Department for Interdisciplinary Life Sciences

University of Veterinary Medicine Vienna

Research Institute of Wildlife Ecology
(Interim Head: PD. Dr. rer. nat. Claudia Bieber)

MHC class II DRB Variation across Chamois populations (*Rupicapra rupicapra*) in the Eastern Alps: Comparing endemic to non- endemic regions of sarcoptic mange (*Sarcoptes scabiei* var. *rupicaprae*)

Diploma thesis

University of Veterinary Medicine Vienna

submitted by

Patrick Bilic

Vienna, August 2022

Supervisor

Dr. phil. Franz Suchentrunk

Department for Interdisciplinary Life Sciences

University of Veterinary Medicine Vienna

Table of contents

Acknowledgements:

First and foremost, I would like to express my deepest gratitude to Dr. phil. Franz Suchentrunk for his guidance and support during this work as well as for fortifying my motivation to pursue a career in science through many insightful and exciting debates and his seemingly all-encompassing knowledge about natural sciences. I would also like to thank Anita Haiden for supporting me with the lab work and for making it an entertaining and memorable experience.

Furthermore, this endeavor would not have been possible without the assistance of the team of the pathology lab of the Research Institute of Wildlife Ecology, who harvested the tissue samples, as well as all the people who helped with the organization and provided the necessary samples including Christine Miller, Martin Janowsky and all the hunters that were involved in this undertaking.

I would be remiss in not mentioning my parents, my brother and my grandparents for their everlasting love and support. Lastly, my special thanks go to my wife who stood by me through all of the good times, the rough moments, and everything in-between and who always knew how to lighten my mood, no matter how hard life got.

Abbreviations

MHC	Major histocompatibility complex
VUI	Vorarlberg and upper Inn Valley
UA	Upper Allgäu
SW	Mountain massifs west of the Stubaital
SEZ	Central Alps mountain massifs east of Stubaital and west of the Zillertal
ATK	Achental and Karwendel mountain massif
CAK	Chiemgau Alps and Kaisergebirge
ET	East Tyrol
HT	Hohe Tauern National Park – Salzburg part
mtDNA	Mitochondrial DNA
RFLP	Restriction fragment length polymorphism
cds	coding sequence
CD4 T cells	T-Lymphocytes that express the glycoprotein cluster of differentiation 4 on their surface
Dn/ds	ratio of the number of nonsynonymous substitutions to the number of synonymous substitutions
AICc	Corrected Akaike Information Criterion
HWE	Hardy-Weinberg Equilibrium
LD	Linkage Disequilibrium

1 Introduction

During the last century there have been plenty of reports regarding outbreaks of sarcoptic mange (i.e., infections with *Sarcoptes scabiei var. rupicaprae*) in chamois (*Rupicapra rupicapra*) from the Eastern Alps, resulting in steep population declines, with mortality rates up to around 80 % in some cases (Rossi et al. 1995). Most interestingly, these severe mortality rates seem to decline after initial outbreaks to around a maximum of 25 % (Rossi et al. 2019) before rising again every 10 to 15 years. Such a wave-like pattern strongly suggests a potential genetic advantage of surviving individuals that might be reduced after a new strain of the responsible pathogen has emerged and expanded widely throughout populations. Previous genetic studies have either focused on the genetic background of the parasite (Oleaga et al. 2013, Rasero et al. 2010) or the host (Mona et al. 2008, Schaschl et al. 2012, Cavallero et al. 2012). In terms of host genetics there has been special interest in major histocompatibility complex (MHC) genes (i.e., genes of the so-called major histocompatibility complex, see below), being the most likely candidate gene family to influence the specific immune response to infections with sarcoptic mites and potentially also the individual chance of survival.

In the eastern Alps, specifically in Austria, Germany, Italy, and Slovenia, the spatial distribution of sarcoptic mange seems to have come to a halt historically at two specific landmark systems, namely the river Inn, originating in Switzerland, entering Austria (Tyrol) in the west, then flowing northeastwards to Southern Germany on the one hand, and the Wipptal, a southbound valley in the Province of Tyrol, with its northern begin in the Inn Valley just south of the city of Innsbruck, on the other hand. Obviously, this demarcation of the distribution of sarcoptic mange in chamois is being continued in northern Italy (in the Province of Bozen – Südtirol/Alto Adige) where it has been present historically east of the line formed by the Brenner Pass (Brennero), the Passeier Tal /Val Passirina, the Passer River and the Etsch/Adige River (Fig. 1). Only in the very recent years antibodies specific to sarcoptic mange have been detected in tissue samples of hunted chamois west of that geographical line, too, yet no mange affected animals were found (Janovsky et al. 2018). That geographic divide is formed in essence by physiographical features that do not at all represent barriers to migration for chamois (particularly so in historical times with less anthropogenic segmentation by settlements and traffic infrastructure such as highways and railroads).

Hence, for Austria epidemiologically two distinct geographical partitions can be recognized: A historically mange free area west of the Wipptal and north of the river Inn and a mange endemic area east and south of these landmarks.

This study aims at comparing allelic variation of exon 2 DRB sequences, one MHC class II gene, in chamois from Austria; this gene has been considered important in the context of sarcoptic mange in chamois, and one study found a heterozygosity advantage specifically for adult males at this locus (Schaschl et al. 2012). The authors of the latter study interpreted their main result of a DRB heterozygosity advantage in males that are old enough to participate in the energetically demanding rutting season at the beginning of the winter as indicative of a selective advantage for males. They argued that heterozygosity at that locus might be connected with a lower energy demand in immune reactions and thus leave proportionally more energy available for other metabolic demands. In that way heterozygous adult males may better survive the rutting season and the subsequent harsh winter conditions. The immediate advantage of heterozygous individuals or that of specific alleles at the DRB locus in fighting sarcoptic mange infections could, however, not be proved, but older males heterozygous at that locus exhibited better long-term survival according to survival curves, whereas that was not the case for females (and younger males most likely not engaged in rutting competitions). Body weight, as a proxy of body condition, is significantly fluctuating in older males seasonally, with a pronounced decrease during the energetically demanding rutting season, which likely increases their risk of mortality during the following harsh winter period, at least more than in females and younger males that do not participate in the rut.

The present study tests specifically 1) for different allele compositions/allele frequencies/genotype frequencies between regional chamois populations in endemic vs. non-endemic parts of sarcoptic mange of chamois from the Eastern Alps of Austria, and 2) for seasonal changes in individual heterozygosity of the DRB exon 2. Different allele/genotype frequencies at that locus would accord to the hypothesis of immediate relevance of this MHC gene in survival of sarcoptic positive chamois, specifically, if not paralleled by neutrally evolving population genetic markers, such as the presently used microsatellites; significant seasonal changes of individual DRB exon 2 heterozygosity, specifically increased values after the rutting and the winter season in the absence of a general heterozygosity effect in the neutral marker system would fit the

hypothesis of an overdominance effect and positive selection as already reported (Schaschl et al. 2012). However, an immediate role of the DRB-2 locus in fighting sarcoptic mange infections would be suggested only if significant allele/genotype differences at that locus were paralleled by differences between endemic and non-endemic parts of sarcoptic mange in the Eastern Alps.

Individual body weight data were also available for a fair number of chamois sequenced presently for the DRB exon 2 locus. Thus, following the results of Schaschl et al. (2012), the effect of individual DRB heterozygosity on body weights could be tested as well, expecting higher weights in older males (likely participating in rutting competitions), specifically from the endemic range as opposed to homozygous ones and younger males or females. This has been achieved by generalized additive modelling of individual body weights and by controlling for general levels of heterozygosity as indicated by microsatellite markers.

1.1 Chamois

Depending on the subspecies, chamois weigh between 25 and 50 kg and have a height at withers of 110 to 135 cm. The chamois has a slight sexual dimorphism where the male is larger and has thicker and slightly bigger horns (Nowak 1999), Suchentrunk, pers. data).

The chamois are ungulates and are taxonomically classified in the family *Bovidae* and the subfamily *Caprinae*. Together with several tropic, subtropic (e.g., Serau – genus *Capricornis*), and subarctic/alpine (mountain goat – *Oreamnos americanus*) ungulates they have formally been combined into the taxon *Rupicaprini*, a subgrouping within the *Caprinae*, which is, however, not supported by mitochondrial DNA genome comparisons (Bover et al. 2019, Pérez et al. 2014). Chamois are generally divided into the northern (*Rupicapra rupicapra*) and the southern chamois (*Rupicapra pyrenaica*), which can be subdivided into seven and three subspecies, respectively (Corlatti et al. 2011, Masini and Lovari 1988).

1.1.1 Habitat and geographic range

The northern chamois and its various subspecies can be found in different places worldwide, such as the Balkan chamois (*Rupicapra rupicapra balcanica*) from Slovenia to Greece, the Tatra chamois (*Rupicapra rupicapra tatrica*) in Slovakia and Poland, the Anatolian chamois (*Rupicapra rupicapra asiatica*) found in Turkey, the Caucasian chamois (*Rupicapra rupicapra caucasica*) in Azerbaijan, Georgia, and Russia, the Carpathian chamois in Romania (*Rupicapra rupicapra carpatica*), the Chartreuse chamois (*Rupicapra rupicapra cartusiana*) in France, and the Alpine chamois (*Rupicapra rupicapra rupicapra*), which is relevant in the following text and can be found in the entire Alpine region. With an estimated 500,000 individuals, the Alpine chamois is the most common subspecies (Corlatti et al. 2022).

The chamois prefers mainly steep and rocky habitats in subalpine to alpine regions, but also alpine pastures and meadows. Predominantly they can be found in the range of 1500 up to 2500 meters above sea level. However, populations can also be observed at lower altitudes, usually associated with human retreat from mountain villages. Therefore, it is not uncommon to find chamois at altitudes of 300 to 400 meters. An extreme example is a population of chamois in Trieste, which can be found at an altitude of 30 to 170 m above sea level (Miller and Corlatti 2014).

The habitat can be variable due to different factors. Key factors are the food supply as well as steep and rocky places, which give the chamois the necessary feeling of security. For example, in winter chamois tend to prefer south-facing slopes, lower elevations and forests, where a better food supply is available and easier movement is possible during this period, while in summer they tend to prefer higher elevations and north-facing slopes. Anthropogenic factors such as hunting pressure and tourism in the form of hiking and skiing may cause chamois to respectively move either to higher or lower elevations. Grazing animals such as sheep and cattle may also influence habitat selection. The competition with sheep is particularly strong as the food spectrum of both species overlaps and diseases can easily be transmitted from one species to another (Miller and Corlatti 2014).

1.1.2 Social Structure

The social structure of chamois is characterized by a high variability. First and foremost, there are different herd structures. While the majority of chamois males consists of solitary animals, males at an age of one year, so-called yearlings, can be found in bachelor groups. Adult females and juveniles also group together. Mixed-sex groups are also found but are more likely to occur at high population densities. The number of single males is especially high during spring and summer while this number decreases during rutting time in autumn. During this time the so-called rutting herds, which are important for mating, are formed. However, some males remain alone during this time to avoid conflict situations during the reproductive season. The sex ratio of chamois under natural conditions will vary on average from 1-1.2 to 1-1.4 with a slight excess of females (Miller and Corlatti 2014). The population density of chamois varies according to hunting pressure, season and food supply. For example, in one study (Poubelle et al. 1989), a non-hunted population and a hunted population in the French Alps were each examined for population density. The non-hunted population had a density of 4.7 animals per 100 ha, while the hunted population had 2.3 animals per 100 ha. The highest densities of chamois are reached in protected areas where hunting is prohibited: here, population densities of 17 to 28 chamois per 100 ha can be recorded.

1.1.3 Reproductive behavior

The rutting season takes place between October and December and has its peak in November. The sexual behavior of the animals is assumed to be polygynous but still debated (Corlatti et al. 2022). The sexual maturity of the animals starts at the age of 3.5-4 years for males and at the age of 2.5 years for females. However, males do not have the best chance of reproducing until six years of age during the rutting season. Males follow two different strategies depending on the individual: On one hand, there are territorial males that try to keep their harems together during the rut and protect them from other males, and on the other hand, latter males occasionally try to mate with females in other harems or take the place of the territorial male themselves. The respective strategies are characterized by different success depending on environmental conditions, e.g. territorial males can defend their harem better in cold snowy winters, while migrating males have more success in warmer winters. Regardless of the strategy chosen, the

rutting season is characterized by an enormously increased energy demand for males, which causes them to lose five to six kilograms in weight in some cases (Miller and Corlatti 2014).

The rutting season is followed by a 170-day gestation period of the females, which ends in May to June. A female chamois usually gives birth to only one fawn, with rare exceptions of twin and triplet births. These are weaned after about three months (Nowak 1999). While young females usually remain in the territory of the mother, males migrate from the herd at the age of two to three years.

1.1.4 Ranging and migration

Several studies on the migration behavior of chamois have already been conducted (Lovari et al. 2006, Nesti et al. 2010, Unterthiner et al. 2012). One of these studies (Nesti et al. 2010) showed that the home ranges of the resident males differ significantly with a median of 49ha from those of migrating males with 749ha. Furthermore, the study was able to show that the home ranges of the animals in winter with a median size of 32 ha are significantly smaller than in summer with a median size of 108 ha. The females observed in this study stayed in significantly higher areas in warmer months compared to resident males and have a large home range with a median of 711 ha which could favor reproductive success of migrating males in warmer winters.

1.1.5 Diseases

In addition to chamois mange, which is of particular interest in this study, chamois can also potentially be infected by a variety of diseases from the viral, bacterial, and parasitological pathogen spectrum. The most important pathogens are papillomaviruses, parapoxviruses such as Orf virus, pestiviruses such as border disease virus, bacteria such as *Salmonella enterica*, *Chlamydia abortus*, *Coxiella burnetti* and *Borrelia*, contagious ecthyma or infectious keratoconjunctivitis caused by *Mycoplasma conjunctivae*, which is also common in sheep. Microparasites such as *Babesia* and macroparasites such as roundworms, tapeworms and lungworms can also infect chamois (Miller and Corlatti 2014).

1.2 Sarcoptic Mange

1.2.1 *Sarcoptes scabiei*

Sarcoptic mange is a parasitic disease caused by the mange mite *Sarcoptes scabiei*. It is most commonly known as scabies occurring in humans, being one of the three most frequent skin disorders besides tinea and pyoderma in children (Andrews et al. 2009). Taxonomically, this species of mites belongs to the family of *Sarcoptidae* in the order of *Sarcoptiformes* and the subclass of *Acari* (mites and ticks) (Sayers et al. 2019, Schoch et al. 2020). *Sarcoptes scabiei* can be further subdivided into varieties, e.g., *Sarcoptes scabiei var. hominis* or *Sarcoptes scabiei var. canis*. Varieties of sarcoptic mange mites are host specific but have been shown to also be transmittable to other species in some cases, as was shown with *Sarcoptes scabiei var. rupicaprae*, which is responsible for sarcoptic mange in chamois (Turchetto et al. 2020). The mite is around 0.2 to 0.4 mm in size, has an oval, tortoise-like body, featuring multiple cuticular spines and two blade-like claws on the tarsi. The life cycle consists of egg, larval, protonymphal, tritonymphal and adult life stages (Fain 1968). After finding a suitable host the mite burrows into the skin bypassing the *stratum corneum* to reach the *stratum granulosum* and *stratum spinosum*. There it feeds off the surrounding live cells and tissue fluid in the newly created tunnels. Most of these burrows are located superficially in the *stratum corneum*, containing eggs, cellular debris and feces. Inside the burrows fertilized females lay around 3-4 eggs per day on average which hatch within three days, each egg measuring around 150–200 µm by 175–250 µm. The emerging larvae can easily be distinguished by the missing fourth pair of legs, a feature unique to this stage of development. After hatching, the larvae start to move through the skin, while some migrate to the surface and others remain at their original positions and the connected burrows (referred to as molting pockets). Subsequent development into protonymphal, tritonymphal and adults takes around 2 weeks. After developing into adults, the mites surface to mate and the cycle repeats.

1.2.2 Clinical signs and pathophysiology

Clinical signs of sarcoptic mange infection are intense pruritus and skin lesions of varying severity and distribution including erythematous eruptions, papule formation, seborrhea. As was shown in an experimental setting with two chamois, the first clinical manifestation can start as soon as days post-infection consisting of papules and desquamation (Lavin et al. 2000).

