AN EC-FUNDED PROJECT ON CHARACTERISATION OF GENETIC VARIATION IN THE EUROPEAN PIG. OBJECTIVES, ORGANISATION, BREED SAMPLING, DNA PREPARATION AND CIRCULATION

UN PROJET FINANCÉ PAR LA COMMUNAUTÉ EUROPÉENNE SUR LA CARACTÉRISATION DE LA VARIATION GÉNÉTIQUE DU PORC EUROPÉEN. OBJECTIFS, ORGANISATION, ÉCHANTILLONNAGE DES RACES, PRÉPARATION ET CIRCULATION DE L'ADN

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Pig. Breed. Genetic diversity. Sampling. Genetic marker.

MOTS CLÉ ADDITIONNELS

Porc. Race. Diversité génétique. Échantillonnage. Marqueur génétique.

SUMMARY

The overall objective of the project (PigBioDiv) was to demonstrate the applicability of molecular biology tools for evaluating pig genetic diversity. Use was made of two marker technologies, namely *microsatellites* and *arbitrary amplification of fragment length polymorphism* (AFLP). Altogether, including this project (PigBioDiv) and a previous pilot project (PiGMaP), s*eventeen European teams* and an *international organisation* (FAO) contributed to the work. This paper outlines the various tasks which were shared among the participants and particularly the sampling scheme and the subsequent circulation of the DNA samples among the partners. DNA was extracted from about 50 pigs from each population. The populations considered included 69 European domestic breeds (or lines) and, in addition, the Chinese *Meishan* breed and a sample of European wild pigs. The European populations sampled belonged to 3 categories, namely local breeds, national varieties of international breeds and commercial lines mostly derived from the previous category. Difficulties were met during the project, particularly in sampling rare breeds under extensive conditions. The number of individuals and the diversity of origin recommended could not be reached in a few local breeds. Although the problems encountered significantly compromised the work plan initially intended, it was possible to complete all tasks that were proposed. The project has demonstrated how effectively commercial and public sector entities can work together. Guidelines have been developed in the project in view of more efficient international implementations of the approach.

RÉSUMÉ

Le projet PigBioDiv se donnait comme objectif de démontrer les possibilités d'application des outils de biologie moléculaire à l'évaluation de la

diversité génétique du porc. Deux technologies de marquage ont été utilisées, à savoir les *microsatellites* et *l'amplification arbitraire de polymorphisme de longueur de fragment* (AFLP). En tout, si on regroupe ce projet (PigBioDiv) et un projet-pilote précédent (PiGMaP), *dix sept équipes européennes* et une *organisation internationale* (FAO) ont participé aux travaux. Cet article décrit la répartition des différentes tâches et notamment le protocole d'échantillonnage et la circulation des échantillons d'ADN entre les participants. Les populations considérées incluaient 69 races (ou lignées) européennes domestiques, auxquelles s'ajoutaient la race chinoise *Meishan*, et un échantillon de sanglier européen. Les populations européennes échantillonnées appartenaient à 3 catégories, à savoir des races locales, des variétés nationales de races internationales et des lignées commerciales dérivées en majorité de la catégorie précédente. Des difficultés sont apparues au cours du projet, particulièrement dans l'échantillonnage des races à petit effectif dans des conditions d'élevage extensif. Le nombre d'individus par race et la diversité des origines n'atteignaient pas le niveau recommandé dans quelques races locales. En dépit des problèmes rencontrés, qui ont compromis de manière significative le plan de travail initial, toutes les tâches prévues furent menées à bien. Le projet a montré comment des organismes du secteur public et du secteur privé peuvent travailler ensemble très efficacement. Des recommandations ont été établies durant le projet en vue de mettre en œuvre plus efficacement des projets internationaux similaires.

INTRODUCTION

The critical evaluation of livestock genetic resources and conservation of key populations are important for enabling the European agriculture and food industries to respond to future changes in consumers needs. With this aim in mind, a project was launched by the European Commission (EC) in 1998, entitled *Characterisation of genetic variation in the European pig to facilitate the maintenance and exploitation of biodiversity* (in brief PigBioDiv).

The intention was to evaluate and quantify as accurately as possible the contribution of a large and diverse sample of European pig breeds to the genetic diversity of this species. The purpose of this paper is to give a general presentation of the project, its objectives and an outline of its organisation, with a description of the populations sampled and of the biological material collected and stored.

