ANIMAL MOVEMENT ON SHORT AND LONG
TIME SCALES AND THE EFFECT ON GENETIC
DIVERSITY IN COLD-ADAPTED SPECIES

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Animal movement on short and long time scales and the effect on genetic diversity in cold-adapted species

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The thesis is based on the following papers, which are referred to in the text by their Roman numerals:


Candidate contributions to thesis articles*

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* Contribution Explanation
  Minor: contributed in some way, but contribution was limited.
  Significant: provided a significant contribution to the work.
  Substantial: took the lead role and performed the majority of the work.
I am also a co-author of the following article, which is not included in this thesis but was published during my doctoral studies at Stockholm University:

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Introduction

The genetic diversity within species and the way it is geographically structured is strongly affected by population size and movement across the landscape. In small and isolated populations, neutral variation is lost over time through the random process of genetic drift (Nei et al. 1975) and this stochasticity may also cause differentiation between different parts of the range. Mutations and, most importantly, gene flow are counteracting forces that bring new variation to a population and can disrupt local genetic structures over large areas.

Many northern species today exhibit cyclic population dynamics, with substantial fluctuations in numbers between years. This could have complex genetic outcomes, where on one hand the pronounced drift during the low phase leads to comparatively low diversity (Elton 1924; Nei et al. 1975), while an increased dispersal during peak years potentially could act as pulses of gene flow that maintains a high genetic variation (e.g. Ehrich & Stenseth 2001; Norén & Angerbjörn 2014).

Current patterns of variation are not only shaped by contemporary gene flow, but have also been influenced by movement on longer time scales. This is perhaps most obvious in the arctic and alpine regions that were covered by continental glaciers during the height of the last Ice Age, ca 20 thousand years ago. Consequently, modern populations should all be descendants to immigrants from the surrounding areas that colonised these regions following the ice retreat. The mode of these post-glacial colonisations have had a great impact on modern species diversities, and to get a more comprehensive understanding of taxa it can thus be important to look deeper into their histories (Hewitt 2000).
The past holds the key to the future

The last 2.6 million years of the earth’s history, called the Quaternary, has been characterised by large-scale climatic fluctuations. Long ice ages have been repeatedly interrupted by shorter interglacials, in transitions that during the more recent part of the Quaternary (Fig. 1) came to occur in 100 thousand year cycles (Imbrie et al. 1992). These followed a saw-toothed pattern, where full glacial conditions were reached after long periods of gradual cooling and build-up of ice volume, while the transitions to interglacial conditions have been characterised by rapid warming (Broecker & van Donk 1970). The last switch from the Pleistocene glacial period to the current interglacial period, the Holocene, occurred 11,700 years before the present (yr BP), and ice core data have shown that half of the total 15 ºC temperature increase occurred within less than 15 years (Taylor et al. 1997). Continued climate warming, this time induced by humans since the start of the industrial revolution, is now being suggested to have pushed the Earth into the Anthropocene epoch (Waters et al. 2014).

Figure 1. Past climate changes. (A): Temperature fluctuations over the last four glacial cycles, obtained from the Vostok ice core in Antarctica (modified from Petit et al. 1999). The top shaded area indicates the Pleistocene period in blue, with the Late Pleistocene in darker blue, as well as the Holocene period in red. (B): Northern hemisphere temperature fluctuations over the last 140 thousand years, obtained from several Greenland ice cores (modified from Johnsen et al. 2001).
The Quaternary climate fluctuations caused substantial rearrangements of habitat and species distributions. These range dynamics have generally been regarded as expansions and contractions, where taxa advanced and retreated in a north/south direction over time (Hewitt 1999; Hewitt 2000). However, climate changes often result in a combination of the two contrasting processes, and what determines if the total distribution of a taxa will increase or decrease is simply whether the expanding range is more successful than the contracting one (Jackson & Sax 2009). For instance, cold-adapted species are expected to have had their greatest distributions during periods of climate cooling when they expanded their ranges to more southern and lowland areas, but this would also have been coupled with retreats from some regions at the northern or alpine limits of their distribution as these became covered by the growing ice sheets (Hewitt 2001; Stewart et al. 2010). These increases and declines in species distributions also led to changes in connectivity, and it has been proposed that the recurrent population fragmentation and isolation in refugia led to accelerated evolutionary rates during the Quaternary (Hewitt 1996; Rand 1948). However, this has been difficult to evaluate since modern genetic data and fossil morphology often lack sufficient resolution to specify the exact locations and timings of past speciation events (Ho et al. 2011; Hofreiter & Barnes 2010; Lister 2004).