Subsequently, crusts accompanied by pruritus and alopecia emerged around 3- and 4-6-weeks post-infection, respectively. A potential complication that can occur later in individuals suffering under heavy burdens of sarcoptic mange mites are secondary bacterial infections, for example with *Staphylococcus spp*. and *Streptococcus spp*. This typically happens due to the broken skin barrier (Walton et al. 2004, Walton 2010) or through mite-transported opportunistic bacteria (Shelley et al. 1988). There has also been evidence that mites are able to change the skin microbiome by producing serpin, a complement inhibitor which compromises the function of neutrophilic granulocytes thus promoting bacterial growth (Mika et al. 2012, Swe and Fischer 2014). While potentially self-limiting, bacterial co-infection can result in severe pyoderma, subsequent sepsis and eventually death. Anemia and damage to inner organs have also been shown as an indicator for poorer survival of infected hosts (Arlian et al. 1990). Heavy mite burdens might also lead to dehydration and impaired thermoregulation as was shown in mange affected wombats in another study (Simpson et al. 2016). In turn, the loss of thermoregulation can have a strong negative impact on survival rates, as the alpine chamois is more dependent on it than other species due to its subalpine to alpine habitat.

1.2.3 Epidemiology of sarcoptic mange

Sarcoptes scabiei is the cause of epizootic disease in a wide range of species, such as wild canids (Pence et al. 1981), wild cats (Ryser-Degiorgis et al. 2002), wild boars (Ippen et al. 1995), wild ungulates (Rossi et al. 1995), great apes (including humans) (Kalema G. et al. 1998) and wild bovids (Sachs and Sachs 1968). On the island of Bornholm in Denmark, sarcoptic mange is discussed to be the main driving force behind the extinction of red foxes (Henriksen et al. 1993, Lindström et al. 1994). The disease has also been reported to be among emerging diseases in wildlife (Tompkins et al. 2015). In alpine chamois (*R. r. rupicapra*) *Sarcoptes scabiei var. rupicaprae* is one of, if not the most relevant pathogen to occur, causing various epizootics in populations of the Eastern Alps. Mortality rates in sarcoptic mange outbreaks can get as high as 80 % (Rossi et al. 1995) and the disease is considered to be a concern for conservation of this caprine species even if population levels are stable at the time of writing (IUCN Red List of Threatened Species, 2021).

While the mite's taxonomic name suggests that infection only occurs in chamois, *S. scabiei var. rupicaprae* has been observed in both experimental and natural settings to be transmissible to

other host species, namely to the Alpine ibex, *Capra ibex*, the domestic goat, *Capra aegagrus f. dom.*, domestic sheep and mouflon (as a very basal domesticated form of sheep), *Ovis aries f. dom.*, roe deer, *Capreolus capreolus*, and red deer, *Cervus elaphus* (Turchetto et al. 2020).

First reports of sarcoptic mange in the Eastern Alps date back to the beginning of the 19th century (Deutz 2002). Areas where sarcoptic mange was or is present have been approximated in previous studies (Miller 1986, Rossi et al. 2007) and are shown in Figure 1. Of particular note in terms of spatial distribution are corresponding landmarks such as the river Inn, flowing north-eastwards from Switzerland through the western part of Austria in Tyrol to Bavaria in Germany, and the Wipptal, a valley extending between Innsbruck and Franzensfeste in the "Autonomous Province of Bozen – South Tyrol (Alto Adige)" in northern Italy. Historically and until very recently, outbreaks in Austria seem to have been limited to the area south of the river Inn and west of the Wipptal (Fuchs et al. 2000, Miller 1986, Rossi et al. 2007).

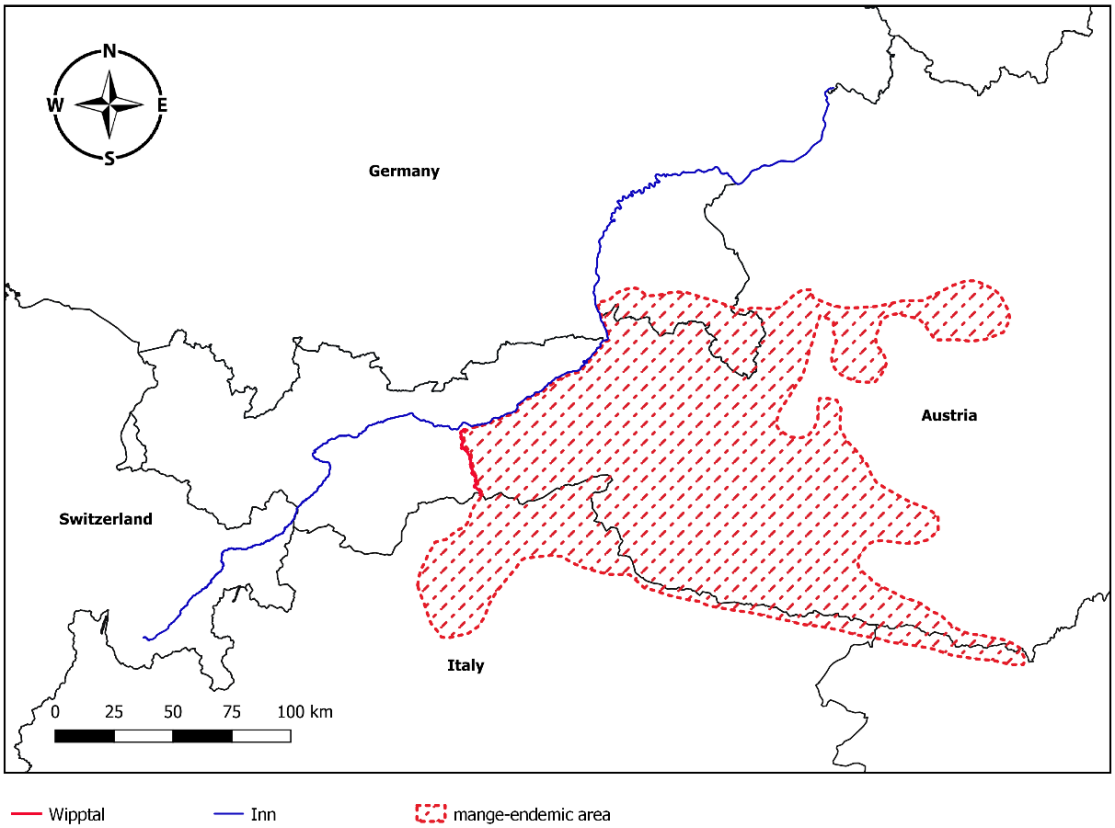

Figure 1: Mange endemic area in the Eastern Alps according to Miller (1986) and Rossi et al. (2007). The sarcoptic mange has come to a halt at the Wipptal in the west (solid red line) and at the River Inn in the north (blue line).

1.3 Genetics

1.3.1 Role of the major histocompatibility complex class II (MHC class II) and the DRB exon 2 locus

The immunological response of mite-infested chamois is still poorly understood, particularly regarding the role of the major histocompatibility complex class II (MHC class II):

MHC class II molecules are found in antigen-presenting cells such as B cells, monocytes, macrophages, and dendritic cells, where they bind to pathogen derived peptides generated in phagolysosomes. These antigens are then presented on the extracellular surface to naïve CD4 T cells which are responsible for further activation of the innate and the adaptive immune system (Luckheeram et al. 2012).

Looking at parasitic infections from a population genetics context, special emphasis has been put on the genetic variability of the corresponding MHC-class II genes, because of their role in parasite resistance of vertebrates and their potential effects on long-term survival of populations (Hedrick et al. 1999, Hedrick et al. 2001).

The Red Queen hypothesis (van Valen 1973) states that two species (for example in a predator prey relationship) antagonizing will lead to their oscillatory coevolution, alternating their relative biological advantage. On a genetic level this is described as negative frequency-dependent selection, which occurs when an advantageous allele is increasingly selected for as its frequency declines, i.e., when it is rare, contrary to positive frequency-dependent selection where selection occurs more likely with increasing frequency (Ridley 2004). In accordance with this, the abundance of common MHC class II alleles in a population leads to the genetic advantage of rare strains of parasites that can evade the defense mechanisms. These rare variants then in turn are rising in frequency, leading to the selection of less frequent MHC class II alleles which have proven to be beneficial against the increasingly common parasite strains, resulting in a wave-like pattern, with one species alternately outcompeting the other. MHC class 2 genes are reported to have one of the highest levels of polymorphism found among vertebrate species and therefore serve as good target genes when studying population genetic differences of hosts between endemic and non-endemic areas of specific pathogens. Reported strong linkage disequilibria of class 2 region genes in mammals lead to the conclusion that the analysis of the presently

studied DRB-locus variability may provide sufficient information about overall MHC class 2 variability (Marsh et al. 2000).

1.3.2 Genetic variation and differentiation in chamois

Several studies have looked at the genetic differentiation and diversity of chamois, using various molecular markers such as mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP), allozymes, and microsatellites (Crestanello et al. 2009, Loison et al. 1999, Lorenzini 2005, Rodríguez et al. 2009, Schaschl et al. 2003, Schaschl et al. 2004, Schaschl et al. 2012, Soglia et al. 2010). Genetic variation of both northern and southern chamois and their various subspecies was summarized in a review by Corlatti et al. (2011). For alpine chamois, which is the subspecies of interest, allelic richness ranged from 4.20 to 7.20, haplotype diversity from 54.0 % to 94.5 %, nucleotide diversity from 0.709 % to 3.740 %, observed heterozygosity from 52.7 % to 71.0 % and expected heterozygosity from 54.0 % to 73.8 %. Looking at genetic differentiation, one study compared the phylogeographic structure of 443 alpine chamois in the eastern alps using both mitochondrial and nuclear markers (Schaschl et al. 2003). Interestingly one of the key findings of this study was that, while mtDNA-RFLP analysis showed a rather high phylogeographic structuring (67.09 % of genetic diversity explained by differentiation among populations), this could not be reproduced by using nuclear markers revealing that only 0.45 % of the allozymic diversity could be explained by the studied population structure. The authors of this study stated that the most likely reason for this difference is the higher philopatry of females compared to males which was described in another study by Loison et al. (1999).

2 Material and Methods

2.1 Samples and study regions

This study is based on 208 tissue samples (liver, spleen, skin) of chamois shot between 2010 and 2018 by professional hunters predominantly in the course of regular hunts in several provinces of the Eastern Alps in Austria and Southern Germany; only some few samples were collected from individuals found dead. The collection of those samples was partly organized by Dr. rer. nat. Christine Miller (Rottach-Egern, Deutschland), the team of the pathology lab of the Research Institute of Wildlife Ecology (FIWI, University of Veterinary Medicine Vienna, headed by Dr. med. vet. Anna Kübber-Heiss) and Dr. med. vet. Martin Janovsky

(Landesveterinärdirektion Tirol), who also collated some metadata (sampling location and date, sex, age, eviscerated body weight) according to the hunters´ information. However, the whole set of the aforementioned individual metadata was available only for a total of 153 chamois (81 females and 72 males). The sampling locations were grouped into eight different geographic regions (hereinafter "sample regions"), i.e., VUI – Vorarlberg and upper Inn Valley, UA – Upper Allgäu, SW – mountain massifs west of the Stubaital, SEZ – Central Alps mountain massifs east of Stubaital and west of the Zillertal, ATK – Achental and Karwendel mountain massif, ET – East Tyrol, part of the "Hohe Tauern", CAK – Chiemgau Alps and Kaisergebirge, HT – Hohe Tauern National Park – Salzburg part (Figure 2). General and specific information on sites and regions where sarcoptic mange outbreaks have been recorded historically and in the recent past were taken from the literature (Miller 1986, Rossi et al. 2007, Fuchs et al. 2000) and local veterinary authorities (Landesveterinärdirektionen Salzburg and Tirol). For graphical depiction of sample sites and the mange endemic area the QGIS software, version 3.12.1 (QGIS.org 2020) was used.

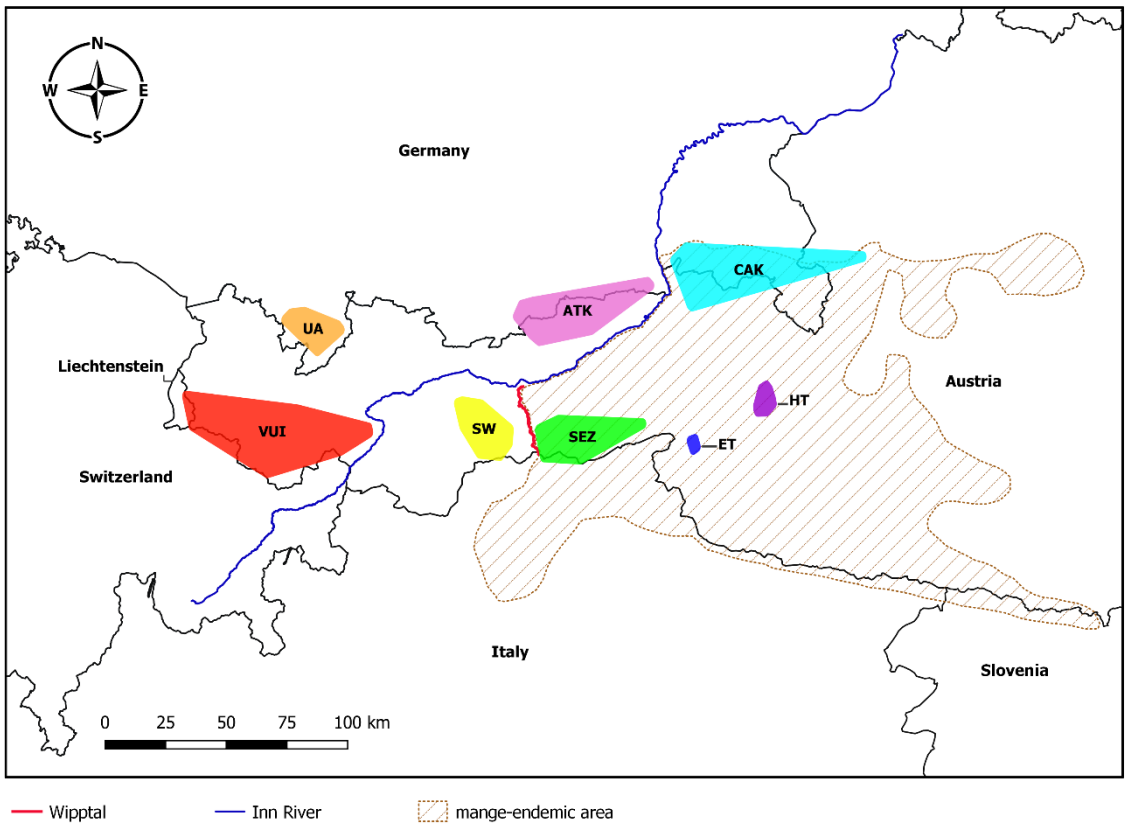

Figure 2: Sample regions: All 208 individuals were grouped according to their respective location of shooting and associated landscape features between sample regions (mainly mountain massifs or ranges and valleys). VUI – Vorarlberg and upper Inn Valley, UA – Upper Allgäu (Germany), SW – mountain massif west of Stubaital, SEZ – Central Alps east of Wipptall and west of Zillertal, ATK – Achental and Karwendel mountain range, CAK – Chiemgau Alps and Kaisergebirge, ET – East Tyrol (Hohe Tauern National part), HT – Hohe Tauern (Salzburg part of National Park).

2.2 DNA Isolation and MHC genotyping

Tissue samples were preserved frozen at -20°C ,and DNA was extracted with the DNA extraction kit GenElute^{TM} Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich Co., St. Louis, Missouri) according to the manufacturer's protocol. We amplified the 236 bp long partial coding sequences of the MHC class II DRB exon 2 using the primers HL030 (5′-

ATCCTCTCTCTGCAGCACATTTCC-3′) and HL032 (5′-TCGCCGCTGCACAG-TGAAACTCTC-3′) (Schaschl et al. 2004).

