

FAO ANIMAL PRODUCTION AND HEALTH



guidelines

MOLECULAR GENETIC CHARACTERIZATION OF ANIMAL GENETIC RESOURCES

COMMISSION ON
GENETIC RESOURCES
FOR FOOD AND
AGRICULTURE



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Recommended Citation

FAO. 2011. *Molecular genetic characterization of animal genetic resources*. FAO Animal Production and Health Guidelines. No. 9. Rome.

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ISBN 978-92-5-107032-1

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Foreword

Animal genetic resources for food and agriculture (AnGR) are an essential component of the biological basis for world food security. Hundreds of millions of poor rural people keep livestock and often rely on their animals to provide multiple products and services. In harsh environments where crops will not flourish, livestock keeping is often the main or only livelihood option available. Livestock currently contribute about 30 percent of agricultural gross domestic product in developing countries, with a projected increase to about 40 percent by 2030. The World Bank has estimated that it will be necessary to increase meat production by about 80 percent between 2000 and 2030. This will require more efficient animal production systems, careful husbandry of natural resources and measures to reduce waste and environmental pollution.

The *State of the World's Animal Genetic Resources for Food and Agriculture* provided for the first time a comprehensive country-driven global assessment of the roles, values and status of AnGR. It clearly shows that the diversity of AnGR is continually decreasing and that potential of the remaining diversity for increasing food security and improving livelihoods is not being fully realized. As a global response to these shortcomings, the member countries of FAO developed the *Global Plan of Action for Animal Genetic Resources (Global Plan of Action)*, which was adopted by the International Technical Conference on Animal Genetic Resources for Food and Agriculture held in Interlaken, Switzerland, in September 2007, and subsequently endorsed by all FAO member countries.

The *Global Plan of Action* contains four Strategic Priority Areas, which provide a basis for enhancing sustainable use, development and conservation of animal genetic resources throughout the world. Its implementation will contribute significantly to achieving Millennium Development Goals 1 (Eradicate extreme poverty and hunger) and 7 (Ensure environmental sustainability). The first of the Strategic Priority Areas is the characterization, inventory and monitoring of trends and associated risks of AnGR. The characterization of AnGR is necessary in order to properly assess the value of breeds and to guide decision making in livestock development and breeding programmes.

The Interlaken Conference called on FAO to continue developing technical guidelines and technical assistance and to continue coordinating training programmes as a means to support countries in their efforts to implement the *Global Plan of Action*. The objective of these guidelines on *Molecular characterization of animal genetic resources* is to help countries to plan and implement effective analyses of the genetic diversity of their AnGR, so the resulting information can contribute to the development of effective plans for sustainable use and conservation of AnGR. They provide countries with advice on how to:

- plan molecular characterization studies;
- collect and evaluate DNA;
- organize and analyse molecular characterization data;
- interpret the results; and
- collaborate internationally to obtain the maximum utility from the information.

The preparation of the guidelines was initiated in accordance with Strategic Priority 2 of the *Global Plan of Action*, which addresses the need to develop international technical standards and protocols for characterization of AnGR. The guidelines were discussed and validated at a series of workshops. In total, more 40 scientists, technicians and policy-makers from all regions had an opportunity to contribute to the process.

The guidelines will be refined and updated periodically as experience with their use in the field is accumulated and as technologies for molecular characterization advance. The assistance of the National Coordinators for the Management of Animal Genetic Resources and their country networks will be particularly important to this process of revision.

Acknowledgements

These guidelines revise and build upon the *Secondary guidelines: measurement of domestic animal diversity (MoDAD)* published by FAO in 1993. They were developed in strict collaboration with the International Society of Animal Genetics (ISAG) – FAO Advisory Group on Animal Genetic Diversity and with GLOBALDIV, “A global view of livestock biodiversity and conservation”, a three year project funded by the European Commission in the framework of the AGRI GEN RES initiative. Paolo Ajmone-Marsan and Hans Lenstra, participants in both of these groups were involved in the planning, writing and review of the guidelines. Other members of the ISAG–FAO Advisory Group and of GLOBALDIV provided critical technical reviews and other contributions, particularly Mike Bruford, Licia Colli, Riccardo Negrini, Ezequiel Luis Nicolazzi, Steffen Weigend and John Williams. The guidelines were presented and evaluated at workshops held in Poland and Austria. They were presented to and endorsed by the Commission on Genetic Resources for Food and Agriculture at its Thirteenth Regular Session in July 2011.

