Serial population extinctions in a small mammal indicate Late Pleistocene ecosystem instability

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The Late Pleistocene global extinction of many terrestrial mammal species has been a subject of intensive scientific study for over a century, yet the relative contributions of environmental changes and the global expansion of humans remain unresolved. A defining component of these extinctions is a bias toward large species, with the majority of small-mammal taxa apparently surviving into the present. Here, we investigate the population-level history of a key tundra-specialist small mammal, the collared lemming (*Dicrostonyx torquatus*), to explore whether events during the Late Pleistocene had a discernible effect beyond the large mammal fauna. Using ancient DNA techniques to sample across three sites in North-West Europe, we observe a dramatic reduction in genetic diversity in this species over the last 50,000 y. We further identify a series of extinction-recolonization events, indicating a previously unrecognized instability in Late Pleistocene small-mammal populations, which we link with climatic fluctuations. Our results reveal climate-associated, repeated regional extinctions in a keystone prey species across the Late Pleistocene, a pattern likely to have had an impact on the wider steppe-tundra community, and one that is concordant with environmental change as a major force in structuring Late Pleistocene biodiversity.

megafauna | palaeogenetics | palaeoclimate | modelling

The end of the Pleistocene witnessed a dramatic series of faunal extinctions resulting in the loss of at least 97 genera (1), with an apparently unprecedented bias toward large (>44 kg) terrestrial mammal species. Numerous causes have been proposed, but the two main hypotheses focus on anthropogenic or climatic drivers, either exclusively or in combination. Overkill hypotheses draw on the inferred characteristics of human colonization (2, 3), while rapid climatically driven environmental change, particularly that associated with the transition from the last glacial cycle into the warmer current interglacial (4), is the main alternative.

Although it has long been recognized that the Late Pleistocene saw significant changes in small-mammal distributions and community richness (5), very few species-level extinction events are known from this period. Previous ancient DNA research has therefore focused almost exclusively on the large-mammal fauna, yet this approach may have exacerbated the inability to differentiate between the effects of human activity and climatic change because of the intrinsic link between larger-bodied species and vulnerability to human predation.

To address this gap in population-focused ancient DNA analyses, we selected a previously unused study system, employing the remains of collared lemmings (*Dicrostonyx torquatus*) from Late Pleistocene sites in North-West Europe. This small rodent is well-represented in the fossil record from the Middle to Late Pleistocene throughout much of Europe (6). A tundra specialist, the collared lemming coexisted with many large mammal species that perished around the Pleistocene/Holocene transition. In contrast, the collared lemming survived and is currently widely distributed across the Palaearctic tundra, from the White Sea in western Russia to the Chukotka Peninsula and Kamchatka, northeast Siberia (7). As a key prey species, it is a major component in the diet of the majority of avian as well as mammalian Arctic tundra predators. However, collared lemmings are not identified as a likely target for human predation (8), and major changes in population structure may therefore be more confidently attributed to the effects of environmental change.

Population History of Late Pleistocene Collared Lemmings

To determine population-level dynamics of collared lemmings during the Late Pleistocene, we used techniques for the analysis of ancient DNA, to sequence 780 bp (Table S1) of mitochondrial DNA (mtDNA; cytochrome *b*) from a total of 88 ancient specimens (Table 1), 76 of which successfully yielded mtDNA for the targeted region (see Table S2 for accession numbers). The samples were sourced from three North-West European archaeological cave sites (Fig. 1), targeting stratigraphic layers spanning ~50–10 Kyr BP (radiocarbon years): Trou Al'Wesse (TAW; Belgium), Caverne Marie-Jeanne (CMJ; Belgium), and Bridged Pot Cave (BPC; United Kingdom). To better support statistical inference of temporal dynamics, a subset (23 individuals) (Table S3) of the DNA sequenced samples were radiocarbon-dated by accelerator mass spectrometry (AMS) at the Oxford Radiocarbon Accelerator Unit, after pretreatment by ultrafiltration, and calibrated using the IntCal09 (9) curve in OxCal (10).

