

Original article:

## On the origin of the Norwegian lemming

Vendela K. Lagerholm <sup>1,2\*</sup>, Edson Sandoval-Castellanos <sup>1,2</sup>, Dorothee Ehrich <sup>3</sup>, Natalia I. Abramson <sup>4</sup>, Adam Nadachowski <sup>5</sup>, Daniela C. Kalthoff <sup>6</sup>, Mietje Germonpré <sup>7</sup>, Anders Angerbjörn <sup>2</sup>, John R. Stewart <sup>8</sup>, Love Dalén <sup>1</sup>

1. Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Stockholm, 10405, Sweden
2. Department of Zoology, Stockholm University, Stockholm, 10405, Sweden
3. Department of Arctic and Marine Biology, University of Tromsø, Tromsø, 9037, Norway
4. Zoological Institute, Russian Academy of Sciences, St. Petersburg, 199034, Russia
5. Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Kraków, 31-016, Poland
6. Department of Zoology, Swedish Museum of Natural History, Stockholm, 10405, Sweden
7. Operational Direction “Earth and History of Life”, Royal Belgian Institute of Natural Sciences, Brussels, 1000, Belgium
8. Faculty of Science and Technology, Bournemouth University, Dorset, BH12 5BB, United Kingdom

*Keywords:* Speciation, colonisation, extinction, Pleistocene, ancient DNA, *Lemmus*

\* Correspondence: Vendela K. Lagerholm, Fax: +46 (0)8 5195 5181,

E-mail: vendela.k.lagerholm@nrm.se

Running title: Norwegian lemming palaeogenetics

## 1 **Abstract**

2 The Pleistocene glacial cycles resulted in significant changes in species distributions, and it has  
3 been discussed whether this caused increased rates of population divergence and speciation. One  
4 species that is likely to have evolved during the Pleistocene is the Norwegian lemming (*Lemmus*  
5 *lemmus*). However, the origin of this species, both in terms of when and from what ancestral  
6 taxon it evolved, has been difficult to ascertain. Here, we use ancient DNA recovered from  
7 lemming remains from a series of Late Pleistocene and Holocene sites to explore the species'  
8 evolutionary history. The results revealed considerable genetic differentiation between glacial  
9 and contemporary samples. Moreover, the analyses provided strong support for a divergence time  
10 prior to the Last Glacial Maximum, therefore likely ruling out a post-glacial colonisation of  
11 Scandinavia. Consequently, it appears that the Norwegian lemming evolved from a small  
12 population that survived the Last Glacial Maximum in an ice-free Scandinavian refugium.

## 13 **Introduction**

14 Changes in the distribution of organisms are thought to be one of the main drivers of speciation  
15 (Hewitt 1996; Mayr 1963). This is because habitat fragmentation and long distance colonisation  
16 events can lead to isolation of conspecific populations, with subsequent evolutionary divergence  
17 due to genetic drift and natural selection. The Pleistocene glacial cycles (*c.* 2.6 million to 12  
18 thousand years ago) are considered to have had a major impact on the distribution and abundance  
19 of species, especially in temperate and polar regions (Stewart *et al.* 2010). It has therefore been  
20 proposed that glacial cycles have led to increased rates of speciation, both due to prolonged  
21 periods of isolation in refugia and due to colonisation of, and adaptation to, previously  
22 uninhabitable regions (Hewitt 1996; Johnson & Cicero 2004; Rand 1948). However, this view  
23 has been a topic of considerable debate in recent decades, since it has been argued that  
24 phylogenetic estimates of speciation rates are not higher during the Pleistocene compared to  
25 earlier time periods (Klicka & Zink 1997). Moreover, the lengths of glacials and interglacials  
26 have been considered too short to permit evolution of reproductive isolation among conspecific  
27 populations (Brochmann *et al.* 2003; Lister 2004). On the other hand, theoretical work (Mayr  
28 1954), as well as some empirical studies (Johnson *et al.* 1996), suggest that speciation can occur  
29 rapidly under certain conditions.

30 One of the main problems in investigating whether the Pleistocene climate fluctuations  
31 influenced speciation stems from the difficulty in identifying the precise location and timing of  
32 such events. This is partly due to that morphological divergence is expected to be low during  
33 early stages of speciation, and thus typically invisible in the fossil record. In addition, inference

34 using modern DNA data and fossil-based molecular clocks usually lack sufficient resolution to  
35 reconstruct past speciation events (Ho *et al.* 2011a; Hofreiter & Barnes 2010; Lister 2004).

36 One species that likely evolved comparatively recently is the Norwegian lemming (*Lemmus*  
37 *lemmus*). Today, the Norwegian lemming inhabits the mountain tundra of Fennoscandia, a region  
38 encompassing the Scandinavian Peninsula, Finland and the Kola Peninsula (Fig. 1), which is  
39 thought to have been completely covered by the Scandinavian Ice Sheet between approximately  
40 30 to 16 thousand calendar years before present (kyr BP) (Mangerud *et al.* 2011; Svendsen *et al.*  
41 2004). In this paper, we refer to this time period as the Last Glacial Maximum (LGM), although  
42 it should be noted that other more narrow definitions have been used in other studies (Svensson *et*  
43 *al.* 2006).

44 The Norwegian lemming is the only endemic mammal in Fennoscandia, and its origin is  
45 therefore somewhat of a mystery. Previous genetic analyses on modern DNA have shown that it  
46 is too different from its sister species, the Siberian lemming (*L. sibiricus*), to have evolved from a  
47 post-glacial common ancestor (Fedorov & Stenseth 2001). Consequently, the Norwegian  
48 lemming either originates from a non-Siberian source population outside the Scandinavian Ice  
49 Sheet (Østbye *et al.* 2006), or it originates from a small population that survived the Last Glacial  
50 Maximum in a local northern refugium (Ekman 1922).

51 Numerous fossil remains have shown that lemmings of the genus *Lemmus* were common  
52 inhabitants of the vast steppe-tundra of midlatitude Europe and Asia during the Late Pleistocene  
53 glacial period (e.g. Nadachowski 1982). Being members of a cold-adapted genus, these southern  
54 populations disappeared during the transition to the current Holocene interglacial, and it has not  
55 yet been established whether they became extinct or shifted their distribution to more northern  
56 latitudes as the temperature increased. It has been postulated that some of these southern *Lemmus*

57 populations tracked their tundra habitat to the Scandinavian Peninsula as the ice margin retreated,  
58 and subsequently founded the modern Norwegian lemming population (Østbye *et al.* 2006). In  
59 contrast to the post-glacial colonisation hypothesis, it has also been proposed that the species  
60 actually survived the last glaciation *in situ* in an ice-free area of Scandinavia, possibly on Andøya  
61 or on a part of the continental shelf that was exposed during times of low sea level (Ekman 1922;  
62 Fedorov & Stenseth 2001). However, there is no fossil evidence of Norwegian lemmings in  
63 Scandinavia during the Last Glacial Maximum to support this second hypothesis, although  
64 *Lemmus* sp. bones of ~ 36 kyr BP in age have been found in Norway from the Ålesund  
65 interstadial, indicating the presence of lemmings in the area *before* the last glacial advance  
66 (Larsen *et al.* 1987).

67 The aim of this study was to use ancient DNA from *Lemmus* spp. remains to further investigate  
68 the evolutionary history of the Norwegian lemming. More explicitly, we examined the two  
69 contrasting hypotheses (Fig. 2) discussed above to resolve whether the Norwegian lemming is  
70 derived either from a post-glacial colonisation from midlatitude Europe (scenario 1), or from a  
71 population of lemmings that colonised Scandinavia before the Last Glacial Maximum and then  
72 survived locally in an ice-free northern refugium (scenario 2).

73

## 74 **Materials and methods**

### 75 ***Data collection***

76 A total of 54 Late Pleistocene *Lemmus* spp. mandibles, spanning between ~ 12 kyr and 48 kyr BP  
77 in age, were collected from 11 paleontological sites across the genus' glacial range in midlatitude  
78 Europe (Fig. 1; Table S1, Supporting Information). Further, we also included 27 mandibles from

79 early-mid Holocene lemmings (between ~ 3 kyr and 8 kyr BP in age) found in the Sirijorda Cave  
80 in northern Norway. A modified version of protocol C in Yang *et al.* (1998) was used to extract  
81 DNA from the Late Pleistocene samples, whereas Qiagen's QIAamp Tissue kit was used for the  
82 Holocene cave samples as described in Fernández *et al.* (2006). For the modern data set, 17  
83 Norwegian lemming (*L. lemmus*) tissue samples from seven localities along the Swedish  
84 mountain range were extracted at the Swedish Museum of Natural History using the QIAamp  
85 DNA mini kit (Qiagen), with the protocol DNA Purification from Tissues. In order to get an  
86 estimate of the interspecific variation in European *Lemmus* spp. we also extracted DNA from 11  
87 modern Siberian lemming (*L. sibiricus*) bone samples from three localities within the north-  
88 western phylogeographic group (Fedorov *et al.* 1999) using the same protocol as for the Late  
89 Pleistocene *Lemmus* spp. samples. To avoid confusion, the early-mid Holocene Norwegian cave  
90 samples are hereafter called Holocene Scandinavian, while the modern samples of *L. lemmus*  
91 (Norwegian lemming) are called modern Scandinavian.