The guidelines were prepared under the supervision of Paul Boettcher, with the full support of the Chief of FAO’s Animal Genetic Resources Branch, Irene Hoffmann, and of present and former Animal Genetic Resources Officers and FAO volunteers: Badi Besbes, Beate Scherf, Dafydd Pilling, Roswitha Baumung, Maria Grazia Merelli and Joanna Marchewka. The publication was laid out by Carmen Hopmans and Claudia Ciarlantini. Administrative and secretarial support was provided by Kafia Fassi-Fihri and Silvia Ripani.

FAO would like to express its thanks to all these groups and individuals.

User Guidance

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In 1993, an FAO working group proposed a global programme for characterization of AnGR, including molecular genetic characterization, and formulated the *Secondary guidelines: measurement of domestic animal diversity (MoDAD)* (FAO, 1993) with recommendations for the molecular analysis of domestic animal diversity via a research programme to be coordinated by FAO.

FAO has revised its original MoDAD guidelines in close consultation with the International Society for Animal Genetics (ISAG), through the ISAG–FAO Advisory Group on Animal Genetic Diversity, as well as scientists contributing to the GLOBALDIV project (www.globaldiv.eu) sponsored by the European Union. In the present guidelines, the role of the FAO has been redefined mainly to involve the provision of technical support to countries and coordinating networking across countries. The technical recommendations have been adapted to account for the continuing progress of insight and advances in genetic technology. The broad objective of the guidelines is to provide guidance on performing molecular characterization studies on AnGR.

The guidelines consist of three sections. The introduction provides a rationale for characterizing AnGR, along with an overview of the accomplishments of the molecular approach since the release of the original MoDAD guidelines, summarizing realized benefits and future prospects. Section 2 is aimed particularly at scientists who are planning a molecular characterization study. It starts by describing the strategic choices to be made in the planning phase and continues with specific recommendations and technical pitfalls of sampling, genotyping and data analysis. Legal aspects of ownership and transfer of AnGR are discussed, as well as the translation of scientific results into genetic management. Emphasis is given to standardization of data and their integration into international analyses. Section 3 summarizes the recommendations of the FAO and the ISAG–FAO Advisory Group on Animal Genetic Diversity. Appendices provide a glossary of technical terms; examples of questionnaires for gathering information about collection sites, animals and breeds during the process of sample collection; an example of a simple material transfer agreement; a summary of software that can be used to analyse molecular data; and the standard ISAG–FAO Advisory Group panels of microsatellite markers for nine common livestock species.

Abbreviations and Acronyms

AFLP	amplified fragment length polymorphism
AMOVA	analysis of molecular variance
AnGR	animal genetics resources (for food and agriculture)
bp	base pair
CNV	copy number variation
DAD-IS	Domestic Animal Diversity Information System
DNA	deoxyribonucleic acid
EDTA	ethylenediamine tetra-acetic acid
F_{IS}	within-breed fixation index
F_{ST}	across-breed fixation index
GPS	global positioning system
EAAP	European Federation of Animal Science
HW	Hardy-Weinberg equilibrium
IAEA	International Atomic Energy Agency
ICARDA	International Center for Agricultural Research in the Dry Areas
ILRI	International Livestock Research Institute
ISAG	International Society for Animal Genetics
LD	linkage disequilibrium
MAF	minor allele frequency
MHC	major histocompatibility complex
MoDAD	measurement of domestic animal diversity
MRCA	most recent common ancestor
MTA	material transfer agreement
mtDNA	mitochondrial DNA
N_e	effective population size
Nei's GST	Nei's coefficient of gene differentiation
PCR	polymerase chain reaction
RAPD	random amplification of polymorphic DNA
SNP	single nucleotide polymorphism

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SECTION 1

Introduction



Introduction

RATIONALE FOR CHARACTERIZATION OF ANIMAL GENETIC RESOURCES

Domestic animal diversity is an important component of global biodiversity. About 40 species of domestic animals and poultry contribute to meeting the needs of humankind, providing meat, fibre, milk, eggs, draught animal power, skins, and manure, and are an essential component of many mixed farming systems. Within these species, more than 8 000 breeds and strains (FAO, 2011a) constitute the animal genetic resources (AnGR) that are of crucial significance for food and agriculture.

