Synchronous genetic turnovers across Western Eurasia in Late Pleistocene collared lemmings

Running head: Biogreographic history of the collared lemming

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Abstract

Recent palaeogenetic studies indicate a highly dynamic history in collared lemmings (*Dicrostonyx* spp.), with several demographic changes linked to climatic fluctuations that took place during the last glaciation. At the western range margin of *D. torquatus*, these demographic changes were characterized by a series of local extinctions and recolonizations. However, it is unclear whether this pattern represents a local phenomenon, possibly driven by ecological edge effects, or whether these extinctions and recolonizations took place across large geographical scales. To address this, we explored the palaeogenetic history of the collared lemming using a next-generation sequencing approach for pooled mitochondrial DNA amplicons. Sequences were obtained from over 300 fossil remains sampled across Eurasia and two sites in North America. We identified five mitochondrial lineages of *D. torquatus* that succeeded each other through time across Europe and western Russia, indicating a history of repeated population extinctions and recolonizations, most likely from eastern Russia, during the last 50,000 years. The observation of repeated extinctions across such a vast geographical range indicates large-scale changes in the steppe-tundra environment in western Eurasia during the last glaciation. All Holocene samples, from across the species' entire range, belonged to only one of the five mitochondrial lineages. Thus, extant *D. torquatus*populations only harbour a small fraction of the genetic diversity that existed during the Late Pleistocene. In North American samples, haplotypes belonging to both *D. groenlandicus* and *D. richardsoni* were recovered from a Late Pleistocene site in southwestern Canada. This suggests that *D. groenlandicus* had a more southern, and *D. richardsoni* a more northern glacial distribution than previously thought.

Introduction

The Late Pleistocene was a characterized by dramatic shifts in mammal species' ranges (Hewitt, 1999), changes in community composition and richness (Blois *et al.*, 2010, Graham, 1986, Stewart, 2009), loss of genetic diversity (Shapiro *et al.*, 2004, Stiller *et al.*, 2010), population replacements (Hofreiter *et al.*, 2007, Leonard *et al.*, 2007) and local or global extinctions (Martin, 1984, Stiller *et al.*, 2014). Individual taxa have responded differently to the effects of climatic and environmental changes (Lorenzen *et al.*, 2011, Prost *et al.*, 2013, Stewart *et al.*, 2010). Different responses have also been observed within the same species at the population level (Campos *et al.*, 2010). While many studies have focused on a single or a restricted number of populations to explore their dynamics and identify the forcing mechanisms, the identified trends are not necessarily characteristic of the entire species. Specific factors, such as local environmental changes can play an important role in shaping population dynamics. Further, different responses can be expected for core versus peripheral populations due to source-sink dynamics and ecological edge effects. These factors may affect the level of genetic variation and evolutionary potential of populations at the edge of species' geographical ranges, depending on the amount of gene flow from the core population (Brown, 1984, Hampe & Petit, 2005). Species-level investigations require sampling of multiple populations from across the geographic distribution in order to gain a complete understanding of the evolutionary processes that led to current patterns of biodiversity.

Collared lemmings (*Dicrostonyx* spp.) are cold-adapted, small rodents restricted to the dry, treeless environment of the Arctic tundra (Kowalski, 1995). Their present-day distribution is nearly circumpolar with *D. torquatus* occupying a range from western Russia to northeast Siberia, *D. groenlandicus* inhabiting Alaska,the Canadian Arctic and Wrangel Island, and *D. richardsoni* and *D. hudsonius* occupying regions west and east of Hudson Bay respectively. The recognition of one Eurasian species and three North American species is based on

