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Ancient DNA supports southern survival of Richardson's collared lemming (*Dicrostonyx richardsoni*) during the last glacial maximum

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Abstract

Collared lemmings (genus *Dicrostonyx*) are circumpolar Arctic arvicoline rodents associated with tundra. However, during the last glacial maximum (LGM), *Dicrostonyx* lived along the southern ice margin of the Laurentide ice sheet in communities comprising both temperate and boreal species. To better understand these communities and the fate of these southern individuals, we compare mitochondrial cytochrome *b* sequence data from three LGM-age *Dicrostonyx* fossils from south of the Laurentide ice sheet to sequences from modern *Dicrostonyx* sampled from across their present-day range. We test whether the *Dicrostonyx* populations from LGM-age continental USA became extinct at the Pleistocene–Holocene transition ~11000 years ago or, alternatively, if they belong to an extant species whose habitat preferences can be used to infer the palaeoclimate along the glacial margin. Our results indicate that LGM-age *Dicrostonyx* from Iowa and South Dakota belong to *Dicrostonyx richardsoni*, which currently lives in a temperate tundra environment west of Hudson Bay, Canada. This suggests a palaeoclimate south of the Laurentide ice sheet that contains elements similar to the more temperate shrub tundra characteristic of extant *D. richardsoni* habitat, rather than the very cold, dry tundra of the Northern Arctic. While more data are required to determine whether or not the LGM southern population is ancestral to extant *D. richardsoni*, it seems most probable that the species survived the LGM in a southern refugium.

Keywords: ancient DNA, Arvicolinae, mitochondrial DNA, Pleistocene, refugia, Rodentia

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Introduction

At the last glacial maximum (LGM) in North America, *c.* 26–19 thousand years ago (ka), most of what is now Canada was covered by the Laurentide ice sheet and generally uninhabitable. Species displaced by advancing glaciers either dispersed southward or retracted to Beringia or island refugia. More cold-tolerant populations likely persisted in presumably rare northern refugia (Fedorov & Stenseth 2002; Hewitt 2004; Waltari & Cook 2005; Shafer *et al.* 2010). This reshuffling of species distributions resulted in the formation of *nonanalog assemblages* or communities comprising temperate, boreal and arctic species that are not found in association at present (Graham 1986; Hibbard 1960; Semken 1974; for review, Semken *et al.* 2010). Many of these associations ended abruptly at the Pleistocene–Holocene transition *c.* 11 ka (Semken *et al.* 2010), although most micromammals (up to rabbit size) survived to the

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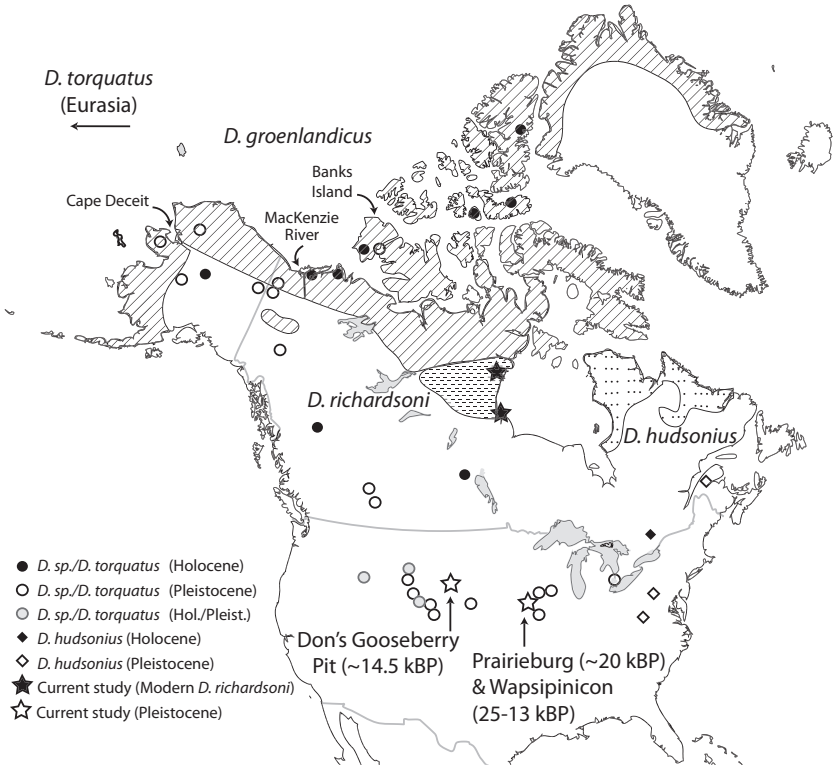

