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# IMAGE

## Innovative Management of Animal Genetic Resources

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Grant Agreement Number: 677353

Horizon 2020 FRAMEWORK PROGRAMME

**TOPIC: MANAGEMENT AND SUSTAINABLE USE OF GENETIC RESOURCES**

Topic identifier: SFS-07b-2015

**Type of Action: Research and Innovation Action (RIA)**

DELIVERABLE D 7.12

Deliverable title: International Conference on Animal Genetic Resources

**Abstract:**

The final public conference aiming to disseminate the main results and outputs of the IMAGE project was organized by UCM partner on February 5<sup>th</sup> 2020 at the Complutense University of Madrid, in Madrid, Spain. The conference was advertised using different communication channels, namely mailing lists, scientific networks, the IMAGE website and social media. The conference was entitled “Gene banks for animal genetic resources: what’s new?” and it was attended by 141 participants, coming from 35 different countries. Although many of the participants were from the academia, several of these were affiliated to other types of stakeholders, namely genebank managers, local breed managers and governance stakeholders from different countries. This broad audience, gathering participants from various countries, was attracted by the very informative program presenting the new knowledge and tools generated by the IMAGE project, which included new technologies for reproduction, better understanding of the value of genetic diversity, in relation with phenotypic variation and conservation strategies, genomic tools for genebank managers with the IMAGE SNP array, the IMAGE diversity browser, the IMAGE portal for genebank managers, and new software to enhance the use of gene bank collections. Overall, the program was composed by six sessions and a total of 18 oral presentations and 14 posters. The video recording of the conference presentations is available through the IMAGE website.

Due date of deliverable: [M44](#)

Start date of the project: March 1<sup>st</sup>, 2016

Organisation name of lead contractor: UCM

Contributors: All partners

Dissemination level: PU

Revision N°: V2

Actual submission date: [M48](#)

Duration: 48 months

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## Executive Summary

<b>Background</b>	<p>T7.2 Dissemination to the Scientific community</p> <p>T7.2 As one of the means to promote the visibility of the IMAGE project and its results among the scientific community, particularly among those working with animal genetic resources, the IMAGE project organized an International Public Conference on Animal Genetic Resources.</p>
<b>Objectives</b>	<p>Promoting and disseminating the results and tools generated by the IMAGE project to the international community targeting the scientific community working with animal genetic resources as well as other stakeholders such as gene bank managers, breeders associations and members of governmental institutions. The organization aimed to target a total of at least 100 participants.</p>
<b>Methods</b>	<p>The lead contractor of the deliverable (UCM), with the collaboration of all IMAGE partners, prepared a one-day program that included a total of six sessions with 18 presentations. This program was prepared aiming to provide an extensive overview of IMAGE outputs to stakeholders, in terms of new knowledge and tools generated by the project, that will have, in the short-term, an impact on the management of gene banks and of animal genetic resources. The conference was entitled “Gene banks for animal genetic resources: what’s new?” and it was scheduled for February 5th at the University Complutense of Madrid, in Madrid, Spain.</p> <p>This event was advertised using different communication channels, namely:</p> <ul style="list-style-type: none"> <li>a) Mailing lists (angenmap, EFFAB, dad-net)</li> <li>b) IMAGE newsletter</li> <li>c) IMAGE website</li> <li>d) Social media (twitter)</li> </ul> <p>All the presentations were recorded in video and were made available through the IMAGE website and IMAGE’s YouTube channel.</p>
<b>Results &amp; implications</b>	<p>The goal of reaching an International audience was successfully achieved. The conference was attended by 141 participants, coming from 35 different countries and from four continents namely, Africa, America, Asia and Europe, thus reflecting the effort of dissemination of IMAGE in conjunction with local organizers among the target audience, and the high interest of the topic attracting different stakeholders (National policy makers, National genebank managers, private genebanks, etc.). Besides the dissemination of new knowledge generated by IMAGE regarding animal genetic diversity and its conservation, this conference was crucial in order to disseminate to the community some key results, including:</p> <ul style="list-style-type: none"> <li>a) gap analysis of European gene banks;</li> <li>b) economic optimization and ethical and social values of gene banks</li> <li>c) new methods and knowledge for the preservation of animal genetic resources and gene bank collections</li> <li>d) databases for the characterization of genetic resources</li> <li>e) discussion of new prospects for the future of animal gene banks</li> </ul>

## Main features of the conference

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### Gene banks for animal genetic resources: what's new?

**Aim:** To promote an efficient dissemination of IMAGE outputs to the scientific community and stakeholders.

**Scope:** With the aim of disseminating IMAGE outputs, a program was prepared in order to present to the community of scientists and stakeholders the following main topics:

- Current analysis of animal gene banks in Europe (gap analysis, economic optimization and ethical and social values)
- New methods for the preservation of animal genetic resources
- New knowledge on animal genetic resources obtained from gene bank collections
- Collecting and connecting data to characterize genetic resources
- Enhance the use of gene bank collections for fitter livestock farming
- Prospects of IMAGE for the future of animal gene banks

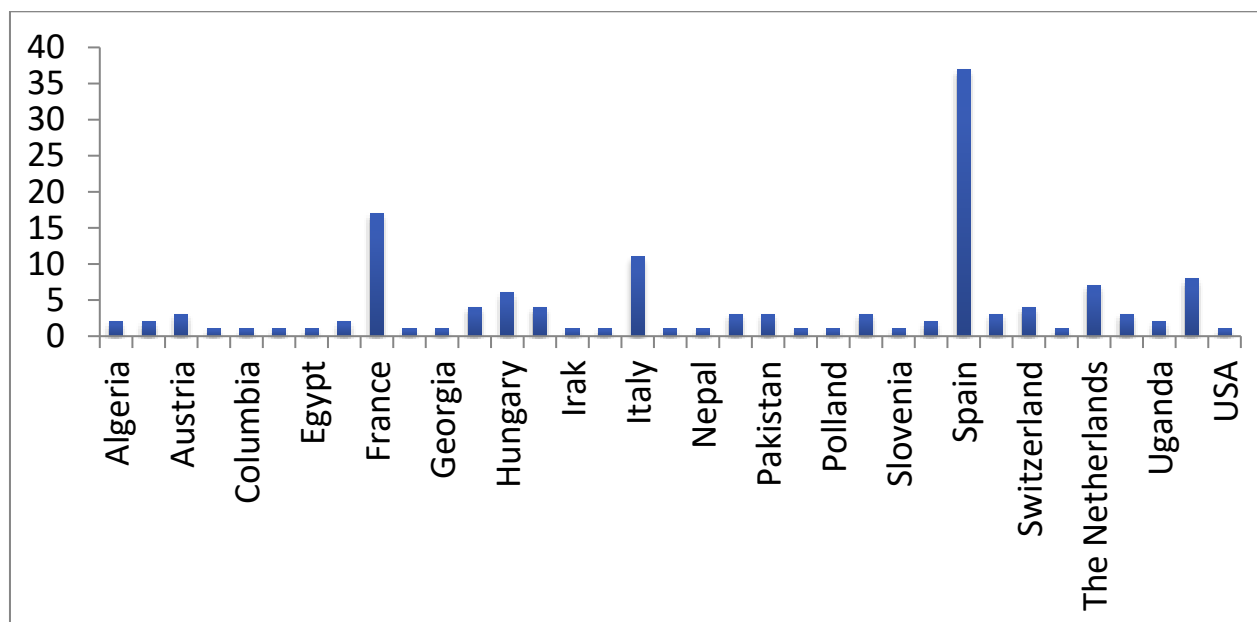
The programme featured six sessions with 18 talks and one poster session showing 14 posters among those developed under IMAGE project that were previously exposed in various scientific conferences. This showed the progression of the work from year 1 to year 4 of the project.

