Open Access Library Journal

Breed Characterization: Tools and Their Applications

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Received 18 March 2015; accepted 2 April 2015; published 8 April 2015

Abstract

During the subsequent history of livestock, the main evolutionary forces of mutation, selective breeding, adaptation, isolation and genetic drift have created an enormous diversity of local populations. Organizing and documentation of basic tools for breed characterization are the major aims of the review with the scope of available markers. This farm animal genetic diversity has a primary requirement to meet current production needs in various environments. In addition, farm animal genetic diversity has a great application of allowing sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objectives. Furthermore, animal genetic diversity provides wider range opportunity for selection and improving. The nondescript breed could be Identified and characterizing by morphological or/and molecular markers to know their potential, to know their special adaptive trait and their status for further actions (improvement, conservation). Markers are conspicuous object used to distinguish or to show variation in population or individual level. Morphological markers normally refer to external animal characteristics which can be obtained by direct visual observation and measurement and used in the identification, classification, and characterization of genetic evolution of different species or populations. Since the measurement and identification of animal morphological traits usually take a long time and limited application in evaluation of qualitative traits, molecular markers have developed quickly, and they are becoming more and more informed. Whatever data type (morphological and molecular data) needs appropriate statistical application. In general, diversity, markers and statistical application are the preliminary tools of breed characterization and breed improvements.

Keywords

Animal Breeding, Animal Genetic Resource Diversity, Markers, Statistics

Subject Areas: **Animal Behavior, Genetics**

How to cite this paper: Hailu, A. and Getu, A. (2015) Breed Characterization: Tools and Their Applications. *Open Access Library Journal*, **2**: e1438. http://dx.doi.org/10.4236/oalib.1101438

1. Introduction

Each living organisms have developed specific genetic characteristics according to its particular natural ecosystem, environmental, and socioeconomic conditions. Collectively, these characteristics constitute the Earth's species diversity. This genetic diversity is used as a tool for breed improvement. However, sufficient markers for evaluating the population structure and other aspects of available animal genetic resources are necessary to assess genetic diversity. In earlier studies, morphological markers and agro-ecological factors were used to represent diversity, and after that, chromosomal karyotyping and biochemical markers were developed. With the rapid development of molecular biology, different types of DNA molecular markers had been explored, 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.

With molecular biology and computer innovations, novel strategies such as whole genome SNP chips and DNA Barcoding have been extensively utilized. All of these markers have played significant roles in the evaluation of genetic diversity in farm animals. DNA molecular marker techniques are widely applied in the fields of germplasm identification, phylogeny, and genetic structural analysis. They overcome the limitations of morphological, cytological, and biochemical markers, namely the small numbers of such markers and the fact they can be environmentally influenced. The expansion in DNA information will facilitate the study of genome-wide diversity; such information is much more precise for the assessment of genetic diversity than previous markers.

Raw data from morphological or molecular markers are important inputs for analysis of different parameters, for identification, characterization and evaluation of breeds/individuals. All data types are meaningful if appropriate statistical analysis is employed. Even though numerous reviews have been done in markers, most of them didn't include application of farm animal genetic resource diversity, morphological markers and statistics. Therefore, the present review is initiated with the objective of reviewing tools of (like diversity, markers and statistics) breed characterization comprehensively and their application in animal breeding

2. Breed Characterization: Tools and Their Application

The term "breed" is difficult to define, but most often understood as "a group of domestic livestock with definable and identifiable external characteristics that distinguish it from other groups within the same species" [1]. Similarly, either a sub specific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other or it could be explained as, defined groups within the same species or a group for which geographical and/or cultural separation from phenotypically similar groups has led to acceptance of its separate identity [2]. Even though, different components should be considered for breed characterization, diversity, markers and statistics were the most notable tools for breed characterization as well improvement.

3. Animal Genetic Resource Diversity

Domestication of livestock species and a long history of migrations, selection and adaptation have created an enormous variety of breeds. During the subsequent history of livestock, the main evolutionary forces of mutation, selective breeding, adaptation, isolation and genetic drift have created an enormous diversity of local populations. In the last centuries, this culminates in the formation of many well-defined breeds used for a variety of purposes with different levels of performance in different production environment [3]. Genetic diversity maintained within a species will thus be correspondingly high when production conditions are diversified and can be correspondingly low if production conditions are highly standardized.

In addition to its requirement to meet current production needs in various environments, farm animal genetic diversity has a great pleasure of allowing sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objectives [4]. Therefore, farm animal diversity has opportunities for active utilization of a wide range of breeds, selection and improvement of their products.

Diversity in animal genetic resource (AnGR) populations is measured in three forms: interpopulation diversity (between breeds), intrapopulation diversity (within breeds), and the interrelationships between populations. Phenotypic characterization is used to identify and document diversity within and between distinct breeds based on their observable attributes (morphological markers). The measurement of genetic relationships between breeds

and genetic heterozygosity within breeds is the task of molecular characterization (by using molecular markers) [5].

