

University of Natural Resources and Life Sciences

Department of Sustainable Agricultural Systems Division of Livestock Science

## MASTER THESIS

# GENETIC VARIABILITY IN THREE HIGHLY ENDANGERED CATTLE BREEDS IN AUSTRIA

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#### STATUTORY DECLARATION

I certify, that the master thesis was written by me, not using sources and tools other than those quoted and without use of any other illegitimate support. Furthermore, I confirm that I have not submitted this master thesis either nationally or internationally in any form.

Vienna, 2016

Signature (Susanne Binder, BSc)

*Dedication* To my beloved sister Sabine for her strength, support and motivation

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# ABSTRACT

To preserve endangered cattle breeds, pedigree analysis is a good method to analyse genetic structure in order to predict the evolution of genetic variability. This study investigated the pedigrees of three highly endangered local cattle breeds of Austria: Pustertal Spotted Cattle, Ennstal Pied Cattle and Original Austrian Brown Cattle. Pedigree files are included from 22,451 animals in Original Austrian Brown Cattle to 1,327 animals in Ennstal Pied Cattle. Breeders follow the conservation programme of ÖNGENE (Austrian Association for Rare Endangered Breeds) by systematically avoiding mating of closely related animals, applying the projected inbreeding coefficient of the potential progeny as indicator. Three reference populations were defined: 1) living population; 2) male living animals of which no semen was available; 3) bulls for which semen was available. PEDIG software was used to calculate genetic parameters in order to evaluate the genetic variability. The significance of the results strongly depends on the quality of the pedigrees. While Original Austrian Brown is derived from a large ancestral population, the two other breeds are based on very small numbers of founder animals. The inbreeding coefficient is less than 1% for Original Austrian Brown Cattle and Pustertal Spotted Cattle. In contrast, the Ennstal Pied Cattle has a percentage of 3.3%. In all three breeds the effective number of founders is unequal to the total number of founders, which implies an unbalanced use of founders. When increase of inbreeding coefficients from the parents of the current population to the current population was calculated, effective population sizes were 238 for Original Austrian Brown Cattle, 82 for Pustertal Spotted Cattle and 34 for Ennstal Pied Cattle. In terms of important ancestors, of the 20 most important ancestors of the living Ennstal Pied Cattle population are mostly female (17) and date back to the seventies. For other two breeds the majority of the most important ancestors are male (18 for Original Austrian Brown Cattle and 13 for Pustertal Spotted Cattle) and for Original Austrian Brown Cattle the bulls date back to the nineteen-fifties. This study confirmed that in small cattle populations special mating programmes and conservation programmes are needed in order to minimize the loss of genetic diversity.

# ZUSAMMENFASSUNG

Die Stammbaumanalyse ist eine gute Methode um die genetische Variabilität der verschiedensten Populationsstrukturen zu analysieren. Die vorliegende Masterarbeit untersucht die genetische Vielfalt von drei hoch gefährdeten österreichischen Rinderrassen (Pustertaler Sprinzen, Ennstaler Bergschecken und das Original österreichische Braunvieh). Der komplette Pedigree des Original österreichischen Braunviehs umfasst 22 451 Tiere und jener der Ennstaler Bergschecken nur 1 327 Rinder. Pro Rasse wurden drei Referenzpopulationen festgelegt.

Die erste Referenzpopulation umfasst zum Zeitpunkt der Datenerhebung für diese Arbeit lebende männliche und weibliche Tiere. Die zweite Referenzpopulation beinhaltet Stiere, deren Sperma in der Gendatenbank der ÖNGENE gespeichert ist und die dritte Referenzpopulation sind lebende männliche Tiere, welche bislang noch nicht in der Genbank erfasst wurden. Die Berechnung der Inzuchtkoeffizienten, der Diversitätskennzahlen und der wichtigsten Ahnen wurden mit dem Softwarepaket PEDIG durchgeführt. Dabei muss berücksichtigt werden, dass die Qualität der Ergebnisse stark von der Vollständigkeit der Abstammungsdaten abhängig ist.

Die Ergebnisse der Arbeit zeigten, dass das Original österreichische Braunvieh im Gegensatz zu den anderen beiden untersuchten Rassen von einer großen Anzahl an Ahnen abgeleitet werden kann. Der Inzuchtkoeffizient beträgt weniger als 1% für das Original österreichische Braunvieh und die Pustertaler Sprinzen. Im Gegensatz dazu weisen die Ennstaler Bergschecken einen Inzuchtkoeffizienten von 3,3% auf. In allen drei Rassen ist die effektive Anzahl an Gründern kleiner als die Gesamtanzahl der Gründer. Das lässt auf unterschiedliche Beiträge der einzelnen Gründertiere schließen. Von den 20 bedeutendsten Ahnen der aktuell lebenden Ennstaler Bergschecken sind die meisten weiblich (17), für die anderen zwei untersuchten Rassen sind die Mehrheit der wichtigsten Ahnen männlich (18 für Original österreichisches Braunvieh und 13 für die Pustertaler Sprinzen).

Diese Studie bestätigt, dass in kleinen Rinderpopulationen spezielle Paarungs- und Erhaltungsprogramme erforderlich sind, um den Verlust der genetischen Vielfalt auf ein Minimum zu beschränken.

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## Abbreviations

DNA	Desoxyribonukleinsäure
e.g.	For example
EUGENA	European Gene Bank Network for Animal Genetic Resources
$\mathbf{f}_{\mathrm{a}}$	Effective number of ancestors
FAO	Food and Agriculture Organization of the United Nations
$f_e$	Effective number of founders
i.e.	In essence
Ng	Effective number of founder genomes
ÖNGENE	Österreichische Nationalvereinigung für Genreserven.
ÖPUL	Das Österreichische Programm für umweltgerechte Landwirtschaft
SAS	Statistical Analysis Software

## **1** Introduction

### 1.1 Structure of the thesis

Chapter 2 provides insight into the history and present situation of endangered and highly endangered cattle breeds in Austria. National and international conservation programmes and subsidies are presented together with the subject of genetic variability estimated from pedigree information.