An underlying assumption to these expansion/contraction dynamics, originally made by Darwin (1859) and later revived by Eldredge (1989), is that taxa are able to track their habitat as it is shifting in space due to climatic changes, thus avoiding the need for adaptation. However, this simplified view has recently been challenged, and it is uncertain to what degree taxa are really able to keep pace when the rate of habitat rearrangements is too high (e.g. Kerr et al. 2015). One important prerequisite for successful shifts to new areas could be the geographic connectivity between suitable habitat patches (Hodgson et al. 2012), especially for species with low dispersal capacity. Further, inter- and intraspecific interactions might also affect the ability to track shifts in habitat, and to do so with small mismatches in relation to climate. The
potential rate of dispersal for taxa is consequently not always equal to their actual rate of range shift. For instance, the expansion of a species into a new area might be slowed down by the preceding response of its prey or the persistence of declining resident taxa (Jackson & Sax 2009), thereby forcing it to linger in areas of unfavourable environmental conditions and increasing the risk of population extinctions. Similarly, the early colonisers could obstruct the subsequent arrival of conspecific individuals from more distant parts of the range through competition (Hewitt 1996; Hewitt 1999). A climate-induced expansion of a dominant species can conversely also drive the contraction of a competitor’s range (Hersteinsson & MacDonald 1992).

The concept of habitat tracking is also problematic from a genetic perspective, especially when it comes to declines in available habitat. More specifically, we cannot assume that a range contraction equals to a population contraction, in the sense that the genetic variation at the trailing range margin survives as the species’ range decreases (Hewitt 1996). For cold-adapted species, this means that populations that have been at the leading edge during a glacial expansion are not necessarily involved in the shifts back to northern areas during the transition from glacial to interglacial conditions. Climate warming might then rather cause extinctions of these southern populations, and instead it is only the individuals at the species’ northern margin that are involved in the shift back to refugial areas (Bennett et al. 1991). The trailing edge is in this case vanishing, more than contracting, and assuming that the genetic variation within the given species is geographically structured, such a pattern of local extinctions and colonisation by few founders would deplete the gene pool (e.g. Hewitt 1996).

A parameter that has greatly influenced the genetic diversity in cold-adapted species is consequently how they coped with the rapid climate warming at the last glacial/interglacial transition. This a key question to resolve, not only to improve the knowledge of our current biota but also to ensure reliable predictions of future changes, since the effects of on-going climate warming are expected to be most severe in northern biomes (Pithan & Mauritsen 2014; Urban 2015). It has historically been difficult to study the genetic effects of past range contractions, since modern phylogeographic analyses
only provides information about the history of surviving lineages. However, recent years’ development in the field of ancient DNA has started to open the black box, and a growing number of studies have retrieved genetic information from the fossil remains of cold-adapted animals that had expanded populations at the end of the last glaciation. Apart from the famous megafauna extinctions that occurred during this time period (e.g. Barnosky et al. 2004), it is becoming clear that also the surviving arctic species were severely affected by the post-glacial habitat transformations (Hofreiter & Barnes 2010). In fact, many studies have revealed unique lineages in the non-refugial areas that did not endure until the present, and large losses of genetic diversity following the glacial/interglacial transition, suggesting that local extinctions of southern populations were the dominant process for cold-adapted species during past climate warming (Campos et al. 2010; Dalén et al. 2007; Palkopoulou et al. 2016).

Objectives
To ensure the future viability of cold-adapted species in an era of climate warming, it is crucial to understand not only their present status and habitat requirements, but also how they respond to environmental alterations. The last transition from glacial to interglacial conditions here provides a unique case study, enabling researchers to analyse genetic changes over time and how these are related to the palaeoclimate record. However, a majority of these studies have focused on charismatic large mammals, and the inferences might then be complicated by the coinciding expansion of humans across Eurasia. Small herbivores, which likely were much less affected by prehistoric hunting, therefore constitute an appealing complement that allows analyses of the pure climate effect on past population changes. In this thesis, I have focused on three types of cold-adapted herbivores; true lemmings (Lemmus), ptarmigan (Lagopus), and hares (Lepus), whose modern distributions stretch from the exposed tundra to the subarctic moorlands and taiga. With their complementing ecologies, they together make a good model system to investigate the effects of climate-induced range shifts in the northern biome. Although their remains are abundant in
the late glacial fossil record of Europe, they have so far received little interest from palaeogeneticists. In fact, birds in general have often been overlooked as a potential source of genetic information due to that their hollow bones are expected to contain poorly preserved DNA. Their flight capability, however, adds an important dimension to the study of habitat tracking, since it could enable a less restricted dispersal across fragmented landscapes.

**Paper I** of this thesis investigates the importance of lemming dispersal on a short time scale in shaping modern patterns of genetic diversity, while climate-induced dispersal and isolation on longer time scales have been the topic for **Paper II** (lemmings), **III** (ptarmigan) and **IV** (hares).

**Study species**

Lemmings constitute the basic resource in many northern ecosystems, and together with co-occurring prey species like ptarmigan and mountain hares they are vital for the function of the Arctic ecosystems. During the last glacial period, these taxa had widespread populations across the vast tundra-steppes of midlatitude Europe, and the rich collections of predator-assembled fossil remains suggest that they played a key role in the food chain also during the last Ice Age (e.g. Averianov 2001; Nadachowski 1982; Tyrberg 1998). While morphological species identification is possible for the ptarmigan (*Lagopus*) and hare (*Lepus*) bones, the identity of the remains from true lemmings (*Lemmus*) is often unclear.

