demographic history that is probably different to that of other Neandertal groups placed in Eastern Europe and in the Italian Peninsula.

The most recent common ancestor of the seven Neandertal sequences has been estimated to be around 250,000 ± 65,000 years of age (assuming an age of around 40,000 years for these specimens) by coalescent methods (Supplemental data). The clade containing the four most similar sequences (Feldhofer 1 and 2, Vindija 75-80 and El Sidrón, between positions 16,076-16,378) sharing the G16,078-C16,154 haplotype, has been estimated to be 130,000 ± 30,000 years of age. Interestingly, this date is roughly concordant with the end of a dramatic glacial maximum around 135,000 years ago [12]. Therefore, it seems that, despite the broad geographic range, the El Sidrón, Feldhofer and Vindija Neandertals may belong to the same group of Neandertals that expanded from a hypothetical southern glacial refugium after a demographic collapse associated with this glacial maximum.

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Supplemental data

Supplemental data are available at http://www.current-biology.com/cgi/content/full/16/16/R629/DC1/

References

1. Krings, M., Stone, A., Schmitz, R.W., Krainitzki, H., Stoneking, M., and Pääbo, S. (1997). Neandertal DNA sequences and the origin of modern humans. Cell *90*, 19–30.
2. Schmitz, R.W., Serre, D., Bonani, G., Feine, S., Hillgruber, F., Krainitzki, H., Pääbo, S., and Smith, F.H. (2002). The Neandertal type site revisited; interdisciplinary investigations of skeletal remains from the Neander Valley, Germany. Proc. Natl. Acad. Sci. USA *99*, 13342–13347.
3. Ovchinnikov, I.V., Götherström, A., Romanova, G.P., Kharitonov, V.M., Lidén, K., and Goodwin, W. (2000). Molecular analysis of Neandertal DNA from the northern Caucasus. Nature *404*, 490–493.
4. Caramelli, D., Lalueza-Fox, C., Condemi, S., Longo, L., Milani, L., Manfredini, A., Saint Pierre, M. de., Adoni, F., Lari, M., Giunti, P., *et al.* (2006). A highly divergent mtDNA sequence in a Neandertal individual from Italy. Curr. Biol. *16*, R630–R632.
5. Krings, M., Capelli, C., Tschentscher, F., Geisert, H., Meyer, S., von Haeseler, A., Grossschmidt, K., Possnert, G., Paunovic, M., and Pääbo, S. (2000). A view of Neandertal genetic diversity. Nat. Genet. *26*, 144–146.
6. Serre, D., Langaney, A., Chech, M., Teschler-Nicola, M., Paunovic, M., Mennecier, P., Hofreiter, M., Possnert, G., and Pääbo, S. (2004). No evidence of Neandertal mtDNA contribution to early modern humans. PLoS Biol. *2*, 313–317.
7. Orlando, L., Darlu, P., Toussaint, M., Bonjean, D., Otte, M., and Hänni, C. (2006). Revisiting Neandertal diversity with a 100,000 year old mtDNA sequence. Curr. Biol. *16*, R400.
8. Beauval, C., Maureille, B., Lacrampe-Cuyaubere, F., Serre, D., Peressinotto, D., Bordes, J.G., Cochard, D., Couchoud, I., Dubrasquet, D., Laroulandie, V., et al. (2005). A late Neandertal femur from Les Rochers-de-Villeneuve, France. Proc. Natl. Acad. Sci. USA 102, 7085–7090.
9. Lalueza-Fox, C., Sampietro, M.L., Caramelli, D., Puder, Y., Lari, M., Calafell, F., Martinez-Maza, C., Bastir, M., Fortea, J., de la Rasilla, M., *et al.* (2005). Neandertal evolutionary genetics; mitochondrial DNA data from the Iberian Peninsula. Mol. Biol. Evol. *22*, 1077–1081.
10. Fortea, J., de la Rasilla, M., Martínez, E., Sánchez-Moral, S., Cañaveras, J.C., Cueva, S., Rosas, A., Soler, V., Julià, R., de Torres, T., *et al.* (2003). La Cueva de El Sidrón (Borines, Piloña, Asturias): Primeros resultados. Estud. Geol. *59*, 159–179.
11. Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. Nature *405*, 907–913.
12. Petit, J.R., Jouzel, J., Raynaud, D., Barkov, N.I., Barnola, J.-M., Basile, I., Bender, M., Chapellaz, J., Davis, M., Delaygue, G., *et al.* (1999). Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature *399*, 429–436.

