CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Expansion of wolves' populations in the Western Palearctic and interactions with dogs

BACHELOR'S THESIS

Prague 2023

Author: Viktoriia Yakushevska

Supervisor: Mgr. Barbora Černá Bolfíková, Ph.D.

Declaration

I hereby declare that I have done this thesis entitled Expansion of wolves' populations in the Western Palearctic and interactions with dogs independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 14.4.2023

....

Viktoriia Yakushevska

Acknowledgements

At this point, I would like to express my greatest thanks to my thesis supervisor Mgr. Barbora Černá Bolfíková Ph.D. for the expert guidance, comprehensive help, a lot of valuable and inspiring advice, recommendations and comments, as well as for the great patience and support during the consultations provided for the preparation of this bachelor thesis.

Abstract

The grey wolf (*Canis lupus*) had high population numbers in history of Europe. However, between the 18th and 19th centuries its numbers began to decline greatly. In particular, there were rapid environmental changes, but also the impact of humans. Due to the fragmentation of the landscape, hunting, poaching, competition for resources, hybridization and insufficient protection, the wolves disappeared, firstly from the European islands, subsequently from Germany and Poland, then from most of the western European countries.

In recent years, the grey wolf (*Canis lupus*) started to recover its numbers in the Western Palearctic, by immigration from eastern or southern populations. Grey wolf started to establish its populations even in countries, where it was extinct. But this recovery wasn't without challenges, because so many factors affected this species. One of the most important factors which can impact the species, is hybridization with domestic dogs.

My literature research is focused on the complex history of the grey wolf, from their extinction in many parts of the Western Palearctic, to their subsequent resurgence. Additionally, my research aims to describe hybridization with domestic dogs and the impact it has had on the genetic structure of wolf populations in certain areas. In the practical part, I had training in a molecular genetics' laboratory, where a sample set analysis was provided to determine the potential presence of hybrids.

Key words: Dog, expansion, genetics, genome changes, grey wolf, hybridization, interaction.

Contents

List of figures

1. Introduction

Wolves have been a part of the Western Palearctic ecosystem for thousands of years, but their populations have undergone significant fluctuations over time. In recent years, there has been an increase of wolf populations, especially in Western Europe.

Overview of wolf populations in the Western Palearctic:

Wolves were once widespread throughout Europe, but their populations were decimated over the centuries by hunting and habitat loss. In the mid-20th century, there were only a few hundred wolves left in Europe, mostly in the eastern parts of the continent. However, during the last decades, wolf populations have started to recover, and their range has expanded westward. In the Western Palearctic region, there are now an estimated 12.000-18.000 wolves, with the majority living in eastern Europe and the Balkans.

Factors driving the expansion of wolf populations:

The recovery of wolf populations in the Western Palearctic is possible because of a combination of factors. One key factor is the protection afforded to wolves by European Union laws and international treaties, which have helped to reduce hunting pressure on the species. Additionally, changes in land use patterns, such as the abandonment of rural areas and the reforestation of abandoned lands, have created new habitats for wolves to colonise. Finally, the recovery of prey species, such as wild boar and deer, has provided wolves with a more abundant food supply.

Interactions between wolves and dogs:

As wolf populations have expanded, there have been increasing reports of interactions between wolves and domestic dogs. These interactions can take several forms, including predation on dogs by wolves, hybridization between wolves and dogs, and social interactions between the two species. The exact nature of these interactions and their impact on both wolf and dog populations is not yet fully understood and is the subject of ongoing research.

2. Aims of the Thesis

- Describe the wolves' population expansion in the selected area.
- Evaluate when was the turning point in the development of wolves in the Western Palearctic, from which countries the wolves began to immigrate to Europe.
- Focus on the occurrence and frequency of the interactions between wolves and domestic dogs in Europe.
- Describe what effects hybridization has on the genetic and phenotypic structure of the wild wolves' populations.

3. Literature Review

3.1. Description of the grey wolf habitat

The grey wolf (*Canis lupus*) is a large carnivore found in many habitats of the Western Palearctic. Its wide distribution and habitat preferences are the result of its ability to adapt to different ecological conditions, which has allowed its population to expand in some areas.

The grey wolf is a habitat generalist, meaning it can live in a wide variety of habitats, including grasslands, forests, mountains, wetlands, and tundra. According to Boitani (2003), wolves are typically found in areas with large land areas, abundant prey, and few people. They prefer habitats with a combination of grasslands, forests, and wetlands because they provide a variety of resources.

Studies have shown that wolves select habitats based on a variety of factors, including prey availability, the presence of burrows, and large expanses (Jedrzejewski et al. 2005; Zlatanova & Popova 2013). Wolves also tend to select habitats with open areas for hunting and dense cover for denning and resting (Fechter & Storch 2014). The presence of cover is particularly important for denning areas because it protects from predators and humans (Kaartinen et al. 2015).

In addition to habitat selection, the presence of food resources is also an important factor in wolf habitat selection. Wolves feed primarily on large ungulates, such as deer (*Cervus*) and elk (*Cervus canadensis*), but will also take smaller prey such as rabbits (*Oryctolagus cuniculus*) and hares (*Leporidae*) (Boitani 2003). They prefer a habitat with abundant prey because it provides a reliable food source.

Finally, wolves tend to avoid areas with high human activity, such as urban areas and agricultural land (Gwynn & Symeonakis 2022). Wolf species are sensitive to disturbance and human presence and often avoid areas where humans are present.

3.2. Phylogeographic distribution of wolves in Europe

The Pleistocene megafaunal extinction was an important era in the history of life on Earth. It was marked by a period with a notable impact on ecosystems and biodiversity worldwide, with rapid environmental changes and significant extinction of large animal species. During this period, a lot of large carnivores, which played important roles in their ecosystems, vanished. After that time, a remaining variety of species developed unique adaptations to survive in harsh environments. These species include the red fox (*Vulpes vulpes*), brown bear (*Ursus arctos*), white bear (*Ursus maritimus*), wolverine (*Gulo gulo*), caribou (*Rangifer tarandus)* and also grey wolf (*Canis lupus*). Holarctic animals that survived the Late Pleistocene megafauna extinction responded to the consequential environmental changes of this era by losing distinct lineages, undergoing phylogeographic changes and domestication (Pilot et al. 2019). It caused a geographic and genetic separation of populations, which made their phylogeographic history very complex (Ginsberg & Macdonald 1990; Wayne et al. 1992). In the case of the grey wolf, the species' survival followed a worldwide sharp decline in population sizes at the end of the Pleistocene (Pilot et al. 2014; Fan et al. 2016). Over time, one lineage of wolves has been successfully domesticated through interaction with humans (Wayne et al. 1992). But this successful adaptation has had negative consequences for the wild population. The major repercussion is hybridization, but also competition and disease transmission (Leonard et al. 2014; Lescureux & Linnell 2014).

During the Middle Ages (Delibes 1990), the wolf population in Europe had already experienced a decline due to various factors, including human impact (Lorenzen et al. 2011). The genetic composition of wolves has been greatly impacted by demographic shifts (Lehman & Wayne 1991; Wayne & Jenks 1991), which can be attributed to the extinction of the species in various European countries such as France, Denmark, Germany, England, Czech Republic, Portugal, Italy (Breitenmoser 1998). Only a small area in the Apennines remained inhabited by the Italian population, with a few individuals, and also the Iberian population occupied a fragmented area (Zimen 1978; Boitani & Mech 2003).

According to the survey Pilot et al. (2010), European wolves have two main haplogroups, called haplogroup 1 and haplogroup 2. In Europe, haplogroup 1 dominates nowadays, especially in Eastern Europe. Currently, the European population has 29 different haplotypes, in Eastern Europe, it is 26 haplotypes. It's interesting that only southern populations have some unique haplotypes. In the Apennine area, there exists one, in the Iberian population, there exist two, in the Balkans are seven unique haplotypes and one of these is shared with the Iberian. All Apennine haplotypes belong to haplogroup 2 and all Iberian belong to haplogroup 1. It was determined that the percentages of variation between haplogroups 1 and 2 are between 76% to 24%. In contrast, during ancient times in Europe, haplogroup 2 was the dominant group, with only one instance of a haplotype belonging to haplogroup 1 in western Russia. The distribution of the haplotypes can be found in Figure 1.

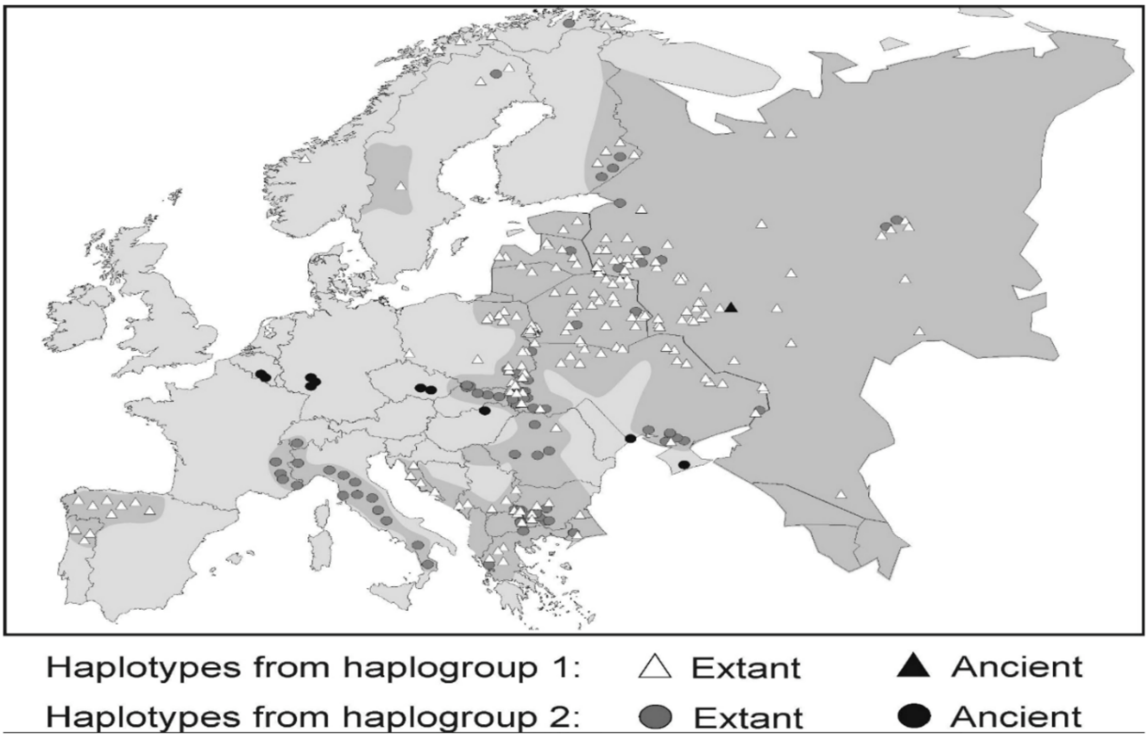

Figure 1: Distribution of haplogroups 1 and 2 in contemporary and ancient European wolves (Pilot et al. 2010).