mitochondrial DNA (mtDNA) diversity, karyotype variation and hybridization experiments, and represents the most widely accepted taxonomic classification (Fedorov & Goropashnaya, 1999). Evidence of a wider geographical range both in Eurasia and North America during the Late Pleistocene has been provided by the fossil record; collared lemmings appear to have expanded southwards during cold periods while they remained restricted to the north during warm interglacial periods, including the present one (Graham *et al.*, 1996, Sher, 1991, Stewart, 2003). It has also been shown that the climates associated with the expanded populations, living in non-analogue communities, are not necessarily equivalent to those associated with modern populations to the North. This may imply that these southern populations were differently adapted to modern ones (Stewart, 2009, Stewart *et al.*, 2003). Historical population fragmentation associated with the glacial/interglacial cycles has been inferred from the observed phylogeographic structure reconstructed from analysis of modern mtDNA data (Fedorov *et al.*, 1999, Fedorov, 1999). Moreover, the observed low nucleotide and haplotype diversity in the mtDNA of *D. torquatus* has been attributed to regional bottlenecks most likely linked to warming events during the Holocene (Fedorov *et al.*, 1999).

Late Pleistocene vertebrate sites are rich in small mammal remains (Markova *et al.*, 1995, Stewart *et al.*, 2003), and these constitute a valuable source of ancient DNA that enables reconstruction of past population histories. Previous genetic studies have exploited the abundance of collared lemming fossil remains collected from regions within the genus' current and past distribution to identify distinct demographic histories in populations from different sites. For instance, genetic continuity during the last 25,000 years has been reported for *D. torquatus* from a single site in the northern Urals (Pymva Shor) with signatures of a severe population bottleneck following the Last Glacial Maximum (LGM) most likely due to climate warming (Prost *et al.*, 2010). Conversely, a series of local population extinctions followed by recolonization events has been documented at the westernmost range of *D.*

torquatus during the last 50,000 years (Brace *et al.*, 2012). These repeatedturnover events were correlated with periods of climatic fluctuation during the last glaciation, and thus likely reflected response to environmental changes. Although both studies demonstrated that climate changes played a major role in driving the dynamics of *D. torquatus*, they indicated the possibility of distinct patterns of response in different parts of the species' distribution, with a single demographic bottleneck in the center and several local extinctions/replacements near the edge of its range.

In North America, species-level identification of fossils of *Dicrostonyx* is hampered by similarities in molar morphology among extant forms, except for *D. hudsonius*, which displays diagnostic morphological characters (Fulton *et al.*, 2013). Evaluation of ancient DNA provides an alternative method for distinguishing morphologically similar taxa but thus far very few North American fossil remains of *Dicrostonyx* have been genetically studied. However, a study on mtDNA from three individuals from Midwest United States dated to ~15,000 and ~24,000 years ago (Fulton *et al.*, 2013) revealed that they belong to *D. richardsoni*. This finding led the authors to hypothesize glacial survival of the species at regions south of the Laurentide ice sheet, similar to what has been suggested for *D. hudsonius* (Macpherson, 1965). However, it remains unknown whether *D. groenlandicus* also was restricted to regions south of the Laurentide ice sheet during the last glacial period or if this type of geographic response was specific to *D. richardsoni* and *D. hudsonius*.

In this study, we broadened the genetic sampling of fossil remains of *Dicrostonyx* to cover most of its Eurasian Late Pleistocene range. We further analysed ancient specimens from two cave sites in Alberta in the Canadian Rocky Mountains, a region that has remained unexplored in terms of small mammal palaeogenetic analyses. Using ancient DNA methods, we generated a dataset of mitochondrial sequences from more than 300 individuals. We combined our dataset with previously published modern and ancient data to address several

questions regarding the past population dynamics and biogeography in collared lemmings. First of all, we evaluated the possibility that local extinctions and recolonizations were widespread across the range of *D. torquatus* during the Late Pleistocene. Moreover, we were interested in exploring whether any observed extinctions and recolonizations were synchronous in time across several geographic sites, since this may indicate extensive changes in the glacial steppe-tundra environment. Finally, we used our data to determine which species of collared lemming occupied southwestern Canada during the last glacial period, thereby providing additional data to assess Pleistocene origins of extant North American populations of *Dicrostonyx*.