The Norwegian lemming (*L. lemmus*) is one of two *Lemmus* species that lives in Europe today. It inhabits the Fennoscandian mountain tundra (Hansson 1999) where it is the only endemic mammal. This restricted range (Fig. 2) has for a long time caused speculations about the species’ origin, since the region is thought to have been completely glaciated during the Last Glacial Maximum, LGM (Ekman 1922). Although this would imply that the Norwegian lemming originated during post-glacial times, the species is morphologically and genetically too distinct to have originated from its closest living relative, the Siberian lemming *L. sibiricus*, at the end of the
Pleistocene (e.g. Fedorov & Stenseth 2001). Apart from the mysterious history, the Norwegian lemming is also famous for its dramatic population cycles. The very rapid increases during peak years are facilitated by the species’ ability to breed all year round which is likely an adaptation to the long winters of the north (Ims et al. 2011). These peaks can occur in synchrony across much of its range, with individuals sometimes moving over 100 km down into forest and farmland regions (Hansson 1999; Henttonen & Kaikusalo 1993). Although dispersing lemmings may overwinter in such lowland valleys, there are no records of uphill return movements to their core tundra habitat, something which would be necessary to enable gene flow among mountain tundra areas separated by forest (Østbye et al. 1993). Another special feature of the Norwegian lemming is the local migrations that usually take place between their summer and winter habitats (Henttonen & Kaikusalo 1993). This has been explained by the strong seasonality in its mountainous habitat and does not occur in the other *Lemmus* species, which live in the relatively flat and moist arctic tundra of Russia and America that allows suitable environmental conditions during both summer and winter. In fact, Ekman proposed in 1920 that this behaviour evolved during the last glacial period when the species was hypothesised to have survived in ice-free coastal areas of Norway (Ekman 1920). Since that time, the Norwegian lemmings’ high mobility has been intensively investigated, but no studies have analysed its impact on the genetic structure and variation of the species.

The *Lagopus* genus is represented by two sister species in Europe, the willow (*L. lagopus*) and rock (*L. muta*) ptarmigan, which are thought to have diverged approximately 1 million years ago (Drovetski 2003). Their modern ranges (Fig. 2) mainly comprise high latitude regions, where rock ptarmigan occupies the more exposed rocky terrains with sparse vegetation, and willow ptarmigan the lower altitudes where birch forest and willow shrubs dominate the landscape (Svensson 1999). In addition, rock ptarmigan also have isolated mountain populations in the high-alpine regions of Southern Europe. Both species are sedentary ground-dwelling birds, and except for willow ptarmigan in the British Isles (*L. lagopus scotica*) they all moult to a white winter plumage (Freeland et al. 2007). This loss in the British Isles is
probably a recent Holocene adaptation to the milder winters in this region where a snow camouflage is often disadvantageous. Interestingly, modern ptarmigan of both species are generally lighter built than their Ice Age counterparts, possibly suggesting differences in diet or degree of sedentariness (Bochenski 1985; Stewart 1999). So far, however, it has not been resolved whether this is due to adaptive changes to post-glacial environmental conditions, or if the fossil remains in fact represent specialised glacial lineages that are now extinct.

The mountain hare (Lepus timidus) has a disjunct modern European distribution alike that of the rock ptarmigan, although the hare has a broader ecological niche that also includes the taiga zone (Fig. 2). Further, all hares except the Irish (L. timidus hibernicus) also develop a white winter coat during the autumn. Along its southern range margin, the cold-adapted mountain hare overlaps in distribution with the temperate European brown hare (L. europaeus), which often results in competitive exclusion of L. timidus by its dominant cousin (Thulin 2003). Having had a continuous glacial distribution across the Mammoth steppe biome (Kahlke 1999), the species has thus experienced large range shifts and contractions during post-glacial times (Thulin 2003). However, even though having a fragmented distribution today and despite having low natal dispersal rates and high site fidelity (Dahl & Willebrand 2005), the mountain hare has a large genetic diversity with surprisingly little structure between the isolated populations (Melo-Ferreira et al. 2007). This has sparked a debate regarding the phylogeographic history of the species (Hamill et al. 2006).
Figure 2. Modern European ranges of Norwegian lemmings (A), mountain hares (B), willow ptarmigan (C) and rock ptarmigan (D).
Methods

Samples and DNA extraction

For Paper I, modern Norwegian lemming samples were collected from throughout the species’ complete Fennoscandian distribution (Fig. 3A). The material mainly consisted of tissue samples stored in ethanol that were taken either from trapped individuals or lemming carcasses between the years 1997 and 2012. In addition, the data set was complemented with museum samples from areas where no fresh material was available, and consisted of bone or tissue material collected between 1960 and 2007. Samples were also gathered from the Norwegian lemmings’ sister species, the Siberian lemming (*Lemmus sibiricus*), to serve as a reference to the level of intraspecific differentiation within Fennoscandia.