Data of animals used in this study and methods are presented in Chapter 3.

Chapter 4 gives the results of the analyses, including presentation of results in tables. Also in Chapter 4 the results are reviewed and discussed, with a conclusion in Chapter 5.

## **1.2** Aim of the thesis

The data derives from three highly endangered cattle breeds in Austria, which have a long history of breeding. The key purpose of the study was to estimate the genetic variability of Pustertal Spotted Cattle, Ennstal Pied Cattle and Original Austrian Braunvieh on the basis of their pedigree information.

# 2 Review of literature

## 2.1 Endangered and highly endangered breeds in Austria

Since the beginning of industrialisation, agricultural production as a dynamic process, developed very efficient breeds of farm animals. In consequence many old breeds from Austria are not economical enough and lost their former relevance. They could not meet the high demands of farmer or the changing consumer wishes. The result was that some breeds are extinct while others are kept in small populations. The first warnings about loss of Animal Genetic Resources in Austria in the early 1970ies remained unheard and just private breeders or associations conserved breeds on a voluntary basis (Schleger, 1983; Fischerleitner, 2007). In 1982 the ÖNGENE – Austrian Association for Rare Endangered Breeds was established, started by a project of conservation measures by the Ministry for Agriculture. The first stock-taking of endangered breeds began and showed that with the exception of Original Pinzgau Cattle and Tyrolean Grey cattle seven out of nine autochthonous Austrian cattle breeds were highly to critically endangered (Fischerleitner and Kienberger, 2005).

BREED	NUMBER OF HEADS (breeding females)
Original Pinzgau Cattle	~ 4000
Tyrolean Grey Cattle	$\sim 4000$
Original Austrian Brown Cattle	~ 1000
Murboden Cattle	~ 500
Carinthian Blond Cattle	~ 100
Waldviertel Blond Cattle	~ 40
Tux-Zillertal Cattle	~ 40
Ennstal Pied Cattle	~ 20
Pustertal Spotted Cattle (Italy – Southern Tyrol)	~ 300

 Table 1: Number of Austrian endangered cattle breeds in 1983

(Fischerleitner and Kinberger, 2005)

From that moment breeders of such endangered cattle breeds were subsidized for keeping cattle of endangered breeds.

Other farm animals (horses, sheeps, goats, pigs) were not supported and still conserved by private breeders or associations (Fischerleitner et al., 2009).

## 2.2 Subsidies and conservation programmes

## 2.2.1 Austrian Agri-environmental Programme (ÖPUL)

Since Austria joined European Union in 1995, the Austrian Agricultural Environmental Programme (ÖPUL) became a tool to promote extensive and environmental actions. ÖPUL has now 30 actions to support farmers and one of these actions is Conservation of rare breeds of farm animals. Since 1995, 4 ÖPUL programmes were implemented through subsidies co financed by the EU (Fischerleitner, 2009).

Table 2: The 4 OPUL - p	rogrammes with	different r	number	of supported	breeds
-------------------------	----------------	-------------	--------	--------------	--------

1995-2000	ÖPUL 1995	20 Breeds			
2001-2006	ÖPUL 2000	30 Breeds			
2007-2014	ÖPUL 2007	31 Breeds			
2015-2020 ÖPUL 2015 29 Breeds					
(Fischerleitner et al., 2009)					

From 2007 - 2014, 4911 farmers decided to join the action.

To take part in this programme the farmers must fulfil the following conditions:

- Holding farmed animals from a list of acknowledged endangered breeds (29 breeds).
- The farmers have to be a member of a responsible breeding organisation for each breed.
- The kept animals are purebred and recorded by an appropriate breeding organisation.
- All breeding animals and in particular rare breeds are registered in an Austrian Control data base with all known pedigree information.
- Implementation of mating proposes for highly endangered breeds and breeds with special programmes for conservation (Fischerleitner et al., 2009).

## 2.2.2 Austrian Association for Rare Endangered Breeds (ÖNGENE)

ÖNGENE was established in 1982 and their primary aims are conserving rare endangered breeds and creating sustainable breeding strategies. From 1983 – 1995 endangered cattle breeds were conserved on farms, public agricultural institutions (research institutes, agricultural schools) and at this time every farmer got subsidies for pure breeding and raising of animals of rare cattle breeds. For the collection of Semen and Embryos ÖNGENE established a national gene bank in Wels (Fischerleitner et al., 2009). The gene bank collects semen of important sires and supports the conservation breeding. In 2000 ÖNGENE developed a conservation programme for endangered breeds. 2015 the programme was updated and their main goals are:

1. Conservation of animals through local farmers,

2. Sustainable use,

3. Conservation and Improvements of biological and genetic diversity of livestock,

4. Conservation and promotion of knowledge on conservation breeding and the use of rare farm animal breeds (<u>WWW.ARCHE-AUSTRIA.AT</u>, visited 12.09.2016).

### 2.2.3 European Gene Bank Network for Animal Genetic Resources

To find a national network of gene banks in the European countries, European Gene Bank Network for Animal Genetic Resources (EUGENA) was established in 2013. EUGENA promotes the ex situ conservation and sustainable use of animal genetic resources in Europe. The main objective of this institution is a realistic, effective, regional and integrated conservation strategy in Europe. The network improves gene bank actions and processes in the European countries by information exchange. By sharing information on gene bank collections in European countries it is possible to optimize monitoring and measurements of Animal Genetic Resources kept in ex situ collections (Hiemstra et al., 2014).

