Abstract

The cave lion (*Panthera spelaea*), the grey wolf (*Canis lupus*) and the brown bear (*Ursus arctos*) all shared an intercontinental distribution across the northern hemisphere during most of the Late Quaternary, and experienced repeated events of climate change. The cave lion went extinct at the end of the Pleistocene and although the wolf and the bear have survived until present day, recent human persecution has caused demographic bottlenecks and local extinctions. In this thesis, mitochondrial and nuclear DNA was analyzed from ancient and modern samples in order to study spatiotemporal changes in genetic diversity in the three species. Mitochondrial sequences analyzed from 48 radiocarbon dated cave lion remains revealed two haplogroups, of which the more genetically diverse seemingly disappeared around 41,000 years BP. Serial coalescent simulations on the data supported a population bottleneck in Beringia between roughly 47-18,000 years BP. Its long duration prevents a specific causal factor to be singled out, but the early onset and overlapping declines of other large mammals in the region suggests that major environmental changes greatly impacted the fauna of Beringia during this time. Using a similar genetic marker, a set of 126 modern wolves and two Siberian wolf remains of Late Pleistocene age were analyzed. The sequences yielded from the latter samples pertained to a basal haplogroup, which contained all Late Pleistocene wolves from previous studies. As data from both modern and ancient wolves were combined, a pattern of decreasing genetic diversity was identified around the Pleistocene-Holocene transition. This decrease was further tested by serial coalescent simulations, which supported a bottleneck in northern North America around this time. Further analyses were applied to one of the ancient wolf remains from Siberia, producing a draft genome sequence and a complete mitochondrial genome. Given the radiocarbon date of the Siberian wolf, a slower mutation rate could be inferred, which pushed back the split between the lineages leading to modern wolves and dogs to at least 27,000 years BP. The Siberian wolf was positioned close to the split but basal to these lineages. A global comparison with modern dogs indicated a closer genetic affiliation between the Siberian wolf and some arctic breeds. For the brown bear, phylogeographic changes in Europe were studied over the last 50,000 years, using radiocarbon dating and mitochondrial sequences. When concatenated and compared with published data, the mtDNA revealed a turnover event just before the LGM, while the dating confirmed a presence of brown bears at relatively high latitudes during this period. Marked shifts in population size were also inferred. Furthermore, data of stable isotope levels confirmed a dietary shift to increasing herbivory around the LGM. Finally, a recent anthropogenic bottleneck among Scandinavian brown bears was studied. While no change in genetic structure could be detected, mitochondrial and microsatellite markers showed a decline in genetic diversity, especially pronounced in the southern subpopulation. ABC simulations supported a bottleneck taking place across all of Scandinavia. Taken together, this thesis have identified and elucidated several impacts on genetic diversity in the past populations of large carnivores. The use of different genetic markers has enabled comparisons with published data, but also revealed their comparatively different benefits and limitations. Overall, the presented studies compose a synthesis of past population dynamics in large carnivores, uniquely revealed by ancient DNA.
List of Papers


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I am also co-author on the following papers, which are not included in this thesis:


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“The long summer was over. For ages a tropical climate had prevailed over a great part of the earth, and animals whose home is now beneath the Equator roamed over the world from the far South to the very borders of the Arctics ... But their reign was over. A sudden intense winter, that was also to last for ages, fell upon our globe.”

— Louis Agassiz
Geological Sketches (1866)

**Introduction**

Climate and chronology

Change is a basis for evolution, and one of the most basic changes affecting the biosphere is that of the climate. Until very recently, global climate has been characterized by a long period of relatively mild and stable conditions that has spanned more or less the last 11,500 years, the entire Holocene era. Looking at a wider timescale, this is however something of an exception. Further back in time, the stable conditions were preceded by considerable variation, as well as long periods of dwindling temperatures. This pattern goes back to the start of the Pleistocene around 2.6 million years ago (Mya), which marked the onset of a relatively colder and drier climate globally (Lowe & Walker 2014). The cold periods, often described as glacials, were dominating from this point in time and only interrupted by shorter interludes of warmer interglacials. The climatic shifts profoundly altered landscapes across the globe, and many geological formations of today can be traced back to these events, which included both continent-wide glaciations and inundations.

The cyclic but irregular pattern of glacials and interglacials has been possible to trace several million years back in time, by studying levels of stable oxygen isotopes in marine sediments and glacial ice cores (Shackleton & Opdyke 1973; Dansgaard et al. 1993). The sequential variation of the isotope levels has been used as a proxy for changing global temperatures and has led to a chronologic division of Marine Isotope Stages (MIS), with odd numbers for interglacials and even numbers for glacials. Sediments from the Atlantic have also revealed layers of coarse gravel originating from terrestrial glaciers, which have been deposited after major discharges of ice bergs into the sea. These so called Heinrich events appear to have been quite infrequent but are thought to have triggered short periods of intense cooling (Heinrich 1988).

The general cooling that took place during the glacials have in many ways been associated with glaciers and ice. Accordingly, the last two glacials (MIS2 & MIS4), together with the intervening but generally cold MIS3, are together often referred to as the last Ice Age. The association with ice derives
from the great ice sheets that expanded across many regions during this time. In Europe, they covered large parts of Scandinavia and the British Isles, while northern North America held two bodies of ice, growing in parallel and merging at the continental divide (Clark & Mix 2002). Around halfway through MIS2, 25-18,000 years ago, the ice sheets reached their maximum extent, and this period has accordingly been termed the Last Glacial Maximum (LGM).

Widespread glaciers that have survived until the present also contain records of oxygen isotopes, which have offered a more detailed record of past climate change (Wolff et al. 2010). Ice cores from Greenland, containing climate records with relatively high resolution, have thus been used to identify shorter phases of cold and warm climate (Fig. 1), which have been termed Greenland Stadials (GS) and Interstadials (GI) (Svensson et al. 2006). A multitude of synonymous terms exist for many of these climate periods and phases, often differing between continents and geographic regions. One example is the last Greenland Stadial (GS1), occurring just before the start of the Holocene, which is often referred to as the Younger Dryas (Kennett 1990). Although it had a limited duration of just over a millennium, this cold phase brought back glacial conditions similar to those prevailing during the LGM along with re-expanding ice sheets.

![Figure 1](image1.png)

**Figure 1.** Chronology of the Late Quaternary with climatic periods and events over the past 60,000 years. The curve indicates levels of oxygen isotopes recorded in the NorthGRIP ice core from Greenland. Glacial Interstadials (GI1-17) and Heinrich events (HE1-5) are indicated. The Last Glacial Maximum (LGM) and the Younger Dryas (YD) are highlighted in blue (modified from Svensson et al. (2006)).
The LGM, although representing a maximum extent of ice sheets, was only the last peak in a long series of intense glacial periods, and ice sheets had lingered both at high latitudes and high altitudes throughout much of the Pleistocene (Lowe & Walker 2014). The accumulation of water into the massive glaciers contributed to a generally cool and dry climate with much lower sea levels than today. Over time, these conditions paved the way for expansive steppes, constituting a biome often referred to as “steppe tundra” or “mammoth steppe” (Guthrie 2001). The steppes stretched across the entire Eurasian continent all the way from the Iberian Peninsula in the west to Siberia in the east. Here they continued across Beringia, a northern landmass bridging Eurasia and North America in the Late Pleistocene.

The mammoth steppe seems to have harbored a varied vegetation with no contemporary analogs (Anderson et al. 1994; Zazula et al. 2003; Willerslev et al. 2014). It has often been described as a mosaic of different vegetation types, which together formed a vast interconnected ecosystem that allowed for a wide dispersal of many species. The fossil record has indeed shown that it contained a high degree of faunal diversity, including mammoths, horses, woolly rhinos and a multitude of other large mammals (Kurtén 1968). Several of these large-bodied animals, often described as megafauna, seem to have been highly adapted for this environment and played important roles in maintaining its ecosystem (Bocherens 2003; Zimov et al. 2012). In recent years, however, it has become increasingly apparent that the composition of the fauna was far from static, likely due to that the fluctuating climate repeatedly altered the environment (Hofreiter et al. 2007; Cooper et al. 2015; Palkopoulou et al. 2015).