To obtain distinct results in subsequent analysis and haplotype phasing, we performed two rounds of PCR, with the first one using both forward and reverse primers and with the second one using either forward or reverse primers in a Sanger sequencing PCR reaction. For the first PCR the reaction mixture contained 7.05 µl distilled water, 1.25 µl 10x PCR-buffer, 1.25 µl of 2mM dNTPs, 10 pmol of each primer, 0.5 µl of 25 mM $MgCl_2$, 1µl of genomic DNA and 0.2 U modified *Taq* Polymerase (FIREPol®DNA Polymerase).

The thermocycler profile consisted of 95°C hot-start for 15 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec, extension at 72 °C for 90 sec, ending with 10 min of extension at 72 °C. Afterwards we performed an enzymatic cleanup of the amplified products using ExoSAP-IT™ Express PCR Product Cleanup Reagent (Applied Biosystems). The second strand-specific Sanger sequencing PCR used the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermofisher Scientific, Applied Biosystems, Waltham, Massachusetts, United States) adding 4 µl distilled water, 5x PCR-Buffer and 50 pmol either forward or reverse primers, with a thermocycler profile consisting of 96 °C for 1 min followed by 25 cycles of 10 sec at 96 °C, 5 sec at 50 °C and 4 min at 60 °C.

Subsequent precipitation to prepare the samples for sequencing was done by adding 10µl distilled water, 6.25 µl of 100mM NaEDTA with a pH of 8 and 60µl of cooled 95 % EtOH to 10 µl of the PCR product. Following this, samples were cooled for 15 min at 4 °C and centrifuged at 3000 rpm for 30 min at the same temperature. After decanting the supernatant, 200 µl of 70 % EtOH were added to the samples following centrifugation using the previous settings. To remove the remaining supernatant the plate was turned and centrifuged with a setting of 300 rpm for 10min at 4 °C and put into a drying cabinet for 5 min at 37 °C. Wells were then refilled with 10 µl of Hi-Di™ Formamide (Applied Biosystems), denatured for 5 min and chilled on ice before sequencing. Sequencing was carried out on an ABI 3130xl genetic analyzer. Sequence analysis was carried out using the software Bioedit version 7.0.9 (Hall 1999).

2.3 Haplotype inference

Instead of inferring haplotypes by cloning (or parental genotyping) we used the PHASE software version 2.1 (Stephens et al. 2001). The used parameters were: MR0, a model that allows for recombination, details to be found in Li and Stephens (2003), d1, which is the setting for a parent independent mutation model, 10000 iterations, a thinning interval of 100 and a burn-in setting of 1000 for 10 runs using different random seeds each run.

All 17 newly found MHC class II DRB exon 2 alleles will be submitted to GenBank (before publication of the manuscript in an international journal). The remaining nine haplotypes found in our samples have already been described previously (Schaschl et al. 2004, accession numbers: AY368455, AY368454, AY368453, AY368451, AY368446, AY368445, AY368443, AY368440, AY368437). Because of higher coverage (236 bp compared to 231 bp) and one polymorphic site on position 235 we decided to continue with the allele nomenclature of the aforementioned study instead of the one published in another study (Mona et al. 2008). The reasoning behind this was that despite some alleles could be aligned with 100 % base identity, the remaining polymorphic sites could still result in a different allele. Translation of haplotypes into amino acids was also performed using BioEdit version 7.0.9 (Hall 1999) checking at the same time for nonsense mutations or stop codons (which were absent) and new protein variants.

2.4 MHC variability

We calculated haplotype diversity, nucleotide diversity, segregating sites, number of haplotypes and the number of mutations of the studied MHC class 2 DRB exon 2 partial coding sequence (cds) locus using DnaSP version 5.10.01 (Rozas et al. 2017). Observed and expected heterozygosity as well as deviation from Hardy-Weinberg-Equilibrium (HWE) was calculated in GenAlEx 6.5 (Peakall and Smouse 2012).

2.5 Selection test

We used the Datamonkey webserver version 2.0 (Delport et al. 2010, Pond and Frost 2005, Weaver et al. 2018), which was last accessed for calculations on 15th of March 2020. Four distinct maximum likelihood approaches for the identification of selection were performed on the allele file: Single likelihood ancestral counting – SLAC (Kosakovsky Pond and Frost 2005), Fixed Effects Like-Likelihood – FEL (Kosakovsky Pond and Frost 2005), Fast Unconstrained Bayesian Approximation – FUBAR (Murrell et al. 2013), and Mixed Effects Model of

Evolution - MEME (Murrell et al. 2012). P values below 0.05 in SLAC, FEL and MEME and a posterior probability of 0.95 in FUBAR were considered as indicating positively selected sites. Furthermore OmegaMap (Wilson and McVean 2006) was used to detect codons under selection, i.e., to determine ω, which indicates positive selection if the ratio of non-synonymous (dN) to synonymous (dS) substitutions (dn/ds) is larger than 1, or negative selection, if dn/ds < 1, or neutrality if dn/ds = 1. The latter tests also accounted for potential recombination. We considered any codon under positive selection, only if identified by at least two tests implemented.

We used the model-based approach of Beaumont and Nichols (Beaumont and Nichols 1996) which was implemented in Lositan version 1.0.0 (Antao et al. 2008) to compare the observed *Fst* values estimated to a function of *Fst* and observed heterozygosity. Using 50000 simulations, and assuming neutral mean *Fst*, an infinite allele mutation model, a 99 % confidence interval, and a false discovery rate of 0.1 %, thirteen microsatellite loci and the DRB exon 2 partial cds locus were examined for neutrality.

2.6 Testing for Recombination

The Datamonkey webserver's GARD technique (Kosakovsky Pond et al. 2006) was used to look for potential recombination partitions. To identify recombination breakpoints, the RDP (Martin and Rybicki 2000), GENECONV (Padidam et al. 1999), MAXCHI (Smith 1992), CHIMAERA (Posada and Crandall 2001), and 3SEQ (Lam et al. 2018) programs included in the RDP3 package (Martin et al. 2010) were all used.

2.7 Evolutionary analysis

Using NETWORK 10.0 (Bandelt et al. 1999) with default properties (equally weighted positions and epsilon set to zero) we created a median joining network for all 25 haplotypes found presently, displaying the frequency of haplotypes in the two study regions (i.e., mange endemic range and historical mange free region). The sizes of pie charts reflected relative frequencies of alleles (i.e., protein variants) and the numbers of mutations between alleles were represented by bars along the connecting lines between alleles. Connecting lines between alleles represented evolutionary trajectories and black dots at converging connecting lines represented median vectors.

2.8 Microsatellite genotyping

Microsatellite genotyping was performed using previously tested microsatellite loci (Lorenzini 2005, Pérez et al. 2000, Schaschl et al. 2012). Following loci were genotyped: RM029, RM026, INRA023, INRA005, INRA011, INRA036, ETH225, ETH10, SR-CRSP1, SR-CRSP5, SR-CRSP9 and SR-CRSP8. We used a Multiplex PCR approach with fluorescently labeled primers and 2 panels. Both panels used the same amplification conditions as previously described in other studies (Lorenzini 2005, Pérez et al. 2000) with the exception in terms of polymerase choice and annealing temperatures at following microsatellites: ETH10: 55°C using FIRE-Pol®DNA Polymerase; INRA023 and SR-CRSP8: 53°C using Phusion® High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA). Allele lengths were measured by an ABI 3130xl genetic analyzer using a custom ROX labeled size standard based on a pUC19-template (DeWoody et al. 2004) and analysed with the software GeneMarker v. 2.7.4. Samples with less than 50 % of loci interpretable data were removed from further analyses.

2.9 Statistical Analysis

The ETH10 primers produced poor amplification results with a genotyping success rate of only 60 %. Successful genotyped individuals had signals of high quality but only few alleles were found at this locus. Thus, we performed population genetic calculations both on all twelve loci and separately by excluding ETH10.

For the calculation of allele frequency, expected and observed heterozygosity per locus and population, and overall F-statistics, the software GENETIX 4.05.2 (Belkhir 2004) was used. We calculated sample region- and locus-specific *Fis* values with their corresponding significance levels in terms of deviation from Hardy-Weinberg Equilibrium by permutation testing, using 1000 permutations per population sample (Weir and Cockerham 1984). We used the same software to calculate *Fst* values for all pairs of sample regions and checked for values significantly higher than zero by permutation tests implemented in the software. Finally, we used GENETIX to calculate Cavalli-Sforza.Edwards (CSE) chord distances of pairs of sample regions with their associated significance levels based on 10 000 permutations.

We used Identix version 1.1.5 (Belkhir et al. 2002) to determine Queller & Goodnight's estimators for pairwise relatedness (Queller and Goodnight 1989) between individuals separately for each sample population and their corresponding confidence intervals given our multilocus

data, to check for accidental sample duplication. We tested for linkage disequilibrium (LD) with a Markov chain method and applying strict Bonferroni corrections for serial testing by using GENEPOP 3.4 (Raymond and Rousset 1995).

FSTAT 2.9.3.2 (Goudet 2001) was used to calculate locus-specific allelic richness (Rs) which takes different population sizes into account through an adapted version of the rarefaction index (El Mousadik and Petit 1996). We used the arithmetic mean of all allelic richness values per locus separately for each sample region to indicate overall allelic richness (AR) per sample region. Rs variation across sample regions and between non-scabies and endemic sample regions was tested by two separate linear mixed effects models based on locus-specific Rs values as dependent variable, locus label (12 loci names) as random factor and sample region/endemic vs. non-scabies region as fixed factor, respectively.

Geneclass version 2.0.h was used to determine the Bayesian probability that an individual's genotype will be assigned to the same population from which it was sampled (Cornuet et al. 1999). In particular, a multilocus method by Rannala and Mountain (1997) was used by applying a resampling technique implemented in GENECLASS 2 0 h INRA/CIRAD© (Piry et al. 2004) to obtain individual likelihood values.

We used Arlequin v3.5 (Excoffier and Lischer 2010) to perform two separate analyses of molecular variance (AMOVA), once based on all eight unstructured sample regions and once based on a structured design with the sample regions grouped into the endemic and the non-scabies regions.

2.10 Structure

Likelihood values for admixture models were calculated using STRUCTURE 2.3.4 (Pritchard et al. 2000) assuming 1-10 genetic clusters (further referred to as K) and correlated allele frequency. The remaining parameter set was as follows: length of burnin: 100 000; number of Markov chain Monte Carlo repetitions: 300 000; number of iterations: 10. Likelihood values were calculated both with and without “population priors” (i.e., information of individual assignment to one of the eight sample regions) using the same parameter set. Because of potential selection effects (i.e., by linkage with unknown loci under selection; see Results) at RM029 and SR-CRSP8 as well as relatively poor genotyping results at the ETH10 locus both models were

repeated without said loci. The STRUCTURE HARVESTER (Earl and vonHoldt 2012) server was used to calculate ΔK (mean(|L″(K)|)/SD(L(K)) (Evanno et al. 2005) as well as mean and standard deviation of estimated Ln probabilities per K (L(K)).

2.11 Statistical modeling of DRB exon2 heterozygosity and of body weight

To specifically test the hypotheses stated in the introduction and the ones resulting from the paper on DRB variability and selection in chamois from the Eastern Alps (Schaschl et al. 2012) we followed the principles of model selection and multimodel inference outlined in Burnham and Anderson (2013) and used the statistical software R v.3.6.3 (R Core Team 2016) to model the individual variation of the DRB exon 2 heterozygosity status and body weights of individual chamois.

Specifically, for individual heterozygosity we used the following linear model syntaxes:

hetzyg ~ sex*age*mange + msathezyg + day + (1|year) (as logistic model),

were "hetzyg" is the individual genotype at the DRB exon2 locus as either homozygous or heterozygous, age is the individual age in number of years as assessed by the horn rings, mange means the individual chamois stemming either from a location in the mange endemic region or out of it, "msathezyg" means the mean heterozygosity as estimated by the putatively neutrally evolving microsatellite loci, day representing the collection date of the individual samples as running calendar day in the year starting with the day on which our first sample has been collected (i.e., 12th August), with only very few samples having been collected later than 31st December, and year representing the year of sampling (i.e., between 2010 and 2018, with many chamois having been collected in 2014). The variable year was used as random factor, as a priori we didn´t have any expectation about possible changes across different years.

The interaction term "sex*age*mange" tested specifically the hypothesis of higher frequencies of DRB exon 2 heterozygosity in older male chamois that are supposedly more often involved in energetically demanding rutting activities than younger males or females (Lovari and Locati 1991, Schaschl et al. 2012). In that global model we corrected for year as random factor and day and "msathezyg" as fixed factors, to account for possible seasonal changes (i.e., from summer to early winter) associated e.g., with changes in parasitic loads. The term "msathezyg" was

included in the global model to discriminate between DRB heterozygosity per se and a possible general overdominance (heterosis, general individual heterozygosity) effect.

In a second (alternative) approach we used the same global model as above, but exchanged the interaction term by rutmale*mange, with rutmale representing either males six years of age or older (i.e., males that are most likely engaged in increased rutting activities) *vs.* younger males and females as alternative category. That variable might even better reflect the biological difference of chamois and their energetic demands particularly during the rutting season (Corlatti et al. 2015, Schaschl et al. 2012, Willisch and Ingold 2007).

Hence, the alternative global model was as follows:

$$\text{hetzyg} \sim \text{rutmale*mange} + \text{msathezyg} + \text{day} + (1|\text{year})$$

We first calculated the corrected Akaike Information Criterion (AICc) values for each global model to identify which one was more informative in terms of information theory to then choose the superior model for model ranking and model averaging (Burnham and Anderson 2013);however, no model was clearly superior to the other (see Results); thus, we performed model ranking and averaging separately for all models based on each of the global models.

To further test the hypothesis of MHC variation affecting specifically the fitness of older males that in principle would participate in the energetically costly rutting competitions, we modeled body weight conditional on various factors and variables including DRB exon 2 heterozygosity (locus heterozygous or homozygous, independent of the allele combination). Moreover, instead of using the factor DRB exon 2 heterozygosity we run alternative models with several alleles found at relative high frequencies. In this context we hypothesized a significant genotype or allele effect for the DRB locus specifically in older males that would be subjected in principle to a pronounced loss of body condition (i.e., body weight) during the rutting season (Schaschl et al. 2012). We also hypothesized an MHC-related effect to occur specifically within the mange endemic range, given the detrimental effect sarcoptic mange potentially has on the body condition of rutting males and under subsequent winter conditions. However, unlike in the above stated heterozygosity models, we included year as factor with a spline as we could not a priory exclude changing ecological conditions from year to year that could have had a certain effect on body weights.

Our global model syntaxes were set up as follows and were compared to their respective AICc values (the random factor year was not considered, as its standard deviation was already proved to be smaller than one percent of the intercept in the above heterozygosity models – see Results section):

bw ~ sex*age*mange*drbhezyg + s(day,by=sex) + msathezyg + s(year,bs="re")
bw ~ sex*age*mange*drbpro1 + s(day,by=sex) + msathezyg + s(year,bs="re")
bw ~ sex*age*mange*drbpro4 + s(day,by=sex) + msathezyg + s(year,bs="re")
bw ~ sex*age*mange*drbpro20 + s(day,by=sex) + msathezyg + s(year,bs="re")
bw ~ sex*age*mange*drbpro21 + s(day,by=sex) + msathezyg + s(year,bs="re")

and

bw ~ rutmale*mange*drbhezyg + s(day,by=sex) + msathezyg + s(year,bs="re")
bw ~ rutmale*mange*drbpro1 + s(day,by=sex) + msathezyg + s(year,bs="re")
bw ~ rutmale*mange*drbpro4 + s(day,by=sex) + msathezyg + s(year,bs="re")
bw ~ rutmale*mange*drbpro20 + s(day,by=sex) + msathezyg + s(year,bs="re")
bw ~ rutmale*mange*drbpro21 + s(day,by=sex) + msathezyg + s(year,bs="re")

with bw being the body weight, age ranging from the first year of life to 18 year old animals, mange meaning collected either within the mange epidemic area or outside of it, “drbhezyg” referring to the DRB exon 2 locus being either homozygous or heterozygous (independent of alleles), and drbpro1, drbpro4, drbpro20, and drbpro21 indicating whether or not the respective protein variant was present or absent in an individual; for drbpro1 we considered three categories of expression, namely protein variant absent, present once (homozygous), or present twice (heterozygous), whereas for all other protein variants we used only two categories of expression (protein variant absent *vs*. present), because of too rare cases of present twice. The variables day, year, and msathezyg were the same as described for the above models, but we considered day separately for the sexes.