The present pattern of diversity of AnGR is the result of a long and complicated history, particularly the process of domestication, which took place 10 000 to 3 000 years ago, depending upon the species. Since then, domestic livestock have spread with human migrations and trading to all inhabited continents. Local adaptation, artificial selection, mutations and genetic drift turned the genetic diversity captured with domestication into a vast array of differences in appearance, physiology and agricultural traits. During recent centuries this differentiation was accentuated by the emergence of breeds – more or less isolated populations that were subject to systematic selection. This development, and the degree of genetic isolation, was most pronounced in the temperate zones where the demands of food supply led to a rationalization of agriculture. The last 50 years saw the global spread of a few highly developed breeds, such as the high-yielding Holstein-Friesian breed of dairy cattle.

The global diffusion of these specialized breeds, which mostly originated in Europe, is endangering or even risking the extinction of many well-adapted local breeds. This trend is particularly evident in marginal areas (Rege and Gibson, 2003) where local husbandry practices are also being abandoned (Köhler-Rollefson *et al.*, 2009). As a consequence, erosion of diversity of AnGR has become a major concern (Hammond and Leitch, 1996; Hodges, 2006; FAO 2007a). The negative consequences of genetic erosion and inbreeding depression have been amply documented and may be manifested by loss of viability, fertility and disease resistance, and the frequent occurrence of recessive genetic diseases (Keller and Waller, 2002; FAO 2007b; Taberlet *et al.*, 2008). According to the report on the *Status and trends of animal genetic resources – 2010* (FAO, 2011a), approximately 8 percent of reported livestock breeds have become extinct and an additional 21 percent are considered to be at risk of extinction. Moreover, the situation is presently unknown for 35 percent of breeds, most of which are reared in developing countries.

FAO has a history of working with its member countries in order to improve the productivity of livestock and the livelihoods their citizens while maintaining AnGR diversity (FAO, 1990a,b,c). Specific priorities for AnGR management are set out in the *Global Plan of Action for Animal Genetic Resources (Global Plan of Action)* (FAO, 2007a), which was adopted at the International Technical Conference on Animal Genetic Resources for Food



and Agriculture held in Interlaken, Switzerland, in September 2007, and subsequently endorsed by all FAO member countries at the 2007 FAO Conference.

One of the Strategic Priority Areas of the *Global Plan of Action* is the characterization, inventory and monitoring of trends in AnGR diversity in order to properly assess the value of breeds and to guide decision making in livestock development and breeding programmes.

The breed is the operational unit in the conservation of genetic resources. However, the use of the breed concept to describe groups of animals having particular genetic characteristics varies with geographical area and species. Most breeds originating from industrialized countries are well-defined and phenotypically distinct and were usually genetically isolated throughout the course of their development. In contrast, Asian and African breeds most often correspond to local populations that differ only gradually according to geographical separation. In addition, breeds with different names may sometimes have a recent common origin, while in other cases their uniqueness has been eroded by cross-breeding.

In the absence of information about the genetic attributes of each breed available for a breeding programme, development of local breeds is often ignored in favour of the introduction of germplasm from exotic breeds, about which more information is generally available. Therefore, characterization of breeds both at the level of animal phenotypes and their interaction with production systems and at the genetic level is most essential.

MOLECULAR CHARACTERIZATION – HISTORY AND PROSPECTS

Since the beginning of the 1990s, molecular data have become more and more relevant for the characterization of genetic diversity (Groeneveld *et al.*, 2010). In 1993, an FAO working group proposed a global programme for characterization of AnGR, including molecular genetic characterization, and formulated the *Secondary guidelines: measurement of domestic animal diversity (MoDAD)* (FAO, 1993) with recommendations for the molecular analysis of domestic animal diversity on a global scale via a research programme to be coordinated by FAO.