**Target:** This meeting aimed at targeting an international audience, guaranteeing a widespread impact of IMAGE project results.

**Results:** In total 141 participants (Figure 1) attended the conference coming from America, Africa, Asia and Europe. The conference attracted members of the international scientific community who work in animal genetic diversity, but also succeeded in attracting other stakeholders, including managers of gene banks, representatives of breeders' associations and officers of governmental bodies related with the management of animal genetic resources. As shown in Figure 1, given the location of the Conference, a strong participation of the Spanish community was observed, but the list of attendants achieved a global widespread distribution, as desired.

Dates: 5 February 2020

Venue: Salón de Actos, Facultad de Veterinaria, Universidad Complutense de Madrid, Spain



**Figure 1- Distribution of participants of the IMAGE final meeting per country.** Bars represent the total number of participants in each country.

### Points from the discussion

There were questions and issues to consider for follow-up studies:

Gene banks are at a crossroad of multiple expectations and opportunities, for research as well as for livestock farming.

Ethical considerations are gaining a growing importance in the preservation of animal genetic resources and need to be considered in the routine operations of gene banks.

Gene banks need to combine different objectives as well as to take into account contrasted expectations from stakeholders

It is recommended to consider all local populations and not only the most endangered ones

It will be useful to enlarge the options to be considered for economic optimization

Coordination across countries is important for the rationalization of genebank collections within and between countries,

Disseminating knowledge to the general public about the preservation of animal genetic resources and the advantages of the new reproductive technologies is a challenge in order to reach informed acceptance of innovations.

Keeping resources on the long term are useful, as demonstrated in several case studies, for breeders as well as for research

Important training needs remain in order to support more innovative use of gene bank collections, going from preservation to dynamic conservation of gene bank collections

## PROGRAM

Wednesday 5 <sup>th</sup> February 2020		
Time	Event	Speaker
09:00	Welcome from the Dean of the Veterinary Faculty	Consuelo Serres
09:05	Introduction	Michèle Tixier-Boichard
<b>Session 1: Current analysis of animal gene banks in Europe and options for optimization</b>		
09:10	Pan-European gap analysis of livestock gene bank collections	Gregoire Leroy et al.
09:30	Economic optimization of national and pan-European gene bank collections	Rafael Silva et al.
10:00	Ethical and social values associated with gene bank collections	Michèle Tixier-Boichard et al
10:30	<i>Coffee break and poster session</i>	
<b>Session 2: New methods to preserve genetic resources</b>		
11:00	Strategy for biobanking avian resources: Advantages and limits in the implementation of a sperm cryobank	Julian Moreno & Elisabeth Blesbois
11:20	Poultry stem cells for biobanking avian genetic resources	Mike Mc Grew et al.
11:30	A hybrid model to restore resources of avian species using PGC or gonadal tissue transfer.	Krisztina Liptoi et al.
11:40	Modelling vitrification for mammal embryos.	Henri Woelders & Florence Guignot
12:00	<i>Lunch</i>	
<b>Session 3: New knowledge on genetic resources obtained from gene bank collections</b>		
13:20	Which genes to trick to grow feathered feet?	Chiara Bortoluzzi
13:40	Reading the history of the Asturiana de los Valles breed in its genome	Susana Dunner et al.
14:00	Deciphering adaptation to environment by landscape genomics	Paolo Ajmone-Marsan et al.
<b>Session 4: Collecting and connecting data to characterize genetic resources</b>		
14:30	A new standard to compare gene bank collections: the IMAGE multi-species SNP chip	Richard Crooijmans
15:00	IMAGE data portal and tools for efficient access and use of information	Alessandra Stella

<b><i>Session 5: Enhancing the use of gene bank collections for fitter livestock farming</i></b>		
15:30	MoBPS – a web-based tool to make optimal use of genetic resources in breeding programs	Henner Simianer & Torsten Pook
15:45	Breeding programs in the South American Creole cattle	Gabor Mezaros et al.
16:00	Trade-offs between genetic diversity and genetic merit when using gene bank bulls	Harmen Doekes & Jack Windig
16:15	Painting eggs in blue	Claudia Dierks
16:40	Coffee break and poster session	
<b><i>Session 6: Prospects from IMAGE for the future of Animal Gene banks</i></b>		
17:10	The role of capacity building and updated FAO guidelines to support gene banking strategies	Luis Telo de Gama
17:30	Expectations from breeders	Manuel Luque
17:45	Protein markers in the individual goat semen for quality / fertility prediction	Elisabeth Blesbois
18:00	General discussion about future animal genetic resources	Sipke Joost Hiemstra
18:15	Concluding remarks	

## Abstracts

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## Introduction

M. Tixier-Boichard<sup>1</sup>, project coordinator

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Animal genetic resources stand at the crossroads of major societal and scientific challenges: erosion of biodiversity, Nagoya protocol, genomic revolution, move towards agroecology... Animal gene banks are one important component of a strategy towards the preservation and sustainable use of genetic resources. An important step made with IMAGE has been to involve semen banks (i.e. gene banks keeping reproductive material) as well as genomic banks (i.e. gene banks keeping blood, DNA, tissues) in the same effort. Some gene banks keep both semen and blood or DNA, but these two types of banks are often separated while they are fully complementary. IMAGE has addressed all important challenges for the management and exploitation of gene banks for research as well as for livestock farming systems. It has fostered pioneer research on several issues in animal breeding and genomics, reproductive physiology, sociology, economics, informatics and bio-informatics, that will be illustrated in this final conference.

The first session will focus on the new knowledge produced on the general landscape of animal gene banks in Europe : inventory and gaps, possibilities for economic optimization, expectations of stakeholders and ethical considerations. The second session will show the progress made on the quality of cryopreserved reproductive material in birds and mammals, in order to hold the promise of breed recovery. The third session will provide striking examples of the new knowledge obtained on farm animals by characterizing gene bank collections with genomics, starting from identifying causal mutations to unravelling past history and adaptation to environment. The fourth session will feature two major innovations of IMAGE for gene bank operations : a new standard molecular tools to facilitate assessment of genetic diversity hold in gene banks, a new framework to connect all data about gene banks and genetic resources via the IMAGE portal and its discovery tool. The fifth session will illustrate the diversity of possible uses of gene banks, illustrating innovative ways of managing genetic diversity, going beyond breed recovery which was often the initial incentive for gene banking. The sixth session will underline the importance of training and updating guidelines for gene banking. It will give the floor to breeding companies, before opening the general discussion on an integrated strategy for genetic resources in agriculture and forestry.

## **Pan-European gap analysis of livestock gene bank collections**

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To assess the gaps in cryomaterial collections, we combine livestock breed-related data from the Domestic Animal Diversity Information System (DAD-IS) and information from questionnaires sent filled by gene banks managers from 15 European and 2 non-European countries on material stored for livestock breeds. Out of the 2949 breeds registered in DAD-IS for these 17 countries, 15.9% have been reported to have material stored in gene banks, but only 4.3% have material sufficient to allow breed reconstitution. The proportion of breeds with stored cryomaterial stored is greater than 20% for ruminants and pigs, between 10% and 20% for equids, and below 10% for rabbit and avian species. There are significant differences between countries in the proportion of populations collected for cryostorage: breeds not-at-risk and transboundary breeds are more likely to have cryomaterial preserved than other breeds. These results highlight the need for increased efforts in collecting material for at-risk local breeds and improved regional coordination of cryoconservation of transboundary breeds.