3 Results

3.1 MHC class II DRB exon 2 sequences

A total of 26 alleles (haplotypes) were found in the sample population of 208 chamois, nine of which were previously detected by Schaschl et al. (2004) namely Ruru-DRB01, Ruru-DRB-04, Ruru-DRB-07, Ruru-DRB-09, Ruru-DRB-10, Ruru-DRB-15, Ruru-DRB-17, Ruru-DRB-18, Ruru-DRB-19. The 17 newly discovered alleles were assigned to the names Ruru-DRB20 to Ruru-DRB36. Each of the newly found alleles translate into a unique amino acid sequence, including the functionally necessary cysteine residues at position 15 and 79 (Schaschl et al. 2004) and without frameshift mutations or stop codons. A list of all alleles including the ones previously described by Schaschl et al. (2004) which were not detected in our sample can be found in the alignment in Figure 3.

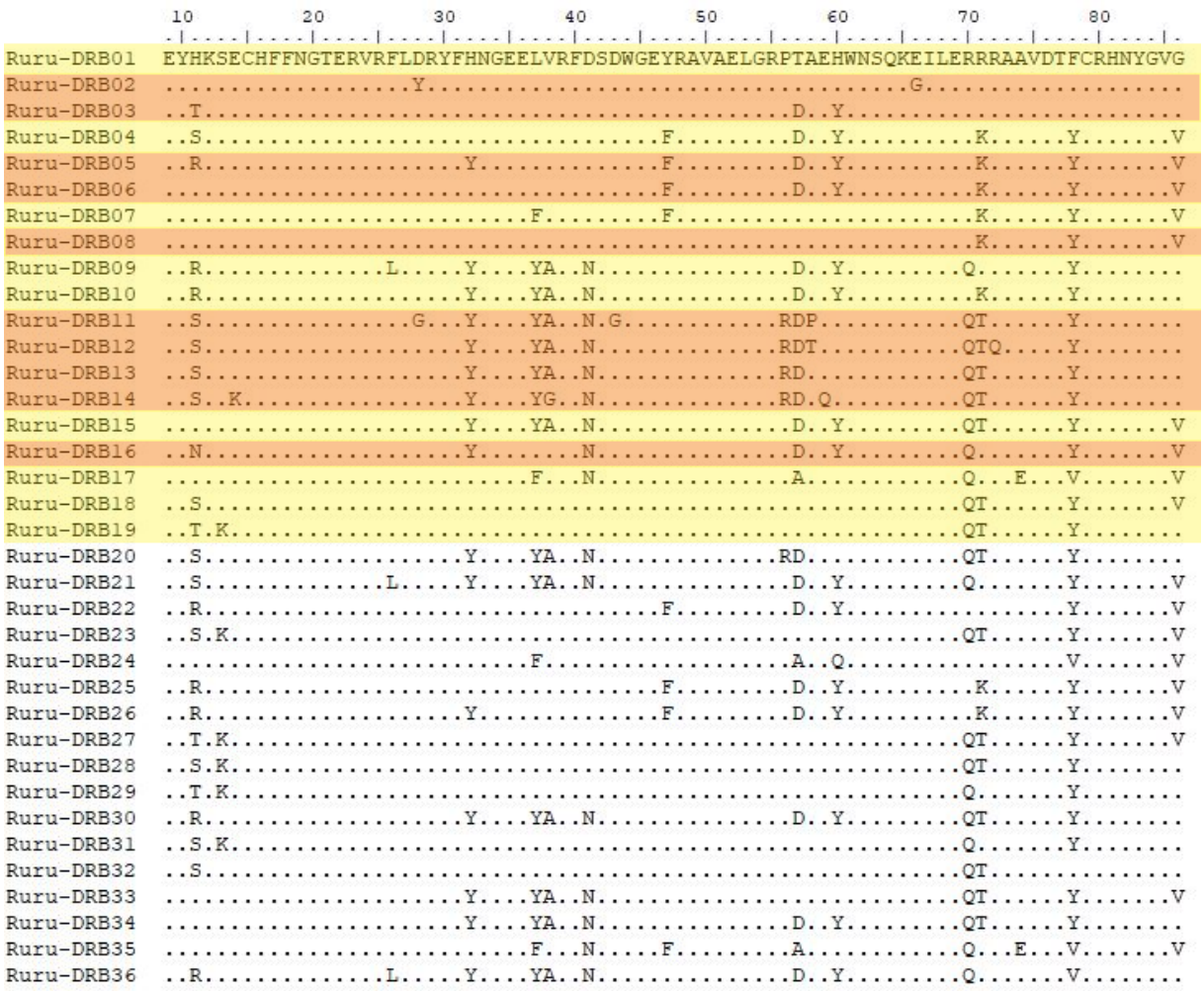

Figure 3: Amino acid alignment of previously published (yellow if recovered in the present study as well, orange if otherwise) and newly discovered MHC class II DRB exon 2 partial cds alleles.

Allele frequencies for the total sample population, for each subpopulation as well as for mange-endemic vs. non-endemic areas are listed in table 1. Allele frequencies across endemic vs. non-endemic regions are shown in Figure 4. The most common allele in both mange endemic and non-endemic areas was Ruru-DRB01 with a frequency of 24.78 % and 33.16 %, respectively, followed by Ruru-DRB20 (11.06 % and 16.84 %, respectively) and Ruru-DRB21 (5.31 % and 8.95 %). Both areas have a respective number of 21 alleles.

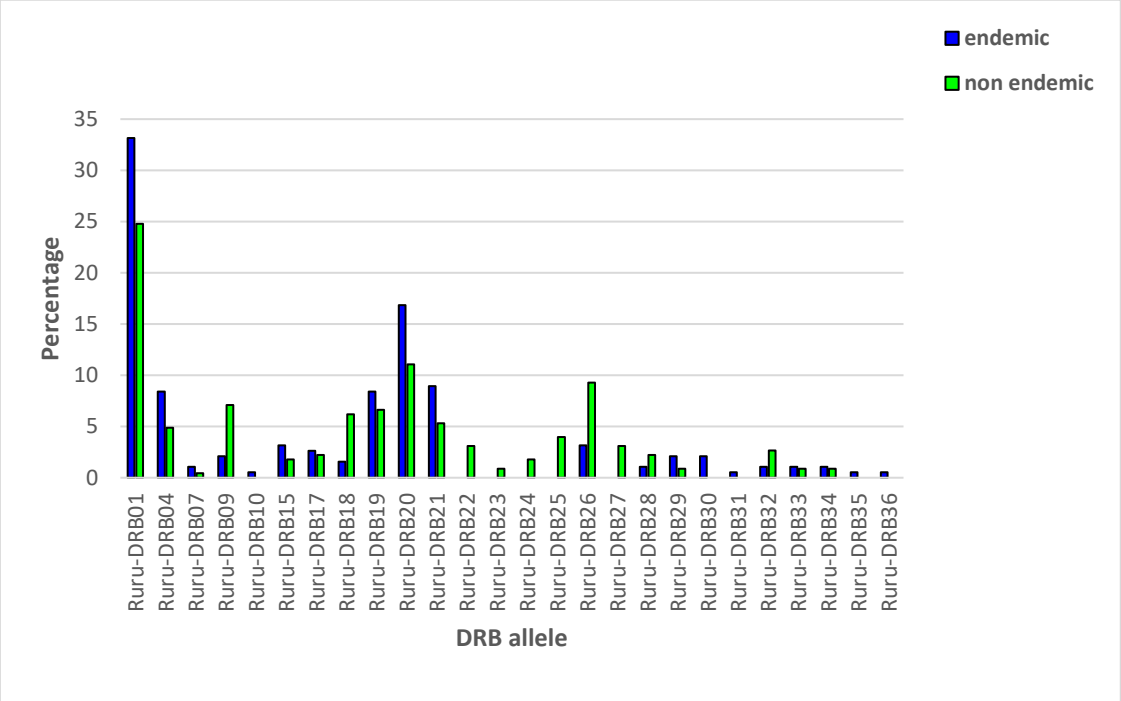

Figure 4: DRB exon 2 partial cds allele frequencies sub-grouped into mange-endemic (blue) and non-endemic (green) regions.

Table 1: Relative frequencies (%) of detected alleles across all regions (see fig. 2), across each of the eight sample regions, and across the non-endemic and endemic regions.

alleles	total	VUI	UA	SW	SEZ	ATK	CAK	ET	HT	non endemic	endemic
Ruru-DRB01	28.61	26.42	6.60	9.43	19.81	10.38	23.58	3.77	12.26	24.78	33.16
Ruru-DRB04	6.49	0.94	0.00	1.89	3.77	7.55	10.38	0.94	0.00	4.87	8.42
Ruru-DRB07	0.72	0.00	0.00	0.94	0.00	0.00	0.94	0.00	0.94	0.44	1.05
Ruru-DRB09	4.81	14.15	0.94	0.00	3.77	0.00	0.00	0.00	0.00	7.08	2.11
Ruru-DRB10	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94	0.00	0.53
Ruru-DRB15	2.40	2.83	0.94	0.00	2.83	0.00	0.00	0.94	1.89	1.77	3.16
Ruru-DRB17	2.40	0.00	0.00	3.77	1.89	0.94	1.89	0.00	0.94	2.21	2.63
Ruru-DRB18	4.09	3.77	1.89	0.00	0.00	7.55	1.89	0.94	0.00	6.19	1.58
Ruru-DRB19	7.45	9.43	0.00	3.77	7.55	0.94	0.00	0.94	6.60	6.64	8.42
Ruru-DRB20	13.70	5.66	2.83	8.49	12.26	6.60	5.66	0.94	11.32	11.06	16.84
Ruru-DRB21	6.97	6.60	0.00	1.89	7.55	2.83	6.60	0.94	0.94	5.31	8.95
Ruru-DRB22	1.68	5.66	0.94	0.00	0.00	0.00	0.00	0.00	0.00	3.10	0.00
Ruru-DRB23	0.48	1.89	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88	0.00
Ruru-DRB24	0.96	2.83	0.00	0.00	0.00	0.94	0.00	0.00	0.00	1.77	0.00
Ruru-DRB25	2.16	6.60	0.94	0.00	0.00	0.94	0.00	0.00	0.00	3.98	0.00
Ruru-DRB26	6.49	9.43	6.60	1.89	0.00	1.89	5.66	0.00	0.00	9.29	3.16
Ruru-DRB27	1.68	2.83	0.94	2.83	0.00	0.00	0.00	0.00	0.00	3.10	0.00
Ruru-DRB28	1.68	0.94	0.00	2.83	1.89	0.94	0.00	0.00	0.00	2.21	1.05
Ruru-DRB29	1.44	0.00	0.00	1.89	1.89	0.00	0.00	0.00	1.89	0.88	2.11
Ruru-DRB30	0.96	0.00	0.00	0.00	1.89	0.00	0.00	0.94	0.94	0.00	2.11
Ruru-DRB31	0.24	0.00	0.00	0.00	0.94	0.00	0.00	0.00	0.00	0.00	0.53
Ruru-DRB32	1.92	0.00	0.00	0.00	0.00	5.66	1.89	0.00	0.00	2.65	1.05
Ruru-DRB33	0.96	0.00	0.00	0.00	0.00	1.89	0.94	0.00	0.94	0.88	1.05
Ruru-DRB34	0.96	0.00	0.00	0.00	0.00	1.89	1.89	0.00	0.00	0.88	1.05
Ruru-DRB35	0.24	0.00	0.00	0.00	0.00	0.00	0.94	0.00	0.00	0.00	0.53
Ruru-DRB36	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.94	0.00	0.00	0.53

Despite private alleles were found in mange-free or mange-endemic areas, no significant differences in allele frequencies between those two regions could be found. Observed heterozygosity ranged from 66.7 % in UA to 100 % in ATK. No sample region showed significant deviation from Hardy-Weinberg-Equilibrium at this locus (table 2).

Table 2: Observed and expected heterozygosity for each of the eight sample regions at the MHC class II DRB exon 2 locus tested for deviation from Hardy-Weinberg-Equilibrium. Key: ns = not significant (after strict Bonferroni corrections, at an alpha of 0.05), Pop = sample population, Ho = observed heterozygosity, He = expected heterozygosity, DF = degrees of freedom.

Pop	Ho	He	Locus	DF	ChiSq	Prob	Signif
VUI	0.868	0.873	DRB	105	102.315	0.556	Ns
UA	0.667	0.799	DRB	36	34.041	0.562	Ns
SW	0.905	0.859	DRB	55	54.966	0.476	Ns
SEZ	0.829	0.838	DRB	66	65.478	0.495	Ns
ATK	1.000	0.877	DRB	91	69.620	0.953	Ns
CAK	0.758	0.797	DRB	66	82.071	0.088	Ns
ET	0.833	0.833	DRB	36	37.500	0.400	Ns
HT	0.810	0.787	DRB	55	19.247	1.000	Ns

The haplotype and nucleotide diversity across all samples and each sample region as well as for endemic and non-endemic regions can be found in table 3. Across the entire sample of 416 sequences (208 individuals), 23 segregating sites, 25 polymorphisms and 26 haplotypes were found. The haplotype diversity for all sequences was 87.5 % and the nucleotide diversity 3.483 %. For the eight sample regions haplotype and nucleotide diversity ranged from 80.6 % to 90.9 % and from 3.141 % to 3.769 %, respectively. The non-endemic region had a higher haplotype diversity, slightly more segregating sites and polymorphisms than the endemic region (89.7 % vs. 83.9 %, 23 vs. 22 and 25 vs. 24, respectively), while nucleotide diversity was slightly higher in the endemic region (3.456 % vs. 3.492 %, respectively). Sample region-specific allelic richness of the DRB exon 2 locus did, however, not differ significantly ($p = 0.087$, t-test, $n = 8$) between the endemic and mange-free regions (mange-free region (mean = 6.82): VUI = 6.922, UA = 6.148, SW = 7.086, ATK = 7.133, endemic region (mean = 6.77): SEZ = 7.034, CAK = 5.892, ET = 9.000, HT = 5.156). Moreover, for the DRB exon 2 locus, mean pairwise *Fst* between regional samples amounted to 0.028 (s.d. = 0.01283, range = 0.0104 –

0.0437) in the mange-free region and to 0.003 (s.d. = 0.0155, range = -0.0278 – 0.0499) in the endemic region, respectively. But the hierarchical AMOVA indicated that no significant (1.24 %, p = 0.1144) portion of the relative allelic variation at the DRB exon 2 locus was due to partitioning among the endemic and the mange-free regions, whereas 4.10 % ($p < 0.0001$) of the variation was due to partitioning between the regional samples within the mange-free and the endemic regions, respectively. By far most (95.21 %, p = 0.0029) of the variation was due to presence within individuals.

Table 3: Haplotype and nucleotide diversity across all samples and each of the eight sample regions (Figure 2) as well as the endemic and non-endemic region; Pop – population (i. e., sample region), ^{E} associated with population acronym denotes a regional sample within the endemic region, n – number of sequences, S – number of segregating sites, Eta – number of mutations, H – number of haplotypes (alleles), Hd – haplotype diversity, Pi – nucleotide diversity. The values for the "endemic" and "non-endemic" regions were calculated by disregarding sample region-specificity, based on respective individual data within each of the two regions.

Pop	n	S	Eta	H	Hd	Pi
Total	416	23	25	26	0.875	0.03483
VUI	106	22	24	15	0.881	0.03468
UA	24	19	21	9	0.833	0.03141
SW	42	22	24	11	0.880	0.03634
SEZ^{E}	70	22	24	12	0.850	0.03550
ATK	54	23	25	14	0.893	0.03198
CAK^{E}	66	20	21	12	0.809	0.03250
ET^{E}	12	21	23	9	0.909	0.03769
THE	42	22	24	11	0.806	0.03379
Non-endemic	226	23	25	21	0.897	0.03456
Endemic	190	22	24	21	0.839	0.03492

A network of DRB exon 2 alleles is shown in Figure 5. The biggest distance between connected alleles was found between Ruru-DRB10 and Ruru-DRB26, consisting of six substitution steps. In general, a subtle division into three different "clades" (i.e., shallow evolutionarily divergent clusters) might be recognized in the network, with Ruru-DRB10 and Ruru-DRB26 and Ruru-DRB01 and Ruru-DRB32 being at the interface between these "clades", respectively. The network also shows the respective allele frequencies depicted by their relative size and their corresponding proportion of individuals belonging to the endemic or non-endemic region. Neither of the "clades" could clearly be assigned to one of the sample regions or to either endemic and non-endemic regions.

