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Molecular Characterization of Animal Genetics Resources, its Potential for use in Developing Countries

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ABSTRACT

The challenges and potentials of application of molecular characterization of animal genetic resources in developing countries were reveiwed; avenues that could be exploited to put to this technology to use were also highlighted. Some of the challenges of the application of molecular characterization of animal genetic resources in developing countries were poor infrastructure, reference genomes, biological background information, population genotyping data are not available, lack of political will, inadequate funding, poor laboratory services and inadequate technical manpower. Potential of molecular characterization of animal genetic resources in developing countries includes better access to genomic resources, accuracy of analytical and data management tools. Most livestock populations in developing nations are poorly characterised into specific breeds. The potential of these ecotypes to increase livestock productivity are yet to be fully exploited due to poor characterization. Molecular characterization to identify variation in desired traits through sequencing will provides avenue for selection, upgrading or crossbreeding to improve performance. This will not only reduce rural poverty, hunger and manutrition, but will also create employment opportunities .The use of marker assisted selection will enhance selection accuracy and the rate of genetic gain. Animal breeders in developing countries would be able to develop local improved breeds, conserve local genetic resources for optimum use and for the future.

Keywords: *developing-nation, genetic-resources, molecular-characterization, potential,*

INTRODUCTION

Recent advances in molecular biology, principally in the development of polymerase chain reaction (PCR) for amplifying dioxy ribonucleic acid (DNA), DNA sequencing and data analysis, have resulted in powerful techniques which are used for the screening, characterization and evaluation of genetic diversity. The extensive number of research information describing the use of these techniques on wide range of animal species and diversity problems, is a testimony to their increasing impact in this field (Karp and Edwards, 1996). The increasing availability of molecular tools for genomic studies had improved genetic information on livestock species for improved utilization and management (FAO, 2015). Genetic improvement of livestock depends on access to genetic variation and effective methods of exploiting this variation. Genetic diversity constitutes a buffer against changes in the environment and is a key in selection and breeding for adaptation and production on a range of environments. In developed countries, breeding programs are base upon performance and molecular evaluation records and this has led to substantial improvement in animal production. Developing countries however, have distinct disadvantages for setting up successful breeding programs. Infrastructure needed for performance testing is normally lacking, herd sizes are normally small, variability between farms, farming systems and seasons are large. Reproductive efficiency is low, due mainly to poor nutrition and management especially in cattle, communal grazing and pasture breeding precluding implementation of systematic breeding and genetic evaluation programs.

The global action plan for Animal Genetic Resources according to FAO (2007) noted that understanding the diversity, distribution, basic characteristics, comparative performance and the current status of each country's animal genetic resources are essential for their efficient and sustainable use. Development and

conservation, complete national inventories, supported by periodic monitoring of trends and associated risks, are basic requirement for the effective management of animal genetic resources. Without such information, some breed populations and unique characteristics they contain may decline significantly, or be lost, before their values are recognized and measures taken to conserve them. According to Sylvester (1998) recombinant deoxyribonucleic acid (DNA) technology has found use in human medicine, in Agriculture and in industry. The application of this technology in human medicine has essentially been in three general areas, greater understanding of cancer-producing genes (onco genesis), which incidentally are carried by everybody. Production of chemically active compounds such as hormones (for example, insulin for diabetics and growth hormone), growth factors, antibodies, vaccines, antibiotics, interferon's and anti-hemophilic factors for example, Factors VIII and gene therapy, (insertion of functional foreign genes to treat or ameliorate certain genetic disorder).

In agriculture, genetically engineered microorganisms are being used to produce feed additives, such as amino acids, vitamins and growth promoters. The greatest potential of this technology in agriculture is perhaps in the production of genetically engineered plants and animals with desirable characters. For instance, in-corporation of the growth hormone gene into the genome of domestic animals to improve their growth rate. Genetically engineered organisms are referred to as transgenics (FAO, 2015). No doubt, the production of plants and animals through recombinant DNA technology raises a number of ethical questions. Moreover, in developed countries where sophisticated biotechnology laboratories and industries which use this technology have increased considerably, there are government regulations governing both research in this area and also the release of genetically engineered organisms into the environment (FAO, 2015). Recombinant DNA technology also has considerable contributed to various industries, such as food processing and chemical industries. Genetically engineered micro-organisms can produce enzymes such as amylases, and also alcohol, amino acids and vitamins. Commercial products like cosmetics, textiles, dyes, detergents and oils may be more cheaply produced by genetic engineering than by synthetic methods (FAO, 2015).

Molecular characterization of animal genetic resources though efficient in developed countries, it applications in developing countries are hindered. The objective of this study is to review the challenges and potential of application of molecular characterization of animal genetic resources in developing countries. To highlight avenues that could be exploited to put these technologies to use in developing countries.