## Economic optimization of national and pan-European *ex situ* gene bank collections

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Commercial livestock production is dominated by a limited number of specialized breeds, displacing many local breeds, which are at risk of extinction. This concentration potentially forecloses future breeding options and the adaptability to changing market and production (e.g. climate) environments. Improvements in storage of genetic and reproductive materials *ex situ*, offers an alternative for breed conservation, but existing collections should be optimised cost-effectively to avoid duplication, and with reference to *in situ* breed status. We develop optimisation models to explore the potential cost savings from collection rationalisation, avoiding duplication and redundancy. A mixed integer programming model is used to identify the least cost collection and storage strategies for 11 European gene banks under a collective budget constraint, and allowing cross-country collections. The results show a potential cost saving of 20% by selecting cryogenic banks that have relatively lower combination of fixed and collection costs, and are geographically closer to collection regions. A second example develops a multi-period chance-constrained optimization model to rationalize Spanish *ex situ* collections under projected *in situ* extinction risk based on census data of 180 Spanish autochthonous breeds. We find that collection costs are sensitive to the accepted level of *in situ* extinction risk up to a threshold value, after which costs plateau. We suggest that an optimisation approach offers guidance on data collection for the design of efficient *ex situ* collections.

## Ethical and social values associated with gene bank collections

M. Tixier-Boichard<sup>1</sup>, A. Doré<sup>2</sup>, D. Laloë<sup>1</sup>, E. Charvolin-Lemaire<sup>1</sup>, W. Kugler<sup>3</sup>

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In comparison to human biobanks, collecting animal gametes, tissue samples or DNA does not seem to be an activity likely to encounter huge ethical problems or issues. Yet, biobanking for domestic animal is tightly connected with society, the economy, politics and, after all, with ethics. IMAGE was challenged to explore how ethics may illuminate the ways biobanks are used and managed. Indeed, biobanks embody different images of the future of animal genetic resources and agriculture, suggest and/or enact particular interactions between research and industry, incorporate conceptions of the living, the animals, the farmers, the technologies and the way they should be used. To capture these dimensions, IMAGE has used three approaches: a dialogue forum, surveys, directed interviews.

The dialogue forum gathered once a year the national coordinators of genetic resources, NGO representatives and breeders representatives. The main topics discussed were the animal health regulations, the economic optimization and the Nagoya protocol. One striking outcome was the possibility to introduce specific measures for gene banks in the delegate act on germplasm of the new EU animal health law. The public was generally well informed about gene banks.

An ethical survey was launched and filled in by the participants at various meetings and events dealing with biobanks and breed conservations. A set of 159 answers was collected, from a variety of profiles, scientists, students, breed managers and biobank managers. Section 1 was aimed at collecting general information about the expertise and the field of activity of the respondent. Section 2 addressed the motivations for breed conservation, the desired decision-making process, the possible balance between cryopreservation and conservation of live populations. Section 3 addressed the acceptance of the technical innovations associated with gene banking with possible trade-offs. Answers supported the perception of genetic resources as a public good. Results also showed that a risk/benefit approach could be considered for the use of invasive technologies or sensitive biotechnologies (cloning, transgenesis). Motivations for gene banking were still very classical, showing that methods for innovative use of gene banks, such as those developed by IMAGE, need explanations, training and success stories. Innovative challenges for animal genetic resources (AnGR) management do not constitute “issues” that circulate out of the social worlds of animal production and conservation.

Two ethnographic studies were conducted by observation and open-ended interviews, with European biobank managers, and French farmers who practice *in situ* conservation. Apart from some of the breed society leaders, the set of technics & practices of *ex situ* conservation was largely unknown and foreign to the farmers practicing *in situ* conservation. These actors keep a local breed in a traditional farming system, used well before the creation of the *ex situ*

technique, and their conservation practices involve the establishment of social links to exchange or purchase animals. They do not catch easily the benefit they may get from gene banks, even though their role remains fundamental for initial sampling and for end-using of the stored material in biobanks. The role of advisers may be crucial at that point.

In conclusion, gene banks are well-known by a specialized public and should be popularized with the support of concrete actions matching the interest of the different types of stakeholder:

- As a key preservation of diversity for NGOs and farmers motivated by local breeds,
- As a support to selection for breeding companies,
- As opportunities for research on genetic diversity and on reproductive physiology,
- As a leverage for a European strategy on animal genetic resources.

## Strategy for biobanking avian resources: advantages and limits in the implementation of a sperm cryobank

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Half of the domestic poultry breeds are currently considered to be endangered. The main causes of threat are the inbreeding, the restructuring of rural areas, the intensive farming and the commercial needs. The cryo-banking has great potential for application in *ex situ in vitro* conservation. Conservation of native breeds with unique characteristics or traits could help with the identification of specific genes involved in natural disease, parasite control, and thermal stress. In addition, they are useful model to basic biological research into physiology, diet, reproduction or climatic tolerance at the physiological and genetic level. Sperm are the most accessible sex cells and are currently the main type preserved in the majority of genetic resource banks. Sperm cryopreservation is the less expensive method available and the most feasible method in birds as cryopreservation of oocyte or embryo is not possible because of large size, high lipid content and polar organization. Sperm banks allow to have precious germplasm for long period of time and/or to restore genetic diversity by mean artificial insemination.

However, several factors limit the implementation of a chicken sperm cryobank. Sperm frozen-thawed still shows very low and variable reproductive success rates following insemination with thawed semen, which limits the use of gene banks. This is mainly due to the high sperm susceptibility to cryodamage during freezing-thawing process. Many traits of rooster sperm determine the high susceptibility to cryodamage: the spindle form with very low cytoplasmic content, the very long flagellum, the low sperm osmotolerance, the oxidative stress sensitivity, and the rapid induction of acrosome reaction *in vitro*. In addition, rooster sperm contains no cysteine residues and lacks the potential stabilizing effect of S-S bonds of mammalian sperm chromatin; this produces a high susceptibility of the DNA to the cryoinjury. Different endogenous and environmental factors may also affect the sperm response to freezing-thawing process. Recent reports have showed that there was an influence of breed on the percentage of viable sperm after freezing-thawing. Thus some breeds return the best freezing-thawing response unlike other with lower cryoresistance. The presence of seminal plasma also affected sperm response to freezing-thawing process, and thus removal of seminal plasma decreased the variability of the results and DNA fragmentation damages. Environmental factors, firstly photoperiod but also temperature, influence on the seasonal variation in freezing damage in free-range rooster sperm. Thus, the semen collection season influences most frozen – thawed sperm motility values: the percentage of sperm showing progressive motility is higher in spring-collected sperm compared to winter-, summer- or autumn-collected samples; the curvilinear velocity, straight-line velocity, and average path velocity values of spring-collected sperm are also higher. Social interactions are also important in the control of sperm quality. Compared to roosters with no-female-contact, the roosters living with hens show higher percentages of progressive motile sperm and increased the



curvilinear velocity, straight-line velocity values; these sperm samples with better initial quality usually return higher post-thaw motility.