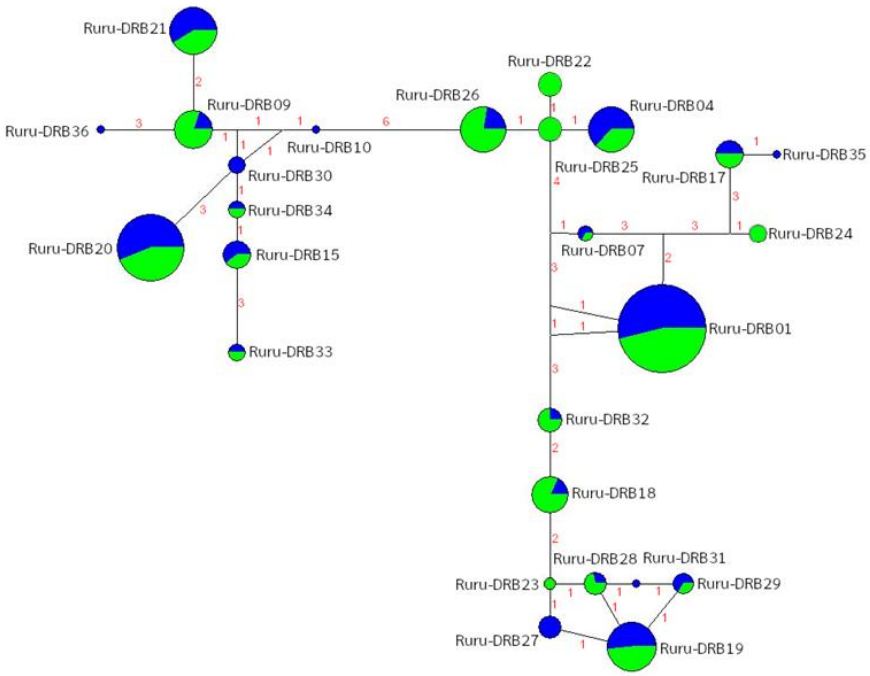

Figure 5: Median joining Network of MHC class II DRB exon 2 based on 26 amino acid haplotypes (alleles). The number of substitutions between alleles are represented by numbers in red. Alleles are represented by pie charts showing their respective allele frequencies in endemic (blue) vs. non-endemic (green) regions. Pie sizes correspond to overall allele frequencies.

3.2 MHC class II DRB exon 2 Selection Test Results

The results of the performed selection tests can be found in Figure 6. Applying a minimum of two tests showing positive selection as criterion for indicating positive selection, the tests performed across the entire sample showed a total of seven codons under positive selection (positions: 11, 12, 57, 60, 70, 71, 86). The non-endemic region and endemic regions showed a total of 11 codons as candidates for positive selection (positions: 11, 13, 38, 41, 57, 60, 70, 71, 74, 78, 86). The endemic region showed one codon less at positive selection (positions: 11, 13, 26, 37, 47, 57, 70, 71, 78, 86). Most codons under positive selection were shared between these 2 regions, however four codons (positions: 38, 41, 60, 74) that were under positive selection in the non-endemic region were not under positive selection in the endemic region and three codons (positions: 26, 37, 47) were not under positive selection, vice versa.

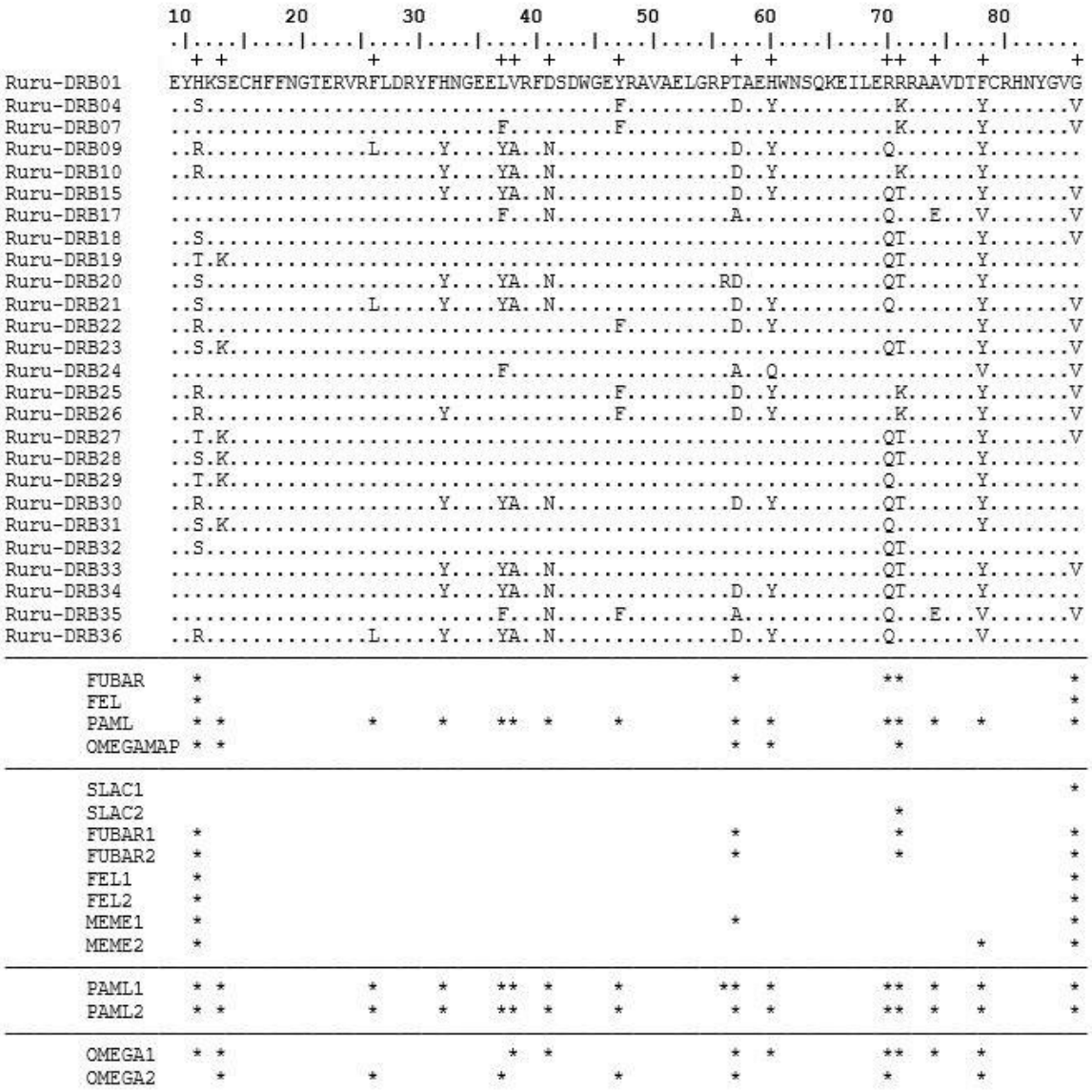

Figure 6: Amino acid alignment and selection tests for the 26 alleles detected in the sample regions. The plus sign (+) above denotes codons that fulfill the criterion of at least two tests detecting positive selection. Asterisks (*) are used to denote positively selected codons that were found using the respective selection tests. The selection tests are assigned according to the tested sample region they have been performed with, using no suffix for the entire sample, 1 for non-endemic and 2 for endemic regions

The additionally performed test for detecting positive or balancing selection using Lositan version 1.0.0 (Antao et al. 2008) did not reveal any type of selection neither for the presumptively neutral microsatellite loci nor for the DRB exon 2 partial cds locus (Figure 7).

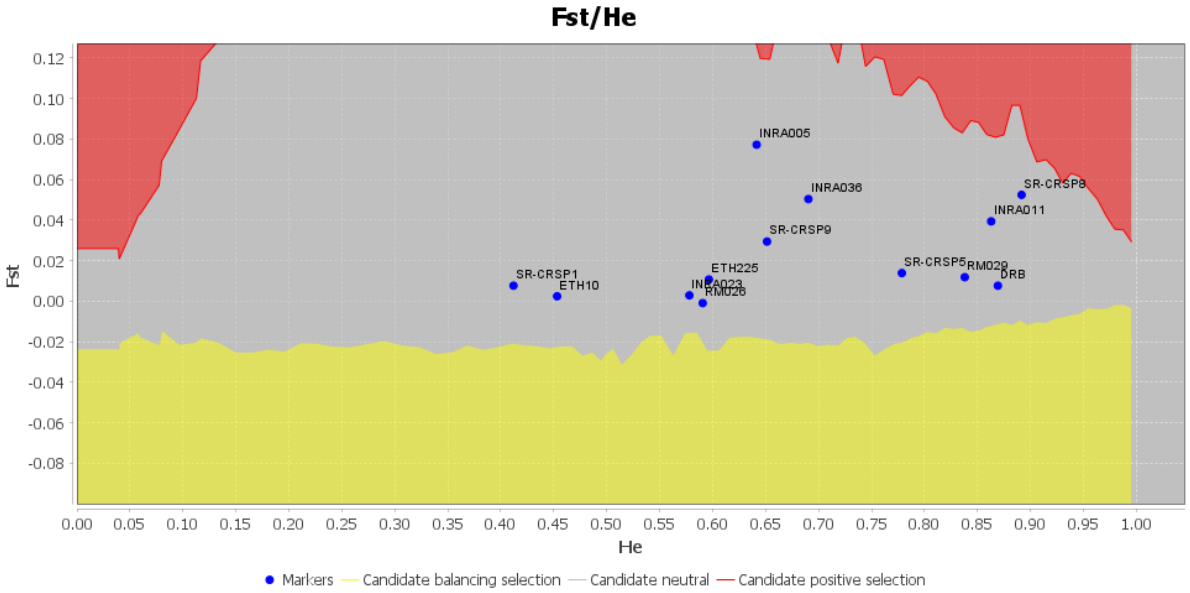

Figure 7: Corresponding *Fst* values for the 12 studied microsatellite loci and the DRB exon 2 partial cds locus as a function of heterozygosity (*Fst*/He), calculated using the software Lositan version 1.0.0. Markers in the gray area of the graph are considered candidates for neutral selection while markers located in the yellow or red area are considered candidates for balancing or positive selection and negative selection, respectively

3.3 Modeling Results of DRB exon 2 heterozygosity

Before running the two alternative models we explored the variance of the random factor year in either model; as the standard deviation was less than one percent of the intercept in each model, we removed that variable from our starting models. The AICc values of the two alternative global models of DRB exon2 heterozygosity, as described in the Material and Methods section but without the random factor year were very similar, namely AICc = 156.76 for the model including the three-way interaction term „sex*age*mange“, and AICc = 149.3 for the alternative model substituting that three-way interaction term by „rutmale*mange“. As the difference between the two AICc values was less than 10, we could not consider the latter model clearly superior over the former (Burnham and Anderson 2013). Consequently, we used both global models in parallel for our subsequent model ranking and model averaging. The relative

variable importance values (RVI) for all independent variables were, however, smaller than 0.7 for each suite of models, and therefore not statistically meaningful (Burnham and Anderson 2013).

3.4 Modeling Results of DRB exon 2 protein variants

Like for the heterozygosity models reported above, all alternative global models including either the four-way interaction term sex*age*mange*drbhezyg or alternatively the three-way interaction term rutmale*mange*drbprotx had similar AICc values. Nevertheless, we selected only the global model with the lowest AICc value, respectively, to run model ranking and model averaging separately for the two suites of models. For the suite of models containing the four-way interaction term it was the one including drbpro21 (i.e., DRB exon2 protein variant 21), and for the suite of models containing the alternative three-way interaction term (i.e., substituting sex*age by rutmale) it was the model including drb20 (i.e., DRB exon2 protein variant 20).

For the suite of models containing the four-way interaction term sex*age*mange*hetzyg or drbpro21 the model averaging resulted in the following relative variable importance values (RVI): age = 1; sex = 1; age*sex = 0.96; mange = 0.92; age*mange = 0.82; day = 0.79.

Hence, only the terms age*sex, age*mange, and day were considered statistically important in affecting body weight. Body weight of chamois carrying or not carrying the DRB exon 2 protein variant 21 did not show statistically meaningful body weight differences, when accounting for the other factors and variables considered in the models. The inspection of the coefficients table and the modeled body weight development along the Julian days could be interpreted as very slight decrease in male body weighs and a slightly stronger decrease of body weights in females from ca. Julian day 100 onward (i.e., last days in November). Generally, with increasing age males displayed a stronger increase in body weight than females during the observation period, independent of whether they were observed in the mange epidemy area or outside of it, and this age-related increase of body weight was somewhat stronger in the mange epidemic area than outside of it (i.e., north and west of the Inntal/Wipptal demarcation).

The model averaging of the suite of body weight models including the rutmale factor and the factor drbpro20 yielded the following values of relative variable importance (RVI):

rutmale = 1; day =0.99; mange = 0.77; very much in line with the interpretation of the above model results including drbpro21, older males (i.e., six years of age or older – those ones that were most likely engaged in rutting) had higher body weights than younger males or females; independently, both sexes experienced a slight but statistically meaningful decrease of body weight with increasing Julian day (i.e., from August to December), and body weights of chamois from the mange endemic range were generally higher than in chamois from the mange-free range, independent of sex, age, or the Julian day.

3.5 Population Genetic Results of Microsatellite Markers

Overall, 94 alleles were revealed at all twelve microsatellite loci, with 28 (29.8 %) of them occurring in each of the eight sample regions, and with eleven (11.7 %) private alleles, occurring in only one of the eight sample regions, respectively. Whereas the average frequency of alleles occurring in all sample regions amounted to 31.36 %, allele frequencies of private alleles had a mean of 3.14 % with a range from 1.0 - 7.5 %. A summary of the sample region-specific allelic variability including expected and observed heterozygosity as well as *Fis* values and allelic richness is given in table 4. According to the model results, there was no significant variation of allelic richness neither when tested across the eight sample regions considered separately ($p = 0.788$), nor when tested between the endemic region and that where mange has never been observed historically ($p = 0.200$).