### 2.2.4 Global plan of action

The global plan of action is the first international framework for the assistance of the management of animal genetic resources for food and agriculture, promoted by local governments (FAO, 2007b). Since the 1960ies, the Food and Agriculture Organization of the United Nations (FAO) set their priorities on plant genetic resources, but since 1990 it has been focused on animal genetic resources for food and agriculture (AnGR). The global plan of action implicates 23 strategic priorities, concentrated on 4 priority areas: Area 1: Characterization, inventory and monitoring of trends and associated risks; Area 2: Sustainable use and development; Area 3: Conservation; Area 4: Policies, institutions and capacity building.

Referring to Area 3, out of 25 European Union countries, 72 % established a cryobank for national Animal genetic resources or have planned their Foundation for 2010/2011. Generally the 25 reporting European countries draw their attention to local small populations. The main emphasis is to build up gene banks, at national level or distributed across the country, and on subsidies to support rare breeds. In connection to the national information system, national gene bank documentation systems are used in 11 countries (Austria, Estonia, Finland, Georgia, Greece, Iceland, Italy, the Netherlands, Slovakia, Slovenia and Switzerland). In summary the countries take appropriate steps at different speeds with different priorities, dependent on their specific terms, conditions and capacities, based on national funds and other funding sources (Hoffmann and Scherf, 2010).

## 2.3 Genetic variability estimated from pedigree information

Within a population, the genetic composition is determined by the genetic variability. It characterizes the total number of genes in a specific population, including the rate of which genes occur. The genetic diversity is perceptible by the phenotype or with the help of DNA sequencing (Falconer, 1984).

Genetic diversity get minimized through extinction of breeds and loss of genes in a population (Baumung and Sölkner, 1999-2001). In the case of stock breeding, genetic diversity is often reduced because of performance oriented livestock breeding (Mitsching, 2011), respectively many local cattle breeds have small populations and for this reason are highly endangered (Fernandéz et al., 2011).

For the assessment of genetic variability, pedigree information can supply various measures. The method of pedigree analysis can be traced back to Wright and McPhee (1925). They examined the genetic structure in British Shorthorn cattle breed. Lacy (1989) improved the method of pedigree analysis and described the effective number of founders and the effective number of founder genomes. The publication by Boichard et al. (1997) on the value of using probabilities of gene origin to measure genetic variability in a population, followed by a paper of Sölkner et al. (1998) gave an insight about pedigree analysis in livestock breeding. Nowadays the pedigree analysis are very useful for horse breeding, sheep breeding, goat breeding and pig breeding (Preinerstorfer, 2009).

The disadvantage of the pedigree analysis are that most of these measures are very sensitive to completeness of pedigree information (Baumung and Sölkner, 2002) and pedigree records from small local breeds are often incomplete because of the mismanagement of the data (Fernández et al. 2011). However, the knowledge about genetic changes within a breed is important in order to take actions if unfavourable trends occur (Hagger, 2005).

Pedigree information has been analysed in Slovak Pinzgau Cattle (Kadlecik et al., (2011), eight Spanish beef cattle breeds (Gutiérrez et al., 2002) and three Italian beef cattle breeds (Bozzi et al. 2006). Mc Parland et al. (2006) performed a study to determine the level of inbreeding and to analyze the pedigree of Irish purebred cattle populations. All of them used pedigree analyses to evaluate the genetic variability.

Many of the rare breeds in Austria were investigated by pedigree analysis so far. Sölkner and Baumung examined (2002) three highly endangered cattle breeds, Druml (2006) evaluated the Austrian Noriker population and Glaeser (2003) explored the Austrian Shagya-Araber horse. Bäckenberger (2011) and Schäfer (2011) explored the pedigree data of Waldviertel Blond Cattle and Murboden Cattle (both highly endangered cattle breeds) in order to describe the genetic variability. Wokac (2003) also used the pedigree analysis to assess inbreeding at Tauernschecken goats.

# 3 Animals, Data and Methods

## 3.1 Animals

In this thesis, the main focus is on highly endangered cattle breeds. For this reason, the pedigrees of the highly endangered Pustertal Spotted Cattle, Ennstal Pied Cattle and Original Austrian Brown Cattle were analysed.

According to the census population size, including only breeding cows, of all cattle in Austria 336 are Pustertal Spotted Cattle, 220 Ennstal Pied Cattle and 832 Original Austrian Brown Cattle (WWW.ZAR.AT, visited 26.06.2016).

## 3.1.1 Pustertal Spotted Cattle

#### History:

This breed is called after a South Tyrol region called "Pustertal".

There are several theories about the origin, e.g. is believed that the Pustertal Spotted Cattle is descended from the Erdringer cattle. Another theory says that under the influence of the Zillertal-, Tux cattle and Pinzgauer cattle the breed found formation. After the First World War and the connecting South Tyrol to Italy, the number of animals of this breed declined. After 1929 the breeding of the Pustertal Spotted Cattle was forbidden and punished for contempt. Some farmers kept to the old breed and bred it illegally. To survive and prevent the controls of the state, they retreated to extreme locations in the mountains. Finally the ban was lifted and in the end of the 1990ies the Pustertal Spotted Cattle found way back from South Tyrol to Austria. A herd book was established in 2000 and was closed five years later. Only animals with registered parents in the herd book are allowed to be in the program for genetic conservation.

#### Phenotype description:

The Pustertal Spotted Cattle is medium to large framed and animals are horned. The primary colour is white with red, brown or black spots at the flanks. The changeover from white to colouring is dissolving into a characteristic sprinkled pattern. The appearance of the animals can be almost white or strongly pied. Special characteristics:

Vitality, robust, high vertility and meat quality.

Based on the good mother properties well suited for suckler cow husbandry (<u>WWW.BMLFUW.GV.AT</u>, visited 10.09.2016).