## Poultry stem cells for biobanking avian genetic resources

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Preserving poultry and avian biodiversity needs the development of new techniques cryoconserve bird species. Avian gene preservation research has recently focused on the use of the early precursors of the reproductive cells, the primordial germ cells. Avian PGCs have a unique migration route through the vascular system which offers easy accessibility in the young avian embryo. These few germ isolated germ cells can be cultured *in vitro*, frozen, injected into a surrogate host recipient embryo and raised to sexual maturity. The efficient recovery of the donor genotype and the frequency of germline transmission from the surrogate host animals are still areas which need further investigation. Here, we will tell you our efforts and successes in the cryopreservation and reestablishment of local chicken breeds in both France and Hungary.

Recently we have developed in France reproductive biotechnologies based on PGCs using as a model a local breed La Poule Noire du Berry (NB), which have benefited from a national program of conservation. The *in vitro* steps that PGCs undergo before their use for reproduction may affect their integrity and reproductive capacities. In this study we investigated the effect of the culture duration and cryopreservation (comprising cell freezing, thaw and re-amplification *in vitro*) on the gene expression, DNA methylation, and germline transmission rate of NB PGCs. RNAseq study and RRBs analysis revealed strong gender differential effect of long term *in vitro* cultures on transcript abundances and DNA methylation of PGCs. Cryopreservation affected DNA methylation in PGCs of both sexes, but not their gene expression. We obtained for the first time in France the progeny from *in vitro* derived and cryopreserved PGCs. The germline transmission rate for female and male NB PGCs was up to 60.6% and up to 42.4% respectively, and seemed to vary according to the culture duration in a gender-specific manner.

In case of the Hungarian poultry breeds, we successfully produced PGC lines from Partridge coloured Hungarian, White Hungarian, Yellow Hungarian, Speckled Hungarian furthermore from Black and Speckled Transylvanian Naked neck breeds. All together 165 PGC lines were established and 990 cryotubes were stored in our gene bank. For every individual cell line at least 6 parallel cryotubes were stored in liquid nitrogen. We chose the Partridge coloured Hungarian to verify the whole gene preservation process. After injecting the donor cell line into the recipient embryos, 24 presumptive chimaeras had been produced and in 4 cases the

germline chimaerism was proved (16,6%). These 4 chimaera birds produced 14 donor-derived hatchlings in total, which is a 4,1% success rate.

These results will serve to better master *in vitro* culture conditions and improve germline transmission rate of avian PGCs.

## **A hybrid model to restore genetic resources of avian species using PGC or gonadal tissue transfer**

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Nowadays - regarding to avian species – semen freezing is the only practically used preservation method. Cryopreservation of oocytes and embryos is impossible, because of their biophysical traits. Furthermore, the females are heterogametic, the males are homogametic therefore the female genome falls out of gene conservation. For the maintenance of it, two suitable methods are available: the orthotopic transplantation of early ovary of day old chicks and the transfer of primordial germ cells (PGCs).

The aim of the recent investigations was to improve the gonadal tissue transfer procedure of newly hatched chicks as well as to create interspecies sterile hybrids as a potential surrogate host of PGCs.

Regarding to the gonadal tissue grafting, successful donor/recipient combinations were formed for 5 indigenous Hungarian chicken breeds. An effective freezing/thawing method was improved, which resulted that the adherence of grafted frozen/thawed gonads is similar to native ones (72% vs. 80%). Donor derived progeny was obtained from frozen/thawed chicken ovarian tissue, which was published for the first time. The original breed can be 100% regained in the first offspring generation.

Referring to interspecies hybrids crossing Guinea fowl with domestic fowl was successful but only with female Guinea fowl crossed with male domestic fowl. The observed offspring from the successful crossing were sterile male hybrids. These sterile hybrids have endogenous germ cells but they cannot develop into sperm cells. The sterile hybrids produced in this study might be suitable recipients for male and possibly for female chicken PGCs as well.

In the future, a sterile hybrid as a general recipient could be a proper solution in the case of tissue transfer too.



## Modelling vitrification of embryos and oocytes

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Osmotic events during cryopreservation may damage cells and tissues. These events include strong changes of solute concentration, the ‘water concentration’ and the volume of the cells. During so-called ‘slow freezing’, the growth of (extracellular) ice leads to very strong increase of solute concentrations and osmotic strength of the remaining ‘unfrozen fraction’, causing efflux of water from the cells, which strongly shrink and will also have extreme rise of osmotic strength in the cytoplasm. But also addition and removal of cryoprotective agents (CPAs), such as glycerol, DMSO, EG, etc. leads to very strong volume changes and levels of dehydration of the cells.

The understanding of these events has led to improved cryopreservation, such as stepwise addition and removal of CPAs. Description of the osmotic events can be made more specific and quantitatively accurate by using mathematical (mechanistic) modelling. Moreover, modelling allows us to ‘look inside cells’ as we can accurately calculate the intracellular concentrations of water or solutes at every point in time. This is possible by using established descriptions of membrane transport and laws of physical chemistry. Central in this are two ordinary differential equations that describe the membrane fluxes of water and CPA. These are ‘linked’ equations, i.e. the result of the first changes variables in the second, and vice versa.

Such equations have been used to predict the chance of lethal intracellular ice formation (IIF) during ‘slow-freezing’ at various assumed (constant) cooling rates (e.g. Mazur, 1963; Liu et al., 2000). We (Woelders and Chaveiro, 2004) have developed a different model that does not assume a pre-chosen constant cooling rate. Instead, it allows cooling to be as fast as possible to prevent ‘slow cooling damage’, within the constraint that it is just slow enough to prevent causes of ‘fast cooling damage’ such as IIF. This results in non-linear (sigmoidal) freezing curves, in line with empirical observations and theoretical cryobiology.

Within IMAGE, we have applied similar modelling to optimize addition of CPAs in protocols for vitrification of oocytes and embryos. In vitrification, very high CPA concentrations are used, which are damaging for the cells. Damage may be reduced by following a two-step addition of CPAs, in which cells are first equilibrated with ‘half strength’ equilibration solution (ES, step 1), and are then placed briefly in ‘full strength’ vitrification solution (VS, step 2). Further ways to reduce toxicity include using lowest possible concentrations and shortest possible duration of exposure. In addition, high sucrose concentrations may be used extracellularly which enables vitrification at lower CPA concentrations. Modelling allows us to see the consequences of certain steps, and to show which empirical experiments seem most meaningful. In this way, the number of (laborious, time consuming, expensive) experiments can be reduced. For example, we showed by modelling that the use of high extracellular sucrose concentrations in VS to replace part of the CPA is likely not beneficial, as the intracellular CPA concentration is not reduced by the use of extracellular sucrose and the cells become severely shrunken and dehydrated at the moment of vitrification. In fact, CPAs that

have entered the cells in step 1 are ‘driven out’ of the cells in the second step if VS contains high sucrose.

For horse oocytes, one of the protocols described in the literature uses very short exposure times (30 seconds in ES, 30 seconds in VS). We have used modelling to see if the short protocol would allow sufficient penetration of CPAs into the cells and would warrant ‘vitrifiability’ of the cytoplasm. The modelling showed that CPA penetration is less in the very short protocol than in the longer protocol, but is still significant. More importantly, due to the efflux of water, intracellular osmolality reaches equilibrium with VS in step 2 within only seconds of incubation (Woelders et al., 2018). This would effectively preclude IIF, i.e. would ensure ‘vitrifiability’ of the cytoplasm. The short protocol was indeed effective and resulted in the world wide first live born foal from vitrified immature horse oocytes (Ortiz-Escribano et al., 2018).

