

Partial Genetic Turnover in Neandertals: Continuity in the East and Population Replacement in the West

Love Dalén,^{*1,2} Ludovic Orlando,³ Beth Shapiro,⁴ Mikael Brandström-Durling,⁵ Rolf Quam,^{2,6,7} M. Thomas P. Gilbert,³ J. Carlos Díez Fernández-Lomana,⁸ Eske Willerslev,³ Juan Luis Arsuaga,^{2,9} and Anders Götherström^{*2,10}

¹Department of Molecular Systematics, Swedish Museum of Natural History, Stockholm, Sweden

²Centro UCM-ISCIII de Evolución y Comportamiento Humanos, Madrid, Spain

³Centre for GeoGenetics, Natural History Museum of Denmark, Copenhagen, Denmark

⁴Department of Biology, Pennsylvania State University

⁵Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden

⁶Department of Anthropology, Binghamton University (SUNY)

⁷Division of Anthropology, American Museum of Natural History, New York

⁸Área de Prehistoria, Facultad de Humanidades y Educación, Universidad de Burgos, Burgos, Spain

⁹Departamento de Paleontología, Facultad de Ciencias Geológicas, Universidad Complutense de Madrid, Madrid, Spain

¹⁰Department of Ecology and Genetics, Section for Evolutionary Biology, Uppsala University, Norbyvägen, Uppsala, Sweden

*Corresponding authors: E-mail: love.dalen@nrm.se; anders.gotherstrom@ebc.uu.se.

Associate editor: John Novembre

Abstract

Remarkably little is known about the population-level processes leading up to the extinction of the neandertal. To examine this, we use mitochondrial DNA sequences from 13 neandertal individuals, including a novel sequence from northern Spain, to examine neandertal demographic history. Our analyses indicate that recent western European neandertals (<48 kyr) constitute a tightly defined group with low mitochondrial genetic variation in comparison with both eastern and older (>48 kyr) European neandertals. Using control region sequences, Bayesian demographic simulations provide higher support for a model of population fragmentation followed by separate demographic trajectories in subpopulations over a null model of a single stable population. The most parsimonious explanation for these results is that of a population turnover in western Europe during early Marine Isotope Stage 3, predating the arrival of anatomically modern humans in the region.

Key words: Neanderthal, demography, climate change, ancient DNA.

A large number of megafaunal taxa went extinct during the latter part of the Late Pleistocene, at both the species (Barnosky et al. 2004) and population level (Barnes et al. 2002; Hofreiter et al. 2007; Leonard et al. 2007). Among these species were the closest known relatives of anatomically modern humans, the neandertals (*Homo neanderthalensis*). While the timing for the disappearance of the neandertals is relatively well known (Finlayson et al. 2006), less is known about their population history in the time leading up to their disappearance.

Neandertals are one of the few hominin species believed to have evolved outside Africa. Although they have been subjected to numerous genetic analyses (Krings et al. 1997; Ovchinnikov et al. 2000; Green et al. 2010), the principal emphasis of these studies has been to investigate possible admixture with anatomically modern humans (*Homo sapiens*) (Belle et al. 2009; Green et al. 2010) as well as relationships with other putatively closely related hominins (Krause et al. 2010). Although genetic data have been used to address geographical population structure (Fabre et al. 2009), the existence of possible local demographic shifts

and population turnover has not been investigated to date; hence, the dynamics of neandertal populations prior to their disappearance are still largely unknown. Over the past decade, an increasing number of studies on both human (Malmström et al. 2009) and other mammalian populations (Barnes et al. 2002; Shapiro et al. 2004; Campos et al. 2010) have demonstrated that serial genetic data sets provide a powerful approach for testing hypotheses regarding demographic changes in populations.

