

deeply buried inside the Earth. The subchondritic Nb/La abundances in both the continental crust and the upper mantle<sup>31</sup>, the 'paradox' that the Pb isotopic composition of nearly all terrestrial samples fall to the right of the geochron<sup>32</sup> and, more recently<sup>26</sup>, the Hf and Nd isotopic and elemental imbalance between chondrites, the upper mantle and the continental crust, have all been used to infer a deep missing reservoir. The Archaean meta-igneous rocks from Greenland may therefore carry geochemical evidence for a so far elusive thermal boundary layer before its processing by 3.8 Gyr of mantle convection.

The nature of this reservoir is uncertain<sup>28</sup>. Neither the survival of a deep layer of primitive material left unperturbed after core segregation nor the segregation of lithospheric plates in the deep mantle<sup>6</sup>, possibly at the core–mantle boundary, could account for the deficit of the heat-producing elements U, Th and K and thereby the 'missing heat'. An alternative model is the segregation of a very dense mineral assemblage rich in Nb, Ta, radioactive, and other elements during an early magma ocean. Although the relative Sm/Nd and Lu/Hf fractionation trends visible in Fig. 2 may help constrain the mineralogy of the segregating phases<sup>26</sup>, too little is yet known about minor high-pressure minerals that could sequester these elements. □

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## Molecular analysis of Neanderthal DNA from the northern Caucasus

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The expansion of premodern humans into western and eastern Europe ~40,000 years before the present led to the eventual replacement of the Neanderthals by modern humans ~28,000 years ago<sup>1</sup>. Here we report the second mitochondrial DNA (mtDNA) analysis of a Neanderthal, and the first such analysis on clearly dated Neanderthal remains. The specimen is from one of the eastern-most Neanderthal populations, recovered from Mezmaiskaya Cave in the northern Caucasus<sup>2</sup>. Radiocarbon dating estimated the specimen to be ~29,000 years old and therefore from one of the latest living Neanderthals<sup>3</sup>. The sequence shows 3.48% divergence from the Feldhofer Neanderthal<sup>4</sup>. Phylogenetic analysis places the two Neanderthals from the Caucasus and western Germany together in a clade that is distinct from modern humans, suggesting that their mtDNA types have not contributed to the modern human mtDNA pool. Comparison with modern populations provides no evidence for the multiregional hypothesis of modern human evolution.

The first successful extraction and sequencing of the mtDNA hypervariable regions (I and II (HVRI & HVRII)) was performed on the Neanderthal-type specimen from Feldhofer Cave, the Neander valley, Germany<sup>4,5</sup>. Phylogenetic analysis of the sequence placed the Neanderthal mtDNA outside the mtDNA pool of modern humans. This was regarded as a breakthrough in the study of modern human evolution, providing molecular evidence that Neanderthals did not contribute mtDNA to modern humans. From this sequence the divergence of Neanderthals and modern humans was estimated to



Stockholm laboratory experienced problems with contamination: most of the cloned PCR products that they analysed contained sequences that are found in the modern human mtDNA pool, with two haplotypes predominant. However, three clones contained DNA that was the same as the sequence determined in Glasgow (two of these contained non-reproducible substitutions).

The preservation of 256-bp DNA fragments in bone that is ~29,000 years old, that has not been preserved in permafrost and that contained sufficient DNA to enable direct DNA sequencing after amplification is unprecedented and may be attributed to specific features of the microenvironment of the limestone cavern<sup>2</sup>. The retrieval of mtDNA showed a positive correlation to the preservation of collagen content and the skeletal morphology.

Phylogenetic analysis using both distance and parsimony optimizations places the two Neanderthal sequences together, in a distinct clade, basal to modern humans. Neighbour-joining analysis supports this separation (Fig. 3a). Parsimony analysis, which makes minimal assumptions about the model of evolution and optimizes the fit between the tree and data, produced similar results (Fig. 3b).

The level of pairwise difference found between the two Neanderthals was higher than the average values found in random samples of 300 Caucasoids ( $5.28 \pm 2.24$ ) and Mongoloids ( $6.27 \pm 2.29$ )—less than 1% of Caucasoid and Mongoloid pairs differ at 12 or more positions—but comparable to a random sample