Materials and methods

Sample collection and DNA analyses

We collected 621 collared lemming mandibles and molars from across Eurasia and western Canada covering a temporal range from present day to more than 50,000 calendar years before present (cal. years BP; Supporting Table S1). To facilitate discussion of the Eurasian dataset we refer to localities in Russia west of the Ural Mountains as western Russia, localities in north-central Siberiaas Central Russia and localities in northeastern Siberia as eastern Russia. DNA extractions were conducted in two laboratories dedicated for ancient DNA, housed at the Swedish Museum of Natural History and the Institute of Genetics and Biotechnology at the University of Warsaw. Amplification and sequencing of the mitochondrial cytochrome *b* (cyt *b*) gene was performed with barcoded primers or library adapters to enable parallel sequencing of pooled individuals using the 454 sequencing technology. For full details on methods and data analyses, see Supporting Information.

Radiocarbon dating

We radiocarbon dated specimens with enough remaining material (more than 50mg after material had been taken for DNA extractions) and for which the entire sequence of targeted mtDNA was obtained. Dating was performed with the Accelerator Mass Spectrometry (AMS) method at the Oxford Radiocarbon Accelerator Unit using methods previously outlined (Brock *et al.*, 2010) (Supporting TableS2). OxCal v. 4.2 (Ramsey, 2009) and the IntCal 13 calibration curve (Reimer *et al.*, 2013) were used to calibrate the radiocarbon dates obtained in this study as well as previously published dates. Calibrated median dates are given in Supporting Tables S2, S3.

Phylogenetic analyses

We aligned sequences generated in this study with previously published ancient and modern complete cyt *b* sequences (see Supporting Table S3, for GenBank accession numbers) in Geneious version 5.5.7. The best-fitting model of nucleotide substitution for each dataset was chosen by MrModetest2 (Nylander, 2004) using a partition scheme with three coding positions. We performed three sets of analyses: *i*) using a dataset comprised of single haplotypes (identical sequences were collapsed), *ii*) using a dataset restricted to dated sequences only (with finite radiocarbon dates), excluding North American sequences and *iii*) using a dataset including radiocarbon dated sequences as well as sequences for which prior information on their age was available. Bayesian phylogenies were constructed in BEAST v.1.8.0 (Drummond *et al.*, 2012) implementing a strict molecular clock with the substitution rate estimated from the data. Analysis of dataset (*i*) was performed under the speciation: birth-death tree prior given that more than one species were included. For dataset (*ii*), we compared three population models, namely constant population size, Bayesian skyline plot (Drummond *et al.*, 2005) and Bayesian SkyGrid (Gill *et al.*, 2013) using marginal likelihood estimation (MLE) (Baele *et al.*, 2013). The Bayesian SkyGrid model was the model of choice from the MLE method and implemented in the final analyses. In datasets (*ii*) and (*iii*) we used the calibrated median of the radiocarbon age of each sequence as tip-dates for internal calibration of the tree, even in cases where the calibrated date extended out of the IntCal13 curve range. Sequences that provided infinite radiocarbon dates were excluded from these datasets. In dataset (*iii*), undated sequences as well as sequences for which radiocarbon dating failed were given a uniform distribution of dates as a prior for age sampling, based on stratigraphic information or associated dated material (field 'Prior age' in Supporting Table S2). Sequences for which prior information on their age was not available were excluded from this dataset. To test whether the temporal span of the dated samples and the sequence information content were sufficient to calibrate the tree and estimate evolutionary rates, we

performed date randomization test as described (Ho *et al.*, 2011) (Fig. S1). The GTR+I+G model of sequence evolution was chosen by MrModeltest2 for datasets (*i*) and (*iii*) using both hierarchical likelihood ratio tests (hLRTs) and the Akaike Information Criterion (AIC). However, due to poor estimates of convergence even after increasing the number of generations to 100 million, the implemented model was changed to HKY+I+G. For dataset (*ii*) we implemented the HKY+I+G model of sequence evolution, which was the best-fitting model according to the AIC (hLTRs recommended HKY+G). Two independent runs of 100 million generations (50 million for dataset [ii]) were performed for each analysis sampling every 10,000 generations (5,000 for dataset [ii]) and discarding the first 10% of the samples as burnin. LogCombiner v.1.8.0 was used to combine log files and tree files, and Tracer v.1.6. (Rambaut *et al.*, 2014) was used to assess convergence between runs.

