

THESIS

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**Comparison of two Zaupel Sheep descendants, Cikta and Bovec,
based on their mtDNA CR sequences**

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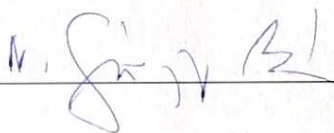
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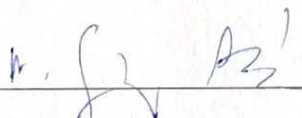
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1. List of Abbreviations

DNA – Deoxyribonucleic Acid

RNA – Ribonucleic Acid

mtDNA – mitochondrial Deoxyribonucleic Acid

ATP - Adenosine triphosphate

Cyt-B – Cytochrome B

MT-CYT – Mitochondrial Cytochrome B

BC – Before Christ

EDTA – Ethylenediaminetetraacetate

CR – Control Region

2. Introduction

We live in a time of acceleration and development. Everything has to be faster, bigger, cheaper or simply more economic. Not only have the standards in technology risen, but also those of animal production. To achieve higher efficiency in livestock, humans started to select and breed different races via artificial insemination or embryonal transfer. Each of them with specialised breeding goals such as meat, milk and wool. To achieve these increasing expectations, selection is driven every day to higher standards. As these breeding aspects become more and more relevant, others such as body conditions and dependant health are pushed to the background. This of course bares a risk of higher infection rates with consequential lower production. The demand for organic animal products has increased not only because of animal welfare aspects but also because of its' effects on global warming. One important contributing factor here is the genetic variety to produce healthy as well as resistant animals. To achieve this goal we try to re-establish autochthonic races, to hybridise standard breeds, and increase their health as well as resistance for future productions.

Considering these aspects, improving the genetic material is not only necessary but inevitable. As such, the research here has to increase.

For this reason, we investigated blood samples drawn from several individuals of different herds, which were thought to be related to the extinct Zaupeel. Specifically, the investigation was based on the comparison of the mutated mitochondrial DNA (mtDNA) and genomic DNA. For this, the analysis of the Cytochrome B region and the CR of the maternal mtDNA was essential. The similarities as well as the alterations in the DNA gave us the possibility to compare the common ancestors and subsequently study the development of sheep races. A vast and thorough research provided us with historical information that was able to supplement our gene analysis, providing us with crucial results to help preserve the genetic variety in the future.

In our case the autochthonic Cikta sheep as well as the Bovec-sheep was used as descendant of the Zaupeel sheep.

In medieval times, the Zaupeel sheep was not only the most widespread sheep in Europe but also used most frequently for all three necessities: meat, wool and milk. Considering the

paucity of the historical information, often containing only the description of the breed, first the phenotype was used as differentiation.

3. Aim

The aim of our study is to recognize and extract the descendants of the Zaupel sheep in the first step by their phenotypic characteristics but then specially to verify them by their genetic components. For this purpose, with help of the latest genetic techniques, the same ancestry and links should be demonstrated and conclude the common origin. To make this possible, we examined the differences in the maternal genetic material from two out of three known Zaupel descendants: Cikta and Bovec. For this purpose, the sequence order of the variable control region (CR) of the mtDNA was examined and evaluated afterwards more detailed. Based on our results we like to prove the same genetic ancestry of the different historical breeds. Above all, however, the goal is to create a basis for further investigation of the genetic characteristics of the Zaupel derivate group in order to conduct more intensive research in this area in the future.

4. Used Gene Components

4.1 Mitochondrial DNA

Mitochondrial DNA (mtDNA) is the DNA inside the mitochondria. The mitochondria appears in high numbers in every living cell and is responsible to supply the body with energy in terms of producing ATP (Adenosine triphosphate). In mammals the structure of the mitochondrial DNA consists of a spherical formed double helix. Sheep possess about 16.500 base pairs in their genome for certain mitochondria, which is used for ancestral research. The mtDNA only gets inherited by the maternal side as the paternal mtDNA gets lost during either penetration or later-on during the cell division. Furthermore, the sheep-mtDNA is used to ascertain and understand the historical background of its domestication and the formation of its different breeds.

For comparison the ancestral line (Haplotype group A, B, C and D) which contains the maternal mtDNA is used. The haplotype group then gets assigned to a Cyt-b-gene. For example according to the haplotype groups, haplotype B occurs primarily in mouflon and European breeds (Tapio et al., 2016). It is still used today.

4.2 Cytochrome B gene

Cytochrome B is a protein of the mitochondrial DNA (MT-CYB) and is one of the eleven components within a protein group of complex III. It plays a fundamental role in the energy transportation chain by converting redox reaction to energy for proton transportation to cross the inner mitochondrial membrane. It is responsible for preventing oxidoreductase. Furthermore, Cyt-B is involved in the process of binding the quinone substrate.

Cytochrome B is a protein that is highly conserved in the gene and is therefore used to determine the degree of relationship within a DNA analysis. Mitochondrial Cyt-B is particularly important when it comes to clarifying the deviations in relation to taxonomy. The mitochondrial Cyt-b-gene is used in systematic studies to verify the differences within several taxonomic levels. It is considered as one of the most effective genes for analysis in mammals since most of the sequencing information is already acquainted. Considering the

multifarious sequence changes, the Cyt-b-gene is a moderate investigation method to compare animals within the same breed (Castresana, 2001).

4.3 Control Region

The control region, also known as the promoter region, is the largest non-coding segment on the mtDNA. It is responsible for controlling transcription and replication of the mtDNA as it contains both transcription start sites. It also has the mitochondrial origin of replication for one strand of the D-loop. This section is the most variable region of mtDNA, enabled by two hypervariable regions in the sequence. Although the variability of the regions is low (1.7%), there is selection pressure on the transcribed secondary structure of the RNA (Stoneking, M., 2000).