THE OBJECTIVES OF THE PROJECT

The main objective of the project was to demonstrate the benefits of an evaluation of genetic diversity in the European pig, considering both commercial populations and local breeds, in order to enhance utilisation of the biodiversity of this species and its preservation for future generations. By using the results of previous EC-funded programmes, such as PiGMaP (1991-1996), the project also intended to ensure that European leadership would be maintained in an area of rapid development, so that European pig breeders would consolidate their world lead position. A significant benefit of the project was therefore its strong technology transfer element, as manifested in particular by the setting up of databases available on the Internet and direct involvement of end

users active in pig breeding and conservation. A new EC pig biodiversity project (PigBioDiv2) was indeed prepared shortly after the completion of this one, including Chinese partners, with the intention of having the European experience extended to China (see Blott *et al.*, 2003, in this Proceedings).

A biodiversity project should provide the reference data necessary to estimate within-breed genetic variability as well as genetic distances between breeds allowing between-breed variability to be evaluated. The design of this project mainly addressed the second issue, which may be seen as the primary focus in the conservation of domestic animal diversity (Barker, 2002). This was achieved by sampling 50 individuals from each of several different breeds and lines and determining diversity at DNA level. The project essentially followed the FAO recommendations, as outlined in the MoDAD report (Barker *et al.*, 1998), putting a strong emphasis on the use of standard DNA marker technologies, such as *simple sequence repeat* (so-called microsatellites) and *arbitrary amplification of fragment length polymorphism* (AFLP), and using high throughput genotyping devices. These aspects will be detailed in the following chapters of these Proceedings.

THE PARTICIPANTS

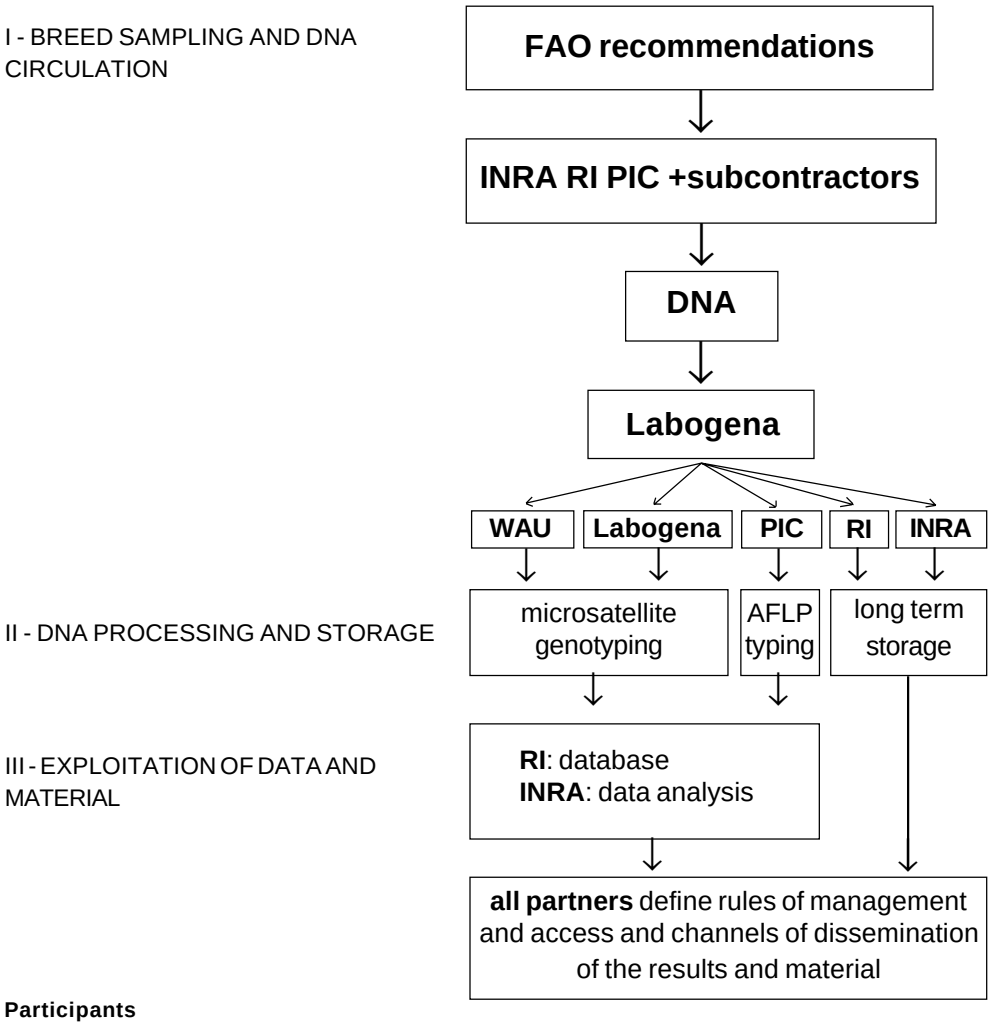
Altogether, including this project (PigBioDiv) and a previous pilot project (PiGMaP, detailed in Laval *et al.*, 2000), eighteen partners were involved in the various tasks necessary for achieving the objectives previously defined. The diversity of status of the participating teams is to be noted, as shown by the following list of participants:

- 10 Universities: Bologna (Italy), Copenhagen (Denmark), Cordoba (Spain), Ghent (Belgium), Göttingen (Germany), Hohenheim (Germany), Milano (Italy), Uppsala (Sweden), Vila Real (Portugal) and Wageningen (The Netherlands);
- 2 Research Institutes: the Roslin Institute (UK) and INRA (France);
- 1 Breeders Organisation: Agence de la sélection porcine (Paris, France);
- 1 Semi-private Laboratory: Labogena (Jouy-en-Josas, France);
- 1 Private Breeding Company: Pig Improvement Company (Abingdon, UK);
- 1 Non-Governmental Organisation: Rare Breeds Survival Trust (Shrewsbury, UK);
- 1 Inter-Governmental Organisation: Nordic Gene Bank (Aas, Norway);
- 1 International Organisation: Food and Agriculture Organisation of the United Nations (Rome, Italy).

More information on the participants in PigBioDiv is given in **figure 1**.

ORGANISATION OF THE TASKS WITHIN THE PROJECT

Five different tasks were defined and shared among the participants, namely (i) breed sampling and DNA extraction, (ii) microsatellite genotyping, (iii) AFLP typing, (iv) Data analysis (genetic distances, phylogenetic trees and genetic diversity), (v) Database set-up and dissemination of



Participants

CONTRACTORS

1 – **INRA**: SGQA-Jouy-en-Josas and Laboratoire de Génétique Cellulaire-Toulouse, France; 2 – **RI**: Roslin Institute, Edinburgh, Scotland, UK; 3 – **PIC**: Abingdon, UK; 4 – **WAU**: Wageningen Agricultural University, The Netherlands; 5 – **Labogena**: Laboratoire d'analyse génétique des animaux, Jouy-en- Josas, France; 6 – **FAO**: Animal Genetic Resources Group, Rome, Italy.

SUBCONTRACTORS

To contractor 1: **Agence de la sélection porcine**, Paris, France; **Animal Genetics Institute**, Gôttingen, Germany; **DIPROVAL**, University of Bologna, Italy, in collaboration with the **University of Milano**, Italy; **Nordic Gene Bank**, Aas, Norway; **University of Cordoba,** Spain; **Universidade de Tras-Os-Montes e Alto Douro**, Vila Real, Portugal.

To contractor 2: **Rare Breeds Survival Trust**, Shrewsbury, UK.

Figure 1. *Overall organisation of the PigBioDiv Project and contributions of the participants (in bold characters).* (Organisation générale du projet PigBioDiv et contributions des participants).

Table I. *Breeds sampled in PigBioDiv and PiGMaP (71 breeds).* (Races échantillonnées dans PigBioDiv et PiGMaP).

Country of origin	Category of population				Total
	Local breed	National variety of international breed	Commercial line	Other	
EUROPE					
Belgium	-	1	-	-	1
Czech Republic	1	-	-	-	1
Denmark	1	2	-	-	3
Finland	-	1	-	-	1
France	5	4	7	-	16
Germany	4	3	4	-	11
Iceland	-	1	-	-	1
Italy	5	3	-	-	8
Netherlands	-	1	-	-	1
Norway	-	1	-	-	1
Poland	1	-	-	1[1]	2
Portugal	1	-	-	-	1
Spain	4	-	-	-	4
Sweden	1	1	-	-	2
UK	7	-	-	-	7
CHINA	-	-	-	1[2]	1
INTERNATIONAL (PIC)	-	-	10	-	10
TOTAL	30	18	21	2	71

[1]Wild pig. [2]Meishan.

results. The diagram of **figure 1** summarises the co-operative steps which took place between the partners throughout the project.

A critical part of the overall organisation was the implementation of a proper sampling scheme of the breeds identified during the preparation of the project, and the subsequent planning of the circulation of DNA among the laboratories in charge of genotyping. Adding the 60 breeds sampled in PigBioDiv to the 11 breeds analysed in PiGMaP, a total of 71 breeds was reached[1]. The 69 European domestic breeds (excluding the European wild pig and the Chinese *Meishan*) were sorted in 3 categories, namely local breeds (30), national varieties of international breeds (18), and commercial lines (21), mostly derived from the previous category. The numbers per category and per country of origin are given in **table I**.

[1]Including the Italian *Mora Romagnola* breed only genotyped for microsatellites on DNA pools.