Fig. 1 Modern *Dicrostonyx* distribution and fossil localities. The ranges of extant *Dicrostonyx* species are illustrated by different patterns. Stars represent the geographic location of samples analysed herein. Geographic locations at which other *Dicrostonyx* fossils (not analysed here) have been found are also indicated, with icon shape reflecting taxonomy and icon colour and fill reflecting age. Locations were taken from FAUNMAP in the Neomap database, http://www.ucmp.berkeley.edu/neomap/search.html). *Dicrostonyx* dated to the most recent 450 years are not included. Some locations discussed in text are noted.

present (Graham & Lundelius 1984). Of those few that became extinct, most occurred on islands (Martin & Klein 1984). Tracking the fate of the populations that survived in these nonanalog assemblages provides a unique opportunity to examine community dissolution in response to climate change, as well as provide data for palaeoclimatic reconstruction.

Today, collared lemmings (*Dicrostonyx*) are tightly linked to the Arctic tundra (Kowalski 1995; Fedorov & Goropashnaya 1999). However, during the last glacial period, *Dicrostonyx* were found across the continental United States (Fig. 1), associated with the narrow band of cold environment along the Laurentide ice front in communities with other arctic and boreal species including *Lemmus sibiricus, Microtus xanthognathus*, *Synaptomys (Mictomys) borealis* and *Phenacomys intermedius* (Graham & Mead 1987). The presence of these cold-adapted taxa has been used to infer a cold, dry palaeoenvironment for these regions at the time. However, these nonanalog faunas also contain species that inhabit warm and temperate environments today including *Scalopus aquaticus*, *Geomys bursarius, Microtus ochrogaster* and *Ictidomys tridecemlineatus* (Semken *et al.* 2010), suggesting that these ice-marginal environments were unlike any existing today.

Living *Dicrostonyx* are divided into one Eurasian species, *D. torquatus*, and at least three North American species, *D. richardsoni*, *D. hudsonius*, and *D. groenlandicus* (Fig. 1), based on mitochondrial DNA, karyotype and hybridization experiments (Scott & Fisher 1983; Engstrom *et al.* 1993; Fedorov & Goropashnaya 1999). *Dicrostonyx richardsoni* is restricted to the western Hudson Bay region, *D. hudsonius* occupies the Ungava area of Quebec, and *D. groenlandicus* is found across the Canadian Arctic and Alaska. Although *D. groenlandicus* has been subdivided into additional species in a variety of ways (see Jarrell & Fredga 1993; Musser & Carleton 2005) (Fig. S1, Supporting information), we treat them as conspecific as in Fedorov & Goropashnaya (1999). For fossil *Dicrostonyx*, taxonomic revisions and highly similar dental morphology have made species assignment challenging. Fossils found across the continental United States have been assigned to either *D. torquatus* or as an unidentified species (*D.* sp.). However, current taxonomy restricts extant *D. torquatus* to Eurasia (Fig. 1). Many North American fossil *Dicrostonyx* were referred to *D. torquatus* based primarily upon geographic assumptions about the distribution of living species and their relationships to the fossil sites at the time (Guilday 1968; Martin *et al.* 1979; Semken & Falk 1987). Of all extant *Dicrostonyx* species, only one Pleistocene taxon, *D. hudsonius*, can be identified consistently by molar morphology (Gromov & Polyakov 1977). Therefore, based upon diagnostic morphological characters, the *Dicrostonyx* fossils identified as *D. torquatus* or *D.* sp from Late Pleistocene sites in the lower 48 United

States and from across Beringia and the Canadian Arctic (Fig. 1) (Eger 1995; Harington 2011) can only be considered as non-*hudsonius*.

