Supporting Information

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SI Text

Archaeological Sites and Samples. *Trou Al'Wesse (Belgium).* A total of 60 collared lemming mandibles were obtained from the current excavations on the terrace of Trou Al'Wesse (TAW; 50.421, 5.294). Located 50 m from the Hoyoux River in the Belgian Ardennes, this is a stratified prehistoric cave site with a long stratigraphic sequence containing Mousterian, Aurignacian, Mesolithic, and Neolithic occupations with intervening periods rich in faunal material (1, 2).

Samples were sourced from meter squares (e.g., M4) dug stratigraphically, each stratum dug in a series of 3- to 5-cm-thick spits. Each square is divided into 50-cm subsquares to control the context of sieved material; the coordinates of artifacts and bones larger than 1 cm are recorded using a Total Station. To examine faunal response to events surrounding the Last Glacial Maximum (LGM), and to remain within accepted limits for radiocarbon dating, collared lemming samples were obtained from pre-LGM strata (16 and 14) as well as post-LGM stratum 12, up to and including transition into the warmer climate of the Holocene (stratum 4b) (3).

Marie-Jeanne (Belgium). A total of 23 collared lemming mandibles were obtained from Caverne Marie-Jeanne (CMJ; 50.130, 4.476), an archeological cave site in the Belgian Ardennes, located 25 m above the right bank of the Féron, a small tributary of the Meuse River near Hastiere-Lavaux. M. Glibert of the Royal Belgian Institute of Natural Sciences initiated excavations of the cave in 1943 and found the deposits to be largely composed of clay, silt, and sand that had been washed into the cave along joints. Paleontological and archaeological objects recovered from the site suggest a Weichselian date, largely pre-Aurignacian in age. Artifacts attributable to the Mousterian were also discovered at the site, but too few to suggest a significant prehistoric human occupation (4). Collared lemming remains however, were found in all horizons, with samples used in this study originating from particularly abundant horizons: 2, 4, 5a, 5b, and 6 (5).

Bridged Pot (United Kingdom). A total of five collared lemming mandibles were obtained from Bridged Pot Cave (BPC; 51.234, -2.680), an archeological site located in Ebbor Gorge, Somerset, England. Excavation of the site was carried out by H. E. Balch, 1926–1929, and continued in 1958 by C. B. M. McBurney (6). Samples were sourced from an established stratigraphic layer (B), rich in small mammal remains and associated with the Younger Dryas ca. 12.8–11.5 Kyr BP (7).

Materials from each of the three locations were sieved on site, sealed in bags, and maintained at a constant temperature to limit contamination and DNA degradation. In the laboratory, mandibular samples were identified to species level through morphological characters of the M_1 , species specific to the collared lemming (8).

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- Miller R, Zwyns N, Otte M, Stevens C, Stewart J (2012) La séquence mésolithique et néolithique du Trou Al'Wesse (Belgique): Résultats pluridisciplinaires. Anthropologie 116:99–126.
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DNA Extraction and Sequencing. A total of 88 collared lemming mandibles were sourced for these analyses. 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). Samples were drilled or ground into a fine powder, with \sim 10–50 mg used in the DNA extraction process. DNA extraction protocol followed Yang et al. (9) using proteinase K to digest bone powders (modified with the inclusion of 1M urea in the extraction buffer) and silica spin columns to purify DNA. Mitochondrial DNA (mtDNA) was amplified using overlapping fragments spanning 780 base pairs of the cytochrome b (cyt b) region. Nine primer pairs were designed specifically for this study, each pair amplifying short (130–180 bp) overlapping fragments (Table S1). An additional forward primer (D2) was used for two samples (44a and 50a) following poor amplification results from an initial primer pair (D). PCR reactions (TAW and BPC samples) were performed using a final concentration of $1 \times PCR$ buffer, 0.2 µM of each primer, 250 µM dNTPs, 2 mM MgSO₄, 1 mg/mL BSA, 1 Unit Platinum Taq DNA polymerase high fidelity, purified water, and 2 µL of DNA extract in a 25 µL mix. PCR conditions were 5 min at 95 °C, followed by 55 cycles of 1 min at 92 °C, 1 min at 49 °C, 50 °C or 51 °C (dependent on primer pair specifications), 1 min at 68 °C, and with a final extension of 5 min at 68 °C. PCR reactions (CMJ samples) were performed using a final concentration of $1 \times PCR$ buffer, 0.2 μM of each primer, 200 µM dNTPs, 2.5 mM MgCl₂, 0.1 mg/mL BSA, 0.4 Units HotStar Taq DNA polymerase (Qiagen), purified water, and 2 µL of DNA extract in a 25 µL mix. PCR conditions were 10 min at 95 °C, followed by 55 cycles of 30 s at 94 °C, 30 s at 49 °C, 50 °C or 51 °C (dependent on primer pair specifications), 30 s at 72 °C, and with a final extension of 7 min at 72 °C.