4. Markers

Markers are some conspicuous object used to distinguish or mark something. Variations in livestock observed at different levels, (*i.e*. at the morphological, cytology, biochemical, chromosomal or DNA level), and all these can serve as the markers for characterization, identification and evaluation farm animal diversity.

5. Morphological Markers

Assessing the diversity of AnGR is made more difficult by the existence of many animal populations that are not assigned to any recognized breed. Even though parts of these "nondescript" populations are known to be multiple crosses of existing breeds, some animals may belong to (relatively) homogenous groups distinguishable from neighboring populations on the basis of identity and stable phenotypic characteristics (among which may be unique and valuable attributes) that warrant their being distinguished as separate breeds. Determining whether or not is one of the roles of phenotypic characterization. Phenotypic characterization is a prerequisite for effective assessment of AnGR diversity and determining whether or not it is being eroded. Phenotypic characterization is therefore fundamental to the establishment of national inventories of AnGR, to effective monitoring of AnGR populations and to the establishment of early-warning and response systems for AnGR [5]. Since morphological markers are easily visible and comparable within the population, they are the primary factors of phenotypic characterization.

The external animal characteristics (*i.e*. coat color, body shape, skin structure, and anatomical characteristics) which can be obtained by direct visual observation and measurement are referred as morphological markers [6] [7]. They are used in the identification, classification, and characterization of genetic evolution of different species or populations especially in developing countries. According to Zewdu [8] characterize and produce a phylogenetic tree of indigenous cattle breeds of northeastern Ethiopia based on their phenotype character.

Even though the evaluation of farm animal genetic resources through morphological markers is based on subjective judgments and descriptions, and the conclusions reached are often not accurate. Furthermore, the measurement and identification of animal morphological traits usually take a long time and limited application in evaluation of qualitative traits, it is still an effective method for the assessment of phenotypic differences [9].

6. Cytological and Biochemical Markers

Cytological markers (like: chromosome karyotypes, bandings, repeats, deletions, translocations, and inversions) have been used for the assessment of farm animal genetic resources [10] [11]. Researchers can trace the origins and evolutionary history of livestock [12], and assess the genetic diversity of domesticated animals by comparing the chromosome number and structure between domesticated animals and their wild ancestors [13].

Furthermore, biochemical markers (e.g. Blood type and isozymes) represent biochemical traits. Though, neither proteins nor isozymes are genetic material, but they are products of gene expression. Amino acid composition of isozymes and soluble proteins were drawn on in investigated the genetic variation within species and phylogenetic relationships between species [14]. The application of these markers was limited because of their vulnerability to environmental impacts and individual growth [15]. Contrariwise, protein electrophoresis is a rapid, economic, and straightforward technique and provides a more detailed representation of polymorphisms than morphological or cytological markers; thus, it is still widely used in elucidating the origin and classification of species [16].

7. Molecular Markers

Molecular markers can be used to scrutinizing genetic variations at the DNA level between poles apart populations and individuals. Molecular markers have developed rapidly, and they are becoming more and more informative. Up to now, numerous types of molecular markers (such as RFLPs, RAPD, AFLP, microsatellite, and SNP) have been exploited to appraise DNA polymorphisms; and widely employed in characterizing animal genetic resources.

8. RFLP Markers

RFLP is a method proven by Grodzicker *et al*. in 1974. It is used to detect DNA polymorphisms among different individuals. Based nucleotide base substitutions, insertions, deletions, duplications, and inversions within the whole genome can eliminate or fashion new restriction sites. The basic principle of RFLP is: genomic DNA of different individuals is processed by known restriction enzymes into DNA fragments of varying size. Then, the digested fragments are separated via electrophoretic analysis. Finally, separated fragments are hybridized with radioactive or chemiluminescent homologous probes and processed by an X-ray film; the different fragments are visible by autoradiography. RFLP was the most widely used markers in AnGR assessments and breeding program development. Even though RFLP is labor-intensive and time-consuming; check out only specific mutations at enzyme cut sites; and polymorphism is relatively low and must be detected by radioisotope, it has the following main advantages:1) high reliability, because it is generated from specific sites via known restriction enzymes and the results are constant over time and location. 2) Co-dominance, which means investigators are able to distinguish heterozygotes from homozygotes. 3) Selective neutrality refers to a situation in which different alleles of a certain gene confer equal fitness.