Bayesian phylogenetic analyses identified five well-supported lineages of collared lemming (Fig. 2). These lineages demonstrate the extensive genetic diversity of Late Pleistocene populations (Fig. S1), a distinct contrast to the genetically depauperate populations reported for this species at present (11). Ancient DNA studies have consistently reported postglacial loss of genetic diversity in northern Holarctic mammal species (12), including collared lemmings (13). However, here we find an unexpected pattern in the spread of diversity through time, as the five lineages are distributed sequentially through the stratigraphy of the archaeological sites from which they were recovered, each generally being found in stratigraphically discrete units within each site (with some haplogroup sharing between sites). We identify

Table 1. Details of the samples of *Dicrostonyx* used in this study; sample localities are indicated according to country, archaeological site and stratigraphic position

Country	Site name	Layer/horizon	Square/level	Spit	No. of samples
Belgium	TAW	4	K4a	5	1
Belgium	TAW	4	K4b	5	1
Belgium	TAW	12 (upper)	M3	1	8
Belgium	TAW	12 (upper)	M3	2	3
Belgium	TAW	12 (lower)	M3	9	6
Belgium	TAW	12 (lower)	M4	5	12
Belgium	TAW	12 (lower)	M4	6	15
Belgium	TAW	14	O7	16	3
Belgium	TAW	14	K6	9	2
Belgium	TAW	14	K7	9	1
Belgium	TAW	15B	M9	8	3
Belgium	TAW	15B	M9	10	1
Belgium	TAW	16	M6	1	1
Belgium	TAW	16	M7	16	2
Belgium	TAW	16	M7	19	1
Belgium	CMJ	2			2
Belgium	CMJ	3			2
Belgium	CMJ	4			4
Belgium	CMJ	5	A		5
Belgium	CMJ	5	A and B		2
Belgium	CMJ	5	B		4
Belgium	CMJ	6			4
United Kingdom	BPC	B			5

distinct, genetic replacements in a mammalian species. All samples from the youngest site (BPC), the upper layers of TAW, and the latest sample from CMJ, together with all modern samples, form a single, albeit unresolved, clade. We interpret the BPC samples as representative of a Late Glacial colonization from the east, rather than a distinct population, because of the clustering of these samples with modern Siberian samples and the periodic land connection that linked the United Kingdom to Europe until ~7,500 cal BP (14). Samples from consecutively deeper layers, across sites with the longest records (TAW and CMJ), continue to demonstrate a significant association between mitochondrial DNA clade and stratigraphic position, forming each of the successive genetic lineages detected in the phylogenetic analyses (Fig. 2). Although there are a small number of exceptions to this pattern, we suggest that the pattern of these mismatches is attributable to a limited degree of reworking between layers; for example, samples H23 and H32, from the lower section of layer 12, return older AMS dates (Table S3) than samples collected from lower layers (e.g., H26 from layer 14).

Regional recolonization implies the presence of a single or several source populations. Our site locations approximate the southern and western extent of the collared lemming's Late Pleistocene distribution, and thus any source population was likely located to the north and east. To test this hypothesis, we combined our data with a collared lemming (modern/ancient mtDNA: 0 to ~25 Kyr cal BP, partial cytochrome *b*) dataset from Pymva-Shor (PS; northern Pre-Urals) (13). Our phylogenetic analysis (Fig. S2) places PS samples within our three youngest clades, but finds little support for PS samples of the same age forming discrete groups. Instead we observe haplotype continuity; for example, haplotype PS H02 (Table S4) is present throughout the temporally sampled range of the PS dataset (0 to ~25 Kyr BP). This structure is compatible with an absence of population extinction events at the PS site, and is consistent with a single/multiple PS-associated source populations for the North-West European sites under investigation.
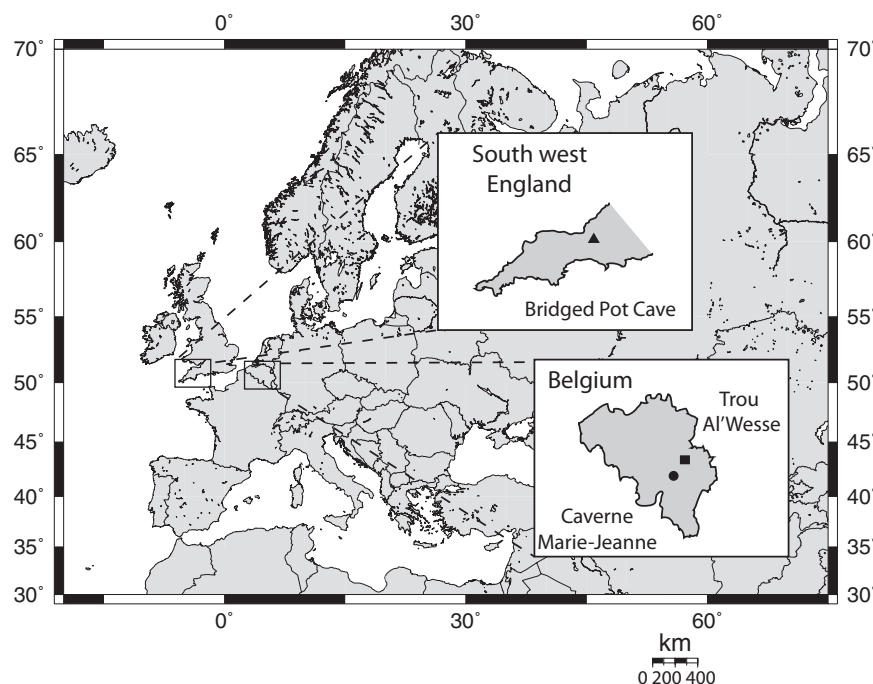
this pattern as representing a series of collared lemming population extinctions followed by recolonization events across the study region, making this a unique example of multiple, chronologically