We also constructed a Bayesian SkyGrid Plot (BSG) (Gill *et al.*, 2013) in BEAST using dataset (*ii*) to infer changes in female effective population size (N_{ef}) through time. Two independent runs of 50 million iterations were run with sampling every 5,000 generations and discarding the first 10% of the samples as burnin. Tracer v.1.6. was used to assess convergence and estimate the BSG.

We reconstructed the spatiotemporal distribution of the mitochondrial lineages in GenGIS (Parks *et al.*, 2009) by assigning dated individuals or individuals for which prior age information was available, to 5 time slices divided according to the approximate temporal distribution of each mitochondrial lineage.

Results

Mitochondrial DNA diversity

When we screened our material for a fragment of 171bp of the targeted mtDNA region, we found that DNA preservation levels varied among different locations. Certain sites exhibited high frequencies of DNA-yielding samples while other sites displayed lower rates. In total 452 specimens yielded amplifiable DNA and 330 of these provided complete cyt *b* sequences (780bp) (Table S2). Only complete cyt *b* sequences were included in the subsequent analyses. We detected 159 novel haplotypes(deposited in GenBank with accession numbers XXXX; Supporting Table S2). When previously published modern and ancient sequences (Supporting Table S3) were combined with our data, this resulted in a dataset that comprised a total of 431 complete cyt *b* sequences.

Radiocarbon dating

Due to the small size of the starting material (not more than 200mg), most of the samples were not treated with ultrafiltration but only gelatinized and filtered for AMS dating. The combination of small sample size and lack of ultrafiltration resulted in a large number of specimens (48 out of 120 or 40%) failing to produce radiocarbon dates because of low collagen yield and/or high carbon:nitrogen ratios, which indicate degradation and contamination of exogenous carbon, respectively. Successfully dated specimens produced a range of dates from ~12,000 radiocarbon years BP to infinite (above ~50,000 radiocarbon years indicating an age beyond the limits of radiocarbon dating). Furthermore, specimens from two sites, Ostrov Bolshevik in central Russia and Kyttyk peninsula in eastern Russia produced very recent radiocarbon dates (less than 1,000 radiocarbon years) confirming that these specimens are modern and of Late Holocene origin respectively (Supporting Table S2).

Phylogenetic structure and demography

Bayesian phylogenetic analyses revealed 8 major mitochondrial lineages (Fig. 1). Of these, lineages EA1 to EA5 were of Eurasian origin and belonged to the clade representing *D. torquatus*. These lineages correspond to lineages 1-5 described in Brace *et al.* (2012). Lineages NA1 to NA3 correspond to the three North American collared lemming species (*D. groenlandicus, D. richardsoni* and *D. hudsonius*). All of the lineages were monophyletic and supported by high to moderate posterior probability values.

Focusing on *D. torquatus*, a temporal structure was observed within the diversity of the Eurasian lineages (Fig. 1), which was present across a large geographical scale ranging from western Europe to western Russia. This temporal division is more clearly observed in the phylogeny with finite radiocarbon-dated sequences (Fig. 2) and is illustrated by the geographical/temporal distribution of the lineages shown in Fig. 3. The two most basal Eurasian lineages (EA1 & EA2) were comprised of the oldest sequences in our dataset with dates greater than~50,000 (infinite radiocarbon dates) up to ~42,300 cal. years BP. These two lineages were followed by lineage EA3, which consisted of specimens that spanned a time range from ~32,000 to ~22,800 cal. years BP. Lineage EA4 was, with one exception, dated to a short time period between 22,200 and ~20,500 cal. years BP, whereas lineage EA5 included Late Pleistocene European and western Russian sequences younger than ~20,500 cal. years old together with all Late Holocene and modern sequences from across Russia. This latter lineage (EA5) covers most of the species' present-day geographical distribution. Within lineage EA5, we observed some evidence of a phylogeographic structure among the modern haplotypes. The haplotypes from Ostrov Bolshevik in Central Russia (H112-H118) were placed within the diversity of modern *D. torquatus* but formed a distinct group (Fig. 1). Contrary to indications from the morphology of their molars that these specimens are relics of

last glacial morphs (Abramson *et al.*, 2004), their mtDNA sequences suggest a more recent origin of this population.