For Papers II, III, and IV, we collected bone remains of lemmings, ptarmigan and hares from a range of palaeontological sites across mid-latitude Europe, situated in the former tundra-steppe region that stretched between the Scandinavian and the southern alpine ice sheets during the Late Pleistocene glacial period (Fig. 3B). These had previously been identified to species or genus level, and spanned an age of between 48 thousand (k) years before the present (yr BP) and up until the final glacial/interglacial transition, ca 10 kyr BP. These remains were found in areas outside, or in exceptional cases at the edge, of the study species’ current distributions and might thus either represent extinct lineages, or direct ancestors of modern arctic and alpine populations that succeeded in tracking their habitats as these shifted at the end of the Pleistocene. In addition to the midlatitude Late Pleistocene samples, we also included early to mid-Holocene (3-8 kyr BP) lemming bones from a northern, previously glaciated, cave site located within the modern range of the Norwegian lemming.
Figure 3. (A) Geographic locations of all modern Norwegian lemming sample sites that produced nuclear DNA for the data analyses of Paper I. (B) Palaeontological sample sites from which successful ancient mitochondrial DNA could be retrieved (Paper II-IV). Lemming sites are shown in yellow, willow and rock ptarmigan in red and blue and mountain hare in green. The shaded areas indicate the maximum extent of the major Scandinavian and alpine ice sheets during the Last Glacial Maximum (Mangerud et al. 2011; Svendsen et al. 2004).

A subset of the Norwegian and Siberian lemming samples included in Paper I was used as modern reference material for the analyses in Paper II. For Papers III and IV, published sequence data from earlier phylogeographic studies provided a comprehensive representation of modern ptarmigan and hare populations across the Holarctic. In addition, I further complemented the ptarmigan dataset with new tissue samples from areas where no published sequences were available.

DNA from the modern tissue material was extracted using either a GeneMole MG10-000 robot with the MoleStrips DNA Tissue kit (Mole Genetics), or manually using Qiagen’s QIAamp DNA mini kit or DNeasy tissue kit. The bone samples were first powdered with a pestle and mortar or sampled with a multi-tool drill. DNA was then extracted from the bone powder following the protocols described in Palkopoulou et al. (2013) or Fernandez et al. (2006). Due to post-mortem degradation, the DNA that is preserved in fossil remains (called ancient DNA, aDNA) is fragmented and occurs in few copy numbers. These extractions therefore followed protocols
specifically developed to purify low quality samples and minimise the presence of inhibitors in later amplification steps.

DNA amplification and sequencing

Microsatellites were chosen as the most suitable marker for Paper I to investigate the genetic structure in modern Norwegian lemming populations. These are short non-coding repeats that occur across the nuclear genome, and their high mutation rates have made them popular to study neutral levels of intraspecific variation. Since there were only a limited number of markers available from related species, we developed twelve new microsatellite primer pairs at Ecogenics (Zurich-Schlieren, Switzerland) from a number of representative Norwegian lemming samples according to the method described by Abdelkrim et al. (2009). The extracted DNA was then amplified with the polymerase chain reaction (PCR) in three multiplexes, each containing four fluorescently labelled primer pairs, using Qiagen’s Type-it Microsatellite PCR Kit. Genotyping of the products was performed on an ABI 3130xl with the GeneScan 500 LIZ size standard, and the alleles were scored using GeneMapper v.4.0 (Applied Biosystems). After evaluations of the obtained genotypes, only nine of the twelve developed loci were used for the final data analyses.

The genetic analyses in Papers II, III and IV are based on mitochondrial (mt) DNA, since this occurs in higher copy numbers than nuclear DNA and thus is very suitable for ancient material (Hofreiter et al. 2001). It is maternally inherited as a single locus, and since variation only arises through new mutations it gives a poor resolution to detect individual-level differences. However, it has been the marker of choice for investigating deeper phylogenetic relationships within species, including those described in this thesis. To enable comparisons with these previously published modern sequences, we targeted the same mtDNA regions for our aDNA datasets. For the ptarmigan study (Paper III) this meant parts of the mitochondrial control region (CR) while for the lemming and hare studies (Papers II and IV) parts of both the CR and the cytochrome b gene (CytB) were analysed. However, due to the fragmented nature of aDNA it was not
possible to use the methods developed for modern samples, and instead the chosen regions had to be amplified in shorter overlapping fragments using specifically designed primers and PCR protocols developed for material with low quantity DNA. The modern samples that had been collected to complement the published sequence datasets were also amplified using these new primers. All PCR products were purified and sequenced for both the forward and reverse strands using an ABI 3130xl (Applied Biosystems) or a MegaBace 1000 (GE Healthcare).

