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GENOMIC CHARACTERIZATION OF ANIMAL GENETIC RESOURCES – DRAFT UPDATED TECHNICAL GUIDELINES

TABLE OF CONTENTS

	Pages
I. BACKGROUND.....	2-3
II. ANNEX - Genomic characterization of animal genetic resources – Draft updated technical guidelines.....	4-118

BACKGROUND

The Commission on Genetic Resources for Food and Agriculture (Commission), at its Thirteen Regular Session,¹ endorsed the *FAO guidelines – Molecular genetic characterization of Animal Genetic Resources*,² which were published in 2011. The guidelines (i) outline the rationale for undertaking characterization of animal genetic resources, including molecular genetic characterization; (ii) describe the strategic choices to be made in planning molecular genetic characterization studies; (iii) provide explanations and recommendations on the steps to take while performing such studies, including animal sampling, genotyping and data analysis, highlighting potential pitfalls; and (iv) encourage standardization of data and integration of national studies into international analyses. The guidelines highlighted the need for their periodic updating and further refinement as experience with their use in the field is accumulated and as technologies for molecular genetic characterization advance.

At its Seventeenth Regular Session, the Commission requested FAO to continue developing and updating guidelines to facilitate the application of new scientific discoveries related to the identification, characterization and conservation of animal genetic resources.³ It further requested FAO to strengthen partnerships with stakeholders and donors to continue technical and policy support for country implementation of the Global Plan of Action for Animal Genetic Resources.⁴

Biotechnologies for the sustainable use and conservation of genetic resources for food and agriculture have advanced substantially in recent years.⁵ Genomics - the study of genes and their functions, and related characterization techniques⁶ - is a field in which technological advancement has been particularly rapid and consequential. Enhancements in procedures for whole-genome sequencing and in high-throughput genotyping have led to greatly decreased costs per unit of genetic information that can be obtained from a single assay. Since the publication of the previous guidelines, thousands of individual animals have undergone whole-genome sequencing and hundreds of thousands of animals have been genotyped with DNA chips, each assaying thousands of single nucleotide polymorphism markers. Application of this information has increased knowledge of breed development and accelerated response to selection. Knowledge of the biology underlying phenotypes has increased as well, as a result. To help countries to benefit from these technological advancements in applications to improve the management of animal genetic resources, FAO has developed new guidelines on genomic characterization, which are given in the annex of this document. The guidelines are intended to update and supersede the *FAO guidelines – Molecular genetic characterization of animal genetic resources*.

The draft guidelines have been prepared in cooperation with the International Society of Animal Genetics (ISAG) – FAO Advisory Group on Animal Genetic Diversity (Advisory Group). ISAG is an academic and scientific organization that focuses on basic and applied research on molecular genetics in domesticated animals.⁷ FAO and ISAG have a long history of collaboration. Members of the Advisory Group have in the past served as editors and/or contributors to the previous guidelines, as well as the *Secondary guidelines: Measurement of domestic animal diversity*,⁸ published in 1993, and the *Secondary Guidelines - Measurement of Domestic Animal Diversity (MoDAD): Recommended Microsatellite Markers*,⁹ which were published in 2004. For the current draft updated technical guidelines, Advisory Group members served as editors and authors of the individual sections. In

¹ CGRFA-13/11/Report, paragraph 79.

² <http://www.fao.org/3/i2413e/i2413e00.pdf>

³ CGRFA-17/19/Report, paragraph 84.

⁴ CGRFA-17/19/Report, paragraph 86.

⁵ CGRFA/WG-AnGR-11/21/8; CGRFA/WG-AnGR-11/21/Inf.11.

⁶ <https://www.who.int/genomics/geneticsVSgenomics/en/>

⁷ <https://www.isag.us/>

⁸ FAO. 1993. *Secondary guidelines: Measurement of domestic animal diversity (MoDAD)*. Rome

⁹ <http://www.fao.org/3/aq569e/aq569e.pdf>

addition, other international scientists were invited to serve as authors and/or reviewers of the document.

The draft updated technical guidelines were developed by using the previous guidelines as a template. Information that is still valid was retained, while in other instances the material was updated. New material was included to explain the relevant recently developed technologies. These developments in biotechnologies have been complemented by advances in methods to analyse and interpret the new information gathered, so the guidelines also contain new material to address the application of genomic methods to the study of animal genetic resources.

In addition to advancements in biotechnologies, important changes in the policy landscape since the publication of the previous guidelines have also occurred. In particular, the *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity*¹⁰ is of relevance for genomic characterization of animal genetic resources, given the importance of international cooperation for understanding national animal genetic resources in a wider context and the frequent movement of biological samples across borders while undertaking associated research. The guidelines explain the relevance of the protocol and share advice on steps for researchers to take to help ensure compliance with applicable national laws on access and benefit-sharing.

A key aim of the guidelines to inform readers on how to undertake from start to finish a genomic characterization study on animal genetic resources. They explain different approaches for genomic analysis and consider different study objectives. The sections of the guidelines address the following topics: (i) rationale for genomic characterization of animal genetic resources; (ii) the basics of carrying out molecular genetic studies; (iii) genomic tools and methods; (iv) genomic applications for assessment of genomic variation within-populations; and (v) overall conclusions and recommendations.

¹⁰ <https://www.cbd.int/abs>

ANNEX**Genomic characterization of animal
genetic resources – Draft updated
technical guidelines**

Table of contents

Foreword	6
Acknowledgements	7
User guidance.....	8
Abbreviations and acronyms	9
SECTION 1 – Introduction	11
SECTION 2 – The basics	17
SECTION 3 – Genomic tools and methods	26
SECTION 4 – Applications of genomics	49
SECTION 5 – Conclusions and recommendations	90
APPENDICES	93

Foreword

Acknowledgements

User guidance

Abbreviations and acronyms

AB	ascertainment bias
ABC	approximate Bayesian computation
ABS	access and benefit sharing
aDNA	ancient DNA
AFS	allele frequency spectrum
AIM	ancestry informative markers
AMOVA	analysis of molecular variance
AnGR	animal genetic resources (for food and agriculture)
bp	base pair
BSAA	breed-specific admixture analysis
CBD	Convention on Biological Diversity
CNV	copy-number variation/variant
DAD-IS	Domestic Animal Diversity Information System
DNA	deoxyribonucleic acid
D_R	Reynolds genetic distance
EAAP	European Federation of Animal Science
FA	factor analysis
GBS	Genotyping-by-sequencing
GWAS	genome-wide association study
HDP	high density panel of markers
H_e	expected heterozygosity
H_o	observed heterozygosity
HWE	Hardy–Weinberg equilibrium
IAEA	International Atomic Energy Agency
IBD	identity by descent
IBS	identity by state
ILRI	International Livestock Research Institute
indels	insertions and deletions (of nucleotides)
ISAG	International Society for Animal Genetics
K	number of genetic groups in model-based clustering analyses
kb	kilobase
LD	linkage disequilibrium
LDP	low density panel of markers
LRS	long-read sequencing
MAF	minor allele frequency
MAT	mutually agreed terms
MCMC	Monte Carlo Markov Chain
MDS	Multidimensional scaling
MLE	maximum likelihood estimate
MSMC	multiple sequentially Markov coalescent method
MSY	male-specific part of the Y-chromosome
MTA	material transfer agreement
mtDNA	mitochondrial DNA
N_b	number of breeding animals
N_e	effective population size
NJ	neighbor-joining
PAR	pseudoautosomal region
PC	principal component
PCA	principal component analysis
PCR	polymerase chain reaction
π	nucleotide diversity
PIC	prior informed consent

PSMC	pairwise sequentially Markov coalescent method
QTL	quantitative trait locus
ROH	runs of homozygosity
SD	standard deviation
SNP	single nucleotide polymorphism
SPA	Strategic Priority Area
SPAG	SPatial Areas of Genotype probability
SRS	Short-read sequencing
SV	structural variation
θ	population mutation rate
WGS	whole-genome sequencing

SECTION 1**Introduction**

Introduction

RATIONALE FOR CHARACTERIZATION OF ANIMAL GENETIC RESOURCES

Domestic animal diversity is an important component of global biodiversity. About 40 species of domestic animals and poultry contribute to meeting the needs of humankind, providing meat, fibre, milk, eggs, draught animal power, skins, and manure, and are an essential component of many mixed farming systems. Within these species, around 8 800 breeds and strains (FAO, 2021) constitute the animal genetic resources (AnGR) that are of crucial significance for food and agriculture.

The present pattern of diversity of AnGR is the result of a long and complicated history that started with animal domestication. Depending upon the species, domestication occurred 10 000 to 1 000 years ago. Since then, domestic livestock have spread with human migration and trading to all inhabited continents. Local adaptation, artificial selection, mutation and genetic drift turned the genetic diversity captured with domestication into a vast array of differences in appearance, physiology and agricultural traits. During recent centuries this differentiation has been accentuated by the emergence and development of breeds – more or less isolated populations that were subject to systematic selection. This development has been most pronounced in the temperate zones where the demands of food supply led to a rationalization of agriculture. The last 50 years have seen the global spread of a few highly developed breeds, most of which originated in Europe. A well-known example is the high-yielding Holstein-Friesian breed of dairy cattle, which has become by far the most widely-dispersed cattle breed in the world.

The global diffusion of these specialized breeds is endangering or even risking the extinction of many well-adapted local populations. This trend is occurring both moderately to highly intensive production environments and in marginal areas (Godber and Wall, 2014, Sponenberg *et al.*, 2018), where local husbandry practices are being abandoned (Köhler-Rollefson *et al.*, 2009). Local breeds are usually much less productive than the highly developed international transboundary breeds when raised in optimal conditions, but are adapted to the local climate (Mirkena *et al.*, 2010) and do perform in a natural environment without intensive management. Indiscriminate crossbreeding and introduction or increased use of the specialized exotic breeds have been reported as the two most important causes of genetic erosion at global level (FAO, 2015).

This erosion of diversity of AnGR has become a major concern (Hodges, 2006, FAO 2007a, Bruford *et al.*, 2015). The negative consequences of genetic erosion and inbreeding depression have been amply documented and may be manifested by loss of viability, fertility and disease resistance, and the frequent occurrence of recessive genetic diseases (FAO 2007b; Taberlet *et al.*, 2008; Howard *et al.*, 2017). According to the report on the *Status and trends of animal genetic resources – 2020* (FAO, 2021), approximately 7 percent of reported livestock breeds have become extinct and more than 25 percent are considered to be at risk of extinction. Moreover, the situation is presently unknown for more than 50 percent of breeds, most of which are reared in developing countries.

Several recent developments have strengthened the interest in local breeds. First, we now realize that their local adaptation will become even more essential in view of the ongoing and escalating climate change (Hofmann *et al.*, 2010). Second, there is a growing interest in the restoration of ecosystems (UN, 2020), including "rewilding" of abandoned agricultural areas, in which domestic ungulates are kept under feral or semi-feral conditions and occupy the niche of mega-herbivores (Carey, 2016). For this purpose, only animals that are adapted to extensive management are suitable. Third, the global COVID-19 pandemic has increased awareness of zoonotic diseases, and the benefits of a high biodiversity of wildlife, livestock and production systems (Liverani *et al.*, 2013).

FAO has a history of working with countries and other stakeholders to improve the productivity of livestock and the livelihoods their citizens while maintaining AnGR diversity (FAO, 1990a,b).

Specific priorities for AnGR management are set out in the *Global Plan of Action for Animal Genetic Resources* (Global Plan of Action) (FAO, 2007a), the internationally agreed policy framework for management of AnGR.

One of the Strategic Priority Areas of the Global Plan of Action is the characterization, inventory and monitoring of trends in AnGR diversity. In brief, this Strategic Priority Area addresses the gathering of information to increase the knowledge about AnGR. Knowledge is necessary to properly assess the value of breeds and to guide decision making in livestock development and breeding programmes. Assessments of the capacity to manage AnGR have revealed that a lack of knowledge is a major constraint (for example, FAO, 2007b; 2015)

The breed is the most common operational unit in the conservation of genetic resources. However, the biological breed concept to describe groups of animals having particular genetic characteristics is not always applicable. Many breeds originating from industrialized countries are well-defined and phenotypically distinct and were largely genetically isolated throughout the course of their development. In contrast, a significant proportion of other breeds, especially those in Asia and Africa, correspond to local populations without structured breeding programmes, and therefore differ only gradually according to geographical separation. In addition, breeds with different names may have a recent common origin (Feliuss *et al.*, 2011) and cross-breeding has been common since the invention of breeds in the 18th century. It is often argued that breeds, by being associated with a group of people, is a social construct rather than a biological concept (“A breed is a breed if enough people say it is”, K. Hammond cited by Oldenbroek and Van der Waaij, 2015).

On the other hand, recent developments in biotechnologies have greatly increased the power of molecular analyses to discern the genetic make-up of organisms and identify differences and similarities among populations. Molecular-genetic analysis can now complement phenotypic characterization and indigenous knowledge to help identify the breeds or groups of breeds that have retained their uniqueness. At the same time, genome-wide analysis is also more and more capable to capture this on the molecular level.

MOLECULAR CHARACTERIZATION – HISTORY AND PROSPECTS

Scientists began use molecular data for livestock in the early 1990s and since then these data have become continually more relevant for the characterization of genetic diversity (Groeneveld *et al.*, 2010; Bruford *et al.*, 2015). In 1993, an FAO working group proposed a global programme for characterization of AnGR, including molecular genetic characterization, and formulated the *Secondary guidelines: measurement of domestic animal diversity (MoDAD)* (FAO, 1993) with recommendations for the molecular analysis of domestic animal diversity on a global scale .

The FAO MoDAD report succeeded in creating awareness of the need to monitor AnGR diversity. In addition, the proposal of the program helped motivate many nationally funded research projects and larger regional and international projects supported by organizations such as the European Commission, the Nordic Council of Ministers, the International Atomic Energy Agency (IAEA), the International Livestock Research Institute (ILRI) and the World Bank.

Scientists in many countries have undertaken studies to characterize locally available breeds, while large-scale international efforts on breed characterization have built comprehensive molecular datasets for most livestock species. The study of genetic diversity of livestock at the molecular level has developed into an active area of research that frequently contributes to the capacity building of young scientists and receives considerable attention in scientific press and at the conferences of organizations such as the International Society for Animal Genetics (ISAG) and the European Federation of Animal Science (EAAP).

The first MoDAD guidelines were followed-up in 2011 by the FAO report *Molecular genetic characterization of animal genetic resources* (FAO 2011). At that time, the state-of-the-art was still largely based on the use of microsatellites as a genetic marker, which after 1990 had revolutionized the science of molecular genetics. However, this category of markers has two major disadvantages for studying genetic diversity. First, allele calling is difficult to reproduce across laboratories. Second, in spite of the efforts of FAO and ISAG towards standardization, too many laboratories continued to use private panels of genetic markers, which precludes the joining of datasets and seriously decreased the impact of the studies.

Starting about 10 to 15 years ago, these markers have for almost all research applications become outdated by the availability for most livestock species of whole-genome sequences. The genomic sequences allowed the identification of millions of single nucleotide polymorphisms (SNPs) and other types of genetic variants such as insertions and deletions (indels), structural variation, copy-number variation (CNV). The invention of “bead arrays” allowed the simultaneous genotyping of 10 000 to 1 000 000 SNPs (Nicolazzi *et al.*, 2015) . Subsequently, whole-genome sequencing (WGS) (Eusebi *et al.*, 2020) became more and more affordable. Section 3 will describe these approaches in more detail.

These developments have generated an ongoing “tsunami” of genotype and sequence datasets and have been accompanied by the development of a multitude of new statistical analyses and programs for data management and analysis (Biscarini *et al.*, 2018). Another consequential development has been the substantial progress in the analysis of ancient DNA (aDNA) (McHugo *et al.*, 2019). All of these developments have contributed to the realization of most of the original MoDAD objectives, albeit with different markers than envisaged originally:

- The wild ancestral species of most livestock species were already identified on the basis of mitochondrial DNA (mtDNA). More detailed studies, often using aDNA, has yielded more details on the interaction of several livestock species and their wild ancestors during and after domestication (MacHugh *et al.*, 2017).
- The differentiation of breeds as well as their homogeneity can now be assessed unambiguously. This is in itself relevant, but also guides downstream analyses that assume breeds to be homogeneous populations.
- The genetic constitution of breeds can now be more accurately assessed via quantitative measures of diversity, admixture or subdivision, inbreeding, introgression and assortative mating.
- The evolutionary history of species and populations can be reconstructed on the basis of the phylogenetic relationships of breeds. The resulting data are commonly visualized by a “tree-like” (hierarchical) topology, but may also indicate gene flow between breeds with different histories.

For the most important domestic species we have obtained a fairly comprehensive global view of diversity – that is, also including Asian, African and South-American breeds as well as those from high-income countries - by integrating national or regional datasets of SNP genotypes or whole genome sequences, even if these datasets originated from different laboratories (in contrast to studies based on microsatellites).

Other positive developments are the realization of more effective cryoconservation programs (Paiva *et al.*, 2016; De Oliveira *et al.*, 2019) and the globalization of the diversity studies with active participation of institutes from all inhabited continents. The genomic characterization of breeds also led to the insight that livestock breeds have never been static phenomena, but rather are the result of a continuously dynamic history that often has included crossbreeding (Feliuss *et al.*, 2015). As a consequence, attempts to establish priorities for conservation of breeds on the basis of neutral genetic markers (Lenstra *et al.*, 2012) have been de-emphasized.

Instead, attention has shifted toward the adaptive variation, SNPs, indels and CNVs that possible that contribute to environmental adaptation or other relevant traits. At least for monogenic traits, most of

the adaptive variation resides in the structural genes (Nicholas and Hobbs, 2014), but there are interesting examples of intergenic mutations (Zappala *et al.*, 2016; Aldersey *et al.*, 2020). In addition, several methods have been developed for detection of a local perturbation of the diversity across the genome that may suggest the effect of selection, the so-called selection signatures (Randhawa *et al.*, 2016; Friedrich and Wiener, 2019). In all these studies, the limiting factor is the validation of the signals found by genome-wide analysis or by studies of gene expression in terms of direct causative effects. From a fundamental-scientific view, it will also be most interesting to link phenotypes to non-coding RNA molecules (Weikard *et al.*, 2016) and to epigenetic phenomena (Giuffra *et al.*, 2019). Because of the multitude of genes, traits and breeds, this promises to remain a large and rewarding area of research.

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SECTION 2**The basics**

The basics

HOW TO CARRY OUT GENOMIC DIVERSITY STUDIES

This section provides a general overview of the steps to be undertaken when conducting genomic studies of animal genetic resources (AnGR). The process of genomic characterization can usually be broken into a series of activities that are distinct in terms of time and space, but coordination among the steps is critical. Although the genotyping technologies have changed substantially since the previous guidelines (FAO, 2011b), the basic steps for any genetic diversity study have remained largely the same.

BEFORE YOU START

Consider the national context for management of animal genetic resources

Genetic characterization is often undertaken as an academic research activity, which has value on its own, but the potential impact of the study will be greatly increased if undertaken in coordination with national framework for management of AnGR (FAO, 2011a). Nearly all countries have identified an organization to serve as its National Focal Point for Management of AnGR and have nominated an individual to serve as a National Coordinator (FAO, 2021). Contacting the National Coordinator prior to the study and keeping him or her informed as the study progresses is recommended for several reasons. Characterization is a key action in national implementation of the Global Plan of Action for Genetic Resources (FAO, 2007) and countries are regularly requested to report their activities to the FAO. Many countries also have national strategies and action plans for management of AnGR (FAO, 2009) and characterization studies should be consistent with such plans. The National Coordinator should also be aware of stakeholders that will be interested in the results and their application in management of the characterized populations. To maximize efficiency of your data collection efforts, phenotypic characterization (FAO, 2012) of the populations and their production system should be undertaken in concert with sampling of biological material for genomic characterization.

Know your breeds

Characterization is itself an information-gathering activity, but efforts should be made to obtain as much background knowledge as possible about the target populations while planning your study. Sources of information will include the comprehensive breed encyclopedia of Porter *et al.*, (2016), scientific literature, national technical reports, popular press articles, breed databases such as the Domestic Animal Diversity Information System (DAD-IS; FAO, 2021) and the Breeds of Livestock (Oklahoma State University, 2015) and websites of breed organizations. This information will help to formulate objectives, plan sampling strategies and develop hypotheses to be tested.

Written information should be complemented with input from local experts, livestock keepers and representatives from breed associations that are familiar with the herdbooks. These stakeholders may be able to share anecdotal information about breeds and provide contacts for sampling of DNA and measurements of phenotypic traits.

Define clear objectives

Genomic analyses can be used to address a wide range of objectives and can be applied in many ways (see Section 4). These objectives are important for deciding on the number and type of animals to be sampled, the optimal genetic marker to be used and complementary information to be collected during sampling. A common objective is to elucidate the relationships of a group of local breeds and their relationships to nearby other breeds, especially if these breeds are ancestral to one of more of the local breeds. For example, Kim *et al.*, (2020) analysed the ancestry indigenous African cattle and compared these cattle with European and Asian breeds. Many livestock breeds have already been subject to genomic characterization and the data from those studies is often in the public domain. Therefore, comparison of targeted breeds with other breeds may not require sampling and genotyping of the

complementary breeds. However, such analyses require preliminary assessment to ensure that genotyping approaches share common markers.

Design the sampling

As noted earlier, genomic characterization will ideally be done in concert with phenotypic characterization and evaluation of the production environment. This will especially be the case when the study represents the first time that a population is characterized, and the objective is to capture the range of variability. To accomplish this, the sampling plan should consider the structure of the production environment, geographic locations and familial relationships (as much as possible). The following recommendations will help ensure a genetically diverse sample:

- preferably, sample in the areas that are close to the site of the development of the breed(s);
- depending on the objectives of the study, populations in other local regions or countries (transboundary breeds) may be sampled as well;
- if applicable, sample animals that represent all different subtypes or subpopulations from different agroclimatic zones (which should be recorded in the complementary data, see below);
- typically, no more than 10 percent of any one herd or village population should be sampled and at most five animals be sampled from any single herd; and
- avoid sampling animals with common grandparents.

When planning for collection of samples from animals in different agroclimatic zones or any large area, a systemic approach is recommended. Two possible options are grid-sampling and sampling along linear transects. With grid-sampling, a “grid” of squares is formed by drawing sets of equally spaced North-South and East-West running lines over a map of the sampling area. Equal numbers of animals are then sampled from each square. With linear transects, straight lines are drawn across the sampling area and animals are sampled (as much as possible) from the farms or herds that fall along (or close to) the lines.

Numbers that count

For reliable estimation of allele frequencies of biallelic markers (such as single nucleotide polymorphisms (SNP), at least 20 animals per breed should be typed. For multiallelic markers at least 40 animals are required. A larger sample size is also recommended for subdivided breeds. For specific objectives such as genome-wide association studies (GWAS) more samples may be needed (see Sections 3 and 4). Collection of more samples than these minimum values will help to increase precision of inferences and provide a back-up in case of low-quality samples.

Know the rules

Genomic characterization involves the collection and analysis of DNA, which is usually regarded as a genetic resource and may generate data of relevance for intellectual property claims. Therefore, it’s recommended that a formal agreement for acquisition and exchange of the material is made with the provider of the material prior to the start of the study. The standard approach is for all involved parties to sign a material transfer agreement (MTA), which stipulates the terms of the material exchange, describes how the material will be used and handled after characterization and analysis. The provider may already have a standard MTA and provide access to the genetic resources only upon acceptance of the terms of the MTA. The MTA should also create legal certainty as to what can be done with the material (such as storage or distribution after the study), as well as with any data resulting from the study. Both parties must be informed about any limits to the rights that the provider can cede to the receiver of the material, in accordance with national regulations. In principle, the providers of the material are to maintain ownership throughout the duration of the study. The providers of the genetic material should be regularly informed about the progress of the study and results obtained. An example MTA is provided in Appendix 2.

Furthermore, international exchange of research material involves a specific set of rules. First, AnGR are considered sovereign to their country of origin. Both the providers of material and their

collaborators must therefore adhere to the terms of each country's regulations implementing the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (Nagoya Protocol) of the Convention on Biological Diversity (CBD), such as the signing of an access and benefit sharing (ABS) contract negotiated on mutually-agreed terms (MAT). More information about the Nagoya Protocol can be found in Box 1 and in other specialized documentation prepared by FAO (2019). Second, because biological material is being exchanged, collaborating scientists must be aware of any sanitary regulations involving international shipments and obtain the necessary permits.

BOX 1**Nagoya Protocol: In brief**

The Nagoya Protocol was developed to ensure the fair and equitable sharing of the benefits arising out of the utilization of genetic resources across countries, one of the three objectives of the Convention of Biological Diversity (CBD, 2011). This requires appropriate access to genetic resources and appropriate transfer of relevant technologies, considering all rights over those resources and to technologies and appropriate funding. The Nagoya Protocol was adopted by the CBD in 2010 and has since been ratified by more than 125 member governments.

As confirmed by the Nagoya Protocol, countries, in the exercise of their sovereign rights over natural resources, may provide that access to genetic resources for their utilization shall be subject to their "prior informed consent" (PIC). Many countries have decided to not restrict access to AnGR. However, where domestic legislation or regulatory requirements require this, users need to obtain permission prior to accessing and using genetic resources for research and development, and share the benefits arising from this utilization of genetic resources on mutually agreed terms (MAT). The Nagoya Protocol, as well as many national ABS laws, do not go into any detail as to what constitutes "utilization" of a genetic resource and therefore requires PIC and MAT. Certain activities, for example taxonomic research, may therefore require PIC and MAT in one country and not in another. It is therefore important to specify in detail the intended activities being undertaken and to clarify with the competent authority of the country that provides the genetic resources if and what kind of permit is required.

In some countries, access to traditional knowledge associated with genetic resources that is held by indigenous peoples and local communities is also subject to their PIC or approval and involvement and MAT have to be established.

Countries that ratify the Nagoya Protocol are expected to ensure that genetic resources utilized within their jurisdiction have been accessed in accordance with PIC and that MAT have been established, as required by the ABS measures of the country from which the resources were obtained. It is therefore crucial to comply with the applicable ABS measures irrespective of where the genetic resources are actually used for research and development. This is because Parties to the Nagoya Protocol are under an obligation to provide that any research and development activity on genetic resources within their jurisdiction complies with the ABS measures under which the genetic resources have been accessed.

Through so-called "checkpoints" Parties to the Nagoya Protocol ensure that research and development on genetic resources within their territory are based on the PIC of the Party that provided these genetic resources, as applicable. Once PIC has been granted and the permit or its equivalent have been made available to the ABS Clearing House, they constitute an Internationally Recognized Certificate of Compliance.

Source: Hartmut Meyer

The ABS contract and MAT define limits of utilization and sharing of benefits. In general, obtaining these contracts involves a phase of negotiation that must be considered before one starts a genomic characterizations study. Box 2 provides key points to consider regarding this negotiation process.

BOX 2

Incorporation of compliance to national ABS frameworks into project planning and implementation

Compliance with ABS measures adds a level of administrative complexity to the execution of research and development projects involving AnGR. The ABS measures require specific planning but may help top avoid eventual disputes regarding the sharing of benefits arising from such projects that could otherwise occur in the course of such a project.

The following points regarding ABS compliance should be considered in preparation for genomic characterization studies involving international exchange of AnGR:

- (i) Account in the project timeline for the time required to negotiate the ABS contract and obtain PIC, the national ABS permit and other applicable permits (typically 2 months to 2 years, depending on the country).
- (ii) Be aware that it may not even be possible to negotiate the required contract.
- (iii) Include in the project budget sufficient funds to cover whatever benefits have been negotiated with the providing country. Expected benefits are variable by country; including provider countries as project collaborators may be advantageous, inasmuch as some of the benefits will be in-kind, rather than monetary.
- (iv) Consider carefully both present and potential future uses of the AnGR samples and prepare the wording of the contract accordingly. If you want to use the resources for a purpose other than that stated in the original agreement, a new agreement will be required.
- (v) Keep all relevant documentation associated with the negotiation of the contract and granting of PIC and the ABS permit. These documents will be crucial for demonstrating compliance with the ABS laws of the providing country if the country in which you are using AnGR has adopted ABS compliance laws (as e.g. the EU and the UK).
- (vi) Keep a record of all benefits (both monetary and non-monetary) obtained by both the provider and user of the genetic resource.

