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ANALYZING THE GENETIC DIVERSITY IN WALLOON AND EUROPEAN PIÉTRAIN PIG POPULATIONS USING PSEUDO-PHENOTYPES, PEDIGREE AND SNP MARKER DATA

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Résumé

Cette étude a pour objectifs de déterminer l'origine du porc Piétrain, d'estimer la diversité des Piétrains wallons et d'analyser la diversité génétique existante chez différentes populations européennes de Piétrain. A cette fin, le pedigree et les pseudo-phénotypes (i.e. les valeurs d'élevage dérégressées) de la population wallonne et les génotypes de différentes populations européennes ont été étudiées. Le positionnement multi-dimensionnel, basé sur les génotypes, n'a pas permis de déduire l'origine des Piétrains avec certitude. Cependant, cette analyse suggère l'implication de différentes races locales anglaises (e.g. le Berkshire) et de différentes races locales tachetées. Ensuite, différents paramètres basés sur le pedigree de verrats dont la descendance a été testée en station ont été analysés pour avoir un aperçu de la diversité génétique de la population wallonne. Le coefficient de consanguinité moyen a été estimé à 2,74%, la taille effective de la population à 223 et le paramètre de diversité génétique à 97,96%. Ces paramètres indiquent que la diversité génétique de la population wallonne semble relativement préservée. Les flux génétiques, peu fréquents entre fermes, ont également été étudiés grâce à un positionnement multi-dimensionnel basé sur l'opposé des coefficients de kinship. Une Analyse en Composante Principale basée sur les pseudo-phénotypes a donné une indication de la trajectoire de la population via les objectifs de sélection actuels. En effet, ceux-ci sont orientés soit vers les traits de croissance, soit vers les traits viandeux. Il peut donc être suggéré au programme Belgian Piétrain, basé sur la cryopréservation de la semence des verrats, d'échantillonner de façon représentative les verrats en fonction des diversités génétique et phénotypique estimées dans cette étude. On peut également recommander aux éleveurs de contribuer de façon plus équilibrée au testage en station puisqu'un seul éleveur a envoyé 55% des verrats testés. Finalement, les génotypes de Piétrains européens et américains ont été analysés. Les estimations de la consanguinité et des segments génomiques en homozygotie ont permis de déduire que les populations néerlandaises et américaines, supposées commerciales, étaient plus consanguines et moins variables. Pour éviter une situation de goulot d'étranglement dans le futur, ces populations devraient favoriser les échanges d'animaux.

Abstract

This study aims to infer the origin of the Piétrain breed, to estimate the diversity of the Walloon Piétrain population and to analyze the existing genetic diversity of different European Piétrain populations. For these purposes, pedigree and pseudo-phenotypes (i.e. deregressed estimated breeding values) of the Walloon population and genotypes of several European populations were analyzed. The Multi-Dimensional Scaling (MDS) based on genotypes did not allow to have an exact assumption of the Piétrain breed origin. However, it suggested the involvement of different local English (e.g. Berkshire) and Spotted breeds. To have an insight of the Walloon genetic diversity, different pedigree parameters of boars provided to progeny testing were then analyzed. The average inbreeding coefficient was 2.74%, the effective population size (N_e) was 223 and the genetic diversity parameter was 97.96%. The genetic diversity found in the Walloon population seemed therefore relatively high. Gene flows, relatively uncommon between farms, were also studied by a MDS based on the opposite of kinship coefficients. A Principal Component Analysis (PCA) based on pseudo-phenotypes provided complementary information about breeding objectives as it was found that owners focused on meat or growth traits. It could therefore be suggested to the Belgian Piétrain program, based on the boar's semen cryopreservation, to sample representative boars in the population regarding its genetic and phenotypic diversities. Moreover, as one owner provided 55% of the tested boars, owners should equally contribute to progeny testing. Finally, different European and an American Piétrain populations were analyzed through genotypes. Inbreeding estimations and Runs of Homozygosity (ROHs) stated that Dutch and American populations, supposedly held by commercial firms, were more inbred and uniform. More exchanges of animals should be done in these populations to avoid bottleneck in the future.

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ADG Average daily gain

AFSCA Federal Agency for the Security of the Food Chain

AI Artificial Insemination

AIC Artificial Insemination Center

ASF African Swine Fever

awé Walloon Breeding Association

BLUP Best Linear Unbiased Predictions

CIAP Interprofessional Center for Animal Improvement and Promotion

CRA-W Walloon Center of Agronomic Research

DNA Deoxyribonucleic Acid

EBV Estimated Breeding Value

FAO Food and Agriculture Organization of the United Nations

FBEP Belgian Pig Breeders Federation

FC Feed Consumption

Fhat1 Inbreeding estimation based on the variance-standardized relationship minus 1

Fhat3 Inbreeding estimation emphasizing minor homozygous alleles frequency

F_{st} Fixation index

GEBV Genomic Estimated Breeding Value

GRM Genomic Relationship Matrix

Hal Halothane susceptibility gene

IBD Identity by Descent

IBS Identity by State

ID Identification

IGF2 Gene responsible for huge muscling

kb kilobase

LD Linkage Disequilibrium

MAF Minor Allele Frequency

MDS Multi-Dimensional Scaling

MHS Malignant Hyperthermia Syndrome

N/n Allele responsible for stress resistance/stress susceptibility

N_e Effective population size

OC Optimal Contribution

PCA Principal Component Analysis

PCI Index of Pedigree Completeness

PRRS Porcine Reproductive and Respiratory Syndrome

PSE Pale Soft Exudative

PSS Porcine Stress Syndrome

SD Standard Deviation

ReHal Halothane Resistant

RN^- Allele responsible for acid meat

ROH Region of Homozygosity

SNP Single Nucleotide Polymorphism

WGS Whole Genome Sequencing

General introduction

In the last decades, several challenges arose in the agricultural field such as to produce enough food to meet the demand, or even to minimize the ecological footprint of agriculture. Conservation of genetic diversity in animal breeding is one of the main concerns agriculture must deal with (FAO, 2015). Different reasons justify the need for animal genetic resources preservation: economic, social and cultural, environmental, reduction of any kind of risks or research purposes (FAO, 2013). According to the FAO (2015), only 20% of the mammalian breeds are not at risk of endangerment, whereas 55% are of unknown status. International transboundary breeds are logically less impacted than local ones.

One of these international breeds is Piétrain, which is widely used as a terminal boar to produce finishing pigs. This breed features muscle hypertrophy that leads to high quality carcass traits. It is therefore not expected to require conservation measures. Nevertheless, there is an important paradox in Piétrain meat production. Despite its use as a terminal boar by industry, pure Piétrain animals can be selected in a traditional manner. Walloon Piétrain breeding is not an exception: with approximately ten traditional breeders nowadays, the pure Piétrain pig population seems to go through a bottleneck in Wallonia, the region of its origin. Moreover, Piétrain breeders are getting old and their natural replacement by heirs or even by new breeders is not in line. In this context, it seems hard to avoid inbreeding in the population, even if some breeders have tried to precisely identify animals by line to avoid it.

Some difficulties are currently preventing the survival of pig farms. Pork meat is, in general, not expensive and its price fluctuates. Exportation is another major issue, since it is blocked by sanitary restrictions. The outbreak of African Swine Fever in September 2018 in Belgium frightened pig breeders, and international trades are now quite challenging. Moreover, welfare issues entangle breeders in a stronger way. As Hanset (1992) precisely reported, a genetic particularity can persist and spread only if the economic environment is advantageous and if bans of any kind do not block its progression. As long as economic, political or even pathogen conditions do not support pig production in general, it is important to determine what measures could be implemented in order to protect the Walloon Piétrain breed. Walloon Piétrain pigs are also at risk not only of being overwhelmed by commercial enterprises but also of losing its specificity. Thus, to preserve this breed, the establishment of the genetic diversity of Piétrain pigs in Wallonia is crucial.

Two first steps were initiated to endorse Walloon Piétrain breeding. The Walloon Breeding Association (awé) established the performance progeny testing around ten years ago to determine which Piétrain boars seem to have high genetic merit. Progeny testing contributes to less biased genetic evaluations (Dufrasne, 2014). In 2017, the Belgian Piétrain program, based on cryopreservation of Walloon Piétrain semen, was launched (awé, 2019). Cryopreservation is often chosen in

conservation programs as it is not expensive (Dumasy et al., 2012). Despite both measures taken to ensure the breed conservation, alternative solutions should be implemented.

From this observation come the objectives of this study:

- To investigate the origin of the Piétrain breed;
- To assess the genetic diversity of the Walloon Piétrain population by pedigree and pseudo-phenotypes;
- To come up with solutions for the preservation of the Walloon Piétrain population;
- To investigate the diversity of different European Piétrain populations by using genomic information.

This study is divided into three main chapters. The first chapter consists of literature review of general pig production, the Piétrain breed and the importance of conservation tools to maintain genetic diversity. The second chapter describes the data access and different computation analyses. Lastly, the third chapter focuses on achieved results and how they can be used in terms of genetic diversity preservation for the Piétrain breed.

Chapter I: Literature review

Introduction

This chapter is divided into five main sections. In the first section, we focus on the social and economic context of pig production and sanitary issues, one of the threats for pig holdings. In the second section, we expand on pig domestication and selection processes. We detail the different breeds as a result from these processes and also crossbreeding, mainly used as a management tool to obtain finishing pigs with desired features. This section paves the way for a breed of special interest for this study: the Piétrain breed. This fourth part describes the supposed origin of this breed, its specific features such as great muscling, the factors implied in its decrease in Wallonia, and current available options to conserve this Walloon heritage. Finally, in the last section, other preservation options as well as the concept of genetic diversity are explored.

1. Context
1.1. Social and economic context

In 2017, 111 million tons of pork meat were produced all over the world. The three greatest producers are China, Europe and the USA, with 55, 24 and 11 million tons, respectively. This meat is the most consumed (38%) in the world (Statista, 2019). The three biggest producers are also the three biggest consumers. China, Europe and the USA consume together almost 80% of the total world production (Planetoscope, 2012). Pork meat consumption does not stop increasing. It increased from 82 to 100 million tons, from 2001 to 2010. This increase was higher in China (49.68%) and Europe (20.05%) (MARM, 2010). It is estimated that China will represent half of the pork meat demand by next year (Planetoscope, 2012). Europe is the main exporter with 42% (Statista, 2019), and has approximately one third of the global pig population (Laval et al., 2000). In Europe, Germany is the first producer and consumer of pork, whereas France is the third and Belgium sits at the eighth place together with Luxemburg (Planetoscope, 2012).

In Belgium, pork consumption in 2015 was of 24 kg/capita/year (Filagri, 2015). It is not a significant number compared to Spain (54.4 kg/capita/year), Germany (53.1 kg/capita/year), or even Italy (39.1 kg/capita/year) (Sukhvinder, 2017). Almost all the production capacity of Belgian pork meat (94.5%) is in Flanders. It means a self-sufficiency of 25% in Wallonia vs. 340% in Flanders. At the national scale, autosufficiency raises up to 190%. In 2013, the absolute number was estimated at 6,351,300 pigs, of which 6,024,914 (~94.9%) were in Flanders (Apaq-W, 2014).

1.2. Sanitary issues

Pig production is highly susceptible to diseases specially because the wild counterpart of the pig, i.e. the wild boar, still lives wildly (Herrero-Medrano et al., 2013). Pigs are particularly susceptible to diseases such as Porcine Reproductive and Respiratory Syndrome (PRRS), or such as diarrhea, very frequent in piglets. The pressure of pathogens does not decrease overtime, and some (zoonoses) are even transmittable to humans such as salmonellosis or influenza (Dourmad & Riquet, 2014). Outbreaks of new diseases are another threat. An example of this is illustrated by the outbreak of African Swine Fever (ASF) in Etalle locality, in the extreme south of Belgium. Even if there were no contaminated domestic pigs, they were eradicated from the infected zone in compliance with the Federal Minister of Agriculture's decision. The AFSCA indicated that this was done to avoid an eventual spread to swine breeds, especially the domestic ones, and also to protect the Belgian Pork Industry. Breeders from this region were financially compensated, since they could not continue their activities at that time. The carcasses of pigs from infected regions entered neither the food chain nor the agroindustry. In addition, control measures were applied to limit the disease's dissemination (AFSCA, 2018).

Besides limiting the occurrence of diseases, another goal is to reduce antibiotic use and to promote vaccination as an alternative because of problems with antibiotic resistance both in humans and animals. Consumers are becoming more demanding regarding the production system aspects such as management and welfare. Particularly, it is more and more demanded that animals be able to express normal behavior. This is why health issues nowadays not only concern the absence of diseases but also the question of animal well-being. Different measures can be applied in order to limit the occurrence of diseases and their spreading: pigpen hygiene, biosecurity measures, vaccination, stress reduction and genetic selection (Dourmad & Riquet, 2014). Research has also a key role to play in the fight against pork diseases for (Dourmad & Riquet, 2014):

- Knowledge of biology of pathogens, their hosts and their interactions;
- Development of diagnostic tools, prophylactic measures such as vaccines, and treatments;
- Determine their feasibility of implementation (efficacy, cost, ease of use, etc.)

In this section, we have observed how important pork meat is to our society. It is appreciated worldwide and is not expensive. Nowadays, the pork industry is faced with the consumers' desire for healthy pork meat from pigs raised in good welfare conditions. This is only one example of the fact that breeding objectives have been changing overtime. For these reasons, in the next section, we will

see how humans have used wild boars and how they later took advantage of genetics to achieve their need for meat and/or other requirements.

2. Pig domestication and selection
2.1. Pig domestication

Sus scrofa (or wild boar) originated from South East Asia and it seems that this species spread in Asia, Europe and North Africa between 3.0 and 3.5 million years ago. During the last ice age, in the end of the Pleistocene, *Sus scrofa* faced a harsh environment and remaining populations endured in warmer zones called refugia (Herrero-Medrano, 2013). The ice age imposed a huge selection pressure on wild boars (Bosse, 2015). Ten thousand years ago, at the end of the ice age, *Sus scrofa* recolonized Europe (Herrero-Medrano, 2013; Bosse, 2015). During this period began the domestication process, caused by different reasons (e.g. religious belief) (Ollivier et al., 2005). The domestication center of this species is still in debate, but scientists have reached a consensus that multiple independent events of domestication took place in Europe and Asia (Bosse et al., 2012; Herrero-Medrano, 2013; Yang et al., 2017). European pigs may come from the Near East, but they were also crossed with European wild boars many times (Amaral et al., 2008; Herrero-Medrano et al., 2013). Apparently, there were regular introgressions of wild boars genes in domesticated animals in both continents (Bosse et al., 2012; Herrero-Medrano, 2013). However, the management of pig populations in Europe and Asia was different. Asian pigs were raised in farms since the beginning of the domestication process, whereas European pigs were used for hunting games and uprooting truffles, and were fed with acorns and other foodstuff found in the forest, where they could also gather to reproduce with wild boars. This latter fact and the European colonization have led to feral or hybrid pig populations. Management differences applied in Europe and Asia led to contrasted pig morphologies. European pigs were relatively similar to their wild counterparts as proven by haplotypes shared by both types of animals (Amaral et al., 2008; Herrero-Medrano, 2013). On the other hand, Asian pigs have a more typical domestic pig morphology, i.e. they still tend to be stockier (Herrero-Medrano, 2013). Both types of pigs are genetically different due to their divergent history (Bosse et al., 2012; Bosse, 2015; Yang et al., 2017). For example, European pigs, especially commercial lines, show a longer Linkage Disequilibrium (LD) and less haplotypes diversity than Asian ones. Moreover, the genetic diversity of Chinese pigs is higher because European wild boars, the ancestors of European pigs, endured stronger bottlenecks, which resulted in longer LD, and also due to selection process that a higher level of inbreeding in Europe implied (Amaral et al., 2008; Groenen et al., 2012).

2.2. Modern breeding

For all types of animal productions, breeding is based on selection criteria/goals, e.g. for milk or meat production breeding purposes. The multiplicity of existing breeds allows breeders to make their first choice according to the kind of productions they desire. After criteria definition, the breeders have to create a mating strategy to achieve their goals, i.e. they have to choose animals that encounter the best breeding objectives and mate them in an appropriate way. During almost all the domestication period, selection was visual, i.e. via phenotypes (Oldenbroek, 2017). However, this way of proceeding is biased due to environment impact on phenotypes (Falconer, 1952). Besides, best animals based on their phenotypes are often genetically related, which could lead to inbreeding depression (Eynard, 2018).

Animal breeding effectively began for all types of animal production in the 18th century, with the use of pedigree as a tool to provide efficient mating strategies (Oldenbroek, 2017). This idea can be illustrated in pigs by the importation of Chinese breeds in England during the domination of China by Great Britain between the 18th and 19th centuries. During that period, the aim was to cross Chinese animals with English ones in order to optimize their prolificacy and weight gain as the demand for fat products was very high (Herrero-Medrano, 2013; Bosse, 2015; Yang et al., 2017). The need for animals that could easily be kept in farms might have arisen from the constant decrease of available forests (Bosse, 2015). This point marks the start of high productive farms in Europe. Animals originated from local English and Asian crosses were the ancestors of English breeds, and therefore, of main breeds currently used all over the world: the Landrace and Large White breeds (Herrero-Medrano, 2013; Yang et al., 2017). Furthermore, haplotypes are shared between Chinese pigs and European pigs such as Large White, Landrace, Duroc and Piétrain (Amaral et al., 2008). During the colonization period, pigs were also introduced in the Americas and Oceania. Evidences show that English modern pigs were crossed with Chinese ones since the mid-eighteenth century in China. However, this appeared to be done to a lesser extent than the crosses observed in England. Therefore, Chinese breeds specificity seems to be better conserved (Yang et al., 2017). Pig populations can therefore be classified in three main groups: 1- commercial or international breeds coming from crosses between Chinese and European pigs, these breeds are genetically close to each other and to local European breeds to a lesser extent; 2- local breeds (Asian or European) that are, in general, genetically close to other breeds of the same continent; 3- wild boars, that tend to be apart from their domesticated counterparts, and feral pigs (Herrero-Medrano, 2013; Yang et al., 2017).

During the second half of the last century, animal breeding became more efficient due to advances in genetic evaluation methodologies. One example is the introduction of the Best Linear

Unbiased Predictions (BLUP) in the 90's (Henderson, 1975). The BLUP method makes Estimated Breeding Values (EBVs) more precise because environmental effects are minimized and, as an animal model (Henderson, 1984), performances of all relatives are taken into account. Then, EBVs for economically important traits are combined into indexes that will help breeders to select young candidates. With the development of statistical methods, data processing progress facilitated less biased genetic evaluations (SanCristobal et al., 2006a). Technical tools and Artificial Insemination (AI) also helped to increase genetic progress (Salaün, 2013).

2.3. Genomics

During the last decade, the use of genomics has increased mainly because of growing throughput and decreasing of its costs (Oldenbroek, 2017). Genomic is the study of genomes through the use of genetic markers. Some objectives of genomics are the mapping of genes and determining their functions i.e. linking them to phenotypes (Gibson & Muse, 2004). There are different kinds of genetic markers (e.g. RFLP, microsatellites, DArT) but some of the most used are Single Nucleotide Polymorphisms (SNPs). They are the most important source of DNA variation: this of one single nucleotide (Meuwissen et al., 2001). Shift from one base to another, insertion or deletion of one nucleotide are SNPs (Gibson & Muse, 2004). These SNPs are usually obtained by chips that can genotype a huge number of them simultaneously. The choice of SNPs chips to be used is made according to their utility, whereas their price increase as the density and coverage increase. Coverage is the average number of reads of each part of the genome (Oldenbroek, 2017). One of the most used porcine chips is the *PorcineSNP60 v2 BeadChip*. It displays 64,232 SNPs distant on average of 43.4 kb and evenly distributed. It is the second version of this chip and it covers a great part of the porcine genome (Illumina, n.d.).