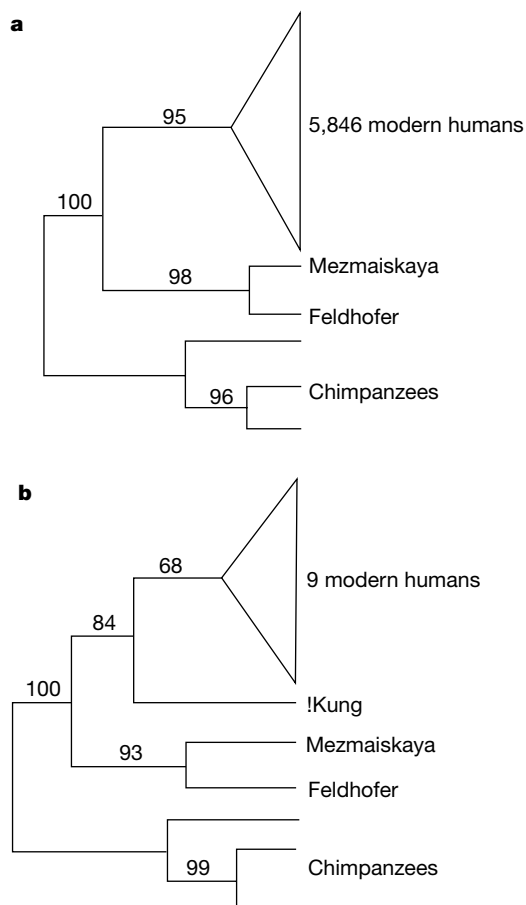
of 300 Africans ( $8.36 \pm 3.2$ ), where 37% of pairs differed at 12 or more positions. When analysing ancient DNA there is the possibility of misincorporating nucleotides in the early stages of PCR, especially when the target DNA is possibly damaged and present in low copy number<sup>12</sup>. As both Neanderthals were analysed in replicate and the results were consistent, however, errors of this type can be discounted.

The Feldhofer and Mezmaiskaya Neanderthals were separated geographically by over 2,500 km. Given that these two individuals contained closely related mtDNA, which is phylogenetically distinct from modern humans, and displays only a moderate level of sequence diversity compared with some primates<sup>13</sup>, these data provide further support for the hypothesis of a very low gene flow between the Neanderthals and modern humans. In particular, these data reduce the likelihood that Neanderthals contained enough mtDNA sequence diversity to encompass modern human diversity.

The 'out-of-Africa' hypothesis for the origin of modern humans predicts equal distances between the Neanderthal sequences and all modern sequences. We observed this in our analysis—the average pairwise differences between the Neanderthals and 300 randomly selected Africans, Mongoloids and Caucasoids were calculated to be  $23.09 \pm 2.86$ ,  $23.27 \pm 4.06$  and  $25.45 \pm 3.27$ , respectively.

We estimated the age of the most recent common ancestor (MRCA) of the mtDNA of the eastern and western Neanderthals to be 151,000–352,000 years. This coincides with the time of emergence of the Neanderthal lineage in the palaeontological records<sup>14</sup>. The divergence of modern human and Neanderthal mtDNA was estimated to be between 365,000 and 853,000 years. Using the same model, we estimated the age of the earliest modern human divergences in mtDNA to be between 106,000 and 246,000 B.P.

The results obtained from this specimen suggest that some other Neanderthal samples may be amenable to molecular analysis. To obtain a more complete picture of the relationship of Neanderthals to modern humans, additional Neanderthals and early modern humans must be analysed, especially from the regions where they may have co-existed. The excellent preservation of this specimen leads to the potential of analysing the entire Neanderthal mitochondrial genome. □



**Figure 3** Phylogenetic relationship of the two Neanderthals and modern humans. **a**, A neighbour-joining tree computed using a total of 1,897 haplotypes derived from 5,846 modern humans<sup>19</sup>. **b**, A maximum parsimony branch and bound search with the two Neanderthal sequences along with the sequences of one !Kung, three other Africans, three Asians and three Europeans, all randomly selected<sup>19</sup>. This result is congruent with four additional data sets that were analysed. In both analyses, three chimpanzee sequences<sup>13</sup> were used as an outgroup. The numbers in both diagrams refer to the bootstrap frequencies (%) obtained from 1,000 replicates.

Methods

DNA extraction, PCR, cloning and sequencing

The DNA extraction methods used in Glasgow<sup>4</sup> and Stockholm<sup>15</sup> have been described. We took precautions to prevent contamination from modern DNA<sup>4</sup>. The Neanderthal-specific primer pairs, NL16,055 and NH16,262, and NL16,209 and NH16,400 (5'-TGATTTCAC GGAGGATGGTGA-3') were used in Glasgow and the primers L16,212 (5'-ATGCTTAC AAGCAAGCACA-3') and H16,332 (5'-TTGACTGTAATGTGCTATG-3') were used in Stockholm. The annealing temperatures for the primer pairs NL16,055–NH16,262, NL16,209–NH16,400 and L16,212–H16,332 were 50 °C, 60 °C and 50 °C respectively; 40 cycles were used for the first two pairs, 55 cycles for the third. AmpliTaq Gold (Perkin Elmer Cetus) was used in all PCRs. PCR products were purified using the QIAquick Gel Extraction kit (Qiagen) before direct sequencing using the Dye Terminator sequencing kit (Perkin Elmer) or cloning into the TA vector (Invitrogen) before sequencing with the same kit using the M13 and T7 primers.