Ancient DNA precautions

Due to the degraded nature of aDNA there is an increased risk of contamination as well as sequence errors. To ensure reliable results it is therefore necessary to take certain precautions. All pre-PCR work on the glacial remains was carried out in the ancient DNA laboratory at the Swedish Museum of Natural History. I contributed to designing and building this laboratory as an initial part of my thesis work, and consequently I did not have to deal with the potential risk of contamination that may result from previous studies of related taxa. Further, the laboratory is physically isolated from the modern DNA and post-PCR facilities, and all lab equipment and working surfaces were frequently sterilised with UV light, bleach or hydrochloric acid. Blanks were extensively used in both extraction and PCR reactions in order to monitor possible contamination, and results from a minimum of two independent amplifications were required to enable the identification of nucleotide misincorporations that otherwise lead to erroneous DNA sequences. The pre-PCR work on the Holocene lemming bones from Norway was performed in the ancient DNA laboratory at Laboratoire d’Ecologie Alpine in Grenoble, France. No rodent samples had been analysed there prior to this study, and equivalent sterilisation procedures and use of negative controls were carried out as in the Swedish laboratory. The sequences obtained from these samples only displayed variation in nucleotide positions that had also been observed to be variable in extant lemming populations. Since such a pattern is unlikely to be caused by
random PCR misincorporation, these samples were therefore not considered necessary to replicate through multiple amplifications.

Radiocarbon dating

The bone remains analysed in this thesis all came from excavated sites whose deposits had been previously studied by palaeontologists and archaeologists. Consequently, the stratigraphic layers were often well described and age determined, both by direct radiocarbon dating of selected remains as well as inferred from the general faunal and palynological composition of the deposits. Nevertheless, we chose to send eleven lemming bones that had produced good quality DNA (included in Paper II) for dating at the Oxford Radiocarbon Accelerator Unit. Due to their small size though, only five samples contained enough collagen to give reliable age estimates.

Data analyses

The modern lemming dataset analysed in Paper I was divided into different geographic sub-regions, for which a number of standard population genetic statistics were calculated. To investigate how well these pre-defined areas represented the true genetic structure of the species we performed different clustering analyses, both using only the “blind” genetic data as well as including geographic coordinates for all the samples. These coordinates were further used to analyse patterns of isolation-by-distance across the Norwegian lemming’s range, and the so called “genetic patch size” which describes the geographic extent of detectable genetic structure.

The mtDNA analyses performed in Papers II, III and IV aimed to clarify the evolutionary relationships between the modern populations and the old bone remains. I performed phylogenetic tree reconstructions and skyline plots of temporal demographic changes using the software BEAST (Drummond et al. 2005) and/or built haplotype networks with the R-script TempNet (Prost & Anderson 2011). The approximate ages of the old samples were used as priors in the BEAST analyses, which allowed for
internal calibrations of the molecular clock (Drummond et al. 2002). This is an additional advantage of using ancient DNA data, since it produces more accurate divergence time estimates than traditional inferences that rely on fossil-calibrated mutation rates (Ho et al. 2007).

In order to evaluate the most likely evolutionary histories of all the study species, we used simulations coupled with Approximate Bayesian Computation (ABC) to evaluate different population scenarios. Just as the BEAST analyses, these simulations are based on coalescent theory, which traces individual sequences backwards in time to the point when they coalesce (Hudson 1990). In brief, we simulated datasets consisting of a large number of genealogies under different evolutionary models. Summary statistics from these datasets were then compared to our observed data, and using a rejection procedure and the proportions of accepted simulations in each model, we could select and further analyse the most probable historic scenario (Beaumont et al. 2002).

The genetic analyses in Paper III were further complemented with species distribution modelling (SDM), in order to investigate in more detail the connections between past habitat alterations and ptarmigan population responses. Climate data from throughout the species’ current ranges, as well as from palaeontological sites with Lagopus spp. remains, was used to calibrate the models and predict temporal changes in available habitat over the last 20 thousand years. Further, we also used SDM to look forward in time and make projections of ptarmigan distributions in a world of continuing climate warming.
Results

Movement on a short time scale

The modern microsatellite study (Paper I) revealed that Norwegian lemmings have a high genetic variation, and a surprisingly weak geographic structure among the analysed sub-regions. The differentiation was mainly seen as a south-west to north-east gradient across Fennoscandia, and the clustering programs identified two major genetic clusters located in southernmost Norway and Sweden, on one hand, and in the Kola Peninsula, north eastern Norway and Finland on the other. The central parts of the lemmings’ distribution comprised a broad transition zone consisting of individuals with gradually admixed assignment proportions (Fig. 4). The existence of a genetic cline was further supported by a pattern of isolation-by-distance observed across the species’ range. Spatial autocorrelation analyses suggested a genetic patch size of approximately 100 km, meaning that lemmings generally are more similar to each other within this range than to a randomly sampled individual from the complete distribution. When comparing the scale of genetic differentiation between the Fennoscandian sub-regions, I found that for a given geographic distance the southern populations were more similar to each other than the populations were in the north-eastern part of the range. In summary, these results are consistent with that lemming dispersal during peak years acts as pulses of gene flow that homogenise the gene pool over vast geographic areas. Further, we propose that the higher differentiation found between northern subpopulations can be explained by the longer periods of interrupted cyclicality reported from this region (Angerbjörn et al. 2001), which have increased the isolation of lemmings in different mountain tundra areas.
Figure 4. Results from the genetic cluster analyses. The upper panel shows the barplot obtained from the software Structure for the selected value of K=2, with individuals ordered along a south-west to north-east gradient. The lower panel geographically display the admixture proportions in the three genetic clusters inferred from the software TESS, with the X and Y axis indicating longitude and latitude.

