***Organised by The BBP &FAIP:***
***The Biotechnology for Biodiversity Industrial Platform***
***and the Farm Animal Industrial Platform***

# Workshop

# on Farm Animal Biodiversity

# *Proceedings*

**Editors:**
**Lydia Smith & Anne-Marie Neeteson**

***Friday, 4 June 1999***
***Veterinary Faculty, Utrecht University, Utrecht, The Netherlands***

**Workshop on Farm Animal Biodiversity, Utrecht, The Netherlands**
**Friday, 4 June 1999**
**Proceedings**

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Cover art work Jill Taylor Whitmore

ISBN 90-76642-01-x AnNePublishers November 1999

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# Introduction

**James Reeves, Chairman of the BBP**

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This well-attended meeting arose through the joint interest of these Industrial Platforms in the particular subject area. FAIP has a much more general and possibly commercial concern in these matters, whereas the main objective of the BBP is to present work and results from the EC Generic Project (Molecular Screening Tools for Biodiversity ) to an extended audience of potential end-users of the technologies and understanding generated therein.

This Generic Project is wide ranging covering studies on both plants and animals and the BBP is structured to reflect these interests. Obviously the focus of this meeting is on the elements of the work coming from the varied animal based research projects. It will be clear from the titles how wide ranging some of these projects are; covering aquatic animals as well as some of the more obvious domesticated farm species.

It was a somewhat daunting challenge for me, as someone concerned mainly with plants, to chair this meeting but I was relieved to discover that many of the problems were common both to the plant and animal fields so I was not totally out of my depth.

This was a very productive collaboration between the two Industrial Platforms which I hope will form a model for others. I must take this opportunity to thank both Dr Lydia Smith of the BBP and Ir Anne-Marie Neeteson of FAIP for bearing the brunt of the organisation of the meeting and putting together such an interesting programme. Thanks too are owed to all the speakers for their excellent and cogently presented contributions.

J.C. Reeves

# Molecular Markers for Biodiversity Studies in Domestic Ungulates

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**Keywords**: Cattle, goat, sheep, ungulates, biodiversity, molecular markers.

In this paper, the utility of molecular markers in the study of genetic diversity among livestock breeds is demonstrated. Molecular techniques allow high resolution analysis of evolutionary relationships from the species level down to that between individuals of the same breed or even of the same flock or herd. The selection of appropriate genetic markers will be discussed and their application to the study of sheep goats and cattle. Some of the analytical difficulties associated with the use of such markers will also be discussed.

## Molecular genetic approaches

The marker of choice is critically dependent on the taxonomic level of the study and the problem to be addressed. The different levels and questions that might be addressed are outlined in Table 1.

## Table 1. Taxonomic Level and Molecular Markers

| Taxonomic Level | Questions | Molecular Tools |
|---|---|---|
| Species/subspecies | Evolutionary differences<br>Hybridisation | Mitochondrial DNA sequence<br>Y-chromosome sequence |
| Breeds/Populations | Breed distinctiveness<br>Genetic substructuring<br>Genetic variation | Microsatellite variation<br>AFLP |
| Individual | Genetic representation<br>Reproductive success<br>Parentage analysis | Microsatellite variation |

## Species questions

The marker of choice for the studies at the species level has been mitochondrial DNA (mtDNA). It is maternally inherited and non-recombining. The evolution of mtDNA sequence is relatively fast compared to the nuclear genome and this allows discrimination of closely related species by analysis of the sequence divergence between them. There are a number of mitochondrial genes that evolve at different rates and which can be applied to the analysis of populations according to the length of time since divergence. When studying evolutionary differences among domestic breeds, the mtDNA control region is the most suitable for studying recent evolutionary events; it is the most rapidly evolving gene of the mitochondrial genome. Control region sequence has been analysed in cattle, sheep and goats and in all cases there is evidence for multiple domestications, as outlined in Table 2.

**Table 2. Domestications of cattle sheep and goat**

| Species | Domestications |
|---|---|
| Cattle | 3 domestications in different geographic regions (Near east, taurine cattle; Asia, zebu cattle; Africa, taurine cattle). |
| Sheep | 2 domestications, probably in the Near East and Asia as for cattle |
| Goat | 1 major domestication, other minor ones, possible later hybridisation with wild species |

These results demonstrate that in all three species, current diversity has ancient roots and that maintenance of present day breed diversity needs to take this ancient divergence into account. Control region sequence can also provide insight into the more recent ancestry of breeds. Some Portuguese cattle breeds, for example, have recently been shown to contain African mitochondrial haplotypes (Cymbron *et al*. 1999), indicating a different ancestry to other European breeds.

Mitochondrial DNA is maternally inherited, but it is also useful to have paternally inherited markers. Analysis of the *ZFY* gene being carried out in sheep (L.J. Lawson pers. comm.) and goat (G. Luikart pers. comm.) and preliminary results suggest that it is a powerful marker for reconstructing phylogenetic relationships within both taxa and especially between wild (sub)species and domestics.

## Population questions

The question that is currently being addressed, is whether breeds are genetically distinct from one another and whether there is any population substructuring. This work is being carried out using microsatellites as genetic markers.

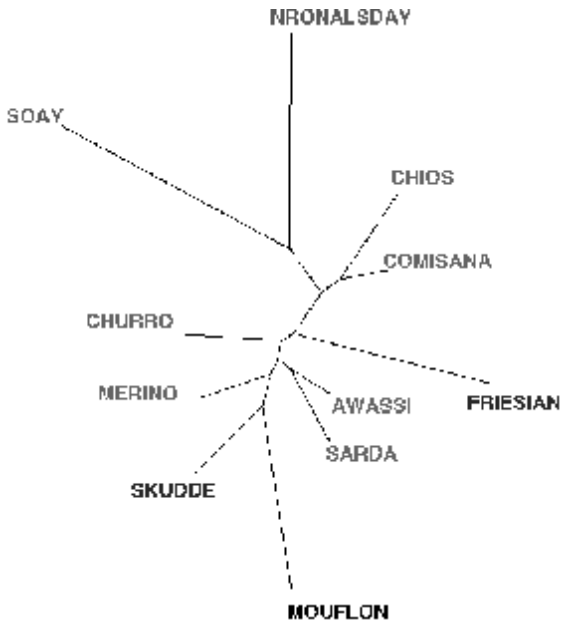


**Figure 1. Star phylogeny**

Analysis of allele frequency data has traditionally been used to calculate genetic distances and from this a tree is generated and relationships between populations are represented. If this type of analysis is applied to breeds then the resulting tree is a 'star phylogeny'. One example is given in Figure 1. The relationships are not resolved and in fact several different trees may be generated none of which is well supported statistically. This indicates that there was a rapid radiation from founder stock and that today's genetic differentiation is mainly the result of fluctuation in population sizes and random genetic drift.