Here, we present mitochondrial DNA data isolated from three *Dicrostonyx* specimens recovered from two locations south of the Laurentide ice sheet: Don's Gooseberry Pit in South Dakota and the Prairieburg and Wapsipinicon local faunas in Iowa (Fig. 1). We compare these data with newly generated sequences for *D. richardsoni* and other available modern data to test whether southern LGM-age *Dicrostonyx* (i) belong to any of the extant species or (ii) represent a unique extinct lineage. If these individuals belong to an extant species and similar ecological tolerance is assumed, then their modern environmental tolerances can be extrapolated to palaeoclimatic models. Alternatively, if these individuals represent a unique *Dicrostonyx* lineage, it would suggest that at least some of the micromammals that comprised nonanalog faunas went extinct with Holocene warming and support a strong role for extinction in the dissolution of nonanalog communities. Ancient DNA provides the first opportunity to test between these hypotheses.

Methods

Sample information: excavation, regional environment, morphological description

We collected fossil material from three Late Pleistocene sites: Prairieburg local fauna, Linn County, Iowa (Foley & Raue 1987); Wapsipinicon Assemblage, Dutch Creek Sample, Jones County, Iowa (Wallace 2008); and Don's Gooseberry Pit, Custer County, South Dakota (Pardi 2010). The Prairieburg local fauna is a specimen-rich micromammal deposit with low species diversity (six taxa) from a small cave along the Buffalo River, a tributary of the Wapsipinicon River, near Prairieburg, Iowa. The fossils of *Dicrostonyx* from Prairieburg were assigned to *D. torquatus* by Foley & Raue (1987). The fauna is a near-tundra analog (Semken *et al.* 2010). Uncalibrated radiocarbon dates on purified bone collagen from two *Dicrostonyx* specimens were 20 045 ± 310, AA 6991 and 20 035 ± 200, AA 5255 (Semken *et al.* 2010).

The Dutch Creek sample from the Wapsipinicon Assemblage is from a small cave along Dutch Creek, a tributary of the Wapsipinicon River near Anamosa, Iowa (Wallace 2008). The fauna contains about 60 mammalian taxa, representing at least 44 distinct species, 15 of which no longer occur in Jones County (Wallace 2008). Again, this site contains a mixture of species that do not co-occur together geographically and consequently represents a nonanalog fauna. Four uncalibrated radiocarbon dates (13 460 ± 20 NZA 10446; 17 280 ± 170 NZA 10444; 17 810 ± 160 NZA 10443; and 25 470 ± 350 NZA 10445) on the lower jaws of *Microtus xanthognathus* (Wallace 2008) place this fauna within the LGM. The *Dicrostonyx* fossils were originally assigned to *D. torquatus* (Wallace 2008).

Don's Gooseberry Pit is located in the southern Black Hills of South Dakota. It is a small pit cave that is being excavated by RWG. Don's Gooseberry Pit contains a mixture of species that do not co-occur today. Even though the excavations have been conducted at 10-cm levels within natural stratigraphic units, it remains to be demonstrated that the various taxa were contemporaneous (Pardi 2010). *Dicrostonyx* individuals directly dated on the basis of purified bone collagen have yielded uncalibrated dates of 14 055 ± 100, NZA 30074; 14 689 ± 170, NZA 30053; and 15007 ± 120, NZA 30177. The younger age of these specimens may relate to the higher elevation of this location than the surrounding area, a topography that allowed the cold-adapted species to remain in the area longer than in neighbouring areas of lower elevation.

All specimens used in this study have been referred to *Dicrostonyx* based upon dental comparisons of the first lower molars of the fossil specimens with modern forms of the collared lemming. *Dicrostonyx* have rootless first lower molars that exhibit six or more closed triangles and, unlike the teeth of other unrooted arvicolines (e.g. *Microtus*, *Lemmus*, etc.), those of *Dicrostonyx* do not contain cement between the re-entrants of the triangles (Semken & Wallace 2002). We examined all specimens to confirm *Dicrostonyx* morphology. As discussed above, species assignment was not possible on the basis of dental morphology. However, none of the specimens had the morphology characteristic of *D. hudsonius* (e.g. Gromov & Polyakov 1977). All radiocarbon dates, although processed by different laboratories, were from purified bone collagen.

To expand the range of modern sequences for comparative analysis, we collected five dried tissue samples from historic specimens of *D. richardsoni* from the Carnegie Museum: four originating from Churchill, Manitoba, and one from Rankin Inlet, Nunavut (Table S1, Supporting information).