5. Breeds

The sheep is one of the earliest domesticated animals in the human history. Along with the goat and dog, the sheep has been appreciated by humans for more than 10.000 years.

Today's domesticated sheep breeds can be traced back to the wild mouflon, living in the mountain area of Asia and Europe. Even the ancestors of North America progenies of Asian immigrants. Earliest fossils date back to the lower Pliocene of Asia at around 5 million years. Today, we have more than 1 billion different breeds of the domesticated sheep all over the world. Depending on each culture and economy, the animals are held for meat, milk, hide and wool production as well as landscaping.

The Zaupel sheep is an indigo Germanic race and no longer exists in its original form. Due to this, there is no complete DNA sequence in possession, held back as file. For further investigations, breed specific phenotypes from the medieval Zaupel sheep records have to be used to select genetic progenies.

In the past, the Zaupel sheep was the predominant breed in south Germany and territories of the Alps. Its' name derives from the German word "Zaupel" which, translated out of the old-Bavarian tongue, means "permissive girl" or "slovenly". The Zaupel was associated with high fertility and was used for its wool as well as for its meat and milk. Over time, the Zaupel was replaced with specialised breeds, i.e., for wool production the Spanish merino sheep stepped in while British breeds were the preferred meat producers, leading to a decrease in Zaupel sheep. Nowadays, four main progeny lines exist: in Germany and Austria the Waldschaf (Sumavska), the Alpine Stone sheep (Bovec), the Tyrol Mountain sheep, and in Hungary the Cikta sheep. They all evolved out of a small group of Zaupel sheep (as seen in Figure 1), which was brought to south Hungary with the emigrating German speaking immigrants e.g., Swabian (Kovács E., et.al., 2017).

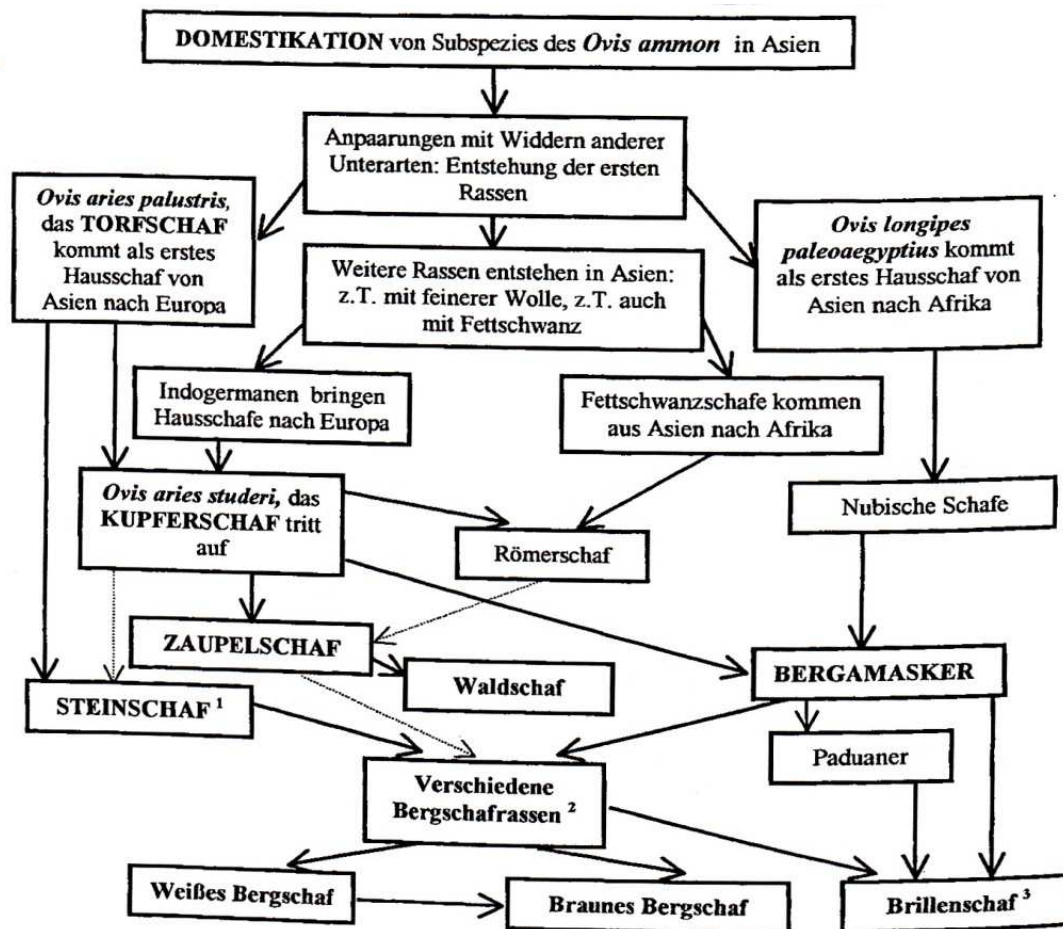


Figure 1: Kinship among some major sheep forms after Wassmuth et al. (2001)

5.1 Extinct breeds

To date, there are four known breeds stemming from the Zaupel sheep. The next section will explore the history and characteristics of the Turf and the Zaupel Sheep for comparisons sake.