3.2.1. Period of reduced numbers

The grey wolf (*Canis lupus*) has always been the most globally spread among the four main large carnivores in the Western Palearctic and historically has been present in almost every country in Europe. It is a very mobile species, which can adapt almost to all conditions, it explains their spread into the tundra, steppes, and even deserts. Grey wolves also expanded to some European islands, such as Sardinia and Ireland. But due to various factors, their numbers began to decline gradually between the 18th and 19th centuries. At first extinct from the islands, then in France, Germany, and Poland. After this time, isolated populations remained in some European countries. But they were very sensitive to all the conditions and also began to collapse. In most Western European countries, wolves were completely extinct, except the Italian and Iberian populations, which remained as small fragmented populations. They mainly stayed in the central Apennine Mountains of Italy and the Cantabrian Mountains of northern Spain (Breitenmoser 1998).

The lowest wolf numbers in Europe were registered during the 1930s and 1960s of the 20th century (Pimlott 1975; Delibes 1990). The gradual reduction in wolf numbers can perhaps be seen with the growth and expansion of human settlements. The extinction of the species was a response to human influence, such as habitat and prey destruction, breeding, and hunting pressure. For all the farmers and hunters, wolves established a major focus, because they attacked farm animals and created competition for hunting other animals. Humans have also changed the ecosystem by destroying forests and opening grasslands and pastures. This forced the wolf out of their natural habitat. The number of natural prey of wolves was reduced as well. The livestock, therefore, provided important prey for the wolves, and this led to even more human persecution.

Crooks et al. (2011) also studied the effects of fragmentation and connectivity of mammalian carnivore habitat on the population dynamics of grey wolves and found that the species had experienced a decrease in population size due to the fragmentation of its habitat.

The effects of anthropogenic environmental changes on the genetic diversity of grey wolves have also been studied. Stronen et al. (2019) conducted a study to assess the impact of human-caused environmental changes on the genetic diversity of grey wolves (*Canis lupus*) in Europe. The study found that areas with higher human population density had lower genetic diversity, indicating the negative impact of human activities on the genetic diversity of grey wolves. The researchers also found that maintaining large, connected habitats is crucial for preserving genetic diversity in grey wolves. Fragmentation of habitats can lead to the isolation of populations, which reduces gene flow and increases the risk of inbreeding. In contrast, maintaining large, connected habitats promotes gene flow and allows populations to exchange genetic material, which helps to maintain genetic diversity.

3.2.2. Repopulation and European distribution

After the decline in wolf numbers in Europe in the 20th century, in recent years, there have been efforts to reintroduce wolves to areas where they were once native and to increase the overall population of wolves in Europe. Consequently, the wolf population is now recovering in many regions of Europe. Recolonization has been successful in some areas, such as France and Spain, where the species has repopulated the area from other parts of Europe, such as Scandinavia and the Balkans (Mech 1995; Boitani 2003).

The main aspects that played a role during the population renewal were the law implementation which prevented the cutting of the forest and also not allowing the pasture of livestock in or next to the forest (Blankenhorn 1990). Thanks to education and raising public awareness, there has been a change in people's mindsets about environmental protection. The forest began to recover together with its wildlife (Baumann 1949). The main prey of the wolf is the ungulates, which spread across Europe from Germany and Austria and rapidly increased their population sizes (Baumann 1949; Schmidt 1976; Kurt 1982). This had the effect of increasing the number of large predators everywhere in Europe. The wolves gradually began to populate the Alps, from which they disappeared completely, then spread also to central Italy (Boitani & Ciucci 1992). Gradually, the population spread to the northern part of the Apennine and the French Alps (Poulle 1995). According to the study of Boitani (1986) the Italian population had risen to approximately 300 individuals. The Spanish population had 1500–2000 individuals (Blanco et al. 1990). Wolves began to inhabit environments not previously seen as their habitat. They have adapted to living near roads, in fields, and closer to people. Which in turn shows their remarkable mobility (Fuller et al. 1992).

The return of the grey wolf to Europe has not been without its challenges, however. Wolves are highly mobile, and their populations can be affected by a variety of environmental and human-related factors, such as habitat loss, hunting, and persecution (Boitani 2003). In addition, the introduction of domestic dogs into the environment can also impact the ability of wolves to establish and maintain viable populations (Pilot et al. 2010).

To ensure the successful long-term repopulation of the grey wolf, public attitudes toward the species must be considered. Research has shown that public attitudes vary across Europe, with some populations being more accepting of wolves than others (Hermann & Menzel 2013; Pereira 2015). In order to ensure successful reintroduction and natural recolonization, it is important to consider the attitudes of local populations and to develop strategies to ensure that the species is accepted in the region (Hulva et al. 2018).

In addition, the genetic structure of wolf populations must also be taken into account. Studies have shown that there is a high degree of genetic diversity among European wolf populations, with some populations being more closely related than others (Hindrikson et al. 2013; 2016). This genetic diversity is important for the long-term success of the species, as it ensures that the population is resilient to environmental and human-related pressures (Hindrikson et al. 2016).

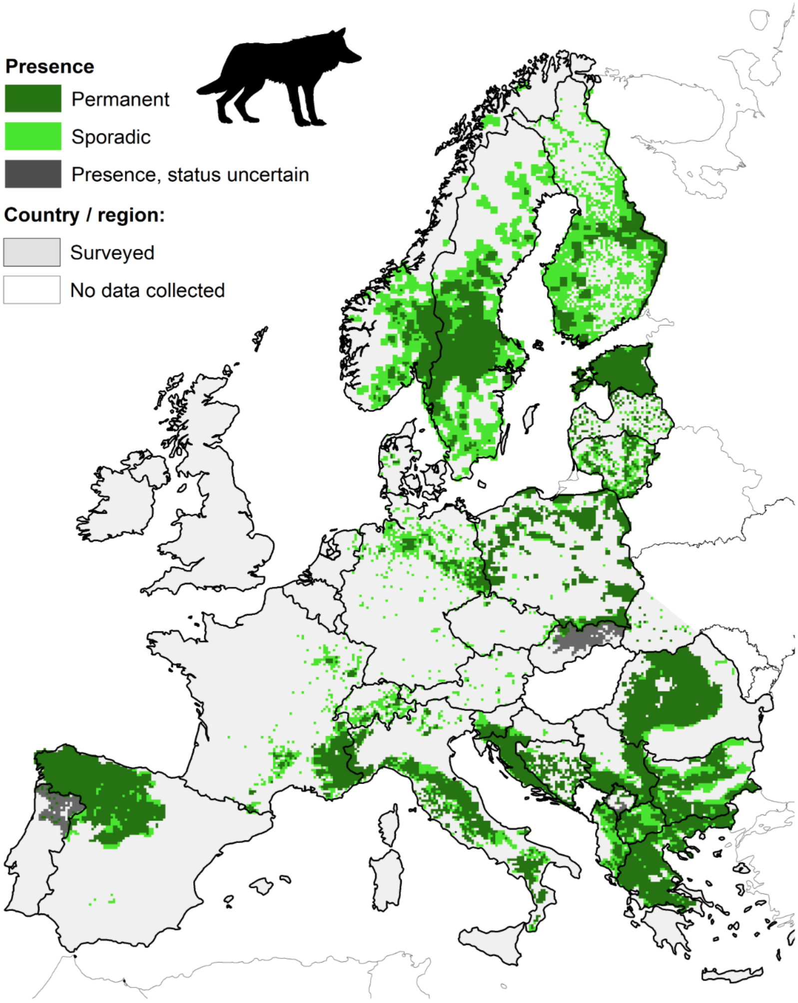

Figure 2: Kaczensky et al. 2021, Distribution of large carnivores in Europe 2012 - 2016

Stronen et al. (2013) provide valuable insight into the European distribution of the grey wolf (*Canis lupus*), focusing on the differentiation of its populations in the north-south direction. As shown in Figure 2, wolves are widely distributed throughout Europe, although their presence is not ubiquitous. The authors explain that the differentiation of wolf populations from north to south is caused by the different environmental conditions. In addition, they discuss the importance of genetic diversity for the long-term survival of the species and suggest that a region of high diversity in the Alps is necessary for the conservation of the species.

3.2.3. Italian population

Italian wolves have experienced extinction due to environmental conditions, lack of protection, and especially hunting. Hunting was the biggest problem for this European population because in those years wolves attacked sheep a lot and farmers had to solve it in some ways. Very often, poisoned baits were used (Apollonio 2006). A study in 2021 found that anthropogenic causes were an important driver of wolf mortality in human-dominated areas in Italy (Musto et al. 2021). In the Alps, wolves were still extinct in the 1920s. Numbers continued to decline across Italy until the 1970s when some 100 individuals still existed in the central Apennines (Zimen & Boitani 1975). Poisoned baits were forbidden in 1976, which was very helpful to the wolves for their recovery (Apollonio 2006). In 1983, the situation improved as well due to better protection of the species and especially the growth of their prey numbers. At this moment, wolves recolonized Western Italy and there were approximately 220 individuals (Boitani 1984). In 2014, a study estimated the total number of wolves in Italy to be around 2,500 individuals (Mattioli et al. 2014). This estimate was confirmed by a 2015 study that estimated the population size to be around 1.500-3.500 individuals (Galaverni et al. 2015).

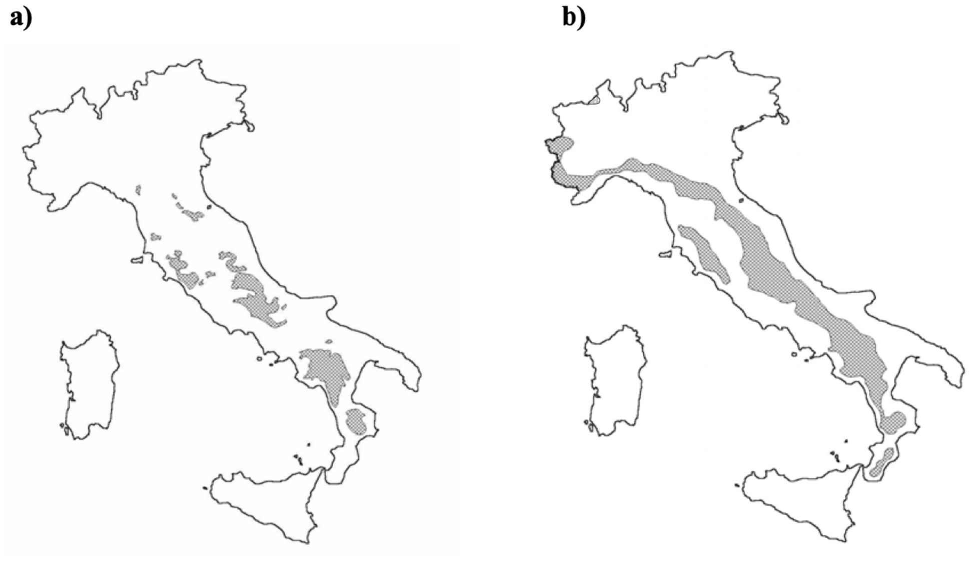

Figure 3: Wolf distribution in Italy (a) 1974 (Cagnolaro et al. 1974); (b) 2005.