Expansions and extinctions

The natural world of today is to a great extent shaped by the climatic upheavals that took place throughout the Pleistocene, including our own species, which emerged from Africa during this time period (Stewart & Stringer 2012). But at the same time as climate change brought opportunities for some, it pushed others to the brink of extinction and beyond. The outcome was mainly decided by the speed, duration and range of the changing environments, but also by the adaptability and ecological requirements of the different species within them (Stewart et al. 2010).

Glacials and interglacials alternatingly favored cold- and warm-adapted species, allowing them to expand their ranges across the continents. As the conditions shifted, this brought demographic changes in form of range contractions or expansions, bottlenecks and extinctions (Hofreiter & Stewart 2009). Many of these population dynamics have been traced in the fossil record, and with the application of radiocarbon dating it has been possible to obtain a more detailed chronology of these events (Stuart et al. 2009).
al. 2004a; Pacher & Stuart 2009). The fossil remains have also served as evidence of the animals that went extinct; mammoths, saber-toothed cats, woolly rhinos and Neanderthals to name a few. The final decline of many of these species of megafauna seem to have coincided and this is most apparently observed at the very end of the Pleistocene around 14,000 years before present (BP) (Stuart 1999). The cause behind this seemingly synchronized event is still a matter of debate, mainly whether it was mediated by humans, climate change or the two in combination (Koch & Barnosky 2006). Other causal factors have also have been put to the table, including hyper-disease or an extraterrestrial impact (MacPhee 1997; Firestone et al. 2007).

Even though the end-Pleistocene extinctions are conspicuous, not all megafauna died out synchronously. Many species disappeared long before the end of the Pleistocene (Stiller et al. 2010), while others survived in scattered and isolated populations into the Holocene (Vartanyan et al. 1993; Stuart et al. 2004b). The nature of these final disappearances and their causes have been the subject of numerous studies and there have been attempts to find common denominators behind them, often related to climate (Cooper et al. 2015). In some cases, the final disappearance seems to have coincided with human expansions (Martin & Steadman 1999). However, the arrival of modern humans has been difficult to pinpoint for many regions, as well as estimating their numbers and impact on the environment.

As many of the large mammals went extinct, this profoundly changed the ecosystems of which they had been a part, including conditions for the species that survived (Hofreiter 2007). The survivors too were directly affected by the climate fluctuations during the Pleistocene, and many species’ current distribution was largely shaped by the most recent shift to warmer conditions after the last glacial. Some of the cold-adapted species were able to track a shrinking habitat (Lagerholm et al. in prep.), while others went locally extinct (Dalén et al. 2007; Campos et al. 2010). Although general models have been proposed for the processes behind the distribution and diversity of modern populations (Hewitt 1996), these have also been opposed by more complex and species-specific scenarios (Stewart 2009).

Large carnivores

Large carnivores play a key role in the ecosystems they inhabit, dictating conditions for entire communities of other species (Flueck 2000; Ripple et al. 2014). This is primarily achieved through predation, creating trophic cascades from the top-down within the food web (Berger et al. 2001; Terborgh et al. 2001). More indirectly, the very presence of large carnivores can affect the behavior of prey and smaller predators (Ripple & Beschta 2004; Prugh et al. 2009; Pasanen-Mortensen et al.}

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Trophic cascades are also going the reverse way, bottom-up: As top predators these animals are highly dependent on the availability of prey and as a consequence, they are ultimately affected by changes at lower trophic levels or by species competing over these resources. Their large size further implies a general requirement for both large prey and large continuous habitats (Carbone *et al.* 1999; Sinclair *et al.* 2003). Together with low reproductive rates, this fact makes them vulnerable to the restrictions of resources, and this is especially true for more specialized species (Rode *et al.* 2015).

Figure 2. Cave lions depicted in Chauvet cave, France, dated to roughly 30,000 years ago (museum replica). By HTO [Public domain], via Wikimedia Commons.

Current distributions of large carnivores have been greatly reduced, and in terms of species, most ecosystems today are impoverished to a large extent (Hofreiter & Barnes 2010). This is in stark contrast to the Late Pleistocene, when many now extinct carnivores were still around. The carnivore guilds present during this time period were not only more diverse, but also included larger and more specialized species. One example is the sabretooth cats (*Smilodon* sp. & *Homotherium* sp.), which seems to have been highly adapted for killing large prey (Antón 2013). Other examples include the dire wolf (*Canis dirus*) and the short faced bear (*Arctodus simus*) of North America, both representing the largest contemporary members of their families. The generally larger size and more robust morphology of many Pleistocene carnivores can be seen as an adaptation for preying and scavenging.
on megaherbivores, which were likely not exempt from predation to the extent that they are today (Van Valkenburgh et al. 2015). The high abundance of different large carnivores also meant more intense competition over carcasses and more thorough consumption of these, which have been implied by a high frequency of dental fractures in carnivore remains from the Late Pleistocene (Van Valkenburgh & Hertel 1993). Even the fossil record of extant carnivores show trends of increased robustness going back in time from the Holocene (Leonard et al. 2007; Marciszak et al. 2015), which might be considered as adaptations to ecological niches that no longer exist.

Anthropogenic impact

To us humans, large carnivores hold a special status, since we among them have identified both our closest companions and most feared adversaries. Hunters have revered them, but also competed with them for prey, and though our view of these animals has changed over time, it has rarely been one of indifference. Cave art and anthropomorphic representations of carnivores from pre-history have indicated their importance in early human cultures (Fig. 2) (Chauvet et al. 1996; Dalton 2003). In many indigenous cultures, cults and rituals surrounding carnivores have prevailed, exemplified by the different kinds of bear worship observed across the Holarctic (Hallowell 1926; Pentikäinen & Tolley 2007). However, when negative attitudes have gained influence, this has led to devastating effects: Human encroachment and outright persecution have severely reduced the populations of large carnivores world-wide, especially over the last centuries (Hunter 2011). Conflicts have often emerged in regions where natural prey species have declined along with the introduction of domestic livestock (Treves & Karanth 2003; Ray et al. 2005). This pattern goes far back in time and seems intimately linked to the spread of agro-pastoralism and increasing human populations (Goudie 2013). In Eurasia, this process most likely started already with the Neolithic revolution in the Holocene. As farmland spread across the continents, large carnivores were forced to track the retreating forests and were confined to less fertile areas with lower human densities. In time, they became intimately associated with the wilderness and were even regarded as a threat to human society, civilization and religion (Pentikäinen & Tolley 2007; Pastoureau & Holoch 2011). Similar associations were made in North America by European settlers, especially farmers and livestock producers, who actively pursued large carnivores on their lands (Kellert et al. 1996). In Sweden, the trapping of large carnivores was encouraged already in legal texts from the Middle Ages (Jansson 1985). Later eradication campaigns were sponsored both by states and local governments, encouraging hunters and offering bounties for killed animals (Matthiessen 1987; Pohja-Mykrä et al. 2005). The eradication efforts in Europe impacted all carnivore populations, with the most dramatic decreases and range contractions taking place in the early 20th century (e.g. Fig. 3). The effects were most notable in the northern and western parts of Europe, where wolves for instance were practically exterminated (Mech & Boitani 2010).
Human impact has not exclusively consisted of hunting and persecution. Events of domestication and translocation have also affected large carnivores and their distribution. The best example being the domestication of wolves, which has not only sparked a wide array of dog breeds, but also mediated a spread of domestic dogs all across the globe. In a few cases, like the Australian dingo and the New Guinea singing dog, domestication was followed by translocations and return to a feral state (Corbett 1995). Beside wolves, few carnivores have submitted to domestication. Bears have for instance never been fully domesticated, but seem to have been occasionally tamed already in Mesolithic times (Chaix et al. 1997). There are also early records of keeping bears in enclosures (O’Regan 2002), and they were frequently used in Roman spectacles along with other wild animals (Kyle 2014). Translocations for this kind of entertainment have been hypothesized as the reason why brown bear remains from North Africa yielded a genotype that had previously only been found in Spain (Calvignac & Hughes 2008). The same purpose was probably behind a translocation of bears to Corsica in the 15th Century, where a population survived for around 200 years (Vigne 1988). Being prized game animals, bears have also been translocated for hunting, for instance to Bulgaria in the 1970’s and 80’s (Nowak et al. 2014).