Table 4: Allelic variation at 12 microsatellite loci in the eight sample regions of chamois (see fig. 2): Overall number of alleles per locus (A acc. to GENETIX), sample region-specific allelic richness (Rs), allelic size range in bp (R), size in bp (S) and frequency (F) of the most common allele, expected heterozygosity corrected for small sample size (He), observed heterozygosity (Ho). Sample region-specific *Fis* values with significant (Bonferroni correction at nominal alpha of 0.05) departure from zero are indicated by asterisk (*). The total number of alleles per locus and the number of private alleles for each population are given in parentheses, respectively.

sample region	index	loci												Mean
		RM029	RM026	INRA023	INRA005	INRA011	INRA036	ETH225	ETH10	SR-CRSP1	SR-CRSP5	SR-CRSP9	SR-CRSP8	
		(8)	(6)	(7)	(5)	(17)	(8)	(7)	(4)	(4)	(7)	(7)	(14)	
VUI (3)	A	7	4	4	6	11	8	6	4	4	6	5	11	6.17
Rs=3.844	R	100-114	90-96	191-203	167-179	201-231	173-187	134-154	204-210	123-129	154-170	124-134	211-243	
Fis=0.0275	S	110	92	197	173	203	179	150	204	125	168	128	237	
	F	0.394	0.580	0.612	0.423	0.440	0.337	0.490	0.595	0.760	0.300	0.726	0.229	
	H_e	0.784	0.569	0.569	0.746	0.769	0.765	0.667	0.537	0.396	0.771	0.452	0.857	0.657
	H_o	0.731	0.600	0.449	0.712	0.700	0.769	0.700	0.514	0.346	0.760	0.431	0.916	0.639
UA (0)	A	5	3	4	4	8	6	4	2	3	4	4	7	4.42
Rs=3.542	R	100-114	90-94	191-203	167-179	203-231	173-187	134-152	204-206	123-127	162-170	124-130	221-241	
Fis=-0.01177	S	110	92	197	179	203	179	150	204/206	125	170	128	229/237	
	F	0.542	0.750	0.500	0.375	0.450	0.417	0.700	0.500	0.917	0.500	0.650	0.200	
	H_e	0.667	0.426	0.696	0.750	0.784	0.757	0.490	0.526	0.163	0.668	0.522	0.884	0.610
	H_o	0.667	0.400	0.750	0.583	0.700	0.917	0.600	0.600	0.167	0.600	0.500	1.000	0.617
SW (1)	A	7	4	6	6	6	8	5	3	3	5	4	9	5.5
Rs=3.753	R	100-114	86-94	187-203	167-179	203-223	173-187	134-154	204-208	125-129	162-170	124-130	219-241	
Fis=0.05667	S	102	92	197	173	203	179	150	204	125	168	128	237	
	F	0.447	0.528	0.441	0.412	0.500	0.368	0.500	0.763	0.842	0.475	0.650	0.333	
	H_e	0.747	0.633	0.754	0.756	0.681	0.788	0.668	0.383	0.286	0.687	0.522	0.824	0.644
	H_o	0.842	0.667	0.765	0.588	0.267	0.842	0.667	0.368	0.316	0.650	0.500	0.833	0.609
SEZ (2)	A	7	3	5	3	10	6	5	2	3	5	4	12	5.5

Rs=3.533	R	100-114	90-94	187-199	171-179	203-231	175-185	134-154	204-206	125-129	154-170	124-136	211-241	
Fis=0.0712	S	114	92	197	173	203	179	150	204	125	166	128	233	
	F	0.214	0.688	0.457	0.786	0.266	0.600	0.656	0.707	0.794	0.424	0.514	0.273	
	H_e	0.841	0.484	0.672	0.352	0.839	0.562	0.542	0.422	0.346	0.674	0.664	0.852	0.604
	H_o	0.686	0.375	0.657	0.315	0.781	0.429	0.500	0.448	0.382	0.667	0.714	0.788	0.562
ATK (2)	A	6	5	5	5	11	5	5	2	3	6	4	10	5.58
Rs=3.654	R	100-116	90-98	187-201	171-179	203-237	175-185	148-156	204-206	125-129	154-172	124-136	219-243	
Fis=0.118*	S	112	94	197	173	203	179	150	204	125	168	128	237	
	F	0.400	0.386	0.808	0.577	0.300	0.519	0.729	0.667	0.712	0.304	0.500	0.260	
	H_e	0.770	0.691	0.343	0.597	0.869	0.637	0.456	0.457	0.459	0.758	0.615	0.862	0.626
	H_o	0.760	0.636	0.346	0.423	0.650	0.615	0.542	0.444	0.423	0.696	0.500	0.609	0.554
CAK (1)	A	7	4	4	4	8	7	5	2	3	6	6	7	5.25
Rs=3.498	R	100-116	90-96	187-199	171-179	203-227	173-185	148-156	204-206	125-129	154-172	124-136	219-241	
Fis=0.128*	S	100	90	197	173	203	179	150	204	125	170	128	231	
	F	0.279	0.462	0.688	0.600	0.385	0.561	0.656	0.750	0.603	0.266	0.470	0.630	
	H_e	0.819	0.667	0.498	0.561	0.786	0.629	0.531	0.381	0.541	0.804	0.658	0.586	0.626
	H_o	0.824	0.539	0.313	0.333	0.577	0.606	0.438	0.433	0.529	0.813	0.636	0.482	0.543
ET (0)	A	5	3	4	3	6	3	4	2	2	4	4	5	3.75
Rs=3.647	R	100-114	90-94	187-199	171-179	211-231	175-183	148-154	204-206	125-127	162-170	124-132	219-241	
Fis=0.0863	S	110	92	197	173	211	179	150	204	125	166	128	241	
	F	0.250	0.600	0.583	0.750	0.400	0.833	0.400	0.600	0.833	0.400	0.500	0.500	
	H_e	0.833	0.622	0.636	0.439	0.844	0.318	0.778	0.533	0.303	0.778	0.712	0.756	0.630
	H_o	0.833	0.800	0.667	0.500	0.800	0.333	0.400	0.400	0.000	0.600	0.833	0.800	0.581
HT (2)	A	5	3	5	4	9	3	6	2	3	5	5	8	4.83
Rs=3.523	R	100-114	90-94	187-201	171-177	203-237	179-183	134-154	204-206	125-129	154-170	124-132	219-241	
Fis=-0.0263	S	102	92	197	173	211	179	150	204	125	166	128	231	
	F	0.310	0.790	0.762	0.700	0.316	0.700	0.575	0.650	0.738	0.395	0.333	0.395	
	H_e	0.771	0.364	0.410	0.468	0.832	0.476	0.636	0.467	0.429	0.762	0.782	0.799	0.600
	H_o	0.571	0.316	0.333	0.600	0.790	0.550	0.650	0.600	0.476	0.895	0.810	0.790	0.615

According to the Rxy values calculated in IDENTIX there was no signal of genetic identity between any two samples analyzed and our test results in GENEPOP indicated absence of any significant departure from linkage equilibrium, when based on the strict Bonferroni criterium. Our analyses in GENECLASS indicated that 73.79 % of all studied overall individual genotypes were assigned to the respective sample region, where they were collected, and the results of a straightforward logistic model of "correctly vs. not correctly assigned individual genotypes" indicated that neither a certain sample region ($p = 0.856$) nor the number of loci ($p = 0.959$) used for an individual composite genotype had an effect on that assignment percentage.

The unstructured AMOVA model indicated that 4.97 % of the relative genetic variability as indicated by the currently used microsatellite markers were due to variation across the eight sample regions ($p < 0.00001$), whereas no (-0.85 %) variation was found between individuals within the sample regions and 95.88 % ($p < 0.00001$) of variability were due to variation within individuals. The hierarchical AMOVA model indicated that no (1.33 %, $p = 0.08700$) genetic variability was due to partitioning into the two "endemic" and "mange-free" regions, whereas 4.16 % ($p < 0.00001$) were due to partitioning into the sample regions, none (-0.84 %, $p = 0.7155$) was due to partitioning into individuals within populations and 95.36 % ($p = 0.01173$) was due to variation within individuals. Concordantly, pairwise *Fst* values between sample regions were low but significant as were pairwise values of Cavalli-Sforza & Edwards distances; for details see table 5.

Table 5: Relative and absolute genetic differentiation between sample regions. Pairwise *Fst* values are given above the diagonal and Cavalli-Sforca & Edwards distances are given below the diagnoal. Values significantly higher than zero (strict Bonferroni criterion, alpha = 0.05) are indicated by asterisks.

	VUI	UA	SW	SEZ	ATK	CAK	ET	HT
VUI		0.0107	0.0203*	0.0442*	0.0465*	0.0553*	0.0432	0.0610*
UA	0.037		0.0464*	0.0695*	0.0592*	0.0823*	0.0709*	0.0900*
SW	0.047*	0.082*		0.0397*	0.0562*	0.0737*	0.0460	0.0722*
SEZ	0.054*	0.081*	0.054*		0.0521*	0.0723*	-0.0043	0.0341*
ATK	0.066*	0.087*	0.072*	0.066*		0.0384*	0.0374	0.0707*
CAK	0.064*	0.083*	0.075*	0.062*	0.050*		0.0504	0.0538*
ET	0.097*	0.115	0.101	0.052	0.096	0.087		0.0064
HT	0.082*	0.107*	0.087*	0.055*	0.093*	0.059*	0.066	

3.6 Structure results

STRUCTURE model runs were performed both with population priors and without, the corresponding STRUCTURE plots generated with CLUMPAK (Kopelman et al. 2015) can be found in Figure 8. Our results indicate that without assuming the eight sampling locations as population priors (referred to as NPP), the most likely level of genetic clustering was returned at K = 3. When including population priors (referred to as PP), the most likely level of genetic clustering remained the same at K = 3, while revealing a clearer image of clusters. Assuming population priors, mange-free populations from VUI and UA were assigned to one cluster, while mange-endemic SEZ, ET and HT were mainly assigned to a second cluster. A third cluster mostly includes chamois from non-endemic ATK and mange-endemic CAK. The remaining sample population of mange-free SW was assigned as being a mixture of the VUI + UA cluster in the non-endemic area and the mange-affected SEZ + ET + HT cluster in the east, representing the geographical situation.

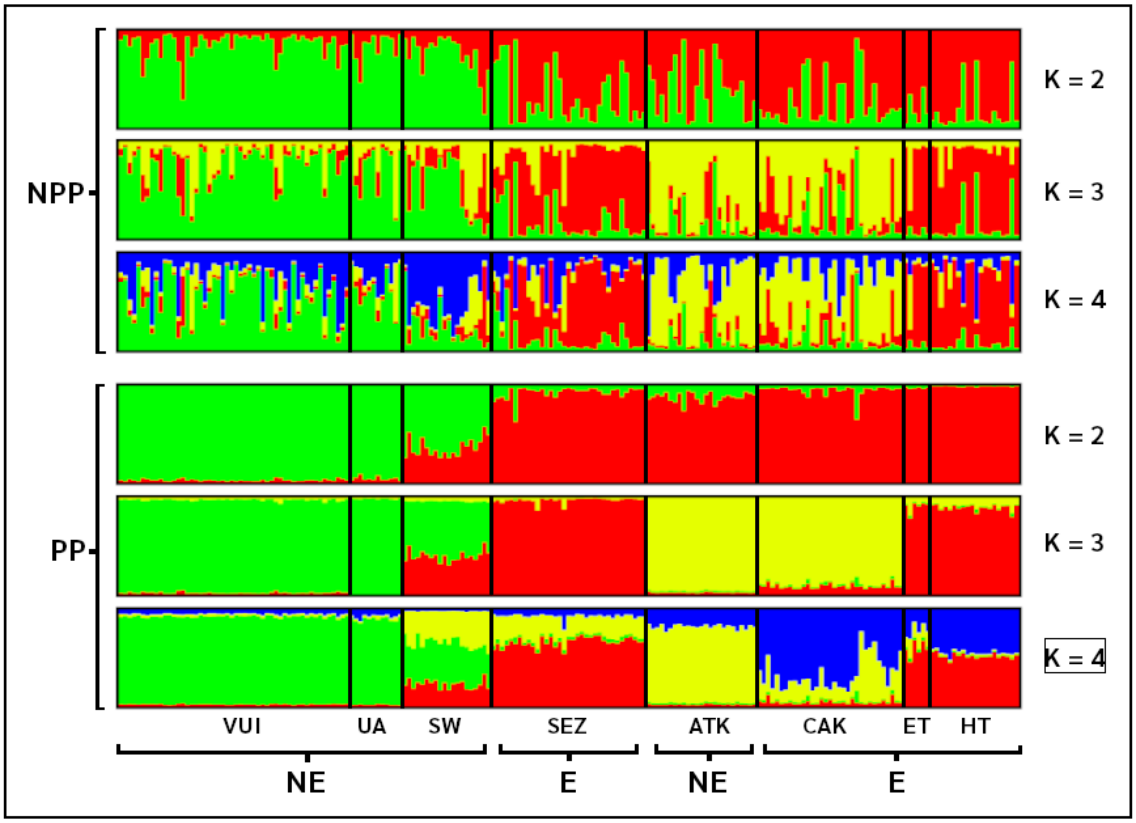

Figure 8: STRUCTURE summary plots generated in CLUMPAK assuming K = 2 - 4 for correlated allele frequencies and admixture models without population priors (NPP – no population priors) and when assuming K = 2 - 4 for corrrelated allele frequencies and admixture models with population priors (PP –population priors); VUI – Vorarlberg and upper Inn Valley, UA – Upper Allgäu, SW – western Stubaital, SEZ – Central Alps east of Stubaital and Zillertal, ATK – Achental and Karwendel, ET – East Tyrol, CAK – Chiemgau Alps and Kaiser Mountains, HT – Hohe Tauern; NE – Non-endemic region, E – Endemic region

Our results for the most likely level of genetic clustering were confirmed for both NPP and PP using Evanno's ad hoc statistic, which can be seen in Figure 9. Looking at distinctive clusters between mange-endemic and non-endemic clusters only one cluster seems to be mainly assigned to the non-endemic region, while the other two clusters appear in both regions.

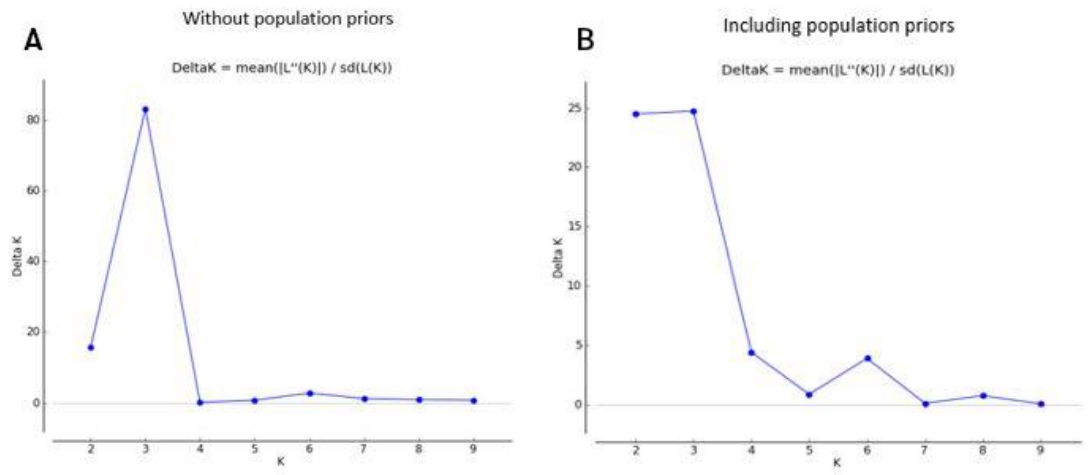

Figure 9: A graphical plot based on delta-K using the method of Evanno et al. (2005), revealing the most likely level of genetic clustering for STRUCTURE models **A)** without assuming population priors and **B)** including population priors.

4 Discussion:

The main aim of the present study was to compare allelic variation at the MHC class 2 DRB exon 2 locus of Alpine chamois, *Rupicapra rupicapra rupicapra*, from local populations (sample regions) within the endemic range of sarcoptic mange (i.e., infection with *Sarcoptes scabiei var. rupicaprae*) and from west and north of it, where mange has never been documented historically in chamois. In the Austrian Alps the historically mange-free region extends westward and northward of a demarcation line formed by the Wipptal, a shallow valley between south of Innsbruck and the Brenner Pass (Brennero) at the Italian border, and the River Inn. Both the Wipptal and the Inn Valley have likely never provided strong barriers to migration of chamois, particularly so in earlier generations when human settlements, infrastructure, and traffic were small and less intensive. So far, the sharp demarcation of sarcoptic mange by those landmarks are not straightforward understandable. Big Alpine valleys, within the mange-endemic region, such as the Ennstal, Murtal, or the Salzachtal, have not prevented mange to spread over generations.

4.1 Variation in the DRB exon 2 locus

Under the assumption of an immediate immunological effect of DRB exon 2 variation on mange infections in chamois, and specifically in view of the sequence variation published by Schaschl et. al. (2012), a marked difference in allele/protein variants or genotypes was hypothesized across the demarcation line between the endemic and mange-free regions. More or less distinct variation was also expected for overall genetic diversity, as reflected by supposedly neutrally evolving microsatellite markers, among sample regions within the endemic region, resulting from (frequent and strong) population fluctuations due to high mange-caused mortality. Severe local population declines, and small effective population sizes increase the chance of reduction of allelic diversity, particularly under little immigration and if lasting over several generations.

Also, particularly under the assumption of a direct immunological effect of the DRB exon 2 locus on mange infections, one or some few protein variants (i.e., the ones best adapted to fight mange infections), should have been enriched in local populations within the endemic region. Thus, higher frequencies of such immunologically favored protein variants were expected in the endemic region, independent from overall genetic background diversity, or perhaps even more in local populations with low general genetic diversity.

Specifically, a clearly higher level of differentiation at the DRB exon 2 locus between the endemic and mange-free regions and particularly across the demarcation line was expected compared to within the endemic and the mange-free regions, respectively, as mange-adapted alleles should have accumulated solely in the endemic region. A high gene flow in the overall nuclear genome, as indicated by the microsatellite markers, across the demarcation line, but distinctly less gene flow at the DRB locus should be indicative of mange-related adaptation and should provide further support to the immediate involvement of the DRB locus in fighting mange infections.