Next, we have also applied similar modelling to porcine embryos, with similar conclusions (Woelders et al., 2018). Empirical experiments were subsequently done to test vitrification of pig embryos, using a long and a short two-step CPA loading protocol. The ‘long’ protocol was 3 min in ES and 1 min in VS (Berthelot et al., 2000; Cuello et al., 2016). The short protocol was 2 min in ES and 0.5 min in VS. In the first two experiments, *in vitro* survival appeared better for the short protocol in the first replicate, but no advantage was seen in the second replicate. Live born piglets were obtained after surgical embryo transfer of embryos vitrified with the short protocol (Guignot et al., AETE, 2019).

In a recent new experiment, embryos vitrified with the long and the short protocol and control embryos were studied after 24h of *in vitro* culture. Real time PCR showed higher expression of two genes, EMP1 and ANXA8, in vitrified embryos compared with control embryos, but no differential expression was seen for the 3 other genes previously found differentially expressed with RNA sequencing (Almiñana et al., 2019). The 2 differentially expressed genes could thus be taken as biomarkers for embryo quality. The *in vitro* survival was lower in embryos vitrified with the short protocol than in embryos vitrified with the long protocol, but the difference in expression levels of the marker genes between the two vitrified groups was not significant. These results do not indicate an advantage of the shorter protocol for pig embryos.

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## Which genes to trick to grow feathered feet?

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Ever since Charles Darwin published *On the Origin of Species*, scientists have been fascinated with understanding the genetic basis of similar phenotypes shared between lineages. Animal evolution offers many examples of traits evolving by parallel evolution. However, the genomic basis of many phenotypes has not yet been fully understood as appropriate model species are often lacking. Domesticated species can provide interesting insights into the genomic architecture of traits that evolved in parallel and the selection that has acted on them. Among vertebrates, domestic chicken (*Gallus gallus domesticus*) is an excellent model, as many of the domesticated phenotypes are common to other domesticated avian species and, in some cases, even to more distantly related species.

Ptilopody (or foot feathering) is a polygenic trait that can be observed in domesticated and wild avian species and is characterized by the partial or complete development of feathers on the ankle and feet. Although recent studies have tried to associate ptilopody to a certain genomic region, it is still unclear which chromosome(s), gene(s), and mutation(s) are directly responsible for the phenotype. A better understanding of the genomic architecture underlying ptilopody comes from a recent study in domestic pigeon. Ptilopody in chicken and pigeon is extremely similar in appearance and this similarity is partly explained by the same genes involved. Even though the same genes are involved, the question is whether a similar underlying mutation has enabled the trait to evolve in both lineages. We here addressed this question from a molecular and evolutionary perspective, providing evidence for a parallel genetic origin of ptilopody in chicken and pigeon.

The molecular mechanisms underlying foot feathering in chicken were unravelled by a combination of whole-genome sequencing, expression analyses, and comparative genomics. DNA of 169 samples from 87 traditional chicken breeds sampled in the Netherlands and Germany was used for whole-genome sequencing (WGS). The loci associated with ptilopody were identified through a standard case/control genome-wide association study. The loci were also subsequently screened for signatures of selection, which were identified by the corrected pooled heterozygosity. To provide more insights into the functional importance of our candidate mutations we identified conserved elements (CEs) throughout the chicken genome using the 23 sauropsids multiple sequence alignment generated by Green al. (2014). We also harvested forelimb and hindlimb buds from 21 chicken embryos sacrificed at Hamburger Hamilton (HH) stage 35 (n=11) and HH39 (n=10) to generate RNA-seq data to investigate changes in expression in our candidate genes.

The genome-wide association analysis identified two genomic loci associated with ptilopody. At both loci, foot feathered individuals displayed elevated levels of homozygosity relative to scaled birds, a clear signature of positive selection.

At one of the loci we identified a 17 kb deletion upstream a gene known to encode a transcription regulator of hindlimb identity and development. The 17 kb deletion in chicken perfectly overlaps the 44 kb deletion found in pigeon by Domyan et al. (2016). The 7 bp microhomology identified at the two deletion breakpoint junctions indicates that this structural variant has emerged in chicken and pigeon multiple times independently.

At the second loci we identified a protein-non-coding variant overlapping a novel lncRNA located upstream a gene involved in forelimb identity and a key determinant of foot feather development. The variant was used to reconstruct a 4 kb haplotype, which was identical in all foot feathered birds, indicating that the trait evolved only once after the domestication event. To understand the evolution of foot feathering, we looked for presence of conserved elements (CEs) in the 23 sauropsids multiple sequence alignment and found that only three variants overlapped a conserved element, suggesting that these variants have a high functional importance.

We further analyzed the gene expression profile of our candidate genes. We found that the gene encoding a transcription regulator of hindlimb identity and development was significantly downregulated in the hindlimb of foot feathered birds at HH35 (*q-value*: 1.79e-03), but not at HH39 (*q-value*: 0.38). On the contrary, the gene involved in forelimb identity was always significantly upregulated in foot feathered birds at both embryonic stages (HH35 *q-value*: 2.49e-14; HH39 *q-value*: 6.87e-03).

Foot feathering is an interesting example of a polygenic trait that has evolved by parallel evolution as its parallel evolution is mirrored in almost every detail at the molecular and, most likely, developmental level. In this study, we showed that, although chicken and pigeon diverged more than 89 million years ago (Myr), in both avian species the exact same number of loci containing the exact same set of genes are involved. This similarity is even more striking as a similar deletion at one of the loci has the same outcome in regulating gene expression.

## Reading the history of the Asturiana de los Valles breed in its genome

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Past events of positive selection leave characteristic signatures in the genetic diversity of a population, which can be detected by genome-wide scans based on present time molecular data. However, determining the adaptive trait or the onset and intensity of selection at a given locus is often difficult from such data. By providing direct access to the temporal evolution of allele frequencies, the analysis of genomic data extracted from gene banks might significantly improve our understanding of selection history in livestock species. The aim of this study is to evaluate whether the analysis of genomic samples collected at different times in the recent past allows (i) detecting recent selection events and (ii) annotating selection signatures found by classical approaches based on present time data only. To answer these questions, we considered the case study of the Spanish bovine breed Asturiana de los Valles (RAV), for which genotyping data was available for 137 animals with birth dates between 1980 and 2010. Fifteen additional RAV animals born in 2008-2013 were sequenced at ~8X coverage. These data were used to detect historical selection signatures in RAV using a classical statistic (nSL) based on a single sampling time. A new statistical approach allowing detection of selection from genomic time series was applied to the combined dataset including nine distinct generations. The time series approach combined with a statistical method allowing to detect clusters of small p-values pointed out several candidate regions with a clear shift in allele frequencies over the few last generations. The time series and nSL approaches detected 13 candidate regions under selection in RAV including genes related to carcass and meat traits (such as *MSTN*, *RBPM2* or *OAZ2*), immunity (*GIMAP7*, *GIMAP4*, *GIMAP8*), olfactory receptors (*OR2D2*, *OR2D3*, *OR10A4*, *OR6A2*) and milk traits (*ARFIP1*). Thus, the combined time series and nSL approach are complementary and should be extended to other populations where temporal data can be extracted from gene banks. These results outline that gene banks represent a great resource for the understanding of breed history and the detection of relevant functional genes and variants.



## Deciphering adaptation to environment by landscape genomics

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The main objective of IMAGE is to fully exploit the potential present and future value of farm animal genetic resources biobanked material. The identification of genes having adaptive value contributes to reach this objective, considering the presently occurring rapid changes in climatic conditions. Climate change affects livestock in multiple ways, directly, in some case decreasing animal welfare and productivity, and indirectly, changing pasture and both feed composition and availability, and parasite, vector and pathogen range. Once identified, adaptive genes can be used to characterize biobanked samples for their potential reserve of adaptive value, to optimize the choice of samples to be newly introduced in gene banks and to set up genotyping and breeding tools to improve livestock adaptation and welfare.