The principle of genomic selection is the following: a reference population, of which genotypes and phenotypes are known, is used to establish a link between SNPs and phenotypes through a predictive formula. This formula can then be used on a candidate population of which phenotypes are unknown to estimate Genomic Breeding Values (GEBVs). The main advantage of genomics is that breeders can select animals based on some traits that are age- or sex-linked or even obtained after slaughter. Before genomics, these kinds of traits were estimated by parental average, which is not very precise because of Mendelian sampling. Genomic selection allows the increase in accuracy and selection intensity, and the decrease of generation intervals. However, accuracy of genomic selection depends mainly on three factors: heritability, number of animals in the reference population and LD (proportional). Correlations among alleles are taken into account by LD (Oldenbroek, 2017). For less

biased genomic predictions, it is therefore important to have the largest and most diverse reference population (Jonas et al., 2017). Unlike Whole Genome Sequencing (WGS), SNPs are not randomly chosen, and are used to fulfil certain purposes which causes bias (Eynard, 2018).

3. Breeds

After discussing the process of domestication and selection in pigs, we will now gain an insight into some of the different breeds resulting from these processes. However, first of all, we will try to define this concept.

3.1. Definition of breeds

Breed is a notion that is commonly linked to the field of animal productions daily use. However, defining breed is complex because the boundary between one breed and another is not always easy to identify (phenotypically and/or genetically). Moreover, this concept is linked to culture and to the breeders' own vision (FAO, 2013).

A traditional definition of breed can be found on the website of Oklahoma State University (1995). They define it as a group of animals whose offspring share some traits and that is relatively homogenous because of selection and breeding processes. This definition is not very clear and some issues arise from it. As they suggest on the website, what distinguished crossbred animals from composite breeds? Moreover, Oldenbroek (2017) pointed out the absence of the role of the breeder and the list of traits passed on offspring.

A better definition, highlighting the role of breeders in the creation, definition and evolution of a breed, was provided by Lush (1994):

"A breed is a group of domestic animals, termed such by common consent of the breeders, ... a term which arose among breeders of livestock, created one might say, for their own use, and no one is warranted in assigning to this word a scientific definition and in calling the breeders wrong when they deviate from the formulated definition. It is their word and the breeder's common usage is what we must accept as the correct definition."[1]

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[1] Lush J.L., 1994. Genetics of population, Genetics Research, Ames, IA, USA.

The FAO definition (FAO, 2001) is less subjective than the one proposed by Lush (1994) and will be used in this study:

"Either a sub-specific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species, or a group for which geographical and/or cultural separation from phenotypically similar groups has led to acceptance of its separate identity."[2] This definition provides some key points for the definition of a breed: 1- a breed is a sub-group of animals of the same species and 2- they can be separated from other sub-groups by their phenotypes.

3.2. Most important breeds of pigs present worldwide

Around 350 pig breeds were described (Salaün, 2013). Therefore, some authors (e.g. Ollivier et al., 2005) consider pigs as one of the most variable species. Nevertheless, nowadays, only four of them are commonly used: Large White, Landrace, Duroc and Piétrain. There are also a lot of small populations of local breeds raised by farmers, especially in developing countries (Salaün, 2013; FAO, 2019).

- The Large White breed, also called Yorkshire, is a pure white pig with erect ears that originated in Great Britain from a local pig breed and Asian pigs (Salaün, 2013; National Pork Board, 2019). It is well-known for its adaptive faculty, reproductive abilities and meat quality (Marcq & Lahaye, 1941; Salaün, 2013; National Pork Board, 2019). Moreover, this breed is the leader as far as growth performances go. It is frequently crossed with Piétrain and Landrace (Salaün, 2013).
- The Landrace is a breed with falling ears and a white coat. It is mainly used for its maternal qualities inasmuch as sows are kind, good milkers and give birth to large piglets (Salaün, 2013; National Pork Board, 2019). There are many different European Landrace populations, sometimes considered as different breeds. British, Danish, Dutch and French Landraces are similar, whereas German and Belgian ones have started later to eradicate Hal animals (Hal gene is explained in section 4.2.3.) (Salaün, 2013).

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[2] FAO, 2001. Working definitions for use in developing country reports and providing supporting data. *In*: Boyazoglu, S.G.& J. ed. *Animal Genetic Resources Information*. FAO, Rome, Italy.

- The Piétrain breed will be explained in more details in the next section as it is the main subject of this study.

- The Duroc, anciently named the Duroc-Jersey, originated in the USA from two red haired breeds: the Duroc and the Jersey (Marcq & Lahaye, 1941; Oklahoma State University, 1995; National Spotted Swine Record, 2019). The main strengths of this breed are its highly robust nature, a high intramuscular fat content, carcass yield and fast growth (Salaün, 2013; National Pork Board, 2019). The Duroc breed has developed both maternal (National Spotted Swine Record, 2019) and paternal lines (Salaün, 2013). This may explain why there is no consensus in the literature about reproductive performances. They are considered less favorable according to Salaün (2013) but it is contradicted by other sources (Marcq & Lahaye, 1941; National Pork Board, 2019).

- The Hampshire breed originated in Great Britain and presents black hair with a white belt including front legs (Marcq & Lahaye, 1941; Salaün, 2013). This breed is known for its lean meat and good maternal abilities (National Pork Board, 2019). The Hal gene is absent and intramuscular fat content is also interesting. However, it owns RN$^-$ allele responsible for acid meat. Today, compared to the other main breeds presented, the Hampshire breed is less common (Salaün, 2013), which could be explained by this latter fact.

3.3. Pig breeds in Wallonia

Besides the breeds aforementioned, some other breeds or breed types are also currently raised in Wallonia:

- Belgian Landrace sows are fertile, maternal and good milkers (Oklahoma State University, 1995). The homozygous NN (stress resistant) Belgian Landrace, selected since 1994, is used as a boar and crossed with Large White sows (naturally stress resistant) to have a F1 sow with heterosis. This sow is used as a parent (maternal line, please see next point in this section for explanation of maternal line). This breed is then crossed with the Piétrain to provide finishing pigs. Nowadays, all Belgian Landrace pigs are stress resistant (Piedboeuf, 2014a).

- Other types of Landrace can be found in Wallonia such as Danish, German, English, Finnish, French, Dutch, Norwegian, Austrian and Swedish (Piedboeuf, 2014a).

- The Berkshire is almost totally black and has erect ears (Marcq & Lahaye, 1941; Piedboeuf, 2014a). It is considered to have interesting growth and reproductive traits (National Pork Board, 2019). In some regions of the world, its sweeter, darker and less acid meat is highly appreciated (e.g. the USA or the UK) (National Pork Board, 2019). Despite producing a lot of

bacon (Piedboeuf, 2014a), this breed was formerly considered as a meat producer (Marcq and Lahaye, 1941). Nowadays, it is rarely used in Belgium.

3.4. Crossbreeding in pigs

There are different types of crossbreeding schemes, each of them for the achievement of different purposes. In this section, we will detail admixture and terminal cross, which are of particular interest in this study.

Even if admixture is often used to improve existing breeds, it can also aim to create a new breed by crossing at least two existing breeds (Handley et al., 2001). An example will be provided in part *4.1. Piétrain history.* On the other hand, terminal crosses only intend to create production animals i.e. pure breeds are crossed to obtain progeny with desired qualities. Terminal crossbreeding is characterized by its number of levels, i.e. the number of generations to obtain the final production animal, and by its number of ways, i.e. the number of breeds involved. The breeds most used for terminal crossbreeding are those mentioned in section 3.2. of the literature review. Crossbred animals are not used for reproduction purposes in this scheme (GSEI, 2019), only potentially to generate the next level. Nowadays, terminal cross is commonly used in pig industry, which implies that there are less and less purebred pigs worldwide (Ollivier et al., 2005). The main advantages pork industry can draw from terminal crosses, i.e. heterosis and complementary effects, explain partially why it is so popular (Sellier, 1976).

Heterosis is mostly due to the interaction between different alleles at the locus level. Indeed, when two different breeds with different alleles for some traits are crossed, a dominance effect appear for these traits on the progeny. It means that progeny is on average better than the average of parents for the trait (Lynch & Walsh, 1998a). One might ask why parents of different breeds carry different alleles. Actually, during the selection process, a breed can lose some alleles by chance or by inbreeding depression. This explains why heterosis is greater when breeds are genetically far (Lynch & Walsh, 1998b).

Besides heterosis, another important aim of terminal crossbreeding is to exploit complementary effects i.e. a combination between two genomes selected for different purposes (Lynch & Walsh, 1998c). It is important to highlight that, in pigs, selection objectives differ across breeds. Moreover, as long as production and reproduction traits cannot be selected simultaneously, finishing pigs resulted from crosses between maternal and paternal lines. Paternal lines, which include Piétrain, are selected for their production traits such as average daily gain (ADG), feed consumption

(FC), carcass quality or meat percentage. Maternal lines such as the Landrace breed, are selected for their maternal qualities and prolificity, but also for their production traits to be transmitted to their finishing offspring (Short et al., 1997; Litten et al., 2004; Dufrasne, 2009; Salaün, 2013). Therefore, maternal and paternal lines in pigs are used to achieve complementarity in the finishing pig.

The same breed can be divided into two lines as it is the case for the Large White and the Landrace. However, in these breeds, paternal lines are nowadays of minor importance. The Hampshire breed is used as a paternal line whereas the Duroc does not belong to any of these lines *a priori* (Salaün, 2013). This point of view is contested by the awé (Piedboeuf, 2014a) that considers the Duroc breed as a paternal line.

4. The Piétrain breed

As populations of purebred pigs used for terminal crosses decrease overtime, it is important to assess and maintain their genetic diversity. The Piétrain breed is one of these breeds and is the main topic of this study.

4.1. Piétrain history

This part explains how this breed, and its particularities, may have been created. Even if the popular history says that the Piétrain breed was originated in Piétrain, Walloon Brabant, Southern Belgium, in the twenties (Hansoul, 1964; Stas & Mougenot, 2009; Stratz et al., 2014), there are several versions of its origin. We now present one main version of the origin of the Piétrain breed as well as some alternative versions, in less details.

4.1.1. Pig production in Wallonia before Piétrain breed

Before World War I, different types of pigs were raised in Wallonia. Among them, the Indigenous white breed, also called the Celtic pig, was the most common. This breed had a white coat, falling ears and produced fat products, highly demanded during the War (Hansoul, 1964). The Celtic pig later became the Belgian Landrace (Leroy et al.,1960). Besides the Celtic breed, other types of unknown animals, more muscular and of better quality, were used, especially for butchery pork purposes. They came from Flanders and Ardennes and were sold at the Tirlemont and Jodoigne

markets. Some Walloon regions also raised crossbred animals from the Celtic and Small White breeds (Hansoul, 1964).

In the twenties, English breeds were imported into Belgium. At this time, crossbreeding became common. Moreover, at the end of World War I, consumers started to demand lean meat (Hansoul, 1964).

4.1.2. Main version

Jules Buis and his father, from the region of Jodoigne, a small town in Belgium, would have tried to improve Walloon pigs by English crosses. For this purpose, they would have bought a young English black-pied boar that may be originating from the Berkshire breed. Their objective was to increase the length of the offspring of this double-muscling boar by crosses with Yorkshire pigs. This first experience was a failure because, even if pigs were muscular, animals were short and fattened as early as eighty kilograms. Some breeders thus gave up on raising this kind of animals and returned to the Indigenous breed that grew faster than black-pied pigs. Nevertheless, black-pied pigs spread in the region and were called at this time "les fleuris" (in English, the "flowered"; this expression was used to refer to their black spots) (Hansoul, 1964).

Between 1925 and 1926, Jules Buis would have tried again to increase the length of black-pied pigs by means of crosses with different White breeds. He would have achieved his goal in 1927 with an Indigenous sow. Piglets from this cross had straight and large snout, horizontal ears, muscular and deep ham, were long and well conformed with a large and flat back, and a groove along the loin. Moreover, they were white and black spotted. Then, the breed type was stabilized with crosses with Large White sows (Hansoul, 1964).

The aforementioned version suggest that Piétrain pigs originated from a three-way cross, first between Berkshire type boars and Indigenous sows, and then the F1 boars with Large White sows (Marcq & Lahaye, 1941; Hansoul, 1964).

4.1.3. Other versions

Another version of the Piétrain origin claims that Jules Kaisin, the grandfather of Henri Stas, a current Walloon Piétrain breeder, would have bought from his neighbor black spotted piglets originated from crosses between two different unknown breeds (Stas & Mougenot, 2009).

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Some authors reported that the Piétrain may originate from Bayeux pigs, which come from a cross between local Norman and Berkshire pigs (Marcq & Lahaye, 1941; Camerlynck & Brankaer, 1958; Stratz et al., 2014). They would have been imported by German officers during World War I. However, German troops never went to Normandy at this time. It was also thought that Piétrain pigs originated from a cross between Berkshire and an Iberic breed (Camerlynck & Brankaer, 1958).

The aforementioned versions remain more or less plausible. However, other colorful scenarios should be considered as rumors. For example, one rumor said that the Piétrain came from wild boars. Two elements supported this hypothesis. First, some Berkshire sows were thought to be freed by German officers during World War I, and therefore, to have met wild boars (Camerlynck & Brankaer, 1958). Secondly, Jules Buis had held a wild sow for a few months and thus would have used it for crossing, a fact he denied (Hansoul, 1964). As Camerlynck & Brankaer (1958) suggested, this is not very plausible as Piétrain pigs already showed a well-developed back. Moreover, they were considered really tame (Camerlynck & Brankaer, 1958).

4.1.4. Expansion of the Piétrain breed

In the 1930's, due to their higher leanness, mottled piglets were sold by merchants from farm to farm and exchanges between breeders became common (Stas & Mougenot, 2009). Moreover, butchers have demanded this type of animals due to their good ratio of lean meat (1/6.8) that involved a better market value. In 1935, it was observed that black spots were bigger and pigs were called "les noirs" (in English, "the black") (Hansoul, 1964).

Around 1940, the breed was popular in the region of Jodoigne. However, during World War II, farms were abandoned and disappeared. The muscular animals also disappeared since, under enemy occupation, fat meat carcasses were desired (Hansoul, 1964; Stas & Mougenot, 2009). The breed was thus considered at the brim of extinction. Then, with the end of the War, the demand for lean meat increased (Rubin et al., 2012) and Piétrain breeding started again. However, the breed was not immediately recognized (Hansoul, 1964; Stas & Mougenot, 2009).

During the fifties, this trend towards lean meat strengthened, which eased again the expansion of the Piétrain breed (Youssao et al., 2002). In 1950, an association of breeders was born and called "Syndicat des éleveurs Piétrain et extension" (Piétrain breeders association and extension). The breed was officially named "Piétrain" since most of the breeders came from the Piétrain locality (Leroy et al., 1960; Hansoul, 1964; Stas & Mougenot, 2009). The first mention of the "porc de Piétrain" (Piétrain pork, in English) appeared in Marcq & Lahaye (1941). Nevertheless, 20 years before its recognition,

this breed used to be called as "race wallonne", i.e. the Walloon breed, at contests. Black-pied pigs were also commonly previously called "les fleuris" or "les noirs", as aforementioned (Hansoul, 1964).

In 1951, boars were admitted to public natural service and the Pigbook was created (Leroy et al., 1960; Hansoul, 1964; Hanset, 1992; Stas & Mougenot, 2009). The first Piétrain boar to enter into the Pigbook was called "Max 7B1" and was born in 1946 (Hansoul, 1964), whereas the first sow was "Espérance de la Sarte" by Jules Kaisin (Stas & Mougenot, 2009). The standard of the breed was also defined (Camerlynck, 1973). In Appendix I, Max 7B1 and other Piétrain pigs from the fifties/sixties are illustrated.

In 1953, the first competition was organized (Leroy et al., 1960; Hanset, 1992; Stas & Mougenot, 2009). In 1955-1956, the breed was recognized in Belgium as a national breed by the Agriculture Ministry, apparently following persuasive arguments of Jules Kaisin at a contest (Leroy et al., 1960; Stas & Mougenot, 2009). At this time, the first breeders association was incorporated into the "Fédération belge des éleveurs de porcs" or "FBEP" (Pig Breeders Belgian Federation) (Stas & Mougenot, 2009). The year 1956 also means first exportations to foreign countries (e.g. France, the Netherlands, Germany). Moreover, boars have been offered to breeders through a mobile pig mating service. First assessments of boars suitable for mating were made (Stas & Mougenot, 2009)

In 1950's, industrialization of agriculture began in Wallonia. However, Piétrain breeding stayed mainly traditional because animals are more stressed by nature, take more time to gain weight and need more care (Stas & Mougenot, 2009). Stas (2009) employed the term of "domestic niche in the core of an industrial world".

At the end of the 1970's, assessments of boars suitable for natural service were stopped by the FBEP because of restructuring. In the 1980's, due to pest epizooties, it was forbidden to provide a mobile pig mating service. Then, gathering pigs for other reasons than for competitions was forbidden. In competitions, sanitary measures became more stringent and participating animals had to be placed in quarantine, which has a cost (Stas & Mougenot, 2009).

Since then, three technological "tools" successively appeared in the Piétrain breeding. First, stress resistant or stress-negative lines (also called ReHal in Belgium for Halothane Resistant) were created (please see point *4.2.3. Muscle hypertrophy and stress susceptibility* of this literature review for further details). Secondly, AI, organized in Artificial Insemination Centers (AIC), increased genetic progress and have limited the spread of sexually transmitted diseases. However, concurrence is normally severe and nowadays there is only one AIC in Wallonia. Finally, despite concerns by some breeders that their own vision of Piétrain pigs might be diluted (Stas et al., 2009), since 2007, a progeny testing scheme has been operating (Dufrasne, 2009).

4.1.5. Origin of the Piétrain breed, what we really know

As a conclusion, most scholars agree that the Piétrain breed arose from Berkshire pigs. Indeed, Berkshire pigs have black or red hair and piglets are sometimes mottled (Camerlynck & Brankaer, 1958). Marcq & Lahaye (1941) thought the Piétrain originated from a cross between Berkshire and Celtic pigs but with Yorkshire influences. Recent genomic studies support this as Yang et al. (2017) also provided evidences that the Piétrain pig originates from White pigs like Landrace and Large White through their Multi-Dimensional Scaling (MDS). Li et al. (2014) moreover suggested that Berkshire and Piétrain breeds are relatively close genetically. The most likely scenario is an initial cross between Berkshire and White breeds (Leroy et al., 1960; Hansoul, 1964; Le Sillon belge, 2000). Mutations and inbreeding would have occurred to achieve the specific double-muscling Piétrain (Camerlynck and Brankaer, 1958; Leroy et al., 1960).

The breed appeared after World War I influenced by English breeds, as supported by recent studies, and almost disappeared during World War II (Hansoul, 1964; Stas & Mougenot, 2009). It was only at the end of World War II that demand and selection for lean meat started (Rubin et al., 2012). This situation was a breeding ground for the development of the Piétrain breed (Hansoul, 1964). In 1950, the name of the breed was officially chosen, and the breeders union created (Leroy et al., 1960; Hansoul, 1964; Stas & Mougenot, 2009). It should be noticed that until 1950, white Piétrain had existed (Marcq & Lahaye, 1941) but were then progressively eliminated to give a specificity to the breed (Camerlynck, 1973). In 1951, boars were admitted to natural public service (Leroy et al., 1960; Hansoul, 1964; Hanset, 1992; Stas & Mougenot, 2009), the standard was defined (Camerlynck, 1973) and the Pigbook created (Hansoul, 1964; Hanset, 1992; Stas & Mougenot, 2009). In 1953, the first competition was organized (Leroy et al., 1960; Hanset, 1992; Stas & Mougenot, 2009). In 1955-1956, the breed had a national recognition (Leroy et al., 1960; Hansoul, 1964; Stas & Mougenot, 2009).

