links between biomarker structure and biochemical function. Similarly, geologists have much to offer evolutionary biology by helping constrain the time period and physical context of the appearance of new life forms.

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Revisiting Neandertal diversity with a 100,000 year old mtDNA sequence

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The cohabitation of Neandertals and modern humans in Europe about 35,000 years ago has stimulated considerable debate regarding hypothetical admixture. Recently, sequences of the hypervariable region-1 (HVR-1) of mitochondrial DNA (mtDNA) from 9 Neandertal specimens dated between 29,000 and 42,000 years ago from dispersed locations have revealed the genetic diversity of Neandertals around the time of the cohabitation [1–4]. The genetic signatures before and after contact with modern humans were found to be similar. They fall outside the range of modern human genetic diversity and show no specific affinity with modern or Paleolithic Europeans [5]. Such observations are generally taken as strong evidence for the 'Rapid replacement' model for the origin of modern humans [4,6], though further evidence is needed to completely exclude admixture [7].

The first presence of modern humans in Europe before 35,000 years ago as well as the survival of Neandertals beyond 30,000 years ago are still controversial issues [9]. Our goal was to recover a Neandertal sequence that unambiguously predates the cohabitation period. A comparison of this sequence with published Neandertal sequences might reveal either the long-time stability of the Neandertal mtDNA-pool or drastic modifications around the time of cohabitation. We, therefore, retrieved 123 bp of the mtDNA HVR-1 from a 100,000 year old Neandertal tooth from the Scladina cave (Meuse Basin, Belgium), which represents the most ancient Neandertal sample analyzed at the DNA level.

The experiments were conducted in a specific laboratory respecting the current authentication standards [10]. The extract was treated with uracil DNA-glycosylase (UDG) to excise deaminated cytosines formed after death, because they lead to artefactual GC→AT polymorphisms during PCR [11,12] and have already been shown to be present in sequences from Scladina fossils [13–15]. We took advantage of previously reported Neandertal sequences to design primers that favor the amplification of Neandertal DNA. PCR was never successful when fragments larger than 173 bp were targeted (Supplemental Data). We amplified four fragments spanning in total 221 bp of the HVR-1. Each PCR product was cloned and the final sequence was deduced from the consensus of 61 clones. Each position was found in at least two amplification products, except for the first 39 and last 59 nucleotides for which PCR replication was not possible. These nucleotides were consequently excluded from the sequence analyses. The remaining 123 bp (Figure 1) fulfilled all standards to guarantee the absence of DNA-damage-induced errors [10]. In addition, we are confident that the conditions in the Scladina cave favour DNA preservation, because an atomic C:N ratio typical of well-preserved collagen was found on the maxillary from the Scladina Neandertal [8], cave bear bones from the same excavation layer have already yielded authentic ancient DNA sequences [13,14] and 60,000–70,000 thousand year old nuclear DNA sequences were successfully amplified from woolly rhinoceroses from Scladina [15].

The Scladina Neandertal sequence has not been found among the 7161 human HVR-I sequences present in the HvrBase++ [16]. It appears more distantly related to the human than to the already reported Neandertal sequences (Figure 1). Of the 123 nucleotides considered, only one polymorphic site (at position 16258) has already been

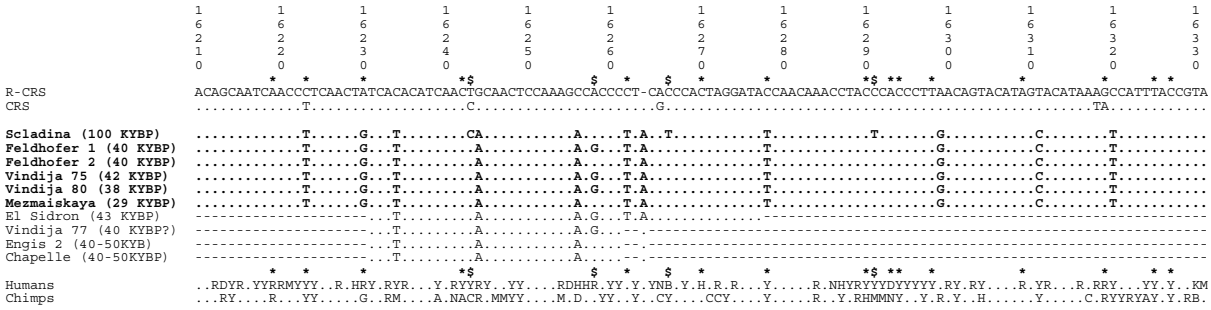

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Figure 1. Alignment of the Neandertal sequences.

The ten Neandertal HVR-I sequences are shown according to their excavation location. Dots indicate identity to the Revised Cambridge Reference Sequence (R-CRS). The Cambridge Reference Sequence is shown and labeled. Insertions and deletions are represented by dashes. The polymorphisms observed in our dataset among the 171 human sequences and the 8 chimp sequences are shown in the two last lines. None of the differences detected between Scladina and other Neandertal sequences ($) is in one of the sites demonstrated by Gilbert *et al*. [12] as being highly affected by *post mortem* degradation in humans (*) or is highlighted as a putative misidentified site (i.e. exhibiting a probability of matching the consensus below 0.95) by the Consensus Confidence program [15]. This suggests that the polymorphism detected here is not artefactual. The six Neandertal sequences used in further phylogenetic and pairwise distribution analyses are shown in bold.

described among Neandertals. The Scladina sequence extends the level of diversity as it exhibits three additional transitions compared to other Neandertal sequences (Figure 1).