Fig. 1. The locations of the three archaeological sites from which samples of *D. torquatus* used in our analyses were recovered. Symbols represent the approximate geographical locations: ▲, Bridged Pot Cave; ■, Trou Al'Wesse; ●, Caverne Marie-Jeanne.
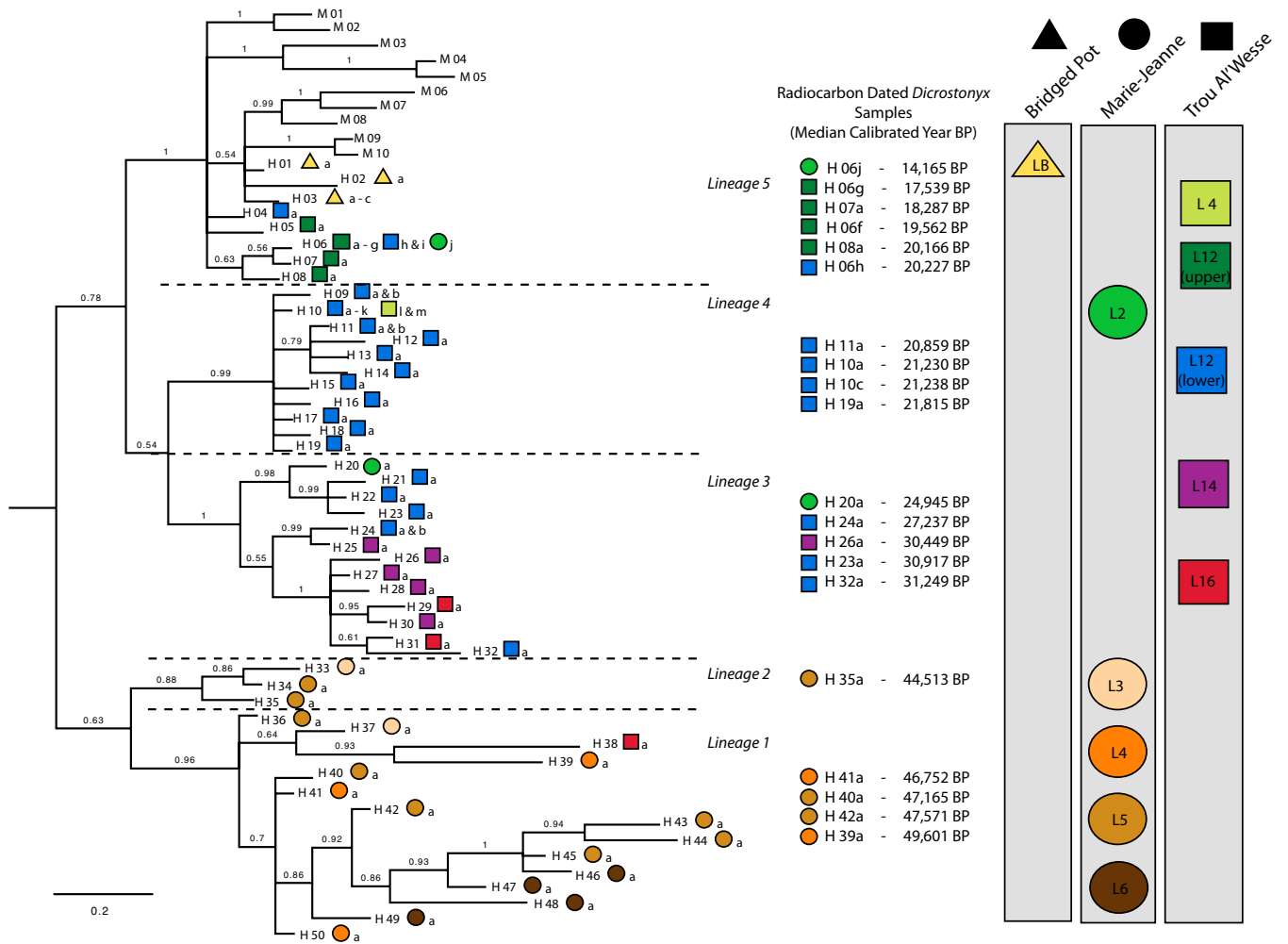
Fig. 2. Phylogeny for mtDNA haplotypes (cyt *b*) of *D. torquatus* with corresponding AMS dates and stratigraphic locations for the samples used in this study. Phylogeny: numbers above branches represent Bayesian posterior probabilities. The outgroup *D. hudsonius* is removed for display purposes. M, modern samples (GenBank); H, haplotype (ancient sample). Symbols indicate the source archaeological site for each sample: triangle, Bridged Pot Cave; circle, Caverne Marie-Jeanne; square, Trou Al'Wesse. Symbol colors indicate the stratigraphic location of the sample within each of the archaeological sites, L, layer (site specific number). Schematic stratigraphic columns represent an approximate position of the layers sampled from each site and the approximate relation of those layers across sites.