DNA extraction and sequencing

We extracted DNA from two elements (both jaws with molars) from Don's Gooseberry Pit: Level 13, Unit 8, Bag 15, Element ILM (PSU extraction IDs: RN023, TF172, TF203) and Level 11, Unit 6, Bag 70 (PSU extraction: RN028) and from two elements received from the University of Iowa: a jaw with teeth SUI 95440 (BC 179) from the Prairieburg Local Fauna (PSU extraction:

TF174) and an isolated tooth SUI 95760 (T 3 17) from Wapsipinicon Assemblage, Dutch Creek Sample (PSU extraction: TF178). We performed DNA extraction following stringent ancient DNA protocols in a dedicated ancient DNA facility at The Pennsylvania State University. For all samples except RN023 and RN028, we first submerged the bone and/or tooth in bleach for 2–5 min to remove any potentially contaminating DNA that may have adhered to the surface of the element during deposition or preservation (Kemp & Smith 2005). This also resulted in the dissolution of each sample. When this occurred, we concentrated the remaining powder by centrifugation and discarded the bleach. We then resuspended the bone powder in sterile water and performed DNA extraction according to the protocol described in either Rohland *et al.* (2010) (TF numbers) or Rohland & Hofreiter (2007) (RN numbers). Following extraction, we added 1 μL of Tween-20 to each of the final 50 μL of eluate to prevent the DNA from adhering to the tube walls.

We extracted DNA from dried tissue samples from the historic Carnegie Museum specimens using a Qiagen DNeasy tissue kit with an additional 20 μL of proteinase K and 20 μL of 1M DTT, rotated overnight during tissue dissolution, and with a final elution into 100 μL of TE with 0.05% Tween-20. Although these samples are comparatively recent (<100 years old), DNA extraction and pre-PCR steps were carried out in a dedicated ancient DNA facility at the University of California Santa Cruz, as museum samples are often degraded and poorly preserved (Wandeler *et al.* 2007).

To facilitate comparison with previously published data, we selected a 498-base pair (bp) region of mitochondrial cytochrome *b* (*cytb*) for sequencing, corresponding to positions 348-845 in GenBank sequence AF119268. We attempted amplification with multiple pairs of primers spanning different fragment lengths in the range of 117-201 bp (Table S2, Fig. S2, Supporting information), with variable success depending on the sample. Amplification conditions are provided in Table S2 (Supporting information). We cleaned each PCR product using a Millipore Multiscreen $PCR_{\mu 96}$ filter plate and directly sequenced the resulting products using Big Dye v3.1 sequencing chemistry (Applied Biosystems) and the same primers as for amplification. For some amplification products, we evaluated the extent of DNA damage by bacterial cloning and by comparison of several overlapping fragments (Table S1, Supporting information). We used the TOPO-TA cloning kit (Invitrogen, USA) according to manufacturer's instructions and sequenced multiple resulting colonies from each PCR product using the M13F primer. We purified all sequencing reactions by ethanol precipitation. Sequences were resolved on an Applied Biosystems 3730xl capillary sequencer at the University Park Genomics Core Facility (The Pennsylvania State University). Carnegie Museum specimens were amplified at UC Santa Cruz and the PCR products cleaned and directly sequenced at UC Berkeley. All sequences were assembled in SeqMan (DNASTAR).

Phylogenetic analyses

To generate a comprehensive data set of available *Dicrostonyx* mitochondrial DNA sequences, we downloaded previously published data from GenBank (Table S1, Supporting information) (Fedorov & Goropashnaya 1999; Fedorov & Stenseth 2002; Prost *et al.* 2010). No molecular data are yet available from the Ogilvie Mountains (*nunatakensis*) or Unalaska (*unalascensis*) populations (treated as species distinct from *groenlandicus* by Musser & Carleton 2005), but all other putative species are represented in our data set comprising 105 individuals and final aligned length of 885 bp. As much of these data stem from different studies, most individuals contain some level of missing data in the analyses, including those sequenced here. We performed phylogenetic analyses using the coalescent-based software BEAST v1.7.4 (Drummond *et al.* 2012). For each analysis, we assumed the Extended Bayesian Skyline Plot (EBSP) coalescent tree prior and the HKY model of nucleotide substitution with gamma-distributed rate variation (Appendix S1, Supporting information). We assigned ages to each of the ancient specimens as prior information, either by extracting this information from publications (fossil *D. torquatus* from Russia, Prost *et al.* 2010) or, for our new data, by estimating the average age of the samples based on direct radiocarbon dates obtained from other individuals of the same species from the assemblage within which the fossils originate as described above. To estimate this average, we first calibrated each available radiocarbon date using the IntCal09 curve (Reimer *et al.* 2009) using OxCal 4.1 online (https://c14.arch.ox.ac.uk/oxcal/OxCal.html). Age estimates were fixed to the mid-point value of the 95% confidence interval for each site, resulting in ages for the two *Dicrostonyx* specimens from the Black Hills of 15 830 calendar years BP (calBP) and an age of 24 000 calBP for the specimen from Prairieburg (Table S1, Supporting information). As we were unable to generate sequence data from the Wapsipinicon sample, it was not necessary to calculate an average age for the assemblage.