Characterization of Animal Genetic Resources

Characterization of animal genetic resources encompasses all activities associated with the identification, quantitative and qualitative description and documentation of breed populations, their natural habitats and production systems to which they are or are not adapted to (FAO, 2007). The objective of characterization is to increase knowledge of Animal Genetic Resources (AnGR), their abundance, and potentials for future uses, in wider environments (FAO, 1984; Rege, 1992). Characterization activities should contribute to objective and reliable prediction of animal performance in defined environments, so as to allow a comparison of the potential performance of different types of AnGR within the various production systems found in a country or region (FAO, 2015). Developing countries are endow with the majority of the global domestic animal diversity-landraces, strains or breeds. Some livestock breeds in these countries are not characterized, their genetic resources are unknown ,such that others are endangered, some had extricated, others that survived are either highly threatened or endangered through indiscriminate crossbreeding or destruction of the environment that enhanced their continued survival. The importance of indigenous livestock breeds lies in their adaptation to local biotic and a biotic stresses and to traditional husbandry systems. However, most of these animal genetic resources are still not characterized and boundaries between distinct populations are unclear. In such case breeds distinctions are defined on the basis of subjective data and information obtain from local communities (FAO, 2015). Reliance on these criteria as a basis for classification for utilization and/or conservation may be misleading, as historical evidences are not always accurate, relying as it often does, on subjective judgments. Archival research can reveal much about the original morphological type of a breed or strain but it's molecular genetic evidence which is factual and precise

cannot be characterize by this method. It is in this sphere that molecular characterization of animal genetic resources has an important role (Rege, 1995).

The term "surveying" is typically used in the context of national efforts to obtain data on the size of breed population (FAO, 1999). Breed identification may involve surveying and characterization. A "survey" may collect a range of different types of AnGR-related data, while characterization, broadly defined, includes the task of obtaining data on population sizes. A survey that provides, for the first time, sufficient data to estimate the size of a national breed population is often referred to as a "baseline survey" (FAO, 2011). At national level, surveying and characterization comprise the identification and description of the respective country's AnGR, including their population sizes and structure, geographical distributions and production environments, as well as environmental threats that change over time. Characterization is typically differentiated into two categories; phenotypic characterization and molecular characterization (FAO, 2015).

Phenotypic Characterization

The term phenotypic characterization of animal genetic resources generally refers to the process of identifying distinct breed populations and describing their external and performance characteristics within given production environments. The process involves desk work in gathering existing data, as well as field work recording information (descriptions, photos and trait measurements) for sampled animals. The term "production environment", in this context, refers not only to the "natural" environment (climate, terrain, etc), but also to management practices and the uses to which the animals are put. Broadly defined, it can also be taken to include social and economic factors such as market orientation, marketing opportunities and gender issues. Recording the geographical distribution of breed populations is considered to be an integral part of phenotypic characterization (FAO, 2015). Phenotypic characterization is used to identify and document diversity within and between distinct breeds, based on their observable attributes (FAO, 2012a). Phenotypic characterization, may includes information on body biometry, performance, strategies for adaptation, unique testing. Unless we know the accurate physical and biometric characters between and within geographic locations, populations, animal husbandry practices, utility of the particular animal breed which influence these traits their overall improvement cannot be properly designed.

Body shapes measured objectively could improve selection for growth by enabling the breeder to recognize early-maturing and late-maturing animals of different size (Brown et al., 1974; 1993). Significant differences in different body measurement traits due to age and sex were reported by many workers in different breeds and species. In cattle (Gilbert et al., 1993; Shahin et al., 1995; Pundir et al., 2007a,b,c, 2008; Singh et al., 2008; and Yakubu et al., 2009), in Egyptian buffalo (Shahin et al., 1993). In horses (Biedermann and Schmucker 1989; Jakubec et al.,1999; Miserani et al., 2002; and Sadek et al., 2006). In sheep. (Salako et al.,2006). The guidelines on phenotypic characterization (FAO, 2012b) offer advice on how to conduct a well-targeted and cost-effective phenotypic characterization study and provide an overview of the concepts and approaches that underpin phenotypic characterization. FAO, (2012) also provides practical guidance on planning and implementing of field work, data management and data analysis. Generic data collection formats for phenotypic characterization of major livestock species, as well as a frame work for recording data on breeds' production environments are also included. It encompasses the following activities:

Describing the geographical distribution of the targeted breeds and if possible the size and structure of their populations;

- Assessing the breeds' phenotypic characteristics, including physical features and appearance, economic traits (e.g growth, reproduction and product yield/quality) and some measures (e.g range) of variation in these traits-the focus is generally on productive and adaptive attributes;
- Obtaining images of typical adult males and females, as well as herds or flocks in their typical production environment;
- Gathering information on the breeds' origin and development;
- Describing any known functional and genetic relationships with other breed within or outside the respective country;
- Describing the biophysical and management environment(s) in which thebreeds are kept;

- Documenting the breeds' responses to environmental stressors such as disease and parasite challenge, climatic extremes and poor feed quality, along with any other special characteristics related to adaptation; and
- Cataloguing and relevant indigenous knowledge (including gender-specific knowledge) related to the breeds and their management.