Source: Karen Marshall

IN THE FIELD

Collecting samples

For this most crucial step, the following considerations are relevant:

- Almost all cells or tissues may be used for DNA analysis: blood, semen, hide, bone, tissue (e.g. ear tissue), plucked hair (only the root cells contain nuclei, but cut hairs can be used for analysis of mitochondrial DNA) and feathers.
- High-quality DNA is most easily obtained from samples of peripheral blood, organs or other tissues. Most convenient are blood samples collected in an anti-coagulant (EDTA or natrium citrate). A protocol for blood collection is provided in Appendix 3.
- Collect enough material for present and future studies (considering ABS agreements for future studies). For most PCR-based applications, including high-density SNP arrays and whole genome sequencing, 5ml of blood is adequate. Note that poultry species have enucleated erythrocytes and, therefore, much less blood (~1 ml) is required.
- Blood samples can be transported at ambient temperatures, but in tropical regions samples should be processed within 36 hours.

- For longer storage, samples can be placed in a room-temperature preservative such as Queen's buffer (0.01 M Tris/HCl, 0.01 M NaCl, 0.01 M EDTA and 1 percent n-lauroylsarcosine, pH 8.0; Seutin *et al.*, 1991).
- Tissue samples of 1 cm squared should be minced to 1 mm squared pieces and placed in Queen's buffer or 70 percent ethanol. Air-drying of ethanol-treated samples allows long-term storage and the easy transport of samples. Alternatively, pieces of tissue may be dehydrated directly by placing in vials on crystals of silica gel.
- Hair samples should be desiccated as soon as possible and stored dry.
- FTA® cards can be used for collection of genetic material with DNA to be amplified by PCR, but special protocols are required to obtain double-stranded DNA and the single-stranded DNA obtained with standard isolation protocols is not suitable for all other applications.
- Samples that are to be used for cloning and Southern blotting require double-stranded DNA of high molecular weight.
- Labelling of samples should be unambiguous and permanent.
- Bank it: store all samples and document all relevant information unambiguously in such a way that it can be retrieved and understood, even by persons not involved in the sampling.

Collecting data

Recording the following information for each sample is essential:

- animal identification, preferably a herdbook registration number;
- date;
- location, preferably based on global positioning system (GPS) coordinates;
- name of collector;
- breed;
- sex of animal; and
- type of sample (blood, hair, etc.).

Collection of the following information is strongly recommended and will be necessary for some types of analyses (e.g. phenotypes will be necessary for studies to determine genomic regions having influence on traits).

- any relevant phenotype(s);
- basic pedigree information (i.e. parents, if known);
- digital photograph(s) of animal, showing any interesting morphological features and including a measuring stick to evaluate body measurements;
- size of herd; and
- notes about any recent change in geographic location of the animal.

An example of a sampling form is provided in Appendix 4.

In addition to the information recorded for each sample, for each breed a form like the example in Appendix 5 should be compiled on the basis of the information available. This form addresses breed origins, farming practices, basic production information, and features of the breed such as productivity, disease resistance or adaptation to local conditions. For further advice on collecting data on breeds' phenotypes and production environments, consult the *FAO Guidelines on Phenotypic Characterization of Animal Genetic Resources* (FAO, 2012).

IN THE LABORATORY

Technological advancement in genomics has been accompanied by the emergence of private companies providing custom genotyping or sequencing services. For many genomic applications, all laboratory procedures subsequent to sample collection can be outsourced, such that a traditional "wet lab" is not strictly needed. Outsourcing saves the investment in expensive and specialized equipment,

which tends to become quickly obsolete. Nevertheless, several issues need to be considered even if the experiments are outsourced.

Extracting DNA

Extraction of DNA is a key step for in-house genotyping and for analyses outsourced to companies that require DNA instead of raw samples. Extracted DNA is also subject to less-stringent sanitary regulations than are biological samples when shipped across borders.

Several reliable protocols for DNA extraction are available. Older protocols are based on Proteinase K/SDS lysis of cells, organic extraction and alcohol precipitation. Salt precipitation avoids organic solvents, but the long-term stability of the DNA samples is problematic. Convenient commercial kits based on the specific binding of DNA to resins are available for several kinds of tissues and usually perform well. Whenever a new DNA extraction procedure is used, a practice run on test samples should be performed to gain experience and ensure it works properly before being applied to the field samples.

DNA assay

Section 3 discusses protocols for genotyping and sequencing in detail. In general, these protocols are highly automated and straightforward, but the indicated procedures need to be followed precisely. The following are general suggestions that will be applicable in many situations:

- Genotyping methods that are sensitive to variation between laboratories, such as microsatellite typing, should include at least one reference sample shared by the laboratories involved.
- The inclusion of a duplicate sample should be considered to permit evaluation of accuracy.
- The quality of the resulting data must be checked critically, even for outsourced analyses.

DATA ANALYSIS

Sections 3 and 4 address specific issues of data analysis according to the type of data (i.e. individual loci or sequence data) and study objective. For the many scientific objectives, software is freely available and greatly simplifies the tasks associated with data analysis. The easy access to software does not, however, relieve the researcher from the responsibility for understanding the genetic and statistical principles underlying the analyses. Each scientist must understand whether and why a specific approach is or is not suitable for his or her data and objective. Instead of copy-pasting computer output into manuscripts, the researcher should evaluate the results critically. For many analyses, multiple options for software are available and repeating an analysis using other options or another program can be informative. When software requires the input of parameters, the effect of parameter changes should be evaluated, especially if true values of the parameters are unknown. Analyses of simulated data is another option for validation of an algorithms. Results of the data analysis should be interpreted in the context of existing biological, genetic and historical knowledge.

PUBLISH IT

Let the world know

Publish your findings in a scientific journal. Explain how the results are relevant in a greater context for management of AnGR. Open-access journals are recommended because of their wide diffusion and free accessibility.

Share the credit

Properly acknowledge contributors of samples and/or data. In general, include all colleagues who supplied samples and/or data as co-authors.

Share the data

After publication, deposit your data in a public database and/or comply with requests to make datasets available as already requested by many journals.

For each of the three tasks listed above, it's important to ensure that all activities are addressed in the MTA.

TRANSLATE THE RESULTS

Genomic characterization studies are excellent capacity building activities and contribute to academic knowledge, but they should be more used to improve the management of the AnGR involved. The country's National Coordinator for the Management of Animal Genetic Resources should be informed about the study and should be provided with data that can be uploaded to the Domestic Animal Diversity Information System (DAD-IS; FAO, 2021). Researchers should communicate their results and conclusions to the main stakeholders of the breeds evaluated and, if request, provide recommendations. The following phenomena can be detected through genomic analyses and may be relevant for breed management:

- **Original diversity.** Breeds located in or having originated near their species' centre of domestication are presumed to have the greatest genetic diversity. The level of diversity can be measured by the amount of heterozygosity, the number of unique haplotypes, or the nucleotide diversity.
- **Unique origin or history.** In many instances, breeds from the same region have relatively high genetic similarity, even if they have distinct phenotypic characteristics, suggesting that they share a common origin or history. Breeds with a distinct origin are assumed to carry unique alleles and thus be of greater value for conservation.
- **Crossbreeding or hybridization.** Breeds that have been subject to recent crossbreeding are typically considered to have decreased conservation value because of lower distinctiveness. This is especially the case when the hybridization involved one or more international transboundary breeds. On the other hand, systematic selection may have created a stable and valuable combination of traits from two breeds, typically a high productivity from one breed and adaptation to a tropical climate from another breed. Well-known examples are the Australian Milking Zebu and the Dorper sheep.
- **Consanguinity.** Conservation activities should avoid inclusion of related animals as much as possible. For example, gene banks should avoid selecting related donors and *in vivo* programmes should select sires that are as unrelated as possible. Genomics can be used to determine the genetic relatedness of animals.
- **Inbreeding depression.** High homozygosity within a breed often leads to decreased fitness and survival. Genomics can be used to diagnose excessive homozygosity and plan matings to decrease the incidence of inbreeding depression. In the worst-case scenario, strategic crossing with another breed may be required to introduce new genetic variation and genomics may be used to identify the most suitable breed.
- **Distinctive phenotypes.** Breeds are often defined by one or more distinctive genetic traits, which may justify their conservation.
- **Genetic defects.** Many genetic defects are determined by mutations of one or a few genes. Identification of the genomic locations of these genes can facilitate elimination of the defect and determination of the precise mutation may allow for the development of control therapies. Deficit or absence of a homozygous genotype for a given locus may indicate lethal recessives that decrease fertility by precluding the development of affected embryos. With the novel CRISPR/Cas technology gene defects can be corrected and the same technology can also introduce mutation that confer desired traits (Menchaca, *et al.*, 2020), although genetic modification is not yet permitted in many countries.

Genomic information will rarely be the only tool available for management of a breed, but can often complement factors such as productivity, breed demographics and cultural significance in making decisions on AnGR management.

INTERNATIONAL COORDINATION

Results from genomic characterization studies have potential high value only for national management of the breed from which the samples originated, but also for other breeds and countries. Thus, sharing of the resources associated with such studies is strongly recommended whenever possible under the terms of use agreements and contracts and the Nagoya Protocol. As already mentioned, the data from such studies should be made available, preferably in a public repository. DNA samples should be stored in a biobank for use in follow-up studies, including international collaborations. Once again, the rules of the source country and conditions imposed by the original owners of the animals must always be respected.

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SECTION 3

Genomic tools and methods

Genomic tools and methods

Since the appearance of the previous version of these guidelines in 2012, there has been a spectacular growth in the use of genomic technologies, which still is becoming more and more powerful and accessible. The first reference genomes from whole genomes sequencing (WGS) allowed the development of the multilocus bead arrays of single nucleotide polymorphisms (SNP) for most domestic species. These fulfilled the objectives originally envisaged for microsatellite genotyping and have remained to be still useful. The next development was a universal adoption of WGS technologies. Reference genomes were considerably improved and approaches as resequencing and genotyping-by-sequencing (GBS) now allow the collection of WGS datasets for tens or hundreds of individuals, to be organized in multilocus genotype datasets. All this is even self-reinforcing, since the availability of high-density genotypes allows an elevation of low- to high density datasets by imputation.

SNP GENOTYPING

Single nucleotide polymorphism

A SNP is a deoxyribonucleic acid (DNA) sequence variation that occurs by substitution of a nucleotide at a specific position in the genome. The SNP is the most common type of polymorphism and is estimated to occur with a frequency of one SNP per 1 kilobase (kb) in most mammalian genomes. SNPs have increasingly become the marker of choice and gradually replaced microsatellite markers to assess genetic diversity, structure and relationship among populations; and to identify genomic regions associated to economic traits. SNPs have several advantages over microsatellites, including (i) stable inheritance; (ii) distribution throughout the genome at a greater density; (iii) location in coding regions that can possibly alter protein function and phenotype expression; (iv) location nearby or within quantitative trait loci of interest; and (v) suitability for high throughput genotyping.

Candidate SNP genotyping methodologies

Discovery of a SNP at the candidate gene level is done by targeted re-sequencing of specific regions of the genome in a subset of unrelated individuals representing the species or the populations of interest. Several methodologies are available to genotype one or a few SNPs identified in different regions of candidate genes like exons, introns, promoters and untranslated regions. Most of these methods are based on conventional or real-time polymerase chain reaction procedures. A few examples include: (i) traditional gel-based approaches, such as amplification refractory mutation system (ARMS; Little 2001), restricted fragment length polymorphism (RFLP; Jarcho 1994), single strand conformation polymorphism (SSCP; Dong and Zhou 2005), denaturing gradient gel electrophoresis (DGGE; Strathdee and Free 2013); and (ii) medium throughput approaches, such as competitive allele specific polymerase chain reaction (KASP assays; He *et al.*, 2014), exonuclease detection (Taqman assay; Holland *et al.*, 1991), mass spectrometry-based primer extension detection (Sequenom) and high resolution melting (Bradić *et al.*, 2011).

SNP microarrays

Advances in next generation sequencing technologies and rapidly decreasing costs of WGS have made SNP discovery at the genome level possible in most of livestock species. The process includes WGS of individuals from a subset of populations of interest and selection of polymorphic loci for additional genotyping in a larger pool of animals, often using high throughput SNP microarray technology. The SNP microarrays consist of allele-specific oligonucleotide probes fixed on a solid support, such as glass slides or silicon quartz wafers. The target DNA samples amplified from fragmented whole genomes are hybridized to these oligonucleotide probes for single nucleotide extension or ligation. The labelled SNPs are visualized by an immunohistochemistry assay to increase the signal intensity and infer the genotypes accurately.

Two major commercial SNP array platforms are currently available for genome-wide SNP typing of livestock species: (i) Illumina's Infinium iSelect Microarray or BeadChip based on single nucleotide extension or allele-specific primer extension (Illumina Inc., 2016) and (ii) Affymetrix's GeneChip or AxiomArray based on molecular inversion probe hybridization (Affymetrix Inc., 2020). The two platforms differ in their array fabrication and protocols. The BeadChip uses 50-mer (i.e. 50 nucleotides) allele specific probes while the GeneChip uses 25-mer and/or 30-mer probes for hybridization with target DNA samples.

The BeadChip is produced by etching out wells on a silicon wafer through photolithography and plasma etching methodologies. The beads linked to "oligos" (oligonucleotides, short strands of DNA) containing 29-mer address tags and 50-mer SNP specific primers are randomly dispersed and assembled into these wells etched on the silicon wafer. The GeneChip is produced by *in situ* synthesis of oligonucleotide probes on a chemically protected array surface. The method relies on photolithographic masking of array surface, ultraviolet light directed deprotection of specific array spots and synthesis of probes through nucleotide coupling, with one nucleotide at a time per spot, for many spots simultaneously. The sequential application of specific lithographic masks determines the order of the probe sequence synthesized on the array surface. At least four photolithographic masks are required to synthesize each nucleotide of the probe sequence (one mask to allow addition of the required nucleotide and three other masks to prevent light from deprotecting the same spot while the other three nucleotides are being added).

SNP array design

The process of SNP array design starts from SNP discovery and includes validation of *de novo* variants, selection of marker loci, validation of draft SNPs and synthesis of arrays.

SNP discovery. The ideal SNP discovery process for designing and developing a diversity array for a given species involves WGS of a panel of unrelated individuals, preferably from diverse breeds across wide geographical locations, ensuring detection of all possible variants within the targeted livestock species. The low coverage sequencing of large numbers of individuals has proven to be more advantageous than deep sequencing of fewer individuals in improving the power of the SNP discovery process (Buerkle and Gompert 2013). SNPs identified from previous studies and databases (e.g., Hapmap, BAC libraries, dbSNP, etc.) can also be utilized to increase the number of SNPs available for the design of the array (Matukumalli *et al.*, 2009).

Selection of SNPs. Errors in genome sequencing can lead to detection of false SNPs. Hence *de novo* variants need to be validated based on quality parameters like: (i) minimum sequencing read depth; (ii) consensus base ratio; (iii) SNP quality score; and (iv) relative distance of the base from the 3'-end of the read, normalized by read length (Shen *et al.*, 2010). Generally, the SNP discovery process results in the identification of several million SNPs after initial validation. Among these, a subset of loci (~2-3 millions) needs to be selected for drafting a marker panel (screening array) based on different criteria (e.g., Fan *et al.*, 2010; Kranis *et al.*, 2013):

- *In silico* design score for likelihood of success in the genotyping assay;
- Minor allele frequency (MAF – frequency of the less-common allele for biallelic SNP);
- Presence of nearby SNPs (e.g., exclusion of SNPs with nearby polymorphisms within 10-15 bases);
- Linkage disequilibrium (LD) estimate with other SNPs included in the panel;
- Physical distribution of SNPs (e.g., equidistant spacing over the genome);
- Polymorphism in multiple populations; and
- Enrichment of specific regions of the genome (genomic regions potentially associated with quantitative trait loci).

Validation of SNPs. The screening array with a draft panel of preselected markers must be validated to accomplish the goal of identifying the second subset of high-performance SNPs that show the potential for various downstream applications, such as diversity, marker-trait association and gene mapping. During the process of SNP validation, the following criteria are utilized to identify marker

loci that are expected to be placed on routine genotyping array (Illumina Inc.; Affymetrix Inc.; Fan *et al.*, 2010; Kranis *et al.*, 2013):

- Performance of SNPs in terms of high call rates;
- Accuracy of genotyping (e.g., efficiency in clustering genotypes, to distinguish homozygous and heterozygous classes);
- Informativeness of SNPs;
- Association with traits of interest;
- Tagging other variants based on LD;
- Imputation of other variants in the genome; and
- Functional significance of SNPs (e.g., exonic/intronic, synonymous/non-synonymous, coding DNA sequence/untranslated region).

Routine genotyping array and marker density. Arrays of different marker density may be used depending on the purpose of downstream applications and the genetic structure of the population investigated. Among the arrays routinely used in livestock species, the marker density ranges from low (<20 000), medium (50 000), medium high (150-200 000) to high (>500 000) SNP density. The cost of per array increases with the number of SNPs, but the cost per marker decreases. The best-performing and most informative markers can be utilized to design smaller, cost-effective arrays for routine genotyping for a specific purpose. Low-density SNP arrays designed with markers having higher MAF and uniform spacing across the genome may be “imputed” (see specific sub-section on this topic below) to higher density (or even complete sequence) if a large set of reference animals are genotyped at higher density or whole-genome sequenced (Boichard *et al.*, 2012). However, with advancements in array fabrications and automated fluidic technologies, the cost of medium density arrays has significantly decreased, resulting in the phasing out of low-density arrays.

Ascertainment bias

When SNPs selected for designing and developing an array are discovered by sequencing only a few samples or from only a few selected breeds, an ascertainment bias (AB) occurs to affect inference about larger and distinct populations. There are two major kinds of ABs in SNPs: (i) MAF bias; and (ii) sub-population bias. MAF bias occurs when the SNPs are preferentially selected based on intermediate frequencies. This results in over representation of common polymorphic loci but under representation of low frequency polymorphisms. Consequently, estimates of population genetic parameters, allele frequency distribution and linkage disequilibrium can be biased (Albrechtsen *et al.*, 2010; Heslot *et al.*, 2013). Sub-population bias occurs when the SNP discovery panel is chosen from individuals belonging to a specific sub-population or a small group of breeds. This results in an overestimation of the variability present in that population but an underestimation of the variability in genetically dissimilar populations. Accordingly, this AB can inflate heterozygosity estimates in populations of breeds that are closely to the breeds in the SNP discovery panel. The AB can increase or decrease the estimates of various genetic parameters as compared to the expected estimates from unbiased data, thus distorting information on population differentiation (Lachance and Tishkoff 2013; McTavish and Hillis 2015).

The phenomenon of AB is not unique to SNP markers alone and may also occur with morphological and microsatellite markers. The AB in microsatellites occurs when the most polymorphic markers are typically selected (Vowles and Amos 2006; Vali *et al.*, 2008). Marker bias influences estimates of genetic variability of populations, but the influence of biased SNPs is much greater than that of microsatellites, by virtue of their large numbers and biallelic nature. One of the potential approaches to mitigate AB is to adopt SNP filtering strategies, such as LD pruning. The basic idea is to remove multicollinearity effects by removing SNPs that are highly correlated with other SNPs within a given genome window, thus reducing LD among the SNPs left after pruning. LD pruning has been effective in reducing AB when estimating genetic differentiation measures among populations including genetic distance, fixation index, inbreeding coefficient, kinship and principal components analysis (PCA) (Malomane *et al.*, 2018). Although LD-based pruning does not fully offset the AB, it does help to reduce its effects. Another potential approach to mitigate AB is to utilize ancestral SNPs (e.g., those

polymorphic SNPs present in wild relatives of a given livestock species) while comparing highly divergent populations. The ancestral loci have relatively higher heterozygosity and are less likely to show population bias, but fine scale patterns of diversity among closely related breeds may be missed (Malomane *et al.*, 2018; Barbato *et al.*, 2020).

How to choose your array?

Choosing a suitable array for genotyping depends on the purpose of the study. Although not exclusive, the following factors may be considered while choosing the array:

- SNP density (e.g., high, medium and low-density arrays);
- potential AB (e.g., genetic/geographic distance between discovery panel and the population to be investigated);
- tagging of specific genomic features (e.g., markers recommended by the International Society for Animal Genetics (ISAG) for parentage testing, copy number variants (CNV), markers for detection of recessive traits);
- SNPs overlapped with existing genotype data;
- cost effectiveness of genotyping (e.g., marker density *vs.* array cost); and
- performance of the array (genome coverage, genotype clustering).

Selecting a high-density array for genome wide association can help in capturing maximum possible variants that have potential influence on the trait (Tsai *et al.*, 2015). In case of genetic evaluation (genomic selection) studies, where genotypes/sequences of several individuals from the reference population are available and population LD is generally high, choosing a low-density array followed by genotype imputation may be cost-effective (Boichard *et al.*, 2012; Georges *et al.*, 2019). With the rapid growth of sequence/marker information over time, newly updated versions of arrays with special features keep evolving for many livestock species across different platforms (See Appendix 7). While choosing a new array for genotyping, checking the overlap of SNP with other available arrays can improve harmonization with existing data for comparative and meta-analysis across studies.

Merging SNP datasets

Unlike microsatellites, SNP markers facilitate merging and harmonizing datasets across different studies, generated in various laboratories and/or different platforms. The genome wide SNP genotypes can also be merged with the genotypes identified by genome re-sequencing data to generate information on specific loci of interest. Datasets are merged based on marker identification numbers and genomic positions, after alignment to a standard reference genome assembly of the species investigated. Care needs to be taken on the strand orientation of markers while merging different datasets. Open source programs, such as PLINK (Chang *et al.*, 2015; Purcell 2010), are available to facilitate merging genotypes from different datasets and optimize resources available for genomic characterization studies in livestock.

GENOTYPE BY SEQUENCING

The principle

Although SNP arrays are arguably the most popular genotyping platform, genotyping-by-sequencing (GBS) can be a method of choice in certain settings, particularly in *de novo* applications (Scheben *et al.*, 2017). The GBS approach is a variant of resequencing that targets a small part of the genome and in one step identifies SNPs, many of which may be novel, and yields the genotypes. The two most common acronyms for genotyping-by-sequencing methods are GBS and RADSeq and their variations.

The principle of GBS is the targeting of a fraction of the genome in such a way that the same fraction is sequenced in all individuals, the so-called “reduced representation” of the genome. This can be accomplished, for instance, by hybrid-capture or size selection of fragments generated by restriction enzyme digestion. The fragments are then sequenced, sequence reads are mapped against a reference genome and alleles called from the reads (Figure 1). There is considerable variation in these steps, which ultimately control the number and quality of resulting markers. Fourteen different methods of

GBS have been described (Scheben *et al.*, 2017) and new ones are continually proposed (e.g., Rowan *et al.*, 2017).

Calling genotypes from alleles is reliable if fragments are sequenced many times. Obviously, the probability of correctly calling a heterozygotes increases with the depth of sequencing (where “depth” refers to the number of times each fragment is sequenced, on average): 0.000 for $x=1$, 0.500 for $x=2$, 0.750 for $x=3$, 0.875 for $x=4$, 0.938 for $x=5$ and 0.998 for $x=10$. Generally, sequencing error is low, 0.01-0.001, but not zero, which makes genotype calling a bit more complex than schematically presented above. Available software tools account for sequencing errors (see Scheben *et al.*, 2017; Lou *et al.*, 2020; and the Software section).

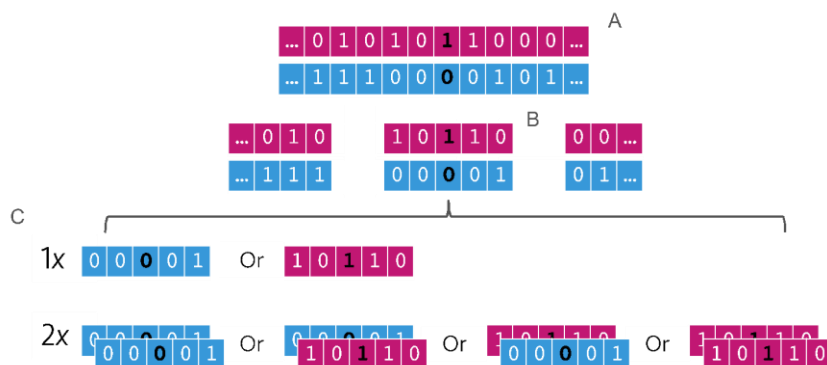


FIGURE 1
Characterizing variation at a fragment of DNA of a diploid individual (A) by focusing on a specific locus (B) and repeatedly characterizing its sequence (C)

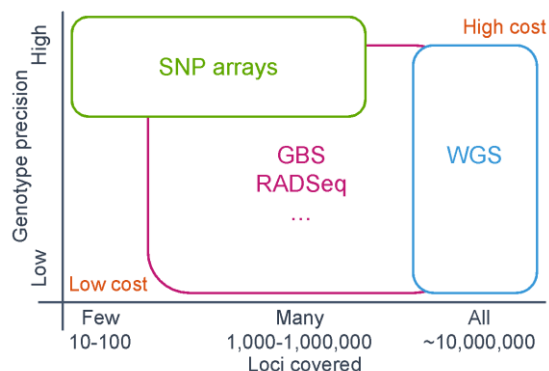


FIGURE 2
Schematic comparison of SNP array genotyping, genotyping-by-sequencing (GBS, RADSeq) and whole-genome sequencing (WGS) in terms of number of covered loci, precision of called genotypes and cost

Advantages and disadvantages

The GBS approach has several main advantages. First, GBS does not depend on a previously chosen set of markers or on the availability of whole-genome data and will reveal genetic variation within any livestock or wildlife population. Consequently, GBS may provide more relevant markers than a commercially available SNP array. Second, GBS yields novel SNPs much faster than can be done by WGS sequencing and also gives immediate information on their minor allele frequencies and phylogeographic pattern within the panel of sample. Third, GBS completely avoids the AB (e.g., Schraiber and Akey, 2015; Simčič *et al.*, 2015; Lou *et al.*, 2020), except for GBS after hybrid capture. Fourth, users can tune GBS to their purpose and budget by choosing the number of sequenced fragments and the depth of sequencing (Figure 2) (see also Section 4). The GBS approach also provides an option for less common species that lack commercial SNP arrays.

There are, however, also disadvantages. First, GBS generally requires purified high-molecular weight DNA. Second, unique alleles in a population can be hard to distinguish from sequencing artefacts, whereas presence-absence variation due to deletions or insertions cannot be scored reliably at low sequencing depth. Third, because the number of markers depends on the sample and the output consists of allele calls instead of genotype calls, GBS requires additional bioinformatic attention in downstream analyses. Note that these considerations also apply SNP calling on the basis of WGS assemblies. However, sequencing methods and tools for downstream analyses are continually improving.

Implementation and applications

Several providers offer continually evolving custom genotyping-by-sequencing. GBS has been adopted actively in several scientific communities (e.g., Buerkle and Gompert, 2013; Scheben *et al.*, 2017; Lou *et al.*, 2020). This adoption was fuelled by a lack of genomic resources or of SNP arrays in target species and/or unacceptably AB in the available SNP arrays. GBS is less expensive than SNP bead arrays for few individuals, but for high numbers of samples the streamlined SNP arrays become cheaper and offers the advantage of a more straightforward data analysis.

Several studies have leveraged GBS for population genetics studies. For example, Dodds *et al.*, (2015) and Bilton *et al.*, (2018) accounted for sequencing depth when estimating relatedness and linkage disequilibrium, respectively. Dodds *et al.*, (2019) and Whalen *et al.*, (2019) applied GBS to parentage assignment. Interestingly, Lou *et al.*, (2020) reviewed a number of standard population genetic analyses, most of which were based on GBS data without imputation and therefore had to develop specific approaches for standard analyses. An alternative approach is to infer genotypes from GBS allele calls from multiple individuals and loci and then follow standard analyses for genotypes obtained with SNP arrays.