Ages of ancient sequences alone were insufficient to calibrate the molecular clock (data not shown). As an additional calibration, we placed a lognormal prior on the root of the tree, representing the divergence between the Eurasian *D. torquatus* and North American *Dicrostonyx* species with mean of 0.9 Ma and 95%

confidence interval spanning 675 ka to 1.41 Ma (lognormal mean 12.9, stdev 0.5, offset 5E5). *Dicrostonyx torquatus* (presumably *D. groenlandicus* based on modern taxonomy) has been identified from Morgan Bluffs near Jesse Bay, Banks Island, Northwest Territories, thought to be ~700 ka (Harington 1990). We assume that this early *Dicrostonyx* fossil from the Canadian high Arctic belongs to the Nearctic lineage, representing a minimum age for the divergence between Eurasian and North American forms. The mean of the distribution is set prior to this point, as genetic divergence between *D. torquatus* and the Nearctic lineages is assumed to have preceded the entry of *Dicrostonyx* into the Canadian Arctic. *Dicrostonyx* sp. are also known from multiple locations in Old Crow, Yukon Territory, Canada, and from Thistle Creek Organic Layers 1 and 2 (Harington 2011). The oldest of these sites, Thistle Creek Organic Layer 1, is thought to be ~740 ka (Harington 2011), similar to the age of the Banks Island specimen and consistent with our calibration. The relationship of the earliest collared lemming in North America, *Predicrostonyx hopkinsi* (Guthrie & Matthews 1971), to *Dicrostonyx* is unknown, so it is not included directly in our calibration. The Cape Deceit fauna (Fig. 1) in which *Predicrostonyx* is found is most likely ~1.8 Ma (Harington 2011), based on the Cape Deceit *Microtus deceitensis* being slightly older than that of the nearby Fort Selkirk fauna, which is securely dated to between 1.5 and 1.7 Ma (Storer 2003). Fort Selkirk also contains *Predicrostonyx* (Harington 2011). *Predicrostonyx* has been suggested as the ancestor to extant *Dicrostonyx* (Guthrie & Matthews 1971), so we set the upper 95% confidence interval bound to be 1.41 Ma to greatly reduce the probability of a divergence prior to the earliest possible appearance of *Predicrostonyx*, but without placing a hard bound.

We could not reject the assumption of a molecular clock, as the distribution of the standard deviation of the clock rate (ucld.stdev) when a relaxed molecular clock was implemented (uncorrelated lognormal distribution) strongly abutted zero. We therefore assumed a strict molecular clock, sampling the evolutionary rate uniformly from values between 10E-9 and 10E-6 to allow for a broad range around substitution rates along Arvicolinae lineages estimated using either multiple fossil calibrations across Rodentia (~2E-8, Horn *et al.* 2011) or tip dates in *D. torquatus* (9.6E-8, Prost *et al.* 2010). We ran three MCMC chains for 80 million iterations each, sampling from the posterior every 4000 iterations. Parameter mixing and convergence of the MCMC chains to stationarity were visualized using TRACER v1.5 (Rambaut & Drummond 2007). The first 10% of iterations were discarded from each run as burn-in and the remainder combined. Finally, to evaluate the influence of the prior settings on the posterior samples, we repeated the analysis as above but without any sequence data. We estimated a maximum clade credibility (MCC) tree using TREEANNOTATOR (distributed as part of the BEAST package) and visualized the MCC tree using FIGTREE v1.3.1 (Rambaut 2009).