Many of these tasks can be accomplished through desk work or by consulting breeders or other stakeholders. The clearest exceptions are items 2 and 3, which require recording of data on a representative sample of live animals directly in their production environments.

Molecular Characterization

Recent advances in the field of genomic technology have constituted a major innovation in livestock production. The increasing availability of molecular tools had deeply affected the studies of livestock species are their management (FAO, 2015). Molecular characterization or genetic characterization therefore, can be defined as the complementary procedures used to unravel the genetic basis of phenotypes, their patterns of inheritance from one generation to the next, within-breed genetic structure and levels of variability, and relationships between breeds (FAO, 2015). The first state of the World's Animal Genetic Resources for Food and Agriculture (first Sow-AnGR) (FAO, 2007c) noted that the main rates of molecular technologies in the characterization of Animal Genetic Resources include:

- Assessing functional and neutral genetic variability within and between populations, including investigation of their history (domestication, expansion or reduction of the population size, migrations, introgression episodes, etc);
- Assessing the current state of a population in terms of risks related to inbreeding and genetic drift, using estimators such as effective population size; and
- Genetic characterization of traits (e.g. physical appearance, productivity, disease resistance and other adaptability traits) specific to given populations.

FAO, (2012) reports highlighted the following three (3) ongoing developments in molecular biology as being particularly relevant to Animal Genetic Resources Management:

- The establishment of whole genome sequences for various livestock species;
- The development of technologies for measuring polymorphisms at loci spread across the entire genome; and
- The development of technologies for measuring gene transcription and expression on a large scale.

Since the first state of the World's Animal Genetic Resources for food and Agriculture was prepared, the list of species whose genomes have been sequenced has continued to grow (FAO, 2015). The costs of genotyping and sequencing have declined sharply during these periods. High density SNP arrays, allowing the simultaneous assay of several tens of thousands to several hundreds of thousands of SNPs, are available for use in livestock species at a cost of US $100 or less if a relatively large number of individuals are sequenced. Genomes can be sequenced for less than US$3,000 each with moderate coverage "eight-fold" (coverage-meaning that, on average, each position in the genome is sequenced eight times). Sequencing smaller fraction of genomes (restriction site associated DNA sequencing –RAD-sequence) can be used directly in the characterization of individual animals (this is termed "genotyping by sequencing") (De Donato et al., 2013). Similarly, the development of tools capable of assaying a high density of transcripts and even direct transcriptome sequencing (also known as "RNA-seq" – short of RNA sequencing), has increased the capacity to study gene expression and hence unravel the complex physiological regulation of target traits (D' Alessandro and Zola, 2012).

The guidelines on Molecular characterization (FAO, 2015) include a short overview of progress in molecular characterization of Animal Genetic resources over the preceding two decades and prospect for the future. FAO, (2015) guidelines also provide practical advice for researchers wishing to undertake a molecular characterization study. The guidelines emphasize the importance of obtaining high-quality and representative biological samples that yield standardized data that can be integrated into analyses on an international scale. With respect to biological samples, the guide-lines suggest the collection of samples from at least 40 animals from across the geographic range of the breed. Blood has traditionally been the most frequently sampled material, but tissue and hair are gaining

popularity. Equipment has been developed for sampling ear tissues during the process of tagging animals for identification purposes. This approach efficiently combines animal identification with sample, collection and links the identification number of the animal to the containers in which the tissue sample is captured and stored. The material in the sampling tubes can also be cryo-preserved and stored in a gene bank for possible use in population regeneration through cloning via somatic cell nuclear transfer (FAO, 2012b). Ideally, for maximum efficiency, phenotypic and molecular genetic characterization activities should be combined, so that body measurements and other relevant traits can be recorded from the same animals on which biological samples are taken. Recording geographic coordinates for each animal from which samples and measurements are taken facilitates the description of their production environments, as the coordinates can be linked to other geo-referenced datasets. A variety of biotechnological tools are available for assaying the DNA collected during molecular characterization. Lists of the standard international society for Animal Genetics-FAO Advisory Group panels of micro-satelite markers for nine common livestock species are included in the guidelines on molecular genetic characterization (FAO, 2011b). These panels are, however, limited to the characterization of neutral genetic variability.

