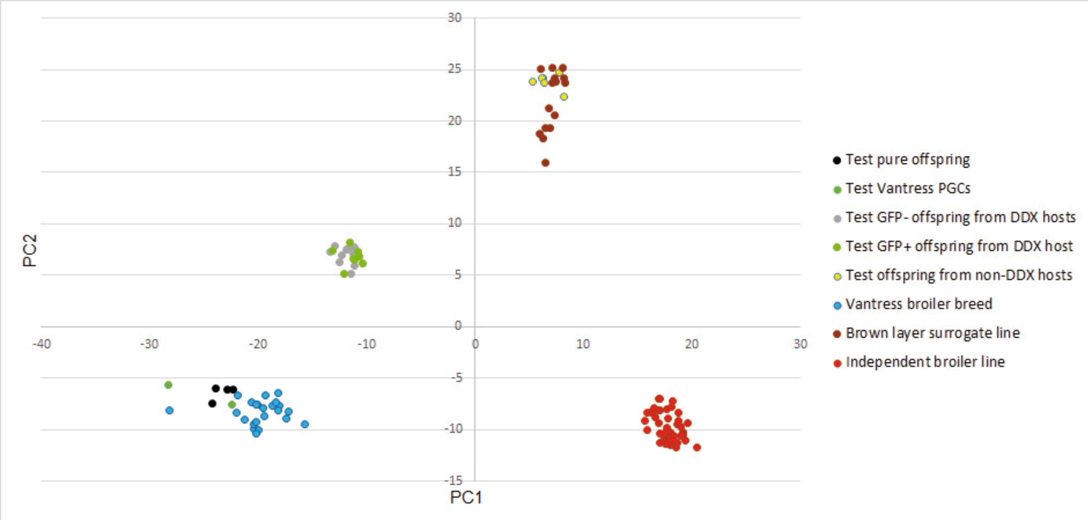

Fig. 1. PC analysis of offspring from *DDX4* Z⁻W and ZW surrogate hosts.

DNA samples were genotyped on a 66K SNP chip and analysed for principal components. Three chicken breeds were analysed, a heritage breed (blue), an independent pedigree broiler line (red), and the Hy-Line brown layer *DDX4* surrogate host line (brown). Offspring (green and grey) from the *DDX4 Z⁻W* hosts clustered between the Vantress breed and the Brown layer line. Offspring (black) from a *DDX4 Z⁻W* host inseminated with Vantress semen clustered with the Vantress breed chicken.

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Enhancing the functioning of farm animal gene banks in Europe: results of the IMAGE project

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Introduction

Globally, commercial livestock production is dominated by a limited number of specialized breeds. The worldwide distribution of a few breeds puts pressure on the majority of local breeds that are *at risk* of disappearing (FAO, 2018). The situation is similar for avian and mammalian species. There is a general consensus that complementary *in situ* and *ex situ* conservation strategies are needed to maintain a broad genetic base for future breeding and adaptation of livestock to changing market, climate and production environments.

The EU Horizon 2020 project IMAGE project (Innovative Management of Animal Genetic Resources; http://www.imageh2020.eu/) focuses on genetic collections in gene banks (*ex situ in vitro*) rather than on live population management (*in situ in vivo*), and on the synergy between gene banks and live population management. The motivation for IMAGE was to show that gene banks are to be managed in a dynamic way in order to go beyond the vision of a frozen ark. Thus, the aim is to enhance the use of genetic collections, to upgrade animal gene bank management, and to further develop genomic methodologies, biotechnologies, and bioinformatics for a better knowledge and exploitation of animal genetic resources.

A European wide survey carried out by Passemard et al. (2017) indicated that about 50% of cryopreserved germplasm collections in Europe also include samples of poultry species. In this survey, a total of 119 cryopreserved breeds of chicken were reported, which is fewer breeds compared to cattle, pig, equids, sheep and goat species. On the other hand, the number of chicken breeds reported in DAD-IS for Europe and the Caucasus is (much) higher than the number of breeds reported for cattle, pig, equids, sheep or goats. The main reason for the lower representation of chicken breeds in genebank collections might be that the use of frozen semen is much less common in the poultry sector. Yet, poultry genebank collections have been growing recently thanks to the improvement of semen cryopreservation techniques and as a result of increased awareness among poultry breeders, particularly for local breeds. Thus, it is timely to discuss the opportunities afforded by gene banks to poultry genetic resources, for both large and small populations.

Rationalization of gene bank collections and gene bank strategies

Many European countries have already established genebank collections for long term conservation of farm animal genetic resources (Passemard et al., 2017). This effort has been encouraged by FAO and supported by national policies. Sharing experiences and comparing strategies and methods has been promoted within the European Regional Focal Point for Animal Genetic Resources (ERFP; https://www.animalgeneticresources.net/). Yet, harmonization of procedures and cooperation between gene banks is limited, so that the further development of a European network of gene banks is now recommended, including rationalization of existing collections and (regular) evaluation of the institutional framework of farm animal gene banks.