As game animals, large carnivores have in some instances paradoxically been saved from extinction by hunters. One example is the legal protection of brown bears in Sweden, which was established on the initiative from hunters in the early 20th Century (Lönnberg 1935; Swenson et al. 1995). Starting around this time, attitudes toward wildlife in general were starting to change, inspiring efforts to
preserve natural environments (Ladle & Whittaker 2011). Although national parks and nature reserves were created as a result in many countries, large carnivores were mostly exempt from protection until the second half of the 20th Century. Reduced hunting and increasing urbanization did have positive effects in many regions, allowing the recovery of some populations. The recovery was also promoted by increased knowledge and appreciation of the ecological functions of large carnivores. However, negative attitudes are still common and controversies have frequently arisen between different interest groups regarding issues like livestock predation, hunting quotas and re-introductions (Dickman et al. 2013; Chapron et al. 2014).

Ancient DNA

The field of ancient DNA (aDNA) arose a mere three decades ago and studies were first applied to samples of historical age (Hiuchi et al. 1984; Pääbo 1985; Hänni et al. 1990). From the very start the focus was on retrieval and comparison of short DNA sequences from the mitochondria, the energy generating organelles within the cell. Amplification of these genetic markers was considerably facilitated by the introduction of the Polymerase Chain Reaction (PCR) method, which together with Sanger sequencing formed the foundation for production of aDNA data for a long time. These techniques opened up for the analysis of even older remains, but were unfortunately also susceptible to contamination, a fact that hampered the reliability of the results from many early studies. To counter these effects, new and higher standards of sterility were introduced as well as more rigorous testing to authenticate aDNA results (Cooper & Poinar 2000).

In spite of some early mistrust against aDNA, its application to genetic studies has revolutionized our insights into the past. By retrieving and analyzing old genetic material, the past diversity within species and populations has become increasingly better understood. While modern DNA has been successfully used to describe current diversity and relationships between extant populations, it has proven limited when inferring estimates of past demographic events and population histories (Hofreiter et al. 2004; Valdiosera et al. 2008). For this purpose, the introduction of ancient DNA has provided invaluable sources of information. Not only has it given access to genetic data from remains of considerable antiquity, but it has also elucidated links between now living species and those long extinct. In combination with radiocarbon dating it has enabled more detailed estimates to be made of past population dynamics both in terms of magnitude and timing. Moreover, by connecting genetic diversity to specific points in time, it has become possible to make more accurate estimates of mutation rates, which has been of great importance for establishing divergence times between lineages (Drummond et al. 2003).
By adding a spatial dimension to comparisons of genetic diversity, questions of phylogeography have also been possible to address. The term was coined in the late 1980’s and signifies the geographical distribution of genetic lineages or species (Avise et al. 1987; Avise 2000). Phylogeographic studies on modern samples have been hugely popular and covered a wide range of organisms. But the use of modern material has failed to describe past diversity and resolve historical processes that have led up to current phylogeographic patterns. However, these limitations have been overcome by targeting aDNA, which successfully has been used to track population dynamics, extinctions and re-colonizations as far back as the Late Pleistocene (Leonard et al. 2000; Shapiro et al. 2004; Thalmann et al. 2013; Horn et al. 2014). Together with data from other disciplines like archaeology, paleoclimatology and geology, aDNA has consequently facilitated connections to be made with possible causal factors behind past shifts in genetic diversity; anthropogenic, climate-induced or others.

Being far more abundant and accessible from ancient remains, mitochondrial DNA (mtDNA) has been used in a majority of past aDNA studies (Hofreiter et al. 2001b). Its relatively high mutation rate, together with its non-recombining mode of inheritance has also made this genetic marker ideally suited for estimating intraspecific diversity. However, the data retrieved from mitochondrial sequences is limited by only providing the evolutionary history of a single, maternally inherited locus. Given the uniparental inheritance, mtDNA consequently only describes female lineages, which might complicate extrapolations to be made for entire populations (Waits et al. 2000).

Since aDNA is available only in small amounts and inherently fragmented, usually to below 100 base pairs (bp) in length (Sawyer et al. 2012), a continuing difficulty has been to amplify longer sequences and thereby achieve greater genetic coverage from a sample. Most often, this has been resolved by amplifying several smaller, sometimes overlapping sequences. Although a straightforward approach, it can imply tedious, costly and time consuming lab work, requiring several re-amplifications in order to obtain reliable consensus sequences. Another way to achieve high genetic resolution that can facilitate comparisons within species and populations has been to target the most variable regions of mtDNA. One example, frequently used for studying intra-specific evolutionary relationships is the hypervariable control region, assumed to be made up of non-coding DNA with a relatively high mutation rate (Moritz et al. 1987). Despite these efforts, amplified sequences can provide varying resolution, which sometimes is not high enough for unambiguous comparisons to be made. For short sequences, the effect of identical mutations arising in different lineages, so called homoplasies, can also cause misinterpretations of population histories. In general, increasing sequence length thus provides better resolution and more well-supported phylogenies (Fig. 4).
Figure 4. The effect of sequence length on phylogenetic resolution, exemplified by median-joining network analyses based on mtDNA from brown bears (modified from (Keis et al. 2013)).

Many of these difficulties have been overcome with the advent of so called next generation sequencing (NGS) methods. Instead of targeting specific DNA fragments and priming sites, these methods, which allow millions of sequences to be analyzed simultaneously, incorporate all the genetic material from an extracted sample (Margulies et al. 2005). According to this principle, all DNA is prepared with specific oligonucleotides, which are attached as adapters to produce sequencing libraries (Meyer & Kircher 2010). Since especially short fragments can be sequenced with this approach, it has proven highly suitable for aDNA. It has also made it possible to sequence the much scarcer and more fragmented nuclear DNA from ancient remains, which has resulted in compilations not only of complete mitochondrial but also nuclear genomes (Green et al. 2010; Miller et al. 2012a; Thalmann et al. 2013; Palkopoulou et al. 2015).

Compared to the mitochondrial genome, data from the nuclear genome can provide significantly more information, not only as a result of its vastly larger size. Since nuclear DNA is inherited from both parental lineages and recombined in the offspring, a wider array of genetic analyses can be performed on this material, also revealing more complex processes like selection and admixture. However, given that sequences from the nuclear genome only exist in two copies per cell, establishing the correct genotypes can be challenging and is highly dependent on the available amounts of well-preserved endogenous DNA. Analyses are also constrained by available data from modern genomes, either from the same or closely related species, which can be used as a reference. The use of reference genomes hold many advantages and can facilitate comparisons, even with genomic sequences of low quality,
but may introduce biases depending on their taxonomic distance to the study species (Prüfer et al. 2010).

The amount of intact DNA available from organic remains is highly dependent on their preservation, and this puts a limit to which sub-fossil material that is suitable for analysis. As a result, obtained data is often suffering from low DNA coverage and uneven sampling. Extremely good preservation, like that found in permafrost or deep caves, along with improved technology have recently contributed to the retrieval of both nuclear and mitochondrial DNA several hundred thousand years old (Meyer et al. 2013; Orlando et al. 2013). However, most remains still yield DNA in low quantities and the ones that do, commonly fall within a timescale of tens of millennia. A similar time frame applies to radiocarbon dating, since the signal from radioactive carbon isotopes is entirely depleted after ca. 60,000 years (Libbey 1952).