BREED POPULATION SURVEY

According to FOA (2015) breed survey provides the bulk of information needed to establish a breed's risk status. An effective baseline survey at national level will establish a reliable estimate of the size, structure and geographical distribution of the breeds population and regular monitoring will record how these change over time. If the breed is present in more than one country (transboundary breed), national surveys in all countries where it is present will be needed in order to obtain an accurate estimate of its global population size (a breed's international distribution and global risk status may be factors to consider in decision making at national level but knowledge of those factors should clearly not be regarded as a pre-requisite for action). Analysis of data from molecular characterization studies allows inferences to be drawn not only on the present genetic structure of a breed population, but also on the breed's history. Molecular characterization can also be used to refine knowledge about trans boundary populations by contributing to the identification of breeds that have different names but show little differentiation at the genetic level. The relative utility value of a breed for food and agriculture will depend on a combination of factors and can be assessed on the basis of the results of phenotypic characterization studies that record performance, adaptability and product quality, along with descriptions of the production environments in which the animals are kept. Phenotypic characterization will also provide an indication of the breed's genetic distinctiveness, as unique traits can be expected to have a significant genetic basis. Molecular characterization can confirm this differentiation with respect to functional genes and extend it to "neutral" areas of the genome that are not subject to the forces of selection. A combination of phenotypic characterization (including information on production environments) and molecular characterization will indicate a breed's adaptive traits and provide some indication of the biological basis for the observed characteristics. Studies will ideally also note any particular historical or cultural significance of the breeds targeted. Molecular characterization can help in the evaluation of a breed's potential for genetic improvement. For simply inherited traits controlled by a single locus or a few well-defined loci, molecular analyses can determine whether a given breed carries the most favourable allele(s) and at what frequency. The situation is more complicated for quantitative traits, because such traits are influenced by many genes-and few of these genes have been identified. However, genetic variation is essential for genetic improvement, and molecular characterization can be used to obtain a general assessment of a breed's genetic variability. An approach of this kind relies on the assumption that overall genetic variation (which includes variation for neutral loci that do not influence traits) is proportional to the variation for trait-influencing loci. As noted above, description of the production environment is an essential element of phenotypic characterization. It can allow inferences to be drawn regarding a breed's potential for improvements, particularly whether or not its genetic potential is being constrained by the environment (natural conditions or management capacity). Describing the production environment in which a breed has been raised for many years can also serve as an indirect means of characterizing its adoptive traits, based on the assumption that, over the years, the breed will have become adopted to the conditions in which it is kept. A description of the production

environment in the broad sense may include an assessment of marketing opportunities and current and potential future demand for product or services provided by breeds and thereby provide information that can be used in planning their future management.

Since the first state of the World's Animal Genetic Resources for food and Agriculture was prepared, the list of species whose genomes have been sequenced has continued to grow. It includes:-

- Chicken - 2004
- Sheep - 2010
- Cattle - 2009
- Horse - 2009
- Pig - 2012
- Rabbit - 2009
- Turkey - 2009
- Goat - 2013.

Source: *FAO, 2015.*

Techniques for Molecular Characterization

Progress in sequencing techniques and the opportunities offered by the development of high-density marker arrays have considerably improved the availability of DNA information over the last ten years, both in terms of number of markers identified and in terms of cost of genotyping. (FAO, 2015). Until recently, microsatellites remained one of the most popular types of marker in genetic characterization studies (Lonstra et al., 2012), used for example in projects such as ("Global Div"), which ran from 2007 to 2010 and combined microsatellite datasets from various diversity studies from different parts of the world (Ajmone-Marsan et al., 2010). Microsatellite data continue to be used especially in developing countries (Abdullah et al., 2012; Azam et al., 2012) and in the context of conservation and priority setting at regional level (Medugorae et al., 2011, Ginja et al., 2013). However, they are increasingly being superseded by the use of SNP marker arrays (FAO, 2015).With the advent of next-generation sequencing, mitogenomics (analysis of the whole mitochondrial genome rather than a limited frequent of mitochondrial DNA) can be routinely used in livestock species, including less intensively studied species such as goats (Doro et al., 2010) and Horses (Achilli et al., 2012). The recent generation of whole genome reference sequences for many livestock species has allowed "population genetics" to become "population genomics". According to FAO, (2015) Population genomics uses large sets of single-nucleotide polymorphisms (SNPs) to study specific variations across the genome and determine how they have been shaped by the history (e.g changes in population size, selection, and cross-breeding) of livestock populations. SNPs can be assigned to various classes (neutral vs genic, intron vs. exon or synonymous vs non-synonymous), which provides opportunities for more detailed analysis of diversity (a) (FAO, 2015). The past decade has also witnessed a revolution in sequencing technologies that has led to the development of various platforms for DNA and RNA sequencing, known collectively as next-generation sequencing technologies (Metzker, 2010 and Davey et al., 2011). These tools can rapidly provide sequence data in the form of short reads (sequenced DNA fragments between 100 and 400 base pairs long on average) that collectively cover the whole genome of a sample (or a transcriptome of a particular organ) several times. Identifying SNPs from this type of data is relatively easy, provided that a reference sequence (Marker) has been established (Nielsen et al., 2011) which is the case for most livestock species (Norman et al., 2013) and these approaches may prove useful for less common livestock species.