The Italian population of the grey wolf (*Canis lupus italicus*) is a distinct morphological and genetic population from other European populations, as evidenced by genetic analyses of the species. The Italian population belongs to haplogroup 2 (Ciucani et al. 2019). A study of fossil remains of the Late Pleistocene wolf (*Canis lupus*) from Italy found that the population was distinct from other Late Pleistocene European populations (Mecozzi & Bartolini-Lucenti 2018). Another study analysed ancient DNA from Late Pleistocene and Holocene Italian wolves and found that the population had undergone a decline in the Late Pleistocene, followed by a population expansion during the Holocene. A study in 2022 analysed ancient DNA from a 1000-year-old wolf cranium from the Po Valley and found that the population was distinct from other European populations (Iurino et al. 2022a, 2022b). The more recent work attributes this unique emergence of a distinct European species to the genetic isolation of Italian populations and drift associated with an extreme bottleneck (Lucchini et al. 2004; vonHoldt et al. 2011; Boggiano et al. 2013; Pilot et al. 2014; Montana et al. 2017a, 2017b). Zimen & Boitani (1974) reported that the Italian wolf population was distinct from other European populations due to a lack of introgression from other European wolf populations. This was further confirmed by a study that found evidence of genetic distinction and long-term population decline in the Apennine wolf population compared to other European populations (Lucchini et al. 2004). In 2009, a study used microsatellite markers to investigate the genetic structure of the Italian wolf population, finding that the population was highly structured (Scandura et al. 2009). This study also found that the Italian wolf population was genetically distinct from other European populations, with a low level of gene flow between them.

3.2.4. Iberian population

The Iberian wolf (*Canis lupus signatus*) is a subspecies of the grey wolf that inhabits the Iberian Peninsula, mainly in the northwestern part. But also have two isolated populations in southern Spain and central Portugal. Now the estimated number of this population is 2000 individuals (Alvares et al. 2005; Blanco et al. 2006; Godinho et al. 2011).

In the early 20th century, the Iberian wolf population experienced a genetic bottleneck due to overhunting and habitat fragmentation. This led to a significant reduction in genetic diversity, making the population more vulnerable to diseases and environmental changes. By the 1970s, the Iberian wolf population had become severely fragmented (Blanco et al. 2006), with only a few small populations remaining in the northwestern regions of Spain and Portugal (Fernández-Gil et al. 2016). Only around 400 individuals remain in isolated pockets across the regions (Blanco et al. 2006). In 1973, Spain declared the grey wolf a protected species, and Portugal followed suit in 1988. Since then, the Iberian wolf population has slowly started to recover. But compared to the repopulation of other European states, the Iberian wolf recovery shows a decline (Torres & Fonseca 2016). Blanco et al. (1992) reported that between 1986–1988 were 294 packs of the Iberian wolf, and in 2012–2014 it was reported 297 packs in a similar area (Magrama 2016). One Iberian wolf population in the northwest is still separated and the southern population in Spain is presumed extinct (Quevedo et al. 2019).

Ramirez et al. (2006) in their study, analysed the wild population and the population from a breeding program that has 15 founders. The results showed that the genetic diversity of the Iberian wolf is comparable to other wild wolf populations. A similar study based on microsatellite markets showed the same results (Luccini et al. 2004). Both studies show relatively high genetic diversity in Iberian wolf populations. All samples for both studies were collected from the northern part of the Duero River. Genetic diversity in the southern part is unknown. The results of individuals from the breeding program showed a diversity comparable to the wild population, which in turn shows that some variance from the wild population is present, even when 5 of the 15 founders are unknown. In a study in which the Iberian population was split into 11 genetic groups (7 in Spain and 4 in Portugal), the results characterised the groups by very low levels of admixture and medium to high genetic differentiation (Silva et al. 2018).

The Iberian wolf is slightly smaller than the wolves in the northern part of Europe. Iberian wolf has distinct white markings on the upper muzzles, dark markings on the tail, and on the front paws (Alcántara & Plana 1999). Vila et al. (1993) study showed differences in skull shape that separate Iberian wolves from wolves in Italy and other populations in Europe. This signature is the result of both historical isolation and adaptation to the Iberian Peninsula's unique ecological conditions. Data on mDNA and microsatellite frequencies showed that Iberian wolves are different from Italian or other European wolves (Vila & Wayne 1999; Lucchini et al. 2004).

But the genetic structure of the Iberian wolf population may be influenced by the hybridization with dogs because the population is small and mainly lives near human settlements. The presence of dogs could lead to the introgression of domestic variants into the wolf population. However, the degree of introgression is still unclear, because wolves have been isolated for a long time and the degree of introgression is probably low (Pires et al. 2017).

3.2.5. Czech population

The population of grey wolves (*Canis lupus*) is located in the Czech Republic, is in the north-central part of the Palearctic region. This population has experienced a long history of persecution. During the 17th century, wolves were widespread in the country. But in the 18th century, wolves were hunted and poisoned throughout the country. As a result, the population rapidly declined to very low numbers. The main period of the wolf population decline is considered to be the second half of the 18th century (Bufka et al. 2005). Studies reported that the last wolf in the Czech Republic was shot in 1747, then it was reported in 1803 and in 1874 (Ševětínský 1895; Baťa 1933; Alberti 1934). But it is assumed that they were either lonely wolves or migrated from somewhere.

However, in recent years, there have been occasional reports of wolf sightings and the occasional individual wolf crossing the border from neighbouring countries. In 2004, a survey confirmed the distribution of wolves in the Czech Republic. The population was distributed in the northwest part, near the border with Slovakia and Poland and in the southwest part of the country, near the Bavarian-Austrian border. The survey also estimated the total population to be between 5–17 individuals and assumed that the population trend in the country depends on the situation in the Western Carpathian (Andìra et al. 2004).

Bufka et al. (2005) wrote that wolves have returned to Šumava and between the years 1990 and 2004, there were 124 recorded wolf appearances, of which 66 were from the Czech Republic. In most cases, the records were of one or two individuals. Everything indicates that the wolves are returning to the Czech Republic, but these are individual cases. Their origin is not clear yet, mostly it is assumed that they come from the Carpathians. To the northern part, a wolf immigrated, probably from western Poland and Saxony.

Overall, the status of the grey wolf in the Czech Republic is still uncertain, with occasional individuals present but not yet an established population. However, the presence of the wolf highlights the potential for the recovery of large carnivores in Central Europe and the need for effective conservation and management strategies.

The Czech population of wolves has also been studied from a genetic perspective. A study conducted in 2021 found that the population was genetically distinct from other wolf populations in the region and that it was a separate conservation unit (Szewczyk et al. 2021). This suggests that the population is unique and should be managed differently from other populations in the region.

A study conducted in 2021 found that wolves in the Czech Republic were infected with a variety of different tapeworm species, some of which were also found in dogs (Juránková et al. 2021). This may assume that there is a certain level of interaction between wolves and domestic dogs in the Czech Republic.

3.3. Interspecies hybridization

In nature often occurs the process of interspecies hybridization. It is an action when two different taxa interbreed in order to produce offspring. In general, this process is considered to be an important mechanism enabling the evolution of new adaptation to new ecological circumstances (Gladieux et al. 2014).

As the number of people increases, the problem of anthropogenically mediated hybridization grows. This happens when the reproductive barrier between two species becomes significantly weaker, leading to interbreeding (Rhymer & Simberloff 1996; Allendorf et al. 2001). Combined since the first domesticated animal until recent ecosystem changes have affected the amount, nature, and density of interbreeding (Crispo et al. 2011; Grabenstein & Taylor 2018). In addition, it also increases the number of domesticated animals, causing a reduction in the population of their wild relatives (Pilot et al. 2018).

It can be a major conservation problem, when it involves interbreeding between defined taxa with invasive, domesticated species or with ancestors (Wayne & Shaffer 2016) and has an important impact on the gene pool and phenotypic characteristics of both groups (Crispo et al. 2011; Grabenstein & Taylor 2018). Hybridization can have several outcomes: may occur hybrid zones that do not affect the parental population; natural introgression; the appearance of a new taxon that does not threaten the wild population. But in the majority of cases, it has influence and leads to the extinction of the parental population. Threats to the parental population may occur, as a result of crosses, between the fertile hybrid and the parental population. This led to the disappearance of natural alleles, reducing the fitness of the parental population and, consequently, to the extinction of the entire wild population (Berger 1973; Clarke et al. 1996). It also happens when hybrids are not very fertile, in which case they provide competition for food and space (Rhymer & Simberloff 1996; Simberloff 1996). Finally, if hybrid numbers are high, it will lead to a small amount of natural individuals or even to extinction (Allendorf et al. 2001).

3.3.1. Dog domestication

Domestication is a long process, during which man takes control of an animal (Zeder 2012). Among the many breeds that have been domesticated, the dog (*Canis lupus familiaris*) takes first place and is one of the human companions during all times. According to the latest molecular genetic studies, it is now known and accepted that the ancestor of the dog is the wolf (Clutton-Brock 1981). The wolf has passed through tens of thousands of years of evolutionary development, as has the dog, but the old lineage that connected them disappeared (Andreska 1991) about 10-40 years ago (Savolainen et al. 2002; Pang et al. 2009; Druzhkova et al. 2013; Freedman et al. 2014; Freedman & Wayne 2017). Therefore it can not be said that today's known wolf is the direct ancestor (Andreska 1991). Also determining exactly, what group of wolves were the direct ancestor of the dog is very challenging (Savolainen et al. 2002; Pang et al. 2009; vonHoldt et al. 2010; Freedman et al. 2014; Frantz et al. 2016; Wang et al. 2017). Wolves and dogs share a common evolutionary history, during which there has been repeated interbreeding since the early stages of domestication (Freedman & Wayne 2017). This ancient introversion has resulted in dog breeds that have retained alleles of their wolf ancestry, while at the same time, most Eurasian wolves have acquired dog alleles (vonHoldt et al. 2010; Fan et al. 2016; Pilot et al. 2018).

3.3.2. Hybridization of wolf with dog

Dogs have undergone successful domestication and the number of dog populations increases with humans (Gompper 2014). However, because of livestock domestication wolves gradually disappeared from human settlements. So, as a result, the two species had completely different historical paths. But in the last years, they have begun to connect their history, to some extent, by hybridization between them. The wolf population has been recovering rapidly in recent times, thanks to the regeneration of their habitat, careful protection and the large expansion of their prey (Breitenmoser 1998). The wolf is a descendant of the dog, and although the last one has experienced different evolutionary processes, both still affect each other in most parts of the Northern hemisphere (Milton 2000). Interspecies hybridization is so widespread among them. Hybridization can be naturally in the wild or as a result of human impact. While natural hybridization has always been considered a process that has positive consequences, anthropogenic hybridization has always been perceived as a negative (Brennan et al. 2014). In addition, this is also accompanied by an increasing threat to the gene pool of species (Randi 2011). Only in Europe, the total density of dogs has been reported to be 18.4 million (Gompper 2014), in this time and the same area the numbers of *Canis lupus* are only 12.0 thousand (Chapron et al. 2014). According to that if the two animals meet, in most cases can be established a hybrid population if the dog is not killed by the wolf

(Lescureux & Linnell 2014). In addition, in some European countries, there is also a problematic factor, such as stray dogs, who live freely (Dufresnes et al. 2019). This process contains two following stages. The first one is when a wolf interbreeds with a dog and as a result, there will be the F1 population that can reproduce. The second stage is breeding between wild wolves and the hybrids, which can cause several issues for wild populations (Hailer & Leonard 2008). But this second stage has been poorly researched because it is a very rare case. That's why the consequences that this can cause are arguable for now (Anderson et al. 2009).