The preservation of DNA shows a clear negative correlation with higher temperatures and as a consequence there is a latitudinal gradient of DNA survival with more favorable conditions at higher latitudes (Hofreiter et al. 2015). This effectively makes the retrieval of aDNA from material collected at lower latitudes and in continents like Africa, Australia and South America more difficult. The geographical delimitation not only excludes a vast number of species to be studied, but also excludes large parts of the current or past range of species with wide distributions.

Even under favorable conditions, animal remains and their DNA molecules degrade over time. The most apparent result is a continuing fragmentation of the DNA strands. This process is very much depending on the surrounding environment and its physical, chemical and microbial composition. These factors, together with the composition of the remains themselves, decide the rate of fragmentation, which has proven to be twice as fast for nuclear DNA compared to mtDNA (Allentoft et al. 2012). Degradation might also involve chemical alterations of the DNA molecules. The most common being cytosine (C) deamination, which produces uracil (U) and leads to misincorporations of nucleotides (C to T & G to A) during amplification (Hofreiter et al. 2001a). These nucleotide replacements may give rise to erroneous sequences and overestimated diversity (Axelsson et al. 2008). One way to overcome misincorporations and ensuing errors is to consistently make independent re-amplifications. Another way is to treat the DNA-extract with uracil-repairing enzymes, which helps to minimize the effects of this chemical damage (Briggs et al. 2007).

Low copy numbers after amplification, which can result from the often limited amounts of aDNA cause yet other problems; misreads and stochastic effects in the PCR that complicate interpretation of the data. Microsatellites are specifically affected by these uncertainties much due to their repetitive and variable structure, and can consequently produce erroneous genotypes as one or both alleles at a
locus are not amplified. This failure to detect alleles, referred to as allelic dropout, often gives an excess of estimated homozygotes, a problem which can only be avoided by confirming re-amplifications.

Along with degradation, ancient remains are also susceptible to contamination of exogenous DNA, and if the contaminating DNA originates from younger or modern material, there is a high risk of it outweighing or obscuring the endogenous DNA within in a sample. This has to be taken into consideration when selecting remains for aDNA analyses, and necessitates careful pre-treatment and cleaning of the material prior to extraction. Another precaution for avoiding contamination is the use of laboratories with sterile environments specifically designed for processing aDNA. Low yields of endogenous DNA from ancient material has also led to evaluations of DNA content in different skeletal remains (Adler et al. 2011). The results have generally favored more compact material, which have been shown to provide better yields of DNA as well as protection from exogenous contaminants (Pinhasi et al. 2015).

The nuclear DNA recovered from ancient remains can be studied in a number of ways, most of which are not relying on complete genomes. A number of more restricted markers have commonly been used to identify polymorphic loci and compare these between populations and individuals. The most basic are Single Nucleotide Polymorphisms (SNPs), which constitute variants of single base pairs at specific positions. SNPs are versatile and can be chosen from across the genome, but do only represent a restricted and a pre-defined range of diversity. Other markers that have been extensively used for studies in population genetics are microsatellites, which consist of sequences of short tandem repeats. These sequences are found throughout the genome and are hotspots for mutations that occur during replication, thus containing great variation within populations (Ellegren 2004). One of the most recent methods developed for selective targeting of DNA, is the use of RNA-baits that can capture selected fragments during a hybridization process, a method which can be applied both to genomic and mitochondrial sequences (Burbano et al. 2010; Carpenter et al. 2013; Enk et al. 2014).

**Stable isotope analysis**

In addition to preserved DNA molecules and data from radiocarbon isotopes (\(^{14}\)C), stable isotopes of different accumulated elements can also be used in the study of ancient remains. Levels of stable isotopes create a signature in tissues that can provide estimates of several factors of an animal’s environment and ecology. Two of the most commonly used isotopes are \(^{13}\)C and \(^{15}\)N, which have principally been used to characterize average diet. Estimates of diet composition may further indicate relations between carnivores and their prey as well as resource partitioning among both carnivores and
herbivores (Darimont et al. 2007). The results from this type of studies are often presented in bivariate graphs, usually showing ratios of δ¹³C and δ¹⁵N, where individuals appear as points. This spatial definition within two isotope gradients can be described in terms of ecological niches – the so called “isotopic niche space” (Newsome et al. 2007). By measuring isotopic ratios of the same tissue from several species within a particular habitat, it is possible to characterize the isotopic (and subsequently ecologic) niche held by these animals, especially considering their diet and trophic level (Bearhop et al. 2004). The use of isotopic niche space can further unravel patterns below the species level, for instance detecting specialization between and within populations (Bolnick et al. 2002; Major et al. 2007; Vander Zanden et al. 2010).

As with aDNA, the properties of the paleontological remains, like contamination and degree of preservation, often restrict analyses of stable isotopes. Additional factors must also be taken into account, most fundamentally being variation in isotope abundance between different environments, which can be influenced by factors like altitude, temperature, precipitation or bedrock type (Bowen 2010). Consequently, geographical regions may differ in terms of isotope signatures, with their own distinct composition, which also varies through time (West et al. 2010).

Data analyses

A great number of different tools are available for analyzing genetic and genomic data, from relatively simple statistical measurements of genetic diversification to simulations of complex population scenarios. To establish phylogenetic relationships and identifying phylogeographic patterns, the most common approach has been to construct phylogenetic trees and networks. In aDNA studies, these have traditionally been based on short mtDNA sequences (Leonard et al. 2000; Barnes et al. 2002), which was also the case for most of the studies presented in this thesis. Since a majority of the samples had directly inferred ages from radiocarbon dating, this also provided reliable connections between genetic diversity and time, which could subsequently be applied in the analyses for estimating mutation rates. This approach thus enables an internal calibration of the molecular clock, which otherwise has to be estimated from divergence times based on the paleontological record (Drummond et al. 2002). The latter method evidently presents a high degree of uncertainty and the two methods have shown considerable disparity, with the former producing comparatively elevated rates. The observed time-dependency in rates estimated using internal calibration has been well established (Ho et al. 2011), with a number of underlying factors proposed (Penny 2005; Debruyne & Poinar 2009).

A fundament for establishing evolutionary relationships based on genetic diversity is the coalescent theory (Hudson 1990), which describes population histories backwards in time. It follows DNA
sequences back to common coalescing points, which represent their most recent common ancestor. The process can accommodate several factors affecting a population, including changes in size and mutation rate. In software simulating the coalescent processes, different settings/priors can thus be applied for testing a variety of population models, which will subsequently be scored according to the fit of the output to that of the observed genetic data. The phylogenetic software BEAST (Drummond et al. 2005), applied in all five studies, contains options for both reconstructing phylogenies and estimating change in effective population size (N_e) over time. Along with input of genetic sequence data, a number of coalescent priors can readily be defined and adjusted for the specific population under study. For estimating population size changes, a set of skyline plot methods are also available, which combine data on lineage coalescent with defined time points to create graphs of effective population size through time (Strimmer & Pybus 2001). These function has been continuously updated with increasingly versatile methods (Minin et al. 2008; Drummond et al. 2012; Gill et al. 2013).

In order to test different population scenarios, including splits, bottlenecks and more elaborate models, coalescent simulations can be performed using Approximate Bayesian Computation (ABC) methods (Beaumont 2010). Output from the simulations in the form of summary statistics is compared to those from the observed data, producing estimates of posterior probabilities. Models are accordingly dismissed or graded according to likelihood with different support values. The addition of temporal data and estimated mutation rates have enabled more reliable and detailed models to be constructed, and several software including this and other flexible functions have proven suitable for aDNA data (Bayesian Serial SimCoal (Excoffier et al. 2000; Anderson et al. 2005), BaySICS (Sandoval-Castellanos et al. 2014) and DIYABC (Cornuet et al. 2008)).