High-density SNP panels are now widely used for Genome-wide Association Studies (GWAS), genomic prediction and population genomic analyses. However, the preliminary phase, i.e SNP discovery or SNP selection from databases, is critical. If data have not been obtained randomly, standard estimators of population genetic parameters should be applied with caution. Non-randon selection may occur if SNP sets are derived for use on a given set of breeds but later used on other breeds or if SNP sets are filtered to meet certain criteria (e.g a minimum allele frequency). Many current tools are affected by both these factors, as they have been developed primarily using widely used international trans boundary breeds and with the use of SNP-filtering criteria. Such protocols bias the distribution of allelic frequencies relative to what would be expected in a random sample (FAO, 2015). The resulting inaccuracy in estimation of genetic parameters is known as "ascertainment bias" (FAO, 2015). Bias caused by problems of this kind is probably present in most commercial and ready-to-use medium-and high-density SNP panels currently available for use in livestock species. Unbiased estimates of the absolute genetic diversity (i.e the nucleotide diversity) of a population can, in theory, be

obtained only via whole genome sequencing. Statistical approaches that explicitly account for the methods used in SNP discovery and sample preparation have been developed for use when undertaking various kinds of population genetics analyses with SNPs (Nielsen et al., 2011; Kofler at el., 2011). Large-scale projects have also started to harvest genome-wide information for use in characterizing livestock population at national or international scale, including studies on cattle (Gautier et al., 2010), sheep (Kijas et al., 2012), horses (Mccoy et al., 2014, Orlando et al., 2013), pigs (Groenen et al., 2012), chickens (Weigend et al., 2015) and goats (Dong et al., 2013)

It is important to note that although cost per SNP is low relative to microsatellites (and decreases with the number of SNPs analysed) the costs of high-density-assays-as of 2015 US$50 to US$200 and depending heavily on the number of arrays purchased – are nonetheless prohibitive for many applications. Costs continue to decline, however, and financially realistic options are likely to eventually become available for most situations. This being said, even if lower cost genotyping assays become available, the bioinformatics infrastructure in most developing countries will still require further development. Both the sheer amount of raw data and the complexity of analytical models are several orders of magnitude larger than those associated with microsatellite-based analyses. This is true for work with SNP arrays data, but even more so for work with sequence data. Further studies are in the process of identifying millions of SNP and haphotypes (specific allelic combinations for a given set of loci) and also other sequence variants such as insertion-deletion polymorphisms (InDels) and copy number variants (CNV) (FAO, 2015). Novel sequencing technologies are continuously evolving, accompanied by a drop in cost per sequenced genome. Allele frequency differences and diversity measurers derived from them can be obtained in-expensively by sequencing pooled DNA from multiple individuals from a population (Qanbari et al., 2012). Sooner or later, sequence-based approaches will become the standard methodology for generating data for use in livestock diversity studies.

Marker information will become even more useful when linked to biological background information available in specialized databases. Information about marked genes and their functions is available in the Ensemble database (among others) for many livestock species. Information on quantitative trait loci (QTL) is collected in the Animal QTL database and genomic pathway information is available through KEGG. In making systemic use of such information will allow a shift from a purely statistical assessment of genetic diversity to a more informative functional approach. Some of the recently developed molecular tools or techniques and their potential application in conservation of animal resources includes:

DNA Sequencing

DNA sequencing is the determination of the order of the nucleotide bases – A (adenine), G (guanine), C (cytosine) and T (Thymine) present in a target molecule of DNA. Early work that was developed for the identification and characterization of clinically important bacterial stains has made it possible to obtain DNA sequences within a few days (Hultman et al., 1989 and Brytting et al., 1992)

Conventional Sequencing Technique

Currently, dye-terminator sequencing technique is the standard method in automated sequencing analysis (Olsvik et al., 1993). The dye-terminator sequencing method, along with automated high-throughput DNA sequence analyzers, is now being used for the vast majority of sequencing work. The basic technique related with dye terminator sequencing and phylo genetic analysis shows that dye-terminator sequence utilizes labeling of the chain terminator NTPs, which allows sequencing in a single reaction, rather than four reactions as in previously used labeled-primer method. In dye-terminator sequencing, the four dideoxynycleotide chain terminators are labeled with fluorescent dyes, each with a different wavelength of fluorescent emission. The main advantages of this technique are its robustness, automation and high accuracy (>98%). On the other hand, the limitations of this technique include dye effects due to differences in the incorporation of the dye labeled chain terminators into the DNA fragment. Such incorporation of dye can result in unequal peak heights and shapes in the electronic DNA sequence trace chromatogram after capillary electrophoresis. Another drawback is its inability to handle long sequences; however, it can be reliably sequence up to approximately 900 nucleotide long DNA fragments in a single reaction. The advent of new generation sequencers with solid state chemistry has significantly overcome these problems. Current interest is in DNA bar coding of plants and

animals with the aim to identify as unknown plant or animal in terms of a known classification. DNA bar coding is a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed-upon position in the genome. DNA barcode sequences are very short relative to the entire genome and they can be obtained reasonably quickly and cheaply (Kress et al., 2005). Integration of recently developed bar coding with techniques such as RAPD, AFLP, microsatellite and SNP seems to provide better resolution.