The interaction between these two species can lead to significant changes in the genetic structure and, thus, affect the future development of wild populations (Randi et al. 2014). Non-native genes that might be discovered in populations can lead to genetic disappearance. Because of reducing genetic diversity and fitness, derange adaptation and balance (Anderson et al. 2009). In addition to the influence of those factors, wolf-dog hybrids are very reproductive, which again can result in a change in the wolf genome and result in genetic extinction (Kopaliani et al. 2014; Hindrikson et al. 2016; Pilot et al. 2019).

Massive interbreeding between wolves and dogs takes place in so-called hot-spot areas. Today in Europe, there are several major zones in Baltic countries, some eastern Europe countries and Scandinavia. Mainly: Italy, Spain, Portugal, and Croatia (also the Czech Republic). Because of the large number of hybridizations in such locations, there is great genetic variability. Mainly new populations are subject for natural selection, which leads to hybrid speciation.

Wolves can have different colours in their coat, especially black, white, brown and grey (Musiani et al. 2007; Caniglia et al. 2013). The grey coat is characteristic of the European populations, but a few years ago an exceptional case was found in the emerging Italian population, which had a black coat (Anderson et al. 2009; Caniglia et al. 2013). The Italian wolf was isolated from other European populations (Lucchini et al. 2004). But this recovery is threatened by hybridization with stray dogs (Lucchini et al. 2004; Caniglia et al. 2013; Randi et al. 2014). The Italian hybridization in most cases was not the result of the recent bottleneck, but its recovery. The same study also confirmed that the Italian population had repeated hybridization episodes in the past (Lucchini et al. 2002; Fabbri et al. 2007; Galaverni et al. 2017).

According to molecular analyses, it was assumed that black coat colour came from wolf-dog hybridization (Anderson et al. 2009; Caniglia et al. 2013). Despite the black coat colour, the wolves had distinct white patches on their paws or chest, they also do not have the white face mask (Randi 2008). Another study also assumed that these phenotypic differences are from the wolf-dog hybridization (Candille et al. 2007). The 2002 study showed that the frequency of hybridization in Italy is 0.9 % (Lucchini et al. 2002), but Verardi et al. (2006) study showed that Italian populations had at least 5 % of hybrids. Finally, Randi (2008) study confirmed that this newly created Italian pack had a hybrid origin.

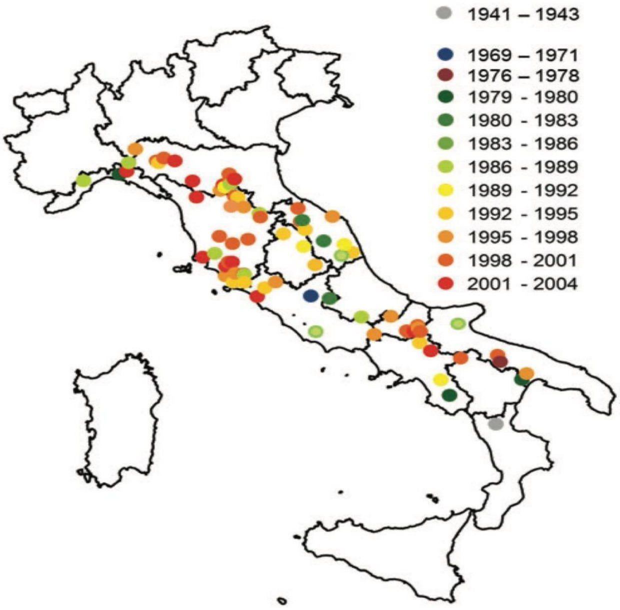

Figure 4: Map visualising the geographical distribution of the wolf x dog admixture events in Italy through time, as reconstructed from PCADMIX results. Locations are plotted where the admixed individuals have been sampled and cannot fully reflect the potential movements from where the first parental hybrids were sampled. (Galaverni et al. 2017)

The Iberian population lived together with dogs for a long period of its history. Godinho et al. (2011) in their study, describe that the Iberian wolf usually occurred near humans and lived near their settlements because at all times its main prey was livestock. Which could have led to easy interaction with the dog. Godinho et al. (2007) study determined that wolves were genetically distinct from dogs because no samples showed hybridization or introgression with dogs. A study by Godinho et al. (2011), found one case of hybridization in northwestern Portugal. Research has shown that the fact of living near a dog did not affect the gene pool of the wolf. And the hybrids in the Iberian population setting 4 %.

The results of the Italian and Iberian populations showed that wolves and dogs in Europe are genetically distinct now. Which may mean that the meaning of hybridization may have been overlooked. But despite this, the mechanics and dynamics of hybridization are far from well understood.

3.3.3. Czechoslovakian Wolfdog

Occurring hybridization can create an introgression of «domestic» alleles into wild populations (Anderson et al. 2009). But man-managed and repeated crossbreeding gave rise to new breeds of wolfdogs such as the Lupo Italiano, the Kunming Wolfdog, the Saarloos Wolfdog and the Czechoslovakian Wolfdog (De Lavigne 2015). Czechoslovakian is the most widely spread among others and its amount reaches 24.0 hundred registered individuals (Smetanová et al. 2015).

The history of the Czechoslovak Wolfdog breed dates, relatively recently, in 1955. This is a unique dog origin from the beginning of attempts to crossbreed a German Shepherd Dog and a wolf (Carpathian). This case represents extreme forms of anthropogenic hybridization (Allendorf et al. 2001). The original object of the experiment was to characterise, for the first time, the genetic composition. If crossbreeding could improve the health, resistance and persistence of dogs. This experiment has confirmed that this combination can produce healthy offspring. In 1965, began the development of a new breed of dog. In 1981 or 1982 the Czechoslovakian Wolfdog was recognized as a national breed by the Fédération Cynologique Internationale (FCI). After this crossing, the organisation prohibited all other crosses between wolf and dog (Hartl & Jedlička 2002).

The resulting breed could create a problem, due to an overly limited number of founders. The biggest issue would be the low genetic variability. But the preliminary genetic studies that have been done have not confirmed such a risk. Research into the diversity of its genome has shown that it differs not only from all other dog breeds but also from both of its parents (Caniglia et al. 2014; Smetanová et al. 2015). Reconstruction methods have identified more than 2.000 genes with dog ancestry that play a role in lipid metabolism, regulation of circadian rhythms, and learning and memory processes. Also, more than 300 genes with wolf ancestry are related mainly to morphological markers (Caniglia et al. 2014).

3.4. Protection

Human opinion is hugely important in wolf conservation planning. If people do not agree to its introduction into the landscape, illegal hunting and poaching will increase greatly (Boitani 2000). Society's attitudes towards the legal protection of carnivore species have shifted with a greater understanding of ecology, allowing for large predators to coexist with humans in favourable political conditions. But humans must avoid a return to regulating numbers by killing, conflicts must be effectively and acceptably prevented. Many methods have been tried to cope with the persistent problems the damage caused to livestock. These included separating these animals from wolves and large carnivores, for example, keeping livestock confined during the night or bad weather, and deterring predators with electric fences, and chemical or visual devices. Another element of protection is guard dogs or intimidation with blank projectiles. If such measures fail, the damage is usually compensated (Rigg et al. 2011).

Improving the minds of humans towards large carnivores is therefore essential for the coexistence of both (Glikman et al. 2012; Bruskotter & Wilson 2014). Information is very important, although it influences public opinion. What is written in the news, media, newspapers and on the internet is rarely discussed, whether it is true or not (Happer et al. 2013; Young et al. 2016a). In most cases, this animal is negatively framed (Jürgens & Hackett 2017) and it has been shown to have a greater impact on people's opinions, than positive information in psychological, social and political studies (Soroka 2006). One hypothesis is that people's beliefs are affected by knowledge and that opinion could therefore be directly or indirectly influenced by the kind of information being provided by different media sources (Guy et al. 2014). Confidence in the origin of the information is an essential component of the position towards wolves. Evidence-based information is necessary for good public conservation decision-making and is central to keeping people's belief in the information they are provided with (Pullin et al. 2004; Cook et al. 2010). Developing trust between stakeholders has proven to be a key element in solving the problem (Young et al. 2016a).

3.5. The importance of *Canis lupus* in the landscape

As a top predator, the wolf plays an irreplaceable role in nature. In addition to the wolf, upper predators include the lynx or bear, meaning that they have no enemy in nature (Kutal 2013), with their position on the highest point of the food web having a cascading influence on their ecological communities and ecosystems through their direct and indirect effects across lower trophic levels (Beschta & Ripple 2006). Also, they are called forest health officers (Chabadová et al. 2016). The absence of a carnivore as the highest link in the food pyramid leads to its disruption, as in the case of food chains. By returning, these relationships are straightened out. Predators themselves have a direct effect on their prey and an indirect effect on the flora.

This highly controversial large carnivore, which has no habitual competitor except humans, brings with it both positive effects on the entire environment, but also negative impacts. The wolf does not change the vegetation directly but contributes significantly by hunting wild boar and mainly large herbivores such as deer and roe deer and prevents them from overpopulating, which is very difficult for nature. Mostly in winter, when there is not enough greenery, animals nibble young trees and graze seedlings, which prevents the natural regeneration of the forest. At the same time, if a predator of a previously dominant species appears, its numbers will be reduced, making space for other species that it had previously oppressed.

The presence of carnivores, therefore, influences the species composition of the forest and restores nature's natural balance. The beast naturally preys on weaker, sick, slow, or old individuals, which are easier to kill, resulting in spontaneous Darwinian natural selection and maintenance of the genetic quality of the population (Kutal 2013).

3.6. Monitoring of *Canis lupus*

Monitoring grey wolves (*Canis lupus*) is an essential component of studying the genetic structure of a population. This is because it allows researchers to track population size, distribution, and movements of the species, as well as to identify any genetic introgression from other species. This information is useful when studying the expansion of wolf populations in the Western Palearctic and their interactions with dogs.

Various methods are used to monitor wolves, such as camera capture, non-invasive genetic sampling, and population assessment methods. Mattioli et al. (2014) studied wolf populations in Italy and proposed a monitoring system using both camera capture and non-invasive genetic sampling. They concluded that a combination of these two methods would be most effective for monitoring wolf populations and distribution in a given area.

Reding and Gräber (2021) studied wolf recolonization in Lower Saxony and concluded that camera capture was the most effective method for estimating wolf population size and distribution in the area. Galaverni et al. (2012) conducted a small pilot study in which they used cameras and non-invasive genetic sampling to monitor wolf populations in Italy. They concluded that camera capture was more effective in estimating population size, while non-invasive genetic sampling was more effective in estimating population genetic structure.

Non-invasive genetic sampling has also been used to study interactions between wolves and dogs in the Western Palearctic. Dufresnes et al. (2019) used non-invasive genetic sampling to study the introgression of dogs into wolf populations in the Alps and concluded that introgression was very limited. This information is useful for understanding wolf population dynamics as well as assessing the potential impact of interbreeding between wolves and dogs.

Camera capture is another important tool for monitoring wolves. Kucera & Barrett (1993), Cutler & Swann (1999), Lucchini et al. (2002), Larrucea et al. (2007) and De

Barba et al. (2010) conducted studies showing that camera capture can be used to monitor wolf populations. The advantage of camera capture is that it is non-invasive and relatively inexpensive, and can provide detailed information on wolf population size, distribution, and movements.