To further test the proposal that the five lineages observed across our dataset represent discrete, nonoverlapping, populations resulting from a history of local extinction-recolonization, we conducted direct AMS dating of 23 samples. Dating multiple samples from each clade provides further support for the proposed pattern, since the dated individuals follow a strictly consecutive order across the five lineages (Fig. 2 and Table S3). We find it unlikely that this pattern can be attributed to local evolution of haplogroups, with replacement by genetic drift because of both the short duration of each population and the generally limited timespan between genetic replacements (Fig. 2). The number of rapid turnover events also precludes recurrent selective sweeps on the collared lemming mitochondrial genome as a likely explanation for the observed pattern. We also reject the possibility that this is an artifact of the use of only a single, sex-linked marker, as we can conceive of no alternative mechanism by which the observed pattern could be generated without local extinction and recolonization events.

As a cold-adapted taxon, collared lemming populations are predicted to decline during warming events because of vegetation change and/or competition from more temperate-adapted fauna (15, 16). We used Bayesian-aged modeling of our AMS-

dated samples to estimate temporal boundaries for the observed genetic turnover events by treating each clade as an independent phase (Fig. 3 and Table S5) and calculated the most likely timing of the start and end of each phase, as well as the most likely timing of turnover events between phases (17, 18). These findings indicate that turnover events (TE:A–D) occurred during periods of climatic oscillation. Climatic warm peaks, Greenland Interstadials (GI)-12 and GI-2 occur during TE:A and TE:C, respectively. The broad boundaries surrounding TE:B include GI-5 to -11 and Greenland Stadials (GS)-6 to -11 (19, 20). TE: D, however, occurs within GS-2, a cold period temporally consistent with approximations of the regional Last Glacial Maximum. Furthermore, when our data are compared with the most thoroughly dated steppe-tundra specialist, the woolly mammoth (*Mammuthus primigenius*), we find that the timing of this most recent turnover event corresponds to the only reported hiatus in mammoth occurrence across central and North-West Europe ca. 21.7–19.4 Kyr cal (21), before its final extinction in the region (14.0 Kyr cal) (22). Thus, the period around 21–19 Kyr appears to have been a time of environmental change significant enough to affect both large and small herbivores in Europe, although the exact mechanism is unclear. Although
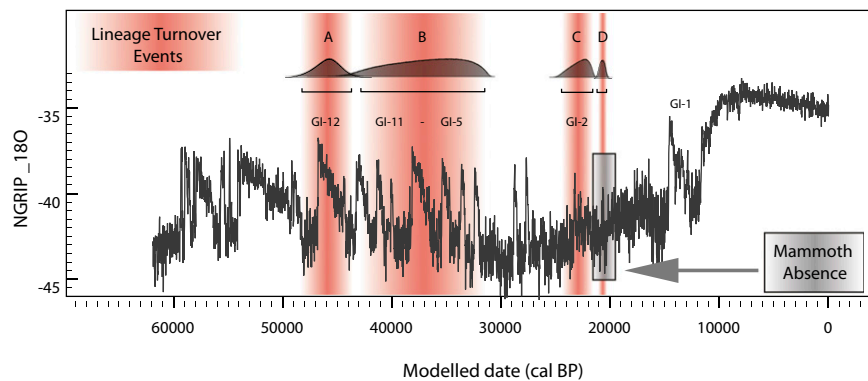
Fig. 3. Bayesian-derived highest probability ranges for the periods of lineage turnover of *D. torquatus*, based on AMS dated samples. Collared lemming lineage turnover events are shaded red and labeled A–D; gray box: *M. primigenius* absence, from AMS dated samples (21). cal BP, calibrated years Before Present; GI, Greenland Interstadials; NGRIP ¹⁸O, NorthGRIP ¹⁸O data (19).