In order to resolve these relationships, it is essential to be able to measure the 'branch lengths' of the tree. This problem has been tackled by K. Dawson & P. Boursot (Montpellier), who used Bayesian analysis of a joint distribution of allele frequencies for pairs of breeds. From this it was possible to determine branch lengths and the amount of genetic drift which has occurred in each population as it as diverged from the ancestral population.

There are other ways of looking at microsatellite variation; geographic variation in allele length was studied across Europe among sheep breeds. Figure 2. shows allele length distributions in two European breeds from the north-west of Europe (Soay) and the south-east (Chios) and it appears that the allele length is longer in the north-west than south-east. The significance of this was tested by spatial autocorrelation for 5 loci and the results suggest that there is a cline in allele length across Europe. This suggests that the population of domestic sheep may be substructured by geographic region. In cattle, a biogeographic analysis of European breeds based on the presence of zebu alleles demonstrates that zebu alleles are present in Hungary but disappear west here. This demonstrates the ability of microsatellites to distinguish breed histories as was demonstrated for mtDNA above.

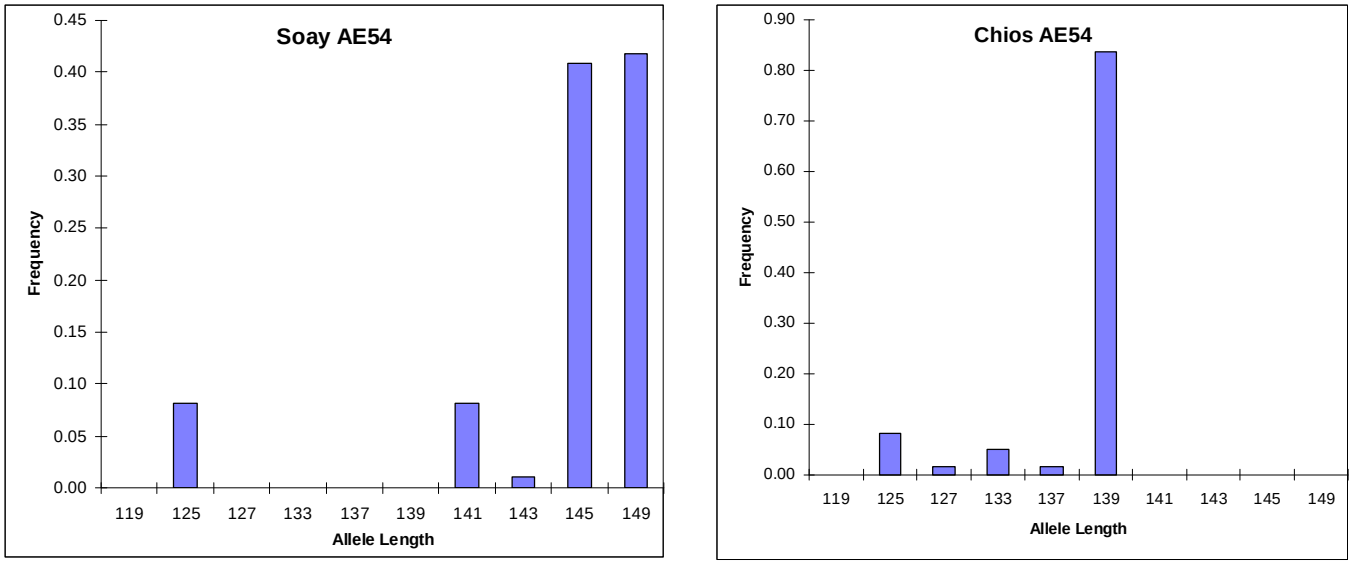


**Figure 2. Allele length distributions**

Microsatellites are also markers for particular genes. In this instance microsatellites have been used to measure variation across the major histocompatibility locus (MHC) in sheep (F. Santucci).
MHC genes are characterised by a high level of polymorphism. Diversity at the MHC loci arises as a result of several processes:

(i) interactions between MHC genes and parasites

- frequency dependent selection (rare allele advantage)
- overdominant selection (heterozygote advantage)

(ii) mating preferences

(iii) selective abortion

Preliminary results show that microsatellites within the MHC have a higher heterozygosity than those in other areas of the genome, suggesting selection for heterozygotes.

## Individual questions

Molecular markers can also be applied within breeds, to answer questions concerning population structure and to issues such as paternity analysis. An approach to paternity assignment in goats (G. Luikart, P. Taberlet, Grenoble). They have developed 22 microsatellites, which can be co-amplified and run in two lanes of a gel.

Testing of these loci in four commercially important goat breeds shows that the probability of identity is as low as $2.3 \times 10^{-19}$ in unrelated individuals and even for sibs as little as $3.6 \times 10^{-8}$. The probability of exclusion of males approaches 1 where the mother is known and even where the mother is unknown is still as high as 0.99.

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# AFLP®: A Multifunctional Tool for the Animal Breeding Industry

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**Keywords:** Pig, AFLP, genetic diversity

This paper introduces AFLP® as a potential tool for animal biodiversity studies and illustrates the technique with a description of recent results obtained with the pig. Archeological studies suggest that the domestication of pigs had already begun 9000 years ago in the Near East and 7000 years ago in China with claims that the domestication of the Sulawesi Warty pig predates these events. From these initial events a broad diversity of domestic breeds developed and there are now around 120 recognized European breeds of pig and over 350 worldwide. The obvious variety in size, shape and colour is clearly exemplified by comparison of Chinese breeds to the European and American types but more importantly beneath this there is a vast diversity of physiology.

Apart from the fact that many of our traditional, now minor, breeds that form part of our agricultural heritage are struggling for their existence, there is a keen interest in biodiversity from a modern commercial perspective. The aim of the modern swine breeding industry is to produce a specific product to address a particular customer or market requirement from a basic set of distinct genetic lines that comprise the raw materials. The ultimate limit on the variety of products and their efficiency and suitability for the various production systems and markets is the underlying genetic material.

Biodiversity can be viewed in two contexts where industry is concerned. Firstly there is the diversity within and among existing in-house lines that forms the basis of current products. Secondly, there is the diversity of lines outside the company in the form of non-commercial breeds and other separate commercial populations. Diversity within a breeding company's lines allows the continual improvement through the selection of healthy vigorous animals with the best performance in particular target areas. The genetic distance between lines can be used to advantage by utilising the heterotic effect in crossbred progeny and the designation of parental combination for particular production scenarios. Current selection practices are based upon today's market needs and this shapes the genetic capacity of current basic lines. However the market is constantly developing with new and more varied demands as illustrated by the current interest in meat quality traits and disease resistance. As such it may be that existing elite lines do not have the capacity to provide such features and that these capabilities need to be sourced externally.