4. Aims of the practical part of the thesis

- Training the principles of DNA extraction, PCR and visualisation of results in a molecular genetics laboratory.

- Learning bioinformatic methods for the analysis of animal samples.

- Analyse a dataset of samples and evaluate the possible occurrence of hybrids.

5. Materials

In this thesis, I was involved in DNA extraction and genotyping of the non-invasive samples which are being routinely processed in the Laboratory of Molecular Genetics at FTZ ČZU. Data generated by me are considered as training data, therefore, any conclusions based on these results should be treated with caution. Due to the sensitivity of the topic, any details about the dataset are not provided. More information should be requested from the supervisor of the thesis.

6. Methodology

Extracting DNA

The NucleoSpin ® DNA Stool kit (Macherey Nagel) was used for DNA isolation from ethanol-stabilised wolf dung. The instruction manual for the isolation was available from the provider and work started and finished strictly according to it. The bright parts have been selected, because it must contain large intestine cells. The samples were then weighed into tubes with glass beads. The most suitable amount of material should be between 0.18 and 0.22 g for higher isolation efficiency. Then, after adding the proteinase, the samples must be left to incubate according to the manufacturer's instructions. After incubation, occurs the centrifugation stage, after which 600 µl of the liquid portion of the samples were pipetted into new tubes. In the following steps, was done a sequential washing with buffer and fixation of the isolated DNA on the silica membrane. The elution buffer was used to separate the nucleic acid. A NanoDrop spectrophotometer was used to determine the concentration of the isolated DNA. The absorbance at 260 nm is directly proportional to the concentration of DNA. Before measuring, the device had to be calibrated using the elution buffer used in the isolation. The results from wolves' samples usually do not come out the cleanest, so it is necessary to monitor the quality of the isolate using an absorbance curve. Individual concentrations had to be recorded on paper.

Preparation of the PCR reaction

The basic principle of PCR is the repetitive controlled denaturation of double-stranded DNA and subsequent renaturation of single - stranded DNA by specific oligonucleotides that are in excess in the reaction mixture. These oligonucleotides subsequently provide primers for the synthesis of the new DNA strand. DNA amplification proceeds in repeating cycles. Isolated wolf DNA was available. For wolves are used three mixtures of primers (primermix A, B, C - this mixture contains primers from A and B). The first step was to illuminate the FlowBox with a UV lamp to sterilise the box and the instruments in it. During the illumination, there should be no chemicals in it, UV radiation can cause it to degrade. Then the chemicals and samples had to be taken out of the freezer and left to stand freely at room temperature to give them time to unfreeze. To strip were pipetted 5 µl of Multiplex PLUS master mix, 3 µl of water, 1 µl of primermix and 1 µl of DNA, for a total capacity of 10 µl. Centrifuged them, so that no droplets remain on the cap and walls. Placed the strips in the BioRad T100 thermocycler and check that the plastic border is positioned on the side corresponding to the top of the tube caps to prevent the caps from sticking or accidentally leaking and evaporating the samples. The appropriate program had to be set on the cycler. When the reaction was complete, the finished PCR samples were transferred to the refrigerator. A negative control should always be performed to ensure that no contamination has occurred. The FlowBox had to be cleaned out after the work and allowed to light up again.

Fragmentation

The next task was fragmentation. For this purpose, a premix containing 8.75 µl formamide and 0.25 µl ladder per sample was prepared, both of which must always be frozen in the refrigerator. After splitting the premix into strips, 1 µl of PCR sample was added. The FR (fragmentation) label and number had to be written on the strip and the bag, then it was transported for control. After the fragmentation results were complete, they could be opened in Geneious. The peaks of the ladders must be ranked and the peaks of each allele labelled with all the colours of the sample. This determined where on the chromosome each allele was located and whether the individual was homozygous (one peak) or heterozygous (two peaks). It is used to identify specific individuals, to determine relatedness, herd membership or simply to determine the species.

7. Results

In Figure 5, the results from the Structure program are displayed. Each column represents one individual and each colour represents the classification of the tested cluster. The orange colour corresponds to the Carpathian population cluster, purple shows the Lowland wolf population. Blue indicates the dog cluster. The green colour in K=4, represents red foxes and golden jackals. At K=4 there was a clear separation of dogs, red foxes + golden jackals and wolves. We can see that our determined identification of the dog or wolf population and the predominant colour of the cluster do not always coincide. In samples 62 and 112, we can see both wolf and dog assignments. In these two cases, it potentially could be an admixture between dog and wolf. However, this approach is not designed to test hybrid categories and only assigns individuals into clusters based on the allele frequencies. Suspected animals have to be tested in a specialised program, which directly tests hybrid origin.

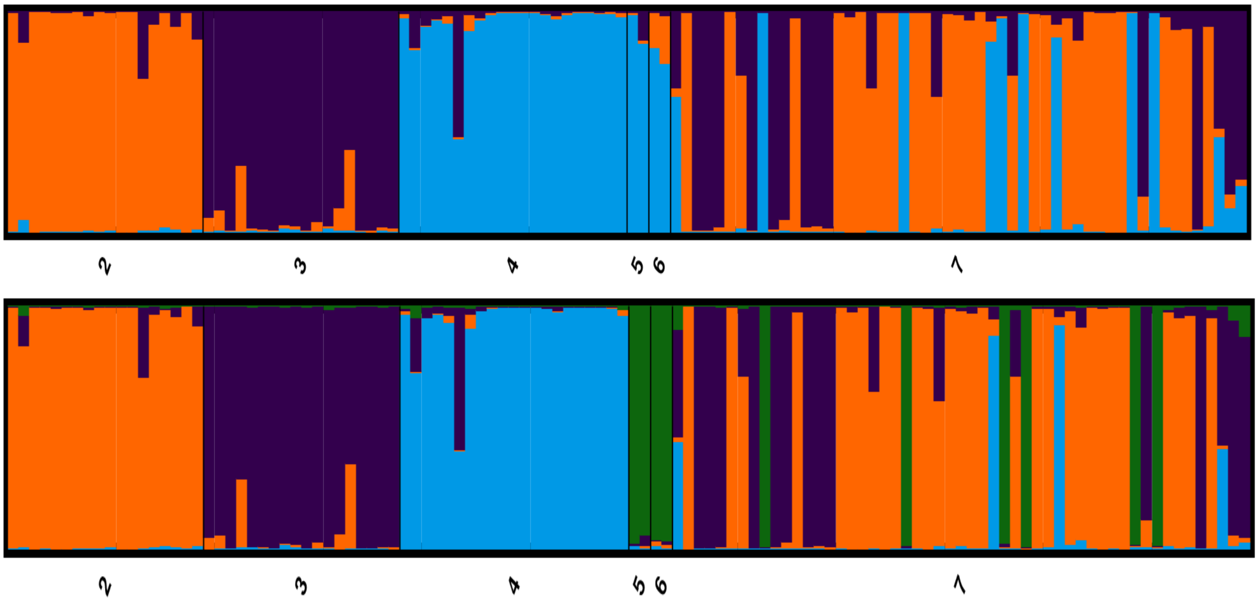

Figure 5: Results in Structure program. The numbers below show populations.

We used the NewHybrid program for more precision in the estimation of admixture between dogs and wolves. The program evaluates the assignment into different pure and hybrid categories. In Figure 8, two different white-grey colours indicate pure wolf and pure dog origin. The green colour shows F1 hybrids and the orange colour shows F2 hybrids. Yellow represents backcrossing into the wolf population, blue represents backcrossing into the dog population. Sample 57 appears to be a hybrid. Samples 86 and 92 partly show the classification of backcrossing.

Samples 62 and 112, which were suspected of an admixed origin based on the Structure program, were not confirmed in the NewHybrid program.

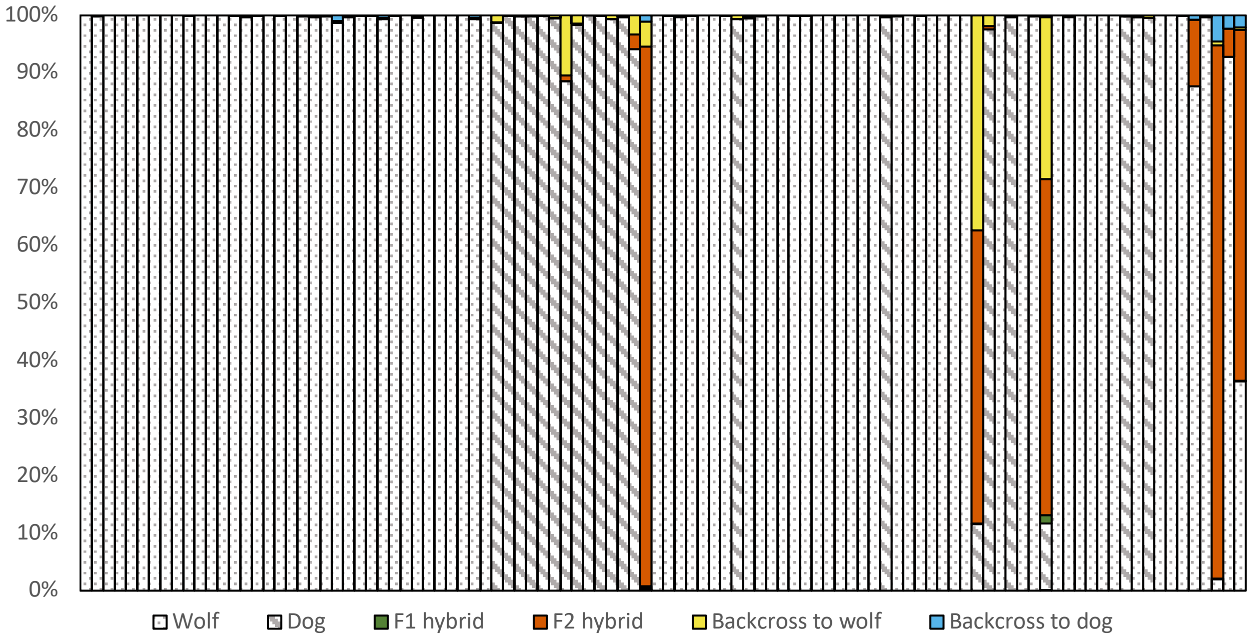

Figure 6: Results in the NewHybrid program.

8. Discussion

First of all, it has to be stated that this is a student training dataset made solely for the purposes of this study, and no management decisions and conclusions should be based on the results of the study. Final evaluations have to be done with more caution, as discussed later.

The results from both the Structure and NewHybrid programs provide insights into the genetic composition of the studied population of canids. During the analysis in the Structure program, the samples were clearly divided into a wolf, dog, red fox and golden jackal populations, which was shown in Figure 5. Results for K=4 had a clear separation and samples were split into a particular group. Only in two samples 62 and 112, was a visible overlap with the domestic dog population.

For a more detailed analysis of hybridization events, a method directly designed for testing hybrid categories in the NewHybrid program was used. The NewHybrid program also had a clear separation of population. It was observed that samples 62 and 112, which showed admixed clustering in the Structure program, were not confirmed it in the NewHybrid program. But the program rated the other sample as admixed and showed backcrossing in two samples.