Imputation of GBS datasets

The availability of GBS and other genotyping platforms has created the need for multiple types of imputation (Figure 3B-D). Imputation of genome-wide data is a large and a fast-evolving field and is in particular suitable for populations with a known pedigree and a small number of parents genotyped by routine SNP array genotyping. For such populations, GBS strategies have been designed that enabled imputation of whole-genome sequences in hundreds of thousands of individuals (Ros-Freixedes *et al.*, 2020a,b). Whalen *et al.*, 2018, 2020) have developed methods, implemented in the programs ALPHAPEEL and ALPHAFAM, that quickly and accurately calls genotypes, carries out phasing and imputes whole-genome sequence data of any sequencing depth in pedigrees. GBS data can also be imputed without pedigrees (BEAGLE, Browning and Browning, 2016; STITCH Davies *et al.*, 2016; and GLIMPSE Rubinacci *et al.*, 2021). Whereas imputation of SNP bead-array datasets to a higher density or to a WGS is at the expense of accuracy, imputation of GBS datasets improves the accuracy of genotypes that in low-depth datasets are incorrect by missed alleles (Figure 3B-D).

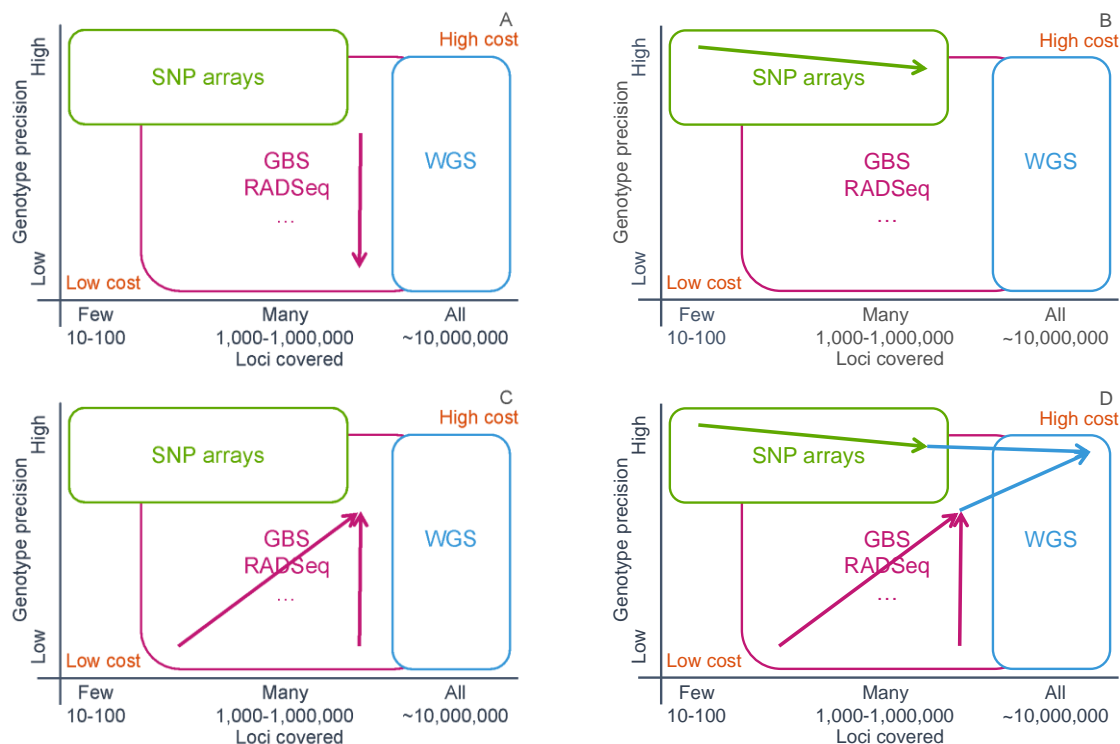


FIGURE 3

Strategies to increase volume of genomic data per unit of cost are indicated by arrows – to enable generating genomic data in the first place or genotyping more individuals at higher densities: A) reducing the depth in genotyping-by-sequencing; B) imputation from low-density to high-density SNP arrays; C) imputation from low-density and low-depth to high-density and high-depth in genotyping-by-sequencing; and D) imputation to whole-genome sequence.

PREPARING A WORKING MULTILOCUS DATASET

Genotyping and sampling errors can occur when performing genomic assays. The analysis of genetic data requires quality checks to identify such errors. Genotyping errors in SNP arrays can be due to low DNA concentration, insufficient quality of the DNA samples, sample contamination, poorly performing genotype probes, poor hybridization of DNA from very divergent populations or related species; and – in the case of WGS and GBS data – to low sequence coverage. Quality checks are performed at both the individual- and SNP-level and guide the “filtering” or the removal of the individuals and/or SNPs. The most common filtering approach attempts to preserve the number of individuals rather than the redundancy of SNPs. However, the optimal procedure and cut-off values for discarding of data should always be evaluated according to the dataset and the objectives of the study.

Although the manipulation and analysis of high-throughput genomic data, and interpretation of the results may require specialized bioinformatic competence (FAO, 2015), several well-documented and freeware bioinformatic tools are available, providing user-friendly tools and routines for the quality checking procedures. Among many, these are the most popular and comprehensive: PLINK (Chang *et al.*, 2015; Purcell, 2010) and KING (Manichaikul *et al.*, 2010).

Merging of datasets

As mentioned previously, a substantial advantage of SNPs relative to microsatellite markers is the option to combine data with existing datasets based on the same SNP panel or panels that have many SNP in common. Since in livestock species most DNA arrays are provided by two major companies (i.e. Illumina and Affymetrix) with considerable across firm and backward compatibility of new

versions, this is often the case. Differences in SNP identification codes and genomic coordinates can be harmonized across the datasets to be merged by using the most updated assembly information for a given species. Differences in strand orientation are identified by four alleles (A, C, G, T) recorded at a given locus, although a strict biallelic locus is generally expected. Such errors can be detected by PLINK during the merging operation and corrected by swapping A↔T and C↔G. However, different strand orientations in A/T and G/C SNPs cannot be detected, and it is advisable to remove all A/T and C/G SNPs if a strand flip has to be carried out. It is also recommended that the datasets to be combined share at least one breed, in order to verify that in the combined dataset *all* animals from this breed cluster together and the diversity patterns are not confounded by systematic differences between the datasets that have been merged.

Mode of inheritance

Mitochondrial DNA (mtDNA) markers are only inherited via the maternal lineages and markers on the male-specific part of the Y-chromosome (MSY) only via the paternal lineages. Both mtDNA and the MSY do not participate in meiotic crossing-over and both constitute a single non-recombining haplotype. As a consequence, they profoundly differ from autosomal markers in terms of mutation rate, evolutionary mechanisms, effect of genetic events and phylogeographic diversity patterns. X-chromosomal markers have a biparental mode of inheritance, but nonetheless differ in several properties from the autosomes:

- They have only one copy in males and thus a 25 percent lower effective population size.
- Genes involved in reproduction are overrepresented on the X-chromosomes.
- X-chromosomes are more involved in meiotic drives than are autosomes, the reasons for which are only partially understood (Hughes *et al.*, 2020).
- X-chromosomes also have a pattern of diversity that differs from the autosomal pattern (Wilson Sayres, 2018; da Fonseca *et al.*, 2019).

As a practical consequence for genetic diversity studies, mtDNA, MSY, X-chromosomes and autosomes should be analyzed separately. This happens automatically if LD pruning is applied, since this removes all three types of SNPs with a special mode of inheritance. For localization of genetic traits by GWAS, autosomes and X-chromosomes can be combined.

Missingness of data

The SNP and sample “missingness” is defined as the percentage of missing genotypes for a given marker or individual, respectively, and is complementary to the “call-rate” (5 percent missingness equates to a 95 percent call-rate). High proportions of SNP missingness can be due to a probe design inadequate for all or a part of the samples and in the latter case can lead to a bias in allele frequencies. A high proportion of missing genotypes for a sample indicates either a poor DNA quality, deviating species origin, or low sequence coverage for WGS- and GBS-based datasets.

For DNA array datasets, SNP-level filtering is typically performed first, followed by sample-level filtering, both allowing 5 percent missingness. A more refined approach can reduce the amount of data lost due to filtering by following a two-step approach. In the first step, a more relaxed threshold (such as 20 percent) to remove SNPs and samples with extremely high levels of missingness, followed by an additional filtering step with a more stringent threshold (e.g. 5 percent). Note that the SNP filtering step removes Y-chromosomal SNPs unless males account for at least 95 percent of the samples.

Sex discrepancy

When the sex of the animal providing the samples is known, discrepancies between the recorded and inferred sex may identify mislabelling of samples. Such an occurrence may indicate poor sample management and should promote a thorough check of the dataset and available metadata. The gender is derived by PLINK from the classical inbreeding coefficient [$F = (\text{expected homozygosity} - \text{observed heterozygosity}) / \text{expected homozygosity}$] for X-chromosomal SNPs; $F > 0.8$ and < 0.2 are often adequate cut-off values for males and females, respectively. Intermediate values may indicate either a mixed sample origin or, for male samples, the presence of SNPs from the pseudoautosomal region

(PAR – region of commonality between sex chromosomes). The PAR can be localized by comparing the frequency of heterozygotes across the X-chromosome in males and females and can be relabelled as a separate chromosome not to be used for sex assessment. It is also essential to perform the sex-check assessments in PLINK on sets of loci in approximate linkage equilibrium (see section LD below). Furthermore, even if the inferred and nominal sex agree, the presence of Y-chromosomal genotypes in a sample labelled as female is another clear warning of a problem.

Minor allele frequency

As explained previously, the MAF for a biallelic SNP is the frequency of the least-commonly occurring allele at a given locus, hence MAF must be ≤ 0.5 . SNPs with a MAF of a few percentage points or less can represent true low-frequency variants but also be the result of genotyping errors. For this reason, it is common to retain only SNPs with $\text{MAF} \geq 0.05$ for many analyses. Alternatively, with large data sets, the threshold $\text{MAF} \geq 10/N$, based on the consideration that a larger sample size (N) reduces the likelihood that a small MAF results from genotyping errors. However, some analyses (such as genome-wide association studies – GWAS – see Section 4) are highly affected by low-frequency variants and thus more stringent thresholds are often applied (e.g. requiring $\text{MAF} \geq 0.05$ even for $N \leq 10,000$).

The MAF is particularly affected by sampling bias and AB. If a dataset has a large degree of homozygosity (hence, a small MAF) a larger sample size is necessary to obtain enough SNPs passing the MAF filtering because of the low overall frequency of heterozygotes. The effect of AB is proportional to the degree of divergence between the discovery and the study populations (Helyar *et al.*, 2011) and leads to an underestimation of the genetic diversity of the study population. This applies for instance to zebu cattle analysed by the popular arrays designed mainly for taurine cattle (Barbato *et al.*, 2020). As discussed above, the AB can be mitigated by filtering loci in high LD and by selection of markers that have a high MAF in the ancestral breeds or in the wild ancestors.

Allelic frequencies are used to calculate expected heterozygosity (H_e) and genetic distances between breeds. For optimizing the breed composition, it should be considered that a low sample size for a given breed (< 20 animals) can lead to underestimating the H_e and thus to inflating the genetic distances to other breeds.

Hardy-Weinberg equilibrium

The Hardy–Weinberg equilibrium (HWE) implies a direct relationship between allelic frequencies (p and q , with $p+q=1$ in the case of a biallelic SNP) and the genotypic frequencies (p^2 and q^2 for the homozygotes, $2pq$ for the heterozygotes), which is supposed to remain constant over generations. The HWE is valid for a Wright-Fisher population of infinite size, with random mating, no selection, mutation, and migration. Significant departure from HWE for individual SNPs can be caused by population subdivision and substructure (e.g. Wahlund effect; Garnier-Géré and Chikhi, 2013), recessive lethal mutations, selection, non-random mating, inbreeding, and genotyping errors (Chen *et al.*, 2017). For datasets of larger populations and datasets aimed at GWAS, it is a common practice to explain strong deviations from HWE by genotyping error and remove the corresponding SNPs.

The HWE can be tested by the so-called exact test, which provides a P -value for the significance of the HWE departure and is fairly robust to deviations from the theoretical assumptions. If the downstream analysis focuses on the identification of selection signatures, it is recommended to use a permissive HWE filtering threshold (e.g. $P < 10^{-10}$) to avoid filtering out too many variants. In the case of GWAS applied to binary traits it is common practice to use more lenient thresholds in cases (e.g. $P < 10^{-10}$) than in controls ($P < 10^{-6}$), because deviations from HWE in cases can be due to true genetic association with the focal trait. When assessing quantitative traits, stricter thresholds can be used (e. g. 10^{-6}).

Non-random mating, which in its most extreme form leads to population subdivision (stratification into different sub-populations), is expected to cause HWE deviations across the whole genome, but

such subdivision is commonly tested by comparing expected and observed heterozygosity (see below), and applying model-free multivariate statistics as principal component analysis or multidimensional scaling.

Heterozygosity

The observed heterozygosity of an individual (H_o) is the proportion of heterozygous genotypes, that is carrying two different alleles at a given locus, preferably considering only autosomal loci. Within populations, individual heterozygosity tends to be variable, but values for individuals with three standard deviations (SD) above the population average may indicate sample contamination or crossbreeds, whereas values three SD below the average can be caused by inbreeding. Filtering H_o outliers is particularly important when the dataset is supposed to be genetically homogeneous, as in GWAS.

For checking the genetic constitution of a population, H_o averaged across the individuals of a population is an indicator of the diversity of the breed, although it should be borne in mind that it can be influenced by AB (see above). It is further useful to compare H_o with H_e , the expected heterozygosity, calculated on the basis of the population allele frequencies. H_o is generally lower than H_e as a consequence of sporadic non-random mating or sampling bias. Large differences between H_o and H_e indicate significant deviations from the Wright-Fisher ideal population, (e.g. $H_o > H_e$) can account for recent population admixture or outbreeding, while $H_o < H_e$ can indicate population subdivision or inbreeding. Both conditions may influence analyses that presume a homogeneous population. The extent of inbreeding/outbreeding can be quantified as $F_{IS} = (H_e - H_o)/H_e$, where F_{IS} represents the inbreeding coefficient.

Linkage disequilibrium

SNPs on the same chromosome are in LD if there is a significant association of their alleles. This is to be expected for loci that are nearby on the same chromosome, for instance within or proximal to the same gene, so that recombination between them is a rare event. LD is also normal for the non-recombining mitochondrial DNA and the male-specific part of the Y-chromosome. However, high LD of well separated genomic SNPs, that is not compatible with the expected recombination rate (about 1 percent per 10^6 base pairs (bp) per generation), may be caused by demographic processes such as admixture and genetic drift or by selective processes such as “hitchhiking” or background selection (McVean, 2007). Occasionally LD is observed between SNPs on different chromosomes (expected recombination rate 50 percent per generation), which is an artefact due to the sampling of a limited number of gametes or individuals.

A consequence of LD is that nearby markers are not independent. Therefore, it is common to implement LD-based pruning procedures to retain markers that are in approximate linkage equilibrium and are thus largely independent. This procedure is required when an analytical model does not explicitly take LD into consideration (see sections on MAF and relatedness). A common PLINK implementation of LD based pruning would calculate correlation coefficients (r^2) within sliding windows across the genome and remove one of each pair of SNPs having LD greater than a given threshold. Typically, markers are selected in 2-Mbp windows that shift 0.2 Mbp forward (~10 percent of the window size) at each iteration while applying a LD threshold corresponding to $r^2 = 0.2$. LD pruning can also be used for data size reduction, as it removes redundant SNPs by selecting only those SNP that are representative of the genetic haplotype blocks. Under this scenario it is possible to apply more conservative LD thresholds (e.g. $r^2 \geq 0.5$), which will remove the highest-correlated loci while preserving moderately correlated SNPs (e.g. due to low-frequency variants). Such an approach will retain the underlying genetic structure of the dataset while reducing the computational burden. Note that LD-pruning removes mtDNA and Y-chromosomal SNPs. It should be carried out after removal of SNPs with low call rates and bad-scoring or contaminated samples. It is beneficial for calculation of genetic distances, principal components analysis or model-based ancestry analysis (e. g. as implemented in the popular software Admixture - Alexander *et al.*, 2009), but not for haplotype-based studies, such as runs of homozygosity (ROH) and local ancestry analyses. An additional benefit of LD pruning is alleviating the AB in DNA array datasets (see above) (Malomane *et al.*, 2018).

Relatedness

Relatedness or kinship indicates the similarity of a pair of individuals due to common ancestry.

Checking the pattern of kinship is essential for several reasons:

- Individuals may be found to be duplicated or identical twins.
- The presence of highly related individuals introduces a bias towards a specific family in the dataset, or reduces the statistical power of GWAS studies. It may be avoided during sampling using pedigree information or a well-considered sampling scheme, but this is not always feasible.
- More in general, kinship may lead to population stratification, which confounds several analyses that assume a homogeneous population. For instance, it may easily cause false positives in single-gene association studies.
- Outliers with a much lower kinship with the other individuals than the population average are either to be excluded from analyses, as likely to be crossbred or mislabelled.
- If genetic distances between animals within breeds are about the same as between neighbouring breeds, genetic clusters do no longer correspond to breeds. As a possible consequence, breed demarcations may be reconsidered, especially if the lack of breed-level differentiation is due to uncontrolled gene flow between local populations kept under an extensive management regime.

A common method for the identification of related individuals is to compute the pairwise proportion of shared alleles inherited from a common ancestor (identity by descent, IBD) across all individuals. The magnitude of the resulting IBD score then reflects the relatedness of two individuals. This classic approach requires approximate linkage equilibrium (see the section on LD) and should be performed on autosomal markers. It relies strongly on the assumption of homogeneous population structure. A matrix of IBD scores may be visualized by plotting it as a heat map.

A more robust and efficient algorithm for inferring relatedness of individuals has been implemented in the software KING (Manichaikul *et al.*, 2010), which has been recently included in PLINK 2.0. The KING algorithm does not require LD among markers or a homogenous population structure. The KING relatedness coefficients are scaled in order to correspond to kinship coefficients (which are $0.5 \times$ the relationships coefficients): monozygotic twins/duplicate samples have kinship 0.5; first-degree relations (parent-offspring, full siblings) have 0.25; etc. As a cut-off value the geometric mean of the values corresponding to two degrees of kinship is used (e.g. a kinship coefficient >0.354 , which is the geometric mean of 0.5 and 0.25) to identify monozygotic twins and duplicate samples.

Alternatively, a neighbour-joining phylogram of allele sharing distances between individuals can be generated to visualise identical samples, variable kinship, outliers, breed-level differentiation and, via the lengths of the terminal branches, the effect of genetic isolation on the diversity within breeds (Cardoso *et al.*, 2018). It will also show the clustering of closely related breeds. For more distant breed relationships genetic distances between breeds instead of between individuals is more suitable. Identical individuals should be removed from the dataset and, if possible, also those individuals with first or second-degree kinship (this is particularly important for GWAS), by removing from a high-related pair the individual with the lowest mean genotype call rate. Because one individual can be involved in several pairs, PLINK 2.0 has implemented an algorithm that maximizes sample size while pruning for relatedness. For calculation of relatedness on the basis of GBS datasets, see Dodds *et al.*, (2015).

Sample size

For diversity studies involving several breeds, availability of samples may be a limiting factor. Having a few samples of an interesting breed is still valuable, but its heterozygosity will be underestimated. Sample size does not have a large influence on the topology of phylogenetic networks or trees, but empirical evidence suggests that having <20 animals inflates the genetic distance to other breeds.

However, 20 animals may not be sufficient to detect low levels of breed admixture or for obtaining meaningful GWAS results (Ball, 2013).

On the other hand, more is not always better. When comparing multiple populations, breeds that are overrepresented may very well distort the results by dominating the principal components and the inference of clusters, respectively, especially if these breeds are also inbred. Hence, for these analyses it is recommended to reduce the number of samples from overrepresented breeds to obtain a dataset with a more balanced representation of breeds.

WHOLE GENOME SEQUENCING

Genotyping arrays are limited to assessing variants that have been converted to assays and are amenable to array genotyping (generally, only SNP), such that the same sample may have to be run with multiple different assays to achieve desired goal(s) or to AB. In contrast, WGS has the advantages that variants across the entire target genome are assessed and it is possible to identify all types of variants including SNP, indels, CNV, and structural variations (SV) like inversions or large deletions. The success of applying WGS for genome characterization depends on several factors including the type and depth of sequencing used and the analysis method(s). Here we present some background on the currently available approaches to using WGS for characterizing variation independent of the target species. Characteristics of the population (breed or species) such as effective population size, or polyploid genomes, may affect the decision on the best approach.

De novo assembly

The most comprehensive evaluation of a genome comes from an independent, haplotype-resolved *de novo* whole genome assembly of the animal. This is the most expensive and technically demanding approach, although the cost has dropped by four orders of magnitude since the first livestock genomes were assembled 15 years ago, and the quality of the assemblies has dramatically increased. The basis of this improvement is the advent of long-read sequencing (LRS) platforms, each of which has particular advantages and drawbacks (Amarasinghe *et al.*, 2020; Logsdon *et al.*, 2020).

The Pacific Biosciences (2015) platform, often referred to as PacBio, can operate in two “modes”, one in which read length is maximized at the expense of read accuracy, and one with relatively shorter reads where read accuracy is maximized (the “HiFi” mode). The former produces contiguous assemblies at lower cost but with higher error rate; the latter produces very high accuracy but somewhat less contiguous assemblies due to the shorter read length. The Oxford Nanopore Technologies (2008) sequencing platforms excel at generating extremely long reads that are very useful for assembling repetitive or duplicated regions of the genome but require relatively large amounts of input DNA and therefore substantial sample size. Both LRS platforms are dependent on availability of suitable DNA template that consists mainly of very long fragments (10s to 100s of kilobases) of undamaged DNA. The characteristics of the two currently available LRS platforms differ sufficiently that a single extraction method has yet to be formulated that is ideal for both platforms.

Short-read sequencing (SRS) (Bentley *et al.*, 2008; Margulies *et al.*, 2005; McKernan *et al.*, 2009; Rothberg *et al.*, 2011) assemblies are the least expensive option and suitable for population level analysis and transcriptomics. However, their contiguity is orders of magnitude lower and all analysis of repetitive content and gene families must be done with consideration of their deficits. There are approaches to improve SRS assemblies through large insert size libraries, or hybrid approaches that combine SRS and LRS. The most effective hybrid approach is linked-read technology (Wang *et al.*, 2019; Zhang *et al.*, 2017; Zheng *et al.*, 2016). In linked-reads, a library production technology is applied that adds barcodes to short fragments derived from a single, longer template molecule. Millions of these independent “micro-libraries” with different barcodes are then sequenced on standard short read platforms. Post-sequencing, the barcode information is used to partially reconstruct the longer DNA molecule and provides longer-range assembly continuity compared to standard, whole-genome short read sequencing.

All platforms are limited in their ability to assemble genomic regions that undergo somatic cell rearrangements in blood cells (immune gene complexes like the T-cell receptor or immunoglobulin loci), if blood is the source of DNA.

Sequence data is first assembled into “contigs”, which are stretches of DNA sequence without gaps. The SRS assemblies have N50 size (the length of the contig where the sum of all longer contigs is >50 percent of the total assembly size) in the range of a hundred kilobases (kb), while the contigs that can be created from LRS data can have N50 size over 70 megabases (Mb), or 700x as long. Generally, the genome sequence coverage target is $\geq 100x$ for SRS assemblies and in the range of 50x for LRS to provide a quality assembly; however, if a haplotype-resolved assembly is desired then more coverage is helpful. Also, if using the HiFi mode of sequencing, coverage can be as low as 20x although haplotype-resolution is steadily improved up to 40x or so. Contigs from LRS need to be “polished” to increase accuracy, with SRS data being useful for this step.

Y chromosomes remain challenging to assemble due to their highly repetitive nature. Even with LRS they often remain highly fragmented. Methods for the identification of these contigs include using the normalized ratio of female to male alignments (Hall *et al.*, 2013), and kmer based approaches (Carvalho *et al.*, 2013; Rangavittal *et al.*, 2019). Prior to polishing an assembly, it’s important to make sure the mitochondrion has been assembled. Failure to do so can result in over-polishing of nuclear insertions of mitochondria (NUMTs) which can lead to difficulties in identifying mitochondrial variants. There are methods to assemble mitochondrial genomes from both SRS and LRS (Al-Nakeeb *et al.*, 2017; Dierckxsens *et al.*, 2017; Formenti *et al.*, 2020; Hahn *et al.*, 2013; Meng *et al.*, 2019). Polished contigs generated from long reads are sufficient to detect the majority of SNV, indels, CNV, and SV in the genome by alignment to reference genome(s). Ideally, a breed- or population-specific reference or a pangenome representation would be used for alignments, when available, to maximize mapping accuracy and variant detection. Alternatively, a full de novo assembly can be achieved by adding scaffolding data, either optical mapping (Hastie *et al.*, 2013; Nagarajan *et al.*, 2008) or chromatin conformation contact mapping (HiC) (Burton *et al.*, 2013; Kaplan *et al.*, 2013).

Resequencing

De novo assembly will always yield the most detailed information about a single genome, however the cost and computational effort required is still impractical for analysis of multiple animals and populations in most circumstances. Resequencing, which involves short or long read genome sequencing at much lower coverage than necessary for assembly and depends on mapping the reads to a reference genome to detect variants, is more amenable to application in larger populations. The goals of resequencing and the platform chosen will determine the depth of sequencing required. For discovery of SNV and indels, the high accuracy and low cost of SRS makes it the common choice. Tenfold mapped genome coverage is typically recommended as the minimum depth, and usually requires about 12-13x raw coverage depending on platform. Extensive testing indicates that this is sufficient for accurate genotyping of ≥ 95 percent of SNV compared to very high coverage sequencing ($\geq 30x$ coverage). The SRS resequencing can also accurately detect small (1-10 base) indels, but much higher coverage is needed to predict larger indels or copy number variants, and some structural variants are very difficult to detect with only short read data. LRS can also produce quality SNV and indel calls but except for HiFi, sequencing will need to be done to a greater depth, around 20x. LRS excels when it comes to CNV and SV. With the same 10x coverage required for SNV and indels, HiFi data will yield many high quality CNV and SV calls. Regular LRS and SRS will benefit from a minimum of 20x coverage. LRS can call these larger variants with much greater frequency and accuracy than SRS and can capture larger variants than HiFi.

Prior to the advent of haplotype-resolved assemblies, it was common to choose female reference animals due to the drop in coverage associated with sequencing the heterologous portions of the sex chromosomes in males. When the sequenced animal is a male and the reference has no Y-chromosome to align to, identifying variants becomes more challenging. In this case, reads that do not map to the

reference can be collected and aligned to an alternate dataset representing the Y chromosome. This can be the Y-chromosome of a closely related animal, or a collection of Y chromosome genes.

The success and accuracy of WGS for detection of variation is also highly dependent on the reference genome to which the reads are mapped. Currently, most studies use a single representative assembly agreed upon by the community of scientists working with the species in question, which provides a common basis for comparison among studies. However, many livestock species are now seeing high quality assemblies of multiple breeds emerging and studies comparing the success of using a breed-specific reference as opposed to “the reference” assembly have not been completed. Early results suggest that variant detection will be improved by breed-specific references, but at the cost of easy cross-study comparison (Crysnanto and Pausch, 2020). Recent efforts in cattle have focused on creation of a “pangenome” reference including all genomic segments globally present among breeds, as a solution to this problem (Heaton *et al.*, 2021).

GENOTYPE IMPUTATION

Imputation: filling up missing values

Missing value imputation is the name given to the process of predicting incomplete values in a set of data. In many instances, missing values cannot be processed in an analysis and thus should be either removed or replaced by a guess beforehand. Although the first approach is simpler (e.g., the missing data could be handled by ignoring the missing information during computations), the second approach may help retain more statistical power in the analysis since it attempts to minimize data loss.

Imputation consists of taking advantage of correlations between variables in order to fill up the empty data. This approach involves the use of prediction methods adapted from statistics and machine learning, or even of deterministic approaches based on heuristics. As for any type of prediction, the imputation of missing values comes with uncertainty, and the accuracy of that prediction is dependent on a series of factors that are inherent to the data.