## 3.1.2 Ennstal Pied Cattle

<u>History:</u>

Investigations showed that the Ennstal Pied Cattle are descended from the red coloured celtic cattle. In the 18<sup>th</sup> century the breed was increasingly displaced by larger breeds, e.g.: Mürztaler, Murbodner, Blondvieh or Pinzgauer. Later on, the focus has been shifted to milk yield and the animals have been crossed with the Simmentaler Fleckvieh. The result was called "Alpenfleckvieh". Around 1935 cattle of this breed were only in the Ennstal valley and around 1950 the Ennstal Pied Cattle had almost disappeared. In 1990 an investigation showed that only 4 farms kept these highly endangered type of cattle. With the help of an organized maintenance breeding and admission in the program for genetic conservation the population became larger again.

### Phenotype description:

The Ennstal Pied Cattle is a slightly built, horned, dainty cattle.

The primary colour is foxy red and dissolving into small spots. The head, neck, legs and belly are white. The ears are often red and the horns and hooves are yellow.

### Special characteristics:

Early mature, good fertility, excellent meat quality and sufficient milk performance. Suitable for extensive regions.

(WWW.BMLFUW.GV.AT, visited 11.09.2016).

## 3.1.3 Original Austrian Brown Cattle

### <u>History:</u>

The Original Brown Cattle is descended from Torfcattle that lived in the Bronze- and Iron Age. In Austria, the Original Brown Cattle found extension in Vorarlberg in the second half of the 19<sup>th</sup> century.

After the Second World War the Brown Cattle was bred as a triple purpose cattle and in the 1960ies the search began after large framed animals with higher milk yields.

This had the consequence of systematically crossing Austrian Brown Cattle with American Brown Swiss. This new breed was established very quickly and displaced almost completely the Original Austrian Brown Cattle. With the help of special breeding systems and genetic conservation programs the highly endangered breed increased in population size again.

#### Phenotype description:

The primary colour of these medium sized and horned animals can be solid brown or grey brown, but in general bulls are of a darker colour than cows. Horn tips, muzzle and strong claws are dark pigmented.

#### Special characteristics:

Dual purpose cattle, well suited for the Alps, has a high vitality and long productive life. Special milk ingredients favour the production and quality of cheese (<u>WWW.BMLFUW.GV.AT</u>, visited 11.09.2016).

#### Table 3: Breed Illustrations





(www.keuschnig.com)

Ennstal Pied Cattle



(www.agraria.org)



(www.originalbraunvieh.de)

## 3.2 Data

### 3.2.1 ZuchtData

The data of the active breeding population and its ancestors were provided by ZuchtData EDV Dienstleistungen GmbH, a subsidiary company of the Association of Austrian Cattle Breeders (ZAR). The data set was provided with a zip-file. The file includes for each animal: the identification number, the identification number of sire and dam, date of birth, sex, different gene share, alive or dead, type of use and artificial insemination identification.

## **3.2.2 ÖNGENE**

The data of the Austrian Association for Rare Endangered Breeds contained all animals (alive or dead) of which semen is available in the genetic data bank.

### **3.2.3 Description of the reference populations**

For the pedigree analysis three types of reference populations were defined, as of 2015: 1) living population; 2) male living animals of which no semen was available; 3) bulls for which semen was available.

	Pustertal Spotted	Ennstal Pied	Original Austrian
	Cattle	Cattle	Brown Cattle
Animals in pedigree	7373	1327	22451
Animals in	1636	688	3524
reference population 1			
Animals in pedigree	3538	814	12690
Animals in	388	154	574
reference			
population 2			
Animals in pedigree	321	149	3240
Animals in	52	50	161
reference			
population 3			

Table 4: Number of animals in the pedigree file and in the reference populations

## 3.3 Methods

### 3.3.1 Pedigree Analysis

Pedigree analyses are a good method to examine the genetic structure of a population. Based on the analyses it is an important tool to describe the genetic diversity within a population. With the help of certain classification numbers the genetic variability of a population can be determined and the use of breeding animals can lead to new trends. Pedigree analyses are a good basis for future breeding decisions. The classification numbers are explained in the subsequent sections (Geng, 2010).

### 3.3.2 Quality of the Pedigree

In order to evaluate the quality of a pedigree, the complete generation equivalent, the average number of ancestors per individual and the percentage of known ancestors per generation are good measures. Maignel et al. (1996) revealed that the number of complete generation equivalent is a very good classification number for describing pedigree information. Boichard et al. (1997) calculate the average complete generation equivalent (g<sub>e</sub>) for a reference population as follows:

$$g_{e} = \frac{1}{N} \sum_{j=1}^{N} \sum_{i=1}^{n_{j}} \frac{1}{2g_{ij}}$$

- n<sub>j</sub> Total number of ancestors of the individual j
- g<sub>ij</sub> Number of generations between individual j and ancestor i
- N Number of animals in the reference population

### **3.3.3** Effective number of founders (f<sub>e</sub>)

Founder animals are animals with no known parents in a population.

$$f_e = 1 / \sum_{k=1}^{f} q_k^2$$

- f<sub>e</sub> Effective number of founder animals
- f Number of founder animals

q<sub>1</sub> Relative share of the founder alleles i in the reference population

If only one parent of an individual is known, the unknown parent animal is defined as founder (Lacy, 1989; Boichard et al., 1997). To calculate the total distribution of the founder animals in a population with respect to the loss of genetic variability due to uneven distribution of founder animals, Lacy (1989) defined the founder-equivalent (fe). The founder equivalent indicates the number of hypothetical equally contributing founder animals producing a population with the same diversity of founder alleles as the reference population (Lacy, 1989).

If all founder animals equally contribute to the distribution of genes in a population the effective number of founder animals is according to the actual number of founders. Otherwise, the effective number of founder animals is less than the current number. The more balanced the expected contribution of founders, the greater is the effective number of founder animals. By spreading the founder genes to only one or a few progeny, the founder allele is may not passed on (Lacy, 1989). Genetic variability may also be lost through genetic drift if all founder animals contribute unequally to a population.