Amplicons were purified using Exonuclease I and Shrimp Alkaline Phosphatase (TAW and BPC samples) and Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (CMJ samples). Sequencing reactions (TAW and BPC samples) were performed by Macrogen using a high-throughput genetic analysis sequencer (ABI3730XL) and at the Molecular Systematics laboratory at the Swedish Museum of Natural History (Stockholm, Sweden) using an ABI3130XL automated sequencer (CMJ samples). Sequencing chromatograms were assembled and analyzed using Sequencher 4.0 analysis software (Gene Codes Corporation) and Geneious 5.5.4 (10). Throughout our procedures, protocols to prevent contamination and ensure accurately coded (undamaged) mtDNA were followed: isolation of work areas, negative controls, reduced fragment length amplification, and repeated PCR amplification and sequencing of fragments.

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Fig. S1. Three-dimensional statistical parsimony network for *Dicrostonyx* mtDNA haplotypes (cyt *b*). Layers represent the different lineages identified by Bayesian phylogenetic analysis. Each circle represents one haplotype. Haplotype color corresponds to the most representative color from each lineage (Fig. 2), with modern haplotype circles M1–M10 colored black. Small black dots represent missing haplotypes. Small clear dots represent haplotypes that are missing from one particular layer but are present in the layer above or below. The size of each circle is proportional to haplotype frequency.



Fig. 52. Phylogeny for mtDNA haplotypes (cyt *b*) of *Dicrostonyx* combining our results and data from Pymva-Shor (1). Numbers above branches represent Bayesian posterior probabilities. The outgroup *Dicrostonyx* hudsonius is removed for display purposes. M, modern samples (GenBank); H, haplotype (ancient sample: present study); PS H and dashed branch line, haplotype from the Pymva-Shor study. Pie charts indicate details of the PS samples corresponding to each haplotype: segment color, sample ages in years Before Present; the number inside each segment represents number of samples per age group. Curly bracket indicates haplotypes that are combined into a single pie chart.

1. 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.

Table S1. Details of the primer pairs used to amplify mtDNA (cytochrome b) from samples of Dicrostonyx

				Product		
Primer name	Forward Sequence 5' to 3'	Reverse Sequence 5' to 3'	Annealing temp (°C)	Start position	End position	Length (bp)
A	CATCTGATACAGCAACAGCATTCTC	GTAGATGCCTCGTCCTACGTGTAA	51	167	286	143
В	TAATAGCAACAGCATTCATAGG	CCCCCTCAGATTCATTCTAC	50	368	481	133
С	GGGGGCTTCTCAGTTGACAA	GGATTTTGTCTGCGTCGGAG	51	496	636	160
D	CTCGGAGACCCAGATAATT	GGAAGGTTATGAGGGCTAG	50	748	901	172
D2	ATATTCTCGGAGACCCAGA	GGAAGGTTATGAGGGCTAG	49	743	901	177
E	GCTCCCTACTTGGCCTATG	AGAATATGGAGGCTCCGTTT	50	101	252	171
F	GGAGCCTCCATATTCTTCATC	AAGGATATTTGTCCTCATGGG	50	256	399	164
G	CTATCAGCAATCCCCTACATC	GTTAGAGCCTGTTTCGTGAAG	51	448	598	171
Н	GGCTCTAACAACCCATCAGGC	TAAGTGGGTTTGCAGGGGTGT	51	610	767	178
I	TAAACTCCGACGCAGAC	TGTGGTGGAGTATTAAGTGG	50	632	781	169

Product start and end positions represent the base pair position of the 3' end of the forward and reverse primers respectively, within the mtDNA cytochrome b gene.