Measurement of the genetic diversity within commercial lines using modern molecular approaches can provide valuable information to enable full utilisation of the potential. The opportunity to both supplement and audit current quantitative genetic approaches to maintaining diversity in the face of commercial selection is of great value and allows evaluation of new management scenarios and approaches. Also valuable is the ability to compare and control diversity between geographically distinct populations enabling the maintenance of competitive populations in commercially advantageous locations. Finally knowledge of the genetic diversity between basic elite lines allows better prediction of crossbreeding strategies. Studies of the diversity and relationships of breeds on a global basis provide an understanding of the origins and development of diversity. Such work also provides information regarding likely sources of valuable germplasm, both in established industry lines and non-commercial breeds, providing the design elements needed to produce the competitive products required.

AFLP is a relatively new genotyping tool that was initially developed for application in plants. Over the last few years we have been investigating its utility for animal applications. It is a very efficient means of identifying polymorphic markers within populations of interest. These markers can then be used in different situations as is appropriate.

The evaluation of AFLP began with an EC Framework IV demonstration project completed in November 1998. The aim was to show the value of this technology for the identification of DNA polymorphisms of value for marker

assisted selection in the pig industry. The approach taken was to combine AFLP with bulk segregant analysis in order to target markers to QTL or genes of interest. The initial model led to the isolation of markers for the simple coat colour trait, *Dominant White.* These markers are now in commercial use. The approach was then applied to evaluate the use of AFLP for the identification of QTL using the complex trait, lean tissue growth rate (LTGR). Pools of animals with high and low LTGR were created from a population of around 1400 animals with a mean LTGR of 408g/d. The animals in the low LTGR pools had a mean score of 270g/d (2.6 standard deviations from the population mean), while the individuals used to create the high pools had a mean LTGR of 513g/d, (2.4 standard deviations above the population mean). The pools were screened with 576 primer combinations and markers associated strongly with either pool evaluated on the individual animals within the groups. Markers associated with the high or low pools with a significance of greater than 95% were then re-screened on a second set of similar pools created from the same population approximately 3 generations later. After two rounds of screening 14 markers remained significant, although two of these markers showed altered association between the two sets of samples. Conversion of the markers to co-dominant forms was followed by the assessment of the association of the markers with LTGR on samples from individuals with values distributed across the population. Despite early positive indications, as the size of the tested sample was increased the significance of the association between the markers and LTGR was lost. The reasons for this are as yet unclear but may be related to the proportion of LTGR variance explained by QTL residing in these regions. If so, validation of individual markers on population samples representing the entire phenotypic spectrum may not be appropriate for markers identified by screening bulked extremes. It is hard to believe that the associations identified in the screening of the high and low pools arose by chance given the reconfirmation with the re-sampled pools. Indeed, the regions of the genome identified by this method were often those already reported to contain QTL for related traits (e.g. growth or fatness). It may be that the QTL identified are real but are too small to be verified across the population and only detectable in a particular small group of extreme animals. We are continuing with this approach in order to determine if the early promise can be brought to fruition as new selection tools.

A further product of this EC funded work is the creation of an AFLP map based upon the Roslin and Uppsala reference families. This map will be combined with the integrated microsatellite map now being completed by the EC PiGMaP consortium led by the Roslin Institute. Such a resource will not only benefit map based cloning and QTL detection programs but also enhance the use of molecular markers for biodiversity studies.

This initial project work identified that AFLP is a powerful tool for developing large numbers of DNA markers. Such a tool may be very useful in biodiversity studies. Although the information content of individual markers is reduced compared to microsatellite markers (the current standard for genetic distance measurement), this is compensated for by the ability to interrogate large numbers of markers per reaction. A second advantage, crucial in species where research funding is restricted, is the limited prior knowledge required for the application of AFLP. Preliminary studies in the pig have demonstrated that AFLP has the capacity to be able to distinguish between commercial lines selected on different indices a relatively few years after separation from a common population.

To thoroughly evaluate the potential of AFLP for such studies and to provide a comparison to a microsatellite based approach a further EC Framework IV demonstration project is underway. This project is coordinated by Louis Ollivier at INRA, Jouy-en-Josas (see accompanying paper). Such technology in combination with methods becoming available for the long-term storage of viable animal germplasm will allow not only rationalisation of commercial breeding but also the preservation of unsupported genetics. Such efforts have not only value in the retention of our natural and agricultural heritage but also in the maintenance of elements for future commercial application. Around 60 breeds are under study in this programme encompassing commercial lines of varying degrees of relatedness from a number of companies, local European breeds, the European Wild Boar and the Chinese Meishan. Fifty animals from each breed will be typed with 3-4 AFLP primer combinations, expected to yield approximately 100-150 markers per breed, and 50 microsatellite loci.

This work will provide a thorough evaluation of the 'fitness for purpose' of AFLP and microsatellite markers for the evaluation of genetic diversity at a range of different distances as well as providing measurement of the diversity of pig breeds within Europe.

*Funding for this work was provided by EC contract Nos. BIO4-CT95-0081 and BIO104-CT98-0188. AFLP® is a registered trademark of Keygene N.V.*

# Genebanks and the Conservation of Farm Animal Genetic Resources

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**Keywords:** genebanks, cryo-preservation, farm animals

## Introduction

Worldwide economic and social developments are the driving forces for the selection of high productive breeds, which are used in intensive animal production systems. This selection threatens the existence of locally developed breeds with lower-input, lower-output levels. A global survey of FAO indicates that 40 animal species are domesticated of which 14 dominant in the production of food for mankind. Within these 14 species 5000 breeds are recorded of which one third is extinct or threatened with extinction. Annually, 50 breeds are lost. This is a serious problem as 50 percent of the total genetic variation within a species is variation between breeds. Awareness is growing that society and breeding organisations can not afford the non-reversible loss of these breeds. Genebanks for plants in which genetic variation is conserved do exist already for more than 30 years. In only a few countries farm animal genebanks exist. Within the management of farm animal genetic resources genebanks can play a crucial role in conserving genetic diversity within species and in its future use.

## Responsibility for conservation

In accordance to the principles of the Convention on Biological Diversity each country is responsible for the management of their own national farm animal resources and for the implementation of conservation strategies. Collaboration between countries is recommended as many breeds are spread over several countries and exchange of genetic material between sub-populations of a breed is very common. FAO developed a global strategy for the conservation of farm animal genetic resources with as a backbone the development of a network of regional and national co-ordinating institutes and focal points. Major products are the development of a database and of guidelines for conservation. The conservation itself is the responsibility of governments, scientists, breeders and breeding organisations. In The Netherlands a genebank for the conservation of farm animal genetic resources was founded in 1993 at the initiative of cattle breeding organisations with a subsidy of the Ministry of Agriculture: the Dutch Foundation for the Conservation of Farm Animal Genetic Resources. In addition to the present sampling activities for cattle, semen from horses, pigs, poultry, sheep and goats will be sampled in the near future.