A relatively low level of differentiation at the DRB locus across local populations (i.e., regional samples) within the endemic region, due to the prevalence of some few favored alleles and despite relatively high differentiation at the microsatellite loci, would be also in line with the interpretation of direct immunogenetic effects of one or more DRB protein variants on mange infections.

However, in the present study no general difference in the allelic variation at the DRB exon 2 locus was found between the endemic region of sarcoptic mange and the historically mange-free region, which does not support the hypothesis of a direct involvement of that locus in mange infections of chamois. None of the 26 presently recovered DRB exon 2 alleles did show any significant frequency difference between the endemic and the historically mange-free regions. Also, both haplotype and nucleotide diversity were very similar in both the endemic and the mange-free region, and allelic richness (accounting for different sample sizes) did not differ significantly between the two regions. Moreover, the hierarchical analysis of molecular variance (AMOVA) for that locus indicated no significant allelic variation at this locus due to partitioning into the endemic or mange-free regions. Altogether, protein variation was rather balanced between the endemic and the mange-free region, even though several protein variants/alleles did occur only in one of the two regions, or even as private alleles only in one of the sample regions. But the frequencies of such protein variants were all at low level, i.e., below 2 % in the case of private alleles and below 4 % in the case of protein variants occurring exclusively either in the endemic or the mange-free region.

Correspondingly, the network of proteins and their associated frequencies showed a fairly balanced distribution of protein variants between the two regions, both in terms of their occurrence (frequencies) and their evolutionary position. In particular, all 25 DRB exon 2 protein variants recovered presently showed a rather even evolutionary differentiation, forming at best three clusters with only shallow divergence between their respective neighbor clusters. The evolutionary divergence between those clusters was in all cases lower than within each of the clusters and none of the clusters was composed of predominantly protein variants exclusively occurring in either the endemic or the mange-free region. All protein variants of any evolutionary cluster that were found exclusively either in the endemic or the mange-free region were present at a low frequency. Thus, there was no particular phylogeographic signal present in the protein variants as regards the endemic and the mange-free regions, which also accords to the above interpretation of no immediate adaptive association of the DRB exon 2 sequences to sarcoptic mange infections.

4.2 General Genetic Diversity, Gene Flow and Population structure

Rather, apart from its generally higher level of allelic diversity (i.e., allelic richness), due to its greater number of alleles, the DRB locus shows in principle very much the same pattern of population genetic structuring with generally high gene flow as indicated by pairwise *Fst* values as the microsatellite loci. Notably, high gene flow among all sample regions can be inferred at the DRB locus, independent from whether sarcoptic mange is endemic or historically absent, and that finding is in accordance with data from chamois from the Italian Alps (Mona et al. 2008).The rather similarly low level of relative (*Fst*) genetic differentiation within the endemic and the mange-free regions, respectively, suggest that local population densities are replenished relatively quickly by immigrating chamois after periods of high mortality due to sarcoptic mange. That would also explain the rather even level of genetic diversity across both the endemic and the mange-free regions. Notably, according to the present finding's migration would have also not been restricted much across the demarcation line between the endemic and the mange-free regions in North Tyrol. The Inn Valley might today indeed represent a somewhat stronger barrier to migration of chamois due to its increased built-up areas and the highway and railroad lines, but those latest landscape developments might be too young to be reflected in the population genetic data as indicated by the currently used microsatellite set. Nevertheless, it should be mentioned that an unpublished study has traced two migrants (one male and one female) across the wide Val Valtellina in Northern Italy between the Rhaetian and the Bergamasque Alps, even though the valley is featuring many built up areas and transport infrastructure (Suchentrunk, pers. comm.). Similarly, according to individual microsatellite genotype data, a small chamois colony on a mountain (Kapuzinerberg) entirely surrounded by built-up areas within the city of Salzburg seem to have received gene flow by natural immigration of at least one or two animals through the built-up area (unpubl. data of F. Suchentrunk). However, migration might be predominantly due to males, as a clearly more differentiated phylogeographic signal was reported for chamois from the eastern Alps (Schaschl et al. 2003), which corresponds to higher phylopatry in females. But the spatio-temporal dynamics of regional sarcoptic mange waves might also prevent too high losses of genetic variability. For chamois in the north Italian Dolomite Mountains an average period of six years was observed from the first registered case of infection to the infection peak, presumably with a maximal mortality rate, and the speed of spreading averaged to 5.5 km per year, with a range between 2.2 and 7 km per

year (Rossi et al. 2007). Hence, immigration into post-mange local populations might suffice to maintain genetic diversity, particularly, if a mange wave has not been regionally too widespread and has not generated too high mortality. In any case, the more or less severe population perturbations caused by local or regional and somewhat periodic sarcoptic mange waves in the endemic region seemingly did not have any significant effect on population-specific diversity and differentiation, when compared to the mange-free region in the Eastern Alps. That suggests that chamois are efficiently capable to cope with local or regional losses in their population density, probably by sufficient migration that can compensate over several generation even severe drops in effective population size. Further studies focusing on genetic diversity of local populations with known differences of long-term population densities (perhaps as indicated by hunting records) might give a better understanding of the currently documented and somewhat surprising maintenance of genetic diversity and shallow differentiation of the overall nuclear gene pool across large areas, as currently studied from Vorarlberg to central Salzburg, and including both populations from silicate and limestone geology as well as different climatic regimes.

4.3 Selection Tests

The Lositan outlier test (Antao et al. 2008) did not return a significant signal of positive or balancing selection for the DRB exon 2 sequences. Rather, the DRB locus was - as all microsatellite loci – positioned within the confidence zone of neutrality in the *Fst* – He plot. The assumption of Wright´s island model of population differentiation was rather met as one precondition for that approach, as judged form the similar pairwise *Fst* values between the eight sample regions. Moreover, evidently, according to our AMOVA results, there was no strong hierarchical structuring in relative genetic partitioning, which could have reduced the power of the model. Rather, the number of sample regions (i.e., populations) for the outlier test might have been too small; e.g., Flanagan and Jones (2017) noted that at least ten populations would be necessary for a reliable test performance. Our low number of sample regions that were used for the *Fst*-simulation could have reduced the reliability of the outlier test performance.

Nevertheless, the codon-specific tests using maximum-likelihood analyses on the Datamonkey webserver returned overall seven positions under positive selection. The finding that some of those overall positively selected codons were confirmed under positive selection only in either

the endemic or the mange-free region, might hint towards different selection pressures on the DRB exon 2 sequences in the two regions, possibly related to sarcoptic mange. However, to explore that possibility, clearly more local samples within each of the two regions, respectively, should be analyzed.

Notably, the three most frequent protein variants are phylogenetically not clustering; rather, they occur on somewhat different evolutionary pathways and are characterized by positions under positive selection next to them, respectively, according to the test results. That means that positive selection among protein variants at the DRB locus does not occur in a particular network cluster, i.e., not concentrated during a certain evolutionary phase, but rather during different periods of the evolution of the protein. Such a pattern of evolutionary distribution of positive selection may be expected particularly under varying pathogenic landscapes over longer evolutionary periods. It would also correspond to a mode of trans-specific evolution, which is common in MHC loci (e.g., for the two chamois species *Rupicapra rupicapra* and *R. pyrenaica* see Schaschl et al. 2005).

The presently obtained statistical test results addressing the hypothesis that DRB exon 2 heterozygosity varies specifically in older males, usually participating in the energy consuming rutting season, did not indicate such an expected effect. Specifically, it was expected that heterozygosity increased in those males during and after the rutting season, in line with Schaschl et al. (2012), who observed longer survival of older males carrying heterozygous DRB exon 2 genotypes. However, currently no significant effect was found neither generally in older males nor when tested specifically for old males (in the terminology of Schaschl et al. 2012) in the mange endemic region. A significant increase of heterozygosity in older males specifically in the mange endemic region would have been in line with the expectation of an overdominance effect at the DRB exon 2 locus, i.e., heterozygosity advantage, in association with sarcoptic mange. However, it should be mentioned that the currently used sample distribution for the statistical models was somewhat biased and unrepresentative for a final conclusion, as relatively few samples of old males were available from the rutting season and basically none from after the subsequent winters that would represent most likely the strongest seasonal selection period for rutting males, when they massively reduce their body weights.

Moreover, individual body weights were not affected by chamois carrying any of the tested DRB exon 2 protein variants, neither in males old enough to repeatedly participate in rutting competitions (i.e., six years of age or older), nor in younger males or females for the mange endemic and the mange-free regions. Specifically, the absence of a statistically significant signal for any of the tested protein variants (particularly DRB exon 2 proteins 20 and 21) in old males/"rutting males" from the mange endemic region were in contradiction to the finding of an overdominance effect of DRB heterozygosity on survival in older males within the endemic region by Schaschl et al. (2012).

In conclusion, all presently obtained population genetic, ecological-genetic and evolutionary results on the DRB exon 2 protein variants of chamois from both the sarcoptic mange endemic region in the Eastern Alps and the historical mange-free region west of it indicate no direct involvement of that locus in fighting *Sarcoptes*-infections in chamois. They are not in line with the finding of a DRB-overdominance effect particularly in old males by Schaschl et al. (2012). This does not support the hypothesis of increased survival of DRB-heterozygous males, mainly due to better immunological reactions to infections with sarcoptic mange. A better survival of old males with heterozygous DRB genotypes, as observed by Schaschl et al. (2012), might be related rather to better fighting other infections particularly during the rutting season and in the subsequent winter period, when energetic budget becomes critical. Selection at the DRB locus may be more related to other (infectious) diseases, mainly of bacterial and parasitic origin, like keratoconjunctivitis, contagious ecthyma, and various endoparasites, and various genotypes at that locus might increase individual life spans or reproductive performance.

5 Summary

Sarcoptic mange is a parasitic disease that has reportedly resulted in steep population declines of chamois population in the eastern alps. However, the spatial distribution of sarcoptic mange seems to have come to a halt by two specific landmarks, namely the River Inn and the Wipptal where sarcoptic mange has not been reported north or west of, respectively. In the present study we tested whether the respective chamois populations of sarcoptic-mange-endemic and the sarcoptic-mange-free areas in the eastern alps show any significant differences regarding their allelic variation at their DRB exon 2 sequences, a MHC class II gene, considered important in the context of immune responses against parasitic infections. We also tested whether a seasonal change of heterozygosity at the aforementioned gene that would fit the hypothesis of an overdominance effect previously reported (Schaschl et al. 2012) can be detected. To this end, we studied 208 tissue samples of chamois which were collected in mange-endemic and mange-free areas during regular hunts and provided with their corresponding metadata (sampling location and date, sex, age, eviscerated body weight). In parallel, genetic variability was studied at 12 microsatellite markers, as indicators of neutral variation in chromosomal DNA. The hypothesis of different allelic DRB exon 2 variability of the regional samples between the mange-free and mange-endemic regions could not be confirmed, as all tested indicators were not statistically significantly different in both regions. The same results were obtained for the neutral microsatellite markers. The statistical models did not reveal significant seasonal variation in DRB exon 2 heterozygosity, and a significant DRB exon 2 heterozygosity effect on chamois body weight, especially that of rutting bucks, could not be detected either. However, no samples were available after the winter season; thus, a possible heterozygosity effect during the demanding winter season on body weight could not be tested. Overall, all currently obtained results could not confirm the previously formulated hypotheses of heterozygosity at the DRB exon 2 locus in relation to mange infections of chamois. Furthermore, the overall genetic variability in regional populations was not significantly different between the endemic region and the mange-free region of the Eastern Alps in Austria.

6 Zusammenfassung

Die *Sarcoptes*-Räude ist eine parasitäre Krankheit, die mehrmals zu starken Rückgängen der Gamswildbestände in den Ostalpen geführt hat. Die räumliche Verbreitung der *Sarcoptes*-Räude scheint jedoch entlang zweier geographischer Demarkationslinien zum Stillstand gekommen zu sein, einerseits entlang des Inns und andererseits entlang des Wipptals, wo die *Sarcoptes*-Räude respektive nördlich und westlich bislang nicht beobachtet wurde. In der vorliegenden Studie wurde untersucht, ob die jeweiligen Gamswildpopulationen in Räude endemischen und Räude freien Gebieten signifikante Unterschiede in der allelischen Variation ihrer DRB Exon 2-Sequenzen aufweisen, einem MHC-Klasse-II Gen, das eine wichtige Rolle im Zusammenhang mit Immunantworten gegen parasitäre Infektionen spielt. Neben dem statistischen Vergleich der Variabilität, wurde auch auf eine mögliche saisonale Veränderung der Heterozygotie am DRB Exon 2-Locus getestet, entsprechend eines früher publizierten Überdominanzeffekts (Schaschl et al. 2012). Es wurden 208 Gewebeproben von Gämsen untersucht, die in Räude endemischen und Räude freien Gebieten im regulären Jagdbetrieb gesammelt wurden, und von denen Abschuss-, bzw. Fundort und -datum, Geschlecht, Alter und ausgeweidetes Körpergewicht bekannt waren. Parallel dazu wurde die genetische Variabilität an 12 Mikrosatellitenmarkern, als Indikatoren neutraler Variation der chromosomalen DNA, dieser Gämsen untersucht. Die Hypothese unterschiedlicher allelischer DRB Exon 2-Variabilität der regionalen Stichproben zwischen der Räude freien und der Räude endemischen Region konnte nicht bestätigt werden, alle getesteten Indikatoren waren statistisch nicht signifikant verschieden in beiden Regionen. Dieselben Ergebnisse wurden für die neutralen Mikrosatellitenmarker erzielt. Die statistischen Modelle ergaben keine signifikante saisonale Variation der DRB Exon 2-Heterozygotie, und ein signifikanter DRB-Exon 2-Heterozygotieeffekt auf das Körpergewicht der Gämsen, insbesondere das der Brunftböcke, konnte ebenfalls nicht festgestellt werden. Allerdings standen keine Proben nach der Wintersaison zur Verfügung; somit konnte ein möglicher Heterozygotieeffekt während der zehrenden Wintersaison auf das Körpergewicht nicht getestet werden. Insgesamt konnten alle gegenwärtig erzielten Ergebnisse die früher formulierten Hypothesen der Heterozygotie am DRB Exon 2-Locus in Bezug auf Räudeinfektionen der Gämsen nicht bestätigen. Darüber hinaus war die allgemeine genetische Variabilität in regionalen Populationen zwischen der Endemieregion und der Räude freien Region der österreichischen Ostalpen nicht signifikant verschieden.

7 References

Andrews RM, McCarthy J, Carapetis JR, Currie BJ. 2009. Skin disorders, including pyoderma, scabies, and tinea infections. Pediatric Clinics of North America, 56 (6): 1421–1440.

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G. 2008. LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. BMC bioinformatics, 9: 323.

Arlian LG, Bruner RH, Stuhlman RA, Ahmed M, Vyszenski-Moher DL. 1990. Histopathology in Hosts Parasitized by Sarcoptes scabiei. The Journal of Parasitology, 76 (6): 889.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular biology and evolution, 16 (1): 37–48.

Beaumont MA, Nichols RA. 1996. Evaluating loci for use in the genetic analysis of population structure. Proceedings of the Royal Society of London. Series B: Biological Sciences, 263 (1377): 1619–1626.

Belkhir K. 2004. Genetix 4.05, logiciel sous Windows TM pour la genetique des populations. Laboratoire Genome, Populations, Interactions, CNRS UMR 5000, Universite de Montpellier II.

Belkhir K, Castric V, Bonhomme F. 2002. identix, a software to test for relatedness in a population using permutation methods. Molecular Ecology Notes, 2 (4): 611–614.

Bover P, Llamas B, Mitchell KJ, Thomson VA, Alcover JA, Lalueza-Fox C, Cooper A, Pons J. 2019. Unraveling the phylogenetic relationships of the extinct bovid Myotragus balearicus Bate 1909 from the Balearic Islands. Quaternary Science Reviews, 215: 185–195.

Burnham KP, Anderson DR. 2013. Model Selection and multi-model inference. A practical information-theoretic approach. Second ed., corr. third printing. New York: Springer New York, 488.