5.1.1 Turf sheep

The Turf sheep was one of the early sheep breeds already known in the Neolithic Period (10,000 BC). As a descendent from the Armenian wild sheep, it is one of the oldest farm animals to be kept by humans. Since then, the worldwide distribution of the turf sheep and similar descendant phenotypes, as well as types of bred wool-sheep held place. They were

all bred for wool, milk or meat production. During the time of stilt houses, the Turf sheep evolved in mid Europe. Excavations revealed numerous records, and with it knowledge, about the breed. It was thought to be a small and delicate sheep with rough mixed wool that had multiple colour variations. Both sexes carried horns. Mainly the wool of the sheep was used for things such as outer garments, curtains and blankets. In certain instances, the meat, hides and horns were also used. Another Name for this breed was Zaupeel sheep. Out of the Turf sheep the medieval Zaupeel sheep evolved. As an additional descendent of the Turf sheep, the Travetscher sheep is acknowledged as such. It became extinct in 1960 despite the effort of preserving the breed (Bündner Oberländer Schaf, 2021). Through further breeding of similar looking sheep, today's Vriner sheep inherits the properties of not only the Travetscher sheep but also the ones of the Turf sheep (see Figure 2).



Figure 2: Female Vriner sheep (Val Lumenzia), 1902

5.1.2 Zaupel Sheep

“Most of the European medieval sheep – as cattle- belong to a single, primitive breed. The physique was small, as cattle were. Animals belonging to this population regarding their outer appearance resembled the becoming extinct Land-sheep.” (Bököny, 1958)

The primal Zaupel-sheep was a descendant of the Land-sheep. It developed into four main breeds: Cikta, Waldschaf (Sumavska), the Mountain sheep and the Stone-sheep.

It was found from middle Germany and bred mostly in Oberschwaben, Ober- and Unterbayern, Steiermark, Kärnten, Krain, Tirol. Its’ range reaches Belgium in the north and as far as Italy in the south. In the east it reaches Hungary and, in the west, even over the Rhein. As the climatic and vegetative conditions increased, the Zaupel was crossed with more economic breeds resulting in today’s ancestral progenies.

A historical description of the Zaupel-sheep was mentioned by Bohm (1878) as well as by Heyne (1909) in each of their textbook regarding sheep breeding. In general, the description appears to be very similar to the one of Cikta. As pictured in Figure 3 the wither height in rams was around 59cm, the adult ewes had a wither height of 55cm. The regular appearance was comparable to the one of a Merino-sheep. Its base was narrow. The head was small, but long, which results in a flat and slender form especially of the forehead. The ears were medium sized, strongly rolled and protruding sideways. The horns, which you could mostly find on the ram, were curved, flat and growing to the back and downwards. It curled up to the same height as eyes and ears, ending blunt. Neck and trunk were covered in strong, shining wool. Most animals had downy wool under the long and rough cover wool. The sheering weight was around 1,5 kg. The colour was mostly dirty white, sometimes also brown or black. White animals often had a black muzzle and black rings around the eyes. The breed was seemingly fertile and had mostly twins (Mitro, S., 2016).

To encounter a more detailed view of the primal Zaupel sheep we will have to lay our focus more carefully onto its closest relatives. The Cikta sheep, together with the Bovec sheep and the Waldschaf in Germany, is mentioned as one of the closest breeds, sharing many similarities with the Zaupel sheep (Schwäbisches Bauernhofmuseum Illerbeuren, 2000, Figure 4).

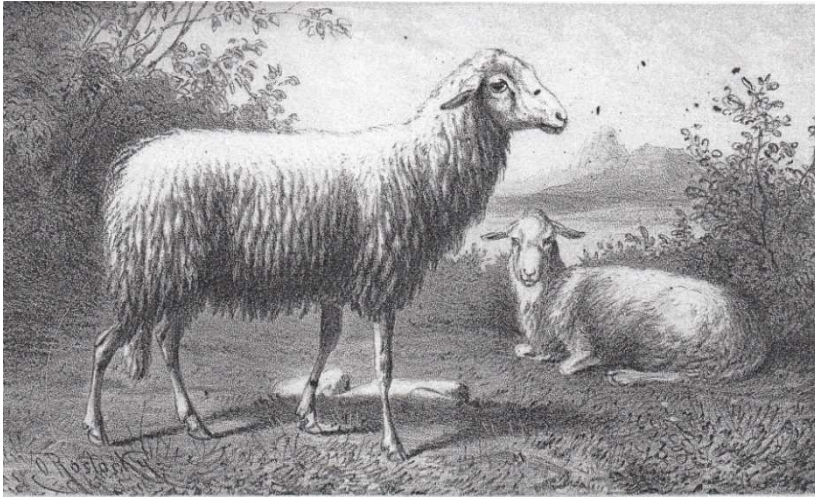


Figure 3: The Zaupel sheep (May G., 1868)



Figure 4: Today's Zaupel sheep
(with kind approval of Schwäbisches Bauernhofmuseum Illerbeuren, Kutter T., 2012)

5.2 Descendants of the Zaupel sheep

The next section will discuss the descendants of the Zaupel Sheep. These include the Cikta, a Hungarian breed, the Waldschaf, stemming from Bavaria in Germany as well as the Bovec sheep from Slovakia and the Tyrol Mountain sheep stemming from the Austrian Alps. Each breed will be discussed for the similarities to the Zaupel sheep, as well as the unique characteristics it has and its range.

5.2.1 The Cikta

The Cikta sheep is a Hungarian breed which evolved from the medieval Zaupel sheep in the early 1900s. They still share many aspects with the Zaupel sheep, including the horns and skeletal construction. The breeders try to keep these unique features. Its' body size is comparable with the one of a Merino. It was formerly held as a meat producing breed. If you compare the description of the Zaupel sheep by Bohm in 1878 and Heyne 1909 it sounds just like the Cikta sheep of today.