The pattern of sequential phylogenetic groups through time appears to have occurred in most of the studied sites. In general, the lineages in western Russia and Europe did not overlap in time, although there were a few exceptions to this pattern. The oldest sequences within lineage EA5 from Biśnik cave in Poland were dated to ~19,000 cal. years BP, although one specimen from the same site with a younger date (16,159 cal. years BP) actually fell within lineage EA4 (Fig. 2). All other sequences within EA4 were tightly grouped in time, ranging from 20,383 to 22,157 cal. years BP. Furthermore, the only finite-dated sequence within lineage EA2 (44,356 cal. years BP) postdated some of the Belgian sequences within lineage EA1 (Fig. 2).

When we included additional sequences in the phylogeny by assigning prior ranges for the age of undated specimens as tip-dates, based on stratigraphic information or associated dated material, this provided further support for the observed temporally-structured mitochondrial diversity (Supporting Fig. S2). There was again an association between the inferred age of the sequences and the mitochondrial lineage they were placed within across nearly all studied sites in Europe and western Russia. One single exception to this pattern was observed in Pymva Shor in western Russia, where the assumed age of sequence E333 (~26,000 cal. years BP) did not fit with the observed temporal range of the lineage it fell within (EA4, up to ~22,200 cal. years BP; Fig. 3, Supporting Fig. S2).

The phylogenetic position of ancient haplotypes from eastern Russia is noteworthy. Haplotypes H169, H196 were basal to lineage EA4, and haplotypes H194, H195, H96 were basal to lineages EA5-EA2 (Fig. 1, Supporting Table S2). However, it should be noted that support for this latter association was very low, and in the phylogeny where sequences with

prior information on their age were included (Supporting Fig. S2) the position of haplotype H96 (represented by sequence E157 from Bison's site in northeastern Russia) changed and became basal to the entire diversity of *D. torquatus* withhigh support value. One more specimen that stood out in the phylogeny is the one represented by haplotype H119, which originated from Batagay close to the Yana River in eastern Russia, and fell at the base of the entire phylogeny including both Eurasian and North American mitochondrial lineages (Fig. 1).

The ancient haplotypes from North America were phylogenetically placed within the mitochondrial diversity currently observed among extant collared lemmings in Alaska and Canada (Fig. 1). Haplotypes H92, H93 were basal to *D. groenlandicus* whereas haplotype H121 was grouped together with *D. richardsoni*, and haplotype H120 was basal to both these lineages.

The Bayesian SkyGrid Plot revealed changes in N_{ef} through time but was not able to capture the population extinction/replacements in any detail (Supporting Fig. S3). This could be attributed to the temporal (albeit not spatial) structure in our data, which violates the assumption of a single panmictic population in BEAST (Drummond *et al.*, 2005, Navascues *et al.*, 2010).

Discussion

We identified five mitochondrial lineages in *D. torquatus* that sequentially replaced each other through time across a large part of the species' distribution (Fig. 2, 3). These five lineages have previously been identified in a regional ancient DNA study on collared lemmings from western Europe, where the successive replacement of these lineages through time was interpreted as a series of local population extinctions followed by recolonizations (Brace *et al.*, 2012). Our results demonstrate that this pattern was not limited to the western edge of the species' range, but extended across a large part of its geographical distribution including Europe and western Russia (Fig. 3). Thus, rather than indicating an edge effect at the western limits of the species' range margin, which could have been driven by small-scale ecological perturbations, our results are more consistent with a scenario of several major environmental changes that affected a large part of the Eurasian steppe tundra biome.