92 We targeted two mitochondrial regions previously used in modern phylogenetic studies of the  
93 *Lemmus* genus, comprising the first hypervariable part of the control region (CR) and parts of the  
94 cytochrome *b* (*cyt b*) gene. Further details regarding DNA extraction, PCR amplification and  
95 sequencing are presented in the Supporting Information online.

96 The pre-PCR work on the Late Pleistocene samples was carried out in the ancient DNA  
97 laboratory at the Swedish Museum of Natural History, where no previous work on *Lemmus* spp.  
98 had been done. For all Late Pleistocene samples, at least two independent amplifications were  
99 done in order to resolve erroneous bases caused by misincorporation during PCR. The Holocene  
100 Scandinavian samples were analysed in the ancient DNA laboratory at Laboratoire d'Ecologie  
101 Alpine in Grenoble, France, where no rodent samples had been analysed before. Since the

102 sequences obtained from Sirijorda cave only displayed variation in nucleotide positions that are  
103 variable in extant lemming populations, it seemed unlikely that the observed variation could have  
104 been caused by PCR misincorporation. The Sirijorda sequences were therefore not considered  
105 necessary to replicate through multiple PCRs. All working surfaces and lab equipment were  
106 regularly sterilised with UV light, bleach or hydrochloric acid, and extraction and PCR blanks  
107 were extensively used to monitor possible contamination. The pre-PCR work on the modern  
108 samples was carried out at the Swedish Museum of Natural History, in laboratories physically  
109 separated from both the ancient DNA and post-PCR facilities.

110 Eleven Late Pleistocene lemming mandibles that gave successful DNA sequences were dated at  
111 the Oxford Radiocarbon Accelerator Unit. Five of these produced radiocarbon dates, which were  
112 calibrated to calendar years before present using OxCal 4.1.7 (Bronk Ramsey 2009) and the  
113 IntCal 09 calibration curve (Reimer *et al.* 2009). The remaining six samples failed due to low  
114 collagen yields, likely owing to the small size of the lemming mandibles rather than poor  
115 biomolecular preservation. All dates, including the inferred ages of the remaining samples in the  
116 data set, are listed in Table S1, Supporting Information.

117

### 118 ***Data analyses***

119 Sequences were aligned and edited using the software SeqMan in the package Lasergene v8.1.5  
120 (DNASTAR). BioEdit v1.7.3 (Hall 1999) was subsequently used to construct a combined data set  
121 of 520 bp, consisting of both CR (168 bp) and *cyt b* (352 bp) sequences. Additionally, we used a  
122 partial data set of 172 bp (96 bp CR and 76 bp *cyt b*) that also included the Holocene  
123 Scandinavian lemming sequences. The Late Pleistocene sample sites were assigned to six

124 geographic regions; England, Belgium, Germany, Poland, Russian plains and Ural Mountains.  
125 Genetic diversity within these, Holocene Scandinavia, and the two modern regions (Scandinavia  
126 and NW Russia) were calculated with Arlequin v3.5.1.2 (Excoffier & Lischer 2010).

127 Temporal statistical parsimony networks were created with the R-script TempNet v1.4 (Prost &  
128 Anderson 2011) to display the haplotypes found in the different time periods (the Holocene and  
129 the Late Pleistocene). The phylogenetic relationships among all samples and the divergence times  
130 for different lineages were calculated with the software BEAST v1.6.1 (Drummond & Rambaut  
131 2007). Initially, the analyses were performed using the nucleotide substitution model GTR+G, as  
132 an analysis in MrModeltest v2.3 (Nylander 2004) showed this to be the most appropriate model  
133 of nucleotide substitution. However, due to poor mixing of the Markov chain Monte Carlo  
134 (MCMC) this was later changed to the simpler model HKY+G. The phylogenetic analyses were  
135 performed using a strict molecular clock with fixed mutation rates. Based on the previously  
136 published rates of 17 % Myr<sup>-1</sup> (CR) and 5 % Myr<sup>-1</sup> (cyt *b*) (Fedorov & Stenseth 2001), and the  
137 relative length of each gene region in our combined data sets, we set the mutation rate to 8.9 %  
138 Myr<sup>-1</sup> and 11.7 % Myr<sup>-1</sup> respectively for the 520 bp and the 172 bp alignments. However, since  
139 the mutation rate could have a strong impact on the subsequent analyses, and concerns have been  
140 raised about biases in mutation rate estimates (Ho *et al.* 2011a), we also ran the analyses using  
141 mutation rates of 30 % and 50 % Myr<sup>-1</sup> which encompass the range of previously published  
142 estimates from ancient DNA data sets on large herbivores, such as saiga (*Saiga tatarica*; Campos  
143 *et al.* 2010a) and bison (*Bison bison*; Shapiro *et al.* 2004). It should be noted that the issue of  
144 elevated mutation rates in ancient DNA data sets is a topic of discussion (Ho *et al.* 2011b;  
145 Navascués *et al.* 2010; Navascués & Emerson 2009), wherefore using a rate of 50 % Myr<sup>-1</sup> in our  
146 analyses may seem unreasonably high. Nonetheless, we decided to include it since the mutation



147 rate is inversely proportional to the split time estimated in the coalescent simulations and we  
148 wanted to exclude the possibility of selecting the wrong scenario due to using a too low mutation  
149 rate. The dates of all ancient sequences, obtained either from direct radiocarbon dating or from  
150 inferred ages based on stratigraphy and published dates, were included in the analyses. However,  
151 due to uncertainties regarding the age of the Ural deposit, the date of these sequences were  
152 instead inferred using the option Tip sampling, with a wide uniform prior encompassing the  
153 proposed age ( $28.6 \text{ kyr} \pm 15 \text{ kyr BP}$ ). To assess the robustness of the analysis, BEAST was also  
154 run using only the sequences that had specific ages, i.e. the modern samples and those of the  
155 ancient remains that were successfully radiocarbon dated. However, this did not affect the overall  
156 topology of the phylogeny, nor the split times among major clades (data not shown). All analyses  
157 were made with a randomly generated starting tree, and the length of the MCMC was set to 30  
158 and 50 million generations for the partial and the complete data sets, respectively, with  
159 parameters logged to file every 1,000 generations. Two independent runs were made for each  
160 analysis, and the results were checked in TRACER v1.5 (Rambaut & Drummond 2007) to ensure  
161 that runs were converging on the same distribution. The sample of trees obtained from a BEAST  
162 run was summarised with TreeAnnotator v1.6.1 to a maximum clade credibility tree with median  
163 node heights, using a burnin of 10 % and a posterior probability limit of 0.5, and the output was  
164 graphically edited in FigTree v1.3.1 (Rambaut 2009).

165 We also constructed a phylogenetic tree in MrBayes v3.2.2 (Ronquist *et al.* 2012) from the  
166 partial data set, in order to investigate the reliability of our topology also without using sample  
167 ages and mutation rate as priors. Using the tundra vole, *Microtus oeconomus*, as an outgroup  
168 (GenBank accession no AY305172; Galbreath & Cook 2004) we ran the analyses for 5 million  
169 generations with the HKY+G substitution model, with a sample and print frequency set to 100.

170 Two independent runs were made, with results checked in Tracer v1.5 (Rambaut & Drummond  
171 2007) to ensure convergence before discarding 10 % as burnin. The combined tree file was  
172 graphically edited in FigTree v1.3.1 (Rambaut 2009).