A highly divergent mtDNA sequence in a Neandertal individual from Italy

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Neandertals are documented in Europe and Western Asia from about 230,000 to 29,000 years ago. Analyses of mitochondrial DNA (mtDNA) from Neandertal samples [1,2] and other analyses [3–5] appear incompatible with the hypothesis that Neandertals are direct ancestors of modern Europeans [6,7]. However, there are broad geographic gaps in the sampling of Neandertal DNA diversity. Here, we describe the sequence of the first mitochondrial hypervariable region

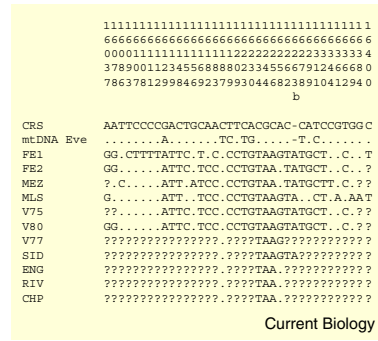

Figure 1. Neandertal diversity.

Comparison of the Cambridge reference sequence (CRS) and the putatively ancestral sequence to all extant human variation (mtDNA Eve) with 11 Neandertal sequences, five of them represented by short fragments. CHP = La Chapelle aux Saints, CRS = Cambridge reference sequence, ENG = Engis 2, FE1 = Feldhofer 1, FE2 = Feldhofer 2, MEZ = Mezmaiskaya, MLS = Monti Lessini, RIV = Rochers de Villeneuve, SID = El Sidrón 441, V75 = Vindija 75, V77 = Vindija 77, V80 = Vindija 80.

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(HVR1) in a new specimen from Monti Lessini (MLS) in Northern Italy. This sequence contains several previously unidentified nucleotide substitutions and comparison to five other complete HVR1 sequences reveals a previously undetected amount of genetic variation among Neandertals.

The MLS individual is represented by an incomplete jaw and by 13 bone fragments, two of which belong to the post-cranial skeleton [8]. The sample is estimated by stratigraphy to be 50,000 years old. Preliminary tests of amino acid preservation showed that there was a good chance to retrieve amplifiable endogenous DNA from the site (Supplemental data). DNA extraction, amplification, cloning and sequencing were replicated independently in two laboratories. The HVR1 was divided into 7 amplicons and 135 clones were sequenced overall. Sequences containing the nucleotide substitutions typical of Neandertals represent the large majority (100 clones out of 135) of the PCR products.

The consensus MLS mtDNA sequence, determined on the basis of 100 clones (Supplemental data) shows 23 substitutions with respect to the Cambridge Reference Sequence (CRS). 16 of these are also present in all previously studied Neandertals (Figure 1), while 7 of them are polymorphic [9,10]. Three polymorphic substitutions are specific for MLS. The MLS sequence also shows two substitutions previously observed only in Mezmaiskaya (MEZ) Neandertals. The six Neandertal samples whose sequence spans at least the region between positions 16083 and 16378 show 35 substitutions with respect to the Cambridge reference sequence (CRS) (Figure 1). Four C>T changes (positions 16107, 16108, 16111 and 16112) have been observed only in the FE1 Neandertal. As these are probably post mortem artifacts [4], we have removed them from the following analyses.

In order to establish the phylogenetic structure of

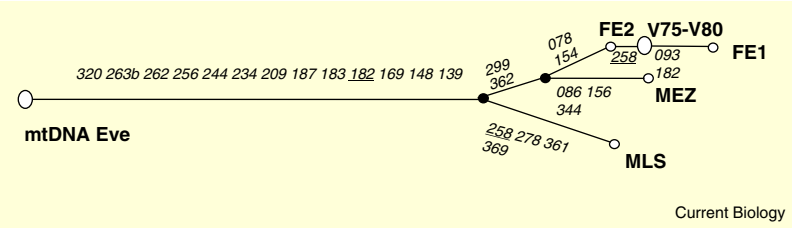

Figure 2. Median-joining network showing the substitutions separating the six Neandertal sequences.