The current gene bank portfolio (amount and type of material stored) should be regularly evaluated. Beyond the issues of technical feasibility of conservation methods, important questions include the extent to which a collection meets the gene bank objectives, whether the collection may be further developed or optimized from a genetic point of view, about the cost efficiency of the collections and how can we reduce costs in different collection and storage and access scenarios. These questions can be informed the opinions and interests of different stakeholders to support and to finance cryopreservation programs.

Gene bank objectives

Passemard et al. (2017) showed that most gene banks have two main objectives. First, almost all gene banks indicated that their collections have been established to be able to support the *in situ* conservation of local/native breeds. And, at the same time, the collections also have a long-term insurance conservation objective, including the ability to recreate extinct breeds or extinct lines. Although with a lower ranking, gene bank managers also said that collections are being established for research or genetic diversity studies. Finally, survey respondents apparently did not consider the potential future use of gene bank material to develop new lines or breeds.

Various types of germplasm

Cryopreservation of semen is applicable for long term conservation of rare poultry breeds, and may also be used for maintaining breeding lines, both for breeding and long-term cryopreservation purposes. As well as semen, IMAGE research also demonstrated the potential of cryopreservation and transfer of gonads to be an effective means in bird and mammalian species, while primordial germ cells (PGCs) offer possibilities and potential advantages in bird and fish species (Blesbois et al., 2019). Current EU guidelines and national legislation do not consider and – thus – do not allow the use of PGCs and gonad transplantation. However, amendment of national regulations is in principle possible, and may be considered, applying a risk-benefit approach, for instance gonad transplantation is invasive but may help to recover a breed.

Gap analysis

FAO defined Sustainability Development Goals indicator 2.5.1: "Sufficient material stored per breed to allow breed reconstitution from genebanks". (SDG; https://www.un.org/sustainabledevelopment/sustainable-development-goals/). Leroy et al. (2019) analysed for how many breeds, registered in DAD-IS (www.fao.org/dad-is/), "sufficient material" is already stored in gene bank collections across Europe. Here, "sufficient" was defined as a minimum of 25 (unrelated) donor animals and a minimum number of semen straws to reconstitute a breed through backcrossing. Although 15.9% of the breeds registered have genetic material cryopreserved in gene banks, only 4.3% of the breeds have sufficient material stored. In particular for avian species there is a very limited amount of genetic material per breed stored in gene banks so far. However, smaller amounts of genetic material stored per breed are still useful, for instance for supporting *in situ* management, and one could argue that complete reconstitution of a lost breed is an unlikely scenario for most breeds represented in gene banks.

Genetic diversity captured in genebank collections

Within IMAGE, several case studies have been undertaken to analyse the genetic diversity captured in genebank collections, to evaluate genetic changes over time, or to assess the genetic representativeness of gene bank collections in comparison to breeding populations kept *in vivo*.

Farm animal genebank collections in the Netherlands include poultry species (Schurink et al. 2019). 31 native Dutch chicken breeds are already represented in the genebank collections of CGN, however the amount of genetic material stored per breed is still limited and some breeds are still missing. For example, the so-called neo-bantam breeds have not been conserved yet in the national genebank collection. Bortoluzzi et al. (2018) showed that large fowls and neo-bantams share a high proportion of their alleles, but bantamisation has also generated unique and identifiable genetic diversity. Therefore it was concluded that neo-bantam breeds should also be part of the portfolio of the national chicken gene bank collection. In France, the CRB-Anim infrastructure project has boosted semen preservation for 15 additional chicken breeds, which all differed from each other according to molecular genotyping (Restoux et al., 2017). A similar effort took place for most research lines of INRA.

Genomic characterization of collections

Genebank collections should be genetically characterized to unlock their genetic potential, to assess the genetic diversity captured in genebank collections, or to better understand genetic changes over time. Within the IMAGE project, a cheap and worldwide available multi-species SNP array has been developed, to facilitate molecular characterization of both *in situ* populations and *ex situ* collections. The multispecies SNP array IMAGE001 contains 10K SNPs for the main agricultural species cattle, pigs, chicken, horse, sheep and goat, and is more focused on traditional breeds.

Different types of SNPs were selected for the 10K SNP array for chicken, including i) informative SNPs in many or in a specific group of breeds (derived from existing SNPs arrays) covering the whole genome, ii) SNPs derived from mitochondrial DNA, the sex chromosomes and SNPs related to specific traits, iii) SNPs in the major histocompatibility complex (MHC), iv) CNVs related to certain traits, and v) ancestral SNPs. The IMAGE001 Affymetrix SNP array will be available before December 2019.