173 Approximate Bayesian Computation coupled with coalescent simulations was carried out using  
174 the partial dataset, in order to test the two contrasting hypotheses regarding the Norwegian  
175 lemming's (*L. lemmus*) glacial history. The statistical inference relied on one single parameter:  
176 the time separating all the Scandinavian lemmings (modern and early-mid Holocene) from their  
177 closest non-Scandinavian glacial relatives. This inference was based on hypothesis testing using  
178 acceptance ratios (Bayes factors) of the simulations of the two proposed scenarios, and the  
179 estimation of the mentioned divergence time. The program Bayesian Serial SimCoal (Anderson  
180 *et al.* 2005; Excoffier *et al.* 2000) was used to run coalescent simulations (Fig. S1, Supporting  
181 Information) for three different analyses: simulations for performing a model comparison  
182 (hypothesis contrast) using Bayes factors, simulations for estimating the parameters of interest,  
183 and simulations for a cross-validation test using pseudo-observed datasets. Pilot simulations were  
184 carried out to test different prior distributions and their effect on the posteriors, as well as to  
185 define proper parameter values. Also, a comprehensive selection of summary statistics was  
186 carried out in order to select an appropriate and informative set. Other pilot simulations explored  
187 alternative population sizes, mutation rates (fixed or sampled from a prior), as well as alternative  
188 scenarios and statistical groups. For the parameters estimation, optimisation simulations were  
189 made in order to improve the fit of the simulations to the data, and thereby increasing the  
190 accuracy of the estimates. Thus, the prior distributions in the final simulations were tuned  
191 according to the obtained posteriors in the optimisation runs (but using wider variances)  
192 (Bertorelle *et al.* 2010; Lopes *et al.* 2009).

193 The model that was simulated (Fig. S1, Supporting Information) consisted of four populations  
194 (Scandinavia, Siberia, glacial England and glacial continental Europe) whose lineages coalesced  
195 backwards in time. Population sizes were simulated with initial exponential priors ( $\lambda=250,000$ ).  
196 This was used because exponential priors sample uniformly in a logarithmic scale, which is  
197 advantageous when parameters have ranges covering several orders of magnitude, as in the case  
198 of lemming populations sizes which potentially can reach millions of individuals. The parameter  
199 value was set to 250,000 for an optimal acceptance rate of the simulations. The Scandinavian  
200 population was also set to have an exponential growth starting 11.5 kyr BP, corresponding to a  
201 post-glacial population expansion into previously ice-covered Scandinavian areas, since that was  
202 expected under both of the hypothesised scenarios. The ages of all Late Pleistocene samples were  
203 assigned from normal prior distributions (around the dates listed in Table S1, Supporting  
204 Information) to account for the uncertainty in the age estimates, both when these were derived  
205 from radiocarbon dating and when inferred from stratigraphic contexts. Generation time was set  
206 to 1 per year. As in the BEAST analyses, the simulations were made with three fixed mutation  
207 rates; 11.7 %, 30 % and 50 % Myr<sup>-1</sup>. Post simulation analyses were made in a custom software  
208 (available upon request) written in the programming language Fortran 95. In order to deal with  
209 the large number of summary statistics employed, the rejection was performed by using a vector  
210 containing the threshold distances for every summary statistic (Table S2, Supporting  
211 Information). In addition, summary statistics were normalised with the distance between the  
212 median of the simulated values and the observed value, which empirically yielded better results  
213 than using the variance. Further details regarding the simulation procedures are given in the  
214 Supporting Information online.

215

## 216 **Results**

### 217 *Data set*

218 The complete 520 bp sequence targeted in this study was obtained from 23 Late Pleistocene and  
219 27 modern samples, while a partial 172 bp fragment was obtained from eight of the early-mid  
220 Holocene *Lemmus* spp. samples (Table S1, Supporting Information). We therefore had two data  
221 sets containing 50 and 58 sequences, respectively (GenBank accession numbers: JX483882-  
222 JX483939).

223

### 224 *Genetic diversity and phylogenetic relationships*

225 There was a high genetic variation in the glacial data set with a total of 19 and 17 unique  
226 haplotypes found in the complete and partial alignments, respectively (Fig. 3; Fig. S2 and Table  
227 S4, Supporting Information). This was also reflected in the estimates of nucleotide and haplotype  
228 diversities, which generally were higher in the overall glacial data set, although regional levels of  
229 diversity in the glacial populations were comparable to those in the modern-day populations.

230 Bayesian phylogenetic analyses showed that the diversity is distributed into three clades (Fig.  
231 4). The first two (clades A and B) include the representatives of each of the two modern species,  
232 whereas the third (clade C) is basal and only includes Late Pleistocene lemmings. The modern  
233 Scandinavian samples form a well supported monophyletic group together with all but one of the  
234 Holocene Scandinavian cave samples (Figs 3 and 4; Fig. S3, Supporting Information). For all  
235 mutation rates used (11.7, 30 and 50 % Myr<sup>-1</sup>), the estimated time to the most recent common  
236 ancestor (tMRCA) for this Scandinavian group and the most closely related Late Pleistocene

237 sequences pre-date the final retreat of the Scandinavian Ice Sheet (100 kyr, 43 kyr and 32 kyr BP  
238 respectively; Fig. 4; Table 1). Very similar results were obtained from the network and  
239 phylogeny constructed from the 520 bp data set (Fig. S2, Supporting Information), and the  
240 tMRCA estimates pre-dated the last glacial retreat also when the 520 bp sequences were used  
241 (Table S5, Supporting Information). Further, the robustness of the overall tree topology estimated  
242 in BEAST was supported by Bayesian phylogenetic analyses that did not incorporate sequence  
243 dates or pre-defined mutation rates (Fig. S3, Supporting Information), although some deeper  
244 internal nodes found in the BEAST analyses could not be resolved.

245

#### 246 *Bayesian coalescent simulations of population divergence times*

247 Consistent with the phylogenetic results, the Bayesian coalescent simulations of the two  
248 hypothesised scenarios strongly supported a population divergence that pre-dated the last glacial  
249 retreat (Fig. 5; Table 2). The acceptance ratio yielded a higher support for this scenario, with  
250 Bayes factors of 7.4, 48.3 and 37.2 (for mutation rates of 11.7, 30 and 50 % Myr<sup>-1</sup>, respectively).  
251 In the pseudo-observed datasets (PODs) analysis, the probabilities of selecting the right scenario  
252 were 0.67 and 0.81 for scenarios 1 and 2, respectively, when a mutation rate of 11.7% Myr<sup>-1</sup> was  
253 assumed. Additionally, the analysis that took into account the observed Bayes factor, in which  
254 the only PODs that were considered were those with a Bayes factor equal to or larger than the  
255 observed one, yielded values of 0.98 and 0.89 for scenarios 1 and 2 respectively. For the mutation  
256 rate of 30% Myr<sup>-1</sup>, the corresponding values were 0.90 and 0.90 in the first run, and 0.97 and 0.97  
257 when considering the observed Bayes factor. The mutation rate of 50% Myr<sup>-1</sup> resulted in values

258 of 0.94 and 0.90 in the first run, which rose to 0.98 and 0.99 respectively after the observed  
259 Bayes factor was taken into account.

260 Two of the summary statistics allowed a good differentiation between our hypotheses; the mean  
261 number of pairwise differences and the  $F_{ST}$  between Scandinavian lemmings (including both  
262 modern and early-mid Holocene cave samples) and their closest glacial relatives (Fig. S4,  
263 Supporting Information). The observed values for both these statistics were too high to  
264 correspond to scenario 1 ( $p$ -value 0.06-0.0098), but were not significantly differentiated from  
265 scenario 2 ( $p$ -value 0.36-0.09).

266 When using coalescent simulations coupled with Approximate Bayesian Computation analysis,  
267 there is always a concern that the true scenario is not incorporated among the models tested  
268 (Templeton 2009). However, one way to address this problem is to assess how well the models fit  
269 to the empirical data (Csillery *et al.* 2010). To address this, we compared the posterior  
270 distributions with the summary statistics of the observed data set. The results showed that the  
271 observed data had a close fit to the non-rejected summary statistics in the simulated data sets,  
272 which indicates that the simulated models provide a good fit to the empirical data (Fig. S5,  
273 Supporting Information).

274

## 275 **Discussion**

276 Our results indicate a large genetic variation in the lemming populations that inhabited the  
277 steppe-tundra region of midlatitude Europe during the Late Pleistocene period. In particular, the  
278 glacial populations in Eastern Europe appear to have had a very high nucleotide diversity, which  
279 could reflect long-term occupation in the region (Table S4, Supporting Information). It should be

280 noted, however, that these diversity estimates may to some extent be inflated due to the  
281 heterochronous nature of the data (Depaulis *et al.* 2009). Nonetheless, the seemingly high genetic  
282 variation in the glacial populations, as well as the large effective population sizes estimated in the  
283 Bayesian coalescent simulations (Fig. S6, Supporting Information), support the view that  
284 *Lemmus* spp. were common in the European Late Pleistocene steppe-tundra ecosystem.