Results

Ancient DNA from fossil Dicrostonyx

We found all ancient samples to be badly degraded, resulting in difficulty generating the complete target mtDNA sequence fragment. However, only one sample, SUI 95760 (T 3 17), from Wapsipinicon Assemblage, failed to yield DNA. In total, we designed and tested 39 primers spanning differently sized segments of the 498-bp target region. Despite this effort, it was not possible to close a 174-bp gap in the middle of the target sequence for either TF174 or RN023/TF172. Additionally, RN028 is missing the first 11 bp, and RN023/TF172 is missing the first 98 bp. In total, we obtained 324 bp from TF174, 226 bp from RN023/TF172 and 487 bp from RN028. Sequencing multiple amplification products following cloning revealed a minimal amount of DNA damage. We observed one C to T transition in TF172, one G to A in TF174 and two adjacent C to T transitions in RN028. Cytosine deamination to thymine, which will manifest as either a C to T or G to A transition, is the most commonly observed postmortem modification in ancient DNA (Hofreiter *et al.* 2001). The Carnegie Museum specimens yielded extremely clear direct sequences and were not cloned. Overlapping fragments did not show any conflicting bases in these samples.

In addition to cloning, all reported sequences are the consensus of multiple, overlapping amplification except the 3′ fragment in TF174 and RN023/TF172 (DICR13F14R). We observed only one mismatch in overlapping fragments: in TF174, Dicr1new contained an A where amplification Dicr2F3R had a G. A third amplification could not be obtained. As *D. groenlandicus* also contains a G at this position, and the observed mismatch is probably due to cytosine deamination, we assumed this position to be a G. No stop codons were inferred for any of the newly generated sequences, supporting their authenticity. Consensus sequences archived in GenBank under the accessions KC469633–KC469637.

Phylogenetic analyses

The four new Churchill, Manitoba, *D. richardsoni* sequences are identical to one another; the Nunavut sequence differed by four of 498 bp (0.8%). Therefore,

two new haplotypes are presented for this species. All three ancient specimens from South Dakota and Iowa belong to the same clade as all *D. richardsoni* (Bayesian posterior clade probability, BPP = 0.9526, Fig. 2). The modern taxa are supported as a clade (BPP = 0.9998), and the ancient individuals are recovered, but not supported (BPP = 0.63), as a single clade, sister to the modern haplotypes. Individual relationships within the ancient *Dicrostonyx* plus modern *D. richardsoni* clade remain unresolved. *Dicrostonyx torquatus* and *D. hudsonius* are each strongly supported (both BPP = 0.9998) as monophyletic. The molecular clock analysis resulted in a mean evolutionary rate of 8.0E-8 mutations per site per year and a mean root height of 732 ka (95% HPD: 572–943 ka).

Discussion

A putative southern LGM refugium for Dicrostonyx richardsoni

All three *Dicrostonyx* individuals from South Dakota and Iowa belong to an evolutionary lineage with extant *D. richardsoni* (Fig. 2, Node A; BPP = 0.95). This close relationship between ancient *Dicrostonyx* from the US Midwest and living *D. richardsoni* supports the hypothesis that *D. richardsoni* survived the LGM in a southern refugium (MacPherson 1965; Eger 1995). The estimated time to most recent common ancestor (tMRCA) of the three ancient sequences and the modern *D. richardsoni* is comparable with both the tMRCA estimated for all *D. hudsonius* individuals (Fig. 2, Node B) and the tMRCA for all *D. groenlandicus* individuals (Fig. 2, Node C). The amount of genetic divergence within each of these three clades (~0-1.2%) is consistent with intraspecific variation within *D. torquatus* (Fedorov & Goropashnaya 1999). These results suggest that the LGM-age *Dicrostonyx* from Iowa and South Dakota belong to *D. richardsoni*.