Although the MoDAD programme recommended originally by the Working Group was not realized, the FAO MoDAD report succeeded in creating awareness of the need to monitor AnGR diversity and in establishing a standard approach for molecular genetic characterization. In addition, the proposal of the programme helped motivate many nationally funded research projects as well as larger regional and international projects supported by organizations such as the European Commission, the Nordic Council of Ministers, the International Atomic Energy Agency (IAEA), the International Livestock Research Institute (ILRI) and the World Bank, which together achieved many of the original objectives of MoDAD. Scientists in many countries have undertaken independent studies to characterize locally available breeds, while large-scale international efforts on breed characterization have built comprehensive molecular datasets for most livestock species. The study of genetic diversity of livestock at the molecular level has developed into a most active area of research, which for example receives considerable attention in scientific press and at the conferences of organizations such as ISAG and the European Federation of Animal Science (EAAP).



So far, most molecular work has been based on the use of neutral genetic marker data, which serve as a proxy or estimate of the likelihood of important functional genetic variation within breeds or breed groups. This work has:

- identified the wild ancestral species of most livestock species and localized the site(s) of domestication;
- provided insight into breed formation and breed uniformity;
- assessed the genetic constitution of breeds via quantitative measures of diversity, admixture or subdivision, inbreeding, introgression and assortative mating;
- partially reconstructed the phylogenetic relationships of populations, unravelling the evolutionary history of species and populations;
- investigated algorithms that can be used to prioritize breeds for conservation using molecular data (this is directly relevant for programmes of conservation, restocking or utilization of AnGR);
- introduced these data into the permanent scientific record and provided the opportunity to publicize the issues surrounding loss of livestock diversity in the international press, the interest of which may be attracted by particularly well-done and intriguing studies; and
- established an informal international network of organizations and institutions interested in molecular studies of AnGR.

Despite these accomplishments, the objective of comprehensively characterizing livestock diversity as a basis for guiding the genetic management of AnGR has not been achieved and will require substantial investment in time and financial resources. Future characterization programmes need to be designed so as to yield the most valuable information with maximum efficiency. More specifically, as a means to overcome the limitations of the data collected to date, molecular characterization of AnGR should target, in the near to medium term, the following objectives:

- obtaining a global view of diversity by integrating national or regional datasets – too many projects have been undertaken independently without coordination with other studies; this has very often led to the use of private panels of genetic markers, which precludes the joining of datasets and seriously decreases the impact of the studies;
- addressing the “white spots” on current phylogeographic maps – most studies have focused on the standardized breeds used in industrialized countries, with relatively less attention given to many local breeds, most notably African and Asian livestock, which has remained largely free of systematic selection and harbours much of the original diversity;
- identifying genomic regions involved in functional diversity: adaptation, disease resistance or productive traits; and
- determining how molecular data may best contribute to the optimal management of AnGR. By discovering unique molecular variants or evidence for independent development, or conversely by identifying breeds that are nearly identical genetically, molecular studies may indicate the relative value of different breeds for conservation.



However, there is no wide consensus on how to use molecular data for selection of AnGR for conservation. This shortcoming is in part because previous characterization studies have mainly considered neutral variation, which represents only a minor and non-functional fraction of the differences between breeds and individuals.

Because of recent technological developments, accomplishing these objectives no longer relies exclusively on microsatellite genotyping, which after 1990 revolutionized the science of molecular genetics. Dense marker panels of single nucleotide polymorphisms (SNP) are becoming available for most livestock species. This technology will require a new range of methods of analysis for inferring population structure and relationships through approaches such as network construction, model-based clustering, coalescent theory, population genomics, and identification of "selection signatures" or regions of the genome subject to selection. In addition, whole-genome sequencing has been completed or is under way for most major livestock species.

The new tools are likely to substitute microsatellites in many applications. The costs and benefits of re-examining characterized breeds must be considered. Models for linking existing information on genetic diversity, largely based on microsatellite markers, to new information generated with SNP markers may be needed.

In anticipation of the advent of low-cost individual genome sequencing it becomes possible to think in terms of DNA variation as a more basic unit of conservation. This variation may have different modes of distribution, ranging from groups of breeds with special phenotypes or native locations within specific geographic or agroclimatic areas, to specific breeds and individual animals and their offspring.



SECTION 2

How to carry out molecular diversity studies