#### **3.3.4** Effective number of ancestors (f<sub>a</sub>)

Another measure of diversity is the effective number of ancestors. It is defined as minimum number of ancestors (founder animals and non-founder animals), necessary to describe genetic diversity in a reference population. This measure takes the effects of a genetic bottleneck into account. Unfortunately, the loss of genes due to genetic drift is not considered and therefore the effective number of ancestors is overestimating true diversity. The ancestors are selected because of their genetic contributions to the population.

The ancestors are not necessarily founders, but a kinship will exist. Therefore, only the marginal gene portions of ancestors are considered. These marginal gene portions can be justified by any other ancestors (Boichard et al., 1997). For precise calculations, each ancestor is determined by having a gene portion above zero. That is difficult if the examined population is very big. To make it easier, Boichard et al. (1997) suggest upper and lower limits.



f<sub>a</sub> Effective number of ancestors

p<sub>k</sub> Marginal share of ancestor k in the reference population

#### 3.3.5 Gene shares of important ancestors

With the help of effective ancestors the most important ancestors and their gene portions can be examined. By calculating the main ancestors the percentage of gene shares can be identified (e.g.: 25%, 50% and 75%). Thereby the most important sires of a reference population give information about the composition of the population.

### 3.3.6 Effective number of founder genomes (Ng)

Another important classification number in addition to the effective number of founders and the effective number of ancestors is the effective number of founder genomes. It indicates the probability that the genes of the founder population are also present in the current population (Boichard et al., 1997). MacCluer et al. (1986) describe the method of gene dropping in order to calculate the remaining founder genomes in the reference population. The pathways of genes are observed from the founder population up to the current population. Each founder animal will be randomized with two hypothetical alleles and with probabilities of 0.5 each, one of the two alleles is transmitted to a progeny. By simulating the transfer of alleles for the entire pedigree, the relative share of founder alleles in a reference population may be calculated.

The effective number of founder genomes considers bottleneck effects, genetic drift and other possible causes for changes in the genetic variability of a population. The calculated values are smaller than the other classification numbers.

The effective number of founder genomes is estimated after Lacy (1989) and Boichard et al. (1997):

$$N_g = 0.5 \cdot \left( 1 / \sum_{i=1}^{2f} q_i^2 \right)$$

- N<sub>g</sub> Effective number of founder genomes
- f Number of founder animals
- 2f Number of founder alleles
- q<sub>l</sub> Relative share of founder alleles i in the reference population

### 3.3.7 Inbreeding, inbreeding coefficient and effective population size

According to Rasch and Herrendörfer (1990), inbreeding occurs with mating of a pair of individuals which are more closely related to each other than the average of the population. In the context of this study, if an animal shares a common ancestor on maternal and paternal side of the pedigree, we consider it inbred.

In livestock breeding a certain amount of inbreeding can be tolerated. By practical approach of line-breeding, the genetic make-up of most important ancestors is widespread in a population. Negative form of inbreeding can lead to inbreeding depression. Inbreeding is determined by inbreeding coefficients. Inbreeding coefficient is defined as the probability that two allele at any locus are identical by descent (Falconer, 1984). The further the relationship is removed, the lower is the inbreeding coefficient. Values between 0 and 1 are possible, these values are often converted in percent of inbreeding by multiplication with 100. The inbreeding coefficient can be calculated after Wright (1925):

$$F = \sum \left(\frac{1}{2}\right)^{n1+n2+1} \cdot (1+F_A)$$

- F Inbreeding coefficient of an individual
- $n_1$  Number of generations between the individual and the common ancestor on the father's side.
- $n_2$  Number of generations between the individual and the common ancestor on the mother's side.
- F<sub>A</sub> Inbreeding coefficient of the common ancestor on the mother's side

With the knowledge of the inbreeding coefficient, the effective size of a population can be defined. The effective population size ( $N_e$ ) is determined as a size of an ideal population presenting the same rate of increase in inbreeding per generation as the non-ideal population (Sölkner et al., 1998). The effective population size may be calculated after Wright (1923):

$$N_e = \frac{1}{2\Delta F}$$

 $\Delta F$  Relative increase in inbreeding by generation

The value of  $\Delta F$  may be conveniently calculated by subtracting from the inbreeding coefficient of each animal of the reference population the average inbreeding of its parents.

#### 3.3.8 Total/marginal contributions of the most important ancestors

The calculation of the effective number of ancestors is based on the marginal contribution of each ancestor. The word marginal presents the gene contribution which cannot explained by an ancestor already chosen before (Baumung and Sölkner, 2002).

## 3.4 Software

The data set necessary for pedigree analysis was prepared with SAS 9.4 and Microsoft Excel. For the calculation of the described indicators of population variability PEDIG from Boichard (2007) was used. This software works with large populations and consists a set of independent programs written in Fortran. Pedig determines various indicators based on the probability of gene origin, kinship and inbreeding coefficients as well as the quality of the pedigree.

The programs used in PEDIG software package are prob\_orig.f, ngen.f, meuw.f and segreg.f. Prob\_orig.f calculates the probabilities of gene origin for a reference population or a group of individuals, which are selected according to their sex and birth period. The effective number of founders is calculated from those probabilities. The result file also shows the number of founders, the effective number of ancestors and the greatest marginal contributions of the most important ancestors. For this thesis, the inbreeding coefficient for all animals was calculated with meuw.f. The program ngen.f determined the average number of known ancestors, the percentage of known ancestors per generation and the number of equivalent complete generations. Finally, the program segreg.f was used to estimate the effective number of founder genomes in the reference population (Boichard, 2007).

In order to work with PEDIG-Software, the data was reformatted with SAS and the following information was included: the identification number of each individual must be a consecutive number, date of birth, a reference code and the identification number of sire and dam.