Movement over longer time scales

Paper II investigated the evolutionary history of the Norwegian lemming, by comparing mitochondrial genetic variation in modern Fennoscandian (L. lemmus) and Siberian (L. sibiricus) individuals with that retrieved from 23 European lemming bone remains of up to 48,000 yr BP in age. The results showed that the glacial populations had a large mtDNA diversity that appeared to be geographically structured, but that they were genetically divergent from the two modern species. Further, both the phylogenetic reconstructions and the coalescent simulations supported a pre-LGM divergence time between the midlatitude populations and modern Norwegian lemmings, therefore making a postglacial colonisation of Fennoscandia from these surrounding areas highly unlikely (Fig. 5). Whereas the star-like L. lemmus haplogroup appears to have been absent in the areas south of the Scandinavian ice sheet up until the final de-glaciation, it is found in a cave in Norway less than 3.5 thousand years later, often in the form of the modal haplotype. Bone remains of Lemmus sp. dating back to a previous interstadial ca 36 kyr BP have previously been reported from Norway.
(Larsen et al. 1987), and the aDNA results from this study strongly suggests that the endemic Norwegian lemming descends from a small population that locally survived the LGM in one or more restricted ice free areas. One could speculate that such a prolonged refugial isolation under extreme climate conditions might have caused rapid evolutionary changes (Mayr 1963; Orr & Smith 1998), and might potentially even represent the speciation event of *L. lemmus*.

**Figure 5.** Temporal haplotype network (upper left) and phylogenetic tree (lower right) of Holocene and Late Pleistocene European *Lemmus* spp. Black dots in the network represent missing haplotypes, while empty circles correspond to a haplotype that is absent from that temporal layer but present in the other. The dashed circles and connecting lines between the two layers indicate the absence of the Fennoscandian haplogroup in the Late Pleistocene data set. In the phylogeny, the positions of the Holocene Fennoscandian samples are highlighted with yellow (modern Norwegian lemmings, *L. lemmus*) or brown (3 to 8 kyr BP old *Lemmus* sp. from Norway) branches. A high mutation rate of 30 % Myr\(^{-1}\) has been used in the analysis, and internal nodes with posterior probabilities above 0.8 are marked with asterix. The time scale is presented in years before the present, with the ages of all samples being proportional to their branch lengths.
In contrast to the lemming paper, I found that the modern European haplogroups of both ptarmigan species (Paper III) were present also in the Late Pleistocene European population (represented by 42 samples), with the modal haplotypes being identical between the time periods (Fig. 6). This pattern is consistent with the type of genetic founder effects expected during postglacial colonisation waves (Hewitt 1999). Moreover, temporal genetic continuity in Europe was also the most highly supported scenario in the analyses of molecular variance (AMOVA). In addition, the habitat tracking scenario also received the highest support in the rock ptarmigan ABC analyses, but unfortunately the willow ptarmigan dataset was not informative enough to give consistent support to any of the simulated scenarios. The species distribution modelling revealed that this long-term survival of both species might have been facilitated by a continuous availability of ptarmigan habitats over the last 20 millennia, even though these habitats have shifted substantially in space since the height of the last glaciation (Fig. 7). The range shifts might also have been coupled with adaptive changes, as shown by the differences in morphology (e.g. Stewart 1999) but not in mtDNA between the Late Pleistocene and today. However, the predictions for the future suggests that at least one third of willow ptarmigans’ current habitat, and nearly half of that available for rock ptarmigan, will disappear by 2070-2100, and the remaining areas will also become increasingly fragmented (Figs. 6 and 7).
Figure 6. Temporal haplotype networks of modern and Late Pleistocene willow ptarmigan (upper) and rock ptarmigan (lower). Barplots to the right show estimated changes in the proportion of available habitat and the degree of habitat fragmentation in Europe, illustrated as number of patches and median patch area, for the respective species. The future projections, averaged for 2070 to 2100, are shown for both IPCC climate scenario B1 (white bar) and A2 (black bar).
Figure 7. Back-casted European distributions of willow and rock ptarmigan for the last 20 thousand years, with occurrence probabilities ranging from 0 (grey) to 1 (red). Also shown is the Lagopus spp. fossil sites used in the model calibrations, and the approximate extent of glaciated land during the different time periods. Panels to the right show future projections of ptarmigan distributions for the years 2070-2100, based on the two different climate scenarios.

The palaeogenetic analyses of 19 European mountain hares (Paper IV) revealed that the complicated phylogeographic structure and high diversity found in the modern species also existed during the Late Pleistocene period. In the less variable cytochrome b gene (CytB), the modal haplotypes were identical in the modern and glacial datasets, whereas a majority of the individuals in both time periods carried unique variants of the control region (CR) sequence. However, the ancient haplotypes were nested within the diversity of the contemporary ones, and the reconstructed phylogeny shows that the glacial hares are intermingled with modern individuals throughout
the evolutionary tree (Figs. 8 and 9). We therefore propose that the ancient midlatitude bone remains belonged to representatives of the same diverse hare population that is currently found in various locations across northern and alpine Europe. Further, the demographic analyses indicated that the mountain hare’s population size has fluctuated over time in concordance with the glacial cycles, with expansions and declines occurring during past periods of climate cooling and warming, respectively. In that sense, the mountain hare consequently appears to be a textbook example of a cold-adapted species, even though it stands out from the majority of previously studied Arctic taxa in that it successfully survived the Pleistocene-Holocene transition without any major declines in diversity.

