

# PAST AND FUTURE ACTIVITIES TO HARMONIZE FARM ANIMAL BIODIVERSITY STUDIES ON A GLOBAL SCALE

ACTIVIDADES PASADAS Y FUTURAS PARA ARMONIZAR LOS ESTUDIOS SOBRE LA BIODIVERSIDAD DE LOS ANIMALES DE GRANJA EN UNA ESCALA GLOBAL

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## PALABRAS CLAVE ADICIONALES

MoDAD. Recomendaciones. Serie de marcadores. Meta-análisis.

## SUMMARY

Triggered by the Convention on Biological Diversity, activities were started to establish the genetic relationships of farm animal breeds. Under the co-ordination of FAO, an initiative called MoDAD (Maintenance of Domestic Animal Diversity) was started to provide technical recommendations for biodiversity studies in farm animals, in order to ensure compatibility of different studies in the same species. After almost ten years of experience, it is noticed that these recommendations only partly have been adopted by the scientific community. Based on this experience, a new working group will review the actual recommendations. In this context, it will be essential to decide whether microsatellites are still the marker system of choice, if multiplexing and analysis of pooled DNA have to be considered, and if Y-specific and mitochondrial markers have to be included to study paternal and maternal ancestries. Given that in some species major studies with only partly overlapping marker sets are available, methods to conduct a joint analysis of such data sets need to be derived. Methodological concepts are presented which could form the basis for such a meta-analysis.

## RESUMEN

Estimulados por la Convención sobre la Diversidad Biológica se comenzaron a realizar actividades para establecer las relaciones genéticas de las razas de animales domésticos. Bajo la coordinación de la FAO empezó una iniciativa llamada MoDAD (Mantenimiento de la Diversidad de los Animales Domésticos) para proveer de recomendaciones técnicas para los estudios sobre la biodiversidad de los animales de granja para asegurar la compatibilidad de los diferentes estudios en las mismas especies. Después de casi diez años de experiencia, se nota que esas recomendaciones sólo han sido adoptadas parcialmente por la comunidad científica. Basados en esta experiencia, un nuevo grupo de trabajo revisará estas recomendaciones. En este contexto, será esencial decidir si aún los microsatélites son el sistema marcador de elección, si el desarrollo de multiplex y el análisis de mezclas de ADN tienen que ser considerados, y si el marcador Y específico y los marcadores mitocondriales tienen que ser incluidos para estudiar la ascendencia paterna y materna. Están disponibles estudios destacados en algunas especies con series de marcadores parcialmente sobrepu-

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tos, es necesario derivar métodos para conducir un análisis conjunto de tales series de datos. Se presentan conceptos metodológicos los cuales pudieran formar la base para tales meta-análisis.

variability is the best insurance to be able to adapt farm animal populations (through selection and breeding) to future, unpredictable needs.

## INTRODUCTION

The value of biodiversity and the need to maintain it was agreed upon in the Convention on Biological Diversity (CBD) which was agreed at the UN world summit in Rio de Janeiro in 1992. This agreement also includes farm animals, and since then a number of activities have taken place under the co-ordination of the United Nation's Food and Agriculture Organisation (FAO).

You cannot manage and conserve what you do not understand. Therefore, there is an obvious need to make an inventory of available genetic diversity in the major farm animal species. Unlike the situation in non-farm animals, genetic diversity in farm animals is stratified in a within and between breeds component. While it was clear that the breed will be the primary object of conservation, breeds as organisational units (with herdbooks, etc.) are a concept that was developed in the 19<sup>th</sup> century in Europe and North America and as such are not well established in other important parts of the world.

There are a number of good reasons for conserving farm animal diversity. As long as production circumstances and consumer demands are variable, diversity is necessary to fulfil the various requirements. And since environmental conditions, disease challenges and markets always will change, genetic

## PAST ACTIVITIES

In 1993, an FAO working group was established to develop an integrated global programme to establish the genetic relationships among farm animal breeds. This work in general is based on four steps:

- collecting a representative sample of the breeds to describe;
- assessing the genotypes for a predefined set of molecular markers and estimating allele frequencies;
- calculating pair-wise genetic distances between the breeds in one species;
- constructing dendrograms and phylogenetic trees to visualise the genetic structure of the breed.

This initiative, called MoDAD (Maintenance of Domestic Animal Diversity) was chaired by Prof. J.F.S. Barker and launched a report with suggestions on the following topics:

- sampling strategies;
- molecular analysis, with the concept of a *parent lab* for each species, which acts as a reference lab for all groups involved in the respective species;
- recommendations on the statistical methods to use;
- establishing a global data base for the genotypes;
- establishing global DNA banks;
- publication policy
- species should be prioritised according to their importance in

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agriculture and food production, explicitly listed are cattle, sheep, pig, buffalo, goat, horse, and ass.

While a number of national or regional (e.g. European) studies were started to assess the diversity within certain farm animal species, it was obvious that general rules must be defined to allow the combination of data and results of these studies to get a global view of the diversity within a given species. For this, it is of primary importance to harmonise sampling strategies, to use a common set of markers, to make lab results comparable, and to provide data and results in a defined format.