285 The modern Scandinavian population displays low levels of nucleotide diversity and a star-like  
286 pattern in the haplotype network (Fig. 3; Table S4, Supporting Information), which indicate a  
287 previous reduction in population size followed by a demographic expansion, as also previously  
288 demonstrated in a mismatch distribution test done by Fedorov & Stenseth (2001). This could  
289 either correspond to a bottleneck during the Last Glacial Maximum in line with the hypothesis of  
290 local glacial survival (i.e. scenario 2), or a post-glacial founder event (i.e. scenario 1). Both these  
291 hypotheses are supported by the observation that all but one of the Scandinavian cave samples  
292 from the early-mid Holocene fall within the diversity of the modern samples (Figs. 3 and 4), thus  
293 making a more recent genetic bottleneck unlikely.

294 The central, and most common, haplotype in Scandinavia (Fig. 3) is likely to represent either  
295 the haplotype that survived the hypothesised LGM bottleneck, or alternatively, the founding  
296 haplotype during a post-glacial colonisation. However, this haplotype was not observed in any of  
297 the glacial populations that surrounded the Scandinavian Ice Sheet, which could have been  
298 expected if the Norwegian lemming (*Lemmus lemmus*) originated from a post-glacial  
299 colonisation from these southern populations. Instead, the most recent common ancestor  
300 (MRCA) to the Norwegian lemming and the most closely related glacial lemmings was estimated  
301 to have lived between 100 kyr to 32 kyr BP. Even for the extreme mutation rate of 50 % Myr<sup>-1</sup>,  
302 the lower bound of the 95% highest posterior density (HPD) interval does not include the time

303 after the final retreat of the Scandinavian Ice Sheet (Table 1). Although the tMRCA may predate  
304 the time of actual population divergence, this difference is reduced when the populations are  
305 small, which likely was the case for the founder population of the Norwegian lemming.  
306 Furthermore, the Bayesian coalescent simulations provided a markedly higher support for a pre-  
307 LGM divergence between Scandinavian and glacial European populations, with an estimated  
308 population divergence time of more than 78 kyr BP (lower 95 % HPD for 50 % mutation rate =  
309 32 kyr BP; Table 2). It should be noted that the coalescent framework takes the temporal  
310 dimension and co-ancestry relationships into account at once. Therefore, the possibility that the  
311 dominant haplotype in extant *L. lemmus* existed outside the ice sheet during the Last Glacial  
312 Maximum, but was not sampled, would not only be the likelihood that it was absent in our glacial  
313 European sample, but the likelihood that it was not in the sample and that it did not coalesce with  
314 any of the sampled lineages. The estimation performed in the Approximate Bayesian  
315 Computation analyses targeted the time of the divergence of the Scandinavian lemmings from  
316 their ancestral population, and not the time to the origin of the lineage. The time window between  
317 those two events (the origin of the lineage and the origin of the population) is therefore where  
318 potentially unsampled lineages could have coalesced, thus producing a more recent origin of the  
319 *L. lemmus* lineage. However, such a scenario was not supported, and instead the results suggest  
320 that none of the populations that lived south of the Scandinavian Ice Sheet during the end of the  
321 last glaciation were the direct ancestors of the Norwegian lemming. Consequently, the most  
322 parsimonious explanation is that the species originates from a population that survived the Last  
323 Glacial Maximum in a northern refugium.

324 The hypothesis of small ice-free refugia in Scandinavia during the Last Glacial Maximum  
325 recently gained support in a study by Parducci *et al.* (2012), which reported paleoecological and



326 genetic data suggesting a local glacial survival of pine (*Pinus sylvestris*) and spruce (*Picea abies*)  
327 in Scandinavia (but see Birks *et al.* 2012). The data presented in this study thus lends further  
328 support to the local northern refugium hypothesis, and suggests that this putative ice-free area  
329 was diverse or large enough to harbour both Arctic taxa like lemmings, as well as Boreal trees.  
330 Alternatively, there might have existed multiple refugia that were inhabited by differently  
331 adapted plant and animal communities, for example at higher altitudes or on the part of the  
332 continental shelf that was flooded by rising sea levels during the Holocene (Nesje *et al.* 2007).

333 As indicated by the divergence time estimates in the phylogeny and the Bayesian coalescent  
334 simulations, it appears likely that Scandinavia was colonised by European lemmings (*Lemmus*  
335 sp.) during an interstadial period sometime between the Karmøy glaciation, which ended ~ 60 kyr  
336 BP (Mangerud *et al.* 2011), and the last glacial advance ~ 30 kyr BP (see Fig. 1a). The  
337 occurrence of *Lemmus* sp. fossil remains in Scandinavia dating to the Ålesund interstadial ~ 36  
338 kyr BP (Larsen *et al.* 1987) also confirms that the region was populated at this time period,  
339 although unfortunately we have no genetic information on these. As the Scandinavian Ice Sheet  
340 started to grow during late Marine Isotope Stage (MIS) 3, culminating in the full glacial  
341 conditions during MIS 2 (Svensson *et al.* 2006), the lemming population in Scandinavia must  
342 have become increasingly small and isolated from the surrounding southern populations. As the  
343 ice sheet melted during the early Holocene, Norwegian lemmings originating from the ice-free  
344 northern refugium likely expanded into the previously glaciated regions of Scandinavia.  
345 Interestingly, the observation in this study of a ~ 8 kyr BP old specimen from Sirijorda Cave in  
346 Norway carrying a haplotype today only found in *L. sibiricus* (Figs. 3 and 4) indicates that  
347 Siberian lemmings may have expanded into Scandinavia as the ice sheet melted. Alternatively,  
348 introgression between the two species may have led to inclusion of *L. sibiricus* haplotypes in the

349 *L. lemmus* gene pool. Haplotypes belonging to *L. sibiricus* have, however, not been observed in  
350 any modern Norwegian lemmings (this study; Fedorov & Stenseth 2001) and the species does not  
351 inhabit the region today. Moreover, this observation is based on one single sample, making  
352 further evaluation of the existence and extent of past gene flow from *L. sibiricus* into Scandinavia  
353 difficult at present.

354 The results presented here indicate that the end-Pleistocene midlatitude European *Lemmus*  
355 populations did not contribute to the gene pool of the contemporary lemming populations in  
356 Scandinavia and northwest Russia. Instead, it appears that the midlatitude populations became  
357 extinct at the Pleistocene-Holocene transition, and that this led to a marked decrease in genetic  
358 diversity that included the loss of a major mitochondrial (mt) DNA clade (Figs. 3 and 4).  
359 Whether this extinct clade represents a divergent population or a separate species is difficult to  
360 ascertain at present, since we have only analysed mtDNA. In any case, the observed loss of an  
361 entire clade adds to a growing body of evidence suggesting that many glacial populations and  
362 species were unable to track the shifts and contractions in habitat that took place at the end of the  
363 last Ice Age (Campos *et al.* 2010a; Campos *et al.* 2010b; Dalén *et al.* 2007).

364 An inability of populations to track reductions in habitat availability implies that a succession  
365 of expansions and contractions in species ranges, such as the ones that likely took place at the end  
366 of the Pleistocene, would have been characterised by a series of population extinctions (Brace *et*  
367 *al.* 2012). This could provide an explanation for the observation that many extant Holarctic  
368 species appear to have lost significant amounts of genetic diversity since the Late Pleistocene  
369 (Hofreiter & Barnes 2010). With the ongoing increases in global temperatures, this in turn raises  
370 concerns about the fate of extant cold-adapted populations that inhabit the southern margins of  
371 the Arctic biome.

372 From an evolutionary perspective, it appears likely that the northern survival of a small and  
373 isolated *Lemmus* sp. population during the Last Glacial Maximum may have contributed to the  
374 evolution of the Norwegian lemming, or possibly even represents the speciation event itself. This  
375 is consistent with the hypothesis that adopting a new refugium provides a mechanism of  
376 speciation (Stewart & Stringer 2012). Both the isolation and the small population size could have  
377 led to rapid evolutionary changes, consistent with the model of peripatric speciation (Mayr 1963).  
378 Such evolutionary changes may also have been reinforced by shifts in local ecological conditions  
379 (Orr & Smith 1998) due to the changes in temperature and precipitation associated with the onset  
380 of the Last Glacial Maximum, as well alterations in the lemmings' realised niche if their key  
381 predators and competitors were unable to persist in the local refugium (Dalén *et al.* 2007; Hewitt  
382 1996; Stewart 2008). Further analyses of autosomal genes, including those under natural  
383 selection, from serially sampled Norwegian lemming specimens could thus constitute a unique  
384 opportunity to study the speciation process in real time.