Simulations and model fitting can be used for most genetic and genomic markers. However, when applied to a wider genomic scale, this approach becomes computationally intensive and less straightforward. A simpler option consists of testing and comparing shared derived alleles between four taxa, where an excess of shared alleles between two taxa indicates gene-flow. This can be detected using D statistics, which is an approach that has been extensively used for genome-wide comparisons of aDNA data (Green et al. 2010; Cahill et al. 2013).

**Study species**

The cave lion (*Panthera spelaea*)

Fossil remains of a lion-like cat found in Tanzania points to an African origin of lions in the late Pliocene (5-1.8 Mya) (Turner & Antón 1997; Werdelin & Lewis 2005; Barnett et al. 2006). In Europe,
The earliest presence of lions dates from around 700 thousand years (kyr) BP, and represents a large form commonly named *Panthera fossilis* or *P. leo fossilis* (Turner & Antón 1997), which constitutes the most probable ancestor of the cave lion. In the Late Pleistocene, the cave lion’s range stretched all across northern Eurasia, and even into North America. Here a separate population was isolated south of the glacial ice sheets, and is referred to as the American lion (*Panthera atrox*) (Turner & Antón 1997; Sotnikova & Nikolskiy 2006).

The cave lion was one of the top predators among the Pleistocene megafauna. Based on its dental characteristics, it has been described as a hypercarnivore (Wroe & Milne 2007) and stable isotope studies have revealed that prey specialization was common (Bocherens et al. 2011). Although decisively larger, it seems in many ways to have been a northern counterpart to its now living relative, the modern lion (*Panthera leo*) (Yamaguchi et al. 2004; Christiansen 2008). Even though little is known about its appearance and social life, some features have been revealed from Paleolithic art and through skeletal remains (Guthrie 2005; Mazák 2010). The most well-preserved remains have also provided genetic material, which has confirmed their close relatedness to modern African and Asiatic lions (Burger et al. 2004; Barnett et al. 2009). These studies, based on mitochondrial DNA, have estimated a divergence between the cave lion and the modern lion going back ca 600 kya. However, in terms of classification there is yet no consensus and the cave lion has either been described as a separate species or as a subspecies (*Panthera leo spelaea*).

During the Pleistocene, a contact zone with modern lions existed in the Near East, but so far no signs of genetic interchange have been discovered. Modern lions did later expand their distribution in this region, into Anatolia and the Balkans in the early Holocene. Their presence here was even testified to well into historical times by Greek writers like Herodotus and Aristotle (Bartosiewicz 2009). By that time cave lions had vacated all of their former range. Along with so many other large mammals, they went extinct at the end of the Pleistocene, around 14 kyr ago. The latest radiocarbon dated remains has been found in Alaska, and suggests eastern Beringia as their last refuge (Stuart & Lister 2011).

**The grey wolf (Canis lupus)**

Wolves are iconic carnivores and the largest extant members of the canid family. Although highly social and pack living, their reproduction within a pack is typically restricted to a dominant pair (Fox 1971). Wolves share a common ancestor with coyotes (*Canis latrans*), and the split between the two species has been estimated to between 4.5 and 1.8 Mya (Nowak 2003). In the early Pleistocene, wolves seem to have shared a common radiation with several related canids across Eurasia (Azzaroli 1983), resulting in a vast geographical distribution, which can still be observed today. The species is
currently spread across most of the northern hemisphere, exhibiting great variation in both size and coat color (Wang et al. 2010), a variability which has given rise to the description of a great number of subspecies. In contrast, their genetic structure seems to rarely conform to the proposed subspecies divisions (Vila et al. 1999). The geographical proliferation still seems to have been accompanied by adaptations to a wide range of environments and prey. With the use of various genetic markers, recent studies have identified populations structured according to specific habitats (Carmichael et al. 2001; Geffen et al. 2004), suggesting that genetic structure in wolves can arise through “isolation by environment” (Leonard 2014; Wang & Bradburd 2014). Analyses of functional genes have further supported this suggestion, which have been most clearly demonstrated in arctic environments (Schweizer et al. 2015).

When mtDNA diversity alone has been studied in wolves, it has revealed little geographic structure on a global scale (Vila et al. 1999). However, the addition of aDNA has shown a discontinuous history of mtDNA lineages, with a distinction of two major haplogroups, one containing all samples from the Late Pleistocene along with a small subset of modern Eurasian wolves (Leonard et al. 2007; Pilot et al. 2010). In North America, modern wolves are conversely affiliated to the other group, together with a majority of current populations. Interestingly, the genetic turnover in American wolves seems to have been accompanied also by a change in phenotype, from a robust to a more gracile form (Leonard et al. 2007).

At some point during the Late Pleistocene wolves came into contact with modern humans, and were incorporated into their societies as the domestic dog (Canis l. familiaris). The evolutionary relationship between wild wolf populations and different dog breeds has been the topic of several genetic studies, often with a focus on trying to identify the location and timing of domestication (Vilà et al. 1997; Savolainen et al. 2002; Germonpré et al. 2009; Larson et al. 2012; Thalmann et al. 2013; Freedman et al. 2014). The results have been contradictory, with a great variation both in estimations of time and space for such events. Some have pointed to a temporal origin of dogs around 16-11 kya (Axelsson et al. 2013; Larson et al. 2014), while others have favored an interval up to ca. 30 kya (Thalmann et al. 2013; Wang et al. 2013). A complicating factor to the relationship between wolves and dogs has been the frequent hybridizations between the two, both in the wild as well as mediated by humans (Muñoz-Fuentes et al. 2010; Godinho et al. 2011). For wolves, hybridization has been confirmed not only with dogs, but also with coyotes (Fain et al. 2010), and several populations have been strongly influenced by this kind of gene-flow. One example is the Eastern/Great Lakes wolf in North America, which shows signs of both ancient and recent admixture with coyotes (Koblmüller et al. 2009).
The brown bear (*Ursus arctos*)

The brown bear is a member of the order Carnivora, sharing a common descent with other mammalian carnivores. In dietary terms, however, it is far from a distinct carnivore and better described as a generalist, exploiting a wide range of nutritional sources, from grass and berries to moose calves, salmon and carcasses (Mattson *et al.* 1991; Mowat & Heard 2006). The brown bear is probably the least specialized ursid, also when it comes to choice of habitat, and has the widest distribution among all living bear species. Its distribution was even wider in the past and has gradually been reduced as a consequence of human expansion and hunting (Servheen *et al.* 1990). Some isolated populations have gone completely extinct in historical times, like those on the British Isles and in North Africa (Hamdine *et al.* 1998; O’Connor & Sykes 2010).

In the Late Pleistocene, the range of the brown bear overlapped with that of the cave bear (*Ursus spelaeus*) in Europe and western Eurasia. As the name suggests, the cave bear seems to have been more dependent on caves for winter hibernation, and many cave sites have yielded overwhelming amounts of its remains (Rabeder *et al.* 2000). Paleontological studies have placed the divergence between these two bear species at around one million years ago (García 2004; Madurell-Malapeira *et al.* 2009), and while some genetic studies have supported similar estimates (Loreille *et al.* 2001; Korsten *et al.* 2009), others have favored a split already at about three million years ago (Krause *et al.* 2008). The most recent split however, is that between the brown bear and the polar bear (*Ursus maritimus*). The timing of this split has been contentious and was initially estimated to a mere 150 kyr ago, based on mitochondrial DNA (Lindqvist *et al.* 2010). However, the mitochondrial DNA of polar bears has proven to fall within the diversity of brown bears and introgression of both mitochondrial and genomic DNA from polar bears have been detected in modern and ancient brown bear populations (Edwards *et al.* 2011; Cahill *et al.* 2013, 2015). More comprehensive genomic studies have recently established the monophyletic division between the two species (Hailer *et al.* 2012; Miller *et al.* 2012b), as well as provided a divergence time of around 400 kyr BP (Liu *et al.* 2014).