## Conservation of genetic diversity in animal species

Conserved genetic variation will secure future food production for a growing global population and future market demands, e.g. a diversification of production systems or consumer demands for specialised foods. Besides, the present socio-economic value of a breed, opportunities for genetic research, cultural and historic reasons and the ecological value of a breed are seen as objectives for conservation. Within actual breeding schemes a large effective population size is a guarantee for the conservation of a large amount of genetic variation. When a breed is threatened by extinction, *in situ* conservation is to be preferred. Then, all objectives of conservation can be met and the development of the breed can continue: selection for economic profit within the limits of a small population and adaptation to changing circumstances. Sometimes a breed is set aside its production environment in a zoo or in a park. Then, only the cultural, historic and ecological value is stored in *ex situ* live conservation. The *ex situ* conservation in genebanks does not contribute to the socio-economic, the cultural and historic and ecological value of a breed. But conservation in genebanks might be less costly when it is expected that it will take many years before an objective of conservation has to be fulfilled. Decreasing the risk of *in situ* conservation schemes is considered as the main value of a genebank for farm animals. Most genebanks store cryo-conserved semen. Embryo's, oocytes and somatic cells also create opportunities for conservation. For the future use of somatic cells, the technique of nuclear transfer should be developed further.

## Selection for conservation

Resources, both in terms of finances and manpower, are scarce in the area of conservation of breeds. However, the number of breeds that require a conservation programme is high. Which ones should be selected for ***in situ*** or ***ex situ*** conservation? Selection criteria are: degree of endangerment, adaptation to a specific environment, traits of economic importance, unique traits, culture or historical value, genetic uniqueness of a breed (based on genetic distances) and the species a breed belongs to. The single most important criterion for breed prioritisation is the degree of endangerment. For the other five criteria it can be recommended that a breed, scoring exceptionally high for one criterion, should be prioritised. The complexity of breed prioritisation for conservation, underlines the importance of good documentation and information.

## Establishing a conservation scheme

The option for a successful re-establishment of a breed set the most demanding requirement for a cryo-preserved genebank. The minimum population size is about 25 males and 25 females. The individuals that form the basis of the conservation scheme are chosen so that the variability amongst them is maximised. In ***in situ*** conservation schemes the cryo-preserved semen can be used as a reserve to guarantee the success of a breeding programme in case of a fatal wide-spread disease or loss of elite breeding animals. Therefore, with ***in situ*** conservation schemes animals should be systematically sampled for cryo-conservation. A conservation scheme is characterised by animal identification, recording of traits, genetic ranking (breeding value estimation), a selection and mating plan and a well-designed cryo-conservation project. For both, ***in situ*** and ***ex situ*** schemes, the detection and conservation of the founder animals is of great importance. In the choice of the founder animals pedigree information and/or information of genetic markers is indispensable.

## Operation of conservation schemes

For a live conservation programme, the following issues are important: the effective population size at which the breed is maintained, the selection of animals within the breed as parents for the next generation, the mating structure of the selected animals, the genetic improvement that needs to be achieved, the recording of traits and the registration of the pedigree. The most relevant topic for cryo-conservation schemes is the replacement of retrievals from the genebank, since retrievals will deplete the genetic material of the genebank. Live and cryo-conserved schemes are combined with two aims: cryo-conserved material serves as a back-up in case the live population runs into genetic problems and cryo-conserved material can actively be used to increase the effective population size and to reduce genetic drift. Prolonging the generation interval can be worthwhile in this respect.

# Development of Strategy and Application of Molecular Tools to Assess Biodiversity in Chicken Genetic Resources (short title: AVIANDIV)

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**Keywords:** Chicken biodiversity, molecular markers

Over more than 7000 years of domestication a vast genetic diversity in chickens has been accumulated, and the chicken has been considerably changed and much differentiated (reviewed by Crawford, 1990). The Red Junglefowl (*Gallus gallus*) is considered as the basis of all domesticated chicken breeds of today whatever size, shape or colour they are. It has a body weight of about 500 to 1000 g, and the hen often lays no more than 10 to 15 eggs. On the other hand, commercial laying hens, for example, are nowadays capable of producing more than 300 eggs a year. From its origin in South East Asia, chickens were spread out to other continents, other cultures, and other environments, and numerous local breeds of fowls throughout the world have been developed. Biodiversity in chickens is displayed by many breeds and strains created during domestication. They may differ in genetic features resulting from development under specific conditions in a given region (climate, diseases, feeding). The poultry meat and egg industry started to develop at the beginning of this century, and has grown into the vast chicken industry. The current breeding strategies for commercial chicken concentrate on a few specialised breeds and involve intense selection for either laying performance or meat production based on distinct lines. Considering the large pool of chicken breeds developed, only a rather limited number of breeds have contributed to the commercial breeding stocks of today. For example, the Single Comb White Leghorn breed has replaced all others as producers of industrial white-shelled eggs.

*The local breeds may contain much of the genetic variation pertaining to adaptation to particular environments. They may become economically important in the future for genetic adaptation to changes in agricultural production and preferences. However, the flock size of many local chicken breeds has been extremely reduced and many are under risk of extinction. A reduction of genetic variability may be expected to occur in commercial lines used by multinational breeding companies in layers and broilers due to the high selection pressure in the current market-orientated breeding strategies.*

*Although decisions on conservation of genetic resources have to rely upon a range of information, molecular markers may serve as an important initial guide.*

The overall goal of the project is to experimentally evaluate strategic questions relating to the assessment of Biodiversity in the chicken using molecular information that can be measured in various ways at the DNA level. In the proposed project we will examine the level of point mutations between and within chicken populations. This will be relevant as a criterion to assess Biodiversity, and at the same time it will provide the background to adopt a completely new technology of DNA chips in poultry. The DNA chips technology can be used for large-scale detection of point mutations and, from the Biodiversity standpoint, may revolutionise the possibilities for assessing genetic differences. Microsatellites are currently widely accepted as the most powerful tool to characterise Biodiversity at the molecular level. By collecting large samples from various breeds we will establish a complex genetic distance matrix to quantify Biodiversity in chickens based on microsatellites. Although theoretical studies have been carried out (Nei et al.; 1983, Nei, 1987), questions related to the sampling strategy, number of markers and statistical analysis need still to be answered experimentally and will be approached in the proposed project. The project will deepen our understanding of Biodiversity not only in chickens, but also in comparative aspects by extending molecular studies of Biodiversity within non-mammalian species. The major objectives addressed in the project are as follows:

1) To assess Biodiversity in a wide range of 50 chicken breeds using a set of 25 microsatellites and DNA pools of 50 individuals per breed for genotyping. This information will give insight in the currently existing Biodiversity in chickens, and by including commercial lines, to what extent they compare to unimproved local breeds and the Red Jungle Fowl (*Gallus gallus*), the progenitor of all chickens. Gene mapping studies using microsatellites are already well established in the chicken. It is logical for that purpose to link the genetic characterisation of breeds to the EU-funded project CHICKMAP (Project co-ordinator Dr. D. W. Burt). Some Participants of our project are far ahead in this technology to guarantee that the most appropriated markers in chickens will be used.