In 2000 P. Neugebauer registered imported Cikta sheep as Zaupel in the Bavarian Sheep Herd register, and disagreements arose among breeders. Today the general opinion is that since the Zaupel breed is extinct, one cannot consider a Cikta sheep as Zaupel in the official Herd register. The phenotype may be the same, but as for today the breed is genetically different. With a non-economical goal to preserve a larger genetic pool, the total existing numbers of Cikta sheep is around 600 animals, divided into a few herds. Besides private breeders, there are also the Duna-Dráva National Park and Schwäbisches Bauernhofmuseum Illerbeuren (handled as Zaupel) who house this species.



Figure 5 - Cikta Sheep, LfL Bayerische Landesanstalt für Landwirtschaft

Nowadays, the Cikta sheep is small to medium sized. The wool colour is mostly white, but generally diverse in colour: brown and black. It is characterized by a small head, flat forehead and small ears. Its' horns are curved backwards (Schafzucht Niedersachsen, 2019). Both male and female can carry horns, with the male horns being more prominent (see Figure 5).

With an aseasonal breeding cycle, the Cikta sheep can conceive twice per year.

Since the early 1700s, big rulers such as Karl III lead German settlers including their Zaupel sheep to Hungary. Today's Cikta sheep has evolved out of these precursors today. Due to explicitly higher quality of wool, in comparison to the native sheep breeds, the shepherds started exchanging their sheep for the newly imported Zaupel. Presuming that Cikta was the woolly land sheep of mid Europe, additional names for it were „Zibben“ (birkás), which was the colloquial name, or the swabian sheep of Tolna and Baranya. In an essay compiled by Andrásfalvy (1975) the writer reports about farmers in Decs (1749) and close to Pilis (1766) complaining about zibben destroying nearby land. Since the word „zibben“ was mentioned instead of „sheep“ one can understand that the farmers were talking about Cikta sheep with high quality wool. To distinguish if the documents really meant the Cikta sheep instead of i.e. Merino sheep you can easily find that the Merino sheep together with its high quality wool was imported for the first time in 1773 by Maria Therese. To understand how the breed reached western Hungary we have to highlight that German shepherd leased Hungarian meadows to maintain their sheep herds. The name „Zibben“ (birka) was already used in the 16.-17. Century formerly for the böhmisch/mährisch breed. It later was used by Moravien

farmers and thereby spread all over Hungary. This eventually led to a clear differentiation between Cikta and Racka sheep (discussed below).

If it hadn't been for the interest in autochthon farm animal breeds, not only the Cikta but also the Racka and Tsigai (Cigája) would have gone extinct. For this reason, the last individuals of their kind were collected and bred in a penitentiary in Bezzeg-pusztá. These measurements were taken by the government in 1974. Since then, a rearing farm for Cikta breeding rams was built in addition in 2014 by the Hungarian breeding association for sheep and goats (MJKSZ).

5.2.2 The Waldschaf: Forest sheep/ Sumavska

The Waldschaf was bred in eastern Bavaria and was/is a cross breed progeny of the Zaupel sheep and the German land race. This breed was well situated in the Alps of Germany and Austria, as well as in the Czech Republic, called Sumavska sheep, and used for wool production. Around 1960, the Waldschaf popularity dropped and finally disappeared. In 1980 scientists began to preserve the dying breed by collecting the last existing individuals of their kind. Due to the interest in keeping genetic diversity, the breed spread almost all over Austria (2009: 1189 registered animals) and the Bavarian forests.

The Waldschaf is a small to medium sized breed. Its fleece is desirably white with a diameter between 35 – 40 Microns. Body parts without wool may be also spotted. Rams (see Figure 6) frequently wear horns whereas ewes do not. The breed is very robust and weatherproof. Its' husbandry is very modest, which makes the Waldschaf perfect for extensive farming, especially in the highlands. High fertility (180-200%) and a non-seasonal breeding are ideal properties for a good production (Schafzucht Niedersachsen, 2019).



Figure 6: Male Waldschaf

5.2.3 Bovec: Bovska Ovca, Alpine/Krainer Stone sheep

The Bovec is a descendant of the Zaupel sheep and can be connected to the Neolithic turf sheep. It corresponds to the original sheep breed of the Alps (LfL Bayern, 2017). Unfortunately, the Bovec sheep is threatened strongly by extinction. In the beginning of the 20th century was commonly seen as pure breed in Bavaria, Germany, specifically in Berchtesgaden, Trauenstein, and Rosenheim. It was also commonly seen in Austria in Salzburg. As crossed breeds it was held in the east and north of Tirol, Kitzbühl and Kärnten. The name Bovec or Bovska originated from a town in Slovenia, situated on the Slovenian – Italian border. The town lies in the Trenta-valley, which lead to another name: Trentarka. Since 1964 the stock decreased dramatically to less than 1000 pieces.

The animals are small and slender with wide and deep bodies and very hard claws. Horned and non-horned animals exist. Females can have short or simple curved horns (LfL Bayern, 2017). The head is generally straight with a slightly bent nose. The ears protrude horizontally away from the head, sometimes are found hanging. Typical for the old breeds is the coarse mixed wool of different colours but the most often seen colors are white, brown or black. Wool cutting can be done twice a year. It is characteristic that head and feet have no wool but just covering hair. The long hairy tail reaches the hook joint and is often curved at the end (LfL Bayern, 2017). The breed is highly adaptable to a harsh environment, living in the parts of high mountains, where other farm animals have no access to. Due to their inclined back legs, they can easily be held on steep meadows. In particular, during the breeding history, one of its characteristics is the excellent feed utilization, an amazing resistance against endoparasites and infections such as foot-rot, as well as the excellent fertility. The animals can have a non-seasonal breeding cycle. As for that, it is possible to extend the breeding season up to two pregnancies per year. Female animals can give birth as early as the age of 12-15 months. The female animals have a very good milk production, providing exceptional nutrition and subsequent healthy development to the lambs (Bayrische Herdenbuch-Gesellschaft für Schafzucht e.V., 2018). The breed is mainly bred for dairy production such as cheese and sour milk (Oklahoma State University Board of Regents. 2001). An ewe (see Figure 7) has a live weight of 45 – 60kg; the ram reaches up to a weight of 60 – 75kg. In 1985 a group of breeders (Dr. Burkl, Haarpainter) paired and started to buy off the remaining sheep in the alpine region. Since 1996, they have increased the size of their herd with additional animals from Nauders and a breeding farm in Ramsau. In 1997, the breeders accomplished to register the Alpine Stone sheep into the herd book and in February