The phylogeography of brown bears has been well studied for several regions, often showing strong geographical structure in mtDNA variation (Taberlet & Bouvet 1994; Miller *et al.* 2006; Hirata *et al.* 2013). One explanation for this is the moderate dispersal exhibited by female brown bears (McLellan & Hovey 2001; Stoen *et al.* 2006). As a consequence, mitochondrial haplotypes often display a marked geographical structure/clustering, since only females propagate the mitochondrial lineages. Given its relatively stable phylogeography, the brown bear has also been used as a model species for genetic studies on more long term changes and the colonization of new territories. The first population
level study on brown bears using ancient DNA was based on ancient remains from North America (Leonard et al. 2000). The results revealed a decreasing diversity over time and no structure among the oldest samples. A similar lack of structure has been proposed for brown bears in Europe during the Late Pleistocene (Hofreiter et al. 2004). In contrast, the current pattern seen in Europe is highly structured; two clades (clades 1 & 3) are clearly separated between the south-west and the north-east. The western clade has been further divided between a western (1a) and an eastern (1b) subclade, which are thought to represent two separate populations that survived the LGM in different glacial refugia; the Iberian and the Italian/Balkan Peninsulas (Davison et al. 2011).

**Research objectives**

The aim of this thesis was to study changes in genetic diversity in large carnivores across space and time, to assess the population dynamics behind these, and identify underlying ecological factors. More specifically, the objectives were to:

I) Formally test a proposed bottleneck taking place in the population of Late Pleistocene cave lions, by including more data and simulating diverse population models.

II) Better describe the global phylogeography of grey wolves and test past population scenarios by adding and comparing mtDNA data from both modern and ancient wolves.

III) Based on the first retrieved draft genome sequence of a Late Pleistocene carnivore, investigate the genetic relationship between an ancient wolf and modern canid populations.

IV) Reconstruct the phylogeography of European brown bears, to test a proposed scenario of current genetic structure being the result of a re-colonization from isolated glacial refugia, and study ecological factors behind observed dynamics.

V) Investigate the genetic consequences of an anthropogenic bottleneck in the Scandinavian brown bear population, by comparing mitochondrial and nuclear diversity in samples from historic and modern bears.

**Summary of papers**

**Paper I**

A previous study on the phylogeography of modern and ancient lions showed indications of a reduced diversity in Late Pleistocene cave lions, explained as potentially the result of a demographic bottleneck (Barnett et al. 2009). To examine the past population dynamics in the species, ancient DNA data from 14 specimens of cave lion were added to 34 previously published sequences. After
extraction, two mitochondrial fragments were amplified following Barnett et al. (2009); a 143 bp long sequence of the ATP8 gene and a 215 bp long sequence of the control region (CR). Bayesian phylogenies were constructed using MrBayes (Huelsenbeck & Ronquist 2001) and a median-joining network was constructed. Both phylogenetic analyses displayed a division into two haplogroups, temporally separated but overlapping to some extent; Haplogroup I contained a comparably higher amount of variation, both in terms of haplotype and nucleotide diversity, and was only represented in samples older than ca. 41 kyr BP. Haplogroup II on the other hand was less genetically diverse and consisted of haplotypes radiating out from a common core haplotype (Fig. 5). From around 50 kyr BP, this second group persisted until the last recorded specimen at the very end of the Pleistocene, and was at this time only represented by two haplotypes (Fig. 5).

A subset of the data taken from Beringia, the most well-sampled region, was tested for different population scenarios with serial coalescent simulations, using Bayesian Serial SimCoal (BaySSC) (Anderson et al. 2005). Out of eight models, the three most supported were further explored with BaySICS (Sandoval-Castellanos et al. 2014). The results favored a model of population decrease of at least a 2-fold magnitude followed by a re-expansion. The timing of this bottleneck was inferred to have taken place between 47-18 kyr BP, which concurs with the pattern revealed from the phylogenetic analyses. Since no human presence in Beringia has been recorded as far back in time as the start of this bottleneck, it seems more likely that a major environmental change was the cause behind this demographic event. It is interesting to note that several other large mammals went through similar declines in genetic diversity in Beringia around this time. Although a more precise synchrony of these declines is not discernable, the period coincides with marine isotope stage (MIS) 3, well known for repeated climate changes of considerable magnitude (Voelker 2002).

![Figure 5. Control region haplotypes in the cave lion distributed in a) a median-joining network and b) a temporal chart with distributions of haplogroups I and II indicated, and estimated extent of the bottleneck shaded.](image)
Paper II

Although numerous, phylogeographic studies on wolves have predominantly been focusing on Europe and North America (Pilot et al. 2006; Weckworth et al. 2010). By adding new samples from geographical areas that had not been well studied previously or at all, we aimed to make a global comparison of genetic diversity within the mitochondrial control region. Two Late Pleistocene wolf remains collected from sites in the Taimyr Peninsula in northern Siberia were also included, which were studied along with data from both modern and ancient wolves in order to examine temporal changes in genetic diversity. 122 samples of contemporary wolves were analyzed from Scandinavia, Russia, China, Siberia, Iran and North America, including six samples (two of which were of historical age) from the previously unstudied Greenland subspecies (Canis lupus arctos). By combining six sequentially overlapping CR-fragments and using primers applied in a previous study (Savolainen et al. 2002), a 582 bp long alignment was created. The ancient wolf samples from Taimyr as well as the samples from Greenland were also amplified and sequenced separately in a laboratory in Copenhagen.

Among the sequences retrieved from the analyzed samples 15 previously undescribed haplotypes were discovered. These were further concatenated with matching sequences of both ancient and modern wolves from previous studies, resulting in three alignments of varying length (57-582bp), which were used for phylogenetic and network analyses. Baysian analyses were employed using the software BEAST (1.8.0) (Drummond & Rambaut 2007), to create a phylogeny including both modern and ancient wolves. Measures of genetic diversity were also calculated and temporal comparisons of haplotype diversity and abundance over time were made.

From the phylogenies and networks a wide spatial distribution of several wolf clades was revealed, but also some showing a more restricted geographical affiliation. All ancient samples predating the Holocene, including the two from Siberia, were distinctly grouped at basal positions in the phylogenies (Fig. 6). These Pleistocene samples exclusively showed a close relationship to a few clades of modern wolves, none of which are currently represented in North America. In order to explore this temporal pattern, coalescent simulations under an approximate Bayesian framework were performed. Three models were tested in BaySSC (Anderson et al. 2005) to explore demographic histories for two subsets of the data representing Eurasian and North American wolves. The output provided support for an end-Pleistocene population bottleneck in northern North America. Since a decrease in genetic diversity associated with the disappearance of a distinct ecomorph has been reported in eastern Beringia at this time (Leonard et al. 2007), the simulation result lends further support for this event.
Figure 6. Maximum clade credibility tree of grey wolves based on alignment A (558 bp). Late Pleistocene samples are noted with numbers 1-10.