Neandertal sequences between 16055 and 16378 were rooted with the putative mitochondrial DNA Eve sequence [10]. The numbers refer to the position in the Cambridge reference sequence, minus 16,000; repeated substitutions are underlined. The problematic positions 16107, 16108, 16111 and 16112 of FE1 are not included. Position 078 is not known for V75, and it is assumed to coincide with V80, from the same site. An alternative solution would be to remove V75 from the tree, which would not change the topology. The more extreme positions (16037, 16380 and 16400) have not been used due to the lack of information in some specimens. Their inclusion would not change the tree's topology.

Neandertal variation, a median joining network was constructed with the sequences from positions 16055 to 16378 (Figure 2). The root of the mtDNA tree inferred from the analysis of modern human diversity, the so-called 'mitochondrial Eve' [11], was included as a reference. Central European sequences cluster to the exclusion of MEZ and MLS, the latter being clearly divergent. The addition of MLS has revealed that 16299G and 16362C are not Neandertal synapomorphic characters, and these two sites should be regarded as polymorphic in Neandertals.

A neighbor-joining tree with the same six sequences (Supplemental data) shows exactly the same topology as the median-joining network. The separation between MLS and the rest of the sequences is supported by 85% of bootstrap replicates. There are 12 polymorphic sites (S) among the 296 sites in the region between 16083 and 16378. Pairs of sequences differ by a number of substitutions (*k*) ranging from 0 (between V75 and V80) to 11 (between MLS and MEZ, including two independent mutations at site 16,154), with an average of 5.76.

How would we estimate the genetic diversity of modern Europeans if we knew only six mitochondrial sequences? We analysed modern samples of size six, chosen in two ways. In a first set of 10,000 experiments, sequences were extracted without replacement from a database selected so as to match the distribution of Neandertal samples (Supplemental data); this distribution is not meant to imply any genealogical relationship between Neandertals and modern people from the same areas, but only to reduce the effect of geography on the comparisons. In a second set of 10,000 experiments, the modern sequences were extracted without replacement from a database comprising 3,917 sequences from 47 European populations.

Neandertals show a lower number of polymorphic sites (*S*), and a higher mean difference between pairs of sequences (*k*), than the whole modern dataset (Supplemental data). However, the values estimated in six modern samples are remarkably close to those observed in Neandertals, and their difference is never significant. As predictable, S is biased more strongly by low sample size than *k*, but more importantly, the *k* value of modern Europeans, marginally lower than observed in Neandertals, implies that modern European trees inferred from small samples would resemble the Neandertals' tree. In brief, Neandertal populations seem to harbor levels of mitochondrial diversity quite comparable to those of modern Europeans. The most recent common ancestor from which the observed Neandertal diversity has originated can be estimated to have lived about 250,000 years

ago [12]; however, the standard error is 65,000 years at these sample sizes.

The MLS sequence documents a greater diversity among the European Neandertals than previously estimated. In particular, the MLS and MEZ sequences appear separated from a cluster of sequences from Germany and Croatia. All sequences in the cluster share two derived alleles, 16078G and 16154C. The analysis of Neandertal genetic diversity confirms that Neandertals were separated from modern humans by several fixed mtDNA differences. However, their internal diversity was rather large. Even members of the same population, such as FE1 and FE2, could differ substantially, and haplotypes in geographically extreme populations also seem to be genetically differentiated. This raises questions concerning the demographic and evolutionary history of Neandertals.

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Supplemental data

Supplemental data and experimental procedures are available at http://www.current-biology.com/cgi/content/full/16/16/R630/DC1/