low temperature alone seems an unlikely driver for this extinction—collared lemmings were still present in the more northerly site at PS—the close timing of this event with the maximum advancement of the Weichselian ice sheet and associated climatic/environmental changes could have significantly impacted herbivores adapted to cold and dry habitats (23, 24).

Any proposed link between collared lemming populations and northern hemisphere climate might imply a further link to vegetation change. Although the taxonomic resolution of pollen may cause problems for paleoecological analyses, there is clear evidence in Europe that vegetation was in part responding to the major climate forcing of Dansgaard-Oeschger (D/O) cycles, Heinrich events, and orbitally driven millennial changes (25). We further note that only a single turnover event (turnover D) can be associated with a similar event in the woolly mammoth. Thus, although our data indicate that the general pattern of Late Pleistocene megafaunal extinction could also be extended to incorporate extirpations in small mammal species, they reinforce the importance of considering intraspecific genetic variation and individual ecologies and life histories in determining response to the events of the Late Pleistocene (26). We further suggest that the observed pattern of extirpation and recolonization is not unique to the collared lemming (27) and is likely characteristic of other small mammal species during this period. However, as one of the keystone prey species in the Arctic ecosystem (28), the localized absences of collared lemming reported here would have had significant bottom-up impacts on the glacial steppe-tundra predator community.

Examination of an under-exploited source of ancient DNA—small-mammal remains—identified previously unreported and unprecedented temporal population structuring of a species within Europe during the end-Pleistocene. That we identify a series of population extinctions throughout the end-Pleistocene from a small-mammal species demonstrates an extensive and prolonged diversity loss and suggests a nonsize-biased reduction in ecological stability during the last glaciation, a pattern consistent with climatic and environmental change as key drivers for changes in Late Pleistocene biodiversity.

Materials and Methods

DNA Extraction and Amplifications. We collected a total of 88 ancient collared lemming specimens from three North-West European archaeological cave sites (TAW, CMJ, and BPC) (Fig. 1). Details regarding the archaeological sites and contexts from which the bones were discovered can be found in the *SI Text*. DNA extractions were conducted in two dedicated ancient DNA laboratories: Royal Holloway, University of London (TAW and BPC samples) and the Swedish Museum of Natural History, Stockholm (CMJ samples). MtDNA were amplified in overlapping fragments using nine primer pairs, spanning

780 bp of the cytochrome *b* (cyt *b*) region. For full details see *SI Text*, and Tables S1 and S2.

Phylogenetic Analyses. Ancient DNA sequence data (see Table S2 for accession numbers) were aligned with modern collared lemming sequences available on GenBank, plus a single North American collared lemming (*Dicrostonyx hudsonius*) sequence (Table S2). In a further analysis this dataset was combined with a dataset from Pymva-Shor (13) (Table S4). The phylogenetic relationships within our dataset (Fig. 2) and our PS combined dataset (Fig. S2) were estimated using Bayesian analyses. For both analyses the DNA substitution model selected with jModelTest 0.1 (29) under Bayesian Information Criterion was Hasegawa-Kishino-Yano (HKY) (30) with γ-distribution (shape parameter: Fig. 2 = 0.073; Fig. S2 = 0.037). Bayesian trees were constructed using MrBayes 3.1 (31) implementing nucleotide substitution model HKY with γ-distribution, using four chains (three heated, one cold) that were run for 1×10^7 generations, sampling every 1×10^4 generations with a burn-in period of 250 trees. Nodal support was determined by approximate posterior probabilities performed in MrBayes. Tracer 1.5 (32) was used to check for stabilization and convergence between runs. *D. hudsonius* (accession no. AJ238437) was used as the outgroup.