Corlatti L, Bassano B, Poláková R, Fattorini L, Pagliarella MC, Lovari S. 2015. Preliminary analysis of reproductive success in a large mammal with alternative mating tactics, the Northern chamois, Rupicapra rupicapra. Biological Journal of the Linnean Society, 116 (1): 117–123.

Corlatti L, Iacolina L, Safner T, Apollonio M, Buzan E, Ferretti F, Hammer SE, Herrero J, Rossi L, Serrano E, Arnal MC, Brivio F, Chirichella R, Cotza A, Crestanello B, Espunyes J,

Fernández de Luco D, Friedrich S, Gačić D, Grassi L, Grignolio S, Hauffe HC, Kavčić K, Kinser A, Lioce F, Malagnino A, Miller C, Peters W, Pokorny B, Reiner R, Rezić A, Stipoljev S, Tešija T, Yankov Y, Zwijacz-Kozica T, Šprem N. 2022. Past, present and future of chamois science. Wildlife Biology, 2022 (4).

Corlatti L, Lorenzini R, Lovari S. 2011. The conservation of the chamois Rupicapra spp. Mammal Review, 41 (2): 163–174.

Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics, 153 (4): 1989–2000.

Crestanello B, Pecchioli E, Vernesi C, Mona S, Martínková N, Janiga M, Hauffe HC, Bertorelle G. 2009. The genetic impact of translocations and habitat fragmentation in chamois (Rupicapra) spp. The Journal of heredity, 100 (6): 691–708.

Delport W, Poon AFY, Frost SDW, Kosakovsky Pond SL. 2010. Datamonkey 2010: a suite of phylogenetic analysis tools for evolutionary biology. Bioinformatics (Oxford, England), 26 (19): 2455–2457.

Deutz A. 2002. Aktuelle Fragestellungen zu Gams- und Steinwildkrankheiten. Matrei.

DeWoody JA, Schupp J, Kenefic L, Busch J, Murfitt L, Keim P. 2004. Universal method for producing ROX-labeled size standards suitable for automated genotyping. BioTechniques, 37 (3): 348, 350, 352.

Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4 (2): 359–361.

El Mousadik A, Petit RJ. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree Argania spinosa (L.) Skeels endemic to Morocco. TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik, 92 (7): 832–839.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular ecology, 14 (8): 2611–2620.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular ecology resources, 10 (3): 564–567.

Fain A. 1968. Etude de la variabilité de Sarcoptes scabiei avec une révision des Sarcoptidae. Acta Zoologica et Pathologica Antverpiensia, (Vol. 47): 1–196.

Flanagan SP, Jones AG. 2017. Constraints on the FST-Heterozygosity Outlier Approach. The Journal of heredity, 108 (5): 561–573.

Fuchs K, Deutz A, Gressmann G. 2000. Detection of space–time clusters and epidemiological examinations of scabies in chamois. Veterinary parasitology, 92 (1): 63–73.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3).

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series, (42).

Hedrick PW, Kim TJ, Parker KM. 2001. Parasite resistance and genetic variation in the endangered Gila topminnow. Animal Conservation, 4 (2): 103–109.

Hedrick PW, Parker KM, Miller EL, Miller PS. 1999. Major histocompatibility complex variation in the endangered Przewalski's horse. Genetics, 152 (4): 1701–1710.

Henriksen P, Dietz H.H., Henriksen S.A., Gjelstrup P. 1993. Sarcoptic mange in red fox in Denmark. A short report. Dansk Vettidsskr, (76): 12–13.

Ippen R, Nickel S, Schröder H-D. 1995. Krankheiten des jagdbaren Wildes. Third., revised and extended edition. Berlin: Deutscher Landwirtschaftsverl., 233.

2021. IUCN Red List of Threatened Species.

Kalema G., Koch RA, Macfie E. 1998. An outbreak of sarcoptic mange in free-ranging mountain gorillas (Gorilla gorilla berengei) in Bwindi Impenetrable National Park, South Western Uganda. In Proc. American Association of Zoo Veterinarians and American Association of Wildlife Veterinarians Joint Conference, Omaha: 438.

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Molecular ecology resources, 15 (5): 1179–1191.

Kosakovsky Pond SL, Frost SDW. 2005. Not so different after all: a comparison of methods for detecting amino acid sites under selection. Molecular biology and evolution, 22 (5): 1208–1222.

Kosakovsky Pond SL, Posada D, Gravenor MB, Woelk CH, Frost SDW. 2006. Automated phylogenetic detection of recombination using a genetic algorithm. Molecular biology and evolution, 23 (10): 1891–1901.

Lam HM, Ratmann O, Boni MF. 2018. Improved Algorithmic Complexity for the 3SEQ Recombination Detection Algorithm. Molecular biology and evolution, 35 (1): 247–251.

Lavin S, Ruiz-Bascaran M, Marco I, Fondevila MD, Ramis AJ. 2000. Experimental infection of chamois (Rupicapra pyrenaica parva) with Sarcoptes scabiei derived from naturally infected goats. Journal of veterinary medicine. B, Infectious diseases and veterinary public health, 47 (9): 693–699.

Li N, Stephens M. 2003. Modeling linkage disequilibrium and identifying recombination hotspots using single-nucleotide polymorphism data. Genetics, (165(4)): 2213–2233.

Lindström ER, Andrén H, Angelstam P, Cederlund G, Hörnfeldt B, Jäderberg L, Lemnell P-A, Martinsson B, Sköld K, Swenson JE. 1994. Disease Reveals the Predator: Sarcoptic Mange, Red Fox Predation, and Prey Populations. Ecology, 75 (4): 1042–1049.

Loison A, Jullien J-M, Menaut P. 1999. Subpopulation Structure and Dispersal in Two Populations of Chamois. Journal of Mammalogy, 80 (2): 620–632.

Lorenzini R. 2005. A panel of polymorphic microsatellites in the threatened Apennine chamois (Rupicapra pyrenaica ornata). Molecular Ecology Notes, 5 (2): 372–374.

Lovari S, Locati M. 1991. Temporal Relationships, Transitions and Structure of the Behavioural Repertoire in Male Apennine Chamois During the Rut. Behaviour, 119 (1-2): 77–103. DOI 10.1163/156853991X00382.

Lovari S, Sacconi F, Trivellini G. 2006. Do alternative strategies of space use occur in male Alpine chamois? Ethology Ecology & Evolution, 18 (3): 221–231.

Luckheeram RV, Zhou R, Verma AD, Xia B. 2012. $CD4^+$T cells: differentiation and functions. Clinical & developmental immunology, 2012: 925135.

Marsh SGE, Parham P, Barber LD. 2000. The HLA factsbook. San Diego: Academic Press, 398.

Martin D, Rybicki E. 2000. RDP: detection of recombination amongst aligned sequences. Bioinformatics (Oxford, England), 16 (6): 562–563.

Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuvre P. 2010. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics (Oxford, England), 26 (19): 2462–2463.

Masini F, Lovari S. 1988. Systematics, Phylogenetic Relationships, and Dispersal of the Chamois (Rupicapra spp.). Quaternary Research, 30 (3): 339–349.

Mika A, Reynolds SL, Pickering D, McMillan D, Sriprakash KS, Kemp DJ, Fischer K. 2012. Complement inhibitors from scabies mites promote streptococcal growth--a novel mechanism in infected epidermis? PLoS neglected tropical diseases, 6 (7): e1563.

Miller C. 1986. Die Gamsräude in den Alpen. Z. Jagdwiss., 32: 42–46.

Miller C, Corlatti L. 2014. Das Gamsbuch. Für Einsteiger und Profis. Second., revised edition. Melsungen: Neumann-Neudamm, 223.

Mona S, Crestanello B, Bankhead-Dronnet S, Pecchioli E, Ingrosso S, D'Amelio S, Rossi L, Meneguz PG, Bertorelle G. 2008. Disentangling the effects of recombination, selection, and demography on the genetic variation at a major histocompatibility complex class II gene in the alpine chamois. Molecular ecology, 17 (18): 4053–4067.

Murrell B, Moola S, Mabona A, Weighill T, Sheward D, Kosakovsky Pond SL, Scheffler K. 2013. FUBAR: a fast, unconstrained bayesian approximation for inferring selection. Molecular biology and evolution, 30 (5): 1196–1205.

Murrell B, Wertheim JO, Moola S, Weighill T, Scheffler K, Kosakovsky Pond SL. 2012. Detecting individual sites subject to episodic diversifying selection. PLoS genetics, 8 (7): e1002764.

Nesti I, Posillico M, Lovari S. 2010. Ranging behaviour and habitat selection of Alpine chamois. Ethology Ecology & Evolution, 22 (3): 215–231.

Nowak RM. 1999. Walker's mammals of the world. Sixth. ed. Baltimore: Johns Hopkins University Press: 837-1936.

Padidam M, Sawyer S, Fauquet CM. 1999. Possible emergence of new geminiviruses by frequent recombination. Virology, 265 (2): 218–225.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics (Oxford, England), 28 (19): 2537–2539.

Pence DB, Custer JW, Carley CJ. 1981. Ectoparasites of wild canids from the Gulf Coastal Prairies of Texas and Louisiana. Journal of medical entomology, 18 (5): 409–412.

Pérez T, Albornoz J, Domınguez A. 2000. A panel of bovine and caprine microsatellites suitable as markers in chamois. Animal Genetics, 31 (5): 345.

Pérez T, González I, Essler SE, Fernández M, Domínguez A. 2014. The shared mitochondrial genome of Rupicapra pyrenaica ornata and Rupicapra rupicapra cartusiana: old remains of a common past. Molecular phylogenetics and evolution, 79: 375–379.

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. The Journal of heredity, 95 (6): 536–539.

Pond SLK, Frost SDW. 2005. Datamonkey: rapid detection of selective pressure on individual sites of codon alignments. Bioinformatics (Oxford, England), 21 (10): 2531–2533.

Posada D, Crandall KA. 2001. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. Proceedings of the National Academy of Sciences of the United States of America, 98 (24): 13757–13762.

Poubelle F, Perrier J, Bantin L, Martinet O. 1989. Etude demographique de deux populations de chamois (Rupicapra rupicapra) dans le massif de la Vanoise (Savoie-France). Comparaison entre une population situee en zone de chassee et une population situee en zone protegee. Gamswild-symp. - Sym. Chamois, Ljubljana, (25/26.X.1988, Paris-Munich): 395–424.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155 (2): 945–959.

QGIS.org. 2020. QGIS Geographic Information System. Open Source Geospatial Foundation Project.

Queller DC, Goodnight KF. 1989. Estimating Relatedness Using Genetic Markers. Evolution, 43 (2): 258.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rannala B, Mountain JL. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the United States of America, 94 (17): 9197–9201.

Raymond M, Rousset F. 1995. An Exact Test for Population Differentiation. Evolution, 49 (6): 1280.

Ridley M. 2004. Evolution. Second ed. Oxford: Oxford University Press.

Rodríguez F, Hammer S, Pérez T, Suchentrunk F, Michallet J, Martinkova N, Albornoz J, Domínguez A. 2009. Cytochrome b phylogeography of chamois (Rupicapra spp.). Population contractions, expansions and hybridizations governed the diversification of the genus. The Journal of heredity, 100 (1): 47–55.

Rossi L, Fraquelli C, Vesco U, Permunian R, Sommavilla GM, Carmignola G, Da Pozzo R, Meneguz PG. 2007. Descriptive epidemiology of a scabies epidemic in chamois in the Dolomite Alps, Italy. European Journal of Wildlife Research, 53 (2): 131–141.

Rossi L, Meneguz PG, Martin P de, Rodolfi M. 1995. The epizootiology of sarcoptic mange in chamois, Rupicapra rupicapra, from the Italian eastern Alps. Parassitologia, 37 (2-3): 233–240.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular biology and evolution, 34 (12): 3299–3302.

Ryser-Degiorgis M-P, Ryser A, Bacciarini LN, Angst C, Gottstein B, Janovsky M, Breitenmoser U. 2002. Notoedric and sarcoptic mange in free-ranging lynx from Switzerland. Journal of wildlife diseases, 38 (1): 228–232.

Sachs R, Sachs C. 1968. A survey of parasitic infestationof wild herbivores in the Serengeti region in northern Tanzania and the Lake Rukwa region in southern Tanzania. Bull. epiz. Dis. Afr., (16).

Sayers EW, Cavanaugh M, Clark K, Ostell J, Pruitt KD, Karsch-Mizrachi I. 2019. GenBank. Nucleic acids research, 47 (D1): D94-D99.

Schaschl H, Goodman SJ, Suchentrunk F. 2004. Sequence analysis of the MHC class II DRB alleles in Alpine chamois (Rupicapra r. rupicapra). Developmental and comparative immunology, 28 (3): 265–277.

Schaschl H, Kaulfus D, Hammer S, Suchentrunk F. 2003. Spatial patterns of mitochondrial and nuclear gene pools in chamois (Rupicapra r. rupicapra) from the Eastern Alps. Heredity, 91 (2): 125–135.

Schaschl H, Suchentrunk F, Morris DL, Ben Slimen H, Smith S, Arnold W. 2012. Sex-specific selection for MHC variability in Alpine chamois. BMC evolutionary biology, 12: 20.

Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, Leipe D, Mcveigh R, O'Neill K, Robbertse B, Sharma S, Soussov V, Sullivan JP, Sun L, Turner S, Karsch-Mizrachi I. 2020. NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database : the journal of biological databases and curation, 2020.

Shelley WB, Dorinda Shelley E, Burmeister V. 1988. Staphylococcus aureus colonization of burrows in erythroderma Norwegian scabies. Journal of the American Academy of Dermatology, 19 (4): 673–678.

Simpson K, Johnson CN, Carver S. 2016. Sarcoptes scabiei: The Mange Mite with Mighty Effects on the Common Wombat (Vombatus ursinus). PloS one, 11 (3): e0149749.

Smith JM. 1992. Analyzing the mosaic structure of genes. Journal of molecular evolution, 34 (2): 126–129.

Soglia D, Rossi L, Cauvin E, Citterio C, Ferroglio E, Maione S, Meneguz PG, Spalenza V, Rasero R, Sacchi P. 2010. Population genetic structure of Alpine chamois (Rupicapra r. rupicapra) in the Italian Alps. European Journal of Wildlife Research, 56 (6): 845–854.

Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. American journal of human genetics, 68 (4): 978–989.

Swe PM, Fischer K. 2014. A scabies mite serpin interferes with complement-mediated neutrophil functions and promotes staphylococcal growth. PLoS neglected tropical diseases, 8 (6): e2928.

Tompkins DM, Carver S, Jones ME, Krkošek M, Skerratt LF. 2015. Emerging infectious diseases of wildlife: a critical perspective. Trends in parasitology, 31 (4): 149–159.

Turchetto S, Obber F, Rossi L, D'Amelio S, Cavallero S, Poli A, Parisi F, Lanfranchi P, Ferrari N, Dellamaria D, Citterio CV. 2020. Sarcoptic Mange in Wild Caprinae of the Alps: Could Pathology Help in Filling the Gaps in Knowledge? Frontiers in veterinary science, 7: 193.

Unterthiner S, Ferretti F, Rossi L, Lovari S. 2012. Sexual and seasonal differences of space use in Alpine chamois. Ethology Ecology & Evolution, 24 (3): 257–274.

van Valen L. 1973. A new evolutionary law. Evol. Theory 1: 1–30.

Walton SF. 2010. The immunology of susceptibility and resistance to scabies. Parasite immunology, 32 (8): 532–540.

Walton SF, Holt DC, Currie BJ, Kemp DJ. 2004. Scabies: New Future for a Neglected Disease. Advances in parasitology, 57: 309–376.

Weaver S, Shank SD, Spielman SJ, Li M, Muse SV, Kosakovsky Pond SL. 2018. Datamonkey 2.0: A Modern Web Application for Characterizing Selective and Other Evolutionary Processes. Molecular biology and evolution, 35 (3): 773–777.

Weir BS, Cockerham CC. 1984. Estimating F-Statistics for the Analysis of Population Structure. Evolution, 38 (6): 1358.

Willisch CS, Ingold P. 2007. Feeding or Resting? The Strategy of Rutting Male Alpine Chamois. Ethology, 113 (1): 97–104.

Wilson DJ, McVean G. 2006. Estimating diversifying selection and functional constraint in the presence of recombination. Genetics, 172 (3): 1411–1425.

8 Table of illustrations

9 Table directory