Next Generation Sequencing Techniques

A new generation of non-sanger based sequencing technologies has been evolving on its promise of sequencing DNA at unprecedented speed, thereby also having enabled impressive scientific achievements and novel biological applications. These techniques have made it possible to conduct robust population – genetic studies based on complete genomes rather than just short sequences of a single gene. Rapid progress in genome sequences of various plant and animal speeles through next generation sequencing will further extend our understanding of how genotypic variation translates into phenotypic characteristics. A comparative genomic approach is extra-ordinarily useful for identifying functional loci related to morphological, geographical and physiological variation, and thus next generation sequencing technology will enable us to better understand the process of plant and animal evolution. Next generation platforms do not rely on sanger chemistry (Sanger et al., 1977) as did the first generation machines used for the last 30 years (Schuster, 2008). The first of this kind of second generation of sequencing technique appeared in 2005 with the landmark publication of the sequencing-by-synthesis technology developed by 454 life sciences (Margulies et al., 2005) based on pyro sequencing (Ronaghi et al., 2006 and Nyren, 2007).

The single-molecule sequencing method (also known as 3rd generation or next-next generation) is independent of PCR (Schuster, 2009 and Blow, 2008). This mode of sequencing protocol was recently developed by Helicas Genetic Analysis System using the technology developed by Braslavsky et al., (2003). Other 3rd generation sequencing systems are being developed by life technologies and Pacific Biosciences SMRT technology and may appear within one or two years. Oxford Nanopore Technology (www.nanoporetech.com) has been developing a label-free, electrical, single molecule genuinely revolutionary DNA sequencing method. This technique is aimed at obviating the need for amplification or labelling by instead detecting a direct electrical signal (Clarke et al., 2009). However, this technique is still in a developing stage. The recently developed Helicos 3rd generation high- through put and low-cost direct single molecule RNA quenching method-without requiring prior conversion of RNA to cDNA-opened the door for a comprehensive and bias-free understanding of transcriptomes (Ozsolak et.al., 2009). By directly sequencing single molecules of DNA or RNA, Helicos True Single Molecule Sequencing (tSMS) technology significantly increased the speed of sequencing, while also decreasing the cost. Briefly, the procedure works by: first capturing billions of single molecules of sample DNA on an application-specific proprietary surface within two flow cells. These captured strands serve as templates for the sequencing-by-synthesis. Polymerase and one fluorescently labeled nucleotide (C/G/A/T) are added. The polymerase catalyses the sequence-specific incorporation of fluorescent nucleotides into nascent complementary strands on all the templates. After a wash step, which removes all free nucleotides, the incorporated nucleotides are imaged and their positions are recorded. The fluorescent group is removed in a highly efficient cleavage process, leaving behind the incorporated nucleotide. The process continues through each of the other three bases. Using Helicos DNA bar coding protocol, scientists at Helicos were able to multiply the system's sample throughout five-fold (from 50-250 samples per run), without compromising accuracy or representational bias (Blow, 2008). DNA sequencing data from next generation platforms typically present shorter read lengths, higher coverage and different error profiles compared with senger sequencing data. A good review on these recent software tools has been published by Miller et al. (Miller and Sutton, 2010).

Microsatellites or Simple Sequence Repeats (SSRs)

Microsatellites are polymorphic loci present in DNA that consist of repeating units of one to six base pairs in length (Bidichandani et. al., 1998) or they are segment of DNA characterized by a variable number of copies (typically 5-50) of sequence motifs of around two to five (2-5) bases (referred to as a repeat unit) (FAO, 2015). At any one locus (site in a genome), there are

usually several different "alleles" in a population, each allele identifiable by the number of repeat units detected via polymerase chain reaction (PCR). Many microsatellites have a large degree of polymorphism. In many species, they were the first standard marker technology used to characterize diversity. However due to their comparatively infrequent presence across the genome, inconsistent reproducibility across laboratories and genotyping platforms, and higher genotyping cost per locus, microsatellites are being replaced by other technologies. The repeated sequence is often simple, consisting of two, three or four nucleotides ('di-,tri-and tetra-nucleotides repeats) and can be repeated many times. It can be amplified for identification by PCR using the unique sequences of flanking regions as primers. Microsatellites have proved to be versatile molecular markers, particularly for population analysis, but are not without limitations. Microsatellites developed for particular species can often be applied to closely related species, but the percentage of loci that successfully amplify may decrease with increasing genetic distance (Jarne and Lagoda, 1996).