SNP genotyping and whole genome sequences were used to associate genome variants of sheep to environmental variables using a landscape genomics approach. Genes that contain or are adjacent to significant SNPs have been identified and analysed for function, involvement in metabolic pathways, association to traits in livestock and other species and investigated for variants likely having a functional effect.

More than 1100 geo-referenced sheep samples from 77 breeds distributed in eight environmental cluster and 32 subclusters have been characterized at 600,000 SNP markers. Landscape genomics analysis identified significant association between environmental variables and more than 483 candidate genes, mostly associated to temperature or temperature associated environmental variables. These are involved in fundamental metabolic mechanisms for adaptation to different environmental challenges as immune response, energy metabolism, morphology and behaviour. The same dataset was analysed with two independent selection signature approaches one based on  $F_{st}$  and the second on the method implemented in PCAdapt software. Overall 26 candidate genes detected by the three methods have strong evidence of having an adaptive role. Among these are CUB, involved in B12 vitamin uptake, ABCG2, associated to milk production, RXFP2, associated to horn development and morphology. Sequence data permitted the identification of candidate variants under selection. Significant SNPs have been included in the low-density SNP array developed in IMAGE and may be used for the characterization of sheep biobanked material, the choice of novel material to be stored and for breeding purposes.

## **A new standard to compare gene bank collections: the IMAGE multi-species SNP arrays**

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Animal genetic material is stored in centres of genetic resources in many countries of the world. What is exactly stored in each centre is not known. Internally the species, breed, sex are known but in most cases no molecular information is available. To benefit from these resources we need to open them by knowing what is stored by transferring sample information to a general public database (BioSamples). In order to compare within or even over countries for similarities and differences between samples molecular profiling is needed. Molecular profiling with single nucleotide polymorphisms (SNPs) is an important tool to perform this comparison but there are too many different arrays per species available which are often biased to commercial population and not always free to use. We developed a multi species SNP array including 6 species (cattle, pig, chicken, horse, sheep and goat), each species with 10K SNPs, more directed to characterize traditional breeds. The DNA markers per species do cover their genome and overlap with existing arrays for around 80% in order to compare already genotyped populations. Per species additional markers are added covering the sex chromosomes, mtDNA, ancestral SNPs, trait markers, MHC markers and variations in genes within QTL regions. This newly developed SNP array can be used without restrictions worldwide at low cost. The genotype information of the samples in BioSamples can be easily connected to the sample info and made public in order to make a real comparison between samples possible.

## **The Innovative Management of Animal Genetic Resources (IMAGE) unified data portal to integrate and represent European gene bank data**

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One key challenge that this project addresses is the integration and transparent use of the vast information stored within more than 60 gene banks/genetic collections spanning 20 European countries, together with the collection of newly generated IMAGE data. This imposed a major strategic challenge for the management and conservation of animal genetic resources due to the huge amount of heterogeneous data distributed across gene banks in different locations, storage formats and languages. The IMAGE web portal integrates data from gene banks and collections with genomics data, geographical information systems data, and other information generated by IMAGE

The solution we implemented comprises 1) a well-defined metadata rule set ensuring high quality and comparable data across the diverse collections originating in different storage formats and languages, 2) development of a single Inject tool helping gene bank managers to enhance, standardise, tag and submit their gene bank data to a Common Data Pool that integrates all gene bank records from across Europe, 3) the sustainability offered by archiving of this data within the EBI BioSamples public archive and 4) a bespoke data portal that integrates gene bank metadata with generated 'omic datasets from within IMAGE and cross referencing to other gene bank and breeding database resources from across Europe such as those hosted by the Food and Agriculture Organisation (FAO). Within the data portal, a Geographic Information System tool is included to assist the user in identifying/storing the geographical origin of the samples as well as displaying individual/population genetic parameters and biological attributes through interactive maps. Querying across all types of data is also expected to facilitate targeted search to identify genetic material of interest residing somewhere in the partner gene banks and collections. Furthermore, starting from data derived from the portal, computing tools and methods have been developed to browse the diversity of sample and/or genomic data. The Diversity Browser is a stand-alone tool that computes principal component analysis (PCA) of a reference dataset and a batch of samples of interest. Finally, an interactive web interface to guide the use of genetic material was created. It allows selective downloading of collection and genotype information to be leveraged using a linked R software package (MoBPS) that provides a computationally efficient and flexible framework to simulate complex breeding programs and compare their economic and genetic impact



## **MoBPS – A web-based tool to make optimal use of genetic resources in breeding programs**

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Breeding and conservation programs are complex by nature. They typically can be represented by boxes (nodes), standing e.g. for cohort of animals of similar type, and arrows (edges), representing breeding activities like selection or reproduction. We developed a conceptual approach on the basis of the assumption, that any breeding program can be constructed in a modular form as a combination of nodes and edges. Based on this theoretical concept, a software ‘Modular Breeding Program Simulator’ (MoBPS) was developed. MoBPS consists of an R-package, which simulates breeding programs under this concept and is publicly available through github (<https://github.com/tpook92/MoBPS>). The R-package is characterised by a high computational efficiency through the use of C/C++ functions in critical steps. To make the package as user-friendly as possible, we developed a web-based graphical user interface in JavaScript, that allows to describe breeding programs in a very intuitive way, accessible through [www.mobps.de](http://www.mobps.de). A MoBPS-job typically consists of four steps: (i) define the ‘breeding setup’, i.e. the number of populations to work with, traits and their genetic characteristics, phenotyping and selection patterns etc.; (ii) draw the breeding program in a very intuitive ‘drag and drop’ type of approach. All information given in the first two steps is combined into a JSON file, being the input for the R-package, which then is executed in step (iii) simulate the breeding program. Finally, in step (iv) results are analyzed and all relevant variables can be plotted. While most functions are automatized, an ‘expert’ mode allows access to all variables and provides opportunities for non-standard evaluations. MoBPS can work both with fully simulated data, but also can read in real data, e.g. genotypes or sequences of existing populations. The work with MoBPS is demonstrated with an example backcrossing scheme taken from the FAO guidelines on cryo-conservation of animal genetic resources, showing that MoBPS not only readily provides results for genetic progress, but also for the development of genetic diversity and inbreeding, and the genetic contributions of recipient and donor populations in an introgression scheme. The respective case is available as a template in the MoBPS system and can be used by logging in as ‘guest’ in the software. MoBPS is an innovative and valuable tool for designing and optimising the use of genetic resources in animal breeding programs. It has been used in a number of demonstration projects within IMAGE and will be maintained, hosted and further developed beyond the end of the project.

## Breeding programs in the South American Creole cattle

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The IMAGE project aims to explore novel ways to manage animal genetic resources. Such management in a data driven society is the most efficient using newly developed or improved software tools. The management of the breed is closely interlinked with a design of a breeding program, for which the two main goals are the increase in production and the maintenance of the genetic diversity.

The objective of this work was to enhance the breeding programs of the Creole cattle populations in two steps. The first step was a simulation of the breeding program and the possible increase in genetic gain, given the country specific characteristics. This was followed up with the second step for the optimization of the breeding program to constrain inbreeding levels.