385

386

387

## 388 **Acknowledgements**

389 We are grateful to field workers within the Scandinavian arctic fox project (SEFALO+), staff at  
390 the Tarfala research station, and Maria Noring (Royal Institute of Technology) for contributing  
391 with modern samples. We thank Eivind Østbye (University in Oslo) for providing the Holocene  
392 Norwegian samples, Ralf W. Schmitz (University of Bonn), Nick Barton (Oxford University),  
393 Christelle Draily (Director of Walou Cave Excavations), Annelise Folie (Royal Belgian Institute

394 of Natural Sciences) and Anne Taylor (Cambridge University Museum of Archaeology and  
395 Anthropology) for giving us access to samples from the Neandertal site excavation, Merlin's  
396 Cave, Walou Cave, Caverne Marie-Jeanne and Bridged Pot Cave, respectively. We also thank  
397 Pierre Taberlet and Christian Miquel (Université de Grenoble) respectively for providing access  
398 to the DNA facilities in Grenoble and for technical support in the same lab. Additional field  
399 collection of modern samples was supported through grants from the Royal Swedish Academy of  
400 Sciences and Riksmusei Vänner to V.K.L. The genetic analyses were funded through grants to  
401 L.D. from the Swedish Research Council (VR) and FORMAS through the FP6 BiodivERsA  
402 ERA-NET program. E.S-C. also acknowledges support from the Strategic Research Programme  
403 EkoKlim at Stockholm University.

## Tables

**Table 1** Median ages, and the 95% highest posterior density (HPD) interval, in thousands (k) of years before present for the phylogenetic tree nodes shown in Fig. 4. The estimated times to the most recent common ancestor of all Scandinavian lemmings and the most closely related Late Pleistocene European lemmings are shown in bold. Based on BEAST analyses of the partial data set, using mutation rates of 11.7 %, 30 % and 50 % Myr<sup>-1</sup>.

	11.7 % Myr <sup>-1</sup>		30 % Myr <sup>-1</sup>		50 % Myr <sup>-1</sup>	
	Node age	95 % HPD	Node age	95 % HPD	Node age	95 % HPD
<i>A</i>	467 k	725 k - 285 k	190 k	284 k - 123 k	130 k	184 k - 87 k
<i>B</i>	260 k	414 k - 144 k	128 k	183 k - 84 k	97 k	133 k - 71 k
<i>C</i>	300 k	476 k - 173 k	112 k	173 k - 69 k	75 k	109 k - 52 k
<i>D</i>	152 k	263 k - 72 k	57 k	90 k - 35 k	41 k	59 k - 28 k
<i>E</i>	<b>100 k</b>	<b>169 k - 49 k</b>	<b>43 k</b>	<b>64 k - 26 k</b>	<b>32 k</b>	<b>46 k - 21 k</b>
<i>F</i>	86 k	164 k - 36 k	38 k	59 k - 22 k	30 k	42 k - 20 k
<i>G</i>	78 k	148 k - 33 k	32 k	53 k - 16 k	22 k	36 k - 12 k
<i>H</i>	65 k	113 k - 33 k	29 k	45 k - 17 k	22 k	33 k - 13 k

**Table 2** Descriptive statistics for the estimated population divergence times between Scandinavian and Late Pleistocene European lemmings, as shown in Fig. 5. The estimates are based on the posterior probability distributions obtained in the Bayesian coalescent simulations of the partial data set, using three different mutation rates.

	11.7 % Myr <sup>-1</sup>	30 % Myr <sup>-1</sup>	50 % Myr <sup>-1</sup>
Mode	101 k	117 k	82 k
Median	93 k	90 k	79 k
Mean	86 k	87 k	78 k
95% HPD Lower	23 k	37 k	32 k