However, F2 hybrids are very unlikely to occur, and more realistically, the signal is arbitrary. Another clue is that the signal was not stable using different approaches. All suspect individuals should be re-genotyped and re-analyzed. Another fact is, that more populations of wolves were included in the dataset, which meant that they share different allele frequencies. In the future, each population (Carpathian and Lowland) should be tested separately.

A major disadvantage of this analysis is the small number of amplified samples and therefore specific predictions and conclusions for the population cannot be made with high confidence.

9. Conclusions

The literature thesis concludes that the wolf population is increasing in the Western Palearctic region, which began in the 20th century. But the expansion of wolf populations is a complex process that is influenced by various factors. One of the main factors is the availability of prey, which has a direct impact on the size and distribution of wolf populations. Other factors include habitat suitability, human activities, and climatic conditions. For instance, the expansion of wolf populations in some areas of Europe has been attributed to the recovery of large herbivores such as deer (*Cervus*), which provide a significant source of food for wolves.

Genetic diversity is another important factor that influences the expansion of wolf populations. The genetic diversity of a population affects its ability to adapt to changing environmental conditions and resist diseases. In this thesis, it was found that the genetic diversity of wolf populations in the Western Palearctic varies depending on the location, with some populations showing higher levels of diversity than others. This information is important for conservation efforts and for understanding the long-term viability of wolf populations in the Western Palearctic.

The interaction between wolves and dogs is a complex and often contentious issue. Domestic dogs can have both positive and negative impacts on wolf populations. Dogs can pose a significant threat to wolves through hybridization and competition for resources. In this thesis, it was described that the impact of dogs on wolf populations varies depending on the location and the management strategies in place. But the results of the studies showed that hybridization in the selected populations does not recently occur frequently.

Overall, this thesis highlights the importance of understanding the complex interactions between wolves and their environment, including other species such as dogs.

10. References

Alberti K., 1934: Beiträge zur Geschichte der Stadt Asch und des Ascher Bezirkes. Aš 1934, I: 256–260.

Allendorf, F. W., Leary, R. F., Spruell, P., and Wenburg, J. K. (2001). The problems with hybrids: setting conservation guidelines. Trends Ecol. Evol. 16, 613–622. doi: 10.1016/S0169-5347(01)02290-XAvise, J. C., Walker, D., and Johns, G. C. (1998). Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc. R. Sco. Lond.B,265:1707–1712.

Alcántara, P., and Plana, M. (1999). The wolf in Spain (Signatus.org).

Alvares F, Barroso I, Blanco JC et al. (2005) Wolf status and conservation in the Iberian Peninsula. Conference ''Frontiers of Wolf Recovery: Southwestern US and the World'', pp. 76– 77. Colorado Springs, EUA.and Landforms 31:1525-1539.

Anderson TM, vonHoldt BM, Candille SI et al (2009) Molecular and evolutionary history of melanism in North American gray wolves. Science 323:1339–1343

Andìra M., Èervený J., Bufka L.,Bartošová D. and Koubek P. 2004. Recent distribution of the wolf (*Canis lupus*) in the Czech Republic. Lynx (Praha), 35: 5–12.

Andreska J. 1991. Lovy bez psů, záhada paleolitu. Čtení o psech, čtení pro dobrou pohodu. Magazín. Praha. Magnet-Press. ISSN 80-85434-00-8.

Apollonio M. A cura del dr. Andrea Gazzola e di Alessia Viviani con la supervisione scientifica, 2006.

Baťa L., 1933: Dosavadní výsledky zoologického výzkumu jižních Čech [Up to date results of the zoological research in the Southern Bohemia]. Jihočeská společnost vlastivědná, České Budějovice, 67 pp. (in Czech).

Baumann, F. (1949) Die freilebenden Saugetiere der Schweiz,Huber, Bern.

Berger, L. (1973). Systematics and Hybridization in European Green Frogs of Rana esculenta Complex. J. Herpetol. 7, 1–10. doi: 10.2307/1562822

Beschta, R. L., and W. J Ripple. 2006. River channel dynamics following extirpation of wolves in northwestern Yellowstone National Park, USA

Blanco JC, Rodrıguez A, Cuesta L, Reig S, del Olmo JC (1990) El lobo en Sierra Morena. In: Blanco JC, Cuesta L, Reig S (eds) El lobo (*Canis lupus*) en Espan˜a. Situacio´n, problema´tica y apuntes sobre su ecolog´ıa. Instituto para la Conservacio´n de la Naturaleza, Madrid, pp 61–68

Blanco, J. C., Cortés, Y., & Virgós, E. (2006). Status, distribution and long-term changes in the conservation of the wolves in Spain. Mammal Review, 36(1), 21-50.

Blanco, J. C., Reig, S., and De la Cuesta, L. (1992). Distribution, Status and Conservation Problems of the Wolf *Canis lupus* in Spain. Biol. Conservation 60, 73–80. doi:10.1016/0006-3207(92)91157-N

Blankenhorn H. J. (1990) Wild und Jagd-Vergangenheit, Gegenwart und Zukunft. In Wild and Jagd 1915-1990, ed. W. Fuchs and H.-P. Breitenmoser. Schweizer Jiiger, Einsiedeln, 9-16.

Boggiano, Ciofi, Boitani, Formia, Grottoli, Natali, Ciucci. (2013). Detection of an East European wolf haplotype puzzles mitochondrial DNA monomorphism of the Italian wolf population. Mammalian Biology 78 (2013) 374–378.

Boitani L (1984) Genetic considerations on wolf conservation in Italy. Bolletino Di Zoologia, 51, 367-373

Boitani, L. (2003). "Wolf conservation and recovery," in Wolves: Behavior, Ecology and Conservation, eds L. D. Mech and L. Boitani (Chicago: University of Chicago Press), 317–340.

Boitani, L. 1986. Dalla parte del lupo. Mondadori, Milano, Italy.

Boitani, L., 2000. Action plan for the conservation of Wolves in Europe (*Canis lupus*). No. 113. 1-85. Bern Convention, Strasbourg Cedex, Coun- cil of Europe, Nature and Environment.

Boitani, L., and P. Ciucci. 1992. Wolves in Italy: critical issues for their conservation. Pages 74–90 in C. Promberger and W. Schröder, edi- tors. Wolves in Europe. Proceedings of the workshop on Wolves in Europe: current status and prospects. Munich Wildlife Society, Oberammergau, Germany.

Breitenmoser U (1998) Large predators in the Alps: the fall and rise of man's competitors. Biological

Brennan, Woodward, Seehausen, Muñoz-Fuentes, Moritz, Guelmami, Abbott & Edelaar. (2014). Hybridization due to changing species distributions: adding problems or solutions to conservation of biodiversity during global change: 476 - 488.

Bruskotter, J. T., and Wilson, R. S. (2014). Determining where the wild things will be: using psychological theory to find tolerance for large carnivores. Conserv. Lett. 7, 158–165. doi: 10.1111/conl.12072

Bufka L., Heurich M., Engleder T., Wölfl M., Èervený J. and Scherzinger W. 2005. Wolf occurrence in the Czech-Bavarian-Austrian border region – review of the history and current status. Silva Gabreta 11(1): 27-42.

Cagnolaro, L.: Rosso. D.: Spagnesi. M.: Ven- TURi. B. (1974): Investigation on the wolf (*Canis lupus*) distribution in Italy and in Canton Ticino and Canton Grigioni (Switzerland). Ric. Biol. Selvaggina 59, 1-75 (in Italian).

Candille SI, Kaelin CB, Cattanach BM, Yu B, Thompson D. a, Nix M. a, Kerns J. a, Schmutz SM, Millhauser GL, Barsh GS. 2007. A b-defensin mutation causes black coat colour in domestic dogs. Science 318:1418–1423.

Caniglia R, Fabbri E, Galaverni M, Milanesi P, Randi E (2014) Noninvasive sampling and genetic vari- ability, pack structure, and dynamics in an expanding wolf population. Journal of Mammalogy 95: 41– 59.

Caniglia R, Fabbri E, Greco C, Galaverni M, Manghi L, Boitani L, Sforzi A, Randi E. 2013. Black coats in an admixed wolf - dog pack is melanism an indicator of hybridization in wolves? Eur J Wildl Res. 59:543–555.

Chabadová, Z., Kameníková, M., Nový, J. (2016). Konfliktní druhy. Český nadační fond pro vydru. https://vydry.org/brozura-konfliktni-druhy/

Chapron G, et al. Recovery of large carnivores in Europe's modern human-dominated landscapes. Science. 2014 Dec 19;346(6216):1517-9. doi: 10.1126/science.1257553. PMID: 25525247.

Ciucani, Marta & Palumbo, Davide & Galaverni, Marco & Serventi, Patrizia & Fabbri, Elena & Ravegnini, Gloria & Angelini, Sabrina & Maini, Elena & Davide, Persico & Caniglia, Romolo & Cilli, Elisabetta. (2019). Old wild wolves: Ancient DNA survey unveils population dynamics in Late Pleistocene and Holocene Italian remains. PeerJ. 7. 10.7717/peerj.6424.

Clarke, B., Johnson, M. S., Murray, J., Hewitt, G., and Wragg, G. M. (1996). Clines in the genetic distance between two species of island land snails: how 'Molecular Leakage' can mislead us about speciation. Philos. Trans. R. Soc. B Biol. Sci. 351, 773–780. doi: 10.1098/rstb.1996.0072. Conservation 83: 279–289.

Clutton-Brock, J. (1981) Domesticated Animals from Early Times, University of Texas Press

Cook, C.N., Hockings, M., Carter, R.B., 2010. Conservation in the dark? The information used to support management decisions. Front. Ecol. Environ. 8, 181–186.

Crispo, E., Moore, J.-S., Lee-Yaw, J. A., Gray, S. M., & Haller, B. C. (2011). Broken barriers: Human-induced changes to gene flow and introgression in animals. Bioessays, 33, 508–518.

Crooks KR, Burdett CL, Theobald DM, Rondinini C, Boitani L. Global patterns of fragmentation and connectivity of mammalian carnivore habitat. Philos Trans R Soc B Biol Sci. 2011; 366: 2642–2651.

Cutler TL, Swann DE (1999) Using remote photography in wildlifeecology: a review. Wildl Soc Bull 27:571–581

De Barba M, Waits LP, Genovesi P, Randi E, Chirichella R, Cetto E (2010) Comparing opportunistic and systematic sampling methods for non-invasive genetic monitoring of a small translocated brown bear population. J Appl Ecol 47:172–181

De Lavigne G (2015) Free Ranging Dogs—Stray, Feral or Wild. Raleigh, North Carolina: Lulu. com. pp. 447.

Delibes, M. 1990. Status and conservation needs of the wolf (*Canis lupus*) in the Council of Europe member states. Nature and Envi- ronment Series 47. Council of Europe, Strasbourg, France.

Druzhkova, A.S., Thalmann, O., Trifonov, V.A., Leonard, J.A., Vorobieva, N.V., Ovodov, N.D., Graphodatsky, A.F., Wayne, R.K., 2013. Ancient DNA analysis affirms the canid from Altai as a primitive dog. PLoS One 8 (3), e57754.

Dufresnes, Christophe & Remollino, Nadège & Stoffel, Céline & Manz, Ralph & Weber, Jean-Marc & Fumagalli, Luca. (2019). Two decades of non-invasive genetic monitoring of the grey wolves recolonizing the Alps support very limited dog introgression. Scientific Reports. 9. 148. 10.1038/s41598-018-37331-x.