# **4** Results and Discussion

## 4.1 Quality of the Pedigrees

Table 4 provides information about the quality of pedigrees for the three breeds. The complete generation equivalent is a good measure to assess pedigree completeness (i.e. number of generations in a homogenous complete pedigree) (Boichard et al., 1997) and the value of 5.43 for Pustertal Spotted Cattle is not so far from that found for the Italian Chianina breed (Bozzi et al., 2006). The other findings for the generation equivalent were above 3.0 generations and allow almost accurate computations (Falconer and Mackay, 1996). The number of maximum known generations ranges from 20 (Original Austrian Brown Cattle, Pustertal Spotted Cattle) to 18 (Ennstal Pied Cattle).

When Bozzi et al. (2006) investigated Italian cattle population and Baumung and Sölkner (2002) examined Austrian cattle breeds, they found lower values for maximum known generations.

The Ennstal Pied Cattle has the lowest number of animals in the pedigree but provides similar values of pedigree completeness as the Pustertal Spotted Cattle.

However, Original Austrian Brown Cattle shows more accurate pedigree information because around 60% and 40% of ancestors were known for generations 8 and 10. The complete generation equivalent for this breed is 8.54. This quite high value is due to the fact that pedigrees have been recorded for a much longer time in Original Austrian Brown Cattle as compared to the two other breeds in this study.

	Pustertal Spotted	Ennstal Pied	Original Austrian
	Cattle	Cattle	Brown Cattle
Animals in the pedigree file	7373	1327	22451
Animals in reference population	1636	688	3524
Average no. of ancestors	204	129	6366
Complete generation equivalent	5.43	5.04	8.54
Maximum no. of generation traced	20	18	20
% known ancestors at generation 2	99.46	99.45	99.75
4	90.02	82.66	97.70
6	45.76	37.82	78.06
8	10.42	06.63	59.85
10	01.23	00.26	40.46

Table 5: Pedigree information available for the three populations

## 4.2 Inbreeding coefficient, effective number of founders, effective number of ancestors and the effective number of founder genomes

## **4.2.1 Pustertal Spotted Cattle**

Table 6 presents different measures to assess the genetic variability of Pustertal Spotted Cattle The average inbreeding coefficient was less than 1% for all reference populations. The values for the effective population size ranged from 41 (reference population 2), 82 (reference population 1) and 89 for reference population 3. The total number of founders (f) were 2456, 1202, 117 and higher than the effective number of founders (f<sub>e</sub>).

The highest effective no. of founder genomes of 14 was found in reference population 1 and as expected the values for  $N_g$  were always lower than those for  $f_a$ . This is so based on the fact that  $f_a$  considers the bottleneck and Ng also considers the random loss of alleles during segregation.

	reference population 1	reference population 2	reference population 3
Average inbreeding coefficient	0.0087	0.0091	0.0092
Inbreeding increase	0.0061	0.0122	0.0056
Effective size (N <sub>e</sub> )	82	41	89
Total no. of founders (f)	2456	1202	117
Effective no. of founders (f <sub>e</sub> )	62.8	53.1	65.7
Effective no. of ancestors $(f_a)$	29.4	24.5	31.6
Effective no. of founder genomes $(N_g)^1$	14	13	12

Table 6: Results of the pedigree analysis for the three reference populations

<sup>1</sup> mean of 1000 replicates, standard deviation = 1.59 to 1.69 according to breed

### 4.2.2 Ennstal Pied Cattle

The average inbreeding coefficient provides poorly values in contrast to Original Austrian Brown Cattle and Pustertal Spotted Cattle.

The average inbreeding coefficient for reference population 1 is >3%, for reference populations 2 and 3 it is >2%. Similar findings were reported by Bozzi et al. (2006) for the Chianina and Marchigiana beef cattle breed. Possible explanation for these high values is the extremely small population size of the Ennstal Pied Cattle breed. Therefore future breeding decisions should be influenced on controlled inbreeding.

The effective population size ( $N_e$ ) are 34, 47 and 60 respectively. The FAO (1998) revealed that an effective population size of less than 50 individuals indicates that this population is at a critical stage. Meuwissen (1999) pointed out that due to mutation and drift the crucial effective size should be between 50 and 100 animals. The total number of founders shows large differences between the reference populations. The largest number was found for reference population 1 and the smallest for reference population 3. The effective number of founders is smaller than the total number of founders, except reference population 3. Here is the effective number of founders 37.3 and the total number of founders 34.

The effective numbers of founder genomes were similar in all three reference populations and range from 6 to 7. According to Lacy (1989), at least 20 effective founder genomes are necessary to maintain genetic variability within a population.

	reference	reference	reference
	population 1	population 2	population 3
Average inbreeding coefficient	0.0332	0.0246	0.0206
Inbreeding increase	0.0289	0.0211	0.0164
Effective size (N <sub>e</sub> )	34	47	60
Total no. of founders (f)	235	197	34
Effective no. of founders (f <sub>e</sub> )	21.5	19.4	37.3
Effective no. of ancestors (f <sub>a</sub> )	15.6	13.5	26.6
Effective no. of founder genomes $(N_g)^1$	7	7	6

Table 7: Results of the pedigree analysis for the three reference populations

<sup>1</sup> mean of 1000 replicates, standard deviation = 1.16 to 1.26 according to breed

## 4.2.3 Original Austrian Brown Cattle

The Original Brown Cattle offers the largest population in sum and the following parameters provide significant classification numbers to evaluate the genetic variability.