2) To investigate basic questions to estimate Biodiversity using microsatellites. Based on (1), 10 breeds representing a wide spectrum of the dendrogram will be selected for genotyping of 30 individuals per breed using 50 microsatellite loci. There have been theoretical studies providing estimates of the number of animals per breed to be sampled and number of microsatellites to be used. (e.g. Barker et al., 1993; Nei, 1987). However, these predictions have not been tested experimentally in chickens to date. This approach will make possible a detailed analysis of the use of microsatellites, their size distribution and frequencies, and be of value to further diversity studies using this type of molecular marker.

3) To evaluate the level of point mutations in random non-coding DNA fragments for two reasons: a) to examine the feasibility of adopting DNA chip technology for the poultry populations and b) to use it as a criterion for the estimation of Biodiversity between and within chicken populations. To approach this question we will select 10 breeds and 10 individuals per breed, and we will sequence 10 random non-coding fragments per individual.

4) To develop a new statistical approach to assess Biodiversity. The statistical analysis of data to estimate genetic distances between populations and genetic variability within breeds is not straight forward and requires specific efforts. So far, no general consensus exists as to which of the many genetic distance estimates would be the best for analysis of within-species populations. Experimental data based on (1), (2) and (3) will provide the basis to determine the most appropriate method for estimating genetic distances.

5) To establish a chicken DNA collection and Poultry BIODIVERSITY database from the populations sampled for further research within the Community. A DNA collection of various breeds will extend the basis to search for further genetic polymorphisms identified in the Chicken gene mapping project. The Poultry BIODIVERSITY database will provide public access to the information collected and the analytical tools developed within the project.

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# Genetic Resources in Cattle

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**Keywords:** Cattle biodiversity, molecular markers

Several bovine species have contributed to the domesticated cattle around the world. Taurine cattle (*Bos taurus*) is the main species, but zebu (*Bos indicus*) is more predominant in the hot and dry climate zones. Yak (*Bos grunniens*) is held at high altitudes in and around Tibet, while Bali cattle and the gayal or mithan are domesticated variants of the banteng (*Bos javanicus*) and the gaur (*Bos gaurus*), respectively. In addition, water buffalo *(Bubalus bubalis*) is raised in large numbers in Asia, Southern Europe, North Africa and South America. With the exception of the water buffalo, cattle species cross-hybridise and several breeds have been derived from more than one species.

In several cases the hybrid origin of these breeds is not completely obvious from the appearance and has not been documented. It has been demonstrated that the species composition of these breeds can be identified by AFLP (amplified fragment length polymorphism) and SFLP (satellite fragment length polymorphism). Several African taurindicine breeds (zebu-taurine cattle, [1]), at least three individual Bali cattle (banteng with a zebu component), Madura cattle (zebu-banteng) [2] and the beefalo breed (bison-taurine cattle) have been examined. For the taurindicine breeds this is in agreement with mitochondrial DNA sequences [3] and microsatellite genotypings [4].

Taurine cattle have differentiated into hundreds of distinct breeds, each with different breeding objectives and adapted to its own local environments and/or management system [5]. Previous studies on genetic distances within and across breeds were based on blood groups and protein polymorphisms [6-8], but current molecular studies on cattle breeds use the DNA methodology: sequencing of mitochondrial DNA [3], microsatellite genotyping [4, 9-12] or AFLP fingerprinting (Ajmone-Marsan *et al.*, unpublished results). How do these genotyping methods discriminate breeds?

As mentioned already, *AFLP* is informative for species composition in exotic breeds. Since AFLP reveals mutations - point mutations in or near restriction sites, insertions, deletions- with the same mode of evolution as functional gene defects, it may be predictive for inbreeding depression. However, recent studies in Piacenza and Utrecht (Ajmone-Marsan *et al.*, unpublished) have indicated that AFLP patterns of individuals of different taurine breeds are remarkably similar and most polymorphisms are shared by the breeds. Pairwise distances within breeds are variable and only slightly lower than distances across breeds. So although AFLP appears informative for genetic distances of animals of the same or different breeds, selective breeding has not led to a divergence of AFLP patterns.

*Mitochondrial DNA* haplotypes are inherited via the maternal lineage and has in many cases been informative for phylogenetic reconstructions or forensic identifications. However, mitochondrial haplotypes are not breed-specific [3] and variation within breeds is about the same as variation across breeds. So breed formation has not split the maternal lineages.

The multiallelic microsatellites are now the most commonly used markers for genetic linkage studies in man and other mammals. However, alleles are again not breed-specific and individuals can only partly be assigned to breeds on the basis of their alleles [11]. Nevertheless, microsatellites are still sensitive indicators of genetic drift, migration and introgression in breeds or populations.

Since breeding of cattle is based on the use of a restricted number of top sires of bulls with desired characteristics, *Y-chromosomal haplotypes* may have become fixed within the males of the breeds. It may be expected that Y-chromosomal divergence correlates with the breed phenotype if sires of other breeds have been used for upgrading. For example, African zebu breeds, which emerged by introgression of zebu bulls into African taurine herds, all have a taurine mitochondrial haplotype [3], but many of the bulls have indicine Y-chromosomes [13]. Y-specific markers that are variable within the taurine breeds are not available yet, but are likely to be informative for breed formation.

We hypothesize that at the DNA level a breed corresponds to a unique combination of homozygosities of gene variants. These alleles have been fixed by selective breeding and may correspond to the breed phenotype (coat color, morphology and production traits) or may have been selected by the environment. Since these homozygosities have not yet been detected by AFLP, the remainder of the genome seems to have retained much of its diversity.

In 1998 our proposal '*Towards a strategy for the conservation of the genetic diversity of European cattle'* [14] has been approved within the RESGEN program of the Directorate General VI of the European Community. It will build on previous activities co-ordinated worldwide (EAAP, FAO) or on the national or European level. Notably, a previous Concerted Action has already led to a standardised set of 30 microsatellite markers (the *Utrecht List*) and to a cattle diversity database [15]. We will closely interact with several laboratories across Europe. The targets of the project are the following:

1. Microsatellite genotypes of 50 animals from 50 breeds with 30 markers of the Utrecht list. This will combine and complement previous work [4-12]
2. AFLP genotyping of 20 animals from 50 breeds with 100 biallelic markers.
3. Analysis of the effect of selection by (a) studying the diversity in loci coding for milk protein, the myostatin locus, regions known to contain QTL and (b) AFLP analysis of pools of animals of the same breed to identify breed-specific markers.
4. Formulation of recommendations for conservation of the genetic diversity of cattle. It is expected that the RESGEN project will generate a unique and valuable set of data, which represents most of the cattle genetic diversity in Europe and will lead to fundamental new insights in the relation between selective breeding and genetic diversity.

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# Molecular Markers as a Tool to Study Genetic Resources in Oysters

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**Key words:** Oysters, genetic resources, molecular markers.