The number of errors that can be tolerated in an imputation procedure is dependent on the missingness rate. If a data set contains very few missing values, imputation with low accuracy could be performed with little impact on subsequent analyses, since the small number of affected values would be diluted over the rest of the data. This is frequently referred to as “minor imputation”. Conversely, if the number of missing values to be imputed is large, accuracy must be high in order to avoid the “garbage in, garbage out” effect - that is, poor quality input leading to faulty output.

If high missingness rates suggest poor quality data, it’s logical to question whether imputing large numbers of missing values will be of value. However, in specific situations, large numbers of missing data are not caused by systematic errors but are rather deliberately planned during experimental design. It may be logical to “plan” to have missing data if a smaller subset of data can predict the missing data with a high accuracy but at a substantially decreased cost. Genomic data are a logical target for imputation, because DNA markers are inherited in linear sequences on chromosomes that remain intact from generation to generation (if recombination does not occur). Because of this, having data for the first and last nucleotide in a DNA sequence often allows accurate prediction of the entire DNA sequence between those nucleotides, based on full genome sequences of genetically related animals.

In a WGS experiment, for example, millions of variants are detected across samples. Despite drastic reductions in sequencing costs in the past decades, sequencing a whole genome may still cost over US\$ 1 000. In parallel, microarray technologies have matured substantially, making it possible to generate reliable data for a subset of these variants in a cost-effective manner. As discussed previously, the number of variants in these microarrays typically ranges from tens to hundreds of thousands. This is substantial data, but still a tiny fraction of the overall number of variants that could be captured by WGS. Therefore, one may design an experiment where a smaller number of animals

have their whole genome sequenced, thus generating a reference panel of millions of variants. In turn, this reference panel is used as a guide to impute the bulk of the data that was genotyped using a microarray. The same rationale can be applied in imputing genotypes between microarrays, where a lower density panel (LDP) of markers is imputed to a higher density panel (HDP), or even to a full genomic sequence.

Imputation workflow

Genotype imputation is a straightforward procedure, albeit the mathematical and algorithmic mechanics behind it can be rather complex. First, the HDP and LDP is selected such that most – if not all – of the variants in the LDP are included in the HDP. Second, a set of animals is genotyped in the HDP in order to compose the reference panel. Third, animals to be imputed are genotyped in the LDP. Fourth, data quality control is applied to both the HDP and LDP sets. Finally, LDP data is imputed to the HDP using one of many methods available for genotype imputation.

Building a reference haplotype library

The selection of animals to be included in the reference panel must be optimized for improved imputation performance. If no genotypic data is available, one can use pedigree relationships in order to select key ancestors capturing a large proportion of the genetic variation in the population (Goddard and Hayes, 2009). Otherwise, reference animals could be selected based on the LDP data such that haplotype diversity is maximized. This is achieved by iteratively selecting individuals carrying large numbers of high frequency haplotypes (Butty *et al.*, 2019). Of note, if high imputation accuracy must be achieved for low frequency variants, selection of animals carrying rare haplotypes should also be considered (Bickhart *et al.*, 2016).

Imputation methods

Genotype imputation is divided into family-based and population-based methods. In the first, haplotypes from close relatives, typically identified via pedigree data, are used to impute the unobserved genotypes of LDP samples. In the second, pairs of individuals are assumed to share a common ancestor, such that LDP samples are interpreted as mosaics of haplotypes that are present in the HDP set. Furthermore, there are methods that take advantage of both paradigms in order to achieve improved performance. Importantly, some methods require genotypes to be phased (i.e., that the parental haplotypes are separated) before imputation, and most phasing algorithms perform minor imputation on the fly.

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SECTION 4**Applications of genomics**

Applications of genomics

This section will describe how genetic and genomic information can be used to describe patterns (such as genetic diversity, differentiation, classification, and genetic clines) or to infer historical processes (expansions, contractions, admixture and gene flow) affecting animal genetic diversity. In the first case, one can quantify how genetic diversity changes per individual, location or environment or by domestication. The second group of approaches aims to identify, quantify and determine the date of ancient demographic events, possibly including direct or indirect selection acting on specific genomic regions. The principles and limits of several methods that have become available in the last couple of decades are noted.

Developments from the genomic era allow novel options to address previously unfathomable questions with increasingly sophisticated methods, including modelling. In some cases, the resulting patterns can only be interpreted by understanding the underlying models and processes. In addition, the analysis of patterns and processes should be integrated. For instance, most available approaches to identify selection typically make strong assumptions about the demographic history that generate “neutral” patterns, while methods reconstructing demographic events often ignore selection. Inferring simultaneously selection and demography, in order to explain spatial patterns, is one of the greatest challenges of evolutionary genomics.

ASSESSMENT OF GENOMIC VARIATION WITHIN-POPULATIONS

Measures of genetic diversity

Local well-adapted breeds are considered reservoirs of genetic diversity that are recognized to contribute to future traits of interest (Bruford *et al.*, 2015; Groeneveld *et al.*, 2010). Assessment of the within-breed component of genomic variation is essential for the effective management of breeds (Boettcher *et al.*, 2010; Caballero *et al.*, 2010; Ginja *et al.*, 2013; Lenstra *et al.*, 2012; Ruane, 2000; Toro and Caballero, 2005; Toro, Fernandez and Caballero, 2009) Assessment allows for monitoring, on one hand the effect of isolation on the genetic diversity and the ensuing risk of inbreeding depression, and on the other hand the effect of crossbreeding on the genetic constitution of breeds. In addition, a comparison of the diversity of domestic and wild populations, and the geographic pattern of within-bred diversity may reveal the serial population bottlenecks that occurred in the past in association with domestication and migration, respectively.

The genetic diversity can be quantified via the observed (H_o) and expected (H_e) heterozygosities, nucleotide diversity (π) and effective population size (N_e , see below). The observed heterozygosity H_o is based on the actual distribution of genotypes, whereas H_e is based on allele frequencies (Nei, 1973; 1978). Both of these measures are easily obtained from datasets of genetic markers. For nucleotide sequence data, nucleotide diversity (π) gives the average number of nucleotide differences per site between two DNA sequences chosen randomly from the studied population (Nei and Li, 1979) and is equivalent to H_o if all nucleotides are considered as loci. The parameter N_e represents the number of reproducing individuals given a number of conditions (see subsection on Effective population size).

At a low genome coverage (<8x), some heterozygous genotypes will be scored as homozygous genotypes and sequencing errors as heterozygous genotypes (Nielsen *et al.*, 2012), thus distorting the estimates of allele frequencies and genetic diversity. To address this problem, a genotype-likelihood based method has been developed to estimate nucleotide diversity for low sequencing coverage (Nielsen *et al.*, 2012).

Whole-genome sequencing (WGS) allows for assessment of genetic diversity across the genome. For instance, analysis of sequences may detect regions of genes with low genetic diversity because they have been targeted by selection or may localize runs of homozygosity (ROH), that is, long continuous tracts of sequence with not (or very few) heterozygous sites.

Inbreeding and runs of homozygosity

Inbreeding results from the mating of related individuals and it is estimated by the probability (F) that two alleles at a locus are identical. Classically, inbreeding was calculated using pedigree records, but genomic data and H_o and H_e values allow for a more accurate quantification of inbreeding without using pedigree data (Curik, Ferenčaković and Sölkner, 2014; Druet and Gautier, 2017; Howrigan, Simonson and Keller, 2011; Purfield *et al.*, 2012). Inbreeding increases the homozygosity in individuals and thus decreases H_o , but it does not change H_e based on the allele frequencies, resulting in a heterozygote deficit and a departure from the Hardy-Weinberg equilibrium (HWE) of homozygous and heterozygous genotypes. Thus, Wright's F -statistics based on comparing H_o and H_e can be used to infer inbreeding (F_{IS}) from molecular data (see Box 3 for more details on F -statistics). Because F_{IS} is insensitive to inbreeding in more ancient generations, the genomic coverage by ROHs has become a commonly used measure for inbreeding if SNP and WGS data are available.

BOX 3

F-statistics

Wright's (1951) fixation indices can be defined in at least three hierarchical levels that measure the probability of two randomly sampled alleles being the same in a set of subpopulations (e.g. farms) that together form a population (e.g. breed). For instance, the probability of sampling the same allele twice in a subpopulation (a measure of inbreeding) can be obtained from the difference between the average observed heterozygosity of the individuals in the subpopulation ($\overline{H_I}$) and the HWE expectation of the heterozygosity for the subpopulation (H_S):

$$F_{IS} = \frac{H_S - \overline{H_I}}{H_S} = 1 - \frac{\overline{H_I}}{H_S},$$

F_{IS} measures deviations in random mating within a subpopulation from one generation to the next, with negative values (minimum is -1) indicating an excess of heterozygotes in the subpopulation, while positive values (maximum is +1) indicate a deficit of heterozygotes. It is worth noting, that in a subpopulation deviating from HWE, a single generation of random mating would restore the HWE proportions, rendering F_{IS} equal to zero. Similarly, the correlation between allele frequencies in a pair of subpopulations can be expressed as the difference in the average expected heterozygosity of the two subpopulations ($\overline{H_S}$) and the expected heterozygosity estimated for the two subpopulations together (H_T , with $H_T = 2\overline{p} - (\overline{1-p})$ and \overline{p} being the average frequency of one of the two alleles in a biallelic locus):

$$F_{ST} = \frac{H_T - \overline{H_S}}{H_T} = 1 - \frac{\overline{H_S}}{H_T},$$

F_{ST} varies between 0 (indicating no differences in allelic frequencies between the two subpopulation) and 1 (indicating that the allelic frequencies in the two subpopulations are completely different), with the latter indicating that each of the two populations is fixed for a different allele, thus, within population diversity must be zero. F_{ST} was originally defined as a measure of fixation, but it is often seen as a measure of genetic differentiation, and some authors even use it as a measurement of divergence that works like a genetic distance. In practice, it cannot be used as a distance since it has a maximum value of 1, however Reynolds *et al.* (1983) suggested a distance measure that can be seen as measuring drift accumulating between populations in the absence of mutations contributing new genetic variation to the population. For small F_{ST} values, Reynolds distance is approximately the same as F_{ST} and linearly increases with time since separation (Reynolds, Weir and Cockerham, 1983; Weir and Cockerham, 1984).

Lastly, the probability of sampling the same allele twice can also be obtained for two samples randomly collected from the total population (i.e. ignoring the breakdown of groups of samples in subpopulations). F_{IT} thus measures deviations between the average observed heterozygosity across all individuals in the population ($\overline{H_I}$) and the HWE expected heterozygosity for the population (H_T):

$$F_{IT} = \frac{H_T - \overline{H_I}}{H_T} = 1 - \frac{\overline{H_I}}{H_T},$$

As for F_{IS} , F_{IT} varies from -1 to +1, with the obtained value representing the deviation from HWE expected genotypic frequencies due to non-random mating and divergence in allele frequencies between subpopulations. The three fixation indices shown above relate to each other following: $1 - F_{IT} = (1 - F_{ST}) * (1 - F_{IS})$. This relationship enables for additional hierarchical levels to be included as required by the study design, e.g. should there be colonies within subpopulations, the relationship between the fixation indices would become: $1 - F_{IT} = (1 - F_{ST}) * (1 - F_{IS}) * (1 - F_{IC}) * (1 - F_{CS})$, with the corresponding F_{IC} and F_{CS} being:

$$F_{IC} = \frac{H_C - \overline{H_I}}{H_C} = 1 - \frac{\overline{H_I}}{H_C} \text{ and } F_{CS} = \frac{H_S - \overline{H_C}}{H_S} = 1 - \frac{\overline{H_C}}{H_S},$$

with H_C being the HWE expected heterozygosity for the colony, and with F_{IC} varying between -1 and +1, while F_{CS} varies between 0 and 1.

Inferring the frequency and length of ROHs in the genomes of farm animals helps to understand population histories, including the occurrence of bottlenecks, estimate inbreeding and identify signatures of selection (Bruford *et al.*, 2015; Ceballos *et al.*, 2018; Meyermans *et al.*, 2020; Peripolli *et al.*, 2017). For example, long ROH segments suggest recent inbreeding (consanguinity) and low genetic diversity. This has been observed for Holstein-Friesian cattle (Doekes *et al.*, 2019; Peripolli *et al.*, 2017; Purfield *et al.*, 2012; Upadhyay *et al.*, 2019) and is possibly associated with deleterious homozygous variants (Pryce *et al.*, 2014). In contrast, relatively many short ROHs with only a few long ROHs indicate a reduced population size in the past and little recent inbreeding, as found for wild boars (Peripolli *et al.*, 2017). Conversely, hybridization reduces the ROH coverage and increases genetic diversity, as in cattle of admixed taurine-zebu ancestry (Kim *et al.*, 2017; Purfield *et al.*, 2012).

A high frequency of short- to medium-length ROH detected around a specific locus in several individuals can be indicative of recent positive selection (Curik, Ferenčaković and Sölkner, 2014; Zhang *et al.*, 2015). ROHs fixed in a population indicated a selective sweep by rapid selection for a beneficial mutation. However, appropriate analyses and demographic modelling are needed to disentangle the processes behind specific genomic patterns and the associated parameters (MacLeod *et al.*, 2013; see sections below on Relationships between breeds and Reconstruction of population history and demographic modelling).

Effective population size

The effective size (N_e) of a population corresponds to the number of individuals in an idealized Wright-Fisher population (i.e. reproducing with random mating, even sex ratio and non-overlapping generations) that would become inbred or lose diversity at the same rate as this population (Frankham *et al.*, 2002). The concept of N_e is central to population genetics, as it quantifies genetic drift and allows for comparisons across species or populations, but is difficult to estimate. The census size of a population and the number of breeding animals (N_b) are usually larger than N_e , because actual populations do not follow the assumptions of an idealized population. Non-random mating and uneven sex-ratios are common in domestic animal populations that are under strong artificial selection, especially with the use of reproductive biotechnologies, and may also result from admixture events. Thus, N_e can be small even for a large population and through fixation of alleles it implies loss of genetic variability, fast genetic drift, a high level of inbreeding and possibly decreased viability (Kristensen *et al.*, 2015; Peripolli *et al.*, 2017).

Estimation of N_e depends substantially on the indicators of genetic diversity considered, such as inbreeding, and on variation of genetic diversity through time. For instance, from a dataset of sequences N_e can be estimated as θ / μ (where θ , is the population mutation rate estimated on the basis of the number of segregating sites; μ , the individual mutation rate). Whole-genome data provide the least biased estimation of diversity (and thus N_e). Linkage disequilibrium (LD) based methods can account for sample size, mutation, phasing (assigning alleles to the paternal and maternal

chromosomes), and recombination rate (Barbato *et al.*, 2015; Orozco-terWengel *et al.*, 2015). ROH may also serve as a robust genome-scale N_e estimator, although interpretation and scaling depend on local recombination (Bruford *et al.*, 2015).

However, computed values should be treated as estimates that are prone to biases, especially if the population is subdivided. For instance, Wakeley (1999) has shown that a structured population in which all subpopulations increase in size while exchanging more migrants will exhibit a signal of decrease of N_e despite the increase in the actual size of the population. Similar results were obtained by Mazet *et al.* (2016) for genomic data from single individuals analysed using the pairwise sequentially Markov coalescent (PSMC) method (see below). If N_e is used for translating various measures of genetic diversity within a specific model, it should be interpreted within that model. Various complex coalescent-based methods have been developed that allow to infer the population demography over time, including the variation of N_e or connectivity, such as the PSMC, the multiple sequentially Markov coalescent (MSMC) method and Fastsimcoal2 (see section on Reconstruction of population history and demographic modelling).

Increasing the N_e of a breed or population is challenging, as it may require a balanced number of breeding males and females contributing to subsequent generations, thus limiting directional selection pressures, and avoiding inbreeding (Curik, Ferenčaković and Sölkner, 2014).

Several program packages available for the analysis of WGS data allow for estimation of within-breed genetic diversity summary statistics. These software packages include the following: *PLINK* (Chang *et al.*, 2015; Purcell *et al.*, 2007); *VCF Tools* (Danecek *et al.*, 2011); R package *diveRsity* (Keenan *et al.*, 2013); *BEAGLE* specifically for detecting IBD (Browning and Browning, 2010); *SNeP* specifically to estimate N_e (Barbato *et al.*, 2015); *ANGSD* (Korneliussen, Albrechtsen and Nielsen, 2014) useful for low coverage WGS data. For more information, see Appendix 6.

The take home message is that estimation of parameters related to within-breed genetic diversity such as N_e is important, but this information needs to be interpreted properly and placed in the proper context. Estimation of such parameters should be complemented with clustering methods and demographic modelling (see subsequent sub-sections) to better understand evolutionary processes. By combining different approaches, it will be possible to use genomics to make a more informed management of breeds and other populations of livestock by monitoring N_e and practices such as crossbreeding, while avoiding high levels of inbreeding and ultimately preserving adaptive variation.

ASSESSMENT OF POPULATION STRUCTURE AND BETWEEN-BREED GENOMIC VARIATION

Principal component analysis

Assessment of the genetic variation within breeds is usually an important objective for all genomic characterization studies. Such studies often involve multiple breeds or populations, however, so the analysis of the structure of the multiple populations and how they relate genetically to each other is also a common research application. The foremost way of visualizing genetic structure of a multi-population (multi-breed) sample is to perform a principal component analysis (PCA) on the individual multilocus genotypes. PCA is a statistical method to capture the variability associated with many variables (such as marker genotypes) into a much smaller number of variables that still contain most of the original variation. The resulting variables (the “principal components” – PC) are ordered in terms descending order according to the amount of variation they explain. Thus, PC1 explains the most variation, PC2 the second most, and so forth.

The theoretical ground of the PCA approach has been described by several studies. In a sample made of two distinct genetic groups, the first principal component separates these two groups and the

proportion of variance explained by this first component corresponds exactly to the F_{ST} of the sample (McVean, 2009). Distinguishing the two groups is possible if (and only if) the product of the number of samples and markers is larger than $(1 / F_{ST})^2$ (Patterson, Price and Reich, 2006). Similar arguments suggest that more complex patterns of population structure also will be detected if the dataset is large enough. For instance, for a sample made of p distinct genetic groups, the number of principal components with significant contribution to the total observed variance is equal to $p - 1$ (Patterson, Price and Reich, 2006). The PCA may also suggest about past admixture events: admixed individuals are on the PCA located between the two parental populations while their distances to these parental populations reflect the percentage of admixture (McVean, 2009; Patterson, Price and Reich, 2006). When population structure arises from a continuous isolation-by-distance model, the PC often show genetic clines that correlate with geographical clines and the two-dimensional patterns may even resemble the geographic map of the area under study (Novembre *et al.*, 2008).

A standard procedure is to plot projections of the samples on the plane defined by two principal components, usually PC1 and PC2 or PC1 and PC3, thus providing a useful survey of the dataset. Although the individual PC have no biological or other significance *a priori*, plotting of the data frequently reveals patterns that suggest a logical interpretation. Figure 4 (P. Ajmone-Marsan, personal communication, 2021) shows an example of a PCA plot of cattle breeds and wild relatives. The wild relatives (Banteng and Gaur) appear at the top of the graph, separated from cattle breeds along the lower part. The Hong Kong feral (HKF) cattle, which seems to include some common ancestry with wild relatives, falls closest to the middle of the plot. The other cattle breeds are separated horizontally according to subspecies. European taurine (EU-tau) breeds group on the far lower left, whereas Asian indicine (AS-ind) are on the far right. African taurine (AF-tau), are just to the right of the European taurine group, whereas Sanga cattle, which are considered to be a composite of African taurine and indicine breeds, are grouped in the bottom centre of the graph, between their two ancestral groups.

However, several limitations, potential sources of bias and associated misinterpretations should be avoided. Because the PCA plots are two-dimensional projections of samples from a multidimensional space, they visualize only a small part of the total variation. For instance, they do not unambiguously indicate a close relationship of samples and do not clearly indicate duplicates. Published plots are often too dense and thus only allow a superficial survey of the dataset. In addition, the use of many different symbols can be confusing. This can be remedied by plotting breed averages instead of individuals and by positioning the breed codes within the plot, just as always done with geographical names on a road map. Unbalanced sample sizes between genetic groups may strongly affect the position of these groups on the plot; those with large sample sizes being typically pushed towards the centre of the plot (McVean, 2009). If the dataset contains a breed that by genetic isolation differs substantially from all others, the major PC mainly reflect this difference, that is, the bias due to inbreeding (Lenstra *et al.*, 2012). This result can be prevented by excluding such breeds while computing the PCs but not from the plot, an approach referred to as “supervised” PCA (Ciani *et al.*, 2020). Note also that several higher-order PCs typically each correspond to the contrast of one breed with the others (Kijas *et al.*, 2012).

Menzio *et al.* (1978) introduced PCA for the study of human genetic variation across continental regions. It has since become a standard of population genetic data analysis since the 2000s, especially with the advent of high density genotyping and WGS technologies. It can be applied using generic R functions or population genetics software such as *PLINK* (Chang *et al.*, 2015) or *eigensoft* (Price *et al.*, 2006). (See Appendix 6.)

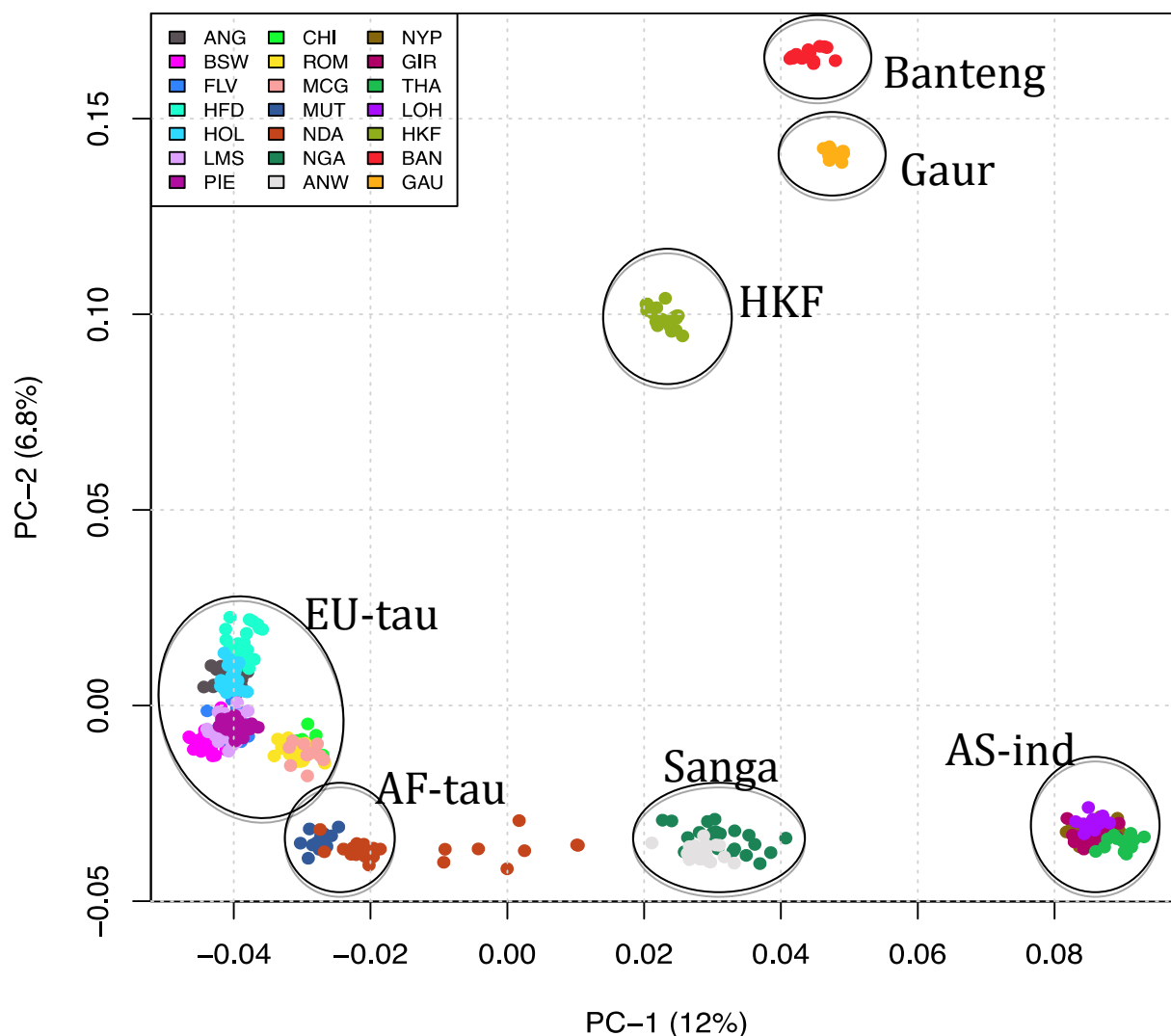


FIGURE 4
A principal components analysis plot of groups of bovine populations, including Banteng, Gaur, Hong Kong feral cattle (HKF) and groups of European taurine (EU-tau), African taurine (AF-tau), Sanga and Asian indicine (AS-ind) breeds.

While genetic clines that correlate with geographic clines often result from past migration waves, alternative explanations should be explored, such as isolation by distance (Novembre and Stephens, 2008). Ancient DNA (aDNA) samples with heterogeneous sample ages may also distort PCA representations, which can be amended by factor analysis (FA) instead of PCA (François and Jay, 2020). Another common method for comparing ancient and modern DNA samples is a supervised PCA in which low-coverage aDNA samples are projected in a plot of coordinates computed on the basis of high-coverage DNA samples (e.g. Daly *et al.*, 2018), but this does not correct all potential biases related to sample age heterogeneity (François and Jay, 2020). For more details on the analysis of ancient DNA specimens see Box 4. Another mode of supervised PCA is the calculation of PCs that show the difference between geographical (or genetic) extremes, such the northern-most and the southern-most breeds, in order to test the clines between these extremes (Ciani *et al.*, 2020).

BOX 4

Archaeogenetics applied to farm animals

The recovery of genome variation from archaeological livestock samples is now eminently feasible, thanks to methods for DNA extraction and high throughput sequencing, particularly using

instruments that give high read numbers from small fragments. Application of these technologies has helped solve the problems of contamination in the field - the presence of modern livestock DNA within an extract is now measurable through bioinformatic analysis of the resulting data and problem samples can simply be excluded. Also, it is possible to identify damage patterns to authenticate aDNA sequences and validate results.

Ancient genomics is an invaluable tool for understanding modern diversity and its origins. Archaeological bones from the cradle of domestication in the Near East have yielded genome data for both cattle and goat (Daly *et al.*, 2018; Verdugo *et al.*, 2019) showing that in both initial domestication must have been followed by secondary input from additional wild populations. A more extreme example is clear in pig ancestry where the Near Eastern genome which first enters Europe was almost completely replaced by introgression from local wild boar (Frantz *et al.*, 2019). Because DNA degrades with time, more recent genomes will be more accessible, and the early development of modern breeds will become visible. For example, in horse the sharp drop in genome heterozygosity within the last 250 years has been shown (Fages *et al.*, 2019). Ancient genomics can also reveal past domestic animal diversity that has since disappeared; for example ancient Iberian horse genomes from over four millennia ago have revealed the presence of a now-extinct lineage which is not ancestral to modern horse populations (Fages *et al.*, 2019).

One enduring aim is to understand genome variation in terms of impact and function and ancient genomics has the potential to add to this in two ways: in giving temporal resolution to the search for genome regions which have been under selection and in ancient epigenomics. A group of 8 000-year-old goat populations from Serbia and Iran showed evidence for selection of genetic variation involved in pigmentation and some production traits; the earliest discovered direct evidence of human manipulation of a herded animals genome (Daly *et al.*, 2018). Inference of the methylation patterns of ancient molecules is also possible, although requiring high coverage sequencing, a route to directly mapping epigenetic markers in the past (Hanghøj *et al.*, 2019).