At least three turnover events were revealed from the reconstructed phylogeny (Fig. 2). In general, the timing of these appear synchronous across different sites in Europe and western Russia (Fig. 3). Replacement of lineages EA1-EA2 by lineage EA3 appears to have occurred between ~42,300 and ~32,000 cal. years BP, after which the climate started to become colder following the end of Greenland Interstadial (GI) 5. Interestingly, this is a time period that has been marked by population replacements in other species within Europe, such as the cave bear (Hofreiter *et al.*, 2007) and the woolly mammoth (Palkopoulou *et al.*, 2013). The two subsequent replacements appear to have had much shorter time ranges, with the replacement of lineage EA3 by EA4 occurring between ~22,800 and ~22,200 cal. years BP, and that of lineage EA4 by lineage EA5 around 20,500 cal. years BP. The time intervals of both these replacements fall within the LGM when tundra and steppe tundra enviroments spread over most of Europe and Western Asia. However, the observed turnovers seem to have been preceded by the short warming stage of GI 2 (Svensson *et al.*, 2008) and moreover they

coincide with short term warmings in the high resolution palynological record from Lago Grande di Monticchio in Italy (Allen *et al.*, 1999) (Supporting Fig. S4). This suggests that short term environmental changes could have affected a large part of the Eurasian steppe tundra biome during the LGM. Moreover, it is notable that the latter replacement occurred within a time period (21,700 – 19,400 cal. years BP) for which a gap has been reported in the fossil record of woolly mammoths from central and northwestern Europe, when the Weichselian ice sheet reached its maximum extent (Nadachowski *et al.*, 2011, Stuart *et al.*, 2004, Ukkonen *et al.*, 2011). As for the two oldest lineages (EA1 and EA2), it is unclear whether these were temporally separated, and hence whether they represent a fourth extinction/recolonization event, since they included sequences that were very close to the limits of radiocarbon dating (many of these specimens provided infinite dates).

Interestingly, we found evidence of mitochondrial lineage replacement as far east as the high-Arctic site of Pymva Shor in the northern Urals. Mitochondrial DNA variation data from this site was previously interpreted as indicating population continuity through time (Brace *et al.*, 2012, Prost *et al.*, 2010). However, our data suggests a replacement of lineage EA4 by lineage EA5 sometime between ~16,000 and ~26,000 cal. years BP (Fig. 3). We hypothesize that the difference between our results and those from previous studies can be explained by higher accuracy offered by the comparatively longer cyt *b* sequences retrieved in our study. Prost *et al.* (2010) used a fragment of similar size (704bp) that included 282bp *cyt b* and 426bp control region (CR). However, CR shows less diversity in collared lemmings than *cyt b* (Prost *et al.*, 2010) and thus a longer fragment of the *cyt b* should result in an increase in resolution. Overall, the finding that lineage replacements were geographically widespread indicates a pattern of repeated population extinctions across much of the collared lemming's Eurasian distribution.

However, there were a few exceptions to the overall pattern of synchronous replacements, and these merit a closer discussion. In Biśnik cave in Poland, the presence of an ~16,000 years old sequence (95% range: 15,901-16,411 cal. years BP) within lineage EA4 (Fig. 2, Fig. 3) could indicate that lineage EA4 coexisted with lineage EA5 (for at least 4,000 years taking into account the error ranges of the calibrated dates). However, this is only a single sequence and more data are needed to resolve whether these two lineages overlapped in time in this region. Moreover, the phylogenetic placement and associated date of the oldest sequence from Pymva Shor in western Russia indicates a larger upper temporal range for lineage EA4, which could further imply that lineages EA4 and EA3temporally overlapped in this region (Fig. 3, Supporting Fig S2). However, the lack of older specimens from Pymva Shor does not allow us to explore the mitochondrial diversity that existed further back in time. Alternatively, temporal discrepancy in Pymva Shor could be due to errors in the assumed age of the specimens, since these were based on associated rather than direct radiocarbon dates (Golovachov& Smirnov, 2009). Sequencing of additional directly-dated specimens from Pymva Shor is needed to unravel the timing of the replacement of lineages EA4 and EA3 at that site. Nonetheless, the phylogenetic placement of specimens from other western Russian sites (e.g. Yudinovo, Studenaya, Betovo), which were directly dated and altogether cover a wider temporal range (Fig. 2, Fig. 3), reinforces the observed pattern of simultaneous lineage turnovers in western Russia and Europe.