Among the historic *D. richardsoni*, the four Churchill, Manitoba, samples yielded identical haplotypes. The two samples from Rankin Inlet, Nunavut (GenBank AJ238435 and one presented here), differed from each other at two of 498 bp (0.4%), and each differed from the Churchill samples by 4 bp (0.8%). The level of variation observed between living individuals is lower than

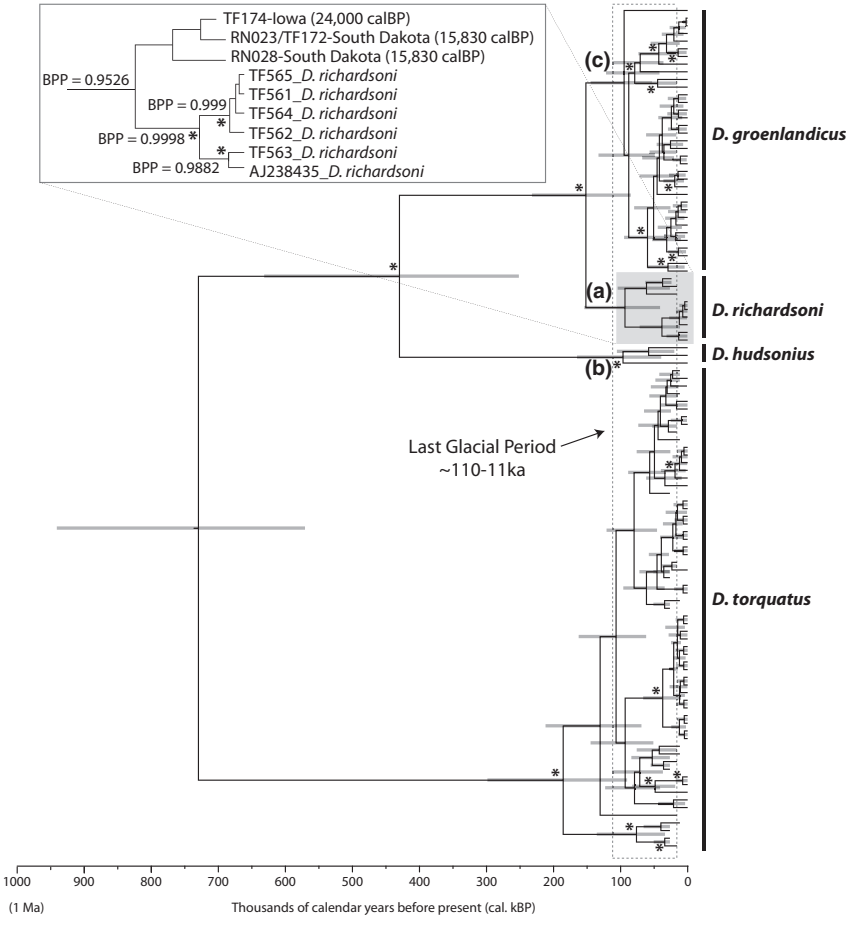

Fig. 2 Maximum clade credibility tree resulting from the combined BEAST analysis of 105 *Dicrostonyx* mitochondrial partial *cytb* sequences. Grey horizontal bars represent the 95% confidence interval for node divergence time; stars indicate nodes supported with Bayesian posterior probability ≥ 0.98. Letters refer to nodes discussed in text. Detailed information about the sequences included in the analysis, including geographic origin, age and GenBank accession numbers, is provided in Fig. S3 and Table S2.

that of other extant North American species (Fig. 2), which could be consistent with a species bottleneck, perhaps resulting from either restriction to a glacial refugium or founder effects during postglacial expansion. Brace *et al.* (2012) illustrated recently that *D. torquatus* in north-west Europe experienced repeated lineage turnover during the last 50 ka, in contrast to the genetic stability observed across Siberia in an earlier study (Prost *et al.* 2010). Like ours, both studies illustrate a loss of genetic diversity towards the present.

Unfortunately, our data are insufficiently informative to resolve whether the LGM-age samples are directly ancestral to the modern *D. richardsoni,* which would strongly suggest a postglacial, northward expansion from a southern refugium. Alternatively, present-day *D. richardsoni* may have expanded from a cryptic refugium near the present range, such as a nunatak. However, given known fossil locations, a southern refugium seems most probable. Additional genetic data from living *D. richardsoni* and from specimens recovered from nearby fossil localities will be needed to directly test this hypothesis. Regardless, our results provide the first genetic confirmation of a southern refugium for *D. richardsoni* and are consistent with the survival and post-LGM dispersal of a small North American mammal population that was displaced by advancing glacial ice.