Because of inherent properties of ancient DNA (particularly low concentration, short fragments, chemical damage) the methods that are useful are limited. Some suggested guidelines are as follows:

- Only high throughput sequencing is regarded as reliable and current state of the art; PCR-based assays including microsatellite size calls and Sanger sequencing are no longer deemed reliable data.
- The best assay is the highest coverage genome retrieved by shotgun high throughput sequencing. This is a standard that the field should aim for, particularly with rare irreplaceable ancient specimens.
- However, many successful population genetic analyses can also be performed using low coverage genomes (i.e. <1x), and some samples may not feasibly be sequenced to higher density because of poor preservation.
- Methods such as RNA bait capture may also be useful (particularly for less well-preserved samples) for target read enrichment prior to sequencing. Examples of useful and achievable bait targets are either whole mitochondrial chromosomes, or specific autosomal loci such as selected genes or panels of thousands of informative SNPs.
- Additionally, it is highly desirable to use uracil–DNA glycosylase (UDG) treatment of sequencing libraries to remove C/G→ T/A misincorporations that have occurred as a result of age-related damage to ancient molecules.

Source: Dan Bradley

Multidimensional scaling (MDS) is an alternative method to survey the dataset by reducing the number of dimensions (Tzeng, Lu and Li, 2008). In practice MDS and PCA patterns are largely identical, but MDS cannot be run in a supervised mode.

Model-based clustering

Another common approach to summarize the genetic structure observed in a set of individuals is the clustering method first developed by Pritchard *et al.* (2000) and implemented in the software *Structure*. This method assumes there are K genetic groups (or clusters) and all polymorphic sites are at HWE and linkage equilibrium within these groups. Each individual i is modelled as a mixture of these K groups with specific proportions q_{ij} with $j = 1$ to K . *Structure* estimates these proportions from the observed individual genotypes using a Monte Carlo Markov Chain (MCMC) algorithm. In the most standard application (unsupervised clustering), the K clusters and their associated allele frequencies are inferred and the biological meaning of cluster number j is deduced *a posteriori* from the set of individuals such that $q_{i,j}$ is close to one. Ideally, this reveals the major ancestral populations, and the composition of admixed individuals or populations. Supervised clustering allows one to define *a priori* the ancestral clusters and infer directly the admixture proportions of the other individuals, similar to assignment methods like *GeneClass* (Piry *et al.*, 2004). The original approach of Pritchard *et al.* (2000) was later extended to account for sampling location (Hubisz *et al.*, 2009), linked loci and shared ancestry between clusters (Falush, Stephens and Pritchard, 2003). More efficient inference approaches of the same model were also developed in the software *Frappe* (Tang *et al.*, 2006) and *Admixture* (Alexander, Novembre and Lange, 2009), allowing the analysis of much larger datasets and larger values of K , albeit with the constraint that loci are independent from each other, that they are in linkage equilibrium.

Again, there are caveats and pitfalls to be avoided. Many scientists choose to interpret the results of model-based clustering in terms of population history. For instance, the separation of one cluster into two sub-clusters at high K values is often interpreted as a past divergence event. More generally, inferred clusters are equated to ancestor populations and any breed that is not assigned to one cluster is assumed to be admixed. However, *Structure* is essentially a clustering approach for genetic data, but does not model evolutionary processes such as drift, mutation, migration or divergence (see sections on Relationships between breeds: genetic distances and Reconstruction of population history and demographic modelling for such methods). The implicit assumption that the ancestral populations are present in the dataset is often not met. Thus, the inferred clusters do not necessarily correspond to real past or present populations and strongly depend on the composition of the dataset. Applying *Structure* to populations along a genetic cline formed by preferential mating of proximate parents (isolation-by-distance) may infer homogeneous clusters that contain the extreme populations and mixed compositions for the central populations (Engelhardt and Stephens, 2010). This may be remedied by modelling continuous spatial variation between (e.g. Corander, Waldmann and Sillanpää, 2003; François, Ancelet and Guillot, 2006; Guillot, Mortier and Estoup, 2005) or within clusters (Bradburd, Coop and Ralph, 2018), the latter option allows the analysis showing both discrete and continuous genetic structure. By the sampling and the inbreeding bias, inferred clusters tend to correspond to overrepresented and inbred breeds, respectively (Lenstra *et al.*, 2012).

However, in spite of a wide-spread over interpretation, model-based clustering does contribute data analysis by showing graphically: (i) groups of related individuals from one or more breeds; (ii) ancestral proportions, provided inferred clusters correspond to ancestral populations, which may be accomplished in the supervised mode; and (iii) subdivision of breeds not inferred by PCA or genetic distances.

As an alternative to the supervised mode, breed-specific admixture analysis (BSAA) is based on the selection of 300 ancestry informative markers (AIM) specific for a breed suspected to be introgressed, for instance by markers that have the highest F_{ST} between the breed and a group of other breeds. It was demonstrated that *Structure* analysis with 300 to 400 AIM markers provides a more sensitive detection of (pre)historic admixture than F_4 analysis (see below).

Structure and PCA both attempt to summarize observed genetic diversity using a reduced number of K latent factors, namely the clusters for *Structure* and the PC for the PCA (Engelhardt and Stephens, 2010). More specifically, both methods decompose the genotype matrix X as $X = QF$, where Q is a

matrix of n individuals \times K factors that quantify the individual compositions. The two methods impose different constraints to the matrices Q and F ; the coefficients of Q are positive and must sum per individual to one with *Structure*, but not with PCA. Other latent factor approaches were proposed to analyse genetic data (Engelhardt and Stephens, 2010; Frichot *et al.*, 2014) and represent interesting alternatives to PCA and *Structure*. For instance, the sparse Nonnegative Matrix Factorization (sNMF) developed by Frichot *et al.*, (2014) and implemented in the *LEA* R package estimates admixture proportions with the same accuracy as the *Admixture* software while reducing computation time by a factor from 3 to 30 and being less affected by the inbreeding of the analysed populations.

Genetic distances

Genetic distances between individuals

The third common approach to visualize genetic structure uses a matrix of genetic distances between all pairs of individuals. For SNPs of a diploid species, distances are based either on the identity by state (IBS) of markers or on genomic relationships of individuals (Grünwald *et al.*, 2017; Yang *et al.*, 2011), which both can be computed using *PLINK*. These distances can be visualized via MDS analysis, the alternative to PCA described earlier (Tzeng, Lu and Li, 2008) or via Neighbour-Joining trees (Saitou and Nei, 1987; see section on Relationships between breeds: genetic distances). Such trees unambiguously show the identity or close relationship of samples and are recommended for quality control of SNP datasets (see Section 3). They also show the level of inbreeding of breeds via short terminal branches. However, individual-based trees can be misleading on the deeper phylogenetic relationships of the breeds, which are better explored by genetic distances based on allele frequencies of breeds (see section on Reconstruction of population history and demographic modelling). A representation of genetic distances in a network that connect each individual to its ten nearest neighbours (“supermagnetic clustering”) shows genetic structure within populations (Neuditschko, Khatkar and Raadsma, 2012), which may be related to the uneven use of breeding sires (Neuditschko *et al.*, 2017).

So far, the approaches described assume markers to be independent, but several recent studies take advantage of haplotype information of phased datasets, that is, data with information about which alleles of different markers are on the same haploid chromosome copy (maternal or paternal). Such information is not provided by dideoxy-Sanger or short-read sequencing. Without data from the (expensive) PacBio or Oxford Nanopore long-read sequencing (see Section 3), phasing must be inferred statistically by exploiting observed linkage disequilibrium (LD) patterns in the population or genotype information from close relatives (Al Bkhetan *et al.*, 2019; Browning and Browning, 2007; Delaneau, Marchini and Zagury, 2012).

The *ChromoPainter* approach implemented in the program *FineSTRUCTURE* (Lawson *et al.*, 2012) builds a coancestry matrix of individuals based on local haplotype similarity along the genome and uses this matrix for PCA and clustering (similar to *Structure*). For both applications, the use of haplotype information improved significantly the resolution of the inferred population structure. The method was developed for high-density SNP datasets and its performance with low-density (<50 000 SNP) datasets is not yet clear. Therefore, when using this method, it is essential to show that the linked mode, using haplotypes, gives better results than the unlinked mode (using separate SNPs). Going beyond this genome-wide description, the *Relate* (Speidel *et al.*, 2019) and *tsinfer* (Kelleher *et al.*, 2019) methods reconstruct the phylogeny of observed haplotypes, i.e. the most-probable inheritance at every SNP position in the genome, accounting for the haplotype diversity observed among sampled individuals around this position. All these methods are either based on or inspired by the model of Li and Stephens (2003), which iteratively considers one sampled haplotype and tries to reconstruct it as a mixture of the others.

In a study from The Bovine HapMap Consortium (Gibbs *et al.*, 2009), genotype data at 37 470 SNPs was available for 497 cattle from 19 geographically and biologically diverse breeds. This dataset was analysed using a *Structure* like (Figure 5A) and a PCA approach (Figure 5B). PC1 separates zebu and

taurine breeds, similar to the *Structure* analysis with $K = 2$ clusters. PC2 further separates African versus European taurine breeds, similar to the *Structure* analysis with $K = 3$ clusters. Breeds resulting from admixture between these three main groups are indicated by dashed rectangles and clearly stand out in both analyses. The *Structure* analysis with $K=9$ identifies several clusters corresponding to single breeds.

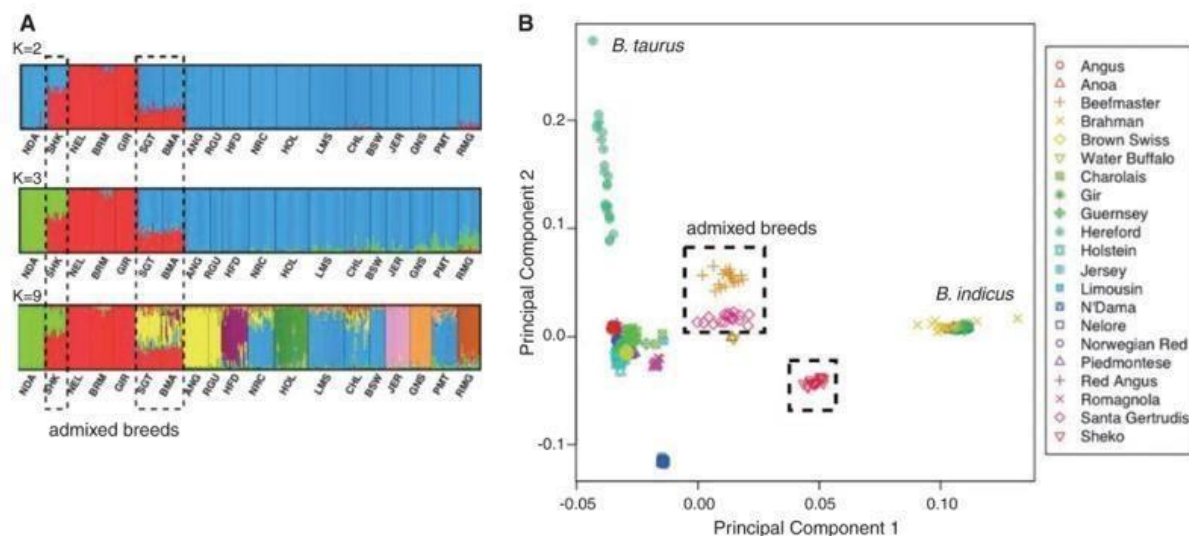


FIGURE 5
Genetic structure of worldwide cattle breeds, according to model-based clustering (A) and principle component analysis (B) (Adapted from Gibbs *et al.*, 2009).

Genetic distances among breeds

Since the development of selective breeding in the eighteenth century, the mating of animals with similar desired characteristics has resulted in the establishment of breeds, which are usually defined by a range of morphological, economic and behavioural traits (Taberlet *et al.*, 2008). Depending on the degree of genetic isolation, animals from the same breed are genetically more similar to each other than to animals from different breeds. These differences among animals from different breeds can be quantified using genetic distances between individuals (see corresponding section) and also by determining the components of the genetic variation within and between breeds using an Analysis of Molecular Variance (AMOVA; Excoffier, Smouse and Quattro, 1992; Michalakis and Excoffier, 1996).

There are two important differences between phylogenies at the species (macroevolution) and at the breed (microevolution) levels. First, the bulk of molecular differences between species are quantified via fixed differences in the DNA sequence. However, the mammalian substitution rate, approximately 1.0 to 1.25×10^{-8} /bases/generation (Altshuler *et al.*, 2010; Kong *et al.*, 2013; Venn *et al.*, 2014), is too slow to generate many of the DNA differences observed between breeds that diverged at most 10 000 years ago and in most cases only 50 to 200 years ago (Laval, SanCristobal and Chevalet, 2002). Instead, the major DNA-based differences between breeds depend on genetic drift, the mechanism behind the change of allele frequencies in genetic markers as a function of the N_e . Second, many species do not interbreed and hybrids of related species are often infertile. As a consequence, the phylogeny of species can be largely represented by a tree topology without connections between branches. In contrast, crossbreeding of breeds is a rule rather than an exception and trees of breeds are at best a practical visualization than an estimation of the true phylogeny of breeds.

The extent of genetic drift separating populations A and B can be quantified as the additive statistic f_2 , or the mean of $(P_A - P_B)^2$ with P_A and P_B corresponding to the frequencies of allele P for a SNP in populations A and B, respectively. The f_2 is the average of the squared differences across all genetic

markers studied in the two populations (Patterson *et al.*, 2012; Peter, 2016). Several genetic distances have been derived from f_2 , such as the Reynolds distance or D_R (Reynolds, Weir and Cockerham, 1983), which is the preferred genetic distance for breed comparisons (Laval, SanCristobal and Chevalet, 2002) and which correlates very well with the F_{ST} fixation index (Wright 1951). Because it approximates the average inbreeding coefficient of the two breeds compared, D_R trees may represent the loss of within group genetic diversity (Laval, SanCristobal and Chevalet, 2002). However, with SNP panels this is likely to be confounded by the ascertainment bias (AB), which may increase the diversity of the industrial breeds.

Note that the accuracy of allele frequencies depends on an adequate sample size per breed. In practice, 20 animals per breed are sufficient for biallelic markers; in fact, a sample size of 20 diploid animals has a probability of 95 percent of including the whole genealogy of the breed (Hein, Schierup & Wiuf, 2003). If this cannot be accomplished, a lower sample size increases the proportion of homozygote genotypes and thus the genetic distances to other breeds but does not alter the topology of a tree (see the following section). The F_{ST} distance as estimated by Weir and Cockerham (1984) accounts for low and uneven sample sizes. Using medium-density array datasets, Ramjak *et al.* (2018) calculated genetic distances between short haplotypes consisting of the alleles of up to four SNPs located within a stretch of 150 kilobases.

Phylogenetic trees and networks. The neighbor-joining (NJ) algorithm (Saitou and Nei, 1987) is the most common and one of the simplest methods to reconstruct phylogenetic trees on the basis of a matrix of $n \times n$ pairwise genetic distances between n breeds. It works by choosing the pair of breeds that after being combined gives the largest reduction of the total branch length of the tree, after which the distance matrix is recalculated and reduced to $(n - 1) \times (n - 1)$ breeds, continuing iteratively until all breeds have been paired. Such a tree complements PCA and model-based methods and represents a hierarchical clustering of breeds, with the length of the terminal branches indicating the degree of genetic isolation of a breed. In contrast to the (non-recommended) UPGMA (unweighted pair group method with arithmetic mean) approach, the NJ algorithm does not assume an even rate of change across breeds, which agrees with the effect of genetic drift being breed specific. The effect of genetic drift can be observed when the NJ tree is visualised like a phylogram where the trees' branch lengths are proportional to the amount of change in each breed. Other visualisations such as cladograms are not suitable to visualise the effect of genetic drift as the branches in such methods are not proportional to the amount of change.

Conceptually, the topology of a tree directly shows genetic events as breed divergence, genetic drift and breed isolation. In practice, this is realized mainly for the most closely related breeds, but is not shown by PCA or model-based clustering. Trees of individuals based on genetic distances also show the genetic drift, but do not show very well the relationships between breeds. However, phylogenetic trees of breeds have a few inherent limitations:

- Testing the significance of clusters is problematic and bootstrapping values are low.
- The deeper bifurcations are supported by short branch lengths and may not be realistic.
- By their hierarchical topology, trees cannot visualize reticulations and are thus confounded by gene flow between breeds leading to admixture. Breeds that have been derived by crossbreeding, such as taurine–indicine composites, tend to cluster in the NJ tree in between the source breeds (Ginja *et al.*, 2019; Kopelman *et al.*, 2013; Mei *et al.*, 2018). Whereas this still may be a useful visualization of a genetic cline with again the terminal branches showing the breed-specific genetic drift (Decker *et al.*, 2014), it should be realized that the genetic differences are dominated by the degree of crossbreeding. Thus, bifurcations and branch lengths no longer correspond to divergence events and genetic drift, respectively. If crossbreeding occurred recently, the genealogy of the alleles depends on the position of the alleles in the genome.

Various methods have been developed to introduce reticulation in a tree by construction of an admixture graph. The most methods popular are the *NeighbourNet* and *Treemix* algorithms.

NeighbourNet graphs (Bandelt and Dress, 1992; Dopazo, Dress and Von Haeseler, 1993; Dress, Huson and Moulton, 1996) can be constructed via the easy program *Splitstree* (Huson, 1998) with many graphical options. It is based on the NJ algorithm, but considers three instead of two breeds before reducing the difference matrix. *NeighborNet* graphs share the main advantages of NJ trees by reproducing the terminal branches and unambiguous clusters of breeds, but replace the often meaningless bifurcations of a tree by networks (Ciani *et al.*, 2020; Kijas *et al.*, 2012; Pitt *et al.*, 2019). These are not to be interpreted in terms of specific genetic events and may correspond to ancestral breed pools, and at least visualize the uncertainty of the phylogenetic reconstruction. However, *NeighborNet* graphs rarely show reticulations corresponding to specific cases of crossbreeding. Kijas *et al.* (2012) and Ciani *et al.*, (2020) showed that deeper phylogenetic splits, i.e. between breed clusters rather than between breeds, are resolved better if (i) the most recent and documented crossbreds are removed, and (b) the unambiguous monophyletic breed clusters are combined in one taxon. All this may contribute to the formulation of specific hypotheses to be tested statistically or by modelling.

The sophisticated program *Treemix* (Pickrell and Pritchard, 2012) constructs a maximum-likelihood tree based on the covariance of the allele frequencies, which overall agrees with NJ trees. Subsequently, the agreement between the covariances explained by the tree and the real covariances can be improved by adding vectors between branches that represent gene flow (migration) between those branches. Taking a user-defined number of migrations, *Treemix* optimizes a new tree topology, the start and endpoint of the migrations and their weights (e.g. Decker *et al.*, 2014; Fonseca *et al.*, 2018; Orozco-terWengel *et al.*, 2015). However, the graphical output has a poor quality that cannot be adapted easily. More importantly, we recommend that the migrations inferred on the basis of an overall match of graph-based and real covariances are tested, for instance using the f_3 or f_4 statistics, which is rarely done. Ciani *et al.* (2020) found that plausible admixture confirmed by both f_4 analysis and by BSAA were only partially reconstructed by *Treemix*. Other methods to construct admixture graphs are discussed in the next section.

Detection of admixture using f_3 , f_4 and D statistics- The availability genome-wide datasets obtained by SNP arrays (e.g. Gibbs *et al.*, 2009; Kijas *et al.*, 2012) and whole-genome sequencing (e.g. Alberto *et al.*, 2018; Bosse *et al.*, 2014; Fan *et al.*, 2020) led to the understanding that hybridization and admixture are more common than previously thought. The density of data now allows for an accurate estimation of the new statistics for the detection of gene flow between well-diverged breeds (Patterson *et al.*, 2012; Peter, 2016). These statistics can be used with both individuals and breeds Figure 6 shows the most common applications of the f_3 and f_4 statistics based on allele frequencies of three (f_3) or four (f_4) breeds. As shown, they can be decomposed as the sum or difference of distances between nodes as quantified via the f_2 genetic drift statistic. For a more extensive theoretical treatment involving path lengths and admixture proportions we refer to Patterson *et al.* (2012) and Peter (2016).

The f_3 statistic using an outgroup is useful for quantifying the relative affinity of animals or breeds, for instance of ancient and modern DNA samples (Daly *et al.*, 2018). The properties of f_3 for detection of admixture can be understood by considering that the average of $(P_C - P_A)(P_C - P_B)$ becomes negative if P_C is intermediate between P_A and P_B for many SNPs, which indeed reveals recent admixture. However, a positive value of f_3 does not exclude admixture, which limits its use.

The statistic f_4 is more predictable since admixture follows directly from systematic differences from zero. These deviations can be understood by considering that sharing of characteristic alleles by source and target or, for a more general case, by A and C (Figure 6), but not by the other possible pairs of breeds, leads to a correlation of P_A and P_C , which makes the average of $(P_A - P_B)(P_C - P_D)$ positive; decomposing f_4 into a function of four f_2 -scaled distances as indicated in Figure 6 results in a total length of zero without gene flow, but becomes greater than zero if gene flow shortens the $f_2(A, C)$ distance.

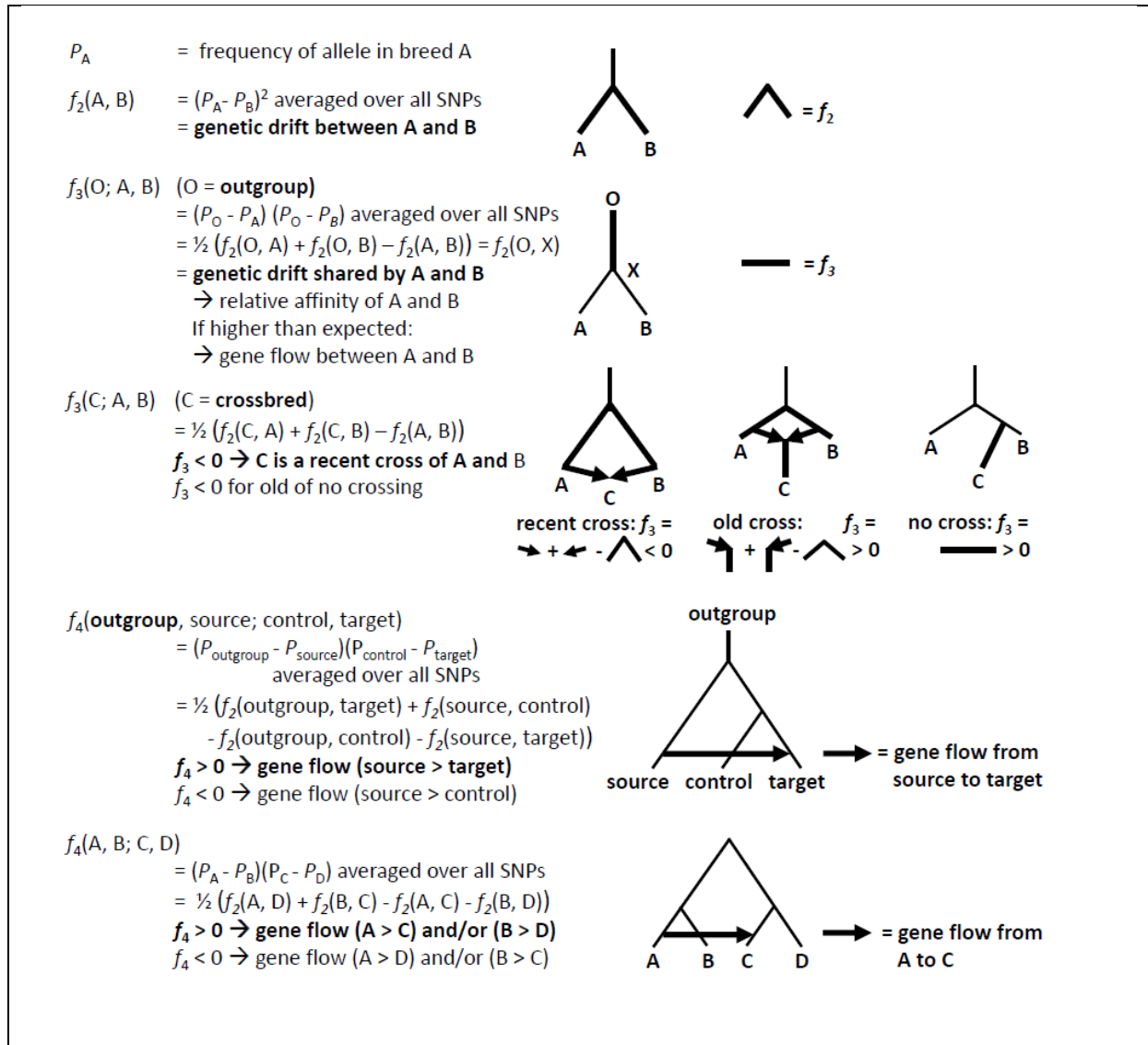


FIGURE 6

Definitions of the f_2 statistic, the outgroup f_3 statistic, the admixture f_3 statistic and the admixture f_4 statistic with an outgroup and the more general case with four breeds. It is shown how the statistics correspond to branch lengths and how they are used as test statistic for the occurrence of gene flow.

The f_4 statistic is related to the D statistic from the ABBA/BABA algorithm. This method considers the sister taxa one and two, the donor three and the outgroup (O). For a given SNP, A is the ancestral allele fixed in O and B the derived allele fixed in three. For a haploid genome, the possible allele combinations for one, two, three and O are AABA, ABBA, BABA and BBBA. Ignoring the non-informative AABA and BBBA, a higher admixture from three into two than from three into one follows from a majority of SNPs with the ABBA pattern:

$D = [n(\text{ABBA}) - n(\text{BABA})] / [n(\text{ABBA}) + n(\text{BABA})]$, in which $n(\)$ are the counts of SNPs with the ABBA and the BABA pattern, respectively. For a collection of samples, the use of allele frequencies instead of counts and an adapted formula allow to consider also SNPs for which A and B are not fixed in the outgroup and the donor, respectively (Patterson *et al.*, 2012). For statistical testing, D is preferred above f_4 (Patterson *et al.*, 2012). However, we stress that both methods strictly depend on a tree-like evolution of the breeds being considered

The f -statistics can be used to construct more realistic admixture graphs than a *NeighborNet* or *Treemix* graph. However, because this procedure is very specialized and only partially automated by

software, the methods described in the literature are not generally used. The programs *QPTOOLS* from the *AdmixTools* package (Patterson *et al.*, 2012) and *ADMIXTUREGRAPH* (Leppälä, Nielsen and Mailund, 2017) take as input a user-specified Admixture graph and all possible f_2 , f_3 and f_4 values and then optimize the branch lengths and admixture proportions. *MIXMAPPER* (Lipson *et al.*, 2013) selects breeds for which a hierarchical tree can be constructed (i.e. no negative f_3 values, additivity of f_2 values) and then sequentially and interactively adds admixed breeds while optimizing topology, sources of gene flow, branch lengths and admixture proportions.

Phylogeny across the genome. So far we compared individuals, or groups, on the basis of genome-wide genetic variation. However, as a consequence of genetic recombination, the genome consists of segments with widely different genealogical histories (e.g. Li and Durbin, 2011). For instance, within crossbred individuals, portions of the genome may originate from different breeds (e.g. Fan *et al.*, 2020), which result from various demographic processes such as migration, inbreeding and selection. Software products have been developed that attempt to overcome the deviating phylogenetic signals from markers and thus establish the true genealogy that reflects the breeds' evolutionary processes. Among these, *SNAPP* (Bryant *et al.*, 2012) uses a likelihood-based approach to estimate the groups' true tree based on the genetic variation of markers across the genome. Hobolth *et al.*, (2007) established a framework that enables simultaneous modelling of divergence or speciation times, and demographic parameters such as the ancestral N_e , which has been successfully applied to model the divergence history of the European bison, *Bison bonasus* (Gautier *et al.*, 2016).