Paper III

In order to more thoroughly investigate the relationship between Late Pleistocene wolves and their modern wild and domestic counterparts, further analyses were applied to one of the ancient wolf samples originating from the Taimyr Peninsula (Paper II). Genomic libraries were constructed and sequenced on an Illumina HiSeq 2500 platform to an average 1-fold sequencing depth of the nuclear genome. The sequencing also yielded the mitochondrial genome, which was assembled to an average 182-fold sequencing depth. When combined with published mitochondrial genomes of modern and ancient canids (Thalmann et al. 2013), the Siberian wolf’s position could be determined within a Bayesian phylogeny, confirming its basal position as seen in the previous study (Paper II). SNP’s identified in the genomic sequence retrieved from the libraries were then compared to matching sets from both dogs and wolves, including data from SNP’s and 6 modern canid genomes (Vonholdt et al. 2010; Vaysse et al. 2011; VonHoldt et al. 2011; Freedman et al. 2014). Applying D statistics (Green et al. 2010) to the data with an Andean fox as outgroup, revealed a symmetrical relationship of the Siberian wolf to both modern dogs and wolves. Further analyses using admixture graph models which
included gene-flow between dogs and wolves, gave consistent support for the Siberian wolf representing the basal lineage, or diverging just before the split between the lineages leading to modern dogs and wolves (Fig. 7). Given the radiocarbon date of the Siberian wolf (ca. 35,000 years BP) its equal distance to the two modern lineages entailed a significantly older divergence between the ancestors of modern dogs and wolves than previously assumed. Based on demographic simulations and the known age of the Taimyr sample, the genome-wide mutation rate was also estimated in order to further investigate the divergence time between modern dogs and wolves. The mutation rate was found to be substantially slower than the one assumed in an earlier study (Freedman et al. 2014), which in turn resulted in a recalibrated split time somewhere between ca. 40-27 kyr BP. $D$ statistics was also used to compare the Siberian wolf’s genome to the most extensive dataset of SNP’s from modern dogs and matching data from 15 modern day wolves (Vaysse et al. 2011). The output clearly indicated a closer relationship between the Siberian wolf and two arctic breeds; Siberian Huskies ($p=9x10^{-6}$) and Greenland Sledge Dogs ($p=1.6x10^{-4}$). Overall, these results provided a first comparison between a Late Pleistocene carnivore and its modern relatives, based on extensive genomic data. Although not defining a domestication event, the results further strengthens previous hypotheses of wolf domestication having taken place earlier than 16 kya, and even before the LGM (Germonpré et al. 2009; Ovodov et al. 2011; Druzhkova et al. 2013).

![Figure 7. Simplified graphical outline of the Siberian wolf’s relationship to modern dogs and wolves.](image-url)
Paper IV

Mitochondrial DNA from modern European brown bears has revealed a limited diversity and a pronounced geographic structure, with two clades occurring in the south-west (clade 1) and north-east (clade 3). Furthermore, a geographic subdivision has been made within clade 1 (1a & 1b), which has been proposed to be the result of a separation between different refugial populations in southern Europe during the LGM. The post-glacial re-expansion of these populations is thought to have created the current phylogeographic structure, and this process has been used to exemplify the Expansion/Contraction model of populations responding to climate change and the availability of habitat (Hewitt 1996, 1999). However, DNA from ancient brown bear remains has cast doubts on this model and indicated a more complex phylogeographic structure before as well as after the LGM (Hofreiter et al. 2004; Valdiosera et al. 2007, 2008).

In order to test these hypotheses and investigate temporal changes of the mentioned mitochondrial clades in Europe, more than 300 samples of European brown bear remains were collected and analyzed. A short sequence from the mitochondrial control region, previously used in a majority of studies on ancient brown bears was amplified. The confirmed sequence data was combined with published sequences, and direct or inferred ages were obtained for 121 of the samples. Both network and phylogenies were constructed and geographical distributions of haplotypes were chronologically analyzed. The results showed a high diversity comprising four distinct mitochondrial clades before the LGM in Europe. Little geographic structure could be distinguished and a significant population turnover was identified just before the LGM. In contrast to the previous assumption of bears being confined to traditional southern refugia during the LGM, radiocarbon dated samples from Belgium and France confirmed the presence of bears here during this period. A Bayesian SkyGrid analysis further revealed several fluctuations in estimated population size (Fig. 8). First a marked increase synchronized with the warming of Greenland Interstadial 1, and later an almost 10-fold decrease, starting in the mid-Holocene. Measurements of stable nitrogen isotopes from the remains indicate a shift towards increasing herbivory around the LGM in Europe. This dietary shift has previously been suggested to result from such a niche becoming available through the extinction of the more herbivorous cave bear (*Ursus spelaeus*) between 30-25 kyr BP (Münzel et al. 2011; Bocherens 2015). More available habitats in the wake of the cave bear’s extinction might also be linked to a slightly increasing trend shown in the SkyGrid plot, starting just before 30 kyr BP (Fig. 8). Thus, Effects from inter-specific competition and human activities may have had a greater impact on European brown bears than climate change.
Figure 8. Bayesian SkyGrid plot of estimated changes in effective population size through time in European brown bears. Highlighted in blue; the Last Glacial Maximum (LGM) and Younger Dryas (YD), in pink; Greenland Interstadial 1 (GI-1). Inset map shows the geographical distribution of radiocarbon dated remains from the LGM along with their haplogroup assignment.

Paper V

During the 19th Century, intensified hunting caused the Scandinavian brown bear to suffer a major decline in population size (Fig. 3), down to an estimated 130 animals as fewest (Swenson et al. 1995). Protection was legally established in the early 20th Century and allowed the bears to slowly recover and re-expand. The present-day population has been the focus of a number of genetic studies, which among other things have revealed a latitudinal division between two mitochondrial lineages that meet at a distinct contact zone in central Scandinavia (Taberlet & Bouvet 1994; Taberlet et al. 1995). Other studies incorporating microsatellite data have further shown the existence of three or four geographically separate subpopulations within Sweden and relatively high levels of diversity (Waits et al. 2000; Manel et al. 2004). However, previous studies have not been able to resolve to which degree the demographic bottleneck in the 19th Century impacted the contemporary genetic structure and diversity. To address this issue, we used mitochondrial and microsatellite DNA markers from historical and modern brown bear samples to estimate genetic changes across the bottleneck.
A multivariate analysis along with simulations showed the same genetic structure before and after the bottleneck, with three subpopulations in southern, central and northern Scandinavia. In terms of allele frequencies, a significant change could however be seen in the southern subpopulation. Mitochondrial diversity was also severely reduced, with falling haplotype numbers across the bottleneck. Except for a decline in allelic richness in the southern subpopulation, the decline in autosomal genetic diversity was less apparent. To test different population scenarios, we used approximate Bayesian computations on the microsatellite data, which showed a clear support for a bottleneck affecting all subpopulations, expressed as a sharp decline in effective population size followed by a recovery. In conclusion, we could show that both mitochondrial and nuclear diversity was negatively affected by the bottleneck and that this was most clearly expressed in southern Scandinavia, where bears were probably more intensively hunted. The static structure of three subpopulations might be better confirmed by more extensive sampling from the central and northern regions, but the data at hand suggests that the genetic structure could have been established by historical ecological processes instead of recent anthropogenic persecution.

Figure 9. Geographical comparison of mtDNA diversity in Scandinavian brown bears, between the 19th/early 20th Centuries and modern times. The dashed lines mark the contact zone between the two lineages (N & S).
Concluding remarks

The study species, which represent three of the most proliferated families of large mammalian carnivores, all emerged and expanded during the Pleistocene. The aDNA retrieved from their remains revealed several marked population dynamics over time on different scales. The dynamics, as characterized by the results, appear both similar and quite different between the three. Due to limited sampling, the declines discerned from the data were supported in restricted geographical regions; Beringia (cave lion), northern North America (wolf) and Europe (brown bear). Nevertheless, some common patterns can be observed within these regions. The estimated timespan of the bottleneck impacting cave lions in Beringia for instance (Paper I), also contained a temporal disappearance of brown bears in the eastern parts (Alaska). The fossil record of cave lions here also display a similar hiatus ca. 40-25 kyr BP. Wolves on the other hand, appear to have suffered a bottleneck only at the very end of the Pleistocene in this region, as described by the simulated models in Paper II. Although the simulations did not support a similar bottleneck in Eurasia, this might be due to a lack of ancient Eurasian samples with high genetic coverage. However, the symmetrical relation between the Siberian wolf at ca. 35 kyr BP and all modern wolves (Paper III), lends support to this scenario, suggesting a widespread bottleneck having taken place at some point between 35 kyr BP and the start of the Holocene, when wolves in North America were isolated through the inundation of the Bering land bridge.

In Europe, cave lion remains are missing between roughly 29-19 kyr BP (Stuart & Lister 2011), which overlaps with turnovers observed in many other species (Cooper et al. 2015), including the brown bear (Paper IV). However, bears were evidently present in Europe throughout this period, with the only distinguishable gap in the fossil record between ca. 32-28 kyr BP. At the end of the last glaciation, cave lions re-expanded across their range (Paper I), but this trend lasted only for a few millennia and was cut short by their final extinction at the end of the Pleistocene (Stuart & Lister 2011). In contrast, European brown bears showed signs of a commencing increase around the same time (Paper IV).