To examine the population dynamics in neandertals, we analyzed mitochondrial DNA (mtDNA) control region variation in 13 individuals (fig. 1), including one previously unpublished sequence from an approximately 48.5 thousand radiocarbon year (kyr) old specimen from Valdegoba, Spain (supplementary fig. S1, Supplementary Material online). Bayesian phylogenetic inference revealed that about half the specimens are grouped into a highly supported monophyletic clade (posterior probability = 0.99), whereas the remaining specimens form a basal paraphyletic group. Interestingly, all specimens in the monophyletic group derive from western Europe and have radiocarbon ages of less

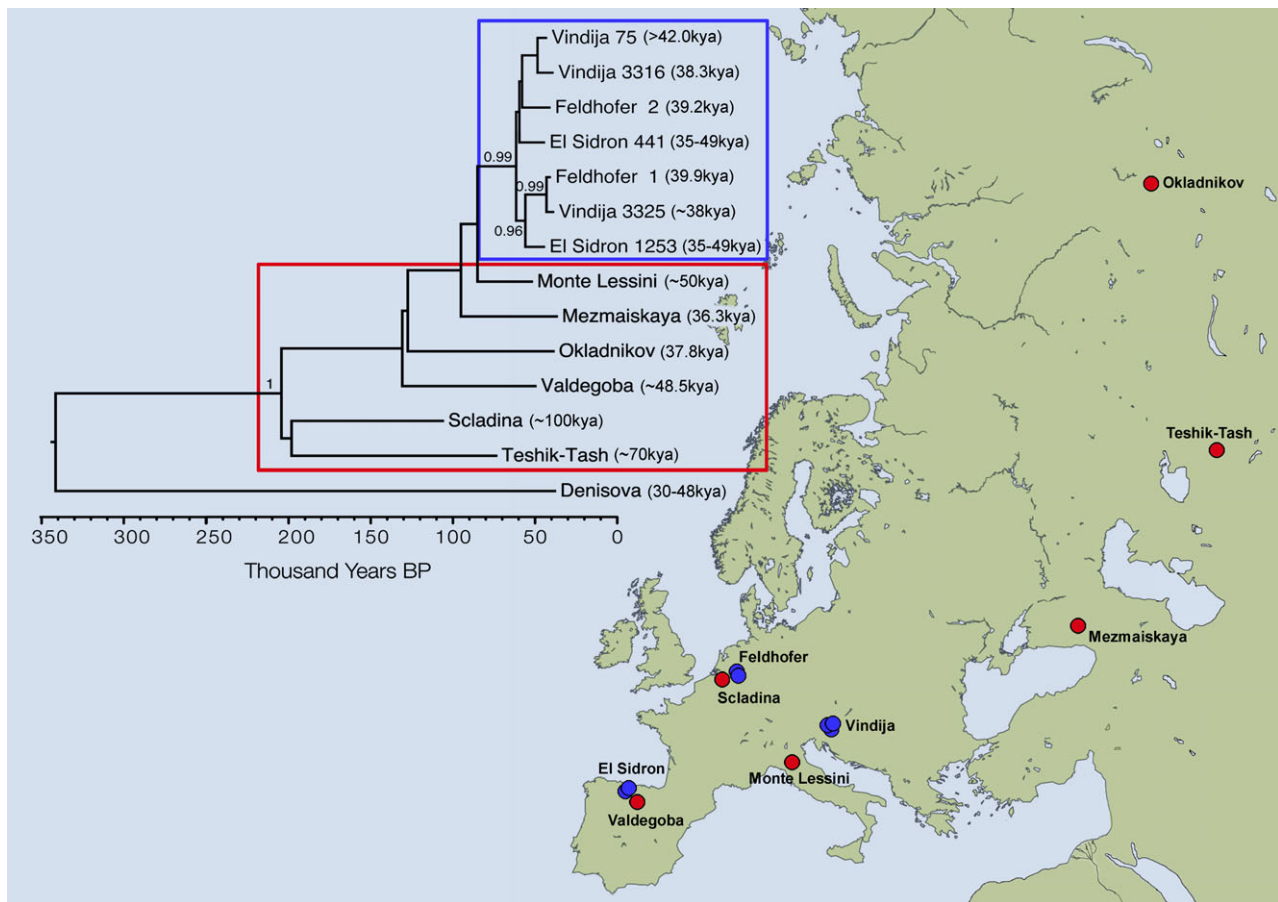


Fig. 1. Phylogenetic relationships (estimated using BEAST v1.6.1; Drummond and Rambaut 2007) and geographic distribution of neandertals. Recent (< 48 kyr) western neandertals are placed within a well-defined monophyletic group (blue box), whereas specimens older than 48 kyr constitute a paraphyletic group together with eastern neandertals (red box). The sampling locations for the specimens are shown with corresponding color coding.