Single-Nucleotide Polymorphism (SNP)

Single-nucleotide polymorphism is a DNA sequence variation that results from a change in the nucleotide at a single location in the genome (FAO, 2015.) In a simpler form it is a DNA sequence occurring when single nucleotide (A,T,G or C) differs among members of a species. SNP is the most abundant marker system both in animal and plant genomes and has recently emerged as the new generation molecular markers for various applications. Being binary or co-dominant status, they are able to efficiently discriminate between homozygous and heterozygous alleles. Moreover, unlike microsatellites, their power comes not from the number of alleles but from the large number of loci that can be assessed (Foster et al., 2010). Most importantly, SNPs are amenable to high throughput automation, allowing rapid and efficient genotyping of large numbers of samples (Tsuchihashi and Dracopoli). SNPs, usually have only two alleles. They may represent either neutral or functional genetic diversity and generally occur throughout the genome. In most species, SNPs occurs on average, one in every 100 to 300 positions in the DNA sequence. For most major livestock species, commercial arrays are available that allow substantial numbers of SNPs (from a few hundreds to over a million) to be genotyped in a single reaction at a low cost per marker. SNP arrays are now routinely used as more informative alternatives to microsatellite panel in genetic diversity studies.

Random Amplified Polymorphic DNA (RAPD)

RAPDS is based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequence (Williams et al., 1990). Those primers detect polymorphisms in the absence of specific nucleotide sequence information and the polymorphisms function as genetic markers and can be used to construct genetic maps. Since most RAPD markers are dominant, it is not possible to distinguish whether the amplified DNA segment is heterozygous (two different copies) or homozygous (two identical copies) at a particular locus. In rare cases, co-dominant RAPD markers observed at different –size DNA segments amplified from the same locus, may be detected (Williams et al., 1990).

The basic technique of RAPD involves

- Extraction of highly pure DNA,
- Addition of single arbitrary primer,
- Polymerase chain reaction (PCR),
- Separation of fragments by gel electrophoresis,
- Visualization of RAPD PCR fragments after ethidium bromide staining under UV light and
- Determination of fragment size comparing with known molecular marker with the help of gel analysis software.

It is important to note that RAPD technique requires maintaining strictly consistent reaction conditions in order to achieve reproducible profiles. In practice, band profiles can be difficult to reproduce between (and even within) laboratories, if personnel, equipment or conditions are changed (Karp et al., 1997). Despite these limitations, the enormous attraction of this technique is that there is no requirement for DNA probes or sequence information for primer design. The procedure involves no blotting or hybridizing steps. The technique is quick, simple and efficient; it requires only the purchase of a thermocycling machine and agarose gel apparatus and relevant chemicals, which are available as commercial kits (e.g Ready-To-Go RAPD analysis beads; G.E. Healthcare Buckinghamshire, UK). Another advantage is the requirement for only small amounts of DNA (10-100ng per reaction) (Karp et al., 1997).

Amplified Fragment Length Polymorphism (AFLP)

The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA (Vos et. al., 1995). The technique involves.

- Extraction of highly purified DNA,
- Restriction endonuclease digestion of DNA (enzyme mixture, usually Eco RI + MseI),
- Ligation of adapters (enzyme adapters),
- Pre-PCR (amplification of the restriction fragments; pre-selective amplification with EcoRI primer + A and MseI primer + C)
- Selective – PCR with labeled primer pair (primer + 3 base pairs; for used labeled, reverse unlabeled), and
- Gel electrophoresis and fragment analysis by automated sequencing machine. The electrophoretograms can be analyzed using programs like GeneMapper (AFLP,2005)

AFLP is applicable to all species and unlike RAPD, this technique is highly reproducible as it combines restriction, digestion and PCR. However AFLP requires more DNA (300-1000ng per reaction) and is more technically demanding than RAPD, however the automation and recent availability of kits means that this technique can be brought to a higher level (Karp et al., 1997).

Markers of Sex-Specific Inheritance

Certain parts of the genome have sex-specific inheritance. Mitochondrial DNA is passed from the mother to the offspring. The Y-chromosome in mammals is inherited from father to son, while the w-chromosome in birds is inherited from mother to daughter. This class of markers can include both SNPs and other sequence variations and has been instrumental in identifying wild ancestors, localizing domestication centres and reconstructing colonization and trading routes (FAO, 2015).