In terms of genetic diversity, all three study species have been previously studied, but to different extent. For the grey wolf and the brown bear, temporal comparisons have been possible to make with modern populations, while the long extinct cave lion has been represented by much fewer samples, all dated to the Late Pleistocene. Thus, a prerequisite for most of our analyses has been genetic data, publicly available from previous studies and online databases like GenBank (www.ncbi.nlm.nih.gov/genbank/). The use of these datasets has also influenced the choice of methods and genetic markers, which have been applied to produce matching data, whether of mitochondrial or autosomal origin. This approach was specifically used for all studies except in Paper
III, where comparisons were also highly depending on the relatively abundant genomic data available from modern canids.

The genetic markers have generally been targeting short mitochondrial sequences. This has imposed certain restrictions on our produced data, especially limited resolution and support for phylogenetic clades/haplogroups. In Paper II, where alignments of different sequence lengths were compiled, the effects on the networks clearly illustrates this problem, as shorter sequences resulted in the merging of haplotypes (see also Fig. 4). A similar effect can be seen when comparing the Bayesian phylogenies in Paper II and Paper III, with significantly stronger support for the latter, based on full mitogenomes. The use of genomic data also enabled analyses including hybridization, which was a necessary component for establishing the relation of the Late Pleistocene wolf to modern canids (Paper III).

In spite of the restricted coverage achieved from short mtDNA sequences, these markers provided sufficient data for many phylogeographic comparisons and estimates of population dynamics. The output from the analyses was also highly affected by the species’ different behavior in terms of dispersal. This is clearly exemplified by the global comparison of mtDNA diversity in wolves, which showed little or no structure (Paper II), whereas brown bears, characterized by strong female philopatry, displayed marked geographical patterns even on regional scales (Paper IV & V). Some populations stood out as especially distinct among the study species and could be linked to specific geographical regions, indicating long-term isolation. Examples were the Himalayan and Indian wolf lineages (Sharma et al. 2004; Aggarwal et al. 2007) as well as the American lion (Panthera atrox) (Barnett et al. 2009). Among European brown bears, clade 1e, unique to the Iberian Peninsula and extinct today, may similarly have formed as a result of prolonged isolation. However, the opposite pattern, with widespread haplotypes, was also common in all three species. Some haplotypes were found across the Eurasian range, a few even co-occurring in Eurasia and North America. Wolf populations were highly characterized by this pattern, but it could also be observed in cave lions (haplotype B) and brown bears (clade 3).

Despite limited genetic resolution in the mtDNA datasets, several demographic scenarios were largely supported by coalescent simulations as well as analyses using other genetic markers. In Paper V for instance, the dramatic decrease in haplotype diversity observed across the bottleneck in southern Scandinavia (Fig. 9) was reflected, albeit to a lesser extent, by the microsatellite data from this subpopulation. Additional support for the decrease has also been found in a recent study, where a similar decline was observed in Y-chromosome haplotypes in southern Norway during the same time period (Schregel et al. 2015).
In some cases, other measurable changes seem to have accompanied those seen in genetic diversity: The genetic turnover observed before the LGM in European brown bears did roughly coincide with an increase in herbivory, as indicated by the stable isotope analyses (Paper IV). A general decrease in body size has also been suggested for brown bears in Europe, comparing Holocene and Late Pleistocene specimens (Marciszak et al. 2015). A more well-supported change in morphology is that observed in Late Pleistocene wolves. Wolves in eastern Beringia have been characterized as more robustly built compared to those of the modern population, and were genetically distinct from the latter (Leonard et al. 2007). The disappearance of this population at the end of the Pleistocene coincided with the bottleneck inferred in Paper II, as well as the extinction of cave lions in this region (Stuart & Lister 2011). Like the cave lions, the Beringian wolves seem to have been adapted for preying on the co-occurring megafauna, which likely restricted them to the mammoth-steppe habitat that supported these species. As a consequence, this may also have rendered them less able to adapt as this ecosystem disintegrated. The changing environment across the northern hemisphere at the end of the Pleistocene might thus be seen as an indirect cause to the demise of both the cave lion and the Beringian wolf. Stable isotope analyses from a recent study have also indicated a more constrained diet in cave lions compared to wolves, and revealed a pattern of more conservative feeding niches of carnivores in Beringia compared to Europe (Yeakel et al. 2013).

Although humans might have competed with all three study species already during the Late Pleistocene, no anthropogenic impacts can confidently be linked to the observed declines during this period. In the Holocene, however, both bears and wolves appear to have been affected by expanding human populations, a process that can be traced as far back as 5,000 years ago for European brown bears (Paper IV), and that clearly escalated as more efficient hunting techniques were invented (Paper V).

Taken together, the recovered data from ancient remains presented in this thesis has revealed a number of declines and changes in genetic structure, which can help to understand the evolutionary processes in large carnivores. The results also highlight the importance of aDNA as a highly useful tool for discovering and describing past events within populations and species, which would not have been detected from modern material alone. These techniques also provide a means to compare past ecosystems to modern ones, and ultimately understand the continuity in between. Ever evolving methods and technology hold the promise for improving this understanding.
**Svensk sammanfattning**

Genom att utvinna och analysera genetiskt material från subfossila lämningar av olika djurarter kan man undersöka en mängd aspekter av deras evolutionära historia. Den här avhandlingen undersöker dynamik, släktsskap och ekologi hos olika populationer av tre stora rovdjur; grotteljon (Panthera spelaea), varg (Canis lupus) och brunbjörn (Ursus arctos). Resultaten är främst baserade på mitokondrie-DNA, men även på nukleärt DNA och stabila isotoper från insamladt benmaterial. Åldern på lämningarna har mer specifikt kunnat bestämmas med hjälp av $^{14}$C-datering och tidsskalan sträcker sig ända till den bortre gränsen för denna metod – ca 50,000 år tillbaka i tiden. Denna senkvartära tidsrymd innefattar både den Holocena, samt senare delen av den Pleistocena tidsepoken, en tid som utmärktes av dramatiska klimatförändringar med låga medeltemperaturer. Genom att använda analyser som jämför lämningarnas geografiska ursprung, den genetiska diversiteten vid olika tidpunkter samt information om samtida ekologiska förhållanden, har avsikten varit att hitta mönster som kan visa på kopplingar mellan populationernas dynamik och förändringar i deras miljö. Även interaktioner mellan arter samt mänsklig påverkan har tagits upp för att uppskatta om/hur dessa faktorer inverkat. I en del fall har vi utgått från tidigare studier, som antytt förändringar i vissa geografiska områden (*Paper I*) eller slagit fast att en flaskhals inträffat (*Paper V*). I andra har vi mer förutsättningslöst undersökt genetisk diversitet på en global (*Paper II*) eller temporal skala (*Papers III & IV*).


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Contributions

Paper I
I took part in the design of the study. All laboratory work was performed by me and I helped with the data analyses. The paper was written by me, with input from the co-authors.

Paper II
I helped to design the study and performed laboratory analyses on the two Late Pleistocene wolf samples. I also conducted all data analyses, except for the coalescent simulations, which were done together with Yvonne Chan. I wrote the manuscript with help from the other authors.

Paper III
This study was based on the DNA extractions I did for paper II. I further helped building the Illumina libraries. The analyses of the mitochondrial genome were performed by me, and I also participated in writing the paper.

Paper IV
For this manuscript I helped to design the study and to formulate hypotheses. I collected samples from museum collections around Europe, on which I did all DNA extractions, amplifications and sequencing. I also conducted all the computational analyses on the data, interpreted the results and wrote the manuscript with input from the co-authors.

Paper V
I helped to design the study and to formulate hypotheses. I collected all the historical samples, and conducted laboratory and data analyses together with George Xenikoudakis. I also took part in the work on the Bayesian simulations as well as in interpreting the results and writing the paper.
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