2000 a standard breed description was settled. In 2008 the number of alpine stone sheep in Bavaria consisted of 203 ewes and rams, in 12 breeding farms. Comparatively, the Slovenian population was at 3500 in 2012.



Figure 7: Female Bovec Sheep with lamb

5.2.4 Tyrol Mountain sheep

The Tyrol Mountain sheep is a descendant of the Stone and Zaupel sheep, especially north-Italian Bergamasca sheep in Bergamon, Lombardy. Through the Austrian region in Lombardy it was introduced to the more northern territories and arrived in Tyrol and the Bavarian Alps. It was used for breeding today's brown mountain sheep. You can distinguish it from the Bavarian Mountain sheep (Kinzelmann, 2008). The size of the Tyrol Mountain sheep is defined as large to medium, with a long and wide dorsum. The foundation is strong with firm ankles and hard claws. The narrow, curved head carries long and wide loop ears and is hornless. The wool is white and free from pigmentation as well as other discolorations. The breeding goal of this sheep is defined by its' very young and extremely high fertility, as it can lamb, aseasonally, twice per year. This results in 1,8 – 2,5 lambs per year. The wither height in ewes (Figure 8) reaches up to 90cm, with a total mass of 90 kg. The rams wither height is up to 100cm, with a maximal weight of 130kg. The wool production per year lies between 2,5 to 5,0 kg. Although the breed is known for its good adaptation to rough and uneven areas in high mountains, where farming is not possible for other animals, it can also be kept on a paddock in small groups. The mountain sheep can resist high precipitation and can therefore be kept in areas with such characteristics. It is not only kept for its' wool, but also for its' good daily weight gain and therefore an acceptable slaughter weight (Berger & Fischerleitner, 2009).



Figure 8: Tyrol Mountain ewe with two lambs

5.2.5 Today's Zaupel Sheep

The Schwäbische Bauernhofmuseum in Illerbeuren is currently the only facility to maintain the Zaupel sheep breed. In addition to old German domestic animal breeds such as Allgäuer Original Braunvieh, Oberland Noriker and many more, the Zaupelschaf has been kept and bred here since 2000.

They currently own 2 herds (Figure 9 and 10), holding a total of 24 individuals. One male, accompanied by eleven female sheep, are kept together in a herd. This type of keeping ensures an annual lambing. The impregnation is done by live covers and has a 90% success rate. Due to the lack of regional breeding animals, the breeding goals are currently not achievable. Furthermore, it is important to note that the Zaupel sheep is a highly adaptable breed with good resistance concerning weather changes. Due to these characteristics, the Zaupel, in contrast to other breeds, has both a low maintenance effort and low veterinary care, of apparently twice a year.

The herds are kept on the pasture from mid-April to the beginning of December, without any further feed additives. In winter they are kept in stables with additional feeding of hay, grummet and mineral licks. Most of the animals bred in the Illerbeuren farm museum are sold to private individuals and are merely used for landscaping and gardening (Schwäbisches Bauernhofmuseum Illerbeuren, 2021).



Figure 9: Today's Zaupel lamb
(with kind approval of Schwäbisches Bauernhofmuseum Illerbeuren, Kutter T., 2021)



Figure 10: Today's Zaupel Ram
(with kind approval of Schwäbisches Bauernhofmuseum Illerbeuren, Kutter T., 2021)

6. Materials and Methods

For this project, first the samples were collected. A thorough statistical evaluation was needed to evaluate the purified sequences to be able to compare the genetic changes between the individuals and subsequently the populations. These tests included the Tajima Test, Fu and Li Test as well as the Jukes and Cantor Method. We finished analysing the work using the Median Joining Network. The details of each step are described below.

6.1 Sample collection

The sample collection was executed by Prof. Dr. Gáspárdy and his scientific members. In 2015, using three herds from different locations, a certain number of individuals were chosen. These herds were either in the hands of governmental supervision or by private persons. One of the private farms is owned by Mr. T. Nagy in Pénzesgyőr, which houses 11 sheep families. The second private farm is owned by Mr. J. Jánosi located in Szécsénke, housing 5 sheep families. The third farm is the state-owned Duna-Dráva National Park located in Nagydorog, which has 20 sheep families. Each animal was carefully chosen to most prominently represent the features of their own herd. This was done to ensure that all the chosen sheep family features are covered while scanning. The sheep had 6-5-4 ancient lines distributed over the 36 oldest sheep families. The samples were collected in the fall of 2015. Out of each ancient family two representatives were selected for specimen collection, leaving a total specimen count of 70 (Rabe A., 2017).