9. RAPD Markers

RAPD amplifies the target genomic DNA with short and arbitrary primers (commonly 10 bp) in a PCR reaction. It can be used to yield relatively convoluted DNA profiles for detecting augmented fragment length polymorphisms between organisms. Since the arbitrary primers match different parts of the genomic DNA, PCR products will differ in number and size (polymorphism). RAPD-PCR fingerprints have been successfully used in defining genetic diversity among different species. For example, the RAPD method was used to generate specific fingerprint patterns of ten different species: including wild boar, pig, horse, buffalo, beef, venison, dog, cat, rabbit, and kangaroo [17]. The main advantage of RAPD markers is: 1) no prior sequence knowledge is necessary for designing the specific primers, which can then be used in different templates. 2) The amount of DNA required is very small because it will be amplified by PCR. 3) RAPDs are simple, quick, and cost effective compared to RFLP [18] [19]. However, RAPDs also have some disadvantages, these include 1) the repeatability and reliability of RAPD polymorphic profiles are poor [20]. 2) Some non-specific and therefore non-reproducible binding of primers occurs. 3) RAPDs are dominant genetic markers which cannot be used to distinguish homozygote from heterozygote genotypes in F2 populations.

10. AFLP Markers

The AFLP procedure is as follows: first, the genomic DNA is digested with a restriction enzyme, and then the digested fragments are ligated to synthetic adaptors and amplified with specified primers that are harmonizing to a selective sequence on the adaptors. Subsequent separation of the amplified fragments is obtained by selective primers and visualized using autoradiography [21]. The main advantages of this market are: not labor-intensive, doesn't consume more time RFLP method and solve the reliability problem caused by non-specific amplifications in RAPDs. Hoda *et al*. used AFLPs to assess genetic diversity and relationships among different breeds of sheep. They analyzed 93 unrelated individuals from three local Albanian sheep breeds markers. The results obtained indicate the high diversity in Albania sheep breeds. AFLPs are notable for their genetic stability, they provide an effective, rapid, and economical tool for detecting a large number of polymorphic genetic markers, that can be genotyped automatic [22] [23]. However, AFLPs are dominant bi-allelic markers [22], and are unable to distinguish dominant homozygous from dominant heterozygous individuals [24]. The AFLP method is an ideal molecular approach for population genetics and genome typing, it is consequently widely applied to detect genetic polymorphisms, evaluate, and characterize animal genetic resources [25] [26].

11. Microsatellite Markers

Microsatellite DNA, also known as short tandem repeats (STRs) or simple sequence repeats (SSRs), are common repeated sequence of living organizms. The contiguous regions of repeated sequences at microsatellite loci are mostly conservative. The recurrence motifs are highly variable between dissimilar species and even different individuals of the same species. Generally they consist of motifs which are made up of 1 - 6 base pairs (bp) tandemly repeated several times (e.g. CACACACACACACACA) [27] [28]. So specific primers could be designed

based on the conserved sequences and amplify the core repeat sequences by PCR. Genetic polymorphisms can then be detected via electrophoresis [29]. Microsatellites were the markers most widely used for genetic diversity, mapping quantitative trait loci of production and functional traits in livestock [30]-[32]. Furthermore, they have also been used for marker assisted selection practices [33]. SSRs don't use radioisotopes; it has higher repeatability and stability than RAPDs; they are co-dominant markers (able to distinguish homozygotes from heterozygotes). The disadvantages of SSRs: time-consuming and expensive to develop, heterozygotes may be misclassified as homozygotes when the null-alleles occur because of mutations in the primer annealing sites, stutter bands may complicate accurate scoring of polymorphisms, underlying mutation model largely unknown, and do not provide information on functional trait biodiversity.

12. SNP Markers and Whole-Genome Sequencing

Single nucleotide polymorphism (SNP), was first discovered by Lander in 1996. It refers to a sequence polymorphism caused by a single nucleotide mutation (single base transitions, transversions, insertions and deletions) at a specific locus in the DNA sequence [34]. The fundamental principle of SNPs is to hybridize detected DNA fragments with high-density DNA probe arrays. Then, the SNP allele is named according to the hybridization results. SNPs are bi-allelic markers, indicating a specific polymorphism in only two alleles of a population [35]. Currently, SNP markers are one of the preferred genotyping approaches, because they are plentiful in the genome, genetically steady, and amenable to high through put automated analysis [36] and distribute in both coding and non-coding regions of genomes [37]. The SNP has the following advantages: 1) they are numerous and widely distributed throughout the entire genome [38]. 2) High genetic stability, excellent repeatability, and high accuracy. 3) Allow for fast, high-throughput genotyping [39]. 4) Expedient for effectively distinguishing heterozygote from homozygote alleles because of its co-dominances. One disadvantage of SNP markers is the low level information obtained compared with that of a highly polymorphic microsatellite, but this can be waged by employing a higher density markers (HD SNP chips) and whole-genome sequencing [40] [41]. Because of their extensive distribution and abundant variations, SNPs plays an important role in livestock population structure, genetic variation, 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. For example, linkage disequilibrium (LD) among different SNPs can be utilized in connotation analysis

With the improvement of sequencing technology, whole-genome/gene sequencing has become available for characterizing genetic diversity among farm animals. It is the most straightforward 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 [9].