RECONSTRUCTION OF POPULATION HISTORY AND DEMOGRAPHIC MODELLING

Several methods presented above can identify evolutionary processes in the history of species, populations and breeds, while representing patterns of differentiation. The subsequent sections will focus on methods that explicitly aim at detecting, dating and quantifying population size changes, and other events such as admixture or population splits. Because the complexity of the evolutionary history of species and breeds probably precludes its full reconstruction, even with genomic data, the interpretation of an inferred demographic history should thus always be done with caution. Also, in the case of domesticated animals one must keep in mind that this evolutionary history integrates the recent artificial selection, the domestication process, and the history of the wild species from which they were derived. Genetic and genomic data enable testing alternative interpretations and models by comparing the genetic consequences of alternative evolutionary scenarios with observed data. This is particularly true with the approximate Bayesian computation (ABC) framework (Beaumont, 1999; Beaumont, Zhang and Balding, 2002).

An initial step is to describe the coalescent theory, which is at the heart of modern population genetics (Hudson, 1990, 2002; Wakeley, 1999). It was developed in the 1980s and 1990s to study the statistical properties of gene trees for non-recombining DNA fragments (Kingman, 1982) evolving under a simple demographic model that assumes panmixia and isolation from other populations (i.e. no population structure), non-overlapping generations, neutrality, and constant population size (e.g. the Wright-Fisher model). It has since been extended to account for population size changes (Griffiths and Tavaré, 1994), population structure (Herbots, 1994), recombination (Griffiths and Marjoram, 1997) and different sampling times to integrate ancient DNA or viral samples. Some types of selection can also be integrated. The coalescent theory takes a retrospective approach that differs from modelling or simulation approaches used before the 1980s. Coalescence and classical simulation are now commonly denoted as backward and forward simulation, respectively. A coalescent approach works backward in time and focuses on the statistical properties of the gene trees of the samples. If a population is large, the probability that lineages coalesce (have a common ancestor) is low, whereas in a small population this probability is high. As a consequence, starting from a particular sample size the length of the branches connecting these samples will depend on the changes in population size, as one goes backward in time, and as the number of remaining lineages decreases. In a more general way, the coalescent theory studies how gene trees depend on the parameters of the demographic models of

interest (past population sizes, migration rates, age of a bottleneck or an admixture event). Coalescent-based methods are sample-based and thus computationally less expensive than the population-based forward methods, which require the simulation of the whole population. The development of programs such as *ms* (Hudson, 2002), allowed the development of simulation-based inference methods including ABC. Forward simulations are still used, for instance for selection, and are being revived after recent improvements in efficiency (e.g. *Slim*, Haller and Messer, 2019).

The coalescent theory played a central role in the development of inferential methods in the 1990s and 2000s. It was shown that bottlenecks and expansions have different effects on the shape of trees, hence allowing the use of coalescence-based inference to distinguish between the two types of events. This led to the development of likelihood-based methods to compute the conditional probability $P_M(Data / Param)$ of generating the observed *Data* under a predefined demographical model *M*, defined by a set of parameters $Param = (Par_1, \dots, Par_k)$, which may correspond to divergence times, population sizes, or migration rates. Likelihood methods apply optimization algorithms, often via a sophisticated search of the parameter space, to find parameter values that best explain the observed *Data*, i.e. the maximum likelihood estimates (MLE) of the parameters. A Bayesian perspective can also be used to estimate probability density functions for the parameters, assuming prior distributions for these parameters, $P_M(Param)$ and multiplying them by the likelihoods to obtain posterior distributions using Bayes formula: $P_M(Params / Data) = P_M(Param) * P_M(Data / Param) / P_M(Data)$. Likelihood-based methods were developed between the mid-1990s and mid-2000s and applied to different demographic models to consider: population size change (Beaumont, 1999); population structure and gene flow (Beerli and Felsenstein, 2001); population split and post-split migration rates (Nielsen and Wakeley, 2001); or admixture (Chikhi, Bruford and Beaumont, 2001).

In the last decade several important methods were developed that allow users to infer either simple or complex demographic models with genomic data. One of the most popular is the PSMC method of Li and Durbin (2011), which uses the genome of a single diploid individual (or two haploid genomes) and infers a histogram or “skyline plot” usually interpreted as a history of population size changes. The PSMC uses information on mutation and recombination rates to analyse and interpret the distribution of heterozygous sites along the genome. Under panmixia (i.e. absence of genetic subdivision) the method estimates the history of population size changes that best fits the distribution of heterozygous sites. Schiffels and Durbin (2014) extended the method by allowing the use of multiple phased genome sequences simultaneously (MSMC). Under panmixia the MSMC also estimates the history of population size changes. It is important to note that both the PSMC and MSMC only use information from the first coalescence time, T_k , where k is the number of haploid genomes and thus ignores most of the information contained in the whole coalescent tree. These methods provide only information on events that took place within the time frame defined by the T_k . Thus, the more haploid genomes one uses with MSMC, the earlier the start and end of the reconstructed curves.

Other methods use the site or allele frequency spectrum (AFS) as a way to summarize genomic data across many independent loci. For instance, the *Fastsimcoal2* (Excoffier *et al.*, 2013) and *dadi* (Gutenkunst *et al.*, 2009) software allow the user to infer the parameters of tree models in which populations are allowed to have different population sizes, and may be connected by gene flow. For computational reasons, the number of populations is limited, typically two to five populations depending on the methods. *Fastsimcoal2* uses a likelihood approach and can thus be used to compare alternative tree models, with or without gene flow or admixture.

A general inferential framework for comparing alternative models is ABC, which is particularly well-adapted to study complex demographic datasets and evolutionary models for which the likelihood may be difficult or impossible to compute. In such cases, the principle of ABC is to use large numbers of simulations under different demographic models and parameter values together with two approximations. First, the data are summarised by a limited number of summary statistics (such as n_A , Tajima’s D or H_e), and the objective is to estimate $P_M(SummData / Param)$, where *SummData* is a vector of the summary statistics. Second, simulated datasets are selected with a *SummData* that,

according to a tolerance threshold, are close enough to the *SummData* of the real dataset. The parameter values corresponding to the selected simulations (usually the best 0.1 or 1 percent) are kept for inference. The number of simulations used to estimate the posterior distributions of the parameters of a particular model is typically $>10^5$ - 10^6 . The coalescent theory played a central role in the development of ABC methods by enabling an efficient simulation of data. The simplest ABC algorithm is usually called “rejection” because it only requires filtering the best simulations according to the tolerance threshold and rejecting the others.

The ABC framework is extremely flexible and can handle complex datasets and other situations for which classical models do not perform well. For instance, Boitard *et al.* (2016) used the AFS together with summary statistics measuring LD at different distances to reconstruct a history of population size changes in large samples obtained from a set of four cattle breeds (Angus, Fleckvieh, Holstein and Jersey). ABC can also be used to compare alternative demographic models by computing the relative proportions, among alternative models, of simulated *SummData* closer to the observed *SummData*. It can also test the quality of the inference process by generation of pods (pseudo observed data) on the basis of the different models and check if an ABC analysis of the pods indicates the corresponding model and generates a meaningful posterior distribution of parameters. However, the large number of simulations required by ABC methods can become computationally intensive with genomic data and complex alternative models.

Beaumont *et al.* (2002) demonstrated that a regression algorithm achieved the same quality of the inference as the rejection algorithm but with ten times fewer simulations, allowing a much lower tolerance threshold. Other approaches have been proposed to improve the regression step by using non-linear regression and neural networks (Csilléry *et al.*, 2010). More recently, a machine-learning approach called “random forests” has been proposed as a way to avoid the selection step of summary statistics, and the need to define a tolerance threshold (Raynal *et al.*, 2019).

MITOCHONDRIAL DNA AND THE SEX-CHROMOSOMES

The genetic diversity of autosomes, sex chromosomes and mitochondrial DNA (mtDNA) is affected in different ways by demography, and their combined analysis provides insight into the various historical processes that shaped present-day populations. Because of their lack of recombination, the evolution of mtDNA and Y-chromosomal DNA is less complex than the autosomal evolution and can be reconstructed largely by obtaining a phylogenetic tree and coalescence analysis. Generally, it reveals links between the domestic species and their wild ancestors, and shows ancient bottlenecks that contributed to differentiated haplogroup distributions, e.g. in (sub)continents (Lenstra *et al.*, 2014), but is considerably less informative than autosomal DNA for reconstructing breed histories. In addition, mito-nuclear discordances occur frequently simply due to incomplete lineage sorting or by sex-biased introgression, such as for the European bison carrying cattle-like mtDNA (Wang *et al.*, 2018), zebu outside Asia carrying taurine mtDNA (Ginja *et al.*, 2019), or possibly the Grey jungle fowl that carries a domestic/Red jungle fowl mitochondrial haplotype (Lawal *et al.*, 2020).

Before the availability of WGS data, the analysis of Y-chromosomal variation was limited by a lack of informative markers. It shows the paternal history, which by the small male population size for most domestic species has led to a considerable breed-level differentiation (see Box 5). For many species, in current genome assemblies the Y chromosomes are partially represented by unplaced scaffolds that are only present in males, and for a large part consist of Y-chromosomal repeats and multicopy genes. Note that single-copy male-specific SNPs are hemizygous, that is, they have one allele per individual without heterozygosity scores (H_o and H_e) and should not have scores in females.

BOX 5**Y-chromosomal variation**

The mammalian Y chromosome is small, carries only a few genes and mainly consists of repetitive DNA. Due to its complex structure it is often omitted from genome assemblies. But the Y chromosome has a powerful property: its male-specific, non-recombining part (MSY) is transmitted as a tight linkage group (a haplotype) from the father to the son. Hence, genetic variation on the MSY reflects the paternal history of populations (Jobling and Tyler-Smith, 1995).

The MSY diversity is best studied in the humans, where deep insights derive from several decades of steady progress in variant discovery and haplotype analyses. A diverse spectrum of polymorphisms on the human MSY, ranging from small- and large-scale rearrangements, SNPs, indels, and CNVs as single tandem repeats, now represent a clear picture of the human male genealogy (reviewed in Jobling and Tyler-Smith 2017). The wealth of MSY markers available in humans, enables the study of evolution over different time scales. The work in humans has also demonstrated that a community accepted MSY phylogeny, based on defined haplotype determining variants, is pivotal to consolidate results from different studies (Van Oven *et al.*, 2014).

The tremendous amount of WGS data becoming available is now empowering comprehensive fine-scaled MSY haplotype analysis in domestic species. To infer the MSY pattern in a population of interest, proper MSY variant discovery is a prerequisite. As a first step, one usually needs to define MSY sequences that can be used as the reference for variant calling from NGS data. The potential of MSY draft assemblies, combined with an approach to define MSY regions appropriate for reliable variant calling, has been recently demonstrated in the horse (Wallner *et al.*, 2017), camel (Felkel *et al.*, 2019) and sheep (Deng *et al.*, 2020). After mapping WGS data to the reference and variant ascertainment, the allelic states of MSY variants can be catenated into haplotypes and a robust haplotype phylogeny can be built under the principle of maximum parsimony. Once the MSY phylogeny is defined, haplotype dispersal should be screened by genotyping haplotype determining markers in larger sample collections (Deng *et al.*, 2020).

Defining the MSY haplotype signatures that are diagnostic for a given breed or even a single influential individual enables to univocally trace their influence. This process has been conducted in the horse to assess the influence of the Thoroughbred breed (Wallner *et al.*, 2013; Cosgrove *et al.*, 2020). From horse studies the importance of an appropriate ascertainment panel has become evident, as haplotypes that are private in rural populations are still not fully defined (Han *et al.*, 2019) and ancient remains enlighten significant turnover of MSY haplotype spectra through time and space (Fages *et al.*, 2019).

Establishing MSY phylogenies is computationally demanding and obtaining inferences on haplotype distribution is labour intensive. The building of international consortia to coordinate MSY research in livestock would be extremely beneficial.

Source: Barbara Wallner

Deviations from the expected levels of genetic diversity and divergence of the X chromosomes have been observed in several species. For mammals, chromosome X is present in two copies in females and one copy in males, therefore the expected ratio between the genetic diversities of chromosome X and the autosomes is 0.75 (Ellegren, 2009; Wilson Sayres, 2018). However, ratio has varied widely in several breeds of European and African cattle (Da Fonseca *et al.*, 2019). Increased levels of X-chromosomal diversity have been shown to result from growth in population size (Van Belleghem *et al.*, 2018; Pool and Nielsen, 2007). Conversely, a reduction in relative diversity follows an overall reduction in effective population size (Ellegren, 2009; Pool and Nielsen, 2007). In livestock populations, this is often a consequence of breeding practices that involve the selection of few individuals with desirable phenotypes. A reduction in population size would result in relatively strong genetic drift of X-chromosomal alleles, which has a lower N_e than on autosomes. In the case of cattle,

molecular signatures of a bottleneck were detected in several cattle breeds (Boitard *et al.*, 2016; Da Fonseca *et al.*, 2019; Ginja, Telo Da Gama and Penedo, 2010; Kim *et al.*, 2017; Martín-Burriel *et al.*, 2011).

Another process that is expected to impact the relative diversity of the sex chromosomes is sex-biased gene flow (Ellegren, 2009; Wilson Sayres, 2018). This is especially relevant for cattle, for which artificial insemination of many cows with semen of very few sires is a widespread practice. Furthermore, it is known that historically female populations were more likely to be geographically constrained and human-driven hybridization may have been carried out mainly using males (Lenstra *et al.*, 2014). Generally, introgression is more efficient in the autosomes than in the sex chromosomes, as observed for the domestic chicken (Lawal *et al.*, 2020). This pattern can be explained by stronger species barriers on the sex chromosomes (Meiklejohn *et al.*, 2018).

As shown by Da Fonseca *et al.* (2019), the use of whole-genome sequences led to two observations that are probably related and may very well be of fundamental significance. First, a ratio of F_{ST} values for autosomal and X-chromosomal sequences depends dramatically on the cattle being compared. For taurine vs indicine cattle, the X-chromosomal F_{ST} values are ~25 percent larger than the autosomal values. In contrast, within taurine cattle, the autosomal F_{ST} values are about twice those of the X chromosome, and for Iberian cattle with autosomal F_{ST} values ranging from 0.1 to 0.26, the X-chromosomal range is 0 to 0.04. Second, in model-based clustering at $K=2$ the clusters correspond to taurine and indicine cattle. In this case, the autosomes of African taurine cattle show an indicine component of 18 to 36 percent, whereas the X chromosomes remained purely taurine. It is not yet clear if these effects can be explained by a relatively ineffective X-chromosomal introgression; there may well be consequences of genetic conflicts underlying the meiotic drive (Meiklejohn *et al.*, 2018). Since LD-based pruning removes X-chromosomal SNPs, this was never noticed with bead-arrays, but it is certainly worth following up.

IDENTIFICATION OF GENOMIC REGIONS SUBJECT TO SELECTION

Domestication, adaptation, breed formation and selective breeding have left specific genetic patterns, “selection signatures”, in genomic regions of farm animal breeds (e.g. Raudsepp *et al.*, 2019). The identification of selection signatures is a central goal in evolutionary and population genetic studies but has importance also in characterization of animal genetic resources (AnGR) for food and agriculture. With methods for the detection of selection signatures it may be possible to identify, for example, genes related to economically important traits or for adaptation to challenging environments (Saravanan *et al.*, 2020; Weldenegodguad *et al.*, 2019). Breeds displaying special adaptive traits have typically high priority in the conservation. Selection signature studies can also provide knowledge of quantitative trait loci (QTL) for production characters and important causal mutations (Saravanan *et al.*, 2020). Moreover, genome-wide searches for regions associated with phenotypic traits may promote our understanding of function of genomes. For example, several whole-genome data sets have shown that a great proportion of SNPs exhibiting selection signatures correspond to non-coding genomic regions, indicating that selection occurs specifically via the regulatory elements of genomes (Librado *et al.*, 2015).

Types of selection

During the domestication process, genomes of animals have been shaped by natural selection and artificial selection. Human-initiated artificial selection (or selective breeding) conducted over generations for productive, morphological, fertility and other economically traits has had remarkable effects on genetic and phenotypic variation within and between breeds of farm animal species (e.g. Felius 1995; Forutan *et al.*, 2018). Natural selection driven by environmental circumstances involves differential reproduction of genetically diverse types so that some types leave more offspring than others (e.g. Falconer and MacKay 1996). Three forms of natural selection are typically classified:

(i) positive selection; (ii) purifying selection (negative or background selection); and (iii) balancing selection.

The various forms of selection have specific effects on allelic and genotypic frequencies, as well as on genomic structural variation in animal populations (de Simoni Gouveia *et al.*, 2014). When artificial selection or natural positive selection increases frequency of an advantageous allele, the frequencies of neutral variants that are physically linked to the advantage allele tend to change (de Simoni Gouveia *et al.*, 2014 references therein). This process is termed “genetic hitchhiking”. When a substantial (or complete) reduction of genetic variation at the target of selection and its surrounding genomic regions occurs, it’s called a “selective sweep” (Maynard-Smith, 1974). Moreover, in these chromosomal regions an increase in the average LD is expected to occur, leading to long haplotypes. These changes can typically be observed in statistical parameters related to genetic diversity within and between populations (de Simoni Gouveia *et al.*, 2014 and the references therein). The within-population diversity is typically decreasing in these regions, while between-population diversity tends to increase. In negative (purifying) selection, the selective pressure operates against disadvantageous alleles at the target of selection, thus not always affecting the surrounding genetic variation, especially when there are multiple advantageous or disadvantageous alleles. However, negative selection can also result in loss of genetic variation at the region of the genome which, although neutral, is linked to the negatively selected sites; this process is known as “background selection” (Charlesworth *et al.*, 1993). The third main form of natural selection, balancing selection, maintains multiple alleles in a locus and high genetic diversity (Oleksyk *et al.*, 2010). Major histocompatibility complex genes are classical examples of genes shaped by balancing selection (Hedrick, 1998). More information on various forms of selection and their effects on nonneutral and linked neutral genetic variation is provided in the literature (de Simoni Gouveia *et al.*, 2014; Horscroft *et al.*, 2019; Saravanan *et al.*, 2020). Figure 7 is a graph showing trends in diversity of the KITLG genic region of sheep and goats (Alberto *et al.*, 2018). This region includes subregion that show decreased diversity in domestic sheep, and decreased diversity in goats, presumably due to species-specific selection processes associated with domestication.

Methods of detecting selected loci

Methods to detect selection signatures have been recently reviewed e.g. by Horscroft *et al.* (2019) and Saravanan *et al.* (2020). The methods commonly used in livestock studies can be classified into two main groups: methods based on intra-population statistics and those on inter-population statistics (Saravanan *et al.*, 2020). In intra-population methods, genomic or DNA-marker data are compared within populations. In this group, methods are based on the site frequency spectrum, linkage disequilibrium or the identification of genomic regions with reduced variation compared to the genome average (de Simoni Gouveia *et al.*, 2014; Saravanan *et al.*, 2020). The inter-population approaches are based on allele frequency differences and the degree of differentiation between the populations (Saravanan *et al.*, 2020). The level of genetic differentiation can be analysed using single site differentiation or haplotype-based differentiation (Saravanan *et al.*, 2020). Coding sequences of orthologous genes among animal species can also be compared and tested based on synonymous and non-synonymous substitution rates applied to detect the neutrality or the form of selection (positive or negative - de Simoni Gouveia *et al.*, 2014). In addition, “landscape” genetics and genomics (Manel *et al.*, 2003; 2010) approaches may reveal associations between genomic data and environmental variables and thereby environmental adaptive signatures (e.g. Mdladla *et al.*, 2018; Vajana *et al.*, 2018). See Box 6 for more information on landscape genomics.

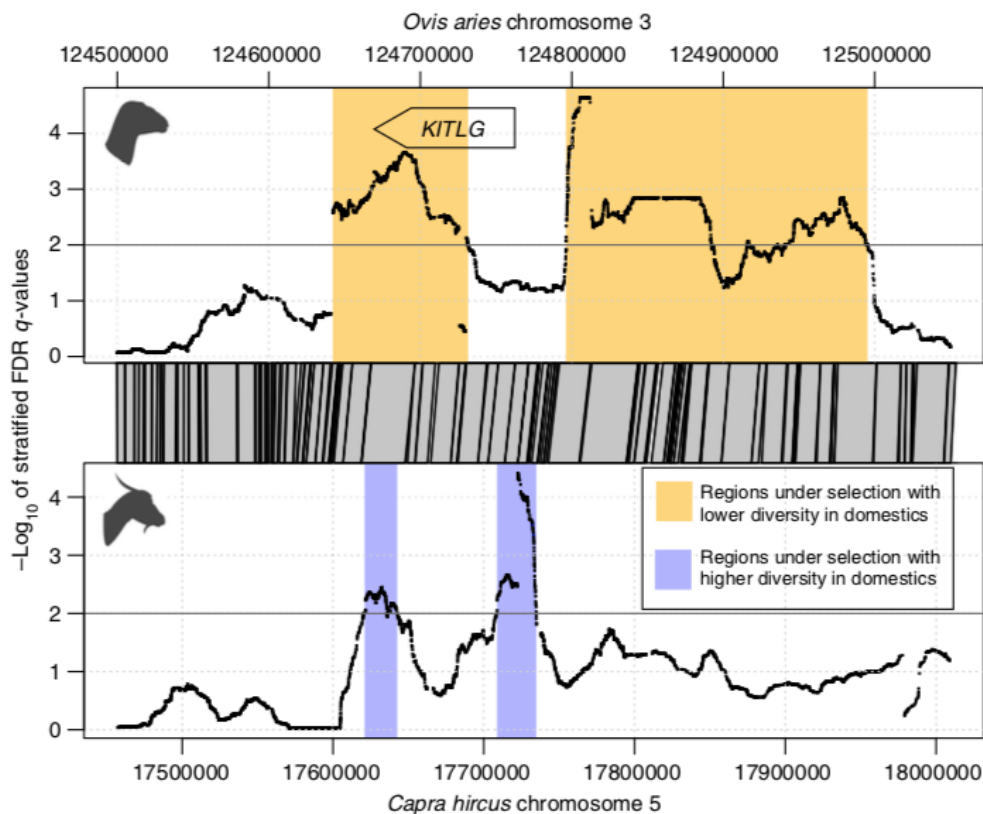


FIGURE 7
Chromosomal regions under selection within the KITLG gene for sheep and goats. The regions have decreased in domestic sheep and a great diversity in goats (from Alberto *et al.*, 2018)

BOX 6

Landscape genomics

Landscape genomics is a research field that combines population genomics, landscape ecology, computer science and bio-geoinformatics to explicitly quantify the effects of environmental heterogeneity on neutral and adaptive genetic variation and underlying processes. Landscape genomics has potential for addressing research questions in various research fields, including ecology, evolution, and conservation of livestock breeds. The discipline is based on the idea that environmental conditions can substantially affect the genetic variation of local populations, with important consequences for evolutionary processes.

The landscape genomics approach consists in linking genome-wide information to environmental variables identify potentially valuable genetic material, like genomic regions conferring adaptive advantages. Typically, data obtained from genome-wide scans carried out on a number of animals from populations living in different habitats or across ecological clines will be compared to geo-environmental information characterizing these habitats (yearly amount of precipitation, monthly temperatures, number of days with ground frost, etc.). Comparisons are carried out by means of association models calculated between allele or genotype frequencies and the values of the environmental variables. The parallel and simultaneous processing of up to billions of statistical models (i.e. hundreds of environmental variables times millions of SNPs) makes it possible to identify genomic regions of interest, for instance linked to genes involved in adaptation to heat, or genes involved in processes of resistance against parasites.

In developing countries, small ruminants play an important role in the livelihood of a large proportion of farmers. In these countries, conserving traditional breeds is essential since the latter are able to survive in changing habitats showing often harsh conditions to which their rich genomic

resources make it possible to adapt. Therefore, landscape genomics is an important tool to identify the key genetic features of local adaptation in order to support sustainable breeding of low-input livestock.

In the current context of global warming, this genetic adaptive potential can be directly integrated into conservation management using SPatial Areas of Genotype probability (SPAGs). This tool is able to transpose landscape genomic results into an adaptive potential spatial prediction framework. SPAGs can be integrated with climate change projections to forecast the future spatial distribution of genotypes. The analysis of the mismatch between current and future SPAGs (“genomic offset”) makes it possible to identify vulnerable populations lacking the adaptive genotypes necessary for future survival.

Environmental data are necessary to compute landscape genomics models. The WorldClim (2020) database contains monthly minimum, maximum and average temperature and total precipitation together with a series of bioclimatic variables computed from these variables. The Climatic Research Unit (CRU, 2021) in Norwich, United Kingdom, a recognised world's leading institutions concerned with the study of natural and anthropogenic climate change, also produces several datasets to be used in the context of landscape genomics studies.

For computation, several open source software implement landscape genomics algorithms. These are provided in Appendix 6.

Source: Stephane Joost

Appendix 6 provides examples of software and includes a section for applications that have been used in domestic animal studies to detect selection signatures. Because various methods have different strengths and weaknesses, it is typically recommended that more than one method is used for analyses of selection signatures (de Simoni Gouveia *et al.*, 2014; Yurchenko *et al.*, 2018; Horscroft *et al.*, 2019; Saravanan *et al.*, 2020). In animal populations, demographic events, such as population expansions, genetic bottlenecks, and population subdivision, can lead to false selection signals that mimic signatures of selection (de Simoni Gouveia *et al.*, 2014). de Simoni Gouveia *et al.* (2014), Ahrens *et al.* (2017) and Horscroft *et al.* (2019) have presented approaches to avoid effects of demography or other factors causing bias in selection signature analyses.

GENOME-WIDE ASSOCIATION STUDIES

The development and application of dense marker panels that can be genotyped at high-throughput using microarray technology (see Section 3) has facilitated the obtaining of large-scale genotype data for many domestic animal species. The most widely applied microarrays provide genotypes for between 50 000 and 800 000 SNPs that are evenly spread across the genome. Millions of domestic animals have been genotyped with these arrays in the past decade with the primary goal to implement genomic selection and predict genomic breeding values (Georges *et al.*, 2019). The availability of dense genotypes and a diverse array of phenotypes, often available over multiple generations, makes domestic animal populations amenable to genome-wide association testing. In genome-wide association studies (GWAS), statistical tests are applied to examine if molecular markers are associated with the expression of phenotypes. Because such analyses effectively involves the testing of multiple hypotheses simultaneously, the markers need to pass stringent significance thresholds to be considered as significantly associated with the trait of interest. Because the marker panels typically do not contain causal mutations, the aim of GWAS is to identify regions likely to harbour causal variants through an association with a physically linked genotyped variant. The precision to localize causal variants through GWAS with anonymous marker panels depends on the marker density. Sparse marker panels may result in large confidence intervals for quantitative trait loci (QTL), rendering the identification of causal variants a difficult task. Due to extensive LD between adjacent markers in populations with small N_e , QTL confidence intervals may also be large when dense marker panels are

employed. Functional investigations are required to differentiate between causal variants and anonymous markers in LD with the causal variants (Karim *et al.*, 2011).

Apart from the density of the marker panel, the power of the GWAS, i.e., the probability to detect trait-associated markers, depends on (i) the LD between molecular markers and QTL; (ii) the heritability of the trait; and (iii) the size of the mapping cohort (Goddard and Hayes, 2009). The most widely applied genotyping arrays in domestic animal species have been developed for cosmopolitan breeds (Matukumalli *et al.*, 2009), thus they are less informative for local breeds that are greatly diverged from the breeds used to develop the marker panels. Moreover, the genotyped markers are often depleted for low-frequency variants. This type of bias, known as “ascertainment bias” (AB – see Section 3) can compromise the power of GWAS, particularly in local breeds. While large mapping cohorts may be established easily in cosmopolitan breeds, the small census size of local breeds may also be a limiting factor for powerful GWAS to detect markers associated with within-breed variation. Across-breed GWAS may reveal variants that are associated with breed differences (Schoenebeck and Ostrander, 2013). However, even in large cosmopolitan breeds, the large sample size required to detect trait-associated variants may not be readily available for novel traits, thus requiring efforts to coordinate collaborative projects to establish powerful mapping cohorts (Lu *et al.*, 2018).