E. Zimen and L. Boitani. 1974. Number and distribution of wolves in Italy

Fabbri E, Miquel C, Lucchini V, Santini A, Caniglia R, Duchamp C, Weber JM, Lequette B, Marucco F, Boitani L, Fumagalli L, Taberlet P, Randi E (2007) From the Apennines to the Alps: colonisation genetics of the naturally expanding Italian wolf (*Canis lupus*) population. Mol Ecol 16:1661–1671

Fan, Z. et al. Worldwide patterns of genomic variation and admixture in gray wolves. (2016) Genome Res. 26, 163–173.

Fechter, Dominik & Storch, Ilse. (2014). How Many Wolves (*Canis lupus*) Fit into Germany? The Role of Assumptions in Predictive Rule-Based Habitat Models for Habitat Generalists. PloS one. 9. e101798. 10.1371/journal.pone.0101798.

Fernández-Gil, A., Álvares, F., Mira, A., & Queirós, J. (2016). The effects of conservation policies on the Iberian wolf populations. In Wolves (pp. 95-110). Elsevier.

Frantz LA, et al. Genomic and archaeological evidence suggest a dual origin of domestic dogs. Science. 2016 Jun 3;352(6290):1228-31. doi: 10.1126/science.aaf3161. Epub 2016 Jun 2. PMID: 27257259.

Freedman AH, Wayne RK. Deciphering the Origin of Dogs: From Fossils to Genomes. Annu Rev Anim Biosci. 2017 Feb 8;5:281-307. doi: 10.1146/annurev-animal-022114-110937. Epub 2016 Nov 28. PMID: 27912242.

Freedman, A.H. et al. (2014) Genome sequencing highlights the dynamic early history of dogs. PLoS Genet. 10, e1004016

Fuller, T. K., W. E. Berg, G. L. Radde, M. S. Lenarz, and G.B. Joselyn. 1992. A history and current estimate of wolf distribution and numbers in Minnesota. Wildlife Society Bulletin 20:42-54. Gese, E. M., and L. D. Mech. 1991. Dispersal of wolves (*Canis lupus*) in northeastern Minnesota, 1969-1989. Canadian Journal of Zoology 69:2946-2955.

Galaverni, Marco & Caniglia, Romolo & Fabbri, Elena & Milanesi, Pietro & Randi, Ettore. (2015). One, no one, or one hundred thousand: how many wolves are there currently in Italy?. Mammal Research. 61. 10.1007/s13364-015-0247-8.

Galaverni, Marco & Palumbo, Davide & Fabbri, Elena & Caniglia, Romolo & Greco, Claudia & Randi, Ettore. (2012). Monitoring wolves (*Canis lupus*) by non-invasive genetics and camera trapping: A small-scale pilot study. European Journal of Wildlife Research - EUR J WILDL RES. 58. 10.1007/s10344-011-0539-5.

Galaverni, Romolo Caniglia, Luca Pagani, Elena Fabbri, Alessio Boattini, Ettore Randi, Disentangling Timing of Admixture, Patterns of Introgression, and Phenotypic Indicators in a Hybridizing Wolf Population, Molecular Biology and Evolution, Volume 34, Issue 9, September 2017, Pages 2324–2339. Genome Biol. 18: 1–13.

Ginsberg JR, Macdonald DW (1990) Foxes, Wolves, Jackals and Dogs. An Action Plan for the Conservation of Canids. International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

Gladieux, P., Ropars, J., Badouin, H., Branca, A., Aguileta, G., de Vienne, D. M., et al. (2014). Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. Mol. Ecol. 23, 753–773. doi: 10.1111/mec.12631

Glikman, J. A., Vaske, J.J., Bath, A.J., Ciucci, P. and Boitani, L. (2012). Residents' support for wolf and bear conservation: the moderating influence of knowledge. European Journal of Wildlife Research 58 (1): 295-302

Godinho R, Llaneza L, Blanco JC, Lopes S, Álvares F, García EJ, Palacios V, Cortés Y, Talegón J, Ferrand N. Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. Mol Ecol. 2011 Dec;20(24):5154-66. doi: 10.1111/j.1365-294X.2011.05345. x. Epub 2011 Nov 9. PMID: 22066758.

Godinho R, Lopes S, Ferrand N (2007). Estudo da diversidade e estruturação genética das populações de lobo (*Canis lupus*) em Portugal. Relatório Final. CIBIO/UP. p 56

Gompper, M.E. (2014). The dog-human-wildlife interface: assessing the scope of the problem. In Free-ranging Dogs and Wildlife Conservation: 9–54. Gompper, M.E. (Ed.). Oxford: Oxford University Press

Grabenstein, K. C., & Taylor, S. A. (2018). Breaking barriers: Causes, consequences, and experimental utility of human-mediated hybridization. Trends in Ecology & Evolution, 33, 198–212. https://doi. org/10.1016/j.tree.2017.12.008

Guy, S., Kashima, Y., Walker, I., O'Neill, S., 2014. Investigating the effects of knowledge and ideology on climate change beliefs. Eur. J. Soc. Psychol. 44, 421–429.

Gwynn, Vashti & Symeonakis, Elias. (2022). Rule-based habitat suitability modelling for the reintroduction of the grey wolf (*Canis lupus*) in Scotland. PLOS ONE. 17. e0265293. 10.1371/journal.pone.0265293.

Hailer F & Leonard J, 2008. Hybridization among three native North American Canis species in a region of natural sympatry

Happer, C., Philo, G., Philo, G., 2013. The role of the media in the construction of public belief and social change. J. Soc. Polit. Psychol. 1, 321–336.

Hartl K, Jedlička J (2002) Československý vlčák. Brno, Česká republika: LOBA. 1–60 p.

Hermann, N.; Menzel, S. Predicting the intention to support the return of wolves: A quantitative study with teenagers. J. Environ. Psychol. 2013, 36, 153–161. [CrossRef]

Hewitt GM: Postglacial recolonization of European biota. Biol J Linn Soc 1999, 68:87-112.

Hindrikson M, Remm J, Ma¨nnil P, Ozolins J, Tammeleht E, Saarma U. Spatial Genetic Analyses Reveal Cryptic Population Structure and Migration Patterns in a Continuously Harvested Grey Wolf (*Canis lupus*) Population in North-Eastern Europe. PLoS One. 2013; 8: 1–12.

Hindrikson M, Remm J, Pilot M, Godinho R, Stronen AV, Baltrūnaitė L, et al. Wolf population genetics in Europe: a systematic review, meta-analysis and suggestions for conservation and management. Biol Rev. 2016;

Hulva P et al. 2018. Wolves at the crossroad: Fission-fusion range biogeography in the Western Carpathians and Central Europe. Diversity and Distributions 24:179-192 interplay of demography and selection during maize domestication and expansion.

Iurino, Dawid & Cilli, Elisabetta & Caniglia, Romolo & Fabbri, Elena & Mecozzi, Beniamino & Ciucani, Marta & Sardella, Raffaele & Davide, Persico. (2022a). On the trail of mediaeval wolves: ancient DNA, CT-based analyses and palaeopathology of a 1000-year-old wolf cranium from the Po Valley (northern Italy). Historical Biology. 1-12. 10.1080/08912963.2022.2071710.

Iurino, Dawid & Mecozzi, Beniamino & Iannucci, Alessio & Moscarella, Alfio & Strani, Flavia & Bona, Fabio & Gaeta, Mario & Sardella, Raffaele. (2022b). A Middle Pleistocene wolf from central Italy provides insights on the first occurrence of *Canis lupus* in Europe. Scientific Reports. 12. 10.1038/s41598-022-06812-5.

Jedrzejewski, W., Niedzialkowska, M., Mysiajek, R.W., Nowak, S. & Jedrzejewska, B. 2005: Habitat selection by wolves *Canis lupus* in the uplands and mountains of southern Poland. – Acta Theriologica 50: (3) 417-428.

Juránková, Jana & Hulva, Pavel & Černá Bolfíková, Barbora & Hrazdilová, Kristýna & Frgelecová, Lucia & Daněk, Ondřej & Modrý, David. (2021). Identification of

Tapeworm Species in Genetically Characterised Grey Wolves Recolonising Central Europe. Acta Parasitologica. 66. 10.1007/s11686-020-00327-7.

Jürgens, U.M., Hackett, P.M.W., 2017. The big bad wolf: the formation of a stereotype. Ecopsychology 9, 33–43.

Kaartinen, Salla & Antikainen, Harri & Kojola, Ilpo. (2015). Habitat Model for a Recolonizing Wolf (*Canis lupus*) Population in Finland. Annales Zoologici Fennici. 52. 77-89. 10.5735/086.052.0207.

Kaczensky, Petra et al. (2021), Distribution of large carnivores in Europe 2012 - 2016: Distribution maps for Brown bear, Eurasian lynx, Grey wolf, and Wolverine, Article, https://doi.org/10.5281/zenodo.5060137

Kopaliani, Natia & Shakarashvili, Maia & Gurielidze, Zurab & Qurkhuli, Tamar & Tarkhnishvili, David. (2014). Gene Flow Between Wolf and Shepherd Dog Populations in Georgia (Caucasus). The Journal of heredity. 105. 10.1093/jhered/esu014.

Kucera TE, Barrett RH (1993) The trailmaster camera system for detecting wildlife. Wildl Soc Bull 21:505–508

Kurt F. (1982) Naturschutz-Illusion und Wirklichkeit: zur Ökologie bedrohter Arten und Lebensgemeinschaften. Parey, Hamburg, Berlin.

Kutal, M. (2013) Velké šelmy v českých lesích: Význam z pohledu ochrany přírody a myslivosti (2nd Ed.). Olomouc: Hnutí DUHA.

Larrucea ES, Brussard PF, Jaeger MM, Barrett RH (2007) Cameras,coyotes, and the assumption of equal detectability. J Wildl Manag71:1682–1689

Lehman N, Wayne RK (1991) Analysis of coyote mitochondrial DNA genotype frequencies: estimation of the effective number of alleles. Genetics, 128, 405-416.

Leonard, J. A., Echegaray, J., Randi, E. & Vilà, C. Impact of hybridization with domestic dogs on the conservation of wild canids in Free-ranging dogs and wildlife conservation. (ed. Gompper, M. E.) 170–184. (Oxford University Press, 2014).

Lescureux, N. & Linnell, J. D. Warring brothers: The complex interactions between wolves (*Canis lupus*) and dogs (*Canis familiaris*) in a conservation context (2014). Biol. Conserv. 171, 232–45.

Lorenzen, E. D. et al. Species-specific responses of Late Quaternary megafauna to climate and humans (2011). Nature 479, 359–364.

Lucchini V, Fabbri E, Marucco F et al (2002) Non-invasive molecular tracking of colonising wolf (*Canis lupus*) packs in the western Italian Alps. Mol Ecol 11:857–868

Lucchini V, Galov A, Randi E (2004) Evidence of genetic distinction and long-term population decline in wolves (*Canis lupus*) in the Italian Apennines. Mol. Ecol., 13, 523–536.

M. Scandura, M. Apollonio and L. Mattioli. 2009. Original investigation Recent recovery of the Italian wolf population: a genetic investigation using microsatellites.

Magrama (2016). Censo 2012-2014 de lobo ibérico (*Canis lupus*, Linnaeus, 1758) en España. Madrid: Ministerio de Agricultura, Alimentación y Medio Ambiente.