than 48 kyr. In contrast, specimens from western Europe older than 48 kyr and from the eastern part of the neandertal distribution belong to the basal paraphyletic group (fig. 1). Based on the strong monophyly of recent western neandertals, we hypothesized that there had been a comparatively recent loss of genetic diversity in this group. To investigate this, we compared nucleotide diversity estimates, corrected for heterochrony (Depaulis et al. 2009). This revealed a more than 6-fold higher diversity in the group containing old western individuals and eastern individuals compared with the recent western group (fig. 2 and table 1). As a comparison, old and eastern neandertals display a diversity comparable to all anatomically modern humans, whereas the recent western group has a markedly lower diversity than modern Eurasian humans (fig. 2). Taken together, these results suggest a genetic turnover in the western European neandertal population approximately 48 kyr ago.

To further explore the hypothesis of population turnover in western European neandertals, we used an Approximate Bayesian Computation approach to test two demographic models that we hypothesize might be consistent with the observed mtDNA data. The software Bayesian Serial SimCoal was used to simulate temporal data under a null model (H_0) consisting of a panmictic population with constant size. We similarly explored an alternative scenario (H_1) of population divergence followed by independent demographic trajectories

in each subpopulation (supplementary fig. S2, Supplementary Material online). Bayes factors were calculated from model posterior probabilities, assuming both models were a priori equally likely. We retrieved 2.5- to 4.1-fold higher posterior support for H_1 when using a data set consisting of samples with longer sequence lengths (Bayes factors were 2.5 and 4.1 for the 191 and 303 bp data sets, respectively; supplementary table S2, Supplementary Material online). Posterior distributions are consistent with intermediate effective female population sizes of approximately 300 and 2,000 individuals in the western and eastern subpopulations, respectively (supplementary fig. S3 and table S3, Supplementary Material online).

Our results suggest that recent western neandertals display significantly less mtDNA variation compared with other neandertals. Furthermore, recent western neandertals constitute a well defined, but shallow, monophyletic group. As illustrated by the demographic simulations described above, these results are consistent with separate demographic trajectories in eastern and western neandertals.

One evolutionary scenario that could explain the genetic data and would be consistent with the simulation results would be an initial divergence between neandertal populations in Eastern and Western Europe approximately 55–70 kyr ago (supplementary fig. S2 and table S3, Supplementary Material online) followed by an extinction of western

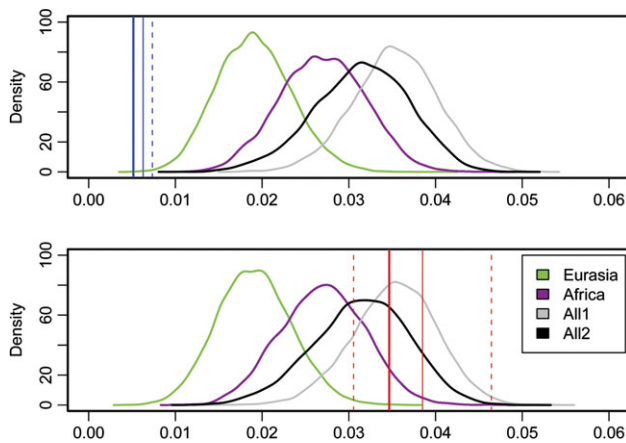


Fig. 2. Mean nucleotide diversity estimates among neandertals and anatomically modern humans worldwide, in Africa and in Eurasia. The distributions among modern human populations are estimated from 10,000 data sets of seven (top panel) and four sequences (bottom panel) of 303 bp. Black: worldwide, data set from Ingman et al. (2000). Gray: worldwide, data set from Gutierrez et al. (2002). Vertical lines are neandertal diversity estimates for the recent western group (blue, top panel) and old plus eastern neandertals (red, bottom panel). Mean values, corrected (thick) or not (thin) for age difference among sequences, are reported with solid lines. Dashed lines: one standard deviation confidence range. Purple: Africa. Green: Eurasia.

neandertals throughout most of their range and a subsequent recolonization of the region either from the east or from a small refugium in the western part of their distribution. In the eastern part of the neandertal distribution, on the other hand, the results suggest genetic continuity through time until the species' disappearance some 28 kyr ago (Finlayson et al. 2006). It is difficult to determine the exact timing of the turnover in the west. The age of the most recently dated specimen in the basal paraphyletic group in western Europe (i.e., Valdegoba) would imply a turnover around 48 kyr, whereas the coalescence time

Table 1. Nucleotide Diversity Estimates of Modern Human and Neandertal Populations.

