], swine [106] and horses [107] were studied. Genetic variability within and among populations is often of importance and may contribute to the selection and preservation of genetic resources.

5.4 High density SNP Assay

Lander [41] proposed a new molecular marker technology named SNP. The more recent SNP concept has basically arisen from the recent need for very high densities of genetic markers for the studies of multifactorial diseases [108]. The fundamental principle of SNPs is to hybridize detected DNA fragments with high-density DNA probe arrays (also called SNP chips); the SNP allele is then named according to the hybridization results [5]. SNPs are third generation molecular marker technology coming after RFLPs and SSRs [109]. It is estimated that when comparing two human DNA sequences, there will be between 1.6 and 3.2 million SNP. They are bi-allelic markers, indicating a specific polymorphism in two alleles only of a population. SNP in coding regions can be directly associated with the protein function and as the inheritance pattern is more stable, they are more suitable markers for selection over time [110]. Currently, DNA chip technology is usually carried out during SNP investigations (Fig. 7). A group of associated SNP loci located on a certain region of the chromosome can form one SNP haplotype. SNPs are third generation molecular marker technology coming after RFLPs and SSRs [109]; it has been successfully used to investigate genetic variation among different species and breeds [111, 112]. Because of their extensive distribution and abundant variations, SNPs play an important role in farm animal population structure, genetic differentiation, origin, and evolution research. Furthermore, we can gain information concerning animal population diversity and population evolution (origins, differentiation, and migrations) via SNP haplotypes among different populations. But this can be compensated for by employing a higher numbers of markers (SNP chips) and whole-genome sequencing [22, 113].

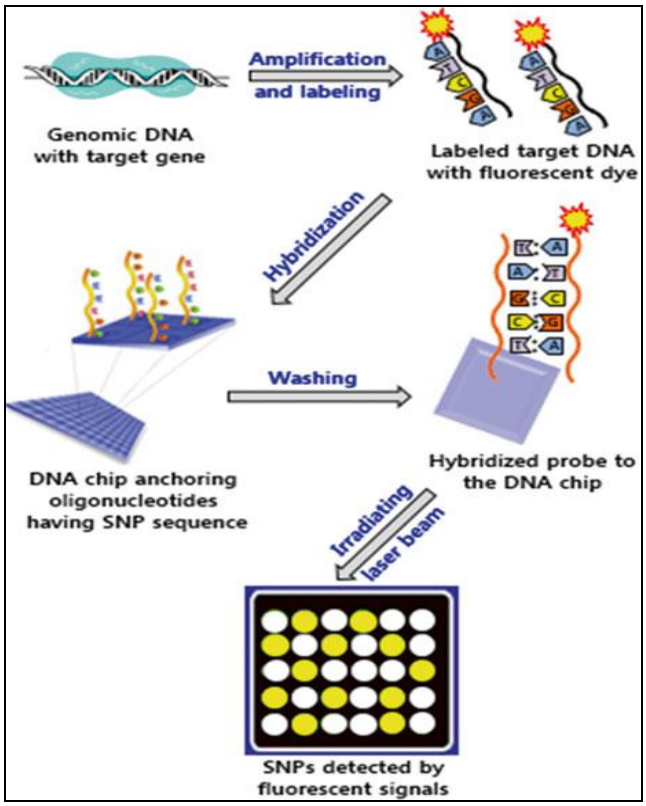

Fig 7: DNA chips to analyze single nucleotide polymorphisms (SNPs). A DNA chip is repaired by attaching DNA fragments of ~25 bp in length from a species to a glass or metal plate. These fragments contain the SNP variants to be analyzed. Fluorescence-labeled probes are hybridized with the DNA fragments on the plate, and the DNA chip is washed. Finally, the probes are exposed to light of a specific wavelength to induce fluorescence.

5.5 Whole genome sequencing

Highest resolution of DNA variation can be obtain by using sequence analysis. Sequence analysis provides the fundamental structure of gene systems of an individual. DNA sequencing is a vital tool in the analysis of gene structure and expression in an animal [10]. A step towards the use of genomic information in livestock improvement is the location of all markers and protein coding genes in the chromosomes [114]. So, the development of genetic maps of the species of interest is required for detecting QTL using molecular markers [88]. Additionally, the construction of physical maps has been favored by means of the development of techniques like the hybrids of somatic cells and fluorescence *in situ* hybridization (FISH) [115]. With the improvement of sequencing technology, whole-genome/gene sequencing has become available for characterizing genetic diversity among farm animals [116]. It is the most straight-forward method and provides more complete information on the genetic variation among different populations because it can detect all the variations within the genome. Currently, the problem with whole-genome sequencing is setting up a high-through data analysis platform to explore useful information for the conservation and utilization of farm animals.

5.6 Species identification and Genetic diversity with the help of DNA Barcoding

Fisher and Smith [117] evaluated the role of DNA barcoding as a tool to accelerate species identification and description of arthropods. Fisher and Smith [117] examined the CO1 DNA barcode morphological analysis of 500 individuals to recognize five species of *Anochetus* and three species of *Odontomachus*. The goal of DNA barcoding is to use a large-scale screening of one or more reference genes in order to assign unknown individuals to species, and to enhance the chances of discovery of new species [22]. Rach *et al.* [118] concluded that the DNA barcodes are able to identify entities below the species level that may constitute separate conservation units or even species units. Fleischer *et al.* [119] have conducted DNA analysis of seven museum specimens of the endangered North American ivory–billed woodpecker (*Campephilus principalis*) whose sequences provided an important DNA barcoding resource for identification of critically endangered and charismatic woodpeckers nearer to that region. So, the COI–DNA barcode has been considered as a tool for species identification, biodiversity analysis and discovery for species like Smith *et al.* [120] that has discovered *Belvosia* parasitoid flies by applying this method. Lorenz *et al.* [121] have suggested that depositing barcode sequences in a public database, along with primer sequences, trace files and associated quality scores, would make this technique widely accessible and applicable for species identification and biodiversity analysis.

6. Conclusion

The developments over the past ten years in molecular genetics have created the potential for the application of functional genomics over a broad field of animal improvement and breeding. These methods provide a large number of markers and opening up new opportunities for evaluating diversity in farm animal genetic resources. Currently, SSR and SNP markers subjected to many researches to compare their validation for map built. In a simulations prediction study, results showed that SNPs are at least two to six times more necessary to achieve the same resolution as microsatellites when used for individual

identification and the study of parentage assessment and relatedness. The SNP markers have promising advantages over microsatellite markers, due to high throughput automated analysis, lower mutation rates and lower genotyping costs. However, SNP markers can only be transferred to different mapping populations within the same species, but not across species. Mitochondrial DNA markers are particularly useful for studying evolutionary relationship among various taxa. DNA barcoding based on mitochondrial genes (most often COI) has emerged as a powerful strategy for species identification. In breeding, QTL will for example be applied in the genotypic selection of superior animals. There is a danger associated with a potentially inadequate use of QTL information, giving an excessively high emphasis to simple molecular information in detriment of the overall economic gain through all traits and their polygenic effects in the population. Dissemination of the information to the industry is therefore a complex issue concerning QTL effects and molecular markers. The characteristics on which the application of the MAS can be effective are those that are expressed late in the life of the animal, or those that are controlled by a few pairs of alleles. Because of its high cost, the use of MAS could be justified, in animal nuclei that allow dilution of the costs when germplasm is extensively used towards the commercial population. So, the use of advanced molecular techniques offers new opportunities and challenges for building and using more predictive and efficient statistical models for livestock improvement.

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