Mattioli, Luca & Forconi, Paolo & Berzi, Duccio & Perco, Franco. (2014). Wolf population estimate in Italy and monitoring perspectives.

Mech, L.D. 1995. The challenge and opportunity of recovering wolf populations. Conserv. Biol., 9, 270–278.

Mech, L. D., and L. Boitani. 2003. Wolves: behaviour, ecology and conservation. The University of Chicago Press, Chicago, Illinois, and London, United Kingdom. 448 pp. ISBN 0-226-51696-2 price (hardbound), $49.00, Journal of Mammalogy, Volume 85, Issue 4, 16 August 2004, Pages 814–815, https://doi.org/10.1644/1545-1542(2004)085<0815:BR>2.0.CO;2

Mecozzi, Beniamino & Bartolini-Lucenti, Saverio. (2018). The Late Pleistocene *Canis lupus* (Canidae, Mammalia) from Avetrana (Apulia, Italy): Reappraisal and new insights on the European glacial wolves. Italian Journal of Geosciences. 137. 10.3301/IJG.2017.22.

Milton, K. 2000. Ducks out of water: Nature conservation as boundary maintenance. In: Knight, J. ed. Natural enemies. New York, NY: Routledge, 229–248.

Montana L, Caniglia R, Galaverni M, Fabbri E, Ahmed A, Bolfíková BČ, et al. (2017a) Combining phylogenetic and demographic inferences to assess the origin of the genetic diversity in an isolated wolf population. PLoS ONE 12: e0176560

Montana L, Caniglia R, Galaverni M, Fabbri E, Randi E (2017b) A new mitochondrial haplotype confirms the distinctiveness of the Italian wolf (*Canis lupus*) population. Mamm Biol 84: 30–34

Musiani M, Leonard JA, Cluff HD et al (2007) Differentiation of tundra/taiga and boreal coniferous forest wolves: genetics, coat colour and association with migratory caribou. Mol Ecol 16:4149–4170

Musto, Carmela & Cerri, Jacopo & Galaverni, Marco et all. (2021). Men and wolves: Anthropogenic causes are an important driver of wolf mortality in human-dominated landscapes in Italy. Global Ecology and Conservation. 32. e01892. 10.1016/j.gecco.2021.e01892.

Pang JF, Kluetsch C, Zou XJ et al (2009) mtDNA data indicate a single origin for dogs south of the Yangtze River, less than 16, 300 years ago, from numerous wolves. Mol Biol Evol 26(12):2849–2864

Pereira HM. Rewilding European Landscapes. Pereira HM, Navarro L, editors. Springer International Publishing; 2015.

Pilot M, Branicki W, Jędrzejewski W, Goszczyński J, Jędrzejewska B, Dykyy I, et al. Phylogeographic history of grey wolves in Europe. BMC Evol Biol. 2010; 10.

Pilot, M. et al. Genome-wide signatures of population bottlenecks and diversifying selection in European wolves. Heredity 112, 428–442 (2014).

Pilot, M. et al. Widespread, long-term admixture between grey wolves and domestic dogs across Eurasia and its implications for the conservation status of hybrids. Evol. Appl. 11, 662–680 (2018).

Pilot, M., Moura, A.E., Okhlopkov, I.M. et al. Global Phylogeographic and Admixture Patterns in Grey Wolves and Genetic Legacy of An Ancient Siberian Lineage. Sci Rep 9, 17328 (2019).

Pimlott, D. H., editor. 1975. Wolves. Proceedings of the first working meeting of wolf specialists and of the first international conference on the conservation of the wolf. International Union for Conservation of Nature and Natural Resources, Morges, Switzerland.

Pires, Ana Elisabete & Amorim, Isabel & Borges, Carla & Simões, Fernanda & Teixeira, Tatiana & Quaresma, Andreia & Petrucci-Fonseca, Francisco & Matos, J. (2017). New insights into the genetic composition and phylogenetic relationship of wolves and dogs in the Iberian Peninsula. Ecology and Evolution. 7. 1-15. 10.1002/ece3.2949.

Poulle, M.-L. (1995) Le suivi des loups dans le Parc national du Mercantour. Bull. mens. Off. Nation. Chasse 201, 36-41.

Pullin, A.S., Knight, T.M., Stone, D.A., Charman, K., 2004. Do conservation managers use scientific evidence to support their decision-making? Biol. Conserv. 119, 245–252.

Quevedo, M., Echegaray, J., Fernández-Gil, A., Leonard, J. A., Naves, J., Ordiz, A., et al. (2019). Lethal Management May Hinder Population Recovery in Iberian Wolves. Biodivers. Conserv. 28, 415–432. doi:10. 1007/s10531-018-1668-x

Ramirez, O., Altet, L., Enseñat, C. et al. Genetic assessment of the Iberian wolf *Canis lupus signatus* captive breeding program. Conserv Genet 7, 861–878 (2006). https://doi.org/10.1007/s10592-006-9123-z

Ramírez, Oscar & Altet, Laura & Ensenyat, Conrad & Vilà, Carles & Sanchez, Armand & Ruiz, Alfredo. (2006). Genetic assessment of the Iberian wolf *Canis lupus signatus* captive breeding program. Conservation Genetics. 7. 861-878. 10.1007/s10592-006-9123-z.

Randi E (2008) Detecting hybridisation between wild species and their domesticated relatives. Mol Ecol 17:285–293

Randi E., Hulva P., Fabbri E., Galaverni M., Galov A., Kusak J., Bigi D., Bolfíková B.Č., Smetanová M., Caniglia R. 2014. Multilocus detection of wolf x dog hybridization in Italy, and guidelines for marker selection. PLoS One 9(1): e86409. DOI: 10.1371/journal.pone.0086409

Randi, Ettore. (2011). Genetics and conservation of wolves *Canis lupus* in Europe. Mammal Review. 41. 99 - 111. 10.1111/j.1365-2907.2010.00176.x.

Reding, Raoul & Gräber, Reinhild. (2021). Wolves back in Lower Saxony - The first 10 years of the recolonization.

Rhymer J. M. a Simberloff, D. (1996). Extinction by hybridization and introgression. Annual Review 31 of Ecology and Systematics, 27(1), 83–109.

Rigg, R., Findo, S., Wechselberger, M., Gorman, M.L., Sillero-Zubiri, C., Macdonald, D.W., 2011. Mitigating carnivore – livestock conflict in Europe: lessons from Slovakia. Oryx 45, 272–280, http://dx.doi.org/10.1017/S0030605310000074.

Savolainen P, Zhang YP, Luo J et al (2002) Genetic evidence for an East Asian origin of domestic dogs. Science 298:1610–1613

Schmidt, P. (1976) Das Wild der Schweiz. Hallwag, Bern, Stuttgart.
Ševětínský J., 1895: Dějiny lesů v Čechách [The history of forests in Bohemia]. Písek, 120 pp.

Silva PM. Historical demography and differentiation of the gray wolf (*Canis lupus*). Universidade do Porto, Portugal. 2016

Silva, P., López-Bao, J.V., Llaneza, L. et al. Cryptic population structure reveals low dispersal in Iberian wolves. Sci Rep 8, 14108 (2018). https://doi.org/10.1038/s41598-018-32369-3

Simberloff, D. (1996). Hybridization between native and introduced wildlife species: importance for conservation. Wildlife Biol. 2, 143–150. doi: 10.2981/ wlb.1996.012

Smetanová M, Černá Bolfíková B, Randi E, Caniglia R, Fabbri E, Galaverni M, Kutal M, Hulva P. (2015). From Wolves to Dogs, and Back: Genetic Composition of the Czechoslovakian Wolfdog.

Soroka, S.N., 2006. Good news and bad news: asymmetric responses to economic information. J. Polit. 68, 372–385.

Stronen, Astrid & Jedrzejewska, Bogumila & Pertoldi, Cino et. all (2013). North-South Differentiation and a Region of High Diversity in European Wolves (*Canis lupus*). PloS one. 8. e76454. 10.1371/journal.pone.0076454.

Stronen, A. V., Ruiz-Gonzalez, A., Jansson, E., Flagstad, Ø., & Andreassen, H. P. (2019). Human-caused environmental changes in European grey wolf populations: genetic diversity, population structure and wolf-dog hybridization. Frontiers in Ecology and Evolution, 7, 448.

Szewczyk, Maciej & Nowak, Carsten & Hulva, Pavel et. all (2021). Genetic support for the current discrete conservation unit of the Central European wolf population. Wildlife Biology. wlb.00809. 10.2981/wlb.00809.

Torres, R. T., and Fonseca, C. (2016). Perspectives on the Iberian Wolf in Portugal: Population Trends and Conservation Threats. Biodivers. Conserv. 25, 411–425. doi:10.1007/s10531-016-1061-6

Verardi A, Lucchini V, Randi E (2006) Detecting introgressive hybridisation between free-ranging domestic dogs and wild wolves (*Canis lupus*) by admixture linkage disequilibrium analysis. Molecular Ecology, 15, 2845–2855.

Vila C, Wayne RK (1999) Hybridization between wolves and dogs. Conserv Biol 13(1):195–198

Vila` C, Castroviejo J, Urios V (1993) The Iberian wolf in Spain. In: Promberger C, Schro¨der W (eds) Wolves in Europe-status and perspectives. Munich Wildlife Society, Ettal, pp 104–109

vonHoldt BM et al. 2010. Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. Nature 464:898–902.

vonHoldt BM, Pollinger JP, Earl DA, Knowles JC, Boyko AR, Parker H, Geffen E, Pilot M, Jedrzejewski W, Jedrzejewska B, et al. 2011. A genome- wide perspective on the evolutionary history of enigmatic wolf-like canids. Genome Res. 21:1294–1305.

Wang, L., Beissinger, T.M., Lorant, A. et al. The interplay of demography and selection during maize domestication and expansion. Genome Biol 18, 215 (2017). https://doi.org/10.1186/s13059-017-1346-4

Wayne RK, Jenks SM (1991) Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. Nature, 351, 565-568.

Wayne RK, Lehman N, Allard NW, Honeycutt RL (1992) Mitochondrial DNA variability of the grey wolf: genetic consequences of population decline and habitat fragmentation. Conservation Biology, 6, 559-569.

Wayne, R. K., & Shaffer, H. B. (2016). Hybridization and endangered species protection in the molecular era. Molecular Ecology, 25, 2680–2689.

Young, J.C., Searle, K., Butler, A., Simmons, P., Watt, A.D., Jordan, A., 2016a. The role of trust in the resolution of conservation conflicts. Biol. Conserv. 195, 196–202.

Zeder MA (2012) Pathways to animal domestication. Biodiversity in Agriculture: Domestication, Evolution and Sustainability, eds Gepts P, et al. (Cambridge Univ Press, Cambridge).

Zimen E, Boitani L (1975) Number and distribution of wolves in Italy. Zeitschrift Fur Saugetierkunde, 40, 102-112

Zimen E. 1978. Der Wolf: Mythos und Verhalten. Meyster Verlag GmbH, Wien, München.

Zlatanova, Diana & Popova, Elitsa. (2013). Habitat variables associated with wolf (*Canis lupus L.*) distribution and abundance in Bulgaria. Bulgarian Journal of Agricultural Science. 19. 262–266.