Despite the world economic importance of oyster farming, genetic improvement has not yet had a great impact on these species. Oyster farming is traditionally based on wild stocks, whose natural populations are often overexploited, and frequently does not fulfil market demand (Goulletquer and Héral, 1997). Up to now, the most effective answer to disease problems or for the improvement of productivity has been the introduction of new species. However, these introductions are constrained by their potential ecological impact and also limited by the availability of suitable species. Proper management of genetic resources is still to be established, but could be of great importance for the long-term sustainability of the shellfish industry. For this reason, molecular markers have been developed and are of great use for the study of these resources, both at the between and the within species levels.

The taxonomy of oysters is not straightforward. Their shell morphology is highly plastic and species determination is often unclear. Furthermore, inter-specific hybridisation can occur (Gaffney and Allen, 1993) and cases of introductions of non-native species are numerous. Consequently, the development of species specific genetic markers has been initiated to ease their identification. These markers are used to trace the origin of non-native stocks (e.g. *Crassostrea angulata* and *C. gigas* in Europe, Boudry *et al*., 1998), or to discriminate morphologically plastic species in the field (e.g. *C. gigas, C. ariakensis* and *C. sikamea* in Japan, Hedgecock *et al.*, 1999).

In areas where natural recruitment of oysters is limited (due to climatic constraints or to decreasing populations), the production of spat can be based in hatcheries. This makes the development of selective breeding programs possible, but such programs have not yet reached a scale enabling proper genetic diversity management, multi-site testing or effective improvement of the commercial stocks. Most of them have remained at an experimental scale, aiming to evaluate the possibility of selection for traits such as growth or disease resistance (e.g. Naciri-Graven *et al*., 1998). Only with the support of the shellfish-farming community will selection programs be able to get past the experimental stage and reach an economic scale, such as the "Molluscan Broodstock Program" initiated in the USA (Hedgecock *et al*., 1997).

Since oysters have a very high fecundity, it is common practice for hatcheries to produce large amounts of offspring from a limited number of parents. The genetic consequences of such practices are of concern, especially if some of these offspring are to be used as parents for the next generation. Inbreeding is likely to occur, leading to a decrease in performance (Bierne *et al*., 1998). Furthermore, a reduced genetic variability limits the possibilities of genetic improvement by selective breeding. Highly polymorphic genetic markers such as microsatellites can be useful tools for the analysis genetic of variability and parental contributions in hatchery-produced stocks. Such analyses have been performed in the progeny of several *in vitro* factorial crosses of the cupped oyster *Crassostrea gigas*. Parentage analysis was eased by the large polymorphism observed at the 3 loci studied. Despite the balanced gametic contribution of each parent before fertilisation, unbalanced parental contributions are frequently observed in the progeny, both at larval and juvenile stages, due to gametic and zygotic competition.

In the European flat oyster, *Ostrea edulis*, *in vitro* fertilisation is not possible, as this is a larviparous (or brooding) species. Genetic variability of 3 strains selected for a resistance to the protozoan parasite *Bonamia ostreae* (Naciri-Graven *et al*., 1998) was analysed using 5 microsatellite loci. The estimation of the effective number of breeders demonstrated the occurrence of bottlenecks and potential inbreeding (Launey, 1998).

We can conclude that, despite the limited impact of genetics in oyster farming, the recent development of molecular markers is likely to have a significant impact on the management of genetic resources in oysters in the near future. Most of these resources are still unexplored and genetics should bring new perspectives to oyster production.

***Acknowledgements** : Some of the results presented here are part of the EC funded project "Genephys" (Contract no. FAIR 95-421).*

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# A Comprehensive Genetic Study of Cultured and Wild Stocks of Gilthead Sea Bream (*Sparus aurata*) and Genetic Assessment of Several Related Species as Candidates for Aquaculture

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Contract No AIR3-CT94-1926

**Key words:** Sea bream, genetic resources, molecular markers.

This study compared samples of gilthead sea bream (*Sparus aurata)* with regard to the amount of genetic variation contained in the sample and the degree of genetic heterogeneity among samples. Five samples were collected from a selected farm from each of five regions: Greece; Italy; the Mediterranean coast of Spain; the Atlantic coast of Spain and Portugal,. The genetic characters used were allozymes, microsatellites and mitochondrial DNA (mtDNA).

All three types of genetic information indicated that the amounts of variation are comparable among samples. There were no obvious signs of inbreeding. Contrary to this, there were substantial but not dramatic genetic differences among samples. The comparison was extended to wild populations using samples from the same geographic area and an additional sample from the Atlantic coast of France. The six wild samples were homogeneous with regard to their genetic make-up, suggesting that there have been no restriction to gene flow among populations of *S. aurata* from Eastern Mediterranean to the Azores. When compared to cultured samples, there was a clear pattern of a small but systematic reduction of genetic variability in the cultured samples vis-à-vis the wild ones. The most parsimonious explanation of these findings is that the founder effect of the aquacultured stocks, amplified by a small effective population size, is responsible for the genetic differences among cultured samples, and that a process of erosion of genetic variability exists in cultured samples but at present, its net effect is small and difficult to quantify because the actual age of the cultured stocks is small.

Three bilateral meristic characters were also scored for each *S. aurata* individual and used to calculate a bilateral asymmetry index. This index was compared to the individual's degree of allozyme heterozygosity. The first observation was that the bilateral asymmetry index was systematically higher in cultured samples than in wild samples. The second observation was that this index was negatively correlated with allozyme heterozygosity. These observations reinforce the view that there is a process of loss of genetic variation and therefore an increase of homozygosity and inbreeding depression, in cultured samples. This provides support for the contentious hypothesis that heterozygosity provides a genetic environment for developmental stability; measurement of its average degree of bilateral asymmetry is thus an indirect yet reliable and at the same time very simple and practical way to assess the genetic quality of a farmed stock.

Seven more species of the same family of Sparidae (*Pagrus pagrus, Diplodus (Puntazzo), Diplodus sargus, Pagellus bogaraveo, Spondyliosoma cantharus, Lithognathus mornyrus and Dentex dentex*) were included in the study. A small aquaculture industry already exists for some of these species (*P. pagrus, P. bogaraveo*) and others are considered as serious candidates (*D. dentex*). Samples from wild populations of these species were examined for differences at both allozyme and mitochondrial markers. The number of populations examined varied according to genetic assay (allozyme or mtDNA) and according to species, but there was always the possibility of comparing Mediterranean with an Atlantic sample.

- Three species (*S. aurata, P. pagrus, D. sargus*) showed no geographical genetic heterogeneity.
- Two species (*S. cantharus and P. bogaraveo*) showed some degree of heterogeneity (or no congruence between allozyme and mtDNA results)
- Three species (*D. pargus, L. mormyrus and D. dentex*) showed a large difference between Mediterranean and Atlantic samples.

In the case of *D. dentex* the differences are as large as one would expected between two different species. At present, there is no obvious explanation of these differences between species and no explanation can be obtained without a further study that would combine the genetic information with information of the population biology of these species for which only fragmentary data exist.