Figure 8. Temporal haplotype networks of modern and Late Pleistocene mountain hares, with CytB variation to the left and CR variation to the right.
Figure 9. Mountain hare phylogenetic tree based on concatenated sequences from both the CytB and CR, and a mutation rate of 7.7 % Myr\(^1\). The European samples are colour coded according to geographic sub-region, and the position of the ancient samples in the phylogeny are indicated with black ovals. The scale and posterior probability levels are identical to those described in Fig. 5.
Discussion

Animals often spend a substantial part of their life on the move, whether it is in search of new resources and potential mates or to avoid competition and unfavourable environmental conditions. These individual movements can occur on different geographic scales depending on landscape barriers and species’ physical abilities as well as behavioural constraints. A well-known type of movement in northern regions is the recurring migration between summer and winter habitats, which allows animals to follow seasonal changes in climate and resource availability. On a short time scale, these periodic shifts can cover vast geographic areas, but since individuals generally return to the same breeding grounds that they left, there are no apparent changes in the longer perspective. However, although migrations do not involve gene flow per se, these periodic movements can sometimes develop into dispersal events. This is seen during peak years of Norwegian lemmings (*Lemmus lemmus*), when their local shifts between wintering and summer habitats continues as mass movements across the mountain tundra and further down into forested valleys (Hansson 1999; Henttonen & Kaikusalo 1993). It should be noted though, that dispersal is in turn just a pre-requisite for gene flow and to have a permanent impact on coming generations, the dispersing individuals also need to locate a new area that fulfil their environmental requirements, as well as to successfully set up a territory and reproduce. For Norwegian lemmings, there are no observations of uphill movements to indicate that the dispersing individuals ever return to their core tundra habitat (Østbye *et al*. 1993). However, the genetic analyses in Paper I revealed that this small rodent displays a pattern of geographically widespread genetic clusters similar to that described for Scandinavian brown bears and moose (Manel *et al*. 2004; Wennerström *et al*. 2016) which strongly suggests that mass movements during peak years indeed result in long-distance gene flow between mountain areas separated by forest.
While there might be substantial amounts of movement and gene flow between different sites across a species’ range, it normally occurs within stable geographic boundaries. However, on a longer time scale, environmental changes can lead to geographic shifts of habitats that require abilities for directional dispersal over many generations. This type of successional movement has been the focus of Papers II-IV in this thesis, where I have investigated post-glacial recolonization dynamics in three types of important prey species in the northern biome. Previous ancient DNA studies on cold-adapted mammal species have generally shown an unexpected lack of habitat tracking during past climate warming, sometimes despite very high dispersal abilities in modern populations (e.g. Dalén et al. 2007). The second paper in this thesis (Paper II) conforms to these discouraging findings, and show that the midlatitude glacial lemming (Lemmus sp.) populations were unable spread northwards at a sufficiently high rate to keep pace with the rapid post-glacial alterations. Together with the previously reported extinctions of Dicrostonyx lemmings (Brace et al. 2012), this lack of habitat tracking in the past presumably would have had large bottom-up effects on the tundra ecosystem, and one could further speculate on the importance of their disappearance in the failure of European arctic fox populations to survive until the present (Dalén et al. 2007).

The intriguing support for a long-term northern glacial survival of lemmings in restricted ice-free refugia highlights the alternative to range shifts, namely staying in place and enduring the new climate. The persistence of such a remnant population would most likely have included severe genetic bottlenecks, and given that genetic diversity is considered vital for long-term survival it could be questioned whether this putative persistence during the whole LGM period is at all possible. However, new genomic data from Channel Island foxes (Urocyon littoralis) have revealed that survival of very small and isolated populations is indeed possible for several thousands of years, despite a near absence of genetic variation and accumulation of deleterious alleles (Robinson et al. 2016). Similar to our suggestions (see Paper II), this remarkable persistence was explained by the habitats’ absence of predators and competitors, and the authors propose that such
decreased environmental stress can allow populations to tolerate higher levels of genetic load.

The results from the two *Lemmus* studies (Paper I and II) illustrate that there are different factors controlling dispersal between existing populations, and the type of directional movement that involves the successional expansion of a range into new territories. In fact, the *Lemmus* populations that inhabited midlatitude Europe during the last glaciation might not have exhibited the strong cyclicity and subsequent mass movements that we see in modern Norwegian lemmings (*L. lemmus*). Further, a pre-requisite for the development of lemming peaks is successful winter breeding, which is in turn is dependent on a long period of continuous snow cover (Ims *et al.* 2011). The climate deteriorations at the last glacial/interglacial transition could thus have led to irregular or even absent outbreaks and consequently less frequent long-distance dispersal events across areas of unsuitable habitat.