Sequence comparisons were conducted with the 171 human HVR-I sequences used in [7] as a representative subset of the overall human diversity. We chose the best-fitting phylogenetic model (among 56) by MODELTEST (Supplemental Data) according to the Akaike criterion. The resulting model (HKY+G+I or HKY+G depending on the inclusion of 8 chimp sequences as an outgroup or not) accurately estimates nucleotide substitution parameters and takes into account rate heterogeneity among sites (as suggested in [7], but lacking in previous phylogenetic analyses involving Neandertals [1–4]). When chimpanzee sequences are used as outgroups, the Neandertals appear as monophyletic (bootstrap support 72.3%). When chimpanzee sequences are excluded, the Scladina sequence still clusters with other Neandertal sequences (bootstrap-support 96.5%), excluding all human mtDNA lineages. This demonstrates that attraction of Neandertal sequences by chimp sequences does not account for the observed topology as suggested in [7].

The pairwise distance distributions within humans, as well as between humans and Neandertals, are closer and overlap more extensively for more recent Neandertals ($p < 0.001$) than for the Scladina specimen (Figure 2). While the diversity of the more recent Neandertals is similar to that of modern humans worldwide, the sequence from Scladina reveals that more divergent Neandertal haplotypes existed before 42,000 years ago. This could suggest that Neandertals experienced genetic drift as demographic bottlenecks eliminated the phylogenetically more recent (i.e. less expanded) haplotypes from populations. Consequently, the most likely conserved Neandertal haplotypes could also be the phylogenetically most ancient (i.e. the most closely related to the common ancestor of modern humans and Neandertals). This could explain the shift towards modern human pairwise distributions observed between 100,000 and 40,000 years ago. Whatever this shift should be related to cohabitation, climatic changes, or any subdivision of populations, the Scladina sequence has revealed that the genetic diversity of Neandertals has been underestimated. Thus, more Neandertal sequences than the six presently available and longer than 100 bp are needed to fully understand the extent of the past diversity of Neandertals.

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Supplemental data

Supplemental data including experimental procedures are available at http://www.current-biology.com/cgi/content/full/16/11/R400/DC1/

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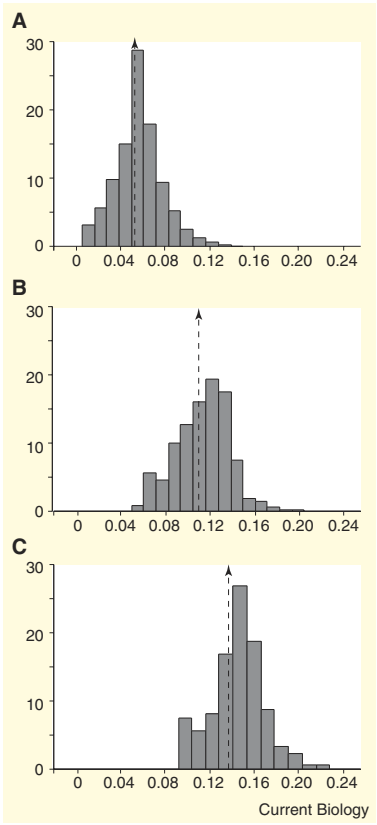

Figure 2. Pairwise distance distribution between modern humans and Neandertals.

The distributions of pairwise distances expressed in terms of number of substitutions per site are estimated from the best-fitting model, within the *Homo sapiens* sequences (A, n = 14535), between the *Homo sapiens* and the first six Neandertal sequences of Figure 1 except Scladina (B, n = 855), between the *Homo sapiens* and the Neandertal sequence from Scladina (C, n = 171). The y-axis shows the percentage of the pairwise counts. The arrows show the means of each distribution. The means of (B) and (C) (0.108 and 0.136, respectively) are significantly different ($p < 0.001$). The distributions in (A) and (B) overlap extensively: 95% (and 99%) of this distribution depicted in (A) overlap with 21% (and 69%) of the distribution in (B). The extent of this overlap is drastically reduced to 7% (and 21%) of the distribution in (C). Actually, 94% of the distribution in (A) does not overlap with (C).

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Do angry men get noticed?

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In humans, the physical differences between the sexes are readily apparent, but possible cognitive and perceptual differences are less obvious. As social animals, humans have specialized mechanisms for recognizing facial expressions, but the extent to which these mechanisms are tuned to differences between male and female faces remains unclear. We measured the efficiency with which emotional expressions conveyed by male and female faces are detected by male and female observers. Angry male faces were detected significantly more rapidly by male than female observers. Moreover, detection of angry male faces by either male or female observers was scarcely affected by the addition of neutral distractor faces to the search display. Our findings are consistent with the notion of a perceptual system in both males and females that has evolved to rapidly detect aggression in males.

In humans, evolution has resulted in marked differentiation between males and females [1,2], including differences in the structural and functional organization of the brain. These differences are reflected in patterns of cognitive and behavioural abilities [3]. For example, females tend to perform better than males at fine motor and perceptual discrimination tasks, whereas males are better at route-finding tasks [3