To define species-specific standards, the International Society for Animal Genetics (ISAG) formed in 1995 an advisory group on animal genetic diversity, which was chaired by Prof. R. Fries. This ISAG/FAO advisory group set up recommended lists of microsatellite markers (about 30 per species) for cattle, chicken, sheep and swine. The choice of marker sets was based on the following criteria:

- markers should be in the public domain;
- markers should have known map locations and should not be linked;
- markers should have more than four alleles and a polymorphism information content (PIC) of at least 60 per cent;
- markers should be usable across species;
- they should have easy to read PCR products.

Experience has shown, that the ISAG/FAO marker lists were only partly accepted in practical diversity studies. Possible reasons for non-

compliance were:

- the ISAG/FAO marker list was not known or the respective primers were not available;
- groups tried to establish an overlap with different marker lists (e.g. for parentage control);
- smaller marker sets had to be used due to financial limitations;
- recommended markers were not polymorphic enough or did not work individually or in multiplexes;
- researchers preferred to use their own marker sets.

In general, the number of larger studies on genetic diversity in a number of farm animal species is encouraging. However, it will be critical to establish the technical and organisational framework to allow a joint analysis of these studies. Only then, it will be possible to get a sufficiently accurate picture of the global diversity, which is a precondition for adequate decision making and conservation policies.

### ACTUAL AND FUTURE ACTIVITIES

In 2002, Prof. H. Simianer was elected as the new chairman of the ISAG/FAO advisory group on animal genetic diversity. There are three major activities to be conducted by the newly formed working group.

#### 1. CRITICAL ASSESSMENT OF TEN YEARS OF MODAD ACTIVITIES

For the major farm animal species, it will be essential to get an overview on the number of larger diversity studies. It will be of special importance to verify, to what extent the ISAG/FAO recommendations were adopted

in these studies, and if (and in which form) results and DNA samples are available for eventual meta-analyses. If studies did not follow the ISAG/FAO recommendations, it will be useful to learn about the reasons. It is planned, that an FAO visiting scientist will compile such a survey in the first half of 2003.

## 2. REVISION OF RECOMMENDATIONS

Based on the experience with the available recommendations, it will be necessary to discuss if the actual recommendations still are applicable or if they have to be modified or extended.

The objective of the co-ordinated activity is to allow a global survey of farm animal biodiversity. However, this will be required to answer very different questions, e.g.

- testing, if different breeds are genetically different or identical;
- reconstructing phylogenetic trees;
- assigning breeds to breed groups;
- deriving breed priorities in conservation activities.

With a given finite budget, all these objectives lead to different optimum designs (e.g. with respect to sample size and number of markers per animal). Therefore it is necessary to discuss the primary goals of this global initiative thoroughly, because some of the major strategic decisions depend on the primary goals.

While it certainly will be necessary to review the lists of recommended microsatellite markers, and eventually replace certain markers by other, better suited ones, it also will be important to discuss a number of more general questions, like:

- Are microsatellite markers still the marker system of choice, or should other types of markers, like RAPD or SNP markers, also be considered?.

- Should the choice of the marker set take into account the possibility of multiplexing?.

- Would it be sufficient to estimate allele frequencies from pooled samples, which is known to be less accurate, but also less expensive than individual genotyping?.

- Is it useful to restrict analyses to anonymous markers or should markers linked to known qualitative traits or QTL also be included?.

- Should the distance studies also account for polymorphisms in the mitochondrial sequence or on the Y chromosome to reconstruct maternal and paternal ancestries, respectively?.

It is expected, that the experiences and recommendations will differ between farm animal species. While in some species (as for example pig as illustrated in the PigBioDiv project), the ISAG/FAO recommendations have been well accepted, this is not the case in other species (e.g. sheep). Also, in some species new standards may have been created by large biodiversity studies using a specific set of markers.

The objective of the new recommendations is to reach maximum acceptance in the scientific community and to allow for an optimal use of already available data and results.