Whereas post-glacial warming appears to have caused local extinctions of lemming populations outside of the northern refugium, the aDNA analyses of ptarmigan (Paper III) and mountain hares (Paper IV) have both revealed a temporal genetic continuity in Europe that supports the hypothesis of habitat tracking. The two latter genera also distinguished themselves from the glacial lemmings by exhibiting no clear phylogeographic structure in the ancient datasets, suggesting that they were not experiencing any major barriers to gene flow in the Late Pleistocene landscape. It could therefore be speculated that the comparatively restricted dispersal of lemmings, as indicated by the observed genetic structure among the Pleistocene midlatitude populations, coupled with a rapid post-glacial fragmentation of the midlatitude tundra habitat contributed to the failure of *Lemmus* populations to shift their ranges. Further, competition with a resident northern population could potentially also have obstructed the southern lemmings to establish in Fennoscandia. The contrasting long-term survival of ptarmigan in Europe could most likely be attributable to their flight capability, which would have made them less dependent on corridors between suitable habitat patches and enabled a more efficient movement across heterogeneous landscapes. While mountain hares are known to have
low natal dispersal rates and high site fidelity (Dahl & Willebrand 2005), it has the broadest ecological niche of the taxa investigated here. This ability to survive in a variety of different habitats was presumably very important during the environmental deteriorations at the Pleistocene/Holocene transition, and could be the key to why mountain hares have successfully shifted their distribution without any losses of unique mtDNA lineages.

As a complement to the genetic analyses, we also included back-casted species distribution modelling (Paper III) to investigate how climate-induced changes in suitable habitat affected the ability of ptarmigan (Lagopus spp.) to shift their ranges. Although these models were based on occurrence data for the two bird species, it might be worth noting that rock ptarmigan (L. muta) share roughly the same modern habitat preferences as the Norwegian lemming (Lemmus lemmus), despite the much more restricted distribution of the latter species (Fig. 2). Consequently, the range changes predicted for L. muta could give a hint on how areas suitable for lemmings have shifted over the last 20 millennia. A similar comparison could perhaps also be made between the willow ptarmigan (Lagopus lagopus) and the mountain hare (Lepus timidus), where the predicted occurrence of willow ptarmigan can give an approximation of the minimum amount of areas suitable for hares. For L. timidus, however, the inference is complicated by the close interaction with the European brown hare (L. europaeus), which is proposed to have led to recent competitive exclusion of mountain hares from many areas along its southern range margin. The back-casted range projections showed that the type of tundra habitat favoured by rock ptarmigan and lemmings only comprised ca 25 % of the comparable areas suitable for willow ptarmigan and mountain hares, and the former habitat type also seemed to have been constantly more fragmented (Figs. 6 and 7). Such a patchy Lemmus environment is in line with the previously discussed genetic differentiation between the glacial midlatitude European sub-regions, and this fragmentation might also have obstructed a subsequent post-glacial range shift. Whereas neither the rock ptarmigan, or the more common willow ptarmigan and mountain hare, appear to have been restricted in their movement by this past habitat structure, they could face problems in the future. In many parts of their current European distributions, they now reside
in isolated Arctic regions or southern alpine “sky islands” (Hampe & Jump 2011) that are already at the geographic limits of possible areas to colonise. As a result of anthropogenic climate warming, our forecasts of the coming 100 years show that these regions will only have remnant, if any, areas left that fulfil the environmental requirements of ptarmigan. These resident birds, and presumably also the corresponding mountain hare populations, will consequently need to adapt in order to survive. Interestingly, both the willow ptarmigan and mountain hare have populations in the British Isles that do not develop the regular white winter colour, which could indicate such an ongoing selection in both species to the milder winters of this region. Further, the previously reported morphological difference between modern and Late Pleistocene ptarmigan (Bochenski 1985; Stewart 1999) suggests that the birds might also have adapted to climate warming in the past, presumably through changes in their diet or the degree of sedentariness (Stewart 1999).

The Norwegian lemming inhabits a region that seems to be comparatively less affected by future climate changes (Fig. 7). However, the increased habitat fragmentation, predicted for both *L. muta/L. lemmus* and *L. lagopus/L. timidus* (Figs. 6 and 7), in combination with a dampened frequency and amplitude of lemming peaks due to milder winter conditions (Ims et al. 2011), could still be expected to increase the genetic differentiation between Norwegian lemming populations in the future.
Sammanfattning

högalpina trakter. Dessa olika utfall skulle kunna härledas till ripornas flygförmåga, vilken möjliggör en friare spridning över fragmenterade områden, samt till att skogshararnas generalistiska natur minskar deras sårbarhet för habitatförändringar. Utbrednings-modelleringar för de kommande 100 åren visade dock att en fortsatt global uppvärmning kan leda till att klimatet i vissa isolerade utbredningsområden blir olämpligt i framtiden, vilket kräver att populationerna där anpassar sig till de nya förutsättningarna för att undgå lokala utdöenden.
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