Both binary (discretely distributed) and complex (continuously distributed) traits may be subjected to GWAS testing. Association testing for binary traits requires genotypes for a cohort of individuals that expresses a particular phenotype (“cases”) and for a cohort of control individuals that don’t express the phenotype (“controls”). Chi-square tests and Fisher tests of allelic association may be implemented to test the association between markers and binary traits (Balding, 2006). It is important though, that both cohorts are matched because systematic differences in allele frequencies between cases and controls due to stratification may confound the GWAS and lead to false-positive associations. The highest-ranking PCs of a genomic relationship matrix can be effectively used to account for population structure and cryptic relatedness in case-control GWAS (Price *et al.*, 2006; Nosková *et al.*, 2020). Case-control association testing of genome-wide markers has been successfully used to reveal causal variants for many Mendelian traits in domestic animals, and most of them are curated at the Online Mendelian Inheritance in Animals (OMIA) database (OMIA, 2021).

Most complex traits are highly polygenic and recent empirical evidence suggests that they are predominantly determined by additive effects (Hayes *et al.*, 2010; Hivert *et al.*, 2020). Each causal variant explains only a small fraction of the phenotypic variation of complex traits. To avoid an inflation of false-positive association signals arising due to population stratification (see above), software (Appendix 6) for complex trait GWAS typically rely on mixed model-based approaches that fit a (genomic) relationship matrix. While QTL that explain a relatively large fraction of the trait variation can be detected in small mapping cohorts (Pausch *et al.*, 2011; Signer-Hasler *et al.*, 2012), the discovery of QTL with small effects usually requires tens-of-thousands of genotyped animals (Bouwman *et al.*, 2018).

The first GWAS were performed in 2005 for monogenic traits in human populations (DeWan *et al.*, 2006; Burton *et al.*, 2007). Likewise, the first SNP-based GWAS in domestic animals were performed for monogenic traits using genotypes for approximately 25 000 SNPs (Karlsson *et al.*, 2007; Charlier *et al.*, 2008). Since then, both the size of the mapping cohorts and the marker density have increased continuously. The largest GWAS in domestic animals have been performed in cattle including the genotypes at more than 25 million variants for more than 94 000 samples (van den Berg *et al.*, 2020). With an ever-increasing size of mapping cohorts and more markers being tested, efficient software tools are required to undertake marker quality control and the association analyses. Since its implementation, the PLINK software has undergone a major overhaul and has become an indispensable tool for genotypic data analysis at the population scale (Purcell *et al.*, 2007; Chang *et al.*, 2015). Moreover, the .bed/.bim/.bam-format and its derivatives used by PLINK to store genotypic data is accepted as an input for most downstream analyses. Basic association analyses may be performed with PLINK. However, more sophisticated statistical methods that are based on Bayesian

or mixed model-based approaches may be conducted efficiently with the EMMAX (Kang *et al.*, 2010), GCTA (Yang *et al.*, 2011) and BayesR (Erbe *et al.*, 2012; Moser *et al.*, 2015) software tools (see Appendix 6).

With an increasing number of animals being sequenced, GWAS nowadays often rely on imputed sequence variant genotypes (see Section 3). Informative haplotype reference panels are required to accurately infer sequence variant genotypes for mapping cohorts that have array-derived genotypes. Such reference panels have been established for a variety of animals including cattle (Daetwyler *et al.*, 2014), sheep (<https://sheepgenomesdb.org/>), goats (<http://www.goatgenome.org/vargoats.html>) and dogs (Plassais *et al.*, 2019). Genotypes imputed from informative haplotype reference panels may readily reveal causal variants in GWAS (Pausch *et al.*, 2017). The integration of functional data is required to fine-map QTL regions and reveal causal variants (Xiang *et al.*, 2019). Novel approaches that are based on low-pass sequencing and imputation-based genotype refinement offer a cost-effective opportunity to perform sequence-based GWAS also in populations that either lack informative haplotype reference panels or for which dense microarrays are not available (Li *et al.*, 2020; Rubinacci *et al.*, 2020; Fuller *et al.*, 2020), which offers opportunities for less common livestock species.

APPLICATION OF GENOMICS IN SUSTAINABLE USE AND CONSERVATION

These guidelines emphasize the use of genomics for the characterization of AnGR and therefore are designed to facilitate implementation of Strategic Priority Area (SPA) 1 of the Global Plan of Action for Animal Genetic Resources (FAO, 2007) on Characterization, Inventory and Monitoring of Trends and Associated Risks. Genomics can also play an important role in the sustainable use and development (SPA2) and conservation (SPA3) of AnGR. Those roles for genomics are somewhat out of the scope of these guidelines, so they will not be addressed in detail. The subsequent sub-sections will briefly introduce some of the opportunities that genomics provide for these SPA. Oldenbroek (2017) addresses the contributions of genomics to management of AnGR in more detail.

Sustainable use and development

The long-term sustainability of a given breed will be enhanced if that breed has greater survival and longevity, is more productive and profitable, or otherwise contributes competitively to the livelihood of its keepers (FAO, 2013). Improvement through selective breeding is one opportunity to increase the average genetic merit of breeds for heritable traits associated with sustainability. Genomic selection (Meuwissen *et al.*, 2001) is an approach that statistically associates the phenotypes of animals within a population to their genotypes, as defined by large panels (typically thousands) of SNP. Operationally, genomic selection can be performed either by predicting the substitution value of each of the alleles of the panels of SNP or by using multi-locus genotypes to obtain more accurate relationship matrices than can be obtained by using pedigrees and replacing the matrices in typical methods based on best linear unbiased predictions (BLUP). When large amounts of historical performance and pedigree data are available, the breeding values of animals without phenotypes can be predicted much more accurately than with pedigrees alone. These “genomic breeding values” may also allow selection to occur prior to sexual maturity, thus increasing response to selection by decreasing the generation interval. Genomic selection is currently being routinely practiced in several livestock species (Georges *et al.*, 2019).

Alas, because the accuracy of genomic selection increases with the amount data available, breeds with small population sizes and/or a lack of historical performance and pedigree data may have less opportunity to benefit than do larger breeds (Mészáros *et al.*, 2015; Obšteter *et al.*, 2019). Breeds with small population size tend to also have small N_b and small N_e , meaning that intense selection to maximize genetic response may quickly decrease genetic variation to unacceptably low levels. Genomic data provides the possibility to balance selection response and maintenance of genetic variation at the molecular level, such as by applying genomic-based optimum contribution selection (Obšteter *et al.*, 2019; Sanchez-Molano, Pong-Wong & Banos, 2016).

Genomics can also contribute to management of genetic diversity as it relates to one or a few loci. Methods for selection signatures and GWAS can be used to identify targets for introgression or purging of deleterious loci. Genomic or marker-assisted selection can then be applied to increase/decrease the frequencies of the alleles associated with the trait in the population (e.g. Gaspa *et al.*, 2015). Genomics may also be used to decrease the proportion of “foreign” genes from a population that had been historically subjected to crossbreeding (Wellmann, Hartwig & Bennewitz, 2012).

Conservation

Genomics may also contribute to reaching conservation objectives. As a first step, genomic estimates of breed-wise genetic distances (distinctiveness) or kinship (diversity) can be used to prioritize genetically breeds for conservation (e.g. Ginja *et al.*, 2013). The methodology for these approaches was originally developed for simpler markers, such as microsatellites, but in general can be applied to genomic data. For a review of these approaches, see Boettcher *et al.* (2010). The genetic assessments may then be considered together with other factors such as risk of extinction and cultural significance for a comprehensive assessment of conservation priority (FAO, 2013).

Toro, Villanueva and Fernández (2014) reviewed the possible contributions of genomics for management of livestock conservation programmes. They discuss genomics-based approaches to evaluate genetic diversity of populations according to identity by descent, rather than identity by state. Approaches applied to *in vivo* populations may also be applied for management of gene bank collections. Ideally, *in vivo* and cryoconserved populations should be managed together.

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SECTION 5

**Conclusions and
recommendations**

Conclusions and recommendations

The FAO and the ISAG/FAO Advisory Group on Animal Genetic Diversity recommend that:

1. Countries continue to characterize their animal genetic resources to provide critical information for their improved management and to contribute to implementation of the Global Plan of Action for Animal Genetic Resources. Genetic characterization should apply the most advanced methods feasible (currently single nucleotide polymorphisms and whole-genome sequencing) and be complemented by phenotypic characterization and description of the production environment and recording of geographic coordinates.
2. National genetic resources are investigated by or in close collaboration with researchers from the same country while (i) respecting the wishes and interests of the livestock keepers; and (ii) complying with legislative, administrative and policy measures addressing access to genetic resources and the sharing of benefits derived from their utilization, as applicable; (iii) complying with measures addressing access to traditional knowledge associated with genetic resources and the sharing of benefits arising from the utilization of such knowledge with indigenous peoples and local communities; and (iv) complying with measures addressing access to genetic resources that are held by indigenous peoples and local communities and the sharing of benefits arising from the utilization of such resources with the peoples and communities concerned.
3. FAO, National Coordinators and any National Advisory Committees for AnGR are made aware of all diversity projects at any geographic level, so that results can contribute to the planning and development of national conservation and sustainable use activities and so that FAO can help facilitate coordination among projects, exchange information and promote funding.
4. Locally adapted genomic resources are considered within an international context, which for marker-based molecular genetic studies implies the use of the same or overlapping sets of genetic markers employed in previous studies, and that, if appropriate, data collected from characterization studies are placed in open-access repositories, including raw data, associated metadata and analysis pipelines and procedures.
5. Studies are guided by one or more specific questions to be answered, recognizing that these may be expanded during data analysis. In addition to establishing the genetic relationships of populations on the basis of neutral genetic markers, the molecular variation involved in phenotypic variation should be searched for, with special emphasis on environmental adaptation and disease susceptibility or resistance.
6. Studies of autosomal markers are complemented by characterization of mitochondrial DNA genomes as markers representing the maternal lineages, and of Y-chromosomal variation as markers of paternal lineages.
7. Studies of the present patterns of genetic diversity of livestock are complemented, where relevant, by the analysis of ancient DNA samples in order to provide a historic context.
8. Genotype data are subjected to a thorough quality check and filtering in order to identify and remove unreliable data, including duplicates, mislabelled samples and outliers associated with breed-level differentiation or undocumented crossbreeding.
9. For all breeds studied, heterozygosities or other indicators of overall genetic variability are calculated to identify breeds with low diversity and anticipate the potential for bias towards these breeds in multi-breed analyses.

10. Researchers understand the statistical methods being applied in data analysis and critically evaluate the results obtained from computer software.
11. Parameters related to within-breed genetic diversity are not simply estimated, but that this information be complemented with that of clustering methods and demographic modelling to better understand evolutionary processes. By combining different approaches, genomics can provide more informed management of populations of by monitoring of within-breed genetic variation and crossbreeding, avoiding high levels of inbreeding and ultimately preserving adaptive variation.
12. Publications of molecular diversity studies are freely accessible.
13. The scientific progress is communicated to breeders, livestock industry, the relevant government agencies and the general public.
14. Results from genomic characterization studies are actively utilized in the monitoring and management of animal genetic resources. Standardized estimates of effective population sizes or other measures of genetic variation can complement data on population size for informing countries on the genetic status and risk of extinction of their national breed populations. Management may include purebred genetic improvement and conservation measures, as well as controlled crossbreeding that preserves the original populations and maintains environmental adaptation.
15. The advances in molecular technology and bioinformatics continue to be closely monitored and relevant innovations be communicated to the animal genetic resources community.

Appendices

Appendix 1

Glossary of technical terms

Approximate Bayesian Calculation (ABC) Modelling approach based on optimizing the agreement between simulated and measured values of summary statistics.

Admixture The presence of genetic influence of multiple breeds in the genome of an individual or a population, due to historical interbreeding.

Allelic richness Measure of genetic diversity obtained by simply counting the number of different alleles within a breed or other population of interest. Allelic richness among populations is only directly comparable if the number of animals genotyped per population is equal.

AMOVA Analysis of molecular variance, estimation of the portioning of diversity over different hierarchical levels: within breeds, among breeds within regions, between regions, etc.

ARMS Amplification refractory mutation system, a polymerase chain reaction (PCR) during which one of the primer covers a single nucleotide polymorphism (SNP) site in such a way that the amplification depend on the SNP allele in the template DNA.

Ascertainment bias Systematic distortion in estimates of molecular genetic parameters (such as allelic frequencies) due to irregularities in the process used to identify the markers. For instance, many SNP in large panels were selected according to their high minor allele frequency in international transboundary breeds and can underestimate the relative diversity in other breeds.

Assembly The process of arranging overlapping individual DNA sequence reads into a contiguous whole-genome sequence.

Autosomes All chromosomes with the exception of the mammalian X or Y or the avian W and Z sex chromosomes.

Bayesian analysis Estimation of a likelihood distribution of model parameters on the basis of the likelihoods of parameter values in the absence of data (the prior) and the likelihoods of the observed data given different values of the model parameters. These estimations depend on a specific model and are often achieved by a strategy (like the Multiple Chain Monte Carlo simulations, MCMC) to explore different plausible values of the parameters (the “parameter space”).

Bead array Silica bead-based microarray for high-density SNP genotyping.

Coalescence analysis Estimation of the divergence times of individual DNA sequences since their descendance from a hypothetical most recent common ancestor, often used to infer present and past effective population sizes.

CNV Copy number variation, a type of structural variation in the genome resulting from differences in the copy number of chromosomal fragments of up to several megabases in length. CNV can be used as a genetic marker and has been associated with differences in human phenotypes.

Contig Uninterrupted DNA sequence assembled by combining separate experimental sequence reads.

Copy number variation (CNV) A type of structural variation in the genome resulting from differences in the number of chromosomal fragments of up to several megabases in length. CNV can be used as a genetic marker and has been associated with differences in human phenotypes.

CRISPR/Cas9 Method for modifying a gene (the target) in a living cell.

Crossing over Mechanism of creating new genetic variability through recombination of chromosomes during meiosis. The newly formed oocyte or sperm chromosome combines segments of the two homologous chromosomes originating from the mother and the father of the individual in which crossing-over takes place.

DGGE Denaturing gradient gel electrophoresis, which generates for a PCR product containing a SNP site a pattern that depends on the SNP alleles.

Depth the approximate number of times that a chromosomal region will be sequenced through a whole-genome procedure that involves sequencing and subsequent assembly of segments.

Diploid Characteristic of a species defined by its containing of two copies of all autosomes, the maternal and the paternal copy.

Effective population size (N_e) Hypothetical population size that would generate observed values of diversity parameters for a given population if mated randomly and not subject to forces such as selection and migration. The N_e corresponds to the number of breeding animals per generation and is usually smaller than the actual population count. It may be calculated separately for males and females.

Fixation Retainment within a populations of only one allele for a give genetic marker which thus becomes homozygous., typically the effect of inbreeding reducing the genetic diversity.

Fixation index (F_{ST}) the proportional increase in homozygosity that occurs through population subdivision (for example, the creation of breeds).

Genetic distance A measure of the genetic differences between two populations (or species) calculated on the basis of allelic frequencies in both populations.

Genetic marker Site in the genome that is variable (polymorphic) within a species. The different variants are called alleles, such as the two (usually) different nucleotides of a SNP.

Genome-wide association study (GWAS) Statistical analysis to detect a correlation between the phenotypes of a group of individuals and the genotypes of a given SNP, calculated for 50 000 to 1 000 000 SNPs spanning the whole genome; values exceeding a significance threshold may indicate that the SNP is near the causative gene.

Genotyping by sequencing (GBS) Sequencing of a subset (reduced representation) of the genome, either by a given class of restriction-enzyme fragments or fragments captured by hybridization to a set of DNA fragments. Sequencing of more or less the same fragments in a panel of animals yields SNPs and at the same time their genotypes. This allows genome-wide SNP genotyping for species for which no bead-array has been developed, such as wild species.

Haplotype Combination of alleles of two or more genetic markers on the same DNA segment. Haplotypes defined by alleles of markers on mtDNA and the non-recombining part of the Y-chromosome are transmitted to offspring. Autosomal and X-chromosomal haplotypes, typically defined by markers in or near the same gene (in which case a haplotype represents a gene variant), are transmitted to the offspring only if there is no recombination between the markers.

Haplogroup Group of closely related haplotypes.

Haploid Containing a single copy of the genome, such as sperm cells and oocytes.

Hardy-Weinberg equilibrium (HWE) Ratio for a given marker and population of the numbers of homozygote and heterozygote genotypes as predicted by random mating in a large population in the absence of selection, migration and mutation.

Heterogametic sex The gender that carries two different sex chromosomes, such as XY mammalian males and WZ avian females.

Heterozygosity State of a genetic marker (or any locus) at which both alleles are different.

HDP High-density panel of SNP on a bead array, typically containing > 500 000 SNPs.

Homogametic sex The gender that carries two identical sex chromosomes, such as XX mammalian females and ZZ avian males.

Homozygosity State of a marker (or any locus) at which both alleles are identical.

Identity by descent (IBD) Identical genotypes of a given marker in two or more individuals because the individuals descend from the same ancestor.

Identity by state (IBS) Identical genotypes of a given marker in two or more individuals.

Imputation Interpolation of greater-density genotypes (or full sequences) for a given (group of) individual(s) on the basis of low-density data for the same individuals and greater-density data for a panel of related individuals.

Introgression Movement of a particular allele or set of alleles from one population (i.e. breed) to another, usually by either deliberate cross-breeding or casual contact between neighbouring populations.

KASP competitive allele specific polymerase chain reaction, a PCR assay of the mutation that generates an allele specific fluorescent signal.

Kinship The probability that a randomly selected allele from two individuals (at the same locus) is identical by descent from a common ancestor (also known as the “coancestry” or “coefficient of coancestry”)

Linkage disequilibrium (LD) Distribution of multilocus genotype combinations in a population for a given pair of markers that is incompatible with independent inheritance, thus indicating genetic linkage of the loci.

Linkage disequilibrium (LD) pruning removal of SNP genotypes from a genome-wide SNP dataset in such a way that there is no linkage disequilibrium between any pair of SNPs that surpasses a user-give threshold.

Locus A distinct region of DNA (often a gene) in the genome.

Meiosis Cell division in the germline leading to haploid sperm or oocyte cell.

Microsatellite Tandem DNA repeat of a 2 to 5 bp unit. In most cases, the repeat unit is the dinucleotide CA. The number of repeats of a given microsatellite is often polymorphic within populations, in which case the microsatellite may serve as genetic marker. Also known as STR (simple tandem repeat) or SSR (simple sequence repeat).

Minor allele frequency (MAF) A metric primarily used to evaluate SNP, corresponding to the frequency of the less common of the two alleles (SNP are usually biallelic). A threshold of $MAF \geq$

0.01 is sometimes considered to define a SNP. Genetic variability and information content of a SNP increases as MAF approaches 0.50 (i.e. the maximum value) and MAF is typically among the criteria for the selection of SNP in commercial panels.

Mitochondrial DNA (mtDNA) the DNA contained in mitochondria, which is widely used in phylogenetic studies because of its variability, lack of recombination and maternal inheritance.

Neighbour joining tree (NJ) Phylogenetic tree constructed on the basis of a genetic-distance matrix.

Nucleotide Any of the four types of molecules that make up the structural units of DNA (and RNA). For DNA, these molecules are adenine, cytosine, guanine and thymine and are often denoted by their first letter (i.e. A, C, G, and T, respectively).

Phylogeny Evolutionary history of a taxonomic group in terms of successive divergence events and mutations or genetic drift.

PLINK Popular and powerful program package for handling and analyzing large SNP datasets.

Polymorphism The presence of at least two different genetic variants or alleles at a given locus.

Primer An oligonucleotide that serves as a starting point for DNA synthesis in PCR. The sequences of the primers define the start and the end of the DNA segment to be amplified and during the PCR reaction bind on the template DNA (often genomic DNA) by complementary base pairing.

Principal component analysis (PCA) A method for analysis of a set of variables, such as allele frequencies, by calculation of a new set of statistically independent coordinates that each correspond to a weighted combination of the original variables in such a way that each coordinate captures as much variation in the original variables as possible. In many data sets, a small number of coordinates may explain a large proportion of the initial variability, thus increasing efficiency. Plotting the distribution of individuals or breeds in a graph of the first two or three coordinates allows for simple visualization of the pattern of diversity.

Quantitative trait locus (QTL) Locus contributing to the variation in a multigenic quantitative trait (such as milk production or carcass weight).

Read the sequence of an individual segment of chromosomal DNA that is obtained through a whole-genome sequencing procedure that involves sequencing and subsequent assembly of segments.

Resequencing Whole-genome sequencing approach assembled by mapping the reads to the genome sequence of a reference genome of the same species.

Runs of homozygosity (ROH) Stretches of DNA, typically of 1 to 15 Mbp, that are (almost) completely homozygous.

Scaffold Non-contiguous combination of contigs that are positioned related to each other on the bases of paired ends, mate pairs, optical mapping or other long-range assembly method.

Selection signature Locus with a deviating pattern of diversity, such as a local high fixation index value in comparison of breeds or a high frequency of a homozygous haplotype within a breed, that can be explained by selection acting on the locus

Sex chromosomes Chromosomes for which the number of copies per cell (in diploid cells normally two) depends on the sex, such as the mammalian X chromosome (two in females, one on males), the

mammalian Y chromosome (one in males, zero in females), the avian W chromosome (zero in males, one in females) and the avian Z chromosome (two in males, one in females).

Single nucleotide polymorphism (SNP) variation resulting from a point mutation and most often corresponding to a biallelic (having two different alleles) marker.

Structural variation DNA sequence variation based on copy number variations (CNV: deletions, duplications and large-scale copy number variants) and on insertions, inversions and translocations.

Taqman assay PCR SNP assay during which two probes each specific for one of the SNP alleles and each carrying two fluorescent groups, bind during amplification to one of the strands of the PCR product depending on the alleles. Any bound probe is degraded by the exonuclease activity of *Taq* polymerase. In the intact probes, one of the fluorescent groups quenches the other. This is lifted by the degradation, which generates an allele-dependent fluorescent signal.

Appendix 2

Example¹¹ Material Transfer Agreement

MATERIAL TRANSFER AGREEMENT (MTA)
for genetic material for genotyping

This Material Transfer Agreement is made by and between,

Name of provider of genetic material ("Provider")

Mailing Address _____

Other contact information – i.e. telephone and fax numbers, email address

and

Name of recipient of genetic material ("Recipient").

Mailing Address _____

Other contact information – i.e. telephone and fax numbers, email address

The parties have agreed as follows:

1. Provider agrees to transfer to Recipient the following (biological) material ("Material"):

Description of the genetic material including type (e.g. DNA, blood, tissue) amount (i.e. number of samples) and other information (e.g. means of preservation)

2. This Material will be used by Recipient solely in connection with the project described as follows:

Description of the project ("Research Project"), including assays to be performed (e.g. genomic characterization using a panel of SNPs), use of the data, context in a larger project and project sponsors.

3. This Material will only be used for research purposes by the Recipient in its laboratory. By requesting the material and signing this agreement, the Recipient is considered responsible for appropriate handling of the material and guarantees that suitable containment conditions are available and will be applied in the Recipient's laboratory. This Material will not be used for commercial purposes, such as production or sale of products or services. Recipient will promptly, after termination of the Research Project, inform Provider of the results of the Research Project.
4. To the extent permitted by law, Recipient agrees to treat in confidence, for a period of XXXX years from the date of its disclosure, any of the Provider's written information about this Material that is stamped "CONFIDENTIAL" (hereinafter "Confidential Information"), except

¹¹ Example kindly provided by the International Livestock Research Institute, Kenya, Nairobi.

for information that was previously known to Recipient or that is or becomes publicly available through no fault of Recipient or which is lawfully disclosed to Recipient without a confidentiality obligation or that is independently developed by Recipient or its affiliated entities without the benefit of any disclosure by Provider. Recipient may publish or otherwise publicly disclose the results of the Research Project, provided that in all such oral presentations or written publications concerning the Research Project, Recipient will acknowledge Provider's contribution of this Material unless otherwise requested by Provider.

5. This Material is considered proprietary to Provider. Recipient therefore agrees to retain control over this Material, and further agrees not to transfer the Material to other parties not under its supervision without prior written consent of Provider. Provider reserves the right to distribute the Material to others and to use it for its own purposes. When the Research Project is completed, the Material will be disposed of as mutually agreed upon by Provider and Recipient.
6. This Material IS BEING SUPPLIED TO RECIPIENT WITH NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Provider makes no representations that the use of the Material will not infringe any patent or proprietary rights of third parties. Recipient agrees to hold harmless and indemnify Provider for all liabilities, demands, damages, expenses and losses arising out of or as a result of Recipient's use of the Material for any purpose.
7. Nothing in this Material Transfer Agreement shall or may be construed as granting Recipient any right or license to the Material for any use other or further than the evaluation described here above.
8. This Agreement shall be governed and construed in accordance with the laws of (*the country where the 'Research Project' was conducted*). All disputes arising out of or in connection with this Agreement shall be settled in first instance by the relevant court of (*the country where the 'Research Project' was conducted*).

RECIPIENT

Place:

Date:

PROVIDER

Place:

Date:

By:

Title:

By:

Title:

Appendix 3

Sampling of blood for DNA

Description

This protocol describes how the collection of blood from the jugular vein into a vacutainer EDTA containing tubes should be organized. The actual sampling should be done by a veterinarian or someone with comparable qualification valid in the country where the sampling is done.

Personnel

Qualified sampler (such as a veterinarian), and ideally at least two assistants (one to hold the animal and second to assist with blood sampling and recording)

Non-veterinary equipment

Labelled tubes for blood samples

Marker pen with permanent ink

Box or other container to collect the samples

Notebook, a pen and a permanent marker for documentation

Digital camera

To be done by the assistance

- Obey strictly instructions of the owner or his representative with regard to hygienic precautions and allowable distance to the animals.
- Photograph the animal, especially relevant morphological traits and, if present, the labels with registration number.
- After receiving the tube, gently invert the tube 4-5 times to mix blood with EDTA, and use the marker pen to write the animal ID, breed and date on the tube. Keep the tube at ambient temperature, but do not expose to the sun or other strong light. Preferably, extract DNA within a few days.

Remember to collect as much information about the animal as possible. Owner of the animal, Animal ID, breed, sampling site and date, age of the animal, animal origin and pedigree information as well as known, short description of appearance, major diseases (or lack of them) or other observations by the owner (see Appendix 4).

Appendix 4**Example questionnaire to be filled during sampling**

Questions in **bold** are generally considered to be mandatory)

Animal code _____

Farm ID _____

Species code _____

AA = *Anser anser domesiticus* (greylag goose)

AC = *Anser cygnoides* (swan goose)

AP = *Anas platyrhynchos* (mallard duck)

BB = *Bubalus bubalis* (water buffalo)

BF = *Bos frontalis* (gayal)

BG = *Bos grunniens* (yak)

BI = *Bos indicus* (zebu)

BJ = *Bos javanicus* (banteng, Bali cattle)

BT = *Bos taurus* (taurine cattle)

CB = *Camelus bactrianus* (Bactrian camel, two-humped)

CD = *Camelus dromedarius* (dromedary, one-humped camel)

CH = *Capra hircus* (goat)

CM = *Cairina moschata* (muscovy duck)

EA = *Equus asinus* (donkey)

EC = *Equus caballus* (horse)

GG = *Gallus gallus* (chicken)

LG = *Lama glama* (Llama)

MG = *Melea gallopavo* (turkey)

OA = *Ovis aries* (sheep)

SS = *Sus Scrofa* (pig)

VP = *Vicugna pacos* (alpaca)

VV = *Vicugna vicugna* (vicuña)

Species name _____

Country _____

Number of the sample _____

Official Animal ID (if available) _____

Animal and sampling information**Sex of animal:** female male **Year of birth of the animal:** _____(YYYY)**Place (locality) of birth of the animal:** _____**Date of collection:** _____ (DD.MM.YYYY)**Breed's full name:** _____**Collector's name:** _____**Collector's institution:** _____Address of the farm and telephone number (if available)**Country of the farm:** _____**Province/county of the farm:** _____**Region of the farm:** _____**Closest town to the farm:** _____

International phone code: _____(4 digits - ex: 0033, 0041, ...)