	Average	Median	SD
<i>n</i> = 7			
Worldwide, data set 1	0.0354	0.0353	0.0049
Worldwide, data set 2	0.0315	0.0316	0.0055
Africa	0.0270	0.0269	0.0049
Eurasia	0.0191	0.0189	0.0043
Western neandertals	0.0063 (0.00514)	NA	0.0011
<i>n</i> = 4			
Worldwide, data set 1	0.0353	0.0353	0.0049
Worldwide, data set 2	0.0314	0.0317	0.0055
Africa	0.0270	0.0271	0.0049
Eurasia	0.0191	0.0189	0.0043
Old/Eastern neandertals	0.0385 (0.3466)	NA	0.0080

NOTE.—The average, median, and standard deviation (SD) values of the distributions shown in figure 2 are reported. Diversity estimates corrected for heterochrony among neandertals are indicated in parentheses. For modern human populations, the distributions were computed from 10,000 random data sets consisting of the same number of sequences as for both neandertal populations in order to quantify possible sampling bias resulting from limited data sets (*n* = 7 and *n* = 4, respectively). NA, not available.

(58 kyr, 95% confidence interval = 54–77 kyr) for the recent western clade appears to be slightly older. However, it should be noted that the age of the Valdegoba specimen lies close to the limits of radiocarbon dating and thus could be an underestimate. On the other hand, it is not unlikely that some variation was retained during the hypothesized turnover, in which case, the coalescence time would predate the turnover. The hypothesis of population turnover proposed here, as well as the timing of it, is likely to be further resolved as genomic data from more individuals becomes available.

Models based on paleontological data that incorporate neandertal migration and demographic changes have largely focused on population movements in a north–south direction (Hublin and Roebroeks 2009). Recently, periods of adverse climate have been argued to have caused local extinction rather than population movements (Hewitt 1999), and this has also been proposed for neandertals (Hublin and Roebroeks 2009). The reduced genetic diversity identified among later neandertals in western and central Europe found in the present study is consistent with this scenario.

The demographic turnover in the west indicated by our analyses would have been followed by a subsequent period of range expansion and recolonization, either from a small refugium within western or central Europe or from western Asia, where the population appears to have been demographically stable. Both of these scenarios find some support in the neandertal fossil record. A north–south geographic pattern has been suggested to characterize neandertal populations, with southern neandertals showing generally wider and shorter faces and presumably representing a more derived condition (Rosas et al. 2006). At the same time, some anatomical differences have been described previously between the southwest Asian neandertals, who are generally argued to show a more primitive morphology, and the “classic” neandertals of Upper Pleistocene Europe (Trinkaus 1983; Condemi 1992; Martínez and Arsuaga 1997; Martínez et al. 2008).

Even given the uncertainties surrounding the exact timing of the inferred demographic turnover, this event clearly predates the arrival of anatomically modern humans in western Europe (Trinkaus 2005). This suggests that the turnover instead may be a consequence of the changes in climate that occurred during early Marine Isotope Stage 3. Notably, several brief periods of extraordinarily low temperatures occurred during this time (Heinrich events 5 and 6; Svensson et al. 2008). Since these cold periods are thought to have been caused by a disturbance of oceanic currents in the North Atlantic, it is possible that they had a particularly strong impact on the environment in western Europe. Although speculative, this could explain why we observe a bottleneck or local extinction in the western part of the neandertal distribution, whereas individuals were less affected in the east. The idea that severe cold spells could have affected neandertals negatively has also found support in morphological analyses (Stewart 2005; Rae et al. 2011), as well as a recent palaeovegetational study which

suggested that the environment in Europe was unsuitable for neandertals during Heinrich event 5 (Muller et al. 2011).