4.2. Phenotypic particularities of the Piétrain breed
4.2.1. Standard of the Piétrain breed

In figure 1 is illustrated a Piétrain boar. The awé has developed the standard of the breed on its website (Piedboeuf, 2014b):

- Hair: pied, irregular with black mottles, sometimes red on the edge.
- Head: relatively light, short and straight, sometimes slightly flat. Cheeks are moderately developed.

- Ears: small, erect and oriented forward.

- Neck: short and light.

- Chest: broad, cylindrical and shallow.

- Shoulder: salient and very muscular.

- Withers: flat and broad.

- Back: relatively long, slightly curved, broad with a little median ridge delimited by highly developed dorsal muscles.

- Loin: highly muscular.

- Abdomen: mildly developed, firmly suspended. Inferior and superior lines are almost parallel.

- Rump: broad with average length. A slight hollow can be seen overhead the tail implantation.

- Tail: moderately thin, low implantation.

- Hams: well developed in depth, well filled, broad and spherical.

- Legs: moderately long, light and solid.

- Posture: correct walk, even hoof leftovers and closed foot.

- Teats: evenly distributed, well developed, at minimum 2x6.

Figure 1. Piétrain boar (Lempereur, 2009)

4.2.2. Coat color of the Piétrain breed

The coat color can be considered as highly specific for certain breeds like Duroc, Hampshire, Berkshire and obviously Piétrain. Seven genes are known to influence the coat color in pigs. Wild boars carry the dominant allele *A* that is typical of the wild coat. Almost all the domestic breeds, except some that are colored, share the recessive allele *a* of this gene. The locus *C* in its recessive form *c* induces an extreme dilution of the coat color that leads to dirt-white tinge. The *D* locus is also linked to dilution and sepia color. The gene *E* is responsible for different colors: E^d for dominant black, *E* for black or black-pied, E^p for black domino and black with six white points, *e* for red and e^h for white head. Smith et al. (1935) consider that the allele *he* is responsible for white head. The allele *I*, which results in color inhibition typical of white breeds such as Landrace and Large White, is dominant. Other alleles of the locus *I* lead to grey, black spots or color. Finally, the white belt of certain breeds like Hampshire is thought to be caused by the *Be* allele (Legault & Chardon, 2000). Lauvergne et al. (1982) also reported a *B* gene responsible for brown color.

The Piétrain is a black spotted pig. This coat particularity is called Domino. The Piétrain thus carry the following genotype: *aa* (no wild-type coat), *ii* (no inhibition of color), E^pE^p (Domino), *HeHe* (white head) and *bebe* (no white belt). The majority of purebred Piétrain have indeed homozygous coat color because this property is fixed in the breed, even for dominant alleles (Legault & Chardon, 2000).

The Berkshire pigs show four stockings and white chamfer and tail end. These animals share the same genotype as Piétrain for hair color. This type of coat can be obtained by expansion of black in Domino animals. This expansion is highly heritable: ~60% (Legault & Chardon, 2000). Some Piétrain animals with a Berkshire hair are found in Wallonia (Figure 2). The origin of the Piétrain coat could therefore be explained as the result from crosses between the Indigenous and Berkshire breeds.

Figure 2. Piétrain piglet with Berkshire type coat (Wilmot, 2019)

4.2.3. Muscular hypertrophy and stress susceptibility in Piétrain breed

One of the first character we see by looking at Piétrain pigs is a huge musculature. Amaral et al. (2011) demonstrated that the Piétrain breed has the greatest nucleotide diversity and was the most selected commercial breed for muscle development. The IGF2 gene, identified by Nezer et al. (1999), is partly responsible for the leanness and muscular hypertrophy of the Piétrain. It explains on average 25% of the differences between Piétrain and Large White pigs for these traits (Nezer et al., 1999). The frequency of occurrence of the gene in the Piétrain population is very high. This gene is carried and transmitted by sire and dam, but is only expressed when transmitted by sires. This is called imprinting. For these reasons, the terminal cross using a Piétrain boar is highly beneficial for finishing pigs (Leroy et al., 2000; Youssao et al., 2002).

However, the Hal gene would also imply in 25% of the difference between Piétrain and Large White. This means that 50% of genes involved in muscle hypertrophy are not yet identified (Leroy et al., 2000; Youssao et al., 2002). The Hal gene (allele n) is responsible for a stress susceptibility (monogenic and recessive trait (Sellier et al., 1989)) called Porcine Stress Syndrome (PSS) (Youssao et al., 2002). The Hal gene (allele n) leads also to Pale, Soft and Exudative (PSE) meat and to Malignant Hyperthermia Syndrome (MHS) (INA P-G, 1999; Youssao et al., 2002). This gene was identified by researchers at Liège Veterinary Medicine University in 1980 (Leroy et al., 1999).

As Hal and IGF2 genes are independent, it is thus possible to select animals to be stress-negative but carrying the interesting allele for the IGF2 gene. The first ReHal Piétrain was produced in 1989 from an absorption cross with a Large White followed by successive Piétrain backcrosses (Leroy et al., 1999; Youssao et al., 2002). The objective was to introduce the allele N of the Hal gene in the Piétrain line to avoid negative effects of this gene (Youssao et al., 2002). This denomination of ReHal Piétrain is used in Belgium. Similar lines exist in other countries and are known as "stress-negative" or "stress-resistant" Piétrain. It is well-known that stress-positive animals shows less backfat thickness, better carcass yield and conformation than stress-negative animals (Youssao et al., 2002; Maquet & Montfort, 2014). However, Leroy et al. (n.d.) reported stress-negative lines that have carcass yield similar to stress-positive and that show meat percentage of 59%, 0.5% less than stress-positive Piétrain. Nowadays, NN homozygous animals with more than 99.7% of Piétrain genetics have been used by farms as sires/dams (Leroy et al., 1999).

4.3. Piétrain disappearance in Wallonia

Even if the Piétrain breed carries interesting genes and shows a particular phenotype, the Walloon Piétrain population seems to be at risk of endangerment according to several Walloon Piétrain breeders (J. Lempereur, personal communication, February 22, 2019; H. Stas, personal communication, March 29, 2019). These breeders (Lempereur, 2011; T., 2012) and Benoît Lutgen (reported by Warnotte, 2009), Minister of Agriculture from 2004 to 2011, stated some reasons of the Piétrain pigs decrease in Wallonia:

1. The size of Belgium and the low number of farrow-to-finish farms in Wallonia compared to the high production capacity of boars in Flanders. Despite the decreasing number of pig herds and breeders, the Belgian supply of pig meat is self-sufficient;

2. The move of the Tienen market towards Antwerp, which makes boar sales more difficult for Walloon breeders;

3. The use of AI that reduces the number of boars used (one boar per one hundred sows or higher). Technological advances have lowered the quantity of ejaculate necessary to make a straw and increased their lifetime. Thus, the number of boars used for the same number of doses have decreased. The price of one boar has dwindled. However, AIC are really helpful to effectively spread genetic progress. It is the main outlet for boar sales;

4. Integrative agricultural systems where porcine selection enterprises provide semen straws to their customers;

5. Customers of breeders are more demanding and take a closer look to zootechnical performances;

6. Sanitary requirements have increased and are sometimes difficult to implement;

7. Administrative tasks are more and more stringent;

8. Fluctuating and often low prices of the pork market.

4.4. The Belgian Piétrain program

This project was launched in 2017 through a tripartite agreement between the awé and the provinces of Liège and Walloon Brabant. It aims to preserve in the long run the genetic diversity of the Piétrain breed and to promote it in an attractive and modern way (awé, 2019). It is a first step for the preservation of the Walloon Piétrain population.

4.4.1. Boars selection, quarantine and sampling

A commission of active Piétrain breeders was created by the awé. This commission is in charge of the definition of breeding objectives and boar choice that will enter the conservation program. If selected boars are free from Classical Swine Fever, Aujezsky's disease, brucellosis and PRRS for at least one month, they stay in quarantine for another month and then are sent to the Interprofessional Center for Animal Improvement and Promotion (CIAP) at Argenteau (awé, 2019). The CIAP is the only porcine artificial insemination center in Wallonia.

Boars stay at CIAP until enough semen straws are produced. Fresh semen is used for genetic evaluation on Landrace (please see next section). If the use of fresh semen is not possible, frozen straws are used. The semen sampled during the stay of the boars at CIAP has three possible destinations (awé, 2019):

1. Frozen straws can be used by breeders.
2. It can be conserved in a cryobank to preserve the breed's genetics.
3. Boars, whose semen has been frozen, are put up for sale.

Finally, boars are sent back to their owners (awé, 2019).

4.4.2. Genetic evaluation of Piétrain in Wallonia

The genetic evaluation of Piétrain boars in Wallonia, set in 2007, is not only earmarked for boars entering the Belgian Piétrain program but for each boar breeders would like to test. This evaluation is performed by Gembloux Agro-Bio Tech and results are published by the awé. The objective is to determine if Walloon Piétrain boars are good enough to transmit interesting production qualities.

Mating plans are organized on two recurring groups of Landrace sows from the Walloon Center of Agronomic Search (CRA-W) with the objective to obtain 18 descendants per boar to be tested at a given moment in time. Their offspring are recorded for carcass weight (kg), ADG (kg/d), backfat thickness (mm), consumption index, conformation index and meat percentage. Moreover, on-farm recording (tested boars and close relatives) is performed for live weight (kg), backfat thickness (mm), meat percentage and loin muscle depth (mm). These criteria are supposed to cover the owners' selection objectives. Based on the aforementioned strategy, a total of 10 traits are available for tested boars; six recorded on crossbred offspring and four own on farm recording. Different models are currently used to generate EBVs for these progeny-tested boars (Dufrasne et al., 2011).

5. Genetic diversity

In the previous section, we saw that the Walloon Piétrain population is potentially at risk. It is therefore important to determine 1- if it is endangered and 2- which measures could be implemented to protect this population. This is why, in this section, the genetic diversity will first be defined. Then, alternatives to preserve an endangered breed will be also put forward.

5.1. Genetic diversity definition and concept

The DNA contains all the genetic information necessary to produce proteins that will, with the environment, generate phenotypes. The diversity observed on populations or species is based on a simple fact: diploid individuals carry two copies, called alleles, of each gene that are subject to mutations. The different alleles and the relationship between both copies produce diversity (Oldenbroek, 2017). Genetic diversity was, thus, reported by Oldenbroek *et al.* (2017) as DNA or phenotypical differences that arise between species, breeds and individuals. Upadhyay (2019) added

that it can refer to individual genome variation and that reflects the variety within a population as it is a sample of it.

Genetic diversity is under constant variation. Mutations occur continuously during population history and some alleles are lost from one generation to another (Oldenbroek, 2017). These mutations are essential for animals to adapt to their environment and, in animal breeding, to select animals with desired traits (Eynard, 2018). Another source of variation of the genome is recombination which occurs during meiosis. This phenomenon can produce new blend of alleles and, as genes can interact, new phenotypes are observed (Upadhyay, 2018).

Moreover, some breeds can maximize more genetic diversity because they carry more within-breed variation (Oldenbroek, 2017). If we downscale this concept, some populations may be more important for genetic diversity of Piétrain pigs. Maybe this is the case for Belgian (Walloon) Piétrain pigs.

5.2. Why it is important to preserve genetic diversity

Nowadays, as previously mentioned, only a few pig breeds are widely used in Europe (Laval et al., 2000; SanCristobal et al., 2006b). The Large White represents 30% of the animals (Laval et al., 2000) while the FAO (2015) reported that 90 pig breeds were declared extinct in Europe. How did we come to such an extreme? During the 20th century, the loss of pig breeds was considerable because high productivity was the main lever of farming (SanCristobal et al., 2006b). The numbers of local pigs thus declined, and some were crossed with major breeds leading to their absorption (Herrero-Medrano et al., 2013). However, a study by Ollivier et al. (2005) demonstrated that 56% of the microsatellite diversity between breeds was due to local breeds whereas commercial breeds are more important for within breed variation. Local breeds are also part of regional culture and history (Herrero-Medrano et al., 2013).

Moreover, this diversity loss within and across breeds could lead to the loss of unknown alleles with possible desirable characteristics such as medical application or specific meat properties, not directly selected so far (Oldenbroek, 2017). These specific or rare alleles could therefore be irreversibly lost (Herrero-Medrano et al., 2013; François et al., 2017; Eynard, 2018). Diversity allows the choice of animals with different, opposite and complementary traits that could be useful if objective goals change according to human needs or even environmental and farming conditions (Laval et al., 2000; Eynard, 2018). For example, to be resistant to certain or novel diseases is of particular interest. To ensure food security in a context of global warming and environmental issues is also highly desired

(Herrero-Medrano et al., 2013). Besides, the increase of inbreeding rates highly favors health and fertility problems. The genetic diversity loss and increased inbreeding levels overtime constitute what is known as genetic erosion (Herrero-Medrano et al., 2013; Bosse et al., 2015; Eynard, 2018).

The need for genetic diversity preservation in pig production in Europe is undeniable (Laval et al., 2000). It is thus critical to establish the genetic diversity of local breeds and to implement solutions to preserve them (Eynard, 2018). The Piétrain breed could in part apply to this issue. Moreover, Piétrain pigs have a highly specific phenotype as seen in section 4.2. Even if by keeping genetic diversity at a high level is critical, this cannot be achieved without considering deleterious alleles that must be eliminated or, at least, mitigated. Therefore, the fitness of animals must be considered as well (Bosse et al., 2015).

5.3. How to preserve genetic diversity

One driving factor against genetic diversity is selection for the best animals and therefore their alleles. Optimal Contributions (OC) is one way of limiting long term increase of inbreeding, preserving genetic diversity, but also genetic progress. It is defined as the proportion of total offspring each animal of the current population should generate to limit inbreeding levels while enhancing genetic progress to the next generation (Meuwissen, 1997).

As we are limited by the resources that we can use to preserve breeds, assessing genetic diversity is critical. Kinship coefficients and genetic distances, by establishing genetic links between populations or breeds, help us to understand at which level a population or breed is irreplaceable (Laval et al., 2000; Eding et al., 2002). Inbreeding coefficients state relationships within the population (Upadhyay, 2018). As inbreeding increases, the risk of homozygosity, and thus, the risk of expressing deleterious alleles also increases (Bosse et al., 2012). The effective population size (N_e) is another parameter that can give an insight of sustainability of a breed (Meuwissen & Woolliams, 1994b). These tools are highly precious to determine the degree of endangerment of each breed, and therefore, the ones to be conserved. This way of working involves a ranking of breeds or populations regarding genetic variation. However, the main risk with this method is that limited-size populations or breeds may not be contributing highly to global genetic diversity and thus could be considered as being not really worth it. It is therefore, really important to check if the alleles they carry are rare and specific (Eding et al., 2002).

As we have seen in section 2.3., high-density SNPs are commonly used to select economically important features (Herrero-Medrano et al., 2013), but it is only one way of exploiting the advantages that genomic evaluation can provide. The evolution of genomics during the last decade paved the way for the use of novel preservation tools (SanCristobal et al., 2006a) such as LD. In inbred populations, correlations between alleles are high (and so is LD) because the portions of genome are highly conserved from one animal to another (Oldenbroek, 2017). The LD is thus, proportional to inbreeding, genetic drift, selection, and inversely proportional to the recombination rate of the chromosomal area (Amaral, 2010). Regions of Homozygosity (ROHs) are related to LD. The length of these regions was shown to correspond well to inbreeding coefficients based on pedigree. For example, a past limited-size population which was not under high pressure of inbreeding recently shows a high number of short ROHs (Herrero-Medrano et al., 2013). The ROHs characteristics are therefore due to population history like bottlenecks, genetic drift and selection process (Herrero-Medrano et al., 2013; Bosse et al., 2015). Thereby, N_e is an important parameter to assess genetic diversity as long as it depends of population history events (SanCristobal, et al., 2006a; Bosse et al., 2012). Another parameter of importance to preserve rare and small breeds is to determine marker-by-marker coancestry (or inbreeding) or shared regions in the genome. Bosse et al. (2015) demonstrated that these methods better preserve diversity and fitness. In this way, real inbreeding levels, because crossing-overs are considered, are known and conservation measures as well as mating can be precisely planned (Bosse et al., 2015). The use of genomic tools allows us to detect rare or deleterious genes to be, respectively, conserved in and removed from the population, as well as exogen alleles that result from crosses of the endangered breed with a sister one, of greater importance (Bosse et al., 2015; François et al., 2017; Eynard, 2018).

Despite all the insights genomics can provide in terms of population history and conservation perspectives, their use in small-sized pig breeds is still limited (Herrero-Medrano et al., 2013). Genomic tools are used more often on mainstream than rare breeds, which could increase the gap between them (François et al., 2017; Oldenbroek, 2017). Moreover, small populations are commonly not recorded as their standard breed's counterparts. This involves a need for genomic-based estimators to search for pedigree errors, calculate allele frequencies and know the IBS (Identity by State) (Eynard, 2018).

Once the status of the breed or population is known, conservation measures can be set up. Breeders have obviously a key role to play in the preservation of the breed by their management strategy, i.e. their mating schemes or transfers of animals. However, other institutions can help them, i.e. by cryopreservation of gametes in a gene bank, by research about threatening diseases or about the breed characteristics (FAO, 2013).

Conclusion

We have covered the overall context of the Piétrain breed. As stated, Piétrain sires are nowadays largely used in terminal crosses to obtain a finishing pig with good conformation and high meat percentage. However, the current situation of Piétrain is precarious in Wallonia. Besides the general bad economic and pathogen conditions inherent to every pig holding, pure Piétrain breeding lacks new and young breeders in Wallonia and is at risk of being overwhelmed by commercial firms. Moreover, purebred pig populations are, most of the time, of very limited size whereas crossbred pigs exist in great number to meet the huge pork meat demand. Therefore, as only four main breeds are intensively used worldwide for pork meat production, it is important to maximize genetic diversity of pigs in Wallonia, in Europe and worldwide in general, even within these four main breeds.

To preserve the Walloon Piétrain population, two first solutions were already implemented. Progeny testing allows better genetic evaluations of Piétrain boars whereas the Belgian Piétrain program is based on cryopreservation of the boars considered representative of the genetic diversity.

We have seen that different genetic and genomic parameters could be used to determine the genetic diversity of a population or a breed. Assessing the genetic diversity of the Walloon Piétrain pigs seems therefore to be another step towards the conservation of this population. For this purpose, in the next chapters, the Walloon Piétrain pedigree will be used to determine several genetic parameters and to have an insight of genetic distances between farms. The EBVs will also be analyzed allowing to determine the phenotypic diversity as well as breeding objectives of Piétrain owners. Both types of information will be combined with information about transfers of animals to suggest some conservation measures to the Walloon Piétrain breeders and to the Belgian Piétrain program. Genotypes of other Piétrain populations will then be used to have a glance of the overall genetic diversity found in the breed. Finally, as the origin of the Piétrain breed is not definitely known, positions of different breeds compared to this of Piétrain will be assessed through genotypes to determine which breeds may be involved in its genesis.