References

1. Krings, M., Capelli, C., Tschentscher, F., Geisert, H., Meyer, S., von Haeseler, A., Grossschmidt, K., Possnert, G., Paunovic, M., and Pääbo, S. (2000). A view of Neandertal genetic diversity. Nat. Genet. *26*, 144–146.
2. Serre, D., Langaney, A., Chech, M., Teschler-Nicola, M., Paunovic, M., Mennecier, P., Hofreiter, M., Possnert, G., and Pääbo, S. (2004). No evidence of Neandertal mtDNA contribution to early modern humans. PLoS Biol. *2*, 313–317.
3. Lahr, M.M., and Foley, R.A. (1998). Towards a theory of modern human origins: Geography, demography, and diversity in recent human evolution. Am. J. Phys. Anthropol. Suppl. *27*, 137–176.
4. Schmitz, R.W., Serre, D., Bonani, G., Feine, S., Hillgruber, F., Krainitzki, H., Pääbo, S., and Smith, F.H. (2002). The Neandertal type site revisited; interdisciplinary investigations of skeletal remains from the Neander Valley, Germany. Proc. Natl. Acad. Sci. USA *99*, 13342–13347.
5. Mellars, P. (2004). Neanderthals and the modern human colonization of Europe. Nature *432*, 461–465.
6. Caramelli, D., Lalueza-Fox, C., Vernesi, C., Lari, M., Casoli, A., Mallegni, F., Chiarelli, B., Dupanloup, I., Bertranpetit, J., Barbujani, G., *et al.* (2003). Evidence for a genetic discontinuity between Neandertals and 24,000-year-old anatomically modern Europeans. Proc. Natl. Acad. Sci. USA *100*, 6593–6597.
7. Currat, M., and Excoffier, L. (2004). Modern humans did not admix with Neanderthals during their range expansion into Europe. PLoS Biol. *2*, 2264–2274.
8. Corrain, C. (1968). Resti scheletrici umani del "Riparo Mezzena", Memorie del Museo di Scienze Naturali di Verona *16*, 97–101.
9. Lalueza-Fox, C., Sampietro, M.L., Caramelli, D., Puder, Y., Lari, M., Calafell, F., Martinez-Maza, C., Bastir, M., Fortea, J., de la Rasilla, M., *et al.* (2005). Neandertal evolutionary genetics: mitochondrial DNA data from the iberian peninsula. Mol. Biol. Evol. *22*, 1077–1081.
10. Krings, M., Stone, A., Schmitz, R.W., Krainitzki, H., Stoneking, M., and Pääbo, S. (1997). Neandertal DNA sequences and the origin of modern humans. Cell *90*, 19–30.
11. Kivisild, T., Reidla, M., Metspalu, E., Rosa, A., Brehm, A., Pennarun, E., Parik, J., Geberhiwot, T., Usanga, E., and Villems, R. (2004). Ethiopian mitochondrial DNA heritage: tracking gene flow across and around the Gate of Tears. Am. J. Hum. Genet. *75*, 752–770.
12. Lalueza-Fox, C., Krause, J., Caramelli, D., Catalano, G., Milani, L., Sampietro, L., Calafell, F., Martínez-Maza, C., Bastir, M., García-Tabernero, A. *et al.* (2006). Mitochondrial DNA of an Iberian Neandertal suggests a population affinity with other European Neandertals. Curr. Biol. *16*, R629–R630.

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Chimpanzees use stone hammers in Cameroon

Bethan J. Morgan[1,2] and Ekwoge E. Abwe[2]

All studied chimpanzee populations use tools, with differences in behavioural repertoires between populations implying significant cultural variation, but the only major known tool-using behaviour that is geographically confined to a single contiguous region is the absence of nut-cracking in all populations east of the N'Zo-Sassandra River in Cote d'Ivoire [1–4]. Nut-cracking is the paradigmatic example of a nutritionally high-value, socially transmitted tradition and here we report that chimpanzees in the Ebo forest, Cameroon, more than 1700 km east of the previously proposed riverine 'information barrier' in Cote d'Ivoire, have been observed to crack the hard shelled nuts of *Coula edulis* with stones used as hammers, so as to access the nutrient-rich seeds. This observation challenges the existing model of the cultural diffusion of nut-cracking behaviour [2,3] by implying that it has been invented on multiple occasions; alternatively, if nut-cracking is an ancient trait in the western chimpanzee populations then there have been extinctions of the behaviour in areas between the N'Zo-Sassandra River and the Ebo forest.

The Ebo forest covers almost 1500 km^2 of closed-canopy and disturbed moist forest across three river valleys and a series of steep mountains in south-western Cameroon (N 04.36° E 010.23°). Sounds of nut-cracking were heard for 20 minutes on September 2nd 2005 at 09:05h in mixed-species forest dominated by *C. edulis* on the brow of a hill. Our research team approached a partially obscured male chimpanzee and subsequently saw arm movements and heard cracking sounds on a *C. edulis* branch 8 m from the ground for