Two of the species (*S. aurata* and *P. pagrus*) were used to produce hybrids in both reciprocal crosses. Both types of hybrids were sterile. In terms of growth both types were close to the parent that grew slower (this being *P. pagrus* in the first five weeks of age and then *S. aurata*), but in terms of viability they were close to *S. aurata*, which survives much better under aquacultured conditions than *P. pagrus*. The sterility of hybrids makes them dead ends for further insights into interactions between genes coming from different species. With regard to potential use of hybrids in aquaculture, their slow growth profiles is a negative factor, but their good survival and sterility may provide enough reason for a further consideration of the idea, particularly if marketing conditions for new products become very strong.

# Overview of Current and Completed Projects Covering Biodiversity and the Type of Tools and Products Emerging for the End User

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**Keywords:** rare breeds, molecular tools, farm animal, biodiversity

## I - Historical Overview

1970 Société d'Ethnozootechnie
Rare Breeds Survival Trust

1980 The European Association for Animal Production (EAAP) established a working group on animal genetic resources (AGR)
FAO held its 1st expert consultation

1988 Creation at the Hannover Veterinary University of the first databank on AGR (EAAP - AGDB)
1988 - 1991: global (53 countries)
1991 - present : strictly European

1993 Two important books:
Genetic Diversity of European Livestock Breeds (EAAP: Simon and Buchenauer)
World Watch List for Domestic Animal Diversity (FAO/UNEP: Loftus and Scherf)

1996 The data were made available on the Internet both by FAO (DAD – IS) and by the EAAP – AGDB at Hannover

1997 Support at the European Union to a "permanent inventory of the European AGR" (RESGEN 083)

The European breeds inventories are summarised in Table 1. See also Ollivier (1998) for more details on this historical overview.

**Table 1. European breeds inventories**

| | EAAP surveys | | EAAP-AGDB Hannover | | FAO Rome |
|---|---|---|---|---|---|
| Species | 1982[1] | 1985[2] | 1993[3] | 1997[4] | 1996[5] |
| Cattle | 271 | 148 | 277 | 311 | 332 |
| Goats | 65 | 45 | 68 | 101 | 123 |
| Horse | 206 | 73 | 123 | 139 | 213 |
| Sheep | 275 | 183 | 283 | 338 | 407 |
| Pig | 123 | 64 | 126 | 134 | 156 |
| TOTAL | 940 | 513 | 877 | 1023 | 1231 |

[1]Maijala *et al.*, 1984; [2]Maijala, 1987; [3]Simon and Buchenauer, 1993; [4]Simon, 1997; [5]FAO, 1996.

## II – EU-funded projects on farm animal biodiversity

Projects on farm animal biodiversity have been funded since 1994 by EU in the framework of its Biotechnology programme (DG XII) and of its Regulation 1467/94 on genetic resources in agriculture (RESGEN programme of DG VI). They are summarised in Tables 2 and 3. It can be seen that those 8 projects cover not only biodiversity per se but also more general aspects of genetic resources management through inventories and conservation techniques.

## III - Type of tools and products emerging

The molecular tools for biodiversity are essentially SSR (Simple Sequence Repeat or microsatellite) or AFLP (Amplification of subsets of Fragment Length Polymorphism)

Implementation requires (1) collecting blood samples, (2) extracting DNA and (3) analyzing either individual DNA or DNA pooled in each breed. Allelic frequencies per breed are thus obtained.

**Within-breed diversity** (only when individual genotyping is performed) can be evaluated through heterozygosity and the test of Hardy-Weinberg equilibrium at each locus.

**Between-breed diversity** (based on allelic frequencies) can be evaluated using several methodologies.

- Breed differentiation: see the fixation indices (F) of Wright (1943) and Nei (1977).
- Genetic distances: various measures an available and a global protocol has been proposed by an FAO Working Group (see Barker, 1994).
- Phylogenetic tree construction: various algorithms are available, but phylogenetic interpretations are unsure.
- Measurement of diversity: e.g. see the Weitzman (1993) approach and its application to farm animal breeds suggested by Thaon d'Arnoldi *et al*. (1998).

**Table 2. Projects on biodiversity funded by EU DGXII (Biotechnology programme)**

| | **Gene bank** | **Cattle/sheep/ goat diversity** | **Avian diversity** | **Pig biodiversity*** |
|---|---|---|---|---|
| Type of project | Research | Research | Research | Demonstration |
| Call for offers | 1995 | 1995 | 1997 | 1997 |
| Coordinator | The Netherlands (Lelystad) | UK (Norwich) | Germany (Celle/Mariensee) | France (INRA) |
| Nb of partners | 7 | 6 | 8 | 6 |
| Nb of subcontractors | - | - | 20 | 6 |
| Nb of EU countries | -6 | all | 8 | 12 |
| Nb of non-EU countries | FAO | large | 9 | 4+FAO |
| Nb of breeds | - | 165 | 50 | 66 |
| Nb of individuals/breeds | - | variable | 50 | 50 |
| Nb of microsatellites | - | 25 | 25 (50 in 10 breeds) | 50 |
| DNA typing | - | Individual | - Pooled for microsat.<br>- Individual for microsat.<br>+ SNP<br>(on 10 breeds) | Individual and pooled<br>+ AFLP |
| Contact person | K Oldenbroek | G Hewitt | S Weigend | L Ollivier |

*** Preceded by a pilot trial in the 1994-1996 PiGMaP project co-ordinated by A. Archibald (Roslin,UK)**

**Table 3. Projects on animal genetic resources funded by EU DGVI (RESGEN programme)**

*web site: http://europe.eu.int/comm/dg06/res/gen/index.htm*

| | COMPLETED | CURRENT | | | |
|---|---|---|---|---|---|
| | Inventory (RESGEN 083) | Rabbit (RESGEN 060) | Pig (RESGEN 012) | Cattle (RESGEN 118) | Horse (*) |
| Type of Project | CA | SC | SC | SC | SC |
| Coordination | EAAP (Rome) | INRA (Toulouse) | INRA (Jouy) | Utrecht University | Trinity College (Dublin) |
| No of partners | - | 5 | 15 | 5 | - |
| No of EU countries | all | 4 | 7 | 14 | - |
| No of non-EU countries | -all | 2 | 6 | 6 | - |
| No of breeds | - | - | 23 | 50 | - |
| No of individuals/ Breed | - | - | 50 | 50 | - |
| Microsatellites | - | - | 50 | 30 | - |
| AFLP | - | - | x | x | - |
| **Contact person** | **L Ollivier** | **G Bolet** | **L Ollivier** | **JA Lenstra** | **D Bradley** |

*** Contract under negotiation**

## IV - Benefits of biodiversity evaluations to the industry

- Overall view of European domestic animal diversity
- Basic information for establishing conservation strategies, at country level (e.g. in the framework of the Convention on Biological Diversity)
- Commercial opportunities for breeding companies and breeding associations, e.g. choice of lines in an improvement programme
- Unambiguous identification (traceability) of breeds, lines, etc…