Materials and Methods

A 303 bp mtDNA control region sequence from a novel Iberian neandertal from Valdegoba cave was generated using a multiplex polymerase chain reaction (PCR) approach (Lalueza-Fox et al. 2006). Each distinct PCR amplification ($n = 11$) was performed in triplicate to allow for erroneous nucleotides to be resolved in the downstream analysis. Amplicons from each replicate were deep sequenced using Roche FLX LR25 chemistry. The generated synthetic clonal sequences from each amplicon were sorted bioinformatically, constructed into networks to enable discrimination between true neandertal sequences and contaminant modern human sequences. Identification of the Valdegoba sequence was based on its position in the network, assuming that modal sequences were authentic, whereas peripheral sequences were derived from DNA misincorporation during PCR and sequence errors (Helgason et al. 2007). The Valdegoba sequence (supplementary fig. S1, Supplementary Material online; GenBank accession number JQ670672) as well as 12 previously published neandertal sequences were subsequently used to perform phylogenetic inference and population genetic analyses as well as serial coalescent simulations to evaluate alternative demographic scenarios (for details, see supplementary information, Supplementary Material online).

Supplementary Material

Supplementary information, figures S1–S3, and tables S1–S4 are available at *Molecular Biology and Evolution* online (<http://www.mbe.oxfordjournals.org/>).

Acknowledgments

The authors are grateful to Carles Lalueza Fox for help with the multiplex setup, to John Stewart for discussions and advice, and to Nuria Garcia and María Cruz Ortega Martínez for help with preparation of the Valdegoba specimen. The genetic analyses were funded by Fundación Atapuerca and the Ministerio de Ciencia e Innovación of the Government of Spain Project No. CGL2009-12703-C03-03. L.D. also acknowledges funding from the Swedish Research Council, Fundación Atapuerca, as well as Formas and the Swedish EPA through the FP6 BiodivERSA ERA-NET program. B.S. was funded by National Science Foundation ARC-0909456 and NASA PSARCI. A.G. was funded by the Royal Swedish Academy of Sciences.