BREEDS SAMPLING AND DNA COLLECTION

The sampling objective was 50 animals (25 males and 25 females), obtained by selecting 2 animals of different sex in each of 25 litters, in order to have 25 sires and 25 dams, as unrelated as possible, represented in each breed sample. In small breeds this objective was sometimes difficult to reach. and the pedigree information requested could not always be provided (see **table II**). Consequently, a minimum of 25 individuals, from 13 different litters, had been set for a breed to be eligible in the analyses. Out of the 60 breeds sampled in PigBioDiv, only three were actually below this minimum of 25 individuals. The available information on pedigree diversity also showed that local breeds had a much lower number of different sires and dams represented than the other two categories (see **table II**).

DNA extraction was performed by the phenol/chloroform method (as described by Zhang and Hewitt, 1998), a procedure known to produce pure and high molecular weight DNA. Each contributor was asked to provide 500 µg DNA shared into 5 aliquots, for typing at PIC (30 µg), WAU (30 µg), Labogena (40 µg), and for long-term storage at RI (200 µg) and INRA (200 µg).

As shown in **figure 1**, Labogena took the responsibility of receiving all DNA from the participants (contractors 1, 2, 3 and subcontractors) and of dispatching the proper aliquots to each of the typing laboratories (contractors 3, 4 and 5) and to the DNA repositories for long-term storage (contractors 1 and 2). The samples provided by each participant in PigBioDiv and stored in the DNA bank are detailed in **table III**. More details on the samples from each breed, including pedigree information, can be obtained from the project web address at http://www.projects.roslin.ac.uk/pigbiodiv/.

DISCUSSION AND CONCLUSION

The various difficulties experienced in the preliminary steps described in this article should not be overlooked. Sampling rare breeds kept under

Table II. *Pedigree diversity of the European breeds and lines in PigBioDiv.* (Diversité des pedigrees des races et lignées européennes de PigBioDiv).

Category	Number of breeds		Average number per breed (range in brackets)	
	Total	With pedigree information	Sires	Dams
Local breed	24	16	12.4 (1-29)	18.8 (1-34)
International breed	14	14	28.1 (9-45)	32.1 (15-51)
Commercial line	21	21	20.6 (7-50)	30.5 (24-54)
Total (average)	59	51	19.9	27.3

Table III. *DNA samples provided to the PigBioDiv project by each participant (60 breeds).*
(Echantillons d'ADN fournis par chaque participant au projet PigBioDiv).

DNA provider[1]	Number of populations sampled	DNA delivered			DNA stored	
		Male	Female	Total	Roslin	Toulouse
INRA + Agence de la sélection porcine	12.5[2]	273	320	597[3]	597	597
Institute of Animal Genetics	11	228	319	547	521	521
University of Bologna + University of Milano	8[4]	131	158	289	276	277
Nordic Gene Bank	6	97	162	259	256	256
University of Cordoba	4	108	62	170	169[5]	171[5]
University of Vila Real	1	30	30	60	50[6]	50[6]
Roslin Institute + Rare Breeds Survival Trust	7.5[7]	147	201	348	348	348
Pig Improvement Company	10	248	250	498	498	498
Total	60	1262	1502	2768[3]	2715	2718

[1]See also the list in figure 1.
[2]Half of Meishan DNA, provided by INRA, is included here.
[3]4 pigs of unknown sex.
[4]Including one breed only typed for microsatellites on pooled DNA.
[5]Including several samples unassigned to their breeds.
[6]Different samples form those delivered for typing.
[7]Half of Meishan DNA, jointly provided by RI and PIC, is included here.

extensive conditions was a considerable challenge. The number of individuals and the diversity of origin recommended could not be met in a few breeds, as shown in **table II**. Due to unexpected delays in sampling, the schedule initially intended for the samples' delivery could not be followed, with ensuing difficulties in the circulation of DNA samples among the partners and delays in the typing tasks. In addition, because of insufficient DNA or missing aliquots, some samples had to be shared between different laboratories. The experience also showed the importance of reliable sex identification, needed when sex-linked markers are to be analysed, and of legibility of the tubes labels, in order to minimise the risk of errors in the data files subsequently generated. In spite of these difficulties, nearly all samples prepared by the various DNA providers eventually reached their final destinations and could be processed in the 3 original genotyping laboratories plus Keygene, as detailed in the following chapters of these Proceedings.

In summary, although the problems encountered significantly compromised the work plan initially intended, it was possible to complete all tasks that were proposed. The largest impact was on the time it took to complete the project. The activities pursued during this project have thus been successful in making advances in the basic experi-

mental design, operational modalities and analytical procedures to be implemented when a broad scale genetic distancing of animal genetic resources is planned. The project also demonstrated how effectively research staff, public sector and commercial entities can work together, as shown by the diversity of status of the participants. It was to be anticipated that such an ambitious project could generate a number of difficulties. This situation was turned around by setting out to provide detailed guidelines based on the experience gained, with a view to allow more efficient international implementations of projects of this kind.

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