The average inbreeding coefficient is small for all three reference populations and is in agreement with those found for other Austrian cattle breeds (Sölkner et al., 1998). The effective size ( $N_e$ ) based on increase of the inbreeding coefficient is 238 and 152. The values for the effective number of founders ( $f_e$ ) are very high in all three reference populations but lower than the total number of founders and led us to the knowledge that founders contribute unequally. The most reliable measures of variability are the effective number of ancestors ( $f_a$ ) and the effective number of founder genomes ( $N_g$ ). Both classification numbers take the bottleneck into aoccount and  $N_g$  also considers the random loss of alleles during segregation. In Reference population 3 (bulls for which semen is available) the value for  $f_a$  is 147.8 and for  $N_g$  is 37. The data are higher than Reference Population 1 or 2. This may be due the fact that bulls of reference population 3 are not descended from the period as the bulls from the other populations.

	reference population 1	reference population 2	reference population 3
Average inbreeding coefficient	0.0083	0.0067	0.0025
Inbreeding increase	0.0021	0.0021	0.0033
Effective size (N <sub>e</sub> )	238	238	152
Total no. of founders (f)	5578	3760	1261
Effective no. of founders (f <sub>e</sub> )	387.5	345.5	505.1
Effective no. of ancestors (f <sub>a</sub> )	42.7	37.6	147.8
Effective no. of founder genomes $(N_g)^1$	21	20	37

Table 8: Results of the pedigree analysis for the three reference populations

<sup>1</sup> mean of 1000 replicates, standard deviation = 2.59 to 4.17 according to breed

## 4.3 Marginal contributions of the most important ancestors

Table 9 provides the marginal gene shares of the most important ancestors for reference population 1 of each of the three breeds under investigation. The values for the most important ancestor are between 7 and 14% of the gene pool. For the Ennstal Pied Cattle only 5 ancestors explain 50% of the genes of the population. Sölkner et al. (1998) found similar results for the Tux-Zillertal (also highly endangered cattle breed in Austria) and Maignel et al. (1996) determined for the French cattle Tarentaise also similar findings. The Original Austrian Brown Cattle has the highest number of ancestors (15) contributing 50% to the population. However, for the Pustertal Spotted Cattle 10 ancestors contributed at least 50% of the genetic material.

	Pustertal Spotted Cattle	Ennstal Pied Cattle	Original Austrian Brown Cattle
First ancestor	08.35	13.67	06.86
Second ancestor	06.64	09.73	05.37
Third ancestor	06.49	09.70	04.55
First 10 ancestors	52.26	70.63	40.73
No. of ancestors contributing to the first 50%	10	5	15
No. of ancestors contributing to the first 80%	33	15	54

Table 9: Description of the most important ancestors

## 4.4 Gene shares of the most important ancestors

Tables 10 - 12 show the 20 most important ancestors of reference population 1 (current living population) with name, birth year, sex and marginal genetic contribution for all three cattle breeds.

For Pustertal Spotted Cattle, the most important ancestor (a bull called Ortner) contributes 8.35% to the genetic material which is very similar to the results from the Murboden Cattle (Bäckenberger, 2011). Most of the important ancestors are male (13) and were born between the 1970ies and end of the 1990ies.

	Name	Birth year	Sex	Marginal genetic contribution (%)
1	Ortner PSS	1984	m	8.35
2	Schecke PSS	1988	f	6.64
3	Rosa PSS	1990	f	6.49
4	Preuss PSS	1995	m	6.13
5	Irbis PSR	1982	m	5.30
6	Etzel PSS	1984	m	4.33
7	Ozo PSR	1998	m	3.90
8	Fritz PSS	1984	m	3.66
9	Grones PSS	1980	m	3.30
10	U-Weger PSS	1991	m	3.19
11	Ranke PSR	1992	f	2.90
12	Elze PSS	1988	f	2.31
13	Orco PSS	1996	m	2.14
14	Omar PSS	1978	m	2.03
15	Elster PSS	1989	f	1.88
16	Blieme PSS	1990	f	1.51
17	Origano PSS	1996	m	1.50
18	Lilla PS	1987	f	1.49
19	Eddy PSS	2003	m	1.42
20	Omar PSS	1978	m	1.29

Table 10: The 20 most important ancestors of the Pustertal Spotted Cattle

A small number of ancestors of the Ennstal pied cattle contribute heavily to the current living population. These values are similar to those found in Danish Holstein Cattle (Sorensen et al., 2005). As a consequence, a typical female Ennstal Pied Cattle has 14% of genes from Hans (first most important ancestor). Hazuchova et al. (2013) found same results for first most important ancestor of the Slovak Spotted Cattle .The ancestors of Ennstal Pied Cattle were born between 1970ies and the end of the 1990ies.

There are no younger bulls in this list who could pass their genetic share to the progeny. The listing indicates that most (17) of the 20 most important ancestors of the living Ennstal Pied Cattle population are female.

	Name	Birth year	Sex	Marginal genetic contribution (%)
1	Hans	1991	m	13.67
2	H1	1975	m	9.73
3	Sachsa*93	1994	f	9.70
4	Bacherin/S*9	1987	f	9.15
5	Gustav	1996	m	7.95
6	Marika*89	1987	f	5.93
7	BSR-Kreuzung	1981	f	4.37
8	Dachstein/*9	1991	f	4.31
9	Moasa*95	1995	f	2.98
10	Glücka*91	1990	f	2.84
11	Senga	1985	f	2.48
12	Romy*93	1997	f	2.18
13	Monika*93	1993	f	1.87
14	Oberwölz	1985	f	1.73
15	Gundi*85	1985	f	1.53
16	Mina	1994	f	1.39
17	Mora*93	1993	f	1.20
18	Irdning**92	1992	f	1.20
19	Schwoaga	1995	f	0.82
20	Edelweiß*93	1993	f	0.79

Table 11: The 20 most important ancestors of the Ennstal Pied Cattle

The most important ancestor of the Original Austrian Brown Cattle contributes 6.86 and the majority of the important ancestors are male (18 out of 20). The values found for this breed are similar to the Italian Romagnola breed (Bozzi et al., 2006) In contrast to the Ennstal Pied Cattle, the animals were born between the nineteen-fifties and 2007.