Area phone code: _____

Phone number: _____

Type of biological material: blood tissue hair other (specify)

GPS coordinates _____

Appendix 5

Breed questionnaire

(To be completed once per breed – complete all questions that are relevant and for which information is available)

COMMON NAME OF BREED: _____

NAME OF SPECIES: _____

Transboundary or brand name			
Local breed name			
Main location			
Breed society?	Circle: Yes No	Year established:	
Description of origin and development			
Population size	Year:	N° of animals:	
N° of reproductive animals	Males in natural service		
	Males used for AI		
	Breeding females		
	Trend in breeding females	Increase <input type="checkbox"/>	Steady <input type="checkbox"/>
Females mated pure (%)			
Adult size (male/female)	Withers height (cm)	M	F
	Live weight (kg)		
N° of farmer/breeders			
Main uses (e.g. meat, milk)			
Typical management conditions	Type		
	Housing		
	Feeding		
Conservation activities	In situ: Y / N Ex situ: Y / N Cryo: Y / N		
Performance comparison	Relative to which breed:		
much higher in:	(e.g. milk yield)		
higher in:			
equal in:			
lower in:			
much lower in:			

Appendix 6

Software for genomic analysis

This is a (incomplete) list of genetic analysis programs with a short specification, grouped according to purpose. Although the software are grouped, many of them can be applied to multiple purposes. Most programs can be downloaded freely from the internet, along with detailed instruction manuals. If not given in this list, details, literature references and URL of programs can be found by search engines. Appearance on this list does not constitute an endorsement of the software by the Food and Agriculture Organization of the United Nations.

Software for managing and/or analyzing large SNP datasets

AlphaPeel software package for calling, phasing, and imputing genotype and sequence data in pedigree populations. <https://github.com/AlphaGenes/alphapeel>

AlphaFamImpute genotype calling, phasing, and imputation algorithm for large full-sib families in diploid plants and animals which supports individuals genotyped with SNP array or GBS data <https://github.com/AlphaGenes/alphafamimpute>

ANGSD utility program for low to medium coverage WGS data <http://www.popgen.dk/angsd/index.php/ANGSD>

diveRsity R package (<http://www.r-project.org>) that calculates a range of genetic diversity statistics. <https://cran.r-project.org/web/packages/diveRsity/index.html>

KING v 2.2.6 toolset to explore family relationship inference and pedigree error checking, quality control, and population substructure identification in genome-wide data. <https://people.virginia.edu/~wc9c/KING/>

PLINK 1.8 & 2.0 Clearly documented high-density SNP handling and analysis program. Requires special format. Outputs to formats compatible with many other applications. Performs a variety of data handling operations and calculations, such as allele-sharing between individuals and coordination analysis. <https://www.cog-genomics.org/plink>

Software for handling genomic data

Samtools suite of programs for working with high-throughput sequencing data, particularly data stored in SAM format files. <http://www.htslib.org>

VCFtools utility program for working with VCF files (Variant call format, a common system for storing of genetic variation information) <https://vcftools.github.io/index.html>

Genotype by sequencing tools

DECONVQC repository that contains scripts used to process and manage output sequence data and metadata related to Illumina hiseq and miseq machines, including running a number of GBS-specific Q/C steps for predominantly GBS-related sequencing output. This project is focused on immediate upstream Q/C and sequence delivery, rather than custom downstream analyses. <https://github.com/AgResearch/DECONVQC>

KGD kinship (genetic relatedness) using GBS (genotyping-by-sequencing) with depth adjustment. <https://github.com/AgResearch/KGD>

TASSEL software package used to evaluate traits associations, evolutionary patterns, and linkage disequilibrium. – it can work with genotyping-by-sequencing data. <https://www.maizegenetics.net/tassel>

See also a range of software described in tutorials for analysis of low-coverage whole genome sequencing data. <https://github.com/nt246/lcwgs-guide-tutorial>

Phasing and imputation

AlphaImpute software that combines heuristics and HMM to perform both family-based and population-based imputation. Requires phased data, but presents the AlphaPhase algorithm embedded into the software for phasing. <https://alphagenes.roslin.ed.ac.uk/wp/software-2/alphaimpute/>

AlphaPhase software that performs genotype phasing and minor imputation through a heuristic algorithm that employs the concepts of surrogate parents and Erdős distance. <https://alphagenes.roslin.ed.ac.uk/wp/software-2/alphaphase/>

Beagle v4.1 (current v5.1 does not handle genotype likelihood input from sequencing) Pedigree-free imputation of genotyping-by-sequencing data. https://faculty.washington.edu/browning/beagle/b4_1.html

Eagle highly efficient and fast genotype phasing and minor imputation software that uses an HMM-based algorithm combined with a positional Burrows-Wheeler transform. <https://alkesgroup.broadinstitute.org/Eagle/>

fastPhase performs genotype phasing and minor imputation using HMM fitted via an EM algorithm. <http://scheet.org/software.html>

FImpute implements a deterministic algorithm that performs both family-based and population-based imputation. Does not require phased data. <https://animalbiosciences.uoguelph.ca/~msargol/fimpute/>

GLIMPSE phasing and imputation method for large-scale low-coverage sequencing studies <https://odelaneau.github.io/GLIMPSE>.

IMPUTE fast population-based imputation through HMM and positional Burrows-Wheeler transform. Requires phased data. <https://jmarchini.org/impute5/>

Minimac population-based imputation using HMM with state space reduction. Requires phased data. <https://genome.sph.umich.edu/wiki/Minimac4>

SHAPEIT fast genotype phasing and minor imputation via HMM and positional Burrows-Wheeler transform. <https://odelaneau.github.io/shapeit4/>

STITCH R package for Sequencing To Imputation Through Constructing Haplotypes <https://github.com/rwdavies/STITCH>

Genetic distances, trees and plots

Beast performs Bayesian MCMC analysis of molecular sequences, inferring rooted, time-measured phylogenies using strict or relaxed molecular clock models. Provides a framework for testing evolutionary hypotheses without conditioning on a single tree topology.

http://beast.bio.ed.ac.uk/Main_Page

Mega calculation of a wide variety of population genetics statistics and convenient tree reconstruction program by the most common algorithms except the Bayesian method. <http://www.megasoftware.net/>

MrBayes command-line operated for handling nexus sequence files for Bayesian tree reconstructions. <http:// mrbayes.csit.fsu.edu/>

neighborNet R package for construction of phylogenetic networks based on the Neighbor-Joining algorithm. <https://www.rdocumentation.org/packages/phangorn/versions/2.5.5/topics/neighborNet>

Network constructs Median-joining networks of haplotype data. Generates evolutionary trees and networks from genetic and other data. <http://www.fluxus-engineering.com/sharenet.htm>

Netview R package for network visualization of individuals of breeds using maximally k nearest neighbors <https://github.com/esteinig/netview>

Paup Classical command-line operated comprehensive package handling nexus files for tree reconstruction according to the most common algorithms (not cost free).

<http://paup.csit.fsu.edu/>

Phylip classical command-line comprehensive package requiring its own file format for tree reconstruction according to the most common algorithms, but offering fewer options than PAUP.

<http://phylip.com>

QPTools package within **AdmixTools** <https://github.com/DReichLab/AdmixTools> (or <https://github.com/uqrmaie1/admixtools>) and **ADMIXTUREGRAPH** an R package https://github.com/mailund/admixture_graph optimize the branch lengths and admixture proportions from Admixture graphs.

Relate estimates genome-wide genealogies in the form of trees, based on inferred haplotypes.

<https://myersgroup.github.io/relate/>

SNAPP uses a likelihood-based approach to estimate the “true” phylogenetic tree of populations based on the genetic variation of markers across the genome. Built upon the Beast software.

<https://www.beast2.org/snapp>

SplitsTree constructs neighbor-joining tree, SplitsTree graphs and NeighborNet graphs. Accepts Nexus files. Many graphical output options.

<http://www-ab.informatik.uni-tuebingen.de/software/splitstree4/welcome.html>

Treemix constructs maximum-likelihood trees based on the covariance of allelic frequencies. Attempt to infer the number of admixture events among the populations.

<https://speciationgenomics.github.io/Treemix>

Tsinfer develops a tree sequence based on inferred haplotypes.

<https://github.com/tskit-dev/tsinfer>

Principal component analysis

Eigensoft Analyses population structure by combining statistical genetics with principal components analysis (**Eigenstrat**) to explicitly model ancestry differences between cases and controls along continuous axes of variation <http://genepath.med.harvard.edu/~reich/Software.htm>

Population assignment and cluster analysis

Admixture Produces results for unsupervised clustering (i.e., without prior population information) comparable or identical to **Structure** but faster by means of a more efficient algorithm. <http://www.genetics.ucla.edu/software/admixture/>

Baps comparable to **Structure** but with increased flexibility in the definitions of levels at which genetic structure may exist. <http://web.abo.fi/fak/mnf/mate/jc/software/baps.html>

Clumpp accepts the output of **Structure** or other clustering programs in order to align the output of different runs. <http://rosenberglab.bioinformatics.med.umich.edu/clumpp.html>

Distrupt reads in tables of genomic components from the **Structure** output and files of options set by the user in order to provide graphical output of the **Structure** clustering. <http://rosenberglab.bioinformatics.med.umich.edu/clumpp.html>

fineStructure clustering program on the basis of ChromoPaint-based haplotype sharing https://people.maths.bris.ac.uk/~madjl/finestructure/finestructure_info.html

Frappe estimates individual ancestry proportions - comparable to **Admixture** <https://med.stanford.edu/tanglab/software/frappe.html>

Geneland clustering program that can make use of both geographic and genetic informations to estimate the number of populations in a dataset and delineate their spatial organisation. <http://www2.imm.dtu.dk/~gigu/Geneland/>

Instruct joint inference of population structure and inbreeding rates, eliminating the assumption of Hardy-Weinberg equilibrium and especially applicable in cases of self-fertilization or inbreeding. <http://cbsuapps.tc.cornell.edu/InStruct.aspx>

LEA R package - estimates admixture proportions similar to **Structure** or **Admixture** software, but with reduced computation time. Less affected by the inbreeding of the analysed populations. <http://membres-timc.imag.fr/Olivier.Francois/LEA/software.htm>

Spaida and Spaing assign individual animals to genetic clusters based on spatial autocorrelations. <http://notendur.hi.is/~snaebj/programs.html>

Structure popular and user-friendly program for an informative visualization of patterns of diversity. Reconstructs model-based subdivision of individual genotypes into a user-specified number of clusters (k) by optimizing of Hardy-Weinberg equilibrium and minimizing linkage disequilibrium within clusters; estimates proportion of individual genomes derived from the inferred clusters. Clusters may correspond to ancestral components, but also to a relatively homogeneous breed or group of breeds. Optionally allows for admixture, linkage between markers, codominant markers, incorporation of prior population information and incorporation of spatial information. It is substantially slower than **Admixture** but has more options. <http://pritch.bsd.uchicago.edu/structure.html>

Whichrun assigns individuals to populations based upon maximum likelihood theory
<http://www-bml.ucdavis.edu/whichrun.htm>

Identification of selection signatures

cgaTOH detection of intra-population selection signatures based on runs of homozygosity.
<https://github.com/hernanmd/cgaTOH.2018>

HapFLK implements the the Lewontin and Krankakauer tests to identify inter-population signatures based on individual loci (FLK) or haplotypes (hapFLK). <https://pypi.org/project/hapflk/>

HierFstat R package for detection of inter-population selection signatures based on fixation indexes (F_{ST}). <https://cran.r-project.org/web/packages/hierfstat/index.html>

Sweed identification of intra-population selection signatures based on the site frequency spectrum
<https://cme.h-its.org/exelixis/web/software/sweed/>

Sweep large-scale intra-population analysis of haplotype structure in genomes for the primary purpose of detecting evidence of natural selection. <https://software.broadinstitute.org/mpg/sweep/>

Selscan detection of intra-population selection based on extended haplotype homozygosity (EHH) and integrated haplotype score (iHS) methods. Detection of inter-population selection based on cross population extended haplotype homozygosity (XP-EHH) <https://github.com/szpiech/selscan>

Landscape genomics

LFMM2 applies latent factor mixed models to test for association between genotypes and explanatory variables that can include environmental or geographical measures.
<https://bcm-uga.github.io/lfmm/>

BAYENV applies Bayesian method to estimate the pattern of covariance in allele frequencies between populations and then uses this as a null model for a test of environmental variables at individual SNPs.
https://bitbucket.org/tguenther/bayenv2_public/

BAYPASS identifies genetic markers subjected to selection or associated with population-specific covariates, including geographical and environmental variables.
<http://www1.montpellier.inra.fr/CBGP/software/baypass/index.html>

BAYESCENV performs F_{ST} -based genome scans to detect local adaptation.
<https://github.com/devillemereuil/bayescenv>

R.SamBada R-package providing a pipeline for landscape genomic analysis, spanning from the retrieval of environmental variables at sampling locations to gene annotation using the Ensembl genome browser. This application standardizes the landscape genomics pipeline, eases the search for candidate genes involved in adaptation processes, and enhances reproducibility of the studies.
<https://github.com/SolangeD/R.SamBada>

Genome-wide association studies

bayesR Bayesian hierarchical model for complex trait analysis, including GWAS.
<https://github.com/syntheke/bayesR>

BOLT applies linear mixed-models for genetic association testing. Includes an application that applies an algorithm for restricted maximum-likelihood estimation of variance components. <https://alkesgroup.broadinstitute.org/BOLT-LMM/>

EMMAX performs large-scale GWAS while correcting for population sampling structure. <https://genome.sph.umich.edu/wiki/EMMAX>

FaST-LMM genome-wide association testing, genomic prediction, heritability analysis. <https://github.com/fastlmm/FaST-LMM/>

GCTA estimates genomic relationships, performs association testing, heritability analysis and variance partitioning. <https://cnsgenomics.com/software/gcta>

GEMMA applies linear mixed models for association testing, multi-trait analyses, heritability analysis. <https://github.com/genetics-statistics/GEMMA>

Special purpose programs

AlphaAssign parentage assignment algorithm that works with SNP array and GBS data <https://github.com/AlphaGenes/alphaassign>

Rannala software multiple packages that perform various specific functions, including LD mapping, data simulation, and detecting migration by using multilocus genotypes. <http://www.rannala.org>

SNeP performs estimations of effective population size trajectories over time by using genome-wide SNP data. <https://sourceforge.net/projects/snepnetrends>

SPAGeDi characterizes the spatial genetic structure of individuals or populations based on genetic marker data. Estimates genetic distance and other basic statistics. <http://ebe.ulb.ac.be/ebe/Software.html>

Simulation, modelling and parameter estimation

ABC and **ABCRCF** R packages that perform approximate Bayesian computation (ABC) for model selection and parameter inference, the latter by utilizing random forests <https://cran.r-project.org/package=abc> and <https://rdr.io/cran/abcrf>

dadi simulates the joint frequency spectrum of genetic variation among several populations. <https://dadi.readthedocs.io/en/latest/>

FastSimcoal2 flexible modelling and simulation software that allows comparison of alternative tree construction models. <http://cmpg.unibe.ch/software/fastsimcoal2/>

PSMC <https://github.com/lh3/psmc> and **MSMC** <https://github.com/stschiff/msmc> and R packages for implementing the pairwise sequentially Markov coalescent and multiple sequentially Markov coalescent methods for modelling population histories.

Multipurpose programs originally developed for microsatellite data

Convert easy program that converts data from an Excel format to files suitable for other software (may not be able to handle large SNP files)

<http://www.agriculture.purdue.edu/fnr/html/faculty/Rhodes/Students%20and%20Staff/glaubitz/software.htm>

GenAlEx estimates of variability based on allele and genotypic frequencies, genetic distances, Principal Component Analysis, Formatting of data for other software. Runs as a Microsoft Excel addin. <http://www.anu.edu.au/BoZo/GenAlEx/>

Microsatellite Toolkit convenient Excel microsatellite data handling tool. Format requires sample names in which letters indicate breed and numerals the individual; easily transformed to Structure format. Exports to Microsat, Arlequin, GenePop and Fstat formats. Check errors in the dataset (missing figures, large gaps between alleles, non-unique sample labels, duplicate samples), converts two column per marker and one line per sample to one column per marker and two lines per individual). Provides summary statistics (observed and expected heterozygosity, number of alleles) and allele frequencies. <http://www.animalgenomics.ucd.ie/sdepark/ms-toolkit/>

Appendix 7

Genotyping arrays available across different genotyping platforms for various livestock species¹²

Platform	Array Name	Species	Marker density	SNPs (N)	SNP ascertainment and/or validation breeds/populations	Special features/potential applications
Affymetrix Axiom	Bos1 Array	Cattle	High	648 855	20 breeds; Bos taurus (Afrikander, Angus, Ayrshire, Boran, Blonde d'Aquitaine, Brown Swiss, Simmental, Hanwoo, Hereford, Holstein, Japanese Black, Jersey, Limousine, Norwegian Red, Rouge des Pres, Romagnola, Tuli); Bos indicus (Brahman, Nelore, Gir).	Useful for GWAS, genetic evaluations, biodiversity research and linkage disequilibrium studies
	BovMDv3 Array	Cattle	Medium	63 648	20 breeds; Bos taurus (Afrikander, Angus, Ayrshire, Boran, Blonde d'Aquitaine, Brown Swiss, Simmental, Hanwoo, Hereford, Holstein, Japanese Black, Jersey, Limousine, Norwegian Red, Rouge des Pres, Romagnola, Tuli); Bos indicus (Brahman, Nelore, Gir).	Useful for dairy evaluation (genomic selection), biodiversity research, copy number variations, parentage verification, markers for deleterious recessive traits, fertility traits and traceability; Consists of 13K Bos indicus SNPs; Includes several hundred SNPs that are optimized for short tandem repeat (STR) imputation
	Buffalo Genotyping Array	Buffalo	Medium	89 988	4 breeds (Mediterranean buffalo, Murrah, Jaffarabadi and Nili-Ravi)	Useful for biodiversity research, GWAS
	ChickenHD Genotyping Array	Chicken	High	580 961	Commercial broiler (Aviagen), commercial layers (Hyline, Synbreed), Experimental inbred (IAH line), Non-selected (J. Line Roslin)	Useful for genetic evaluation of layers and broilers, GWAS, mapping and biodiversity research; Includes markers associated with wild outbred lines

¹² Appearance on this list does not constitute an endorsement of the platform or array by the Food and Agriculture Organization of the United Nations.

Platform	Array Name	Species	Marker density	SNPs (N)	SNP ascertainment and/or validation breeds/populations	Special features/potential applications
Affymetrix Axiom	EquineHD Genotyping Array	Horse	High	670 796	32 breeds (Including Arabian, Belgian, Black Forest, Duellmener, Edlblu-Haflinger, French Trotter, Haflinger, Hanoverian, Icelandic, Lusitano, Marremanno, Mongolian, Morgan, Old-Oldenburger, Quarter Horse, Sorraia, Standardbred, SueddeutschesKatblut, Thoroughbred and Welsh)	Useful for biodiversity research, GWAS, association mapping, genomic prediction of disease risk
	PorcineHD Genotyping Array	Pig	High	658 692	27 domestic and 16 wild boar populations; Commercial domestic European (Large White, Landrace, Pietrain, Duroc, Hampshire); Traditional/Rare domestic European (Angler Sattelschwein, British Saddleback, Bunte Bentheimer, Casertana, Cinta Senese, Gloucester Old Spot, Large Black, Linderodssvin, Mangalica, Middle Wite, Negro Iberico, Retinto, Tamoworth); European wild boar; Asian domestic (Jiangquhai, Jinhua, Leping Spotted, Meihan, Thai Native, Wannan Spotted, Wuzishan, Xiang, Zang); Asian wild boar (China, Japan, Thailand)	Useful for biodiversity research, GWAS, high resolution genetic mapping, QTL analysis, genomic prediction
	Porcine-Breeder Array	Pig	Medium	55 150	Same as Axiom-PorcineHD Genotyping Array, but SNP selection was targeted towards commercial pig breeds	Useful for genomic and trait selection, Parentage testing; Includes 64 ISAG core parentage markers, 42 trait-specific markers selected by USDA and targeted for commercial breeds including Large White, Landrace, Duroc and Pietrain

Platform	Array Name	Species	Marker density	SNPs (N)	SNP ascertainment and/or validation breeds/populations	Special features/potential applications
Affymetrix Axiom	Ovine50K-Genotyping Array	Sheep	Medium	50 000	74 breeds sampled from Asian, African, South-West Asian, Caribbean, North American, South American, European and Australasian sheep	Useful for biodiversity research, GWAS, parentage verification, breed assignment, aneuploidy detection, mating designs, traceability.
	OvineLD-Genotyping Array	Sheep	Low	11 196	Belclare, Charollais, Suffolk, Texel, Vendeen	Useful for biodiversity research, GWAS, parentage verification, breed assignment, aneuploidy detection, mating designs, traceability.
	Bovine	Cattle	Medium	54 560	10 breeds (Holstein, Angus, Jersey, Fleckvieh, Hereford, Limousin, Romagnola, Brahman, Nelore, Gir)	Useful for biodiversity research, GWAS, parentage verification; Consists of 191 ISAG core markers for bovine parentage
	Ovicap Mutispecies Array	Sheep	Medium	54 236	74 breeds sampled from Asian, African, South-West Asian, Caribbean, North American, South American, European and Australasian sheep	Useful for biodiversity research, GWAS, mapping studies
		Goat	Medium	60 034	Milk and mixed breeds (Alpine, Saanen and Creole); Meat breeds (Boer, Katjang and Savanna); Norwegian dairy goat	Useful for biodiversity research, GWAS, parentage verification, mapping studies; Includes 583 markers for parentage testing and traceability
	Camelid-Multispecies Array	Dromedary	Medium	59 958	Dromedary populations from Algeria, Ethiopia, Mauritania, Morocco, Sudan, UAE	Useful for biodiversity research, GWAS, mapping studies
		Bactrian	Medium	59 938	Bactrian populations from Mongolia China	Useful for biodiversity research, GWAS, mapping studies
		New World Camelids	Medium	60 000	Alpaca (Huacaya and Suri), Llama and Vicugna populations from Peru, Chile, Bolivia, Ecuador	Useful for biodiversity research, GWAS, mapping studies

Platform	Array Name	Species	Marker density	SNPs (N)	SNP ascertainment and/or validation breeds/populations	Special features/potential applications
Illumina Infinium	BovineHD-Genotyping-Beadchip	Cattle	High	777 962	20 breeds; Bos taurus (Angus, Bonde d'Aquitaine, Brown Swiss, Charolais, Guernsey, Hereford, Holstein, Jersey, Lagunaire, Limousin, Montbeliad, N'dama, orwegian Red, Piedontese, Red Angus, Romagnola, Senepol, Simental, Wagyu); Bos indicus (Brahma, Gir, Nelore); Crossbreeds (Beefmaster, Brangus, Santa Gertrudis, Sheko)	Useful for genomic selection, GWAS, QTL identification, biodiversity research, Mapping
	Bovine SNP50v3-Beadchip	Cattle	Medium	53 218	17 breeds (Angus, Beefmaster, Gir, Nelore, Brahman, Charolais, Guernsey, Hereford, Holstein, Jersey, Limousin, N'dama, Piedontese, Red Angus, Romagnola, Santa Getrudis, Sheko)	Useful for genomic selection, GWAS, Parentage verification, biodiversity research; Includes 116 parentage markers
	Bovine LD v2.0-Genotyping Beadchip	Cattle	Low	7 931	26 breeds (Angus, Ayrshire, Beefmaster, Blonde d' Aquitaine, Brahman, Brown Swiss, Charolais, Fleckvieh, Friesian, Gelbvieh, Guernsey, Hereford, Holstein, Jersey (US & Denmark), Limousin, Montbeliard, N'dama, Normande, Norwegian Red, Red Angus, Red Dairy (Angier), Red Danish (Denmark, Finland, Sweden), Santa Gertrudis)	Useful for genomic selection, Genotype Imputation, Parentage verification, biodiversity research; Includes 121 parentage markers
	Goat SNP50-Genotyping-Beadchip	Goat	Medium	50 000	Saanen, Alpine, Creole, Boer, Kacang, and Savanna	Useful for biodiversity research, GWAS, genome mapping

Platform	Array Name	Species	Marker density	SNPs (N)	SNP ascertainment and/or validation breeds/populations	Special features/potential applications
Illumina Infinium	Ovine SNP50-Genotyping-Beadchip	Sheep	Medium	54 241	>70 breeds sampled from Asian, African, South-West Asian, Carribean, North American, South American, European and Australasian sheep	Useful for biodiversity research, GWAS, genomic selection, genetic evaluation, linkage disequilibrium studies
	Porcine SNP60 v2 Genotyping Beadchip	Pig	Medium	64 232	Berkshire, Duroc, Hampshire, Landrace, Large White, Meishan, Pietrain, Synthetic (Large White and Pietrain), Wild boar	Useful for biodiversity research, GWAS, genomic selection, identification of QTL, mapping, genetic evaluation, linkage disequilibrium studies
	Equine SNP50	Horse	Medium	54 602	15 breeds (Anadalousian, Arabian, Belgian, Franches Montagnes, French Trotter, Hanoverians, Icelandic, Mongolian, Norwegian Fjord, Quarter Horse, Saddlebred, Standardbred, Swiss Warmblood, Thoroughbred, Hokkaido)	Useful for biodiversity research, GWAS, genomic selection, identification of QTL, mapping.
	GGP Bovine 100K	Cattle	Cattle	100 000	Derived from the original Illumina BovineHD-Genotyping and Bovine SNP50 Beadchip	Useful for GWAS, genetic evaluations, biodiversity research and parentage verification; Has 85% overlap with the Council of Dairy Cattle Breeding (CDCB) virtual evaluation and 35000 SNPs overlap with the Canadian Dairy Network (CDN) Evaluation; Average imputation accuracy to the Illumina Bovine HD is >99.5% in Angus and Holstein populations
GeneSeek Genomic Profiler (Illumina-Infinium)	GGP Bovine 150K	Cattle	Cattle	150 000	NA	Useful for GWAS, genetic evaluations, biodiversity research, parentage, detection of markers for recessive disorders including fetal death and abnormalities that interfere with growth rate in cattle

Platform	Array Name	Species	Marker density	SNPs (N)	SNP ascertainment and/or validation breeds/populations	Special features/potential applications
GeneSeek Genomic Profiler (Illumina-Infinium)	GGP Indicus	Zebu	Medium	35 090	Brahman, Guzera, Gyr, Nelore, Droughtmaster, Santa Gertrudis, Tropical Composite, etc.	Useful for biodiversity research, GWAS, QTL identification
	GGP Bovine-F250	Cattle	High	235 000	NA	Useful for functional genomic studies in cattle, genomic selection, genotype imputation. Includes SNPs enriched for functional variants such as non-synonymous, frameshift and premature stop codons.
	GGP Angus-GS-Beadchip	Cattle	Medium	75 000	Angus	Breed specific SNP array for breeding and improvement of Angus cattle. Includes 22000 new SNP markers with 7800 from Angus sequences that are novel to all available arrays. Also includes markers associated with fertility, feed efficiency, marbling, calving ease and vaccine response. Has markers for detection of genetic conditions like arthrogyrosis multiplex, neuropathic hydrocephalus contractural arachnodactyly, osteoporosis, oculocutaneous hypopigmentation, developmental duplication, BVDV, M1, D2.

Platform	Array Name	Species	Marker density	SNPs (N)	SNP ascertainment and/or validation breeds/populations	Special features/potential applications
GeneSeek Genomic Profiler (Illumina-Infinium)	GGP Ovine50K	Sheep	Medium	50 000	NA	Useful for biodiversity research, GWAS, genomic selection, identification of QTL, genetic evaluation, linkage disequilibrium studies
	GGP Porcine50K	Pig	Medium	51 000		Useful for biodiversity research, GWAS, parentage verification, evaluation of pure line, identification of multi-line reference populations, genetic evaluation; Includes markers for detection of Porcine Stress syndrome and Rendement Napole (RN).
	GGP Porcine80K	Pig	Medium	~80 000	NA	Useful for biodiversity research, GWAS, parentage verification, evaluation of pure line, identification of multi-line reference populations, genetic evaluation
	GGP Equine	Horse	Medium	71 947	Derived from Illumina's Equine SNP50 Beadchip	Useful for biodiversity research, GWAS, genomic selection, identification of QTL, general mapping