For the simulation of the Creole cattle breeding program the MoBPS (Modular Breeding Program Simulator) software was used. The estimated breeding values for weaning weight were used to demonstrate the projected changes within the Colombian Blanco Orejinegro (BON) population, including the bulls from gene banks. The optimization of the breeding program was done utilizing the optimal contribution methodology, using the GENCONT software.

With the implementation of selection in the BON population we achieve the expected increase in production levels. In addition to this positive development, average heterozygosity in the population also decreases, thus the inbreeding levels increase. Such one-sided selection might lead to unsustainable breeding practices. In a simulation we show that the inbreeding levels of the population could be improved by using the gene bank bulls from the conservation program (Figure 1).

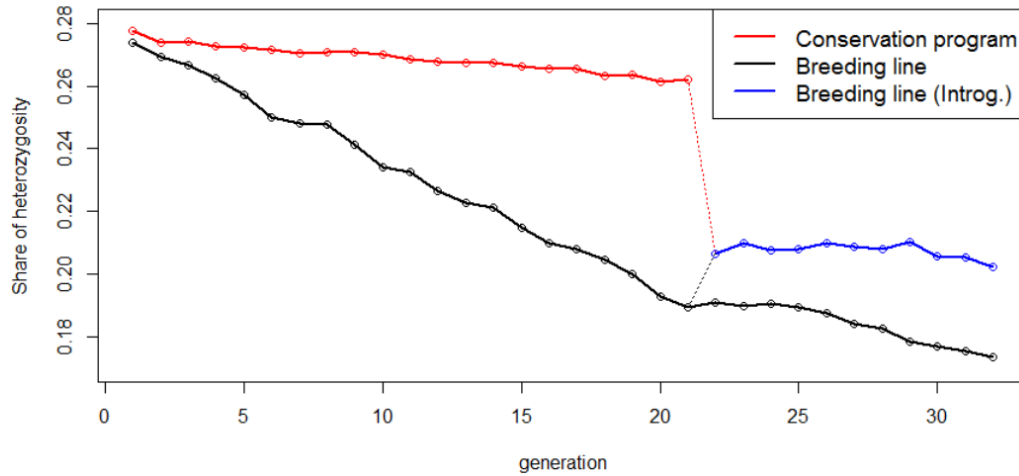


Figure 12 Development of the heterozygosity levels in a simulated conservation and breeding programs

The follow up optimal contribution selection approach was implemented to suggest suitable mating plans for simultaneous increase of production level, while putting constraints on inbreeding levels. The use of gene bank bulls had a clear added value, and enabled an even better management of genetic diversity in the population.

## Trade-offs between genetic diversity and genetic merit when using gene bank bulls

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The aim of sustainable breeding programs should be to improve genetic merit, while maintaining genetic diversity. In this study, we investigated the value of using gene bank bulls (in addition to bulls from the current population) with respect to this trade-off between genetic merit and genetic diversity. The main case study was performed on Dutch Holstein Friesian (HF) cattle. Since the 1990s, semen from HF bulls has been stored in the Dutch gene bank. We assessed the added value of HF gene bank bulls (born before 2010) to the current bull population (bulls born in 2010–2015). In total 5,783 HF bulls were used.

Genetic diversity was defined as 1 minus the mean genomic similarity ( $SIM_{SNP}$ ; this is equivalent to expected heterozygosity  $SIM_{SNP}$ ) or as 1 minus the mean pedigree-based kinship ( $f_{PED}$ ). Genetic merit was defined as the mean estimated breeding value for the total merit index or for 1 of 3 subindices (yield, fertility, and udder health). Using optimal contribution selection, relatedness was minimized or genetic merit was maximized at restricted levels of relatedness. Breeding schemes with only current bulls (born 2010–2015) were compared to schemes in which cryobank bulls were also included.

When relatedness was minimized, inclusion of genotyped cryobank bulls decreased mean  $SIM_{SNP}$  by 0.7% and inclusion of both genotyped and non-genotyped cryobank bulls decreased mean  $f_{PED}$  by 2.6% (in absolute terms). When genetic merit was maximized at restricted levels of relatedness, inclusion of cryobank bulls provided additional merit at any level of relatedness. Additional merit from cryobank bulls depended on (1) the relative emphasis on genetic diversity and (2) the selection criterion. Additional merit was higher when more emphasis was put on genetic diversity. Additional merit was low to nonexistent for the total merit index and higher for the subindices, especially for fertility.

In conclusion, Dutch HF cryobank bulls can be used to increase genetic diversity in the current population. When considering the trade-off with genetic merit, gene bank bulls have limited added value when selecting for the current total merit index, but more value when selecting for specific subindices. The latter is especially important, because breeding goals have changed in the past and will continue to change in future (e.g. weights of specific traits will change and new traits will be included).

## Painting Eggs in Blue

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The objective of this case study is to give an example how to use a specific trait of a genetic resource population conserved in a gene bank (blue eggshell color of Araucana) by transferring it into a contemporary breeding line (high performing White Leghorn, WL) by marker-assisted introgression. The trait of interest (blue eggshell color) is inherited in a dominant way. The causal mutation is a large retroviral insertion on chromosome 1 upstream of *SLCO1B3* at 65.17 Mb. The insertion induces overexpression of *SLCO1B3* in the oviduct. As *SLCO1B3* encodes a biliverdin transporter, biliverdin concentration increases in the oviduct and is being stored in the eggshell. The eggs of homozygous hens are more intensely colored than eggs of heterozygous hens due to a dosage effect. From 2016 to 2019, an initial F1 generation, two marker-assisted backcross generations (BC1 and BC2) and a final intercross generation (IC) were generated, aiming at a high performing WL-like line that is homozygous for blue eggshell color. Selection criteria were heterozygosity/homozygosity at introgression locus, high similarity to White Leghorn and high genetic diversity. For determination of sex, blue eggshell color genotype and 24 breed/line specific single nucleotide polymorphisms (SNPs) at introgression locus newly developed KASP<sup>TM</sup> assays were used. The 24 markers were developed based on genomic data of the founder animals. Furthermore, birds of all crosses were genotyped with a custom 52K SNP array. By analyzing haplotypes at introgression locus with Merlin, recombinant animals were detected. For BC2 crosses, only recombinant BC1 cocks were used. Further selection was based on analyses with Meksafe and MoBPS. The mean WL genome content in total increased up to 91.92 % in the BC2 animals, which is 4.42 % higher than the expected 87.5 % in a BC2 generation without marker based selection. In November 2019, the intercross population hatched (751 animals in total). 188 animals were homozygous carrier of the introgressed gene. Currently, the genotyping of the intercross generation is underway. Performance tests for the IC has not been finished yet, but first analyses of performance data of the BCs and commercial WL were promising. For comparison of laying rates of the back crossing generations, the mean laying rates of week of life 29 until 44 were included. The mean laying rate increased in the BC2 up to 94.7 % and was 2.4 % higher than of the BC1 (92.3 %), but 1.9 % lower than the White Leghorn recipient line (96.6 %). The mean egg weight was 62 g in the BC1, 61.5 g in the BC2 and 63 g in the control lines. The eggshell strength increased from BC1 to BC2 up to 45 N on average and was still significant lower than in recipient WL line (~50 N). In our crosses, a touch of cream color is present in some families, which leads to green eggs but was inherited probably independent from the blue eggshell color. Performance tests of the IC and genotyping results will enable to evaluate the success of the marker-assisted introgression regarding performance data. Selection efficiency will be evaluated by comparing simulated and real data. Homozygous intercross chicken are the basis for a new blue layer line that is highly similar to White Leghorn.