Figure 11: Taking samples on 10th of November 2015 in Nagydorog from a Cikta ewes



Figure 12: Taking samples on 22nd of October 2015 in Szécsénke from a Cikta hogged with pigmented fleece

The second comparative breed examined in this study was the Bovec sheep. Samples were taken from a flock in Slovenia. Blood was collected from 21 individuals for this purpose. It was ensured that the purebred pedigree of the animals had existed for three generations. The pure male Bovec sheep are all descended from different dams and are considered potential breeding rams, included in the regular prion genotyping program, determined by order of the Ministry of Agriculture, Forestry and Food of the Republic of Slovenia (Gáspárdy et.al., 2021).

The blood was taken out of the jugular vein and preserved in an EDTA blood tube.

6.2 Statistical Evaluation

To evaluate the purified sequences several tests were used to compare the genetic changes between the individuals/ populations. Each test will be discussed below in detail.

6.2.1 Tajima's Test

The Tajima's Test was invented by Fumio Tajima. It is based on the detection of different mutation sequences and their correlation. The test concentrates on the distinction between random mutation and non-random mutation, as non-random mutation is critical for the genetic selection and variation, which shows in the constitution of every individual. The selected individuals are tested on their equal mutation sequences and sorted by non-equal mutations within their DNA. This will pinpoint specifically where the genetic drift was initiated that caused the difference in each breed.

6.2.2 Fu and Li's Test

The test by Fu and Li is based on the convergence between the genetics on an individual and are important to distinguish the history of populations. The statistics depend on the fact that unique individuals exclusively affected by things such as polymorphism are decisive for different evolution of populations. The mutations are classified into external and internal mutations. The external mutations, independent from the sample size, differ as opposed to the internal mutations, which affect the presence of selection.

6.2.3 Jukes & Cantor Method

The Jukes and Cantor method is a simplified equation and is used to correct distance data within the sequence alignment of the DNA. It is based on the mutation rate as well as on the backwards mutation of nucleotides within a pedigree. Its' principles are based on the idea that all nucleotides have the same value and mutate equally among the ancestry.

6.2.4 Median Joining Network

Based on informative mutations of haplotypes, the connections among them can be graphically presented on a median-joining network. It uses large datasets such as amino acid sequences and creates an intermediate – sized network with the help of combined minimum spanning network and quasi -median network algorithms. For the demonstration of distances among Cikta haplotypes and Bovec-haplotypes gene bank sequences were used as controls with the help of software PopART (PopART) (Sungsik K., 2015).

7. Results

Table 1 shows the evaluated individuals and their mutations in each breed.

Table 1: Individuals and mutations per breed

Parameters	Cikta	Bovec
Number of evaluated sequences	70	21
Number of polymorphic sites (mutations)	108	52

Regarding the overall population, with the exclusion of alignment gaps, the number of evaluated sites in the CR genome amounts to 1179. Comparatively, the count of polymorphic (mutated) bases was 118. The mutations are polymorphic in the Cikta population, but monomorphic in the Bovec population with a total of 66. The mutation in the Bovec was polymorphic but monomorphic in the Cikta population with a total of 10.

We can conclude that the population of Cikta and Bovec shares 42 polymorphic sites with each other, therefore the genetic identity between the breeds can be considered as significant. The total number of the (individual) sequences came to a total of 91, whereas the average nucleotide diversity (π) was 0.01814. It determines the genomic diversity of individuals between breeds. The nucleotide diversity (π) is the count of various nucleotides at a definite base-sites in two random chromosomes in one population. Haplotype diversity (Hd) was 0,7143 (Kovács, E. et.al. 2019).

The verification of the average count of nucleotide differences (k) and of nucleotide diversity (π) is especially useful in the assessment of different populations.

Table 2 shows that each of the indicators give information about the degree of diversity within a population.

Table 2: Values of k and π per breeds

Parameters	Cikta	Bovec
Average number of nucleotide differences, k	21.251	17.152
Nucleotide diversity, π	$18.02 \cdot 10^{-3}$	$14.55 \cdot 10^{-3}$
Nucleotide diversity with Jukes and Cantor, π	$18.37 \cdot 10^{-3}$	$14.81 \cdot 10^{-3}$
Standard deviation of π	$1.86 \cdot 10^{-3}$	$2.29 \cdot 10^{-3}$

It is apparent that the herd of Cikta is marked as the population with the larger diversity and higher count of average nucleotide differences (21.251) and a nucleotide diversity ($18.02 \cdot 10^{-3}$). In comparison the values were lower in Bovec with an average nucleotide difference of 17.152 and a nucleotide diversity of $14.55 \cdot 10^{-3}$.

Keeping in mind that the diversity results are approximately the same amount as of humans ($3-11 \cdot 10^{-3}$) and 1/10 of the diversity of a vinegar fly (*Drosophila melanogaster*) populations (Li and Sadler, 1991).

Table 3 shows the results of the average count of nucleotide differences (k) and nucleotide diversity (π) comparing breeds.

Table 3: Results of k and π in breed comparison

Parameters	Between Cikta and Bovec
Average count of nucleotide difference, k	22.204
Average nucleotide diversity, π	$18.14 \cdot 10^{-3}$
Average number of nucleotide substitutions per side between breeds, Dxy	$18.83 \cdot 10^{-3}$
Dxy with Jukes and Cantor, Dxy (JC)	$19.18 \cdot 10^{-3}$
Standard deviation of Dxy (JC)	$2.36 \cdot 10^{-3}$

In comparison of Cikta and Bovec the average count of nucleotide differences totals 22.204. As for the corrected calculated numbers according to Jukes and Cantor (Dxy (JC)) the base substitutions amount up to $19.18 \cdot 10^{-3}$ with a standard deviation of $2.36 \cdot 10^{-3}$.