## Figures

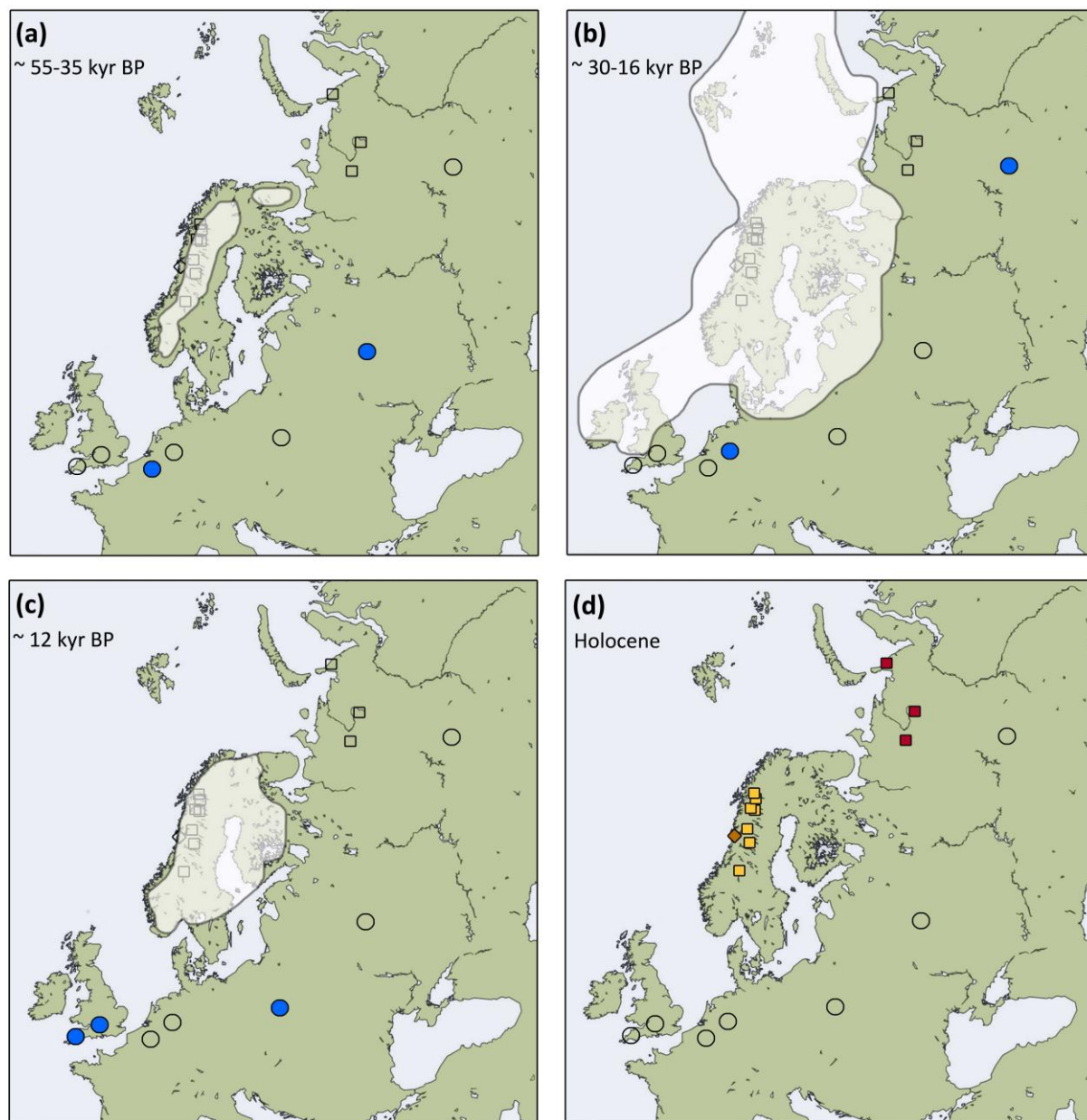


Fig. 1

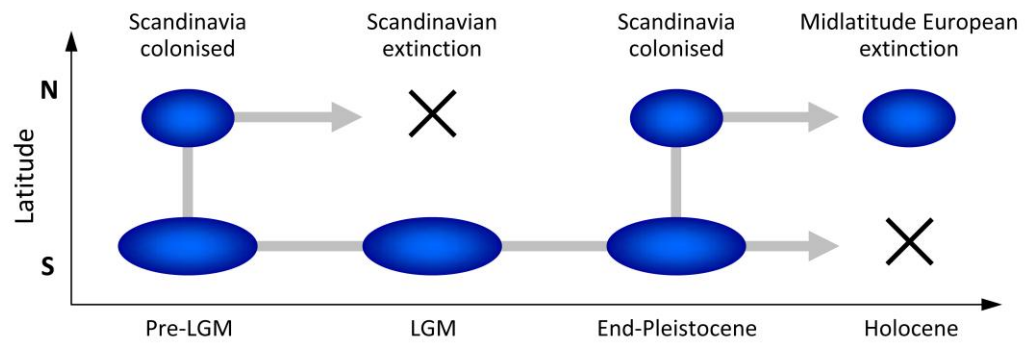
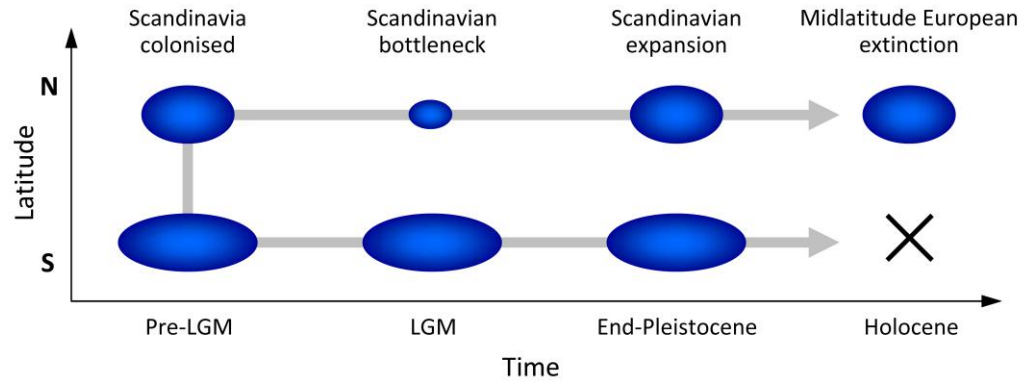
**Scenario 1****Scenario 2**