	Name	Birth year	Sex	Marginal genetic contribution (%)
1	Singau*OB	2007	m	6.86
2	Wenzel*OB	1994	m	5.37
3	Merlo*OB	1995	m	4.55
4	Kronos*OB	2007	m	4.52
5	Siegfried*OB	2005	m	4.23
6	Duefur*OB	1960	m	3.91
7	Munot*OB	1976	m	3.04
8	Asterix*OB	2009	m	2.77
9	Idan*OB	1997	m	2.76
10	Roland*OB	1969	m	2.72
11	Winzer*OB	1970	m	2.58
12	Tristan*OB	2002	m	2.40
13	Gral*OB	1995	m	1.83
14	Klaerli*OB	1956	f	1.81
15	Nussl*OB	1987	f	1.73
16	Zucki*OB	1987	m	1.73
17	Sero*OB	1986	m	1.61
18	Kastor*OB	1998	m	1.38
19	Voeris*OB	1994	m	1.35
20	Glueck*OB	1961	m	1.24

Table 12: The 20 most important ancestors of the Original Austrian Brown Cattle

## 4.5 Comparison with other rare cattle breeds of Austria

Similar studies with other endangered cattle breeds in Austria are compared with this study. The findings about the quality of pedigree are presented in Table 13.

The measure for the complete generation equivalent varied between 1.73 for Carinthian Blond Cattle to 8.54 for the Original Austrian Brown Cattle. The low values for Carinthian Blond and Tux-Zillertaler Cattle are descendend from 2002 (Baumung and Sölkner) and at present the pedigrees are probably better built up. In comparison with the examined breeds in this study, Pustertal Spotted Cattle, Ennstal Pied Cattle and Original Austrian Brown Cattle had the best results.

Breed	Animals in the pedigree file	Complete generation	Maximum no. of generations
		equivalent	traced
Murboden Cattle <sup>3</sup>	10156	4.14	15
Carinthian Blond Cattle <sup>1</sup>	1194	1.73	9
Original Pinzgauer Cattle <sup>1</sup>	176992	5.33	13
Tux-Zillertaler Cattle <sup>1</sup>	1235	2.52	6
Waldviertel Blond Cattle <sup>2</sup>	2474	3.41	5
Pustertal Spotted Cattle	7373	5.43	20
Ennstal Pied Cattle	1327	5.04	20
Original Austrian Brown Cattle	22451	8.54	18

Table 13: The quality of pedigree in compare with other rare Austrian cattle breeds

<sup>1</sup>Baumung and Sölkner (2002) <sup>2</sup>Schäfer (2011) <sup>3</sup>Bäckenberger (2011)

In order to examine the genetic variability of the eight rare Austrian cattle breeds, Table 14

presents the different classification numbers.

The largest number of animals in the reference population showed the Murboden Cattle (10156) and the Original Pinzgau Cattle (9760). The smallest reference population was found for the Tux-Zillertal Cattle (471), followed by the Carinthian Blond Cattle (230). The quite high value of 387.5 for the effective number of founders ( $f_e$ ) was found for the Original Austrian Brown Cattle The Murboden Cattle as the biggest reference population has 47.7 effective number of founders and the Pustertal Spotted Cattle as a rather small population shows an higher number of effective founders (62.8).

Breed	Reference population	Total number of founders (f)	Effective number of founders (fe)	Effective number of ancestors (fa)	Effective number of founder genomes (Ng)
Murboden Cattle <sup>3</sup>	10156	4997	47.7	26	19.4
Carinthian Blond Cattle <sup>1</sup>	230	305	30	29	22.6
Original Pinzgau Cattle <sup>1</sup>	9760	6495	65	32	19.5
Tux- Zillertal Cattle <sup>1</sup>	471	112	21	14	9.7
Waldviertel Blond cattle <sup>2</sup>	2474	970	17.7	14.68	9.52
Pustertal Spotted Cattle	1636	2456	62.8	29.4	14
Ennstal Pied Cattle	688	235	21.5	15.6	7.0
Original Austrian Brown Cattle	3524	5578	387.5	42.7	21.8

Table 14: Results for the pedigree analysis in compare with other rare cattle breeds

<sup>1</sup>Baumung and Sölkner (2002) <sup>2</sup>Schäfer (2011) <sup>3</sup>Bäckenberger (2011)

The smallest  $f_a$  was found for Tux-Zillertal and Waldviertel Blond Cattle. The results for the effective number of founder genomes, which accounts all losses of genetic diversity during segregation, ranges from 7.0 for Ennstal Pied Cattle to 22.6 for Carinthian Blond Cattle. In case of the Carinthian Blond Cattle the values for  $f_e$ ,  $f_a$  and  $N_g$  are very similar and possible explanations are that the conservation of genetic material and recording of pedigree data is in an early stage (Baumung and Sölkner, 2002).

# 5 Conclusion

Pedigree analysis is an important tool to examine the genetic diversity of populations.

The study highlights that the Ennstal Pied Cattle is the smallest population in total numbers and in terms of genetic variability. The result could be attributed to the history of the Ennstal Pied Cattle. The breed was almost extinct and consequently no large genetic diversity can develop from a base of very few founder animals. The Pustertal Spotted Cattle have a somewhat bigger population size but far lower inbreeding coefficients. This may be attributed to a much larger number of founder individuals in this breed. In contrast, Original Austrian Brown Cattle population is more diverse due to the recent use of many bulls from Austria, Germany and Switzerland.

The results of this study lead to the conclusion that for future breeding decisions the best choice of young bulls for artificial insemination will be crucial to minimize loss of genetic diversity in those three breeds. The genetic diversity of the smallest population in this study, the Ennstal Pied Cattle and the Pustertal Spotted Cattle are still at risk and need further monitoring. This study is indicating that sustainable breeding strategies and conservation programmes are absolutely essential for the survival of highly endangered breeds in Austria.

With regard to the future, if subsidies for endangered breeds will be stopped, it will be difficult to preserve these rare breeds because of the trend to intensive Agriculture, including animals with strong milk or meat performance.

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