Table S2. Details of the samples of *Dicrostonyx* that successfully yielded the targeted 780 base pair region of mtDNA (cytochrome *b*) and modern *Dicrostonyx* sequence data obtained from GenBank

			Sito	Stratigraphic position			
UI	Accession no.	Country	name	Layer/horizon	Square/level	Spit	
01 _a	JX867564	United Kingdom	BPC				
02 _a	JX867565	United Kingdom	BPC				
03 _a	JX867566	United Kingdom	BPC				
03 _b		United Kingdom	BPC				
03 _c		United Kingdom	BPC				
04 _a	JX867567	Belgium	TAW	12	M4D	6	
05 _a	JX867568	Belgium	TAW	12	M3	1	
06 _a	JX867569	Belgium	TAW	12	M3	1	
06 _b		Belgium	TAW	12	M3	1	
06 _c		Belgium	TAW	12	M3	1	
06 _d		Belgium	TAW	12	M3	1	
06 _e		Belgium	TAW	12	M3	1	
06 _f		Belgium	TAW	12	M3	2	
06 _a		Belgium	TAW	12	M3	2	
06 _h		Belgium	TAW	12	M4	5	
06 _i		Belgium	TAW	12	M4	5	
06 _i		Belgium	CMJ	2			
07 _a	JX867570	Belgium	TAW	12	M3	1	
08 _a	JX867571	Belgium	TAW	12	M3	2	
09 _a	JX867572	Belgium	TAW	12	M4	5	
09 _b		Belgium	TAW	12	M4D	6	
10 _a	JX867573	Belgium	TAW	12	M4	5	
10 _b		Belgium	TAW	12	M4	5	
10 _c		Belgium	TAW	12	M4	5	
10 _d		Belgium	TAW	12	M4	5	
10 _e		Belgium	TAW	12	M4	5	
10 _f		Belgium	TAW	12	M4D	6	
10 _a		Belgium	TAW	12	M4D	6	
10 _h		Belgium	TAW	12	M4D	6	
10 _i		Belgium	TAW	12	M4D	6	
10 _i		Belgium	TAW	12	M4D	6	
10 _k		Belgium	TAW	12	M3	9	
10 ₁		Belgium	TAW	4	К4	5B	
10 _m		Belgium	TAW	4	К4	5A	
11 _a	JX867574	Belgium	TAW	12	M4	5	
11 _b		Belgium	TAW	12	M4D	6	
12 _a	JX867575	Belgium	TAW	12	M4	5	
13 _a	JX867576	Belgium	TAW	12	M4D	6	
14 _a	JX867577	Belgium	TAW	12	M4D	6	

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Table S2. Cont.

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Ul Accession no. Country name Layer/horizon Square/level Splt 15s, JX867579 Belgium TAW 12 M4 5 17s, JX867579 Belgium TAW 12 M4D 6 17s, JX867581 Belgium TAW 12 M4D 6 18s, JX867581 Belgium TAW 12 M4D 6 19s, JX867582 Belgium TAW 12 M3 9 20s, JX867585 Belgium TAW 12 M3 9 21s, JX867586 Belgium TAW 12 M4 6 24s, JX867587 Belgium TAW 14 D7 16 25s, JX867588 Belgium TAW 14 K6 9 24s, JX867591 Belgium TAW 14 K6 9 24s, JX867592 Belgium TAW 14 <th></th> <th></th> <th></th> <th>Site</th> <th colspan="2">Stratigraphic position</th> <th></th>				Site	Stratigraphic position		
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O AJ238437 Canada	M10	AJ238422	Russia				
	0	AJ238437	Canada				

UI M, modern; UI O, modern (used as an outgroup).