13. DNA Barcoding Markers

Barcoding is an automatic scanning and identification technology, which has emerged from practical computer technologies. A DNA barcode is a Short DNA sequence from a standardized region of the genome used for identifying species. The intent of DNA barcoding is to use large-scales creening of one or more reference genes in order to 1) assign unknown individuals to species, and 2) enhance discovery of new species [42] [43]. Researchers can compilea public library of DNA barcodes linked to named specimens, which can provide a new master key for identifying species diversity [44]. Compared with time-consuming and inefficient traditional morphological classification [42], DNA Barcoding has a high accuracy of 97.9% [45]. In addition, it provides new, quick, and convenient identification strategies foranimal genetic diversity [42]. However, as with the other markers a formantioned the DNA Barcoding technique also has some disadvantages: 1) the genome fragments are very difficult to obtain and are relatively conservative and have no enough variations. Some organisms cannot be identified with COI because of the low evolution rates of COI sequences in any species. 2) COI is an mtDNA sequence of maternal origin, which could bias species diversity [46] [47]. The above disadvantages can be compensated for by using one or more nucleargene barcodes together to make a standardized analysis of AnGR [9]. Biological taxonomists apply this principle to species classification, referring to a DNA barcode [9]. Tautz *et al*. were the first researchers to use the DNA sequences in systematical biological taxonomy (also called DNA taxonomy). Subsequently, Hebert *et al*. proposed the concept of DNA Barcoding and suggested its use for

a single mtDNA gene, mitochondrial cytochrome c oxidase I (COI), as a common sequence in animal DNA Barcoding studies.

14. Statistics

Genetic improvement programs for livestock aim to maximize the rate of increase of some merit function expected to have a genetic basis. Merit can be a linear or nonlinear combination of genetic values for several traits that are economically important. Genetic merit cannot be observed, so it must be inferred from the data. Therefore, Relevant statistical application has been conducted for: 1) assessing whether traits have a genetic basis, 2) developing accurate methods for inferring merit ("genetic evaluation"), and 3) designing breeding programs [48]. Morphological as well molecular data's were important inputs for evaluation of a particular breed. In case of characterization, identification and evaluation, the particular breeds might fall under previously characterized breed or stand as separate breed based on statistical analysis.

Accurate prediction of breeding values is of great importance for animal improvement programs. The prediction of breeding values requires knowledge of the magnitude of the variances and covariances of random effects [49]. The statistical models in animal breeding consist of: 1) a mathematical function relating observations to location parameters and random effects (Bayesians view all unknowns as random), 2) genetic and environmental dispersion parameters, such as components of variance and covariance; and 3) assumptions about the joint distribution of the observations and of the random effects [48]. Therefore, statistics are the basic tool in animal breeding, including: 1) sample size determination, 2) suiting data for analysis (data transformation), 3) to calculate the descriptive statistics, ANOVA, variance, covariance, 4) to cluster breeds into appropriate category, 5) to evaluate their breeding value, heritability and repeatability, and 6) for selection (index calculation) of the best performed individual/ group.

15. Conclusions and Recommendations

The main evolutionary forces of mutation, selective breeding, adaptation, isolation and genetic drift have created an enormous diversity of local populations. This Genetics resource diversity is wider in diversified production environments. The primary importance of this diversified genetics resource is meeting production needs in various environments. Further is has an impact breed improvement program (selection and mating).

The diversified genetics resource should be characterized for the sake of breed status, production value, effective monitoring, and to establish early warning and response for AnGR. Different criteria should be considered for characterization of AnGR. Of that morphological and molecular markers are the fundamental distinguishing criteria's. Any external animal characteristics which can be obtained by direct visual observation and measurement (*i.e*. Coat color, body shape, skin structure, and anatomical characteristics) are morphological markers.

However, since the evaluation of farm animal genetic resources through morphological markers is based on subjective judgments and descriptions, and the conclusions reached are often not accurate. Therefore, molecular markers were invented and they are becoming more and more informative. Molecular markers can be used for investigating genetic variations at the DNA level between different populations and individuals. RFLPs, RAPD, AFLP, microsatellite, and SNP are molecular markers widely utilized in characterizing animal genetic resources. Of these microsatellites DNA markers are widely utilized for Animal genetics identification and characterization.

All Animal genetics resource data's collected via morphological markers or molecular markers would be invalid if an appropriate statistical analysis is employed. Therefore, since a statistical application has great impact on our data, all data types should pass appropriate statistical analysis. Generally, diversity, markers and statistical application are important tools of breed characterization as well animal breeding.

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