Institutional, economical and ethical considerations

To develop a sustainable long term strategy, gene banks will have to address and to involve different stakeholder groups, including public bodies, science, breeders and NGOs. First, the needs, attitudes and preferences of stakeholders should be identified, followed by building a proper collaboration model.

Genebanks also raise "ethical questions" such as: i) what are the criteria for choosing breeds for cryopreservation? ii) who should pay? and iii) what cryoconservation methods are needed and acceptable? The IMAGE ethical survey showed that most respondents and stakeholders believe that a multi-stakeholder approach is needed for decision making, prioritization and funding of gene bank operations. Governments are generally seen as the main source of funding for gene banks. Respondents to the ethical survey were rather positive about the potential and acceptability of new reproductive and cryopreservation technologies, except cloning. We should however realize that most respondents were generally well informed about the latest technologies, which is not the case for the general public who could not be included in the survey.

An economic optimization model was developed within IMAGE (De Oliveira Silva, et al., 2018) with the aim to minimize costs in a pan-European gene bank network. The work suggested that costs could be reduced by 20% when comparing the current national collections with an optimal distribution of collections across countries, in terms of number of breeds. The authors also concluded that one European genebank would not reduce total costs, also due to the fact that there was little redundancy in breeds across countries. Further work is recommended to refine the optimization model, and to attach a genetic component to the economic model, e.g. determining optimal contributions of specific breeds to total genetic diversity at European level.

Quality Management

To guarantee the quality, legal certainty and accessibility of the materials stored, a substantial number of gene banks in Europe have implemented (formal) Quality Management Systems (Zomerdijk et al. 2019). 35% of respondents to the IMAGE survey said that they have defined formal cryoconservation goals, and 50% indicated that the genebank has identified the major risks for their collections and cryopreservation program. 89% follow specific Standard Operation Procedures for freezing and processing, 49% has a database for monitoring collections and 50% use Material Transfer Agreements for (part of) incoming samples. One remarkable outcome of the survey was that a large majority of gene banks (76%) do not have formal procedures for access to material (distribution policy). Further work is needed to strengthen genebank policies, procedures and protocols, by exchange of experiences and knowledge between countries.

Conclusion

Many countries have started cryopreservation programs for livestock species, but further development and rationalization of collections and programmes is needed. In particular avian species and breeds are currently underrepresented in national germplasm collections, whereas efficient techniques become available. The European Genebank Network for Animal Genetic Resources (EUGENA), governed by ERFP (https://www.animalgeneticresources.net/) will further enhance and professionalize national cryopreservation activities, in particular by exchanging knowledge and experiences within the network. Scenarios of use should also be developed to value gene bank collections.

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From wild populations to global diversity – Insight into the Synbreed Chicken Diversity Panel

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Introduction

When a small group of individuals migrate from a single large founder population to establish their own colony, this results in reduction of genetic diversity by genetic drift (Provine 2004). Furthermore, theory of genetic isolation by distance (Malécot 1969) predicts that the new population will genetically differentiate from the founder population. The theory of genetic isolation by distance refers to cases where the genetic differentiation increases with the geographic distance between populations. This is because the exchange of genetic material between the populations (i.e. mating opportunities) is confined by the distance (Cavalli-Sforza *et al.* 1964, Malécot 1969). The consequences of genetic drift act more rapidly to differentiate the populations than the potential or chances of an individuals' interaction under dispersal (Aguillon *et al.* 2017). Consistent with that, a model of expansion from a single founder predicts that patterns of genetic diversity in populations can be well explained by their geographic expansion from the founders, which is correlated to the genetic differentiation (Prugnolle *et al.* 2005, Ramachandran *et al.* 2005, Deshpande *et al.* 2009). Starting from the chicken wild populations, we aimed at investigating the patterns of genetic diversity in the global set of chicken breeds, represented by the Synbreed Chicken Diversity Panel (SCDP) (Malomane *et al.* 2019). The SCDP is a collection of various chicken breeds with various backgrounds from different parts of the world. In the SCDP the geographical location of the sampling often does not coincide with the geographical location of the breed development, since e.g. many samples of Asian breeds were collected from German fancy breeders. Therefore, we used the genetic distance of the sampled breeds to the wild ancestors as a proxy for geographic distance, and verified, whether the reduction of diversity also can be found with increasing genetic distance to the domestication center.

Material and Methods

In this study, data taken from the SCDP consisted of 3,235 chicken individuals from 172 domesticated chicken populations and two wild populations (*Gallus gallus gallus* and *Gallus gallus spadiceus*). The chicken samples were collected in Asia, Africa, South America and Europe, and were genotyped with the 600K Affymetrix® Axiom™ Genome-Wide Chicken Genotyping Array (Kranis