The sequence data from this study were additionally used to construct a 3D statistical parsimony network using the script TempNet (33) in R (34). Samples were assigned to one of five network layers according to the lineage identified through the Bayesian phylogenetic analysis (Fig. S1).

Radiocarbon Dating. The size of the starting materials in this study (collared lemming mandible weight, approximately 60–200 mg) precluded our ability to radiocarbon date all of the samples under investigation. Following successful sequencing, the remaining bone materials were assessed for suitability for radiocarbon dating on the basis of weight and likely collagen preservation. A total of 28 collared lemming samples were identified as suitable to attempt radiocarbon dating. AMS dating was carried out at the Oxford Radiocarbon Accelerator Unit, where samples were chemically pretreated and ultra-filtered to remove potential contaminants before radiocarbon measurements were taken on the AMS system.

Dating Analyses. Directly dated samples were assigned dates in uncalibrated radiocarbon years BP (Before Present AD 1950) format. Calibrated calendar ages were generated using Oxcal v4.1 (10) with IntCal09 calibration curve (9) (Table S3). To develop an appropriate model for the timing of the transitions between different groups, as well as the timing of population turnover events, a Bayesian Phase model was applied. Such methods are well-established for estimating the timing of archaeological groups and of stratigraphic events (35–38). Because there was stratigraphic information to suggest that each of the stratigraphic groups was sequential the phases were organized to respect this and start and end boundaries were applied to calculate the most likely age range for the appearance and end of the group in the study sites (Table S5). A date function was then used to estimate the most likely age range of the periods when lineage turnover events could occur through regional abandonment (Fig. 3 and Table S5). An exponential prior was applied assuming the most likely age for the start or end of a group to be immediately before and after the dated evidence, with an exponentially diminishing likelihood that the age is very much older or younger.