## **The role of capacity building and updated FAO guidelines to support gene banking strategies**

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The key objectives of Work-package 7 of IMAGE (Outreach) were to: a) demonstrate the benefits provided by animal gene banks, and promote their visibility and transparency; b) disseminate new knowledge integrating genetic, technological and socio-economic aspects, to optimise the conservation and sustainable utilisation of livestock genetic resources in the EU; c) strengthen human resources responsible for managing Animal Genetic Resources conservation programs, both in Europe and in third countries.

From the beginning of the project, it was clear that there is a major demand for training in areas related with the management of Animal Genetic Resources (AnGR), particularly as they relate to conservation programs using novel technologies, especially genomic information. Therefore, training and capacity-building programs were developed by IMAGE partners, designed to strengthen the activity of gene bank managers and other stakeholders involved in managing AnGR. These programs were undertaken in Europe and in third countries, considering that there are very diverse groups/needs in terms of the role play (gene bank managers, PhD students, researchers, etc.), scientific background (from highly skilled to practical technicians), geographical location (possible existence of a language barrier), etc.

By ensuring the collaboration of several IMAGE partners, the project has successfully developed various training activities, with eight formal courses organized in six countries, including the Netherlands and Colombia (with 2 courses each), and France, Argentina, Egypt and Morocco (1 course each). These were usually one-week courses, with high quality standards, and efforts were made for lectures to be presented in a language best fit to each group. Overall, nearly 240 students from 36 countries were trained in these courses.

The programs differed somewhat between training courses, but the common focus was the evaluation of genetic diversity by conventional and genomic information, GWAS, selection signatures, population structure and relationships, AnGR and climate change, etc. The majority of the courses provided hands-on experience to the students, who were required to conduct a research project with their own or with publicly available data, and give an oral presentation of their results by the end of the course. The success of these training activities was confirmed by the evaluation carried-out by the students and by the need to organize additional courses than initially planned, to address the demand from candidates.

One additional Task foreseen in WP7 was the development of Guidelines aimed at supporting gene banking strategies. These Guidelines are intended to provide an updating of the FAO guidelines, including those on “Cryoconservation of AnGR”, which were published in 2012. This updating should thus reflect the developments in various areas over the last several years, and is currently receiving contributions from the different partners of IMAGE, based on their own progress in the project. The NORDGEN group is coordinating this effort, and all partners



are deeply involved in putting together a comprehensive report reflecting the contribution of IMAGE to enhance AnGR conservation programs.

## Expectations from breeders in Spain

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Feagas is the Spanish Federation of Livestock pure bred Associations which was founded in 1982; it is the largest organization grouping 113 livestock pure breeds (cattle, sheep, goats, swine, horses etc) in Spain and is recognised officially by the Ministry of Agricultura and the local administrations of the Comunidades Autónomas. The breeds included in Feagas are divided into promoted breeds and trans boundary breeds on one hand, and on the other hand breeds which are at risk. Feagas is responsible of the preservation and improvement and the sustainable use of the livestock breeds in Spain, collaborating with universities and other institutions for the conservation, breeding, management of these breeds.

In Spain, breeders associations have paid some attention to the characterisation of their breeds, making efforts not only for *in situ* conservation but also for *ex situ* programs creating gene banks some of which are coordinated by the Ministry of Agricultural in the Censyras (Animal Breeding and Reproduction centres), universities and research centres. Regulation of the small regional breeds is in charge of the regional administration and the Ministry of Agriculture regulates all breeds located in more than one region. The breeders associations are fully implicated in the characterisation of their breeds and in *in situ* and *ex situ* activities which are supported by the administration which approves the conservation programs, maintains a backup copy of the gene banks in the Censyra and allows geneticists to endorse the conservation programs.

The breeder's opinion is that it is essential to have a complete gene bank for their local breeds and so they do following the recommendations of the FAO and of both the central and regional administrations. They also think that a backup copy in the Censyra is important and there is a clear effort to maintain a good representation of their breeds in national gene banks. The main problems they face is a) the financial support due to the huge number of Spanish local breeds which are of small number and most of them at risk of extinction, b) the lack of agreement between the breed societies and the local administration or the research center conservation criteria, c) the difficult management of some of the breeds to be able to conserve germplasm and d) the poor samples fertility in some cases like in donkeys.

In conclusion, Spain is a country with one of the highest diversity in livestock breeds, over the 80% of the recognised breeds are local, the breeder associations of local breeds promote the storage of their genetic material, the Spanish sector believes in the importance of *Ex-situ* Conservation and over 50% of the Spanish cattle breeds have sufficient genetic material stored and the rest is in process to be completed by the Breeder associations.

## Proteomic approach of the variability of fertility in the male goat

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A multi-partner approach has been set up in the last months of IMAGE, in order to develop new proteomic tools of semen quality evaluation in the buck. Three different companies were involved: Idele, the French technical Institute of Ruminant that provided a manager for the project; Capgenes, a company that conducts goat Genetic Improvement and was involved in the biological material; and INRAE, a French National Research Institute that developed the methodology.

New proteomic tools applied to semen (Intact-cells maldi ToF Mass Spectrometry (ICM-MS) and bottom up high-resolution Mass Spectrometry (HR-MS, orbitrap) have previously proven their efficiency to identify new semen markers of fertility in the chicken (Labas et al 2015; Soler-Vasco et al., 2016; Thélie et al., 2019). Since each species is different, and since the variability of quality of individual ejaculates is quite high in the buck, we found highly relevant to develop this proteomic approach in this new species: the goat. We focused our work on defining protein profiles of cryopreserved semen in order to identify semen quality and fertilizing ability markers. Using Alpine and Saanen bucks provided by Capgenes, we characterized *in vitro* sperm viability and motility parameters (microscopy, CASA) and ejaculates fertilizing ability based on pregnancy rates (ultrasonography) and kidding rates after on farm artificial insemination. For the ICM-MS studies, 15 bad and 15 good ejaculates (respectively < 45 and >75 % fertility) were selected per breed. For the bottom up HR-MS, 5 bad and 5 good ejaculates (respectively < 45 and >75 % fertility) were selected in the Saanen breed. The results of ICM-MS showed a mean of 200 peaks/ejaculate and 85% common peaks between Saanen and Alpines. The results of bottom-up HR-MS allowed identifying 623 proteins. Nineteen of them were differentially abundant in the good and bad fertile ejaculates. They are new candidate markers of fertility. These preliminary results will now be followed by global analyses of the two breeds with further modeling for fertility prediction, and by the validation of the new candidate markers of fertility. This study shows a highly efficient collaboration of the three partners involved in the project.



## **Towards an integrated strategy for conservation and sustainable use of genetic resources in Europe**

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One of the main aims of the GenRes Bridge project, funded by the Horizon 2020 Framework Programme of the European Union, is to develop an integrated strategy for the conservation and sustainable use of genetic resources in Europe. Expectations by the European Commission for GenRes Bridge are to progress in the development of i) individual domain specific roadmaps for management and use of plant, animal and forest genetic resources, ii) cooperation and synergies between the various networks (ECPGR, EUFORGEN, ERFP; for plant, forest and animal genetic resources respectively), iii) a wider agrobiodiversity (integrated) strategy. The integrated strategy will be developed and finalized in the year 2020. Messages and recommendations from the IMAGE project will be included in the integrated strategy development process. Moreover, all relevant stakeholders, including stakeholders involved in the network of the European Regional Focal Point for Animal Genetic Resources, will be involved in this process.