CHALLENGES OF APPLICATION OF MOLECULAR CHARACTERIZATION IN DEVELOPING COUNTRIES

Developing countries are rich in animal genetic resources and housed over 80% of the global domestic animal diversity. However, most of these animal genetic resources are still not characterized and boundaries between distinct populations are unclear. Breeds are defined on the basis of subjective data and information obtained from local communities on historical evidence which may always not be too accurate as they relied on subjective judgments (Rege, 1995). Applying these criteria as the basis for classification for utilization and/or conservation may be misleading. Developing countries have distinct disadvantages for setting up successful breeding programmes. Infrastructure needed for performance testing are lacking, herd sizes are normally small, phenotypic variability between farms are not assessed correctly, production systems and seasons are varied, reproductive efficiencies are low, pasture mating (cumunal grazing) precludes implementation of systematic breeding and animal health management programmes (FAO, 2015). In developing countries the most important resource limitation is lack of funds to set up facilities to support research. Even the existing research facilities, adequate funding for maintainance are not there. Other limitations include inadequacy of scientific equipments to support research and technical manpower. Molecular genetic research is highly sophisticated and also required skilled manpower as well as technologist. Without an established scientific culture, it is almost impossible to engage in and keep abreast of developments in molecular biology research. Other challenges include poor essential utilities like power and water, no support services such as genebank, in vitro storage facilities, storage facilities, animal holding facilities, radiation huddling, disposal facilities and computing facilities and ICT services (Rashid, 2010). In sub-Saharan Africa (excluding the Republic of South Africa), only ILRAD (based in Kenya) is actively involved in biotechnology research, though ILRAD'S biotechnology work is focused on only two (2) diseases, *Trypanosomiasis* and *Theilerosis.* Because the well equipped and adequately funded laboratories doing research in molecular biology are found almost exclusively in developed countries, the gap between the industrialized and developing countries in technical expertise and relevant research capacity is getting wider and motivated scientists from developing countries with the expertise to carry out sophisticated research are opting to work in laboratories in industrialized countries. The importance of molecular characterization of animal genetic resources and its relevance is only slowly being accepted by policy makers in developing countries. In the presence of economic crisis, fiscal constraints, rapid social change and constant political instabilities, the difficulty associated with major

policy changes in developing countries are enormous (Rege, 1995).

Potential and Prospects of Molecular Characterization of Animal Genetic Resources in Developing Nations

According to Jean-Marcel et al. (2010), molecular breeding holds great promise for developing countries. Developing countries with mid-level economies are testing markers applications and taking initial steps towards adoption in their countries. This is a step in the right direction even with limited human resources and inadequate laboratory infrastructure. Through collaboration and virtual platforms aided by the information and communication technology (ICT) revolution, breeders in developing nations now have access to genomic resources, advanced laboratory services and robust analytical and data management tools. These developments are bound to have impact on both animal and crop improvement in developing countries. Also genetic uniqueness of populations of livestock breeds that were classified as ecotypes are now been measured using molecular characterization techniques to determine the relative genetic distances between such populations. Polymorphisms in gene-products such as enzymes, blood group systems and leukocyte antigens which were traditionally used for measuring genetic distances are being rapidly replace by polymorphism at the level of DNA, both nuclear (Jeffreys and Mortan, 1987) and mitochondrial (Loftus et al., 1994) as a source of information for the estimation of genetic distances. This had not only provided information on breed diversity, but also provided a window for genetic improvement of indigenous breeds in developing nations.

Molecular characterization of animal genetic resource is offering unprecedented opportunities for increasing agricultural productivity and for protecting the environment through reduce used of chemicals for fumigation and control of external parasites. The major thrust in molecular research is currently directed at solving immediate problems of industrialized countries, with major investment coming from transnational companies. However, many of the new discoveries and products will find their biggest markets in developing countries where the potential for improvement in agricultural productivity and health are greatest. Developing countries are faced with the challenge to rapidly increase agricultural productivity to help feed their growing population without depleting the natural resource base. The importance of indigenous livestock breeds lies in their adaptation to local biotic and abiotic stresses and to traditional husbandry systems. Marker identification will help to enhance selection of superior genetypes for breeding to improve important traits as tolerance to diseases and resistance to environmental stresses. Identification of carrier genes and their propagation and introduction into the populations will improve resistance against diseases (Gogolin-Ewens et al., 1990).

Molecular markers are indispensable tools for measuring the diversity of animal species. Low assay cost, affordable hardware, throughput, convenience and ease of assay development and automation are important tools that developing nations could employ to fast track livestock improvement for yheir fast growing populations (Rafalski and Tingey, 1993; Rafalski, 2002). Databases based on a large number of potential characters are readily available for inferring relationships using sequence data. Further advantage of sequencing includes substitutions within structural genes that produce differentiation from changes in morphology (Wilson, 1985). Information from the sequences themselves can be useful for specifying parameters of the model of sequence evolution, which in turn, influences the topology of the inferred tree. To date, next generation sequencing technologies have been applied in a variety of contexts, including whole-genome sequencing, targeted resequencing, discovery of transcription factor binding sites and noncoding RNA expression profiling (Morozova and Marra, 2008).

Conclusion

Improvements in sequencing and genotyping technologies have already provided standards that can be used as reference for further genotyping and sequencing studies. Developing countries can take advantage of these and other sequencing efforts currently underway in laboratories around the world. Developing nations must take full advantage of the opportunities that advances in genomics have created to characterize livestock genetic resources within their domain for effective utilization. Enviromental impact detectates animal genetic resource efficiency and utilization, hence developing nations must key into advances in genomics and develop methods for integrating molecular information into breeding programmes and conservation

techniques peculiar to different environmental, agricultural and socio-economics circumstances in their Nations.

RECOMMENDATION

It is recommended that developing nations should key into advances in genomics to facilitate the identification and characterisation of their livestock ecotypes for effective selection reflecting local adaptation to diseases and other environmental conditions.

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