## 3. APPROACHES TO JOINT ANALYSES OF DIFFERENT STUDIES

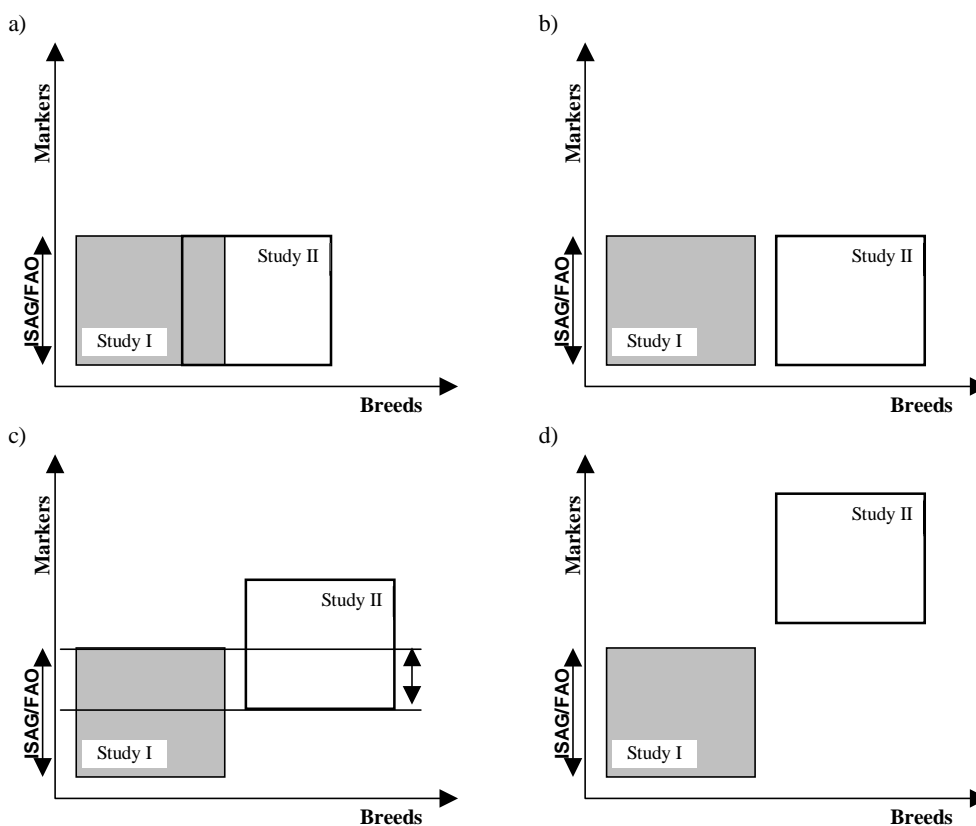
The ideal assumption of the present recommendations was that different studies make use of the same recommended set of markers. If other

requirements are fulfilled (e.g. standardised allele lengths, comparable sample sizes and designs), it is easy to combine results of different studies in a joint analysis, even based on estimated allele frequencies only.

This situation is depicted in **figure 1 a) and b)**. There, the range of breeds included in a study is measured on the X-axis, while the set of markers used is indicated on the Y-axis. In all cases, study I has used the ISAG/FAO marker set. In **figure 1 a) and b)**, a second,

independent study uses the same marker set. Regardless if the range of breeds overlaps, i.e. some breeds are included in both studies (**figure 1a**) or the two sets of breeds are discrete (**figure 1b**), a fully informative joint analysis is possible.

In **figure 1c)**, study II has not used all markers from the ISAG/FAO list, but some additional ones. In a straightforward analysis, only the marker set common to both studies (indicated by the arrow between the



**Figure 1.** Different possible constellations of sets of markers and breeds used in two different diversity studies in the same species. (Diferentes constelaciones posibles de series de marcadores y razas usadas en dos estudios de diversidad en la misma especie).

two lines) is fully informative for estimating distance between breeds of the two studies, respectively.

In **figure 1d**) finally, there is no overlap of the marker sets used, hence it is not possible to estimate a distance between the two sets of breeds.

A general recommendation certainly will be that joint analyses or a meta-analysis of different studies will be the more informative the more markers are in common between the different studies.

Nevertheless, given the situation that in some species large studies have been done using more or less different marker sets, research is needed to find approaches to make optimum use of this information.

There are basically two approaches that can be used to combine different studies using only partly overlapping marker sets:

#### *The missing data approach*

Consider the situation in **figure 1 c**). Assume that markers are numbered, and study I has used markers 1 to 25 and study II has used markers 11 to 35. Then, one could estimate the distance based on the combined set of markers (1 to 35), with observations missing on marker 26 to 35 in study I and on marker 1 to 10 in study II. Dealing with missing observations is a standard problem in statistics and a number of useful concepts, like the EM-algorithm are available.

#### *The probability approach*

Most of the common distance measures depend on the number and specification (e.g. polymorphism) of markers used. That means, that e.g.

Nei's standard distance has a different scale, if markers 1 to 25 or markers 11 to 35 are used. That is also why it is not appropriate to make a direct comparison of distance estimates derived from different marker sets.

Other measures of distance or similarity have been suggested which are scale independent, like the IBD probability, which tries to estimate the probability of two alleles being identical by descent. For this, marker genotypes are only observations, while being IBD or not is the true state of nature, on which inferences are made. Estimating the respective probability can be based on varying numbers of common markers, which, however, should only affect the accuracy of the estimation, but not the scale of the similarity metric, which is the inverse of a distance.

In both cases, the estimation can be improved by genotyping the complete set of markers at least for a subset of breeds or animals within breeds from the studies to combine. However, it is difficult to predict which number of additional genotypings will be necessary and which design will be appropriate to achieve a significant improvement of the situation.

## CONCLUSIONS

Although not being fully accepted and adopted by the scientific community, the ISAG/FAO recommendations have been a useful guideline and have helped to stimulate a number of valuable studies on farm animal diversity. The newly formed group will critically assess the achievements and the shortcomings of the present recom-

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recommendations and will come up with improved concepts in due course. Nevertheless, it will be necessary to discuss steps to make the optimum use of available results and information to get a better understanding of farm

animal diversity on a global scale. For this, a number of methodological developments are necessary, which have to be carried out by individual research projects linked to the activities of the ISAG/FAO advisory group.