Dicrostonyx richardsoni is not the only *Dicrostonyx* species to survive the LGM in a southern refugium. *Dicrostonyx hudsonius*, which now resides east of Hudson Bay, has also been identified near the southern extent of Laurentide ice (Fig. 1), suggesting that both of the more 'southerly' species of collared lemming, *D. richardsoni* and *D. hudsonius*, re-established their northern range from southern LGM refugia (MacPherson 1965; Eger 1995). Genetic examination of the fossils found in western Canada (Fig. 1) will be very enlightening as to whether or not all southern *Dicrostonyx* represent *D. richardsoni*, or if the pattern is similar to that of *D. hudsonius*, where the populations simply move north and south with environmental change. If the latter is true, the western North American fossils could represent additional refugia for the populations that reside in Alaska and the Yukon today.

Inferring the palaeoenvironment along the southern edge of the Laurentide ice

Palaeoenvironmental inferences that draw upon the present environmental tolerances of the animals that inhabited them are based on the assumption of niche conservatism. Such inferences depend on accurate species identifications, which may change as new morphological or molecular data become available, and assume that species niche preferences do not change as species adapt to a changing climate. Martinez-Meyer *et al.* (2004) illustrated that niche conservatism is widespread in mammals, with little change in niche parameters since the LGM. Similarly, Faunmap (1994) documented successful recolonization by many mammal species following long-distance northward dispersal with postglacial warming. Faced with climate change, many species have been shown to shift their geographic range, rather than evolve new adaptations (Chen *et al.* 2011).

Palaeoecological reconstructions based upon the assumptions that all of the *Dicrostonyx* fossils west of the Great Lakes were *D. torquatus* or *D. groenlandicus* suggested elements of a high Arctic tundra environment (reviewed in Mead & Mead 1989). *Dicrostonyx* are commonly used as a strict tundra indicator (Kowalski 1995), as they most currently reside in high Arctic, sub-Arctic and forest tundra environments. However, *D. richardsoni* live in a region of more temperate tundra and taiga: the Taiga Shield and southernmost end of the Southern Arctic ecozones. The more northerly distributed (Fig. 1) *D. groenlandicus* also resides in the Southern Arctic ecozone, but also the Northern Arctic and Arctic Cordilleran ecozones where they experience consistently cooler summers and harsher winters, comparatively (5–10 °C vs. 8–18 °C in July; −25 to −35 °C vs. −18 to −30 °C in January) (Wiken 1986). As such, our results support the hypothesis that the *Dicrostonyx* individuals living in these nonanalog communities were not associated with high tundra, but instead lived in more temperate conditions such as tundra transition, steppe tundra or boreal forest (Mead & Mead 1989; Seymour 2004). Although this does not change the inferred widespread nonanalog environmental conditions observed during the Wisconsin, it does indicate that the inferred tundra-like environment along the ice margin was less harsh than previously supposed. This interpretation is also more consistent with the reconstructions of LGM vegetation in this region of the upper Midwest, which also suggest more temperate tundra (Baker *et al.* 1986; Garry *et al.* 1990). These results highlight the importance of directly linking fossil populations to living ones for palaeoclimatic reconstructions and have direct implications for interpreting nonanalog palaeoenvironments.

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T.L.F., R.W.N., R.W.G. and B.S. designed the study; T.L.F. and R.W.N. performed the research; H.A.S., Jr. collected, identified, and provided the Wapsipinicon and Prairieburg samples; R.W.G. collected, identified, and provided the Don's Gooseberry Pit specimens; and all authors contributed to manuscript preparation.

Data accessibility

DNA sequences: GenBank accessions KC469633–KC469637. Fossil information: Locations and associated radiocarbon dates for all fossil specimens are detailed in the Methods section and in Table S1 (Supporting information). These data will also be made available in Neotoma (http://www.neotomadb.org). PCR primers and amplification conditions are found in Table S2 (Supporting information).

Supporting information

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

Fig. S1 Some alternatively recognized *Dicrostonyx* species/subspecies for comparative purposes.

Fig. S2 Relative positions of primer sets used to generate final sequences.

Fig. S3 Maximum clade credibility tree for *Dicrostonyx* shown in Fig. 1 including specimen identification.

Table S1 Detailed information for specimens included in this study.

Table S2 PCR primers and amplification conditions.

Appendix S1 BEAST xml input file employed.