1. Barnosky AD, Koch PL, Feranec RS, Wing SL, Shabel AB (2004) Assessing the causes of late Pleistocene extinctions on the continents. *Science* 306(5693):70–75.
2. Martin PS (1973) The Discovery of America: The first Americans may have swept the Western Hemisphere and decimated its fauna within 1000 years. *Science* 179(4077): 969–974.
3. Martin PS (1984) in *Quaternary Extinctions: A Prehistoric Revolution*, eds Martin PS, Klein RG (Arizona Univ Press, Tuscon), pp. 354–403.
4. Guthrie RD (2003) Rapid body size decline in Alaskan Pleistocene horses before extinction. *Nature* 426(6963):169–171.
5. Blois JL, McGuire JL, Hadly EA (2010) Small mammal diversity loss in response to late-Pleistocene climatic change. *Nature* 465(7299):771–774.
6. Maul LC, Markova AK (2007) Similarity and regional differences in Quaternary arvicolid evolution in Central and Eastern Europe. *Quat Int* 160(1):81–99.
7. Corbet GB (1978) in *Mammals of the Palaearctic Region: A Taxonomic Review* (British Museum of Natural History, London and Ithaca, NY).
8. Smith EA (1991) *Inujjuamiut Foraging Strategies: Evolutionary Ecology of an Arctic Hunting Economy* (Aldine de Gruyter, Hawthorne, New York), p 213.
9. Reimer PJ, et al. (2009) IntCal09 and Marine09 radiocarbon age calibration curves, 0–50,000 years cal BP. *Radiocarbon* 51(4):1111–1150.
10. Ramsey BC (2009) Bayesian analysis of radiocarbon dates. *Radiocarbon* 51(1):337–360.
11. Fedorov VB, Fredga K, Jarrell GH (1999) Mitochondrial DNA variation and the evolutionary history of chromosome races of collared lemmings (*Dicrostonyx*) in the Eurasian Arctic. *J Evol Biol* 12(1):134–145.
12. Hofreiter M, Barnes I (2010) Diversity lost: Are all Holarctic large mammal species just relict populations? *BMC Biol* 8:46.
13. Prost S, et al. (2010) Influence of climate warming on arctic mammals? New insights from ancient DNA studies of the collared lemming *Dicrostonyx torquatus*. *PLoS ONE* 5(5):e10447.
14. Smith DE, Harrison S, Firth CR, Jordan JT (2011) The early Holocene sea level rise. *Quat Sci Rev* 30(15–16):1846–1860.
15. Hersteinsson P, Macdonald DW (1992) Interspecific competition and the geographical distribution of red and arctic foxes *Vulpes vulpes* and *Alopex lagopus*. *Oikos* 64(3): 505–515.
16. Callaghan TV, et al. (2004) Biodiversity, distributions and adaptations of Arctic species in the context of environmental change. *Ambio* 33(7):404–417.
17. Bronk Ramsey C (2008) Deposition models for chronological records. *Quat Sci Rev* 27 (1–2):42–60.
18. Blockley SPE, Pinhasi R (2011) A revised chronology for the adoption of agriculture in the Southern Levant and the role of Lateglacial climatic change. *Quat Sci Rev* 30(1–2):98–108.
19. Svensson A, et al. (2006) The Greenland ice core chronology 2005, 15-42 ka. Part 2: Comparison to other records. *Quat Sci Rev* 25(23–24):3258–3267.
20. Svensson A, et al. (2008) A 60 000 year Greenland stratigraphic ice core chronology. *Clim Past* 4(1):45–57.
21. Stuart AJ, Kosintsev PA, Higham TFG, Lister AM (2004) Pleistocene to Holocene extinction dynamics in giant deer and woolly mammoth. *Nature* 431(7009):684–689.
22. Lister AM (2009) Late-glacial mammoth skeletons (*Mammuthus primigenius*) from Condover (Shropshire, UK): Anatomy, pathology, taphonomy and chronological significance. *Geol J* 44(4):447–479.
23. Kowalski K (1995) Lemmings (Mammalia, Rodentia) as indicators of temperature and humidity in the European Quaternary. *Acta Zool Cracov* 38(1):85–64.
24. Guthrie RD (1990) *Frozen Fauna of the Mammoth Steppe: The Story of Blue Babe* (Univ of Chicago Press, Chicago).
25. Wohlfarth B, et al. (2008) Rapid ecosystem response to abrupt climate changes during the last glacial period in western Europe, 40-16 ka. *Geology* 36(5):407–410.
26. Stewart JR, Lister AM, Barnes I, Dalén L (2010) Refugia revisited: Individualistic responses of species in space and time. *Proc Biol Sci* 277(1682):661–671.
27. Dalén L, et al. (2007) Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox. *Proc Natl Acad Sci USA* 104(16):6726–6729.
28. Schmidt NM, et al. (2012) Response of an arctic predator guild to collapsing lemming cycles. *Proc Biol Sci* 279(1746):4417–4422.
29. Posada D (2008) jModelTest: Phylogenetic model averaging. *Mol Biol Evol* 25(7): 1253–1256.
30. Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J Mol Evol* 22(2):160–174.
31. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12):1572–1574.
32. Rambaut A, Drummond AJ (2009) Tracer v1.5, URL http://beast.bio.ed.ac.uk/Tracer. Accessed January 10, 2012.
33. Prost S, Anderson CNK (2011) TempNet: A method to display statistical parsimony networks for heterochronous DNA sequence data. *Methods Ecol Evol* 2(6):663–667.
34. R Development Core Team (2008) *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria).
35. Blackwell PG, Buck CE (2003) The Late Glacial human re-occupation of north-western Europe: new approaches to space-time modelling. *Antiquity* 77(296):232–240.
36. Blockley SPE, Ramsey CB, Pyle D (2008) Improved age modelling and high-precision age estimates of late Quaternary tephras for accurate palaeoclimate reconstruction. *J Volcanol Geotherm Res* 177(1):251–262.
37. Blockley SPE, Pinhasi R (2011) A revised chronology for the adoption of agriculture in the Southern Levant and the role of late glacial climatic change. *Quat Sci Rev* 30(1–2): 98–108.
38. Higham T, et al. (2011) The earliest evidence for anatomically modern humans in northwestern Europe. *Nature* 479(7374):521–524.