Fig. 2

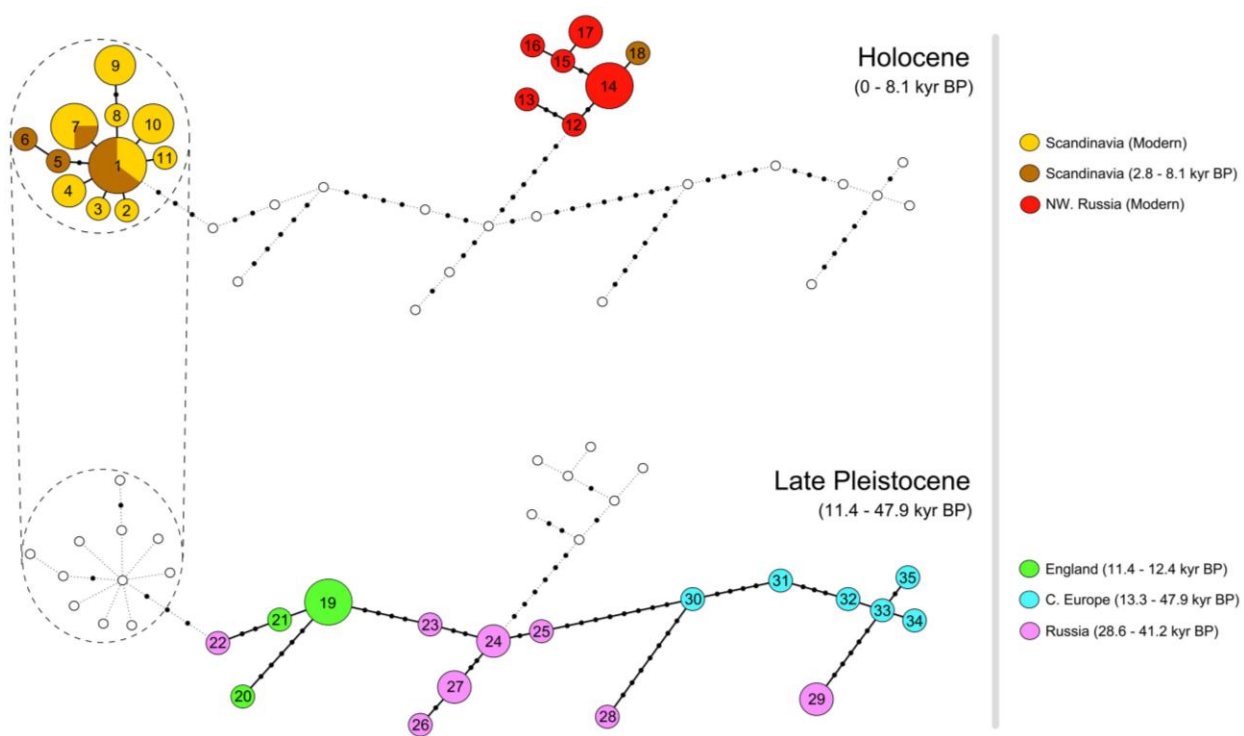


Fig. 3



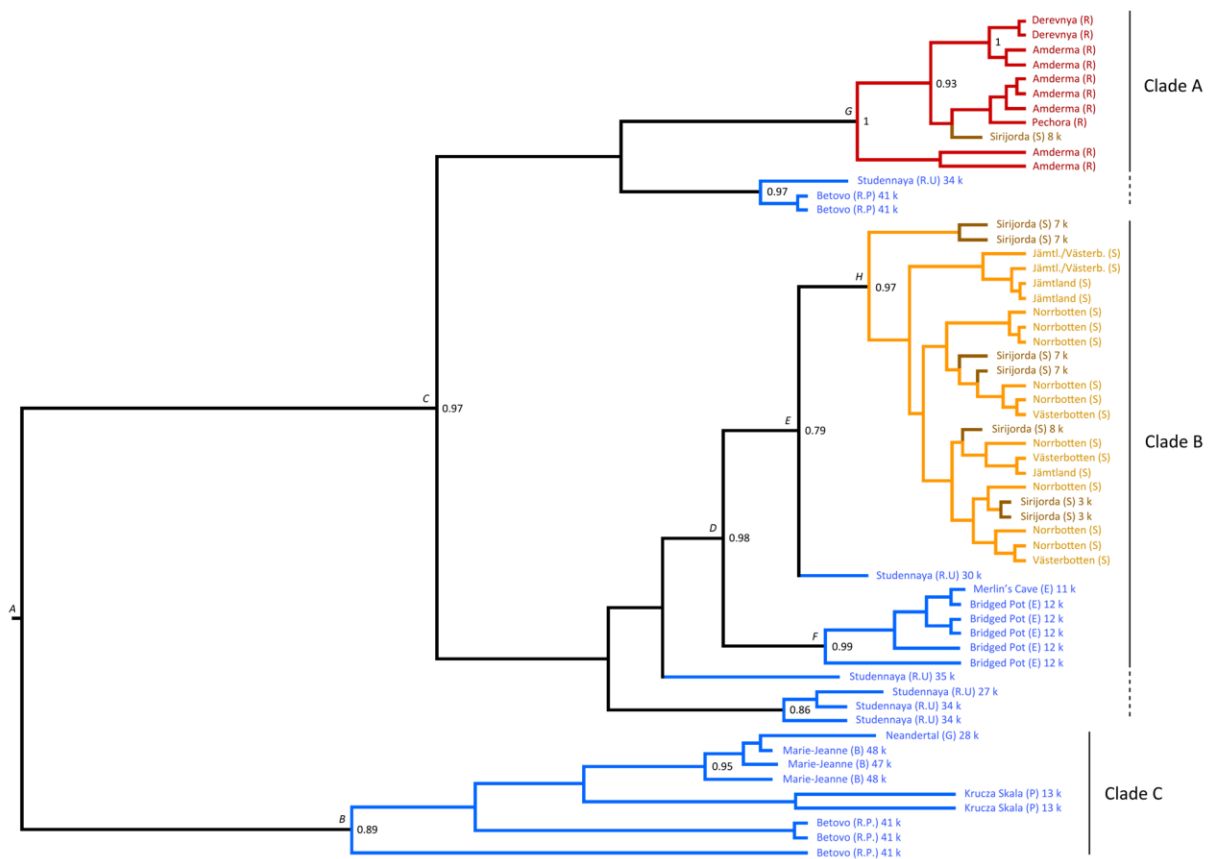


Fig. 4

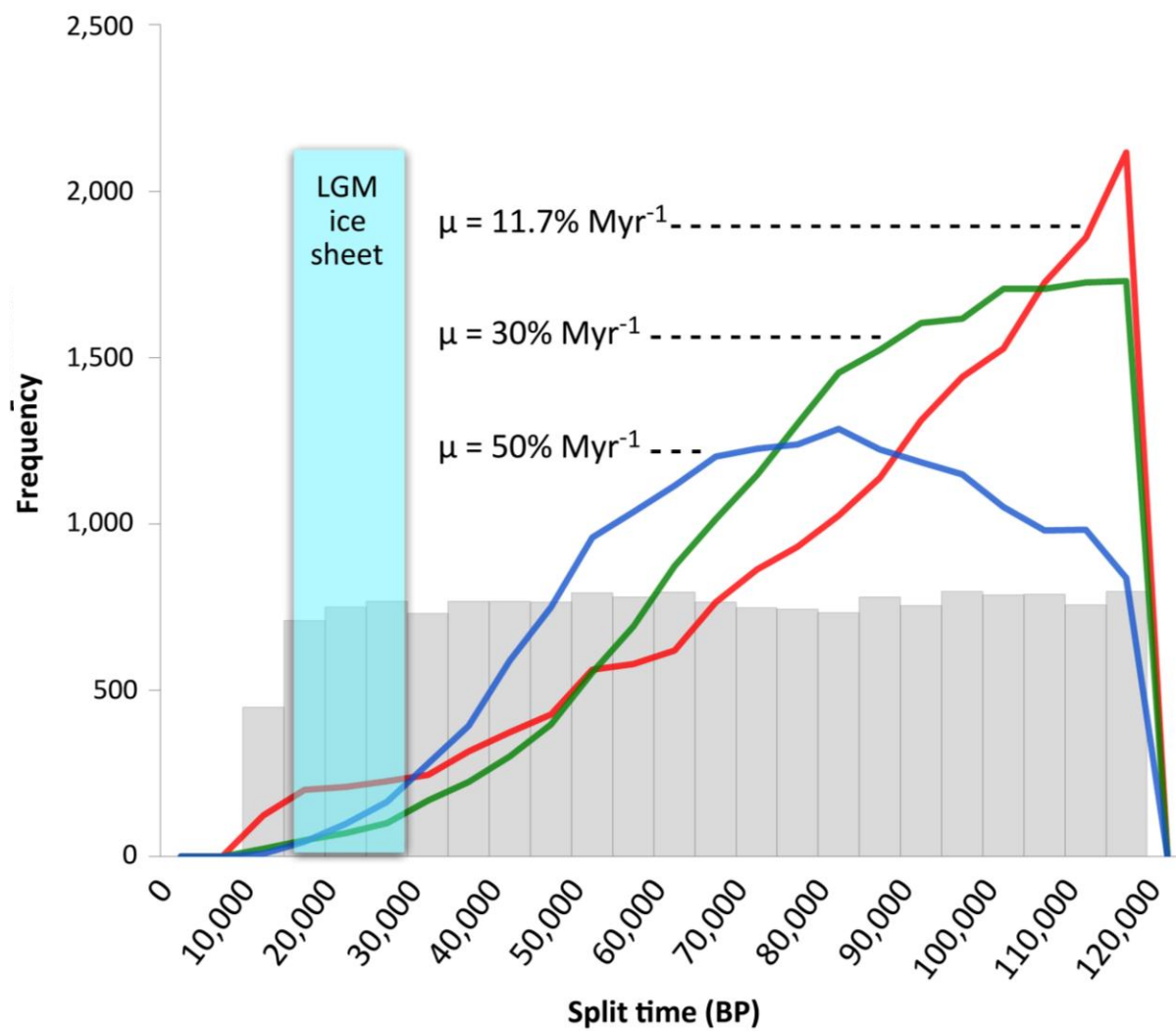


Fig. 5

## References

- Anderson CNK, Ramakrishnan U, Chan YL, Hadly EA (2005) Serial SimCoal: A population genetics model for data from multiple populations and points in time. *Bioinformatics*, **21**, 1733-1734.
- Bertorelle G, Benazzo A, Mona S (2010) ABC as a flexible framework to estimate demography over space and time: some cons, many pros. *Molecular Ecology*, **19**, 2609-2625.
- Birks HH, Giesecke T, Hewitt GM, *et al.* (2012) Comment on "Glacial survival of boreal trees in Northern Scandinavia". *Science*, **338**, 742.
- Brace S, Palkopoulou E, Dalén L, *et al.* (2012) Serial population extinctions in a small mammal indicate Late Pleistocene ecosystem instability. *Proceedings of the National Academy of Sciences*.
- Brochmann C, Gabrielsen TM, Nordal I, Landvik JY, Elven R (2003) Glacial survival or tabula rasa? The history of North Atlantic biota revisited. *Taxon*, **52**, 417-450.
- Bronk Ramsey C (2009) Bayesian analysis of radiocarbon dates. *Radiocarbon* **51**, 337-360.
- Campos PF, Kristensen T, Orlando L, *et al.* (2010a) Ancient DNA sequences point to a large loss of mitochondrial genetic diversity in the saiga antelope (*Saiga tatarica*) since the Pleistocene. *Molecular Ecology*, **19**, 4863-4875.
- Campos PF, Willerslev E, Sher A, *et al.* (2010b) Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 5675-5680.

- Csillery K, Blum MGB, Gaggiotti O, Francois O (2010) Approximate Bayesian Computation (ABC) in practice. *Trends Ecol Evol*, **25**, 410 - 418.
- Dalén L, Nyström V, Valdiosera C, *et al.* (2007) Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 6726-6729.
- Depaulis F, Orlando L, Hänni C (2009) Using Classical Population Genetics Tools with Heterochroneous Data: Time Matters! *PLoS ONE*, **4**, e5541.
- Drummond A, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Ekman S (1922) *Djurvärldens utbredningshistoria på den Skandinaviska halvön* Albert Bonniers Förlag, Stockholm.
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564-567.
- Excoffier L, Novembre J, Schneider S (2000) SIMCOAL: A general coalescent program for the simulation of molecular data in interconnected populations with arbitrary demography. *Journal of Heredity*, **91**, 506-509.
- Fedorov V, Goropashnaya A, Jarrell GH, Fredga K (1999) Phylogeographic structure and mitochondrial DNA variation in true lemmings (*Lemmus*) from the Eurasian Arctic. *Biological Journal of the Linnean Society*, **66**, 357-371.
- Fedorov VB, Stenseth NC (2001) Glacial survival of the Norwegian lemming (*Lemmus lemmus*) in Scandinavia: inference from mitochondrial DNA variation. *Proceedings of the Royal Society B: Biological Sciences*, **268**, 809-814.

- Fernández H, Hughes S, Vigne J-D, *et al.* (2006) Divergent mtDNA lineages of goats in an Early Neolithic site, far from the initial domestication areas. *Proceedings of the National Academy of Sciences*, **103**, 15375-15379.
- Galbreath KE, Cook JA (2004) Genetic consequences of Pleistocene glaciations for the tundra vole (*Microtus oeconomus*) in Beringia. *Molecular Ecology*, **13**, 135-148.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95-98.
- Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**, 247-276.
- Ho SYW, Lanfear R, Bromham L, *et al.* (2011a) Time-dependent rates of molecular evolution. *Molecular Ecology*, **20**, 3087-3101.
- Ho SYW, Lanfear R, Phillips MJ, *et al.* (2011b) Bayesian estimation of substitution rates from ancient DNA sequences with low information content. *Systematic Biology*.
- Hofreiter M, Barnes I (2010) Diversity lost: are all Holarctic large mammal species just relict populations? *BMC Biology*, **8**, 46.
- Johnson NK, Cicero C (2004) New mitochondrial DNA data affirm the importance of Pleistocene speciation in North American birds. *Evolution*, **58**, 1122-1130.
- Johnson TC, Scholz CA, Talbot MR, *et al.* (1996) Late Pleistocene desiccation of Lake Victoria and rapid evolution of Cichlid fishes. *Science*, **273**, 1091-1093.
- Klicka J, Zink RM (1997) The importance of recent ice ages in speciation: a failed paradigm. *Science*, **277**, 1666-1669.
- Lagerholm VK, Sandoval-Castellanos E, Ehrich D, *et al.* Data from: On the Origin of the Norwegian Lemming. Dryad Data Repository. doi:10.5061/dryad.jp8r1.