Genetic replacements, indicating population extinctions and recolonizations, have been observed in several other taxa (Barnes *et al.*, 2007, Hofreiter *et al.*, 2007, Leonard *et al.*, 2007, Stiller *et al.*, 2014). However, most of these studies were conducted on samples from limited geographical distributions, and it is unclear whether these events were site-specific or more widespread. In contrast, our study demonstrates several lineage replacements, which indicate a series of extinctions and recolonisations across a vast geographical area covering

Europe and western Russia. It is therefore possible that population replacements occurred at a wider spatial extent in other taxa too. To assess this hypothesis, further analyses on additional samples from other taxa covering a broader geographical range are needed.

In contrast to the pattern observed in Europe and western Russia, we did not observe a similar temporal separation of lineages in the eastern range of *D. torquatus* (Fig. 3, Supporting Fig. S2). Although all modern eastern Russian haplotypes were phylogenetically placed together with all other modern haplotypes within lineage EA5, the phylogenetic placement of Late Pleistocene eastern Russian sequences do not seem to correspond to their age. Based on dates from associated material, sequences L260, L261 have an inferred age between ~30,000 to ~34,000 cal. years BP. Despite this, these sequences were basal to lineage EA4, where all other sequences have ages ranging from ~20,500 to ~22,200 cal. years BP (Supporting Fig. S2). Although our sampling across eastern Russia was limited compared to western Eurasia, both in terms of sample size as well as temporal coverage, it is interesting to note that all Late Pleistocene eastern Russian sequences occupy basal positions in their respective mtDNA lineages. This indicates that eastern Russia was the source population from which founders recolonized western Russia and Europe following each of the inferred extinctions. The seemingly high genetic diversity in Late Pleistocene samples from eastern Russia is also consistent with the hypothesis that Beringia served as an interglacial refugium for the cold-adapted *D. torquatus* (Fedorov & Goropashnaya, 1999).

One of our samples, from a site near the Yana river in eastern Russia, yielded a highly divergent haplotype (H119, represented by sequence E313) that was basal to the entire phylogeny including both Eurasian and North American mitochondrial lineages (Fig. 1). This specimen yielded an infinite radiocarbon date (>50,299; Supporting Table S2). It is tempting to interpret the basal placement in the phylogeny in combination with the specimen's ancient origin as representing ancestral variation that was present prior to the split between Eurasian

and North American collared lemmings. Alternatively, this haplotype could represent an undiscovered extinct *Dicrostonyx* species. Further sequencing of this specimen including both mitochondrial and nuclear DNA, as well as sequencing of additional collared lemming fossil remains from eastern Russia, may help to resolve the identity of this haplotype and the role of northeastern Siberia as a refugium during periods of unfavorable environmental conditions.

Moving towards the present, we found some evidence of contemporary phylogeographical structure in *D. torquatus* (Fig. 1) in congruence with earlier findings (Fedorov *et al.*, 1999, Prost *et al.*, 2010). It is noteworthy that most of the contemporary phylogeographic clades across the Palearctic have also been found to exhibit variation in chromosomal numbers, although the distributions of mtDNA clades and chromosomal races do not agree in absolute terms (Fedorov *et al.*, 1999). These were hypothesized to have resulted from isolation during previous glacial cycles while regional population reductions during the Holocene were implicated for the limited mtDNA variation observed within each clade (Fedorov *et al.*, 1999). Our phylogenetic analyses demonstrate that the modern mtDNA clades diverged much more recently than previously thought, within the last 20,000 years and that each of the clades coalesced during the last 10,000 years (Fig. 2, Supporting Fig. S2). Thus, the current phylogeographic pattern may be a consequence of population contraction and isolation during warming periods at the end of the last glacial period. This hypothesis is consistent with observed changes in tooth morphotype frequencies (Smirnov & Fedorov, 2003), which also occured during that time. Interestingly, the observation of a recent origin for the present day phylogeographic groups also implies that the current chromosomal variation among extant *D. torquatus* populations evolved recently, possibly as a consequence of Late Pleistocene bottlenecks and the ensuing recolonization of the Eurasian Arctic.