Sequence analyses

The neighbour-joining and the maximum parsimony branch and bound trees were both constructed using PAUP\* 4.0 (ref. 16). For the neighbour-joining analysis, the Tamura-Nei DNA substitution model<sup>17</sup> was used with a gamma distribution of 0.4 (ref. 18), for all other parameters the defaults provided by PAUP\* 4.0 were used. The MRCA was calculated using the described methods and assumptions<sup>5</sup>. PAUP\* 4.0 was used to calculate pairwise differences between sequences: the data sets used for this were constructed by randomly selecting appropriate samples from a published data set<sup>19</sup>.

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**Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest**

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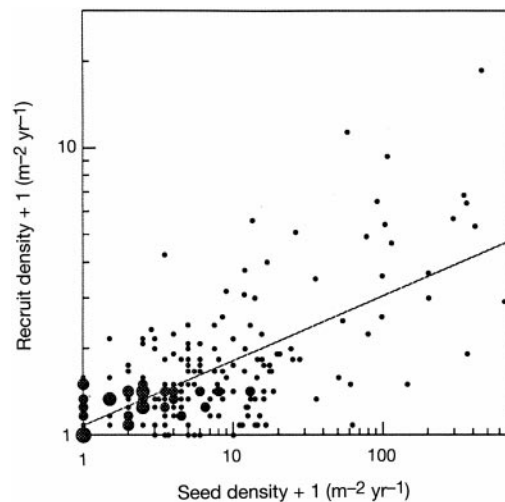
Negative density-dependent recruitment of seedlings, that is, seeds of a given species are less likely to become established seedlings if the density of that species is high, has been proposed to be an important mechanism contributing to the extraordinary diversity of tropical tree communities<sup>1–3</sup> because it can potentially prevent any particular species from usurping all available space, either in close proximity to seed sources or at relatively larger spatial scales<sup>1–18</sup>. However, density-dependent recruitment does not necessarily enhance community diversity<sup>14</sup>. Furthermore, although density-dependent effects have been found at some life stages in some species<sup>3–13</sup>, no study has shown that density-dependent recruitment affects community diversity<sup>14,15</sup>. Here we report the results of observations in a lowland, moist forest in the Republic of Panamá in which the species identities of 386,027

seeds that arrived at 200 seed traps were compared with the species identities of 13,068 seedlings that recruited into adjacent plots over a 4-year period. Across the 200 sites, recruit seedling diversity was significantly higher than seed diversity. Part of this difference was explained by interspecies differences in average recruitment success. Even after accounting for these differences, however, negative density-dependent recruitment contributes significantly to the increase in diversity from seeds to seedling recruits.

Here, both within and between species, we link seed input to initial seedling recruitment in the diverse tropical forest on Barro Colorado Island, Panamá (see Methods). With these data, we first estimate the strength of density-dependent recruitment for individual species and show that it is both pervasive and strong across the community. Then, to determine the influence of these effects on diversity, we compare the observed diversity of seedling recruits to (1) the observed diversity of seeds, (2) the expected diversity of seedling recruits after accounting for average differences in recruitment success among species, but in the absence of density dependence, and (3) the expected diversity of seedling recruits after incorporating our species-specific estimates of density dependence. We thereby test the central and previously unexamined issue of whether density-dependent recruitment increases species diversity.

Seedling recruitment was strongly, negatively density-dependent in our 53 focal species (data for *Trichilia tuberculata*, the most common canopy tree at the site, are shown in Fig. 1). Slopes for the seed–recruit regressions were less than 1 for every species, with a median value of just 0.23 (Fig. 2). Nevertheless, high initial seed densities often overwhelmed density-dependent differences in per seed recruitment so that recruit density was greatest where seeds were initially most numerous; regression slopes were weakly positive for 41 of the 53 species (Fig. 2). To determine the net effect on diversity of these potentially conflicting influences, we therefore conducted the following comparisons.

We first compared the observed diversity of seeds with the observed diversity of seedling recruits at each station. Considering only the 53 focal species, the average Shannon–Wiener diversity index was significantly lower for seeds than for recruits (1.33 and 1.95, respectively, paired *t*-test = 14.7, *P* < 0.0001; Fig. 3a). In addition, the local diversities of seeds and recruits were unrelated



**Figure 1** The relationship between the seed density and recruit seedling density for *Trichilia tuberculata* (Meliaceae). The slope of the log–log relationship is less than 1, indicating that recruitment is negatively density dependent. Nonetheless, recruit density increases with seed density. Each symbol represents a census station(s) consisting of one 0.5-m<sup>2</sup> seed trap and three 1-m<sup>2</sup> seedling plots. Symbol size is proportional to the number of stations (ranging from one to six) with identical counts of seeds and recruits.