While comparing these 1186 sites within the 91 sequences of Cikta and Bovec sheep we have found that according to the Tajima-test which was carried out on the whole population, the average number of paired nucleotide differences (k) reached 21,38315 and the nucleotide diversity (π) amounted to $18,14 \cdot 10^{-3}$ (Kovács, E. et.al., 2020).

7.1. Results in Tajima D - Test

The results of the Tajima's D-test were -0,2639, which is statistically not significant ($P > 0,10$). We can conclude that the breeds are considered as one population hence in equilibrium. A significant negative result would be an indicator for recent (advantageous) selective mutations, concluding in an expansion of the population after a recent bottle neck narrowing, whereas a significant positive result would be an indicator for a genetical narrowing to, such as bottle neck effect and /or a fragmentation into subpopulations.

7.2. Results in Fu & Li's - Test

The results of the D*-test of Fu and Li's gave the value 1,48897 which is found insignificant as $P > 0,10$. For the F*-test of Fu and Li's a result of 0,86572 which is found non-significant as $P > 0,10$ in the whole population.

The Fu- and Li's test result for the haplotype diversity (H_d) was 0,97143, with a standard deviation of $6,0 \cdot 10^{-3}$. The Fu's F_s -statistic was -1,404 ($P = 0,058$), which is close too, but not non-significant ($P > 0,10$). A significant negative value is evidence for an excess number of alleles, which is expected to be seen in a recent expansion of the population or a genetic drift. While a positive value is evidence for a deficiency of alleles, which is expected to be seen in a recent bottle neck effect in a population (Kovács, E. et.al. 2019).

For comparison, the breed Tsigai (Pásztor, 2016) detected that the haplotype diversity (H_d) 0,911 with a standard deviation of 0,077, was $\pi = 2,26 \cdot 10^{-3}$. Due to these indications the Cikta sheep has a smaller genetic variety than the Tsigai sheep.

7.3. Results in Median Joint Network

Figure 13 shows the Cikta sheep individuals (yellow) and the Bovec sheep (blue) CR haplotypes based on informative mutations. The size of the circles is related to the number of individuals representing a haplotype. Regarding the Cikta breed, we keep the probed flocks (Szécsénke, Pénzesgyőr, and Nagydorog) in mind. It is well visible that these Cikta haplotypes are located close to each other. The haplotypes of the Bovec breed are creating a different, but closely related, group.

The haplotypes of Urial (URIAL - black) and Argali (ARGALI - black) are close to each other, but far from Bovec and Cikta, showing a very distant connection to each other. The Mouflon breed (MOUFLO - black) will be settled close to the Cikta groups, showing a more close relationship in between these breeds (Hiedleder et al., 2002). Further reference samples in black are: A-HM236174, B-HM236176, C-HM236178, D-HM236180, E-HM236182 (Meadows et al., 2005).

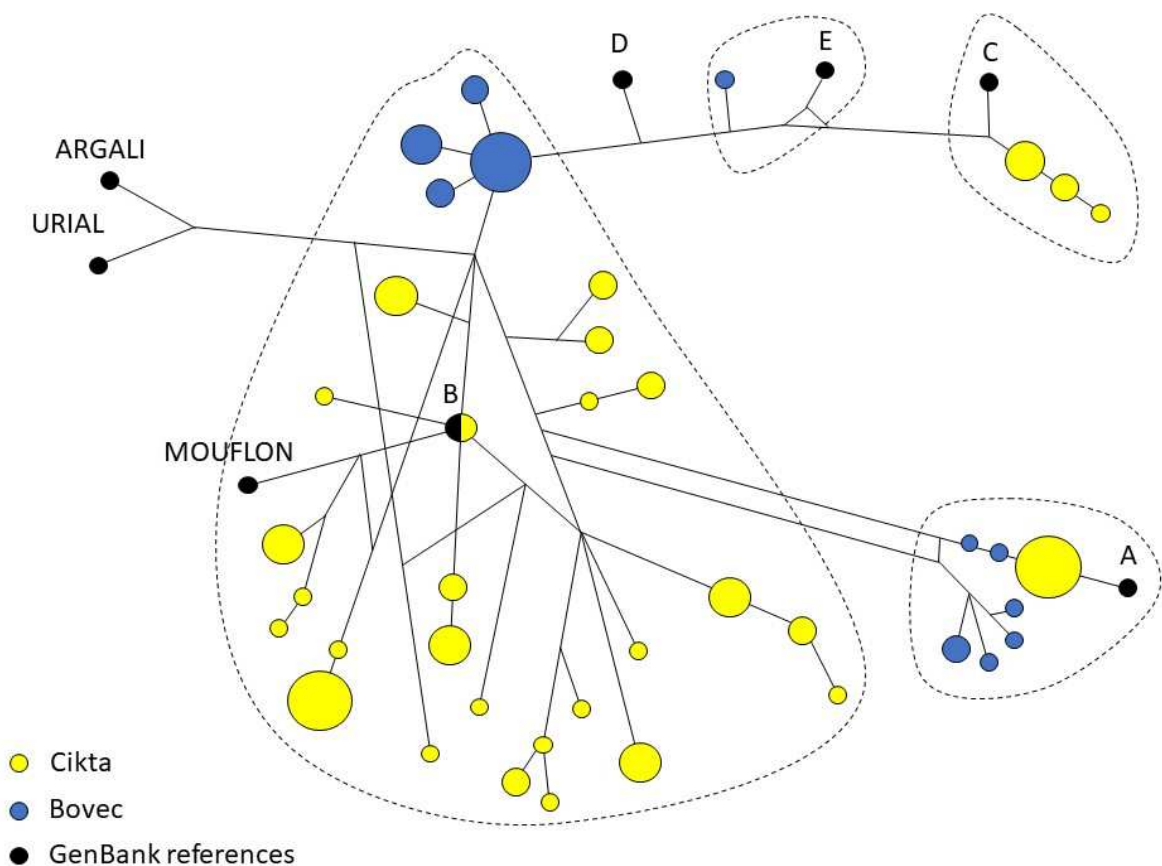


Figure 13: Connection between sample CR haplotypes and reference CR haplogroups as median-joining network (see legend in the text)