References

- Barnes I, Matheus P, Shapiro B, Jensen D, Cooper A. 2002. Dynamics of Pleistocene population extinctions in Beringian brown bears. *Science* 295:2267–2270.
- Barnosky AD, Koch PL, Feranec RS, Wing SL, Shabel AB. 2004. Assessing the causes of Late Pleistocene extinctions on the continents. *Science* 306:70–75.
- Belle EMS, Benazzo A, Ghirotto S, Colonna V, Barbujani G. 2009. Comparing models on the genealogical relationships among Neandertal, Cro-Magnoid and modern Europeans by serial coalescent simulations. *Heredity* 102:218–225.
- Campos PF, Willerslev E, Sher A, et al. (20 co-authors). 2010. Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. *Proc Natl Acad Sci U S A*. 107:5675–5680.
- Condemi S. 1992. Les Hommes Fossiles de Saccopastore et leurs Relations Phylogénétiques. Paris: CNRS Editions.
- Depaulis F, Orlando L, Hänni C. 2009. Using classical population genetics tools with heterochronous data: time matters! *PLoS One* 4:e5541.
- Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol*. 7:214.
- Fabre V, Condemi S, Degioanni A. 2009. Genetic evidence of geographical groups among Neanderthals. *PLoS One* 4:e5151.
- Finlayson C, Pacheco FG, Rodriguez-Vidal J, et al. (26 co-authors). 2006. Late survival of Neanderthals at the southernmost extreme of Europe. *Nature* 443:850–853.
- Green RE, Krause J, Briggs AW, et al. (56 co-authors). 2010. A draft sequence of the Neandertal genome. *Science* 328:710–722.
- Gutierrez G, Sanchez D, Marin A. 2002. A reanalysis of the ancient mitochondrial DNA sequences recovered from neandertal bones. *Mol Biol Evol*. 19:1359–1366.
- Helgason A, Palsson S, Lalueza-Fox C, Ghosh S, Siguroardottir S, Baker A, Hrafnkelsson B, Arnadottir L, Porsteinsdottir U, Stefansson K. 2007. A statistical approach to identify ancient template DNA. *J Mol Evol*. 65:92–102.
- Hewitt GM. 1999. Post-glacial re-colonization of European biota. *Biol J Linn Soc*. 68:87–112.
- Hofreiter M, Munzel S, Conard NJ, Pollack J, Slatkin M, Weiss G, Pääbo S. 2007. Sudden replacement of cave bear mitochondrial DNA in the late Pleistocene. *Curr Biol*. 17:R122.
- Hublin JJ, Roebroeks W. 2009. Ebb and flow or regional extinctions? On the character of Neandertal occupation of northern environments. *C R Palevol*. 8:503–509.
- Ingman M, Kaessmann H, Pääbo S, Gyllenstein U. 2000. Mitochondrial genome variation and the origin of modern humans. *Nature* 408:708–713.
- Krause J, Fu QM, Good JM, Viola B, Shunkov MV, Derevianko AP, Pääbo S. 2010. The complete mitochondrial DNA genome of an unknown hominin from southern Siberia. *Nature* 464:894–897.
- Krings M, Stone A, Schmitz RW, Krainitzki H, Stoneking M, Pääbo S. 1997. Neandertal DNA sequences and the origin of modern humans. *Cell* 90:19–30.
- Lalueza-Fox C, Krause J, Caramelli D, et al. (15 co-authors). 2006. Mitochondrial DNA of an Iberian Neandertal suggests a population affinity with other European Neandertals. *Curr Biol*. 16:R629–R630.
- Leonard JA, Vila C, Fox-Dobbs K, Koch PL, Wayne RK, Van Valkenburgh B. 2007. Megafaunal extinctions and the disappearance of a specialized wolf ecomorph. *Curr Biol*. 17:1146.
- Malmström H, Gilbert MTP, Thomas MG, et al. (12 co-authors). 2009. Ancient DNA reveals lack of continuity between neolithic hunter-gatherers and contemporary Scandinavians. *Curr Biol*. 19:1758.
- Martinez I, Arsuaga JL. 1997. The temporal bones from Sima de los Huesos Middle Pleistocene site (Sierra de Atapuerca, Spain). A phylogenetic approach. *J Hum Evol*. 33:283–318.
- Martinez I, Quam R, Arsuaga JL. 2008. Evolutionary trends in the temporal bone in the Neandertal lineage: a comparative study between the Sima de los Huesos (Sierra de Atapuerca) and Krapina samples. In: Monge J, Mann A, Frayer D, Radovic J, editors. New insights on the Krapina Neandertals: 100 years after Gorjanovic-Kramberger. Zagreb (Croatia): Croatian Natural History Museum. p. 75–80.

- Muller UC, Pross J, Tzedakis PC, Gamble C, Kotthoff U, Schmiedl G, Wulf S, Christanis K. 2011. The role of climate in the spread of modern humans into Europe. *Quat Sci Rev.* 30:273–279.
- Ovchinnikov IV, Götherström A, Romanova GP, Kharitonov VM, Lidén K, Goodwin W. 2000. Molecular analysis of Neanderthal DNA from the northern Caucasus. *Nature* 404:490–493.
- Rae TC, Koppe T, Stringer CB. 2011. The Neanderthal face is not cold adapted. *J Hum Evol.* 60:234–239.
- Rosas A, Martinez-Maza C, Bastir M, et al. (18 co-authors). 2006. Paleobiology and comparative morphology of a late Neanderthal sample from El Sidron, Asturias, Spain. *Proc Natl Acad Sci U S A.* 103:19266–19271.
- Shapiro B, Drummond AJ, Rambaut A, et al. (27 co-authors). 2004. Rise and fall of the Beringian steppe bison. *Science* 306:1561–1565.
- Stewart JR. 2005. The ecology and adaptation of Neanderthals during the non-analogue environment of Oxygen Isotope Stage 3. *Quat Int.* 137:35–46.
- Svensson A, Andersen KK, Bigler M, et al. (14 co-authors). 2008. A 60 000 year Greenland stratigraphic ice core chronology. *Clim Past.* 4:47–57.
- Trinkaus E. 1983. *The Shanidar Neanderthals*. New York: Academic Press.
- Trinkaus E. 2005. Early modern humans. *Annu Rev Anthropol.* 34:207–230.