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**B**iotechnology
for **B**iodiversity Industrial
**P**latform

## Biotechnology for Biodiversity Industrial Platform (BBP )

### This Industrial Platform was established in 1995 to achieve the following objectives

1. **To establish a forum for a free-flowing dialogue between molecular biologists and end-users to**:
   - Increase awareness and understanding of the molecular techniques available and their potential applications to different end-users (i.e. what are they? what can they do? and where can they be found?
   - Increase awareness of the end-users requirements to the producers of the technologies (i.e. what are the specific problems different end-users' face, can these be addressed by the molecular techniques and if so, how?)
   - Provide end-users with fast access to the latest technological developments and their applications
2. **To set into motion mechanisms for training and technology transfer.**
3. **To provide 'after-care' for trained end-users establishing the techniques in-house.**
4. **To achieve wider dissemination, promote education and increase public**

To achieve these ends, the BBP is subdivided into 6 Satellite Groups, each with its own chairperson.

| ***Satellite Group*** | ***Contact*** |
|---|---|
| Molecular characterisation & identification | Robert Cooke NIAB, Cambridge |
| Natural & domesticated populations – monitoring & management | M.Lefort, Bureau de Resources Genetique Paris<br>P.Pamilo, Institute for Genetics, Uppsala |
| Collections, germplasm & ex-situ management | T.Hodgkin, IPGRI, Roma &<br>L.Alderson Rare Breeds Survival Trust |
| Sustainable ulilisation & breeding | Stephen Hall, De Montford University, Milton Keynes |
| Technology transfer & training | W.Spek ALW, Den Haag |
| Dissemination & awareness | Sarah Ball, HDC, East Malling |
| Chairman | James Reeves NIAB, Cambridge |
| Treasurer | John Parker, Cambridge Botanic Garden |

## *More details about the BBP can be found on the WebPages at*
www.niab.com/bbp

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Details of the Generic Project may be accessed at
http://www.lars.bbsrc.ac.uk/biodiversity/posthtml.htm

# Farm Animal Industrial Platform *(FAIP)*

The Farm Animal Industrial Platform (FAIP) is an independent European forum for farm animal reproduction and selection organisations (both industry and farmer's cooperatives), including companies not involved in genetic improvement, reproduction and related technologies. In 1995, the major European breeding industries joined forces, because of their common interest in precompetitive research at European level.

Research in the area of farm animals has an impact beyond species. For this reason, the industries of a wide range of farm animals - cattle and other ruminants, pigs, poultry and aquaculture - are represented within FAIP, directly or through their umbrella organisations. The majority of companies active in this sector are Small and Medium Enterprises.

The aim of the Platform with regard to breeding and reproduction of farm animals is:
(1) to stimulate research and research funding at European level,
(2) to indicate the direction of the research that is important for the industry to the European Commission,
(3) to disseminate research results to industry, beyond the relatively few industries participating in EU projects as full partners, and
(4) being a forum for farm animal industry interested in and/or related to reproduction and selection.

The Platform has regular contacts with project holders of EU granted farm animal reproduction and selection projects. These contacts are important to avoid duplication of efforts, ensure relevance, and optimise use of European funds. FAIP informs members about funding possibilities at European level, and stimulates participation of industries in research projects through partnering of industry and scientists in international research projects.

FAIP is a forum for the development and expression of opinions on research related topics (e.g. patent directive and new breeding technologies).

FAIP aims at explaining breeding and reproduction to a wider audience. Transparency and a continuous dialogue with society are vital for mutual understanding of animal breeding and reproduction and animal products.

## Steering Committee

Dr. Jan Merks, IPG - Institute for Pig Genetics B.V.. Pigs. Chairman.
Dr. Ole Andersen, The Danish AI Societies. Cattle and other ruminants.
Dr. Pierrick Haffray, SYSAAF. Aquaculture.
Dr. Cliff Nixey, BUT - British United Turkeys. Poultry.

## Secretariat

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## FAIP Members

*Farm animals (several species)*
Finnish Animal Breeding Association (FABA), Vantaa, Finland
U.N.C.E.I.A. (Union of French A.I. Cooperatives), Paris, France. Cattle, pigs
Arbeitsgemeinschaft Deutscher Tierzüchter, Bonn, Germany. Ruminants, pigs, poultry
ELPZOO, Zorlesco di Casalpusterlengo, Italy. Cattle, pigs, rabbits
Semenitaly S.r.I., Saliceta S.Giuliano Modena, Italy. Cattle, pigs
Van Haeringen Laboratorium B.V., Wageningen, The Netherlands
Meat and Livestock Commission, Milton Keynes, UK. Beef cattle, sheep, pigs

*Cattle and other ruminants*
CIA Linalux, Ciney, Belgium
Federation of Danish A.I. Societies, Aarhus, Denmark
Besamungsverein Neustadt ad Aisch e.V., Neustadt ad Aisch, Germany
The AI Cooperatives of Ireland, Enfield, Ireland
Laboratorio di Tecnologie della Riproduzione (CIZ), Cremona, Italy
Altapon, Garnwerd, The Netherlands
Holland Genetics V.O.F., Arnhem, The Netherlands
GENO (Norsk Rødt Fe), Hamar, Norway
Svensk Avel, Skara, Sweden
Arbeitsgemeinschaft Schweizerisher Rinderzüchter, Zug, Switzerland
Genus, Newcastle upon Tyne, UK

*Fish*
SYSAAF, Noirmoutier, France: Fish and Poultry
Selonda Aquaculture, Kallithea Athens, Greece
Aquagen, Kyrksaeteröra, Norway
Intervet, Boxmeer, The Netherland.

*Pigs*
Seghers Gentec, Buggenhout, Belgium
Federation of Danish Pig Producers and Slaughterhouses, Copenhagen, Denmark
France Hybrides, Saint Jean de Braye, France
Schaumann Besitz-Hülsenberg GmbH & Co KG, Wahlstedt, Germany
Zentralverband der Deutschen Schweineproduktion e.V., Bonn, Germany
ANAS - Associazione Nazionale Allevatori Suini, Rome, Italy
IPG - Institute for Pig Genetics, Beuningen, The Netherlands
Dalgety PLC/PIC Group, Cambridge, UK
UK MASCP - UK Marker Assisted Selectium Consortium, Edinburgh, UK

*Poultry*
Scanbrid Int. A/S, Bjaeverskov, Denmark
Hubbard-ISA, Lyon, France
Lohmann Tierzucht, Cuxhaven, Germany
Euribrid B.V., Boxmeer, The Netherland: Poultry, fish, pigs
Hendrix Poultry Breeders, Ospel, The Netherlands
Association of British Primary Breeders & Exporters, UK
BUT - British United Turkeys, Broughton, UK
Ross Breeders, Midlothian, UK