8. Discussion

Nowadays it is crucial to obtain and revive as many genetic material concerning breeds as possible. Not only it will help to increase performance and resistance against diseases but also help to reduce genetic miss breeding which often results in genetic/inherited diseases. This study helps to not only conserve but reconstruct genes of sheep breeds with the aim of rebreeding the primal sheep as similar as possible to the historical documentations. During laboratory investigation we have received our final results regarding the comparison of Bovec and Cikta breed. As concluded above neither the Tajima D-test value (-0,26385), nor the value of the FU's Fs-statistic (-1,404) of the Cikta sheep population was statistically confirmed. This shows that the breed is in a genetic equilibrium. A deviation may not endanger the community of the two Zaupeľ derivatives.

It is apparent that the herd of Cikta is marked as the population with the largest diversity and highest count of average nucleotide differences (21.251) and a nucleotide diversity ($18.02 \cdot 10^{-3}$). Followed by Bovec with an average nucleotide difference of 17.152 and a nucleotide diversity of $14.55 \cdot 10^{-3}$.

The numbers show that the population of Cikta and Bovec shares 42 polymorphic sites with each other, therefore we can confidentially say that the genetical identity between the herds can be considered as significant.

Concluding, in our study partial mitochondrial sequences of 70 individuals of the Cikta sheep breed from Hungary and 21 Bovec sheep from Slovakia were examined. With regard to the nucleotide and haploid diversity in the Cikta sheep, a genetic narrowing can be seen. This effect is due to the bottle neck effect which must have happened in the past. However the number of pairwise nucleotide differences are relatively significant / high. It is suggested that there are differences in the genetic characteristics of the sheep families on the individual farms.

For our breeders it is still of great importance to find common genes within the Zaupeľ successors in the future, but this goal will require further research.

9. Abstract

The investigation of endangered and extinct animal breeds is currently among the main tasks of preservation work all around the world. The ultimate goal of this and further studies is to obtain a genetic background and ancestry of the autochthonous breeds, and to preserve this acquired knowledge in the genetic databank. In the case of extinct Zaupe sheep for example, there is a certain circle of today living breeds which is recognized as its closest descendants. It includes the Cikta sheep, the Bovec sheep and the Bavarian Waldschaf. In the current study, the focus lies on the comparison of two of them, the Cikta and the Bovec. For this purpose, their genetic material was compared, with the help of control region in mtDNA of a total of 91 unrelated individuals. Biological samples were collected from 70 Cikta sheep (Hungary) and 21 Bovec sheep (Slovenia) between 2015 and 2017.

In the comparison of Cikta and Bovec the total of polymorphic sites was 118 (while the number of shared mutations was 42) which shows a significant genetic identity between breeds. The results of the Tajima's D-test was -0.2639, showing no significance ($P > 0,10$) as well as the negative value of FU's F_s -statistic (-1.1404, $P = 0.058$) which is also considered to be not insignificant. The research points out that a deviation does not endanger the community of the two Zaupe derivatives and the breeds were not subject to a genetic drift, thus are in genetic equilibrium.

This study confirms the common ancestry between Cikta and Bovec.

Összefoglalás

Két Zaupel juh leszármazott, a Cikta és a Bovska összehasonlítása mtDNS CR szekvenciáik alapján

A veszélyeztetett és kihalt állatfajták vizsgálata egyik fő jelenlegi feladata a fajtafenntartásnak az egész világon. Ennek és a további kutatásoknak a végső célja az autochton fajták genetikai hátterének és eredetének megismerése, és ezen megszerzett ismeretek eltárolása a genetikai adatbázisban. Például a kihalt zaupel juh esetében a ma élő fajtáknak van egy bizonyos köre, amelyet a zaupel legközelebbi leszármazottainak ismerünk el. Ide tartozik a cikta juh, a bovaska juh és a bajor waldschaf. A mostani tanulmányban a hangsúly kettőnek, a cikta és a bovaska fajtáknak az összehasonlításán van.

Ebből a célból genetikai anyaguk a mtDNS-ben lévő kontrollrégió segítségével került összehasonlításra összesen 91, egymással nem rokon egyed mintázásával. A biológiai minták 70 cikta juhtól (Magyarország) és 21 bovaska juhtól (Szlovénia) kerültek levételre a 2015 és 2017 közötti években.

Cikta és bovaska összehasonlításában a polimorf helyek száma összesen 118 volt (míg a közös mutációk száma 42 volt), ami jelentős genetikai átfedést mutat a fajták között. A Tajima D-teszt eredménye $-0,2639$ volt, statisztikailag nem szignifikánsan ($P > 0,10$), valamint az FU-féle Fs-statisztika enyhén negatív értéke ($-1,1404$, $P = 0,058$), szintén nem szignifikánsan azt mutatja, hogy a két fajta közös genetikai pool-ja genetikai egyensúlyi helyzetben van, nincs kitéve divergenciának és genetikai sodródásnak.

Ez a tanulmány megerősíti cikta és bovaska fajtatörténetből ismert közös eredetét.

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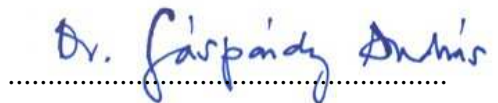
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