		AMS 14C Date		Calibrated age range	Median		
UI	Site	(yr BP)	±	BP (95.4%)	yr BP	Reference no,	Lineage
06 _i	CMJ	12,275	55	14,850–13,925	14,165	OxA-24122	5
06 _g	TAW	14,415	70	17,865–17,185	17,539	OxA-2354-15	5
07 _a	TAW	15,150	120	18,641–18,021	18,287	OxA-2352-39	5
06 _f	TAW	16,440	100	19,903–19,403	19,562	OxA-23565	5
08 _a	TAW	16,980	110	20,443–19,609	20,166	OxA-2352-41	5
06 _h	TAW	17,030	90	20,467–19,876	20,227	OxA-2354-14	5
11 _a	TAW	17,520	120	21,329–20,422	20,859	OxA-23564	4
10 _a	TAW	17,780	130	21,535–20,548	21,230	OxA-2352-40	4
10 _c	TAW	17,780	120	21,533–20,553	21,238	OxA-23563	4
19 _a	TAW	18,260	120	22,217–21,452	21,815	OxA-23567	4
20 _a	CMJ	20,930	140	25,456–24,497	24,945	OxA-24123	3
24 _a	TAW	22,500	190	27,894–26,327	27,237	OxA-2354-16	3
26 _a	TAW	25,650	450	31,101–29,584	30,449	OxA-2352-11	3
23 _a	TAW	26,300	380	31,351–30,356	30,917	OxA-2352-42	3
32 _a	TAW	26,830	360	31,805–30,735	31,249	OxA-23566	3
35 _a	CMJ	40,500	1,400	47,501–42,364	44,513	OxA-24119	2
34 _a	CMJ	> 43,700				OxA-24121	2
39 _a	CMJ	47,600	3,300	63,308–42,622	49,601	OxA-24115	1
41 _a	CMJ	43,000	1,900	49,972–44,453	46,752	OxA-24117	1
40 _a	CMJ	43,600	2,100	NA-44,756	47,165	OxA-24118	1
42 _a	CMJ	44,400	2,300	NA-45,131	47,571	OxA-24120	1
46 _a	CMJ	> 43,000				OxA-24124	1
50 _a	CMJ	> 43,900				OxA-24116	1
45 _s	TAW	Failed no yield					
28 _a	TAW	Failed no yield					
25 _a	TAW	Failed low yield					
38 _a	TAW	Failed no yield					
31 _a	TAW	Failed low yield					

Dates were calibrated using Oxcal v4.1 with the IntCal09 calibration curve. Dates in bold may extend the calibration range. NA, not available because of likely extension beyond the calibration range.

PS H		Haplotype frequency				
number	Accession no.	Modern	11,500 (yr BP)	15,200 (yr BP)	25,200 (yr BP)	
01	HM009006			2	2	
02	HM008998	7	14	1	3	
03	HM008999	3				
04	HM009000		2	7	1	
05	HM009001		1			
06	HM009002		1			
07	HM009003		1			
08	HM009004		1			
09	HM009005		1			
10	HM009008			8	2	
11	HM009007			1	3	
12	HM009009				2	
13	HM009010				1	

Table S4. Haplotype frequency (per age range years BP) of Pymva-Shor samples

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Table S5.	Bayesian derived timing of lineage presence (1–5) and turnover events (A–D) between
lineages o	f Dicrostonyx from North-West Europe based on AMS dated samples

Lineage	Turnover event	Upper boundary (95.4%)	Lower boundary (95.4%)	Range for turnover events
1		50,766–46,002	49,168–44,733	
	А	49,168–44,733	47,624–42,990	48,386–43,817
2		47,624–42,990	45,613–33,777	
	В	45,613–33,777	37,577–30,931	42,938–31,631
3		37,577–30,931	25,243-22,043	
	С	25,243–22,043	22,894–21,300	24,479–21,564
4		22,894–21,300	21,344–20,423	
	D	21,344–20,423	21,062-20,114	21,200–20,269
5		21,062–20,114	14,907–11,396	

Dates are calibrated calendar ages (years BP) generated using Oxcal v4.1 with the IntCal09 calibration curve.

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