The almost instantaneous turnovers of genetic lineage in *Dicrostonyx torquatus* in Europe and western Russia during the Late Pleistocenehas implications for relative dating in the Quaternary. Turnovers, particularly ones involving evolutionary lineages, are considered to be some of the most reliable means of biostratigraphic dating available (Lister, 1992). However, an over-riding concern has been that turnovers are time transgressive and hence cannot be used beyond relatively narrow geographical areas (Lister *et al.*, 2005). The present results showing three rapid turnovers in collared lemmings taking place across more than a 1000 km in Europe and western Russia during the Late Pleistocene may suggest that some of that concern is unnecessary. However, the other implication is that when using turnovers, they may be reliable in the region where the species in question is in its expansive phase but not where it is in its refugium.

The ancient sequences from North America suggest that both *D. groenlandicus* and *D. richardsoni* probably coexisted in southwestern Canada during the Late Pleistocene (Fig. 1). Identification of *D. groenlandicus* in the Canadian Rocky Mountains is consistent with the most recent morphological identification of collared lemmings from Alberta (Burns, 2004), butrecognition of haplotypes indicative of *D. richardsoni* in the Canadian sample reflect greater diversity in Late Pleistocene collared lemming populations than previously recognized. Dates from associated material placed the sequences from January Cave and Eagle Cave between ~33,000 and 40,000 cal. years BP, and >25,000 cal. years BP, respectively (Burns, 1991). Thus, in addition to having a southern distribution as shown by Fulton *et al.* (2013), *D. richardsoni* appears to also have inhabited southwestern Canada during the last glacial period. The glacial origin of *D. groenlandicus* is unresolved but it has previously been proposed that it was restricted to one or more glacial refugia located in the northern part of North America (Ehrich *et al.*, 2000, Fedorov & Goropashnaya, 1999, Fedorov & Stenseth, 2002). Several locations have been proposed for such putative glacial

refugia, including ice-free regions in eastern Beringia and the Canadian Arctic Islands or the coastal part of North Greenland, based on paleoecological data (Macpherson, 1965) and modern mitochondrial diversity data (Fedorov & Goropashnaya, 1999, Fedorov & Stenseth, 2002). However, in contrast to previous inferences, our findings of *D. groenlandicus* in southwestern Alberta in Canada during the last glaciation suggest that this species may have colonized the high Arctic from a location south of the Laurentide ice sheet following the end of the last glaciation.

Conclusions

Reconstruction of the evolutionary history of *Dicrostonyx* across a broad geographic and temporal range represents a unique contribution to our understanding of Pleistocene faunal dynamics. This study demonstrates the potential to reconstruct the evolutionary history of a taxon in detail by analyzing samples from a broad geographical and temporal range. Our analyses unveiled a higher level of genetic diversity across the Late Pleistocene range of the Eurasian collared lemming compared to that observed today. Moreover, an unparalleled pattern of serial genetic replacements through time was revealed across the species' western range during the last 50,000 years. Geographically wide-spread losses of genetic variation and local extinctions during the Late Pleistocene have previously been documented mainly in large-bodied mammals (Barnett *et al.*, 2009, Campos *et al.*, 2010, Lorenzen *et al.*, 2011, Palkopoulou *et al.*, 2013, Stiller *et al.*, 2014). The repeated replacements of collared lemming genetic diversity through time across a large part of its geographical distribution are consistent with a hypothesis that large-scale environmental changes during that time period had a significant impact on the dynamics of cold-adapted small mammals. Additional research is needed to more fully understand the nature of the North American record, and genetic analyses of fossil material from other ecologically similar species will enable us to assess whether such repeated extinctions and replacements were a general pattern among cold-adapted small mammals, which likely would have had cascading effects on the food web of the entire Pleistocene Arctic biota.

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