- Larsen E, Gulliksen S, Lauritzen SE, *et al.* (1987) Cave stratigraphy in western Norway; multiple Weichselian glaciations and interstadial vertebrate fauna. *Boreas*, **16**, 267-292.
- Lister AM (2004) The impact of Quaternary Ice Ages on mammalian evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **359**, 221-241.
- Lopes JS, Balding D, Beaumont MA (2009) PopABC: a program to infer historical demographic parameters. *Bioinformatics*, **25**, 2747 - 2749.
- Mangerud J, Gyllencreutz R, Lohne Ø, Svendsen JI (2011) Glacial history of Norway. In: *Quaternary glaciations - Extent and chronology. A closer look* (eds. Ehlers J, Gibbard PL, Hughes PD), pp. 279-298. Elsevier, Amsterdam.
- Mayr E (1954) Change of genetic environment and evolution. In: *Evolution as a Process* (eds. Huxley J, Hardy AC, Ford EB), pp. 157-180. Allen & Unwin, London.
- Mayr E (1963) *Animal species and evolution* Harvard University Press, Cambridge.
- Nadachowski A (1982) *Late Quaternary rodents of Poland with special reference to morphotype dentition analysis of voles* Panstwowe Wydawnictwo Naukowe, Warszawa.
- Navascués M, Depaulis F, Emerson BC (2010) Combining contemporary and ancient DNA in population genetic and phylogeographical studies. *Molecular Ecology Resources*, **10**, 760-772.
- Navascués M, Emerson BC (2009) Elevated substitution rate estimates from ancient DNA: model violation and bias of Bayesian methods. *Molecular Ecology*, **18**, 4390-4397.
- Nesje A, Dahl SO, Linge H, *et al.* (2007) The surface geometry of the Last Glacial Maximum ice sheet in the Andøya-Skånland region, northern Norway, constrained by surface exposure dating and clay mineralogy. *Boreas*, **36**, 227-239.

Nylander JAA (2004) MrModeltest v2, p.

<http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html>

Orr MR, Smith TB (1998) Ecology and speciation. *Trends in Ecology & Evolution*, **13**, 502-506.

Parducci L, Jørgensen T, Tollefsrud MM, *et al.* (2012) Glacial survival of boreal trees in northern Scandinavia. *Science*, **335**, 1083-1086.

Prost S, Anderson CNK (2011) TempNet: a method to display statistical parsimony networks for heterochronous DNA sequence data. *Methods in Ecology and Evolution*, **2**, 663-667.

Rambaut A (2009) FigTree v1.3.1: Tree figure drawing tool, p.

<http://tree.bio.ed.ac.uk/software/figtree/>.

Rambaut A, Drummond AJ (2007) Tracer v1.4: MCMC trace analyses tool, p.

<http://tree.bio.ed.ac.uk/software/tracer/>.

Rand AL (1948) Glaciation, an isolating factor in speciation. *Evolution*, **2**, 314-321.

Reimer P, Baillie M, Bard E, *et al.* (2009) IntCal09 and Marine09 radiocarbon age calibration curves, 0 - 50,000 years cal BP. *Radiocarbon* **51**, 1111-1150.

Ronquist F, Teslenko M, van der Mark P, *et al.* (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, **61**, 539-542.

Shapiro B, Drummond AJ, Rambaut A, *et al.* (2004) Rise and fall of the Beringian steppe bison. *Science*, **306**, 1561-1565.

Stewart JR (2008) The progressive effect of the individualistic response of species to Quaternary climate change: an analysis of British mammalian faunas. *Quaternary Science Reviews*, **27**, 2499-2508.

- Stewart JR, Lister AM, Barnes I, Dalén L (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 661-671.
- Stewart JR, Stringer CB (2012) Human evolution out of Africa: The role of refugia and climate change. *Science*, **335**, 1317-1321.
- Svendsen JI, Alexanderson H, Astakhov VI, *et al.* (2004) Late Quaternary ice sheet history of northern Eurasia. *Quaternary Science Reviews*, **23**, 1229-1271.
- Svensson A, Andersen KK, Bigler M, *et al.* (2006) The Greenland ice core chronology 2005, 15-42 ka. Part 2: comparison to other records. *Quaternary Science Reviews*, **25**, 3258.
- Templeton AR (2009) Statistical hypothesis testing in intraspecific phylogeography: nested clade phylogeographical analysis vs. approximate Bayesian computation. *Molecular Ecology*, **18**, 319-331.
- Yang DY, Eng B, Wayne JS, Dudar JC, Saunders SR (1998) Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology*, **105**, 539-543.
- Østbye E, Lauritzen S-E, Moe D, Østbye K (2006) Vertebrate remains in Holocene limestone cave sediments: faunal succession in the Sirijorda Cave, northern Norway. *Boreas*, **35**, 142-158.



## Figure legends

**Fig. 1** Temporal and geographic sample distribution. The Scandinavian Ice Sheet's approximate extent is shown for the four time periods, corresponding to (a) the Bø and Ålesund interstadials ~55 kyr to 35 kyr BP (without the short Skjonghelleren stadial ~ 39 kyr BP); (b) the Last Glacial Maximum ~30 kyr to 16 kyr BP; (c) the Younger Dryas stadial ~12 kyr BP (Mangerud *et al.* 2011; Svendsen *et al.* 2004); and (d) the Holocene. The Late Pleistocene sites that yielded successful ancient DNA sequences are illustrated with circles, which are filled blue at their respective time period. Sampling locations for modern specimens are shown as yellow (*L. lemmus*) and red (*L. sibiricus*) squares. The brown diamond represents the cave site from where early-mid Holocene samples were obtained.

**Fig. 2** Schematic illustration of the two hypotheses regarding the evolutionary history of the Norwegian lemming (*Lemmus lemmus*). In scenario 1, the modern population is derived from a post-glacial colonisation from midlatitude Europe following the retreat of the Scandinavian Ice Sheet, whereas the population in scenario 2 has survived in Scandinavia since before the Last Glacial Maximum (LGM). In both scenarios, the lemming populations that inhabited midlatitude Europe ultimately went extinct during Holocene climate warming.

**Fig. 3** Temporal statistical parsimony network. Haplotypes are temporally divided into the Holocene (including both modern and Holocene cave samples) and the Late Pleistocene, with empty circles indicating a haplotype that is missing in one temporal layer but is present in the other. Black dots represent missing haplotypes in the total data set. The number of individuals sharing a haplotype is reflected by its size. The dashed circles and connecting lines between the two temporal layers illustrate the absence of the Scandinavian haplogroup in the Late Pleistocene data set. The analysis is based on the partial data set. The

haplotypes are coloured according to their sample region, with numbers referring to the specific haplotype identifiers listed in Table S1 (Supporting Information).

**Fig. 4** Bayesian phylogeny. Modern NW Russian *L. sibiricus* are shown in red, modern Scandinavian *L. lemmus* in yellow, early-mid Holocene Scandinavian samples in brown and Late Pleistocene European samples in blue. The ages of all ancient samples are shown in thousands (k) of years before present, with those from the Studennaya site referring to the calculated median ages obtained from BEAST. S = Scandinavia; R = Russia; R.P = Russian plains; R.U = Russian Urals; P = Poland; G = Germany; B = Belgium; E = England. Posterior probabilities of internal nodes above 0.8 are shown, with letters A to H referring to the estimated divergence times listed in Table 1. The analysis was performed in BEAST, using the partial data set and a mutation rate of 30 % Myr<sup>-1</sup>.

**Fig. 5** Posterior probability distributions for the population divergence time between Scandinavian lemmings and their closest glacial relatives, based on the Bayesian coalescent simulations of the partial data set, using three different mutation rates. The posterior distributions are truncated at 120 kyr BP, which represents the start of the Late Pleistocene glaciation. The uniform prior distribution is shown with grey bars.

**Author contributions**

V.K.L. and D.E. designed and performed the DNA analyses, and V.K.L. also computed population-genetic statistics, carried out Bayesian phylogenetic analyses and co-wrote the paper; E.S.-C. performed and wrote the text on Bayesian coalescent simulations; N.A., A.N., D.C.K., M.G. and A.A. contributed with material and data; J.R.S. contributed with material and information, and helped interpret the data; L.D. conceived and designed the project and co-wrote the paper. All authors discussed the results and contributed to the preparation of the manuscript.

**Data Accessibility**

All sequences have been deposited in GenBank under the accession numbers JX483882-JX483939. The sequence alignments, as well as the input files and resulting tree files from BEAST and MrBayes have been deposited in the Dryad Data Repository, doi:10.5061/dryad.jp8r1.

**Supporting Information**

Additional supporting information can be found in the online version of this article.

**Text S1** Materials and methods.

**Table S1** All samples included in the study.

**Table S2** Summary statistics employed in the Bayesian coalescent simulations.

**Table S3** Genus-specific primers developed for the study.

**Table S4** Genetic diversity within modern, Holocene and Late Pleistocene sample regions.

**Table S5** Phylogenetic tree node ages.

**Fig. S1** Bayesian coalescent simulation methodology.

**Fig. S2** Temporal statistical parsimony network and Bayesian phylogeny constructed in BEAST.

**Fig. S3** Bayesian phylogeny constructed in MrBayes.

**Fig. S4** Posterior distributions of the employed summary statistics for each scenario separately.

**Fig. S5** Posterior distributions of the employed summary statistics for both scenarios combined.

**Fig. S6** Obtained posterior probability distributions for different effective population sizes.