Chapter II: Data, material and methods

Introduction

This chapter was divided into five main parts: historic review, pedigree, pseudo-phenotypes, transfers and genotypes analyzes. The main objective was to obtain an overview of the Piétrain breed diversity.

1. Historic review

To obtain information about the origin of Piétrain pigs, Jean Lempereur, breeder of Piétrain pigs in the region of Grosage (Wallonia, Belgium) was interviewed. He provided a lot of archival documents that were very useful to reconstruct the probable origin of the breed. Henri Stas, breeder of Piétrain in Piétrain itself, was also interviewed about the origin of the breed.

2. Pedigree
2.1. Data and pre-processing

The pig pedigree, provided by the awé, is essential to analyze genetic links of animals like inbreeding or kinship coefficients. It contains notably ID of the animal, its tattoo, ID of its sire and its dam, breed, sex, birthdate, culling date, province and exploitation. These data were dispersed in different files and the first step was therefore to merge all the interesting files to extract important information. With SAS software (SAS group, 2014), ID, ID of the sire and the dam and group of pigs were extracted. In pedigree study, boars were assigned to a group according to their breeder (hereafter called breeder-group). The hypothesis was that breeders are responsible for mating choices, i.e. the genetic history of boars. File of boars' breeders was provided by the awé.

Detecting errors in the pedigree was done with CFC v.1.0 software (Sargolzaei et al., 2006). Some animals appeared as both sire and dam. According to their sex, the information was corrected. Others were appearing as their own ancestor (cycling pedigree) and were therefore corrected. Finally, some animals appeared twice in the pedigree as the merging process implied on farm-measures where animals can sometimes have been measured twice. Duplicates were thus removed.

2.2. Pedigree parameters

Pedigree can be used to assess different parameters such as N_e or inbreeding coefficient. The knowledge of these parameters is the first step to come up with conservation measures (Eding et al., 2002; Bosse et al., 2012).

Total pedigree number of animals, sires, dams, founders, number of generations and inbreeding coefficients distribution were computed via CFC v1.0 software (Sargolzaei et al., 2006). A total of 219 progeny-tested boars were used for further analysis. The last batch of these 219 boars (n=5) entered the station in March 8th, 2018. Their piglets were born two months earlier (between January 3rd and 4th), and slaughtered between June 27th and August 2nd. Maximum and average inbreeding coefficients and number of unique ancestors per breeder-group were also computed via CFC v1.0 software (Sargolzaei et al., 2006). For all tested boars, kinship coefficients, average inbreeding coefficient, completeness, N_e and genetic diversity parameter were assessed by optiSel v.2.0.2 R package (Wellman, 2018).

Inbreeding coefficients were defined as the probability that two alleles, randomly chosen from sire and dam, are Identical by Descent (IBD) whereas kinship coefficients were defined as the probability that two alleles, randomly chosen from two individuals, are IBD (Eding et al., 2002; Wellman, 2018). The completeness was defined as proposed by MacCluer et al. (1983):

$$\frac{2*C_{sire}*C_{dam}}{C_{sire}+C_{dam}} \tag{1}$$

and,

$$C=\frac{1}{d}\sum_{i=1}^{d}g_i, \tag{2}$$

in which, C is the contribution of sire or dam, g_i is the proportion of ancestors present in generation i, and d is the total number of generations. In this study, d was fixed to four generations as proposed by Wellman (2018). Completeness is a very important parameter: if ancestors are sufficiently known, other parameters such as genetic diversity or kinship coefficients would be more reliable (Li et al., 2011).The genetic diversity parameter was computed as 1-mean of kinship coefficients.

N_e, defined as the theoretical number of non-inbred parents that would give birth to the current population (Wright, 1931), was:

$$\frac{1}{2\overline{\Delta c}}, \tag{3}$$

where $\overline{\Delta c}$ was the average of Δc_{ij} defined as:

$$\Delta c_{ij} = 1 - \sqrt[\frac{g_i + g_j}{2}]{1 - c_{ij}}, \tag{4}$$

c_{ij} is the kinship coefficient between individuals i and j, and g_i, g_j are the numbers of equivalent complete generations of individuals i and j (Wellman, 2018). Some assumptions were made for N_e: 1- the population is isolated; 2- mating are panmictic; 3- the population growth is linear (Herrero-Medrano et al., 2013). Finally, the number of equivalent complete generations was characterized as the sum of the proportion of known ancestors over all traced generations (Wellman, 2018).

2.3. Multi-Dimensional Scaling

A classical MDS analysis was performed by using the cmdscale R function (Kabacoff, 2017). MDS allows to structure the population within and among farms (François et al., 2017). Genetic diversity might be accessed by using the opposite of kinship coefficients as long as they reflect genetic distances among animals (Laval et al., 2000). The MDS is based on a dissimilarity matrix and, thus, 1 - kinship coefficient.

3. Transfers of animals among breeders

Based on information given by the awé, the breeders and current owners of the 219 boars were determined. The reason why breeders and owners are different definitions is due to "breeders" are responsible for the genetic origin of the animal whereas "owners" are considered nominators of animals to progeny testing. Additionally, to interpret correctly the different analyses, Walloon pure breeders were interviewed in a semi-directive manner about their transfers of animals within Wallonia and also across other regions of Europe.

4. Pseudo-phenotypes

4.1. Data and pre-processing

The EBVs of boars of which progeny was tested at station (n=219) as well as their reliabilities were obtained from Gembloux Agro-Bio Tech where genetic evaluations are performed. Six at-station EBVs were available: ADG (g/j), carcass weight (kg), backfat thickness (mm), meat percentage, conformation index and consumption index. Four on-farm EBVs were also used: backfat thickness (mm), meat percentage, live weight (kg) and loin muscle depth (mm). To have more information about Piétrain genetic evaluations in Wallonia, please refer to section *4.4.3. Genetic evaluation of Piétrain in Wallonia* in the literature review.

Boars were assigned to a group according to their current owner (hereafter called owner-group), also considered the nominator for testing. The hypothesis was that the animals reflect the breeding objectives of this nominator. Information about owners was provided by the awé.

The EBV file was merged with others to obtain one file with ID of the animal, its owner, EBVs and their reliabilities. In order to avoid any bias because of differences in reliabilities due to EBV regressed nature, corrected phenotypes should be used. Moreover, direct phenotypes of purebred boars are not directly usable for two reasons: they are not always directly recorded on farm and do not reflect performances in crossbreeding. To overcome these limitations, EBVs were deregressed; i.e. they were divided by their reliabilities (Garrick et al., 2009), in order to obtain pseudo-phenotypes.

4.2. Correlations, PCA, Mahalanobis distances and separation power

Correlations between traits (i.e. deregressed EBVs) were computed. By using the FactoMineR v.1.42 R package (Lê et al., 2008), two PCA were further performed to assess the distribution of boars according to their pseudo-phenotypes values. The first PCA focused on distribution of boars among breeder-groups whereas the second one focused on their distribution according to stress status. For these PCA, only six pseudo-phenotypes were exploited, i.e. offspring performances. This allowed to use traits that were more uniformly tested and more reliable. For each animal, to weight PCA, we took the mean of its six at-station traits reliabilities. Weighting was applied because, even if values are on the same scale for each trait, there are still some major differences due to reliabilities.

Mahalanobis distances (Whitfield et al., 1987) among owner-groups were then assessed using the HDMD v.1.2 R package (McFerrin, 2013) and a dendrogram established. The objective of this test was to discriminate owner-groups. Separation power of the six different traits tested at station were

finally computed to determine for which traits the different owners were the most dissimilar. Separation power was defined as the variance among owner-groups divided by the variance within owner-groups (Coghlan, 2019). This analysis was performed by means of MASS v.7.3-51.3 R package (Ripley et al., 2019).

5. Genotypes
5.1. Data

Piétrain genotypes from the Netherlands and the USA were obtained from the Dryad Digital Repository (Yang et al., 2017) as well as some German Piétrain genotypes. Genotypes from Baden-Württemberg, North Rhine-Westphalia and Schleswig-Holstein were provided by the University of Hohenheim (Germany). The KULeuven University, provided a merged binary file with both data. The Bavarian State Institute for Agriculture (LFL), provided Bavarian Piétrain boars genotypes. Genotypes from all other pig breeds came from the Dryad Digital Repository (Yang et al., 2017). All these populations were genotyped by the *Porcine60kBeadchip v2* (Illumina, n.d.), defined in section *2.3. Genomics*.

5.2. Pre-processing

The pre-processing of genotypes was made via PLINK v.1.9 (Purcell et al., 2007, 2019; Chang et al., 2015). First, the different data were merged. For this purpose, when necessary, XY chromosome, which corresponds to pseudo-autosomal region of X chromosome, was encoded as X chromosome. The X and Y chromosomes were then encoded as chromosomes 19 and 20, respectively. This was made through "–chr-set 18" and "–merge-x" functions. Pigs carry 18 pairs of chromosomes without the sexual pair (Guru Vishnu et al., 2015). Family and individual IDs as well as the sex were recoded, when necessary, by using "–update-ids" and "–update-sex" functions. Triallelic SNPs (due to real trialleles or to differences of encoding between the two data sets) were swapped via "–flip" function. The remaining triallelic SNPs were excluded from further analysis by using the "–exclude" function. Finally, the data sets were merged according to Illumina *Porcine60kBeadchip v2* specifications (Illumina, n.d.) through "–bmerge" function.

The quality control of genotypes, by using "chr 1-18" function in PLINK v.1.9, was then implemented to remove SNPs in sexual and 0 chromosomes. Chromosome 0 refers to problem probes or sequences used for chip quality control (Eccles, 2011). Call rates (genotype and individual) < 0.90

and Minor Allele Frequencies (MAF) < 0.01 were also removed. The MAF threshold was chosen to keep the maximum genetic diversity among samples. These filters were applied by "–maf 0.01", "–geno 0.1" and "–mind 0.1" functions. Table 1 provides the thresholds reported in the literature.

Table 1.

Genotypes quality control thresholds reported in literature

Filter	Threshold	Reference
MAF	0.05	Bosse et al., 2012 Herrero-Medrano, 2013
	0.03	Stratz et al., 2014
	0.01	Bosse et al., 2015 François et al., 2017 Yang et al., 2017
Genotype call-rate	0.95	Bosse et al., 2012 François et al., 2017 Stratz et al., 2014
	0.90	Bosse et al., 2015 Yang et al., 2017
Individual call-rate	0.90	Stratz et al., 2014 Yang et al., 2017

5.3. Investigation of the Piétrain breed origin

A MDS was performed for all the European breeds available in the Dryad Digital Repository (Yang et al., 2017) to determine which ones were the closest to Piétrain breed. Then, a MDS was reperformed by using only the closest breeds in order to have a better overview. These MDS were performed with PLINK v.1.9 (Purcell et al., 2007, 2019; Chang et al., 2015) by using "–cluster" and "–mds-plot" functions. The results were plotted via Rstudio v1.2.1335 (RStudio team, 2015). The MDS were based on genetic distances, i.e. Hamming distances. Hamming distances, applied to genetics, are simple counts of differences between two strands of DNA (Hamming, 1950). Only SNPs in Hardy-Weinberg equilibrium (–hardy midp and –hwe midp options; Wigginton et al., 2005; Graffelman & Moreno, 2013) were kept for MDS. Hardy-Weinberg equilibrium supposed notably the absence of selection and migration (Wigginton et al., 2005). It is thus more relevant to determine the origin of a breed when erasing the effect of selection. The threshold was fixed to 1.10^{-16} because it allowed to

keep enough SNPs (4,601 variants instead of 413 with 1.10^{-6} threshold). Thus, variants completely not in Hardy-Weinberg equilibrium were removed for further analysis.

5.4. Analyzing the genomic diversity of European Piétrain populations

These analyses were performed through PLINK v.1.9 (Purcell et al., 2007, 2019; Chang et al., 2015) and plotted in Rstudio v.1.2.1335 (RStudio team, 2015). A MDS based on Hamming distances as previously defined (Hamming, 1950) was performed on the different Piétrain populations. However, the Hardy-Weinberg filter was not applied as long as the objective was not to investigate the origin of Piétrain but to determine the effects of selection process on these populations. To avoid overweighting due to LD, which are alleles non-randomly associated (Amaral et al., 2008), a filter was applied through "–indep-pairwise" function. The different thresholds were defined according to Yang et al. (2017): 50 for window size, 10 for step and 0.2 for r². The r² is used to define a threshold of correlation between SNPs to be considered as LD. To determine the genetic differentiation degree among Piétrain populations, the F_{st} statistic (also known as fixation index) was determined. It was defined as in Weir & Cockerham (1984):

$$\frac{\sigma^2_p}{\bar{p}\,(1-\bar{p})}, \tag{5}$$

where σ^2_p and $\bar{p}$ are the variance and the mean of allele frequencies, respectively.

Inbreeding coefficients were investigated through two coefficients (Fhat1 and Fhat3) proposed by PLINK v.1.9 (Purcell et al., 2007, 2019; Chang et al., 2015). Fhat1 is based on the variance-standardized relationship minus 1. Fhat3, based on Genomic Relationship Matrix (GRM), is clearly defined in Bérénos et al. (2016):

$$\frac{x^2_{ij} - (1+2p_i)x_{ij} + 2p^2_i}{2p_i(1-p_i)}, \tag{6}$$

p_i is the allele frequency of the ith individual and x_{ij} is the number of copies of the reference allele for the ith individual and the jth SNP marker. Homozygous minor alleles have more weight with this method than homozygous major alleles. The underlying interpretation is the correlation among uniting gametes. These two coefficients were obtained through "–ibc" function with the "–read-freq" function used for adequate estimations through loaded MAF. The same LD pruning as for the previous MDS was made to avoid overweighting of SNPs. The Fhat2 coefficient, also proposed by PLINK v.1.9, was not used for studying inbreeding levels as it does not weight more homozygous minor alleles than major ones.

Another way to have an insight of inbreeding is through ROHs. Runs of homozygosity are homozygous segments of the genome (Herrero-Medrano, 2013). The "–homozyg" function was used to determine them. The same parameters as Yang et al. (2017) were chosen: a sliding window of 50 SNPs, 5 missing calls maximum, 1 heterozygous SNP maximum, a minimum of 50 SNPs to be defined as ROH ("–homozyg-snp function") as well as a minimum length of 500 kb ("–homozyg-kb function"). Pruning of LD was not performed before this analysis as ROHs and LDs are correlated.

Chapter III: Results and discussion

Introduction

In this chapter are developed the results and discussion of pedigree, transfers, pseudo-phenotypes and genotypes analysis. Moreover, before the section related to genotypes, the usefulness of assessing diversity by both pedigree and phenotypes as well as conservation recommendations based on them are discussed.

1. Pedigree analysis
1.1. Pedigree parameters
1.1.1. Overall pedigree parameters

The number of animals in the overall pedigree was 777,321. These animals, of which 12,148 boars and 45,307 sows had offspring, were born between 1970 and 2018. In 1980, the electronic Pigbook recording started in Belgium and the number of animals registered increased up to 1992. A decreasing trend has been observed since then. This may be the first indicator of pure breeding activity decline. This aforementioned behavior was also reported by Welsh et al. (2010) working with several pedigrees from different pig breeds in the USA. It indicates a pig production worldwide trend, probably due to the emergence of breeding companies. In 2003, there was a sharp decrease of the number of animals registered in Belgium. This was the result of the disengagement of Walloon and Flemish Pigbooks caused by the reorganization of Belgian agriculture. Between 2011 and 2014, it was observed a slight increase likely due to healthy economic conditions (SPF Economie, 2015), but also to good dynamics in the Walloon pig breeding. From 2014 to 2017, due to low income earned by breeders (SPF Economie, 2015), the number of animals registered halved (from 2,144 to 1,181). Therefore, the Walloon Piétrain population is currently under a strong bottleneck.

The analysis of the longest ancestral path indicated that there were 30 generations of animals. This number was greater than the number of generations for breeds reported in other studies (e.g. 17-19 range in Welsh et al., 2010). In the overall pedigree, a total of 10,557 animals were founders, of which 8,031 had offspring (2,220 boars and 5,811 sows). For the non-founders, more than 99% of animals had both known sires and dams. None of the animals had an inbreeding coefficient higher than 41% and around 99% of the animals had inbreeding coefficients smaller than 20%. The maximum level of inbreeding was smaller compared to other breeds in the USA: 51% for Landrace and 65% for Large White (Welsh et al., 2010). The average inbreeding was estimated to 4.15%. These values indicate that inbreeding is not yet an issue in the studied population. This can be explained partially by the fact that the number of animals recorded in the Pigbook was relatively high. Moreover, pedigree

information from 1950 to 1970 were not available, which could underestimate inbreeding coefficients. A study from Hanset (1973) assessed the inbreeding coefficient of animals of this earlier part of the pedigree. For boars of which both sire and maternal grand-sire were known, it decreased from 15.62% in 1951 to 5.28% in 1960. At the beginning, some remarkable boars were intensively used by breeders leading to high levels of inbreeding. Hanset (1973) added that this inbreeding level was however smaller than those observed for other breeds (e.g. Large White, Danish Landrace, Berkshire). The Piétrain breed seemed therefore to undergo less inbreeding than other breeds. Therefore, three key elements could explain these low levels of inbreeding: 1- a missing part of the pedigree; 2- a former large population; 3- less inbreeding strategy than for other breeds.

1.1.2. Boars pedigree parameters

The mean Index of Pedigree Completeness (PCI) for the 219 boars was 0.95. The vast majority of these boars had PCI higher than 0.60. According to Li et al. (2011), this value is considered sufficient. However, some boars, especially imported ones, had limited information due to incomplete pedigree transfer. The mean number of equivalent complete generation for boars was 9.97. This latter parameter seemed to be the most appropriate for pedigree completeness investigation (Wellman, 2018). The pedigree of the 219 boars seemed therefore to be sufficiently complete for further analyses. In general, Walloon tested boars had a deep pedigree. It seems logical as Walloon boars' progeny tested pedigree should be sufficiently complete.

There were 19 breeder-groups of boars: five from Flemish (n=12 animals), one from German (n=4 animals) and 13 from current and former Walloon (n=203 animals) breeders. Flemish and German breeder-groups were kept to determine relationships of these populations with Walloon Piétrain pigs. The average kinship coefficients within each breeder-group, by excluding self-relationships, ranged from 0.01 to 0.40. The mean inbreeding coefficient for the boars was relatively small (2.74%). All breeder-groups had average inbreeding coefficients under 10%. However, by looking at the maximum inbreeding coefficient, five out of 19 breeder-groups (2, 4, 7, 8 and 13) provided boars with inbreeding coefficients equal or higher than 10%, with the maximum inbreeding coefficient equal to 21% (breeder-group 4, n=11 boars). This means that some breeders, by purpose or not, leant towards higher levels of inbreeding. This fact can be seen as a management strategy since breeders may tend to send optimal boars for crossbreeding. In this situation, high level of individual inbreeding coefficient of the parent would not be apparent on offspring. It seemed however that the majority of breeders paid particular attention to inbreeding and succeeded in its management. As explained before,

inbreeding levels from 1950 to 1970 could not be computed since this part of the pedigree was not available. It could underestimate inbreeding estimations. It seemed also that inbreeding coefficients of boars, relatively low, were the result of a former large population.

The number of unique ancestors for each breeder-group ranged from 2, for a breeder-group of one animal, to 4,386 for a breeder-group of nine boars. It appeared that another breeder-group was composed of only one boar but this had more unique ancestors (1,220) than the one with two unique ancestors. The group with only two unique ancestors was imported and had limited pedigree (only parents). Also, a breeder-group composed of 55% of animals did not have the higher level of unique ancestors. This can be explained by two points: 1- after a certain number of animals provided by the breeder, the number of unique ancestors of his animals reach a plateau because the pedigree is limited and 2- there is a certain variability between breeders for the number of unique ancestors. A high number of unique ancestors can be explained by the deepness of pedigree and it reinforces the idea of a former large population.

The genetic diversity parameter among boars was 97.96%. The N_e was 223, thus, inside the minimum range 31-250 suggested by Meuwissen & Woolliams (1994b) for sufficient fitness. The different genetic parameters and the PCI mean can be considered as reliable values. It seemed that a high genetic diversity exists in the Walloon Piétrain population. It should however be noticed that this high N_e was maybe due to Flemish and German animals of which ancestors could be different from Walloon animals. Besides, some imported animals had cut pedigree which involved that the assumption of ancestors missing at random for the N_e formula was not observed (Wellman, 2018). Moreover, as inbreeding effects due to intensive use of one boar can be seen a few generations after (Robertson, 1961), it would be important to avoid a current excessive use of one boar in the population.

As a single breeder provided 55% of the boars, potentially not all the diversity was actually captured. Ideally, each Walloon farm should provide 10% (n= 10 current breeders) of the tested boars. As long as different breeders have not the same number of animals available, the boars in progeny testing should at least be proportional. This point is crucial for the breed preservation, and especially for the Belgian Piétrain program success. This program should be able to have access to the most diverse boars as well as to a balanced number of animals coming from different farms.

1.2. Multi-Dimensional Scaling

The Figure 3 points out four main clusters of boars. The first one presented the greatest diversity even though all boars came from the same breeder (breeder-group 1, who provided 55% of the boars). These animals might have an influence from German boars as some of them were near to breeder-group 14 (different German breeders not identified in the data base). In general, the second cluster was composed by breeders from the Walloon Brabant province and seemed to have genetic links within them mainly due to transfers (breeder-groups 2, 4, 7 and 10). This cluster can be divided in two sub-clusters that did not transfer animals but were somehow linked to the core cluster. The third cluster was composed by animals from breeders from Liège and Walloon Brabant provinces (breeder-groups 8 and 13). Some animals from breeder-groups 1, 7, 16 and 19 seemed to have links with animals from this cluster. At last, it was observed a core cluster that grouped mainly Hainaut province, Flemish (breeder-groups 5, 12, 16, 17 and 18) and German breeders (breeder-group 14). In general, genetic links among groups fitted well with statements from interviewed breeders. This MDS therefore seemed reliable.

The core cluster would indicate higher rates of inbreeding. However, by looking at the maximum inbreeding coefficients of these breeder-groups (from 0.00%, for boars of limited pedigree, to 3.96%), in general, it did not actually seem the case. Note that the first two components had a goodness of fit of 7.14%. This small percentage was expected since a 219 x 219 matrix was reduced to a two-dimension one. This could explain why these breeder-groups did not express high inbreeding levels. They only seemed to be close in the first two-dimension plan but they were not on other plans.

Currently, members of all four clusters are active, even if breeders 7, 11, 13 and 15 put an end to their activities. The MDS analysis can help us to develop conservation measures regarding genetic diversity and relationships among farms (Herrero-Medrano et al., 2013). It would be important to preserve diversity from each cluster. As previously mentioned, the MDS was only a glance of the genetic diversity existing in Walloon Piétrain pig population as boars of certain breeders were more represented at progeny testing. In addition, all boars did not enter progeny testing but only those owners considered as being superior. Breeder 1 was the only exception since it seemed that almost all of his boars entered progeny testing.
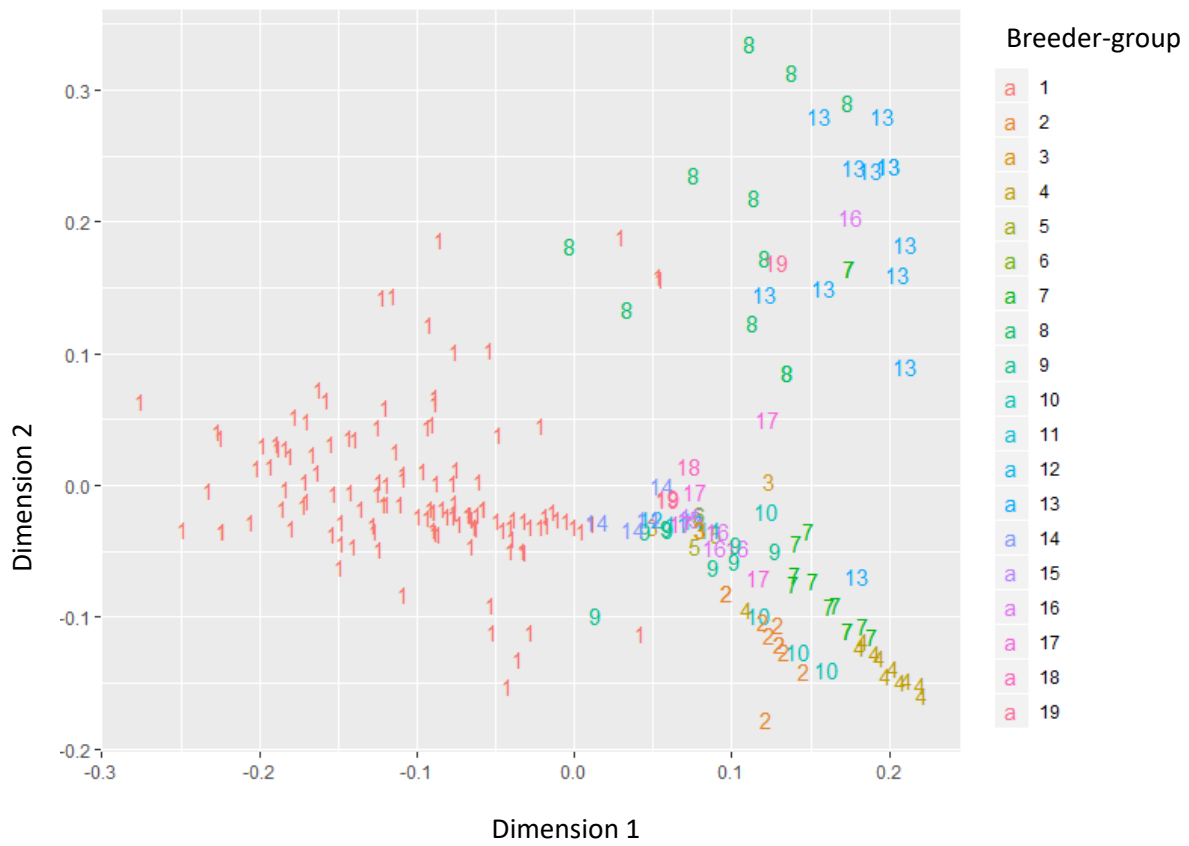
Figure 3. Multi-Dimensional Scaling between 219 progeny tested boars from 19 breeder-groups.

2. Transfers of animals

The Figure 4 depicts transfers of animals from breeders to owners. As a reminder, breeders are responsible for the origin of the pig (birthplace) whereas owners are responsible for boars' progeny testing selection. In order to clarify, identical numbers were used to identify the same person (same number for breeder and owner in the graph). Furthermore, the CIAP and the Belgian Piétrain program were considered as owners.

Boars' transfers from Walloon breeders to Walloon owners were almost inexistent. Only one transfer appeared between breeder 13 and owner 6. By combining this information with semi-directed interviews, we can infer that, even if transfers of pigs have occurred among Walloon farms during the last ten years, they were relatively scarce. In addition, it seemed that owners/breeders have bought/sold animals from/to the same breeders/owners. Therefore, some nuclei of breeders were created as already seen on MDS analysis (Fig.3).

On the other hand, some owners have bought boars in Flanders, and therefore, it seemed that there were two types of situations in Wallonia: 1- breeders who did not transfer animals (or very few); 2- breeders/owners who sometimes sold/bought animals in Flanders. These two strategies were also seen in the MDS (Fig.3) where breeders from type two were in the core cluster whereas breeders from type one were spread all around. The fact that transfers were an uncommon practice can explain the great diversity previously found in the studied population as boars sent to progeny testing came from different lines. It also means that excessive use of one boar in the current population is not expected. Moreover, the limited transfers from foreign regions could mitigate inbreeding coefficients at the farm level and relatively increase the overall genetic diversity. In a thoughtful manner, transfers of animals between Walloon farms may increase genetic variability among them (Gomes Arandas et al., 2017). An increase of genetic diversity in each farm would therefore be expected as different boars would be added to each farm pedigree while the overall N_e of boars would decrease as boars sent to progeny testing will share more ancestors. This N_e decrease would allow better genetic progress (Meuwissen & Woolliams, 1994a). Low transfers also partially explain the high N_e observed. Some farms showed relatively high levels of inbreeding which involved less heterozygotes. As aforementioned, boars which are progeny tested are expected to be used in a balanced manner in the population. Thus, even if the population is undergoing a bottleneck, a low genetic drift could be inferred from these two latter facts resulting in high N_e (Caballero & Toro, 2000).

The specificity of the Walloon population might also be under threat by transfers from Flanders. This kind of animals should not be privileged in the Belgian Piétrain program since its main

objective is to maintain genetic diversity of Walloon Piétrain population germplasm. The Figure 4 also shows that Flemish breeders sold regularly boars to the CIAP. In this figure, German breeders did not transfer any animals at all. It did not mean that they have never bought or sold animals in Wallonia but it was not the case for animals sent to the CIAP.

Besides transfers between breeders and owners, another type of gene flow existent is due to artificial insemination. This kind of gene flow was evident in the MDS analysis (Fig.3). In summary, close breeder-groups of animals share common ancestors, in recent or even distant past, due to animal transfers between farms as well as artificial insemination.
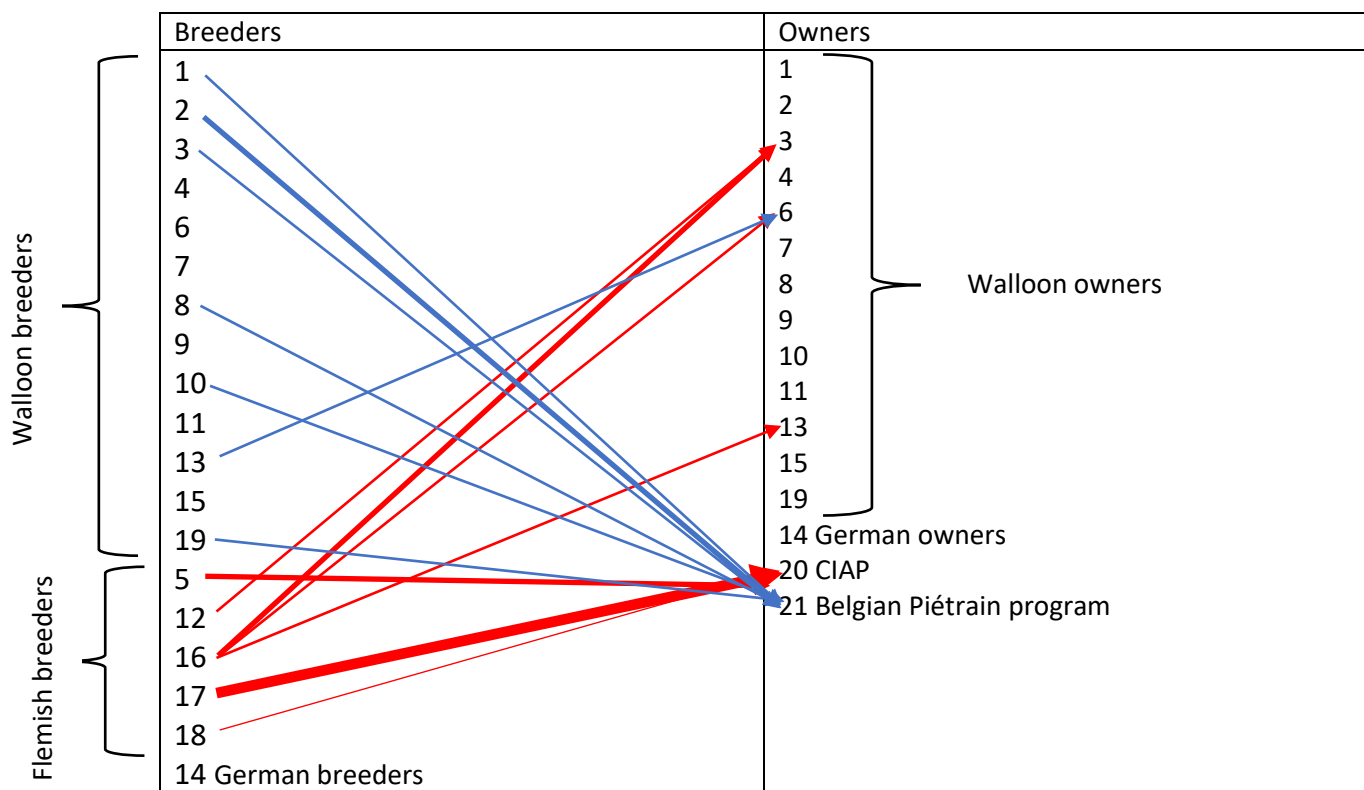

Figure 4. Transfers of boars. The thickness of arrows is proportional to the number of boars transferred. Blue arrows represent transfers from Walloon breeders. Red arrows represent transfers from Flemish breeders.

3. Pseudo-phenotypes analysis

3.1. Descriptive statistics

A total of 16 different owners sent boars for progeny testing. A large variation in the number of boars selected per owner was noticed: there was an owner that sent less than 1% whereas another one provided 55%. The latter provided good and lesser good boars which mitigated this imbalance. This disparity in the number of boars provided by owners to progeny testing could be explained by differences in the total number of animals found in farms and/or by the fact that some breeders did not think to benefit from providing more boars to progeny testing. As some breeders can be considered as close to retirement, their investment could also have decreased. Moreover, 13 Walloon owners have sent boars for progeny testing in 2007. This means a loss of 4 owners in ten years for progeny testing program (4 former owners, 9 current owners and 1 farm that has never sent any boar to progeny testing).

The graph of correlations (Fig.5) indicates several strong correlations between certain traits which justified the realization of a PCA (Gomes Arandas et al., 2017). The ADG was negatively correlated with consumption index and positively correlated with live weight. Indeed, the consumption index represents the quantity of feed necessary for an animal to gain 1 kg: lower means better. Obviously, if ADG is more important, the boar has better chances to have a higher live weight. Backfat thickness was negatively correlated with meat percentage, on farm or at station. It seems logical: a boar with more fat has a lesser meat percentage. Conformation was not really correlated with any of the traits. The good development of muscles seemed thus to be not correlated to muscle, fat or growth traits.
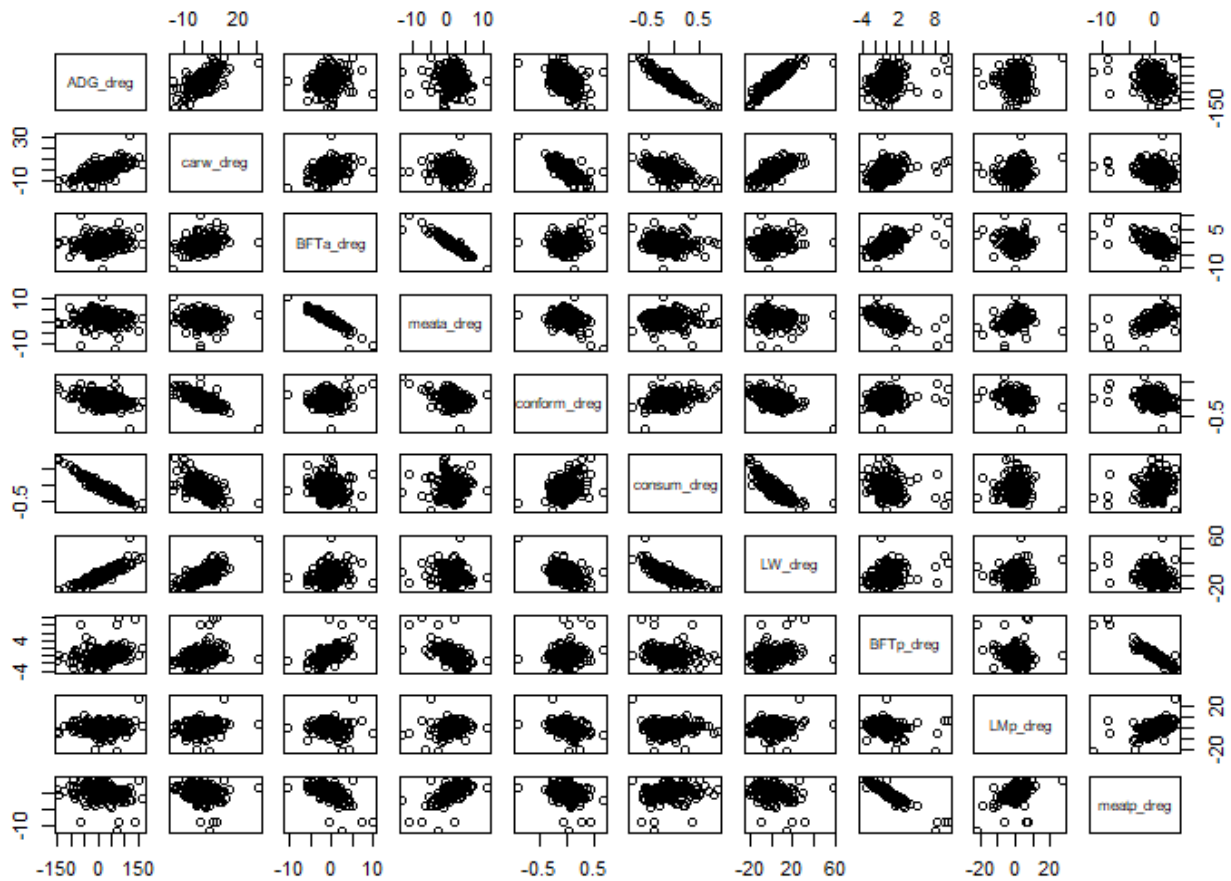
Figure 5. Correlation graph of traits measured on farm and at station. ADG_dreg: deregressed EBV of average daily gain; carw_dreg: deregressed EBV of carcass weight; BFTa_dreg: deregressed EBV of backfat thickness at station; meata_dreg: deregressed EBV of meat percentage at station; conform_dreg: deregressed EBV of conformation index; consum_dreg: deregressed EBV of consumption index; LW_dreg: deregressed EBV of live weight; BFTp_dreg: deregressed EBV of backfat thickness on farm; LMp_dreg: deregressed EBV of loin muscle depth; meatp_dreg: deregressed value of meat percentage on farm.

3.2. Principal Component Analysis

Even if the pedigree analysis allows us to detect past gene flow, it does not illustrate current breeding decisions that reflect breeding objectives. Trajectory of the Walloon population can be determined by means of PCA. Eigenvalues and percentage of variance are presented in Table 2. The first two components were chosen with the criteria of eigenvalue superior to 1 and a minimum of 70% of the variance explained (Gomes Arandas et al., 2017). The first component explained 49.28% of the variance and the second 34.70% (83.98% in total). They seemed therefore to be sufficient to explain the pseudo-phenotypic variation in this population. Figure 6 illustrates relationships between traits and components of the PCA. Table 3 presents the correlations of the different traits with the first two components and their p-values whereas Table 4 shows the contributions of these traits to the PCA. Figure 7 depicts positions of boars, colored by owner-group, on the PCA whereas Figure 8 depicts the same PCA but colored by stress status. In Figure 8, group 1 represent stress-positive animals (nn), group 2 heterozygotes stress-negative (Nn) and group 3 homozygotes stress-negative (NN). In Figures 6, 7 and 8, it was observed that the first component was related with growth whereas the second one with meat traits. Conformation was explained by both components. From Figure 7 and 8, it can be seen that animals of the first quadrant (clockwise) had a high backfat thickness. Boars on the first two quadrants and near the X-axis had high carcass weight and ADG whereas in the third quadrant, they had a high meat percentage. Finally, in the last quadrant, they had high conformation and consumption indexes.

While MDS based on pedigree highlighted gene flows among farms, often linked to geographic distances, the first PCA (Fig. 7) displayed selection objectives of farms. Animals from owner 1 were well sparse but almost all were located on the first two quadrants which implied growth traits selection. By excluding owner-groups 1, 11, 14 and 19, we can see that most owners have selected more for meat or conformation traits, the specificities of the Piétrain breed. It means that breeders chose one of the following strategies: 1- minimizing input costs and increasing the total price perceived by carcass or 2- optimizing the price perceived by kg of carcass. As feed represents around 70% of production cost (Leroy et al., 2000), the first strategy seems cost saving.

In Figure 7, it can also be seen that some owner-groups (6, 9, 10, 11, 14, 20 and 21) presented a great dispersion. Owner-groups 20 and 21 represented respectively the CIAP and the Belgian Piétrain program. This explains the dispersion observed in these owner-groups. Owner-group 14 regrouped different German owners which also clarifies the distribution among this owner-group. Owner-group 11 presented very high backfat thickness which is particularly uncommon in Piétrain pigs. However, this owner put an end to his business and caused biodiversity losses. Owner-group 6 was an outlier

and had distinguishable characteristics that could be preserved. However, this owner was closely linked with Flanders and therefore with less Walloon characteristics. Moreover, owner-group 6 was only composed of two animals, not raised by the same breeder, which also explains why boars from this owner-group were so far from each other. As owner-group 11 no longer exist, the diversity of owner-groups 9 and 10 should be preserved, especially for group 9 that was distinct from other owner-groups. The Belgian Piétrain program (owner-group 21) seemed to choose high conformation boars. Our results may be useful and could suggest that the inclusion of animals with greater meat percentage (e.g. from owner-group 4) is important due to its current lack.

From Figure 8, we can observe that there was a great diversity of pseudo-phenotypes for stress-positive animals. They were distributed in all directions on the graph and the mean of this group was very close to the graph center. Some animals even showed better growth traits than stress-negative animals. It is thus possible to select Piétrain stress-positive boars with good ADG and carcass weight. Heterozygous boars were also distributed among all the quadrants. However, they were less numbered and tended less to the graph center. There were a few homozygous stress-negative and they seemed to be more selected for growth traits. This is logical as stress-negative animals tend to be less conformed and showed lower meat percentage (Leroy et al., 2000; Youssao et al., 2002). As stress-positive boars show better meat characteristics they transmit by imprinting to their offspring (Leroy et al., 2000; Youssao et al., 2002), it seems logical that breeders mostly provide this kind of animals for progeny testing of which one of the commercial outlets is crossbreeding. Stress-negative animals may be mainly used for reproduction purposes in the purebred core, to mitigate excessive effects of n gene or for on-farm distribution of Piétrain meat (H. Stas, personal communication, March 29, 2019). Meat of stress-negative animals is most of the time better appreciated by consumers (Leroy et al., 2000). The number of stress-negative animals is also lower than stress-positive ones in Wallonia.

At last, by looking at the genetic diversity parameter, inbreeding coefficients, N_e and PCA of pseudo-phenotypes, we may infer that the overall diversity of Walloon Piétrain pigs seems to be so far well preserved. However, given the limited size of the population, different threats can break this precarious balance such as germplasm losses, the compromise of the Walloon population specificity, (i.e. by the excessive use of external boars) or the spread of any diseases.

Table 2.

Eigenvalue, percentage of variance and cumulative variance of the principal components.

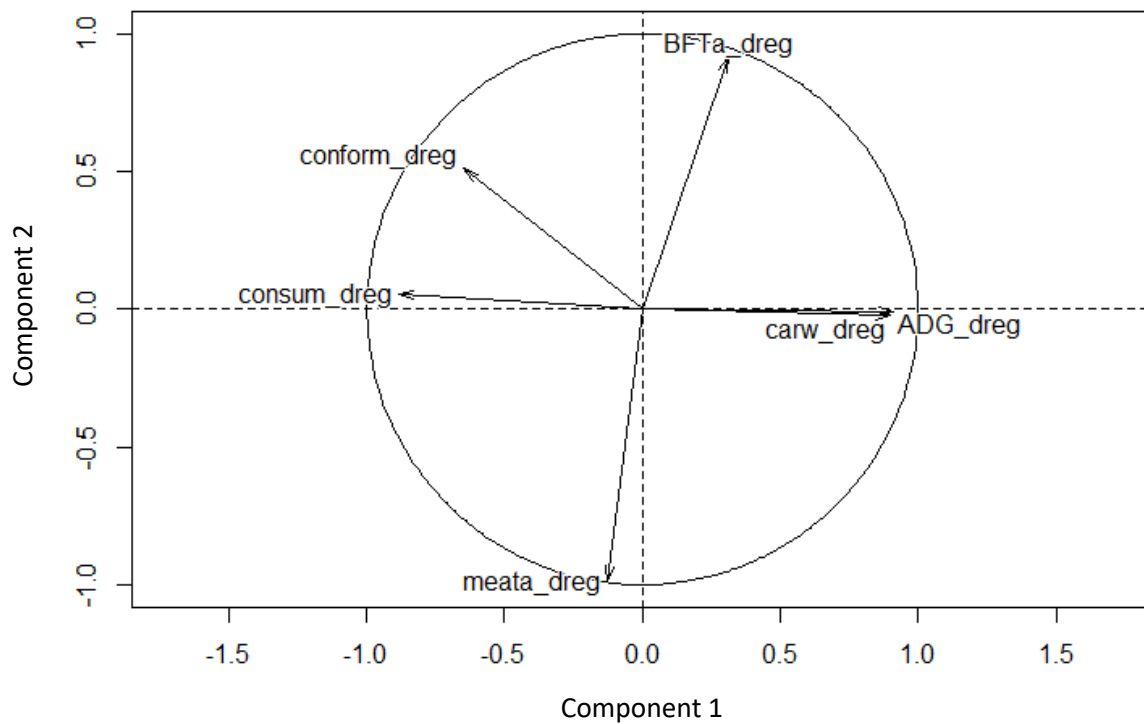| Component	Eigenvalue	Variance (%)	Cumulative variance (%)
1	2.96	49.28	49.28
2	2.08	34.70	83.98
3	0.81	13.43	97.41
4	0.11	1.81	99.21
5	0.03	0.58	99.80
6	0.01	0.20	100.00

Figure 6. Traits factor map. ADG_dreg: deregressed EBV of average daily gain; carw_dreg: deregressed EBV of carcass weight; BFTa_dreg: deregressed EBV of backfat thickness at station; meata_dreg: deregressed EBV of meat percentage at station; conform_dreg: deregressed EBV of conformation index; consum_dreg: deregressed EBV of consumption index.

Table 3.

Correlations between the traits tested in the station and the first two principal components and their respective p-values.

Traits	Component 1	p-value 1	Component 2	p-value 2
Deregressed ADG[a]	0.91	5.60^{-61} (***)	-0.01	$9.13.10^{-1}$
Deregressed carcass weight	0.90	$1.61.10^{-56}$ (***)	-0.02	$7.71.10^{-1}$
Deregressed BFTa[b]	0.31	$9.21.10^{-5}$ (***)	0.92	$1.20.10^{-62}$ (***)
Deregressed meata[c] at station	-0.13	$1.15.10^{-1}$	-0.99	$2.41.10^{-123}$ (***)
Deregressed conformation index	-0.65	$9.76.10^{-20}$ (***)	0.51	$1.1.10^{-11}$ (***)
Deregressed consumption index	-0.88	$3.27.10^{-52}$ (***)	0.05	$5.00.10^{-1}$

[a]: EBV of average daily gain; [b]: EBV of backfat thickness at station; [c]: EBV of meat percentage at station; (***): extremely significant correlation

Table 4.

Contributions of studied traits (%) in the station to the first two principal components.

Traits	Component 1	Component 2
Deregressed ADG[a]	28.17	$3.77.10^{-03}$
Deregressed carcass weight	27.36	$2.68.10^{-2}$
Deregressed BFTa[b]	3.25	40.40
Deregressed meata[c]	0.55	46.82
Deregressed conformation index	14.21	12.61
Deregressed consumption index	16.46	0.14

[a]: EBV of average daily gain; [b]: EBV of backfat thickness at station; [c]: EBV of meat percentage at station.
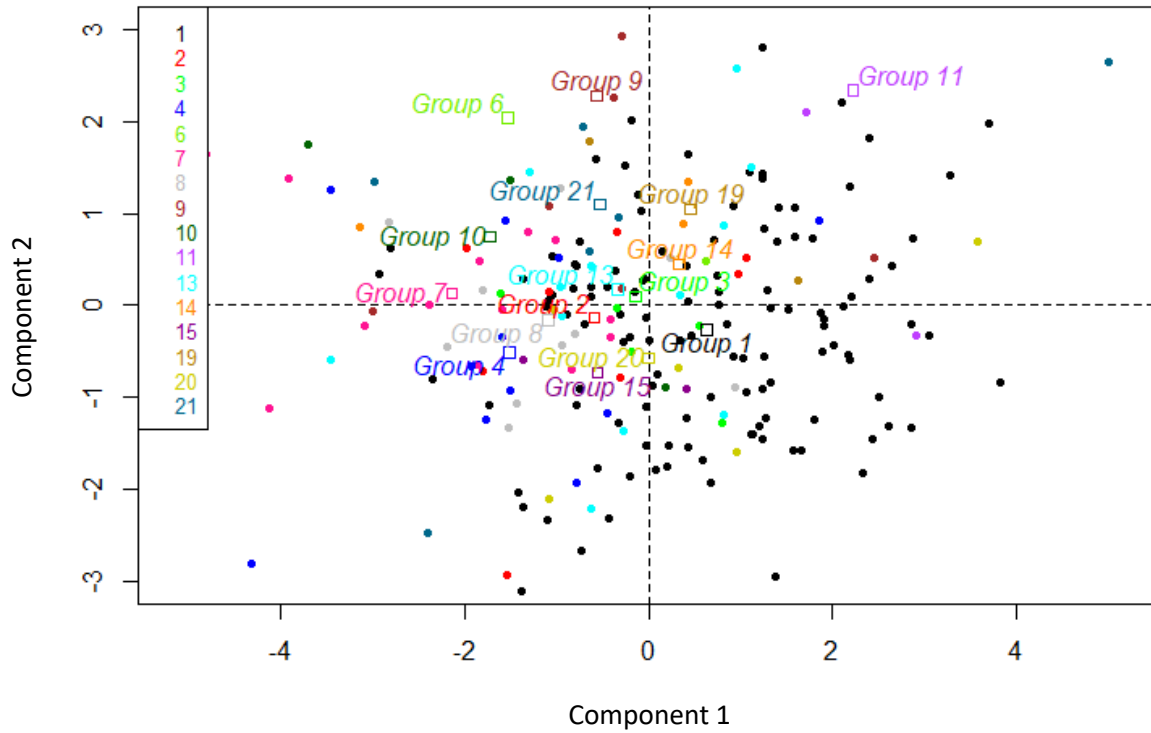
Figure 7. Individuals factor map (Principal Component Analysis) of the 219 boars colored by owner-group. Means of each group are represented by square symbols.
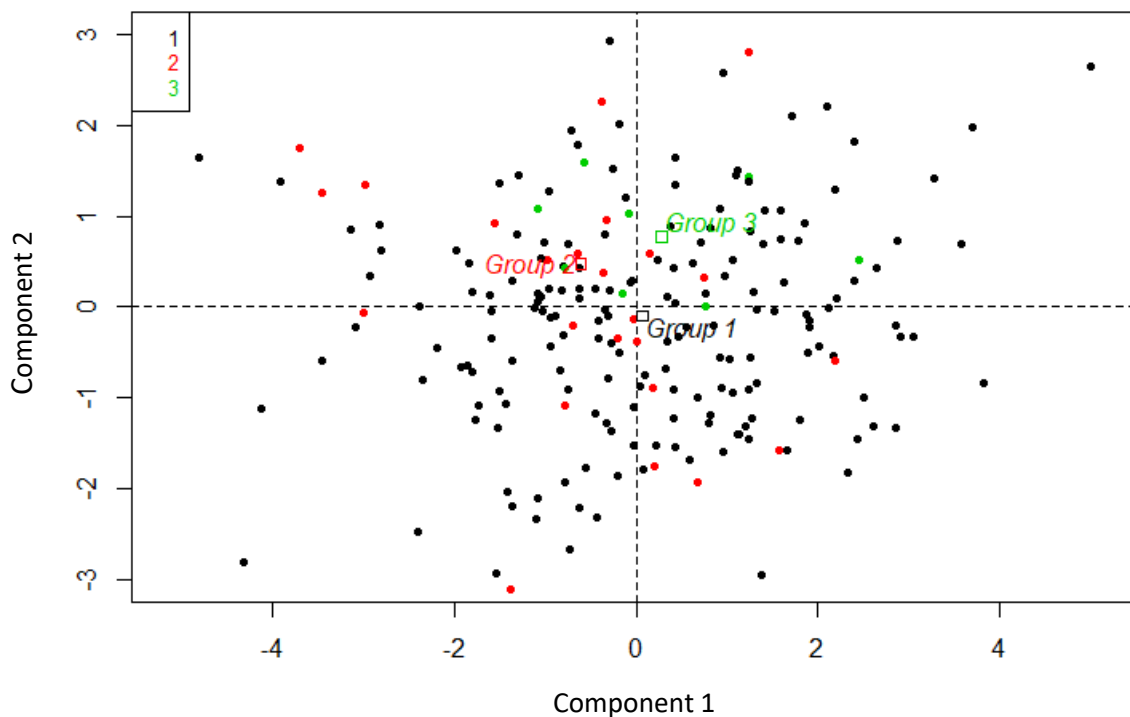

Figure 8. Individuals factor map (Principal Component Analysis) of the 219 boars colored by stress status. Means of each group are represented by square symbols.

3.3. Mahalanobis distances

Mahalanobis distances are illustrated in Figure 9. The distance of owner-group 11, who put an end to his business, in relation to others can also be clearly seen in this graph. The loss of this owner-group can therefore be considered as a problem. Owner-groups 4, 7 and 10 formed a first owner-group in the dendrogram that seemed to be linked with the worse consumption index when we combine this information with the PCA. A second cluster was made of owner-groups 1, 3, 14 and 19 that seemed to select for efficient growth traits. The third cluster with owner-groups 2, 8, 9 and 21 seemed to select for lesser good consumption index (but better than owner-groups 4, 7 and 10) and in general for better conformation. As two boars from owner-group 2 entered the Belgian Piétrain program, it was logical that the owner-group 21 was closer from owner-group 2. Other groups were less evident to characterize. Both pairs (breeder-groups 6 and 15 and breeder-groups 13 and 20) were not really close on the PCA first plan but they were maybe close on other PCA plans. Mahalanobis distances seemed therefore to reflect more similar breeding objectives among owners than transfers of animals or genetic links. There was an exception for breeder-groups 2 and 21 for which transfers appeared recently.
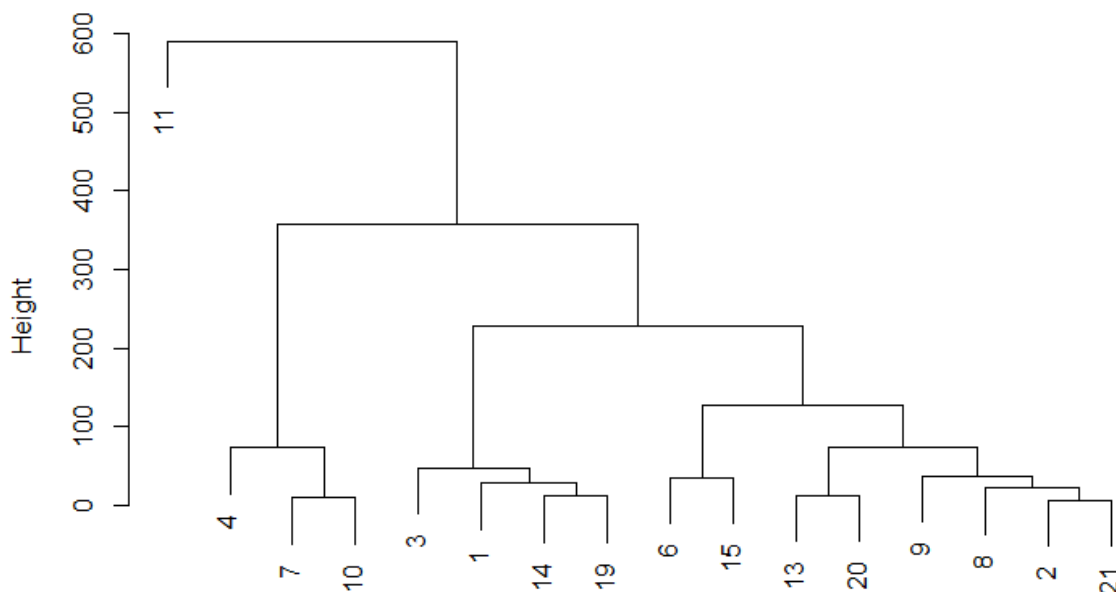

Figure 9. Dendrogram of Mahalanobis distances among owner-groups.

3.4. Separation power of traits

As a great dispersion of individuals was seen in the first PCA (Fig. 7), separation powers of traits were computed in order to determine which traits discriminate the most different breeders. The different separation powers are presented in Table 5. In general, owners seemed to be the more divergent for ADG and for consumption index (growth traits). That is why the Belgian Piétrain program should ensure representative samples for these traits. Moreover, animals with superior meat performances should be tested and enter the program as they seem to be rare. Note that the Piétrain breed shows already exceptional meat characteristics compared to other breeds (Camerlynck & Brankaer, 1958; Youssao et al., 2002). In the owner point of view, it is totally justified to keep a superior animal. However, lesser good animals may also be kept since this kind of animals could maybe carry unknown genes of importance (genetic variability). It can also have excellent growth traits and it is only at this condition that it will enter the station. It is the mission of the Belgian Piétrain program to conserve both types of animals.

Table 5.

Separation power of the tested traits in the station.

Traits	Separation power
Deregressed ADG[a]	6.44
Deregressed carcass weight	4.16
Deregressed BFTa[b]	2.79
Deregressed meata[c]	3.67
Deregressed conformation index	2.63
Deregressed consumption index	7.61

[a]: EBV of average daily gain; [b]: EBV of backfat thickness at station; [c]: EBV of meat percentage at station.

4. Use of pedigree and phenotypes simultaneously

The use of both pedigree and phenotypes in this study was interesting as groups that were genetically close did not have similar performances based on breeder-groups PCA (Fig.7). For example, groups 3, 6, 11 and 15 were in the core cluster of MDS (Fig.3) but spread out in PCA (Fig.7). Pedigree information is very important to determine levels of inbreeding and gene flow between populations whereas phenotypic measures allow to discriminate in our, due to breeding preferences, rather heterogeneous population, owner-groups having animals that carry interesting or rare traits. It indicates that the selection work of the breeder has an important place by studying the diversity of an endangered or not breed. Our results support other authors emphasizing the importance of using physical traits for preservation purposes since some of them cannot be captured by genetic distances computations (Fabuel et al., 2004; Ruane, 1999). The use of phenotypes for conservation purposes is not usual in animal breeding (e.g. Gomes Arandas et al., 2017; Tocci et al., 2018) contrary to ecology (e.g. Amano & Yamaura, 2007; Jones et al., 2009; Turnhout et al., 2010). We therefore suggest to add phenotypic measures by studying genetic diversity of a breed as it adds a certain amount of information to pedigree. Genotypes can complete the diversity overview and conservation measures stated.

5. Conservation recommendations based on pedigree and pseudo-phenotypes

Until now, the Walloon Piétrain population seems to maintain sufficient diversity. The MDS and the PCA analyses demonstrated that boars spread well considering genetic and phenotypic diversities. However, different threats can break this precarious balance: 1- In the last ten years, 4 breeders out of 14 have retired without any successor, the germplasm of their animals mostly lost in the process. If this situation continues at this pace, in a few years, it is very likely that there will be any Walloon Piétrain breeder left. 2- The importation of foreign or Flemish boars or straws might compromise the specificity of the Walloon population. 3- The spread of diseases like ASF leading to compulsory culling, could lead to the extinction of the Walloon Piétrain.

To preserve better the Walloon Piétrain breed, also by doing some adaptations to the Belgian Piétrain program, different solutions exist: 1- Currently, many boars come from the same breeder. To have a better management of the genetic diversity and improving the choice of boars by the Belgian Piétrain program, other breeders should send more boars for progeny testing. 2- The Belgian Piétrain program could choose more diverse boars, i.e. different for growth traits and greater meat percentage.

This study showed manners to detect phenotypically different animals as those from owner-groups 9 and 10 that should be sampled by the program. However, it should be noticed that only one batch of Belgian Piétrain boars could be analyzed in this study since EBVs for further batches were not yet available. Considering other batches could help to determine if the Belgian Piétrain program follows these advices. 3- Financial assistance by the government for breeders could help to mitigate economic conditions that are not favorable nowadays. The government should ease the installation of new and young breeders, who might have rather limited numbers of purebred animals, and separate this support from the support for larger pig producing. 4- For small purebred populations like Walloon Piétrain, diseases like ASF are a large threat. In addition to the current strategy based on limiting the spread of ASF, the government should support specific measures to protect the purebred animals and provide exemptions from immediate culling. 5- The Walloon population has to stay competitive to foreign or Flemish importation of boars. Only through a strong progeny testing this can be achieved. 6- As shown by the fact that some breeders seemed to rely relatively heavily on the use of inbreeding, adapted mating tools provided by the awé could help to plan mating. Such a tool could be based on OC theory therefore optimizing simultaneously genetic and phenotypic variability, i.e. genetic improvement. 7- Transfers of animals between breeders should be favored but it should also be ensured that groups can keep their specificity. Breeding circles proposed by Windig & Kaal (2008) could be one manner to organize these transfers.

6. Genotypes analysis

Previous results have focused on analyzing the genetic diversity of the Walloon Piétrain population. In this section, we will increase the discussion to an international level by 1- investigating its origin; and 2- assessing the diversity of several Piétrain populations.

6.1. Multi-Dimensional Scaling of European pig breeds

The Appendix II shows the MDS with all the European pig breeds. Figure 10 shows the MDS with the closest breeds to Piétrain. Three main clusters can be seen: this of Landrace, Large White and Piétrain populations/strains. In between these three clusters, different local Spotted breeds of pigs can be seen: the Angler Sattleschwein and Bunte Bentheimer breeds from Germany, Pulawska Spot from Poland, Poltava from Ukraine, National Spotted Swine from the USA (but that comes from Poland China breed; National Spotted Swine Record, 2019), and to a lesser extent the Byelorussian pork breed. Another cluster close to these Spotted breeds is composed of local English breeds such as Berkshire, Tamworth and Gloucester Old Spot. Appendix III illustrated local English breeds whereas Appendix IV showed some local Spotted breeds.

A recent study from Gorssen (2018) also analyzed data from Dryad (Yang et al., 2017) and some Piétrain populations to infer about the origins of the Piétrain breed. These results were similar to those obtained in this study, however it seems that this author did not apply a Hardy-Weinberg filter to remove selection process. This author did not capture the close link between Piétrain and English breeds as well, since this link could not be seen without a Hardy-Weinberg filter.

The study of Gorssen (2018) proposed three hypotheses to explain the close relationship of Piétrain populations and local Spotted breeds: 1- Piétrain and local Spotted breeds were all originating from the local English breeds; 2- Piétrain genes were introgressed in local Spotted breeds or vice-versa; 3- Piétrain populations were close to local Spotted breeds because of independent path of selection based on spots.

As mentioned (please, see section *4.1. Piétrain history* in literature review), it is supposed that Piétrain animals came from crosses between Berkshire and White breeds (Camerlynck & Brankaer, 1958; Li et al., 2014; Yang et al., 2017) in the decade of 1920. Regarding our results, this hypothesis cannot be neglected. However, we may also consider that the Piétrain breed may originate from Tamworth and Gloucester Old Spot (Fig. 10).

Some local Spotted breeds were created before Piétrain. The National Spotted Swine was created around 1880 (National Spotted Swine Record, 2019) and Bunte Bentheimer, mid-nineteenth century (Zwick, 1990). From that, we cannot exclude the fact that Piétrain could originate also from these breeds. On the other hand, the Poland China, ancestor of the National Spotted Swine, was not exported during these decades according to Marcq & Lahaye (1941) and may not be one of the origins of Piétrain pigs. Likewise, the Pulawska, Angler Sattleschwein and Byelorussian pigs, originated in the twenties (Heiner Iversen, 1997; Porter, 2002; Slow food fundation for Biodiversity, 2019), as well as the Poltava breed, recognized in the forties (Koziner & Shtakelberg, 1989), cannot be related to the origin of Piétrain. Moreover, the Piétrain breed is more susceptible to have genetic links with geographically close populations (as German local breeds) which reinforces the aforementioned statement.

In addition to these hypotheses, same breed introgressions are presumed for local Spotted breeds. For improvement purposes, the Bunte Bentheimer breed was crossed with the Berkshire breed in the beginning of the 19th century (Zwick, 1990) and the Angler Sattleschwein with Berkshire and Tamworth breeds (Förderverein Angler Sattleschwein e.V., July-2-2019). The Pulawska pig would originate from local and Berkshire breeds (Slow food fundation for Biodiversity, 2019) and the Poltava breed from an Ukrainian native pig crossed with Berkshire, Large White and Tamworth breeds (Koziner & Shtakelberg, 1989). The Byelorussian pig breed came from a native pig breed crossed with Berkshire, Large White, Large Black and Middle White (Porter, 2002).
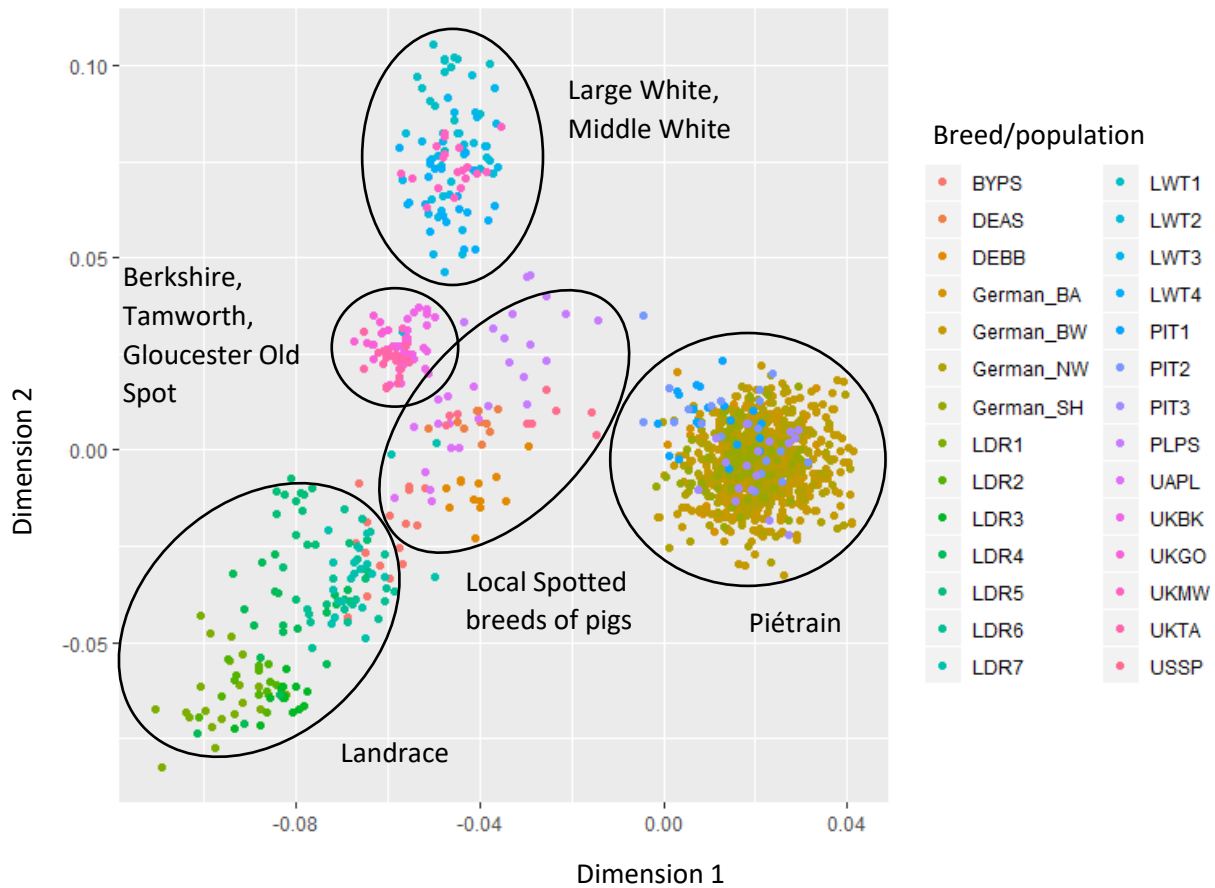
Figure 10. Multi-Dimensional Scaling among different European breeds/populations close to the Piétrain breed. BYPS: Byelorussian Pork Swine; DEAS: Germany Angler Sattleschwein; DEBB: Germany Bunte Bentheimer; German_BA: Germany Bavarian Piétrain; German_BW: Germany Baden-Württemberg Piétrain; Germany_NW: Germany North Rhine-Westphalia Piétrain; German_SH: Germany Schleswig-Holstein Piétrain; LDR1: Denmark Landrace; LDR2: Norway Landrace; LDR3: Finland Landrace; LDR4: China Landrace; LDR5: USA Landrace; LDR6: Spain Landrace; LDR7: Netherlands Landrace; LWT1: Denmark Large White; LWT2: China Large White; LWT3: USA Large White; LWT4: Netherlands Large White; PIT1: USA Piétrain; PIT2: Netherlands Piétrain; PIT3: "Germany others" Piétrain; PLPS: Poland Pulawska Spot; UAPL: Ukraine Poltava Swine; UKBK: UK Berkshire; UKGO: UK Gloucester Old Spot; UKMW: UK Middle White; UKTA: UK Tamworth; USSP: USA Spot (or National Spotted Swine)

6.2. Multi-Dimensional Scaling of Piétrain populations

In Figure 11 is illustrated the MDS of Piétrain populations. It is important to remind that for this analysis, the Hardy-Weinberg filter was not applied to have an insight of selection path for the different Piétrain populations. Moreover, a LD filter was applied to avoid overweighting of some SNPs selected jointly. It is difficult to determine clear clusters among different populations even if some preferential orientations of selection can be seen. American, Dutch and "German others" Piétrain populations tended to split up from other populations, which can be partially explained by geographic origins. It could also be explained by the fact American, Dutch and "German others" could originate from commercial firms. These three populations were genotyped earlier than Bavarian, Baden-Württemberg, North Rhine-Westphalia and Schleswig-Holstein populations as well. This could imply a greater genetic gap between both kind of populations as the selection path was not the same. As the same chip was used for genotyping, these differences could not be explained by genotyping artefacts. Bavarian Piétrain tended to cluster in the center of the figure. Baden-Württemberg and North Rhine-Westphalia populations were well spread even if a direction of selection seemed to be followed (i.e. dimension 2 and 1, respectively). These differences in terms of within-population diversity between Baden-Württemberg/North Rhine-Westphalia and other populations are expected to be partially due to differences in sample size.

It can also be observed from Figure 11 exchanges among different German populations. This is why Bavarian, Baden-Württemberg, North Rhine-Westphalia and Schleswig-Holstein populations were particularly mixed up. Population from Baden-Württemberg tended to be less similar than those from North Rhine-Westphalia and Schleswig-Holstein, which was already observed by Stratz et al. (2014). However, the F_{st} was estimated to 0.03, which implies little genetic drift according to thresholds defined by Hartl & Clark (1997). This seems to be logical: different Piétrain populations did not substantially discriminate.
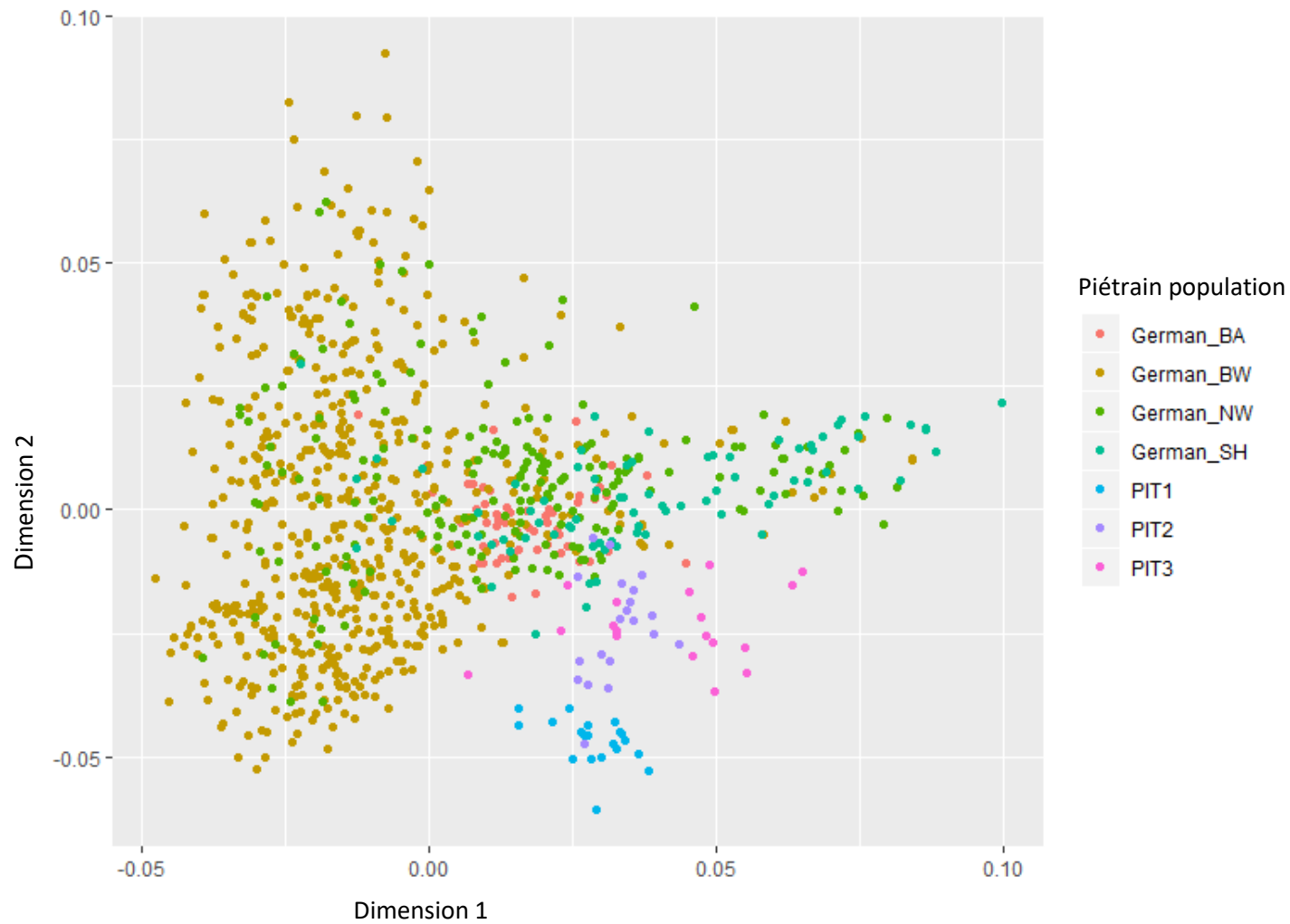
Figure 11. Multi-Dimensional Scaling among different Piétrain populations. German_BA: Germany Bavarian Piétrain; German_BW: Germany Baden-Württemberg Piétrain; Germany_NW: Germany North Rhine-Westphalia Piétrain; German_SH: Germany Schleswig-Holstein Piétrain; PIT 1: USA Piétrain; PIT2: Netherlands Piétrain; PIT3: "Germany others" Piétrain.

Table 6 illustrates different genomic inbreeding estimations for each Piétrain population, whereas Table 7 shows ROH parameters for each population. Negative values of Fhat1 involves less relatedness within population than positive ones. Greater Fhat3 values mean higher inbreeding levels because rare homozygous appear at a higher frequency. We can therefore infer that Dutch and American populations had higher levels of inbreeding (Table 6). Note that the Dutch population seemed to carry rarer homozygous than American due to its higher Fhat3. Bavarian, Baden-Württemberg, North Rhine-Westphalia and Schleswig-Holstein showed low levels of inbreeding whereas the "German others" population had intermediary ones.

It can be seen that the Dutch Piétrain showed the highest number of ROH segments and the highest standard deviation (SD) (Table 7). The total number of kilobases in ROH was relatively high with the highest SD. However, their average number of kilobases in ROH and their SD were not far from other populations. It means that they showed more ROH segments but their length was on average similar to other populations. American Piétrain also showed a relatively high number of ROH segments with the lowest SD. For this population, the total and average number of kilobases in ROH was the highest. However, the average length of ROHs seemed not to differ enough from other populations to infer that the American population underwent a more recent inbreeding pressure (Herrero-Medrano et al., 2013). Populations from Bavaria, Baden-Württemberg, North Rhine-Westphalia and Schleswig-Holstein showed similar values for ROH parameters. Population of "German others" Piétrain showed low levels for all parameters. These results were aligned with inbreeding estimations except for "German others" population where higher ROHs were expected. Yang et al. (2011) also analyzed proportion of ROHs in the same American, Dutch and "German others" populations. The ranking of these populations was the same: American showed higher proportion of ROHs followed by Dutch and then "German others" populations. These levels were in general lower than other commercial populations even if their SD was also lower.

These results suggested that there was a greater variability in German populations (supposedly traditionally raised) compared to Dutch and American populations (expected to be commercial lines). The American population seemed to be particularly inbred and uniform. This can be due to management strategies applied for industrial lines. However, it is indicated to increase exchanges and the number of animals used for reproduction in this population to avoid inbreeding depression in the future. Clark et al. (2013) suggested the use of GEBVs for the determination of OC as it can increase more genetic merit than pedigree-based OC for the same inbreeding restriction. Other tools can be used as marker-by-marker coancestry or coancestry based on ROHs (Bosse et al., 2015). If the hypothesis of traditional *vs.* commercial lines is confirmed, it means that traditionally raised animals have a key-role in the preservation of Piétrain genetic resources. A study of Ollivier et al. (2005)

demonstrated that the Belgian Piétrain line could contribute relatively highly to between-breed variation. This has to be determined for the current population. A further step will therefore be to include Walloon animals and other European ones to confirm the specificity of each population.

Table 6.

Inbreeding estimations for different Piétrain populations.

Population	Fhat1[h]	Fhat3[i]
German_BA[a]	-0.02	0.03
German_BW[b]	-0.10	0.01
German_NW[c]	-0.06	0.01
German_SH[d]	-0.04	0.02
PIT1[e]	0.70	0.25
PIT2[f]	0.86	0.23
PIT3[g]	0.26	0.14

[a]: Germany Bavarian Piétrain; [b]: Germany Baden-Württemberg Piétrain; [c]: Germany North Rhine-Westphalia Piétrain; [d]: Germany Schleswig-Holstein Piétrain; [e]: USA Piétrain; [f]: Netherlands Piétrain; [g]: Germany Piétrain; [h]: inbreeding estimation based on the variance-standardized relationship minus 1; [i]: inbreeding estimation emphasizing minor homozygous alleles frequency.

Table 7.

Runs of homozygosity parameters for different Piétrain populations.

Population	NSEG[h] (± SD)	KB[i] (± SD)	KBAVG[j] (± SD)
German_BA[a]	36.42 (± 6.74)	190,016 (± 47,808)	5,213 (± 831)
German_BW[b]	34.70 (± 5.24)	174,798 (± 39,442)	5,043 (± 886)
German_NW[c]	34.61 (± 5.56)	176,618 (± 44,813)	5,101 (± 973)
German_SH[d]	34.79 (± 5.92)	179,827 (± 46,396)	5,155 (± 963)
PIT1[e]	37.60 (± 4.02)	329,441 (± 43,331)	6,406 (± 1,181)
PIT2[f]	39.30 (± 9.38)	211,280 (± 65,991)	5,284 (± 919)
PIT3[g]	32.61 (± 4.72)	163,140 (± 29,899)	5,008 (± 675)

[a]: Germany Bavarian Piétrain; [b]: Germany Baden-Württemberg Piétrain; [c]: Germany North Rhine-Westphalia Piétrain; [d]: Germany Schleswig-Holstein Piétrain; [e]: USA Piétrain; [f]: Netherlands Piétrain; [g]: "Germany others" Piétrain; [h]: mean number of ROH segments; [i]: mean total number of kilobases in ROH; [i]: mean of mean number of kilobases in ROH.

General conclusion and perspectives

The breeding of Piétrain purebred animals is currently done in two different contexts: 1- by breeding companies that keep private industrial lines of limited size, 2- by the remaining individual purebred breeders, whose numbers have been strongly decreasing overtime (e.g. Welsh et al., 2010). Even if the Piétrain breed is normally not considered as endangered (Henson, 1992), Piétrain populations raised outside breeding companies are scarce. Two solutions were already implemented in Wallonia for the preservation of the Piétrain breed raised by traditional breeders. First, the progeny testing of Piétrain boars was set up in 2007. Then, the Belgian Piétrain program, based on cryopreservation of the best boars' semen, was launched in 2017 (awé, 2019).

The objectives of this study were therefore to investigate the origin of the Piétrain breed, to assess the genetic diversity of the Walloon Piétrain pig population and to determine the genomic differences between some European populations.

The investigation of the origin of the Piétrain breed did not allow to determine absolutely which breeds were involved in its genesis. The hypothesis of an initial cross of a Berkshire boar with an Indigenous sow followed by crosses with the Large White breed could not be excluded. However, some other local English and/or local Spotted breeds could have been involved (e.g. Tamworth and Gloucester Old Spot). Some local Spotted pig breeds, supposedly with the same origins as Piétrain and "born" more or less at the same period, were close to the Piétrain population. Further investigation is however required to search for more accurate facts about the Piétrain breed's origin. The neighbor-joining tree and the admixture methods could maybe help to solve this issue.

Besides the study of the breed origin, the genetic diversity of the Walloon population was assessed through pedigree. The N_e was 223, the genetic diversity parameter 97.96% and the average inbreeding coefficient 2.74%. As confirmed by these main pedigree parameters, the genetic diversity seemed to be sufficiently high in the Walloon Piétrain population. Inbreeding did not either seemed to be an issue. These good results can be due in part to a former large population or management strategy.

In addition, the PCA, based on deregressed EBVs, reinforced the idea that a high diversity exists in the current Walloon population. Actually, the breeding objectives were found to highly vary, implying that owners tried to differentiate from each other. Moreover, by separation power computation, it seemed that owners diverged the most in growth traits. Two main strategies can therefore be observed: meat or growth traits. Most owners focused on the first type of traits, which was the primary objective when the breed was created.

However, some conversation measures could be advised as the diversity found in the Walloon population could be under threat. Different analyses and statistics consolidated this statement. 1-Gene

flows between farms were uncommon, as observed in the MDS based on the opposite of kinship coefficients and in the analysis of animal transfers. 2- Only one breeder provided 55% of the boars tested, which means that, potentially, not all the Walloon diversity is known. 3- Four breeders put an end to their activity, which means that the diversity loss is already a fact. 4- Dutch and American populations, supposedly from commercial lines, seemed to be more inbred and with greater ROHs as found by studying their genotypes. If the traditionally raised Walloon Piétrain population is overwhelmed by commercial firms, an increase in diversity loss could therefore be expected.

Some adaptations should thus be done in genetic diversity management of the Walloon Piétrain population. Improvements through the choice of boars for efficient mating plans and strong progeny testing are a first solution. In addition, advanced tools (e.g. OC which is based on EBVs and pedigree; Meuwissen, 1997) as well as transfers of animals among breeders (e.g. through breeding circles proposed by Windig & Kaal, 2008) should be considered by the Belgian Piétrain program. This program can also play a very important role in finding an equilibrium between keeping all genetic diversity and the best germplasm.

As for other local breeds in Wallonia, Piétrain could be included into agro-environmental measures. These measures provide a framework for financial assistance by the government to breeders to support genetic diversity. They are very efficient and could maybe be implemented, by requiring the European Commission to accept national strategy plans that includes support for endangered breeds. Finally, governments in general should also support specific measures to protect rare purebred animals against diseases. Currently, culling strategies during diseases outbreaks are not designed to protect animals with high genetic value.

Another important advice when studying the status of a breed could be drawn from the results of this study. Physical traits can add a substantial amount of information about breeding objectives and breed particularities that cannot be covered by genetic distances and other parameters. However, there are not commonly used for conservation purposes. Therefore, pedigree, (pseudo-)phenotypes and genotypes should be used simultaneously to have an overall picture of the status of a breed.

One perspective of this study would be to add new batches of boars from the Belgian Piétrain program to check if the different advises provided in this study were followed. The pedigree study of sows born a few years ago could also be done to check if the different pedigree parameters are actually similar for this other part of the population. An index, currently lacking, based on the first two dimensions of the PCA could also be elaborated. This index would help owners to achieve breeding goals by synthetizing the 10 EBVs currently used.

As a further study, it is planned to add other European Piétrain populations (Walloon, French and Austrian genotypes are under acquisition) to analyze their differentiation degree through a MDS and the F_{st} parameter. By the analysis of inbreeding estimations, ROHs, LD and other genomic parameters, it is expected to determine if the Walloon Piétrain population is unique and if it highly contributes to the genetic diversity of the Piétrain breed. The development of genomic evaluations, suitable for Walloon Piétrain pigs, could also be of particular interest as it does not exist currently.

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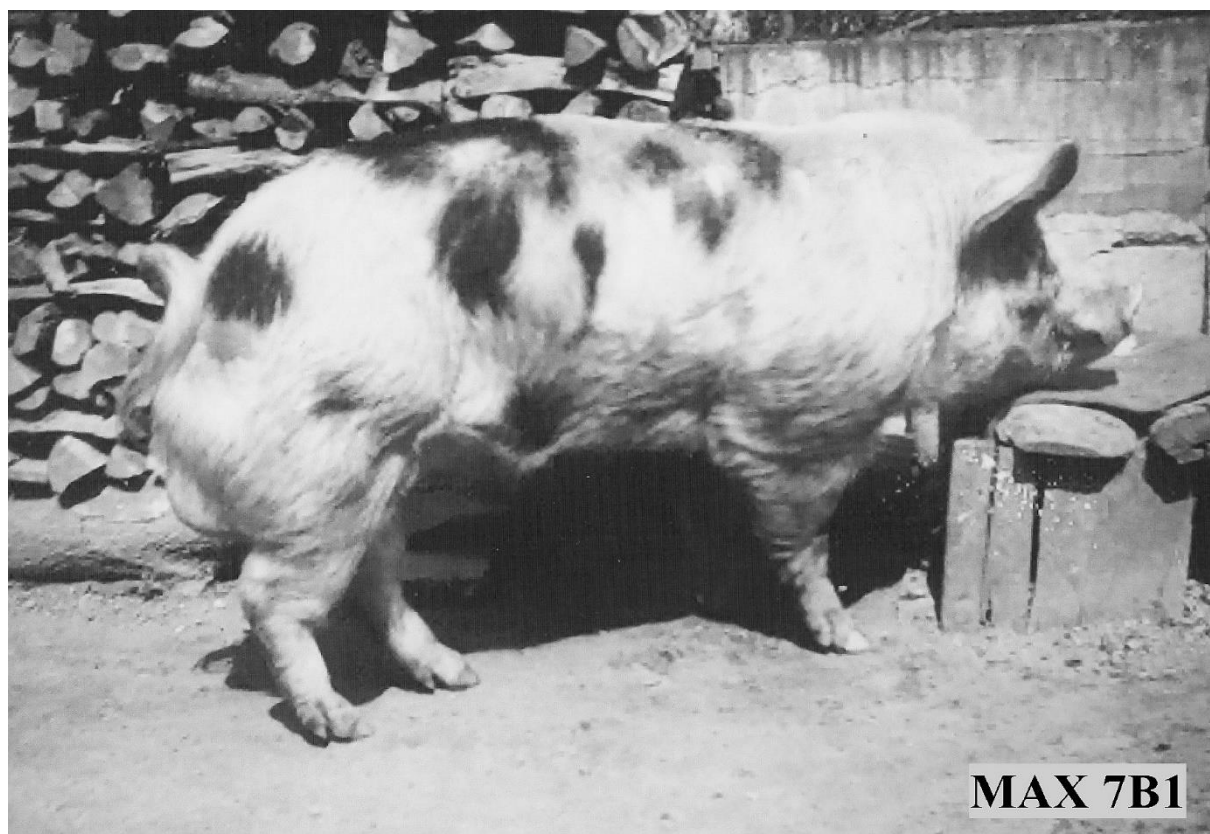
Appendixes

Appendix I. Old pictures of Piétrain pigs
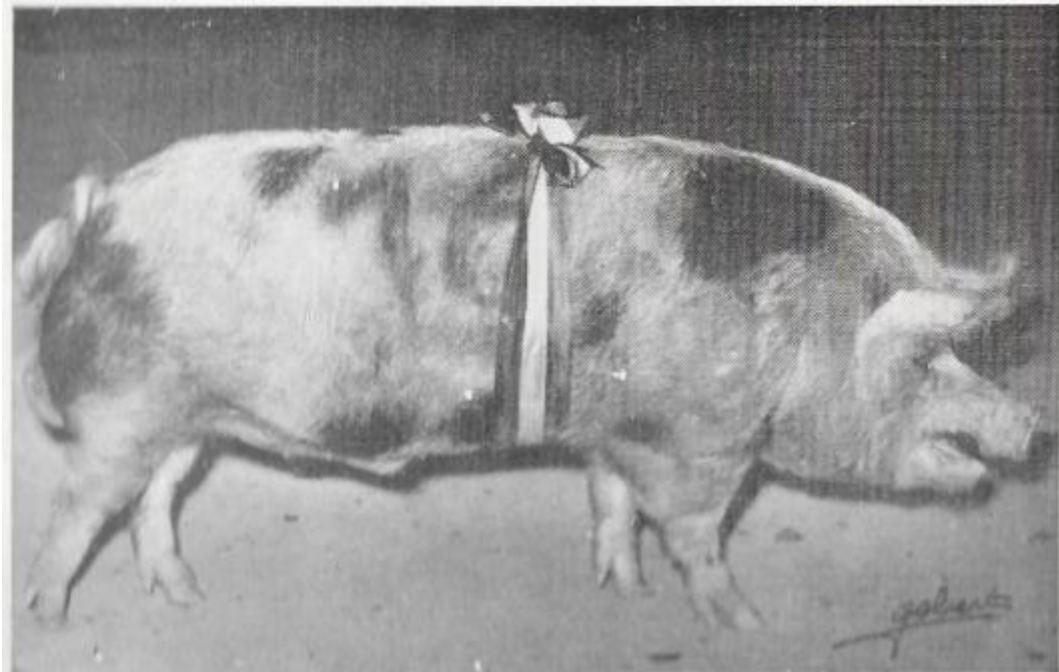
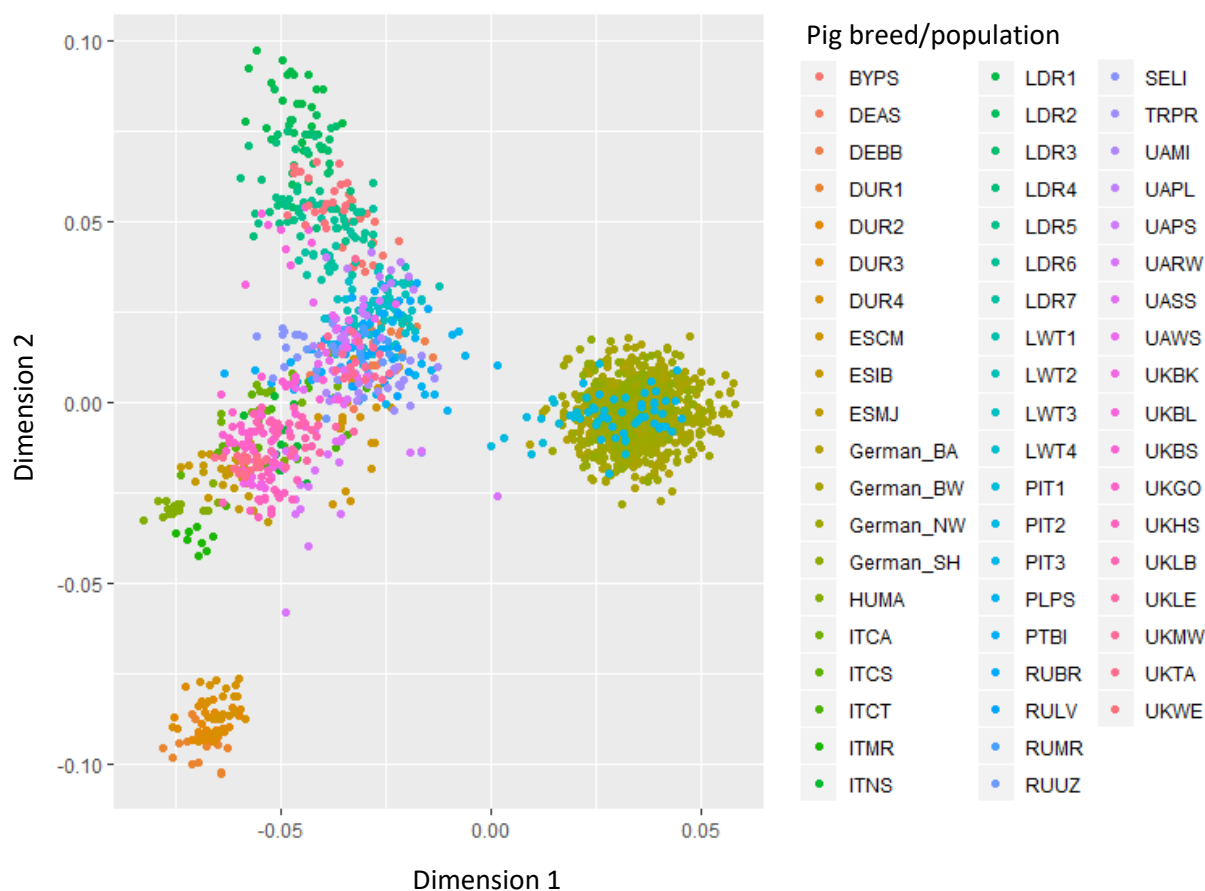**Appendix Ia.** Champion boar of year 1955. Source: Camerlynck & Brankaer (1958).

Appendix Ib. Piétrain sow and her litter. Source: Van Snick & de Lantsheere (1961).

Appendix Ic. Max 7B1, first boar to enter the Pigbook. Anonymous source.

Appendix II

Appendix II. Multi-Dimensional Scaling among different European breeds/populations. BYPS: Byelorussian pork swine; DEAS: Germany Angler Sattleschwein; DEBB: Germany Bunte Bentheimer: DUR1 to DUR4: Duroc populations; ESCM: Spain Chato Murciano; ESIB: Spain Iberian; ESMJ: Spain Manchato de Jabugo; German_BA: Germany Bavarian Piétrain; German_BW: Germany Baden-Württemberg Piétrain; German_NW: Germany North-Rhine Westphalia Piétrain; German_SH: Germany Schleswig Holstein Piétrain; HUMA: Hungary Mangalica; ITCA: Italy Calabrese; ITCS: Italy Cinta Senese; ITCT: Italy Casertana; ITMR: Italy Mora Romagnola; ITNS: Italy Nera Siciliana; LDR1 to LDR7: Landrace populations; LWT1 to LWT4: Large White populations; PIT1: USA Piétrain; PIT2: Netherlands Piétrain; PIT3: "Germany others" Piétrain; PLPS: Poland Pulawska Spot; PTBI: Portugal Bisaro; RUBR: Russia Breitov; RULV: Russia Livni; RUMR: Russia Murom; RUUZ: Russia Urhzum; SELI: Sweden Linderoth; TRPR: Czech Prestice; UAMI: Ukraine Mirgorod Swine; UAPL: Ukraine Poltava Swine; UAPS: Ukrainian Pork Swine; UARW: Ukraine Red White Belted; UASS: Ukrainian Spotted Steppe; UAWS: Ukrainian White Steppe; UKBK: UK Berkshire; UKBL: UK British Lop; UKBS: UK British Saddleback; UKGO: UK Gloucester Old Spot; UKHS: UK Hampshire; UKLB: UK Large Black; UKLE: UK Leicoma; UKMW: UK Middle White; UKTA: UK Tamworth; UKWE: UK Welsh.

Appendix III. Local English pig breeds

Appendix IIIa. Champion Berkshire boar at 2005 Royal Adelaide Show. Source: Davis (2005), https://commons.wikimedia.org/wiki/File:Adelaide_champion_Berkshire_boar_2005.jpg

Appendix IIIb. Gloucester Old Spot boar. Source: Slater (2008), https://www.flickr.com/photos/15181848@N02/2803601977/

Appendix IIIc. Tamworth sow. Source: Merrett (2009),
https://www.flickr.com/photos/davehamster/3715904969/

Appendix IV. Local Spotted pig breeds

Appendix IVa. Angler Sattleschwein pig. Source: von Ebbel (2008),
https://commons.wikimedia.org/wiki/File:Angeliter_Sattelschwein.jpg

Appendix IVb. Bunte Bentheimer sow. Source: "Jed" (2013),
https://commons.wikimedia.org/wiki/File:Tiergarten_N%C3%BCrnberg_-_32.JPG

Appendix IVc. Pulawska pig. Source: Polsus (2017),
https://www.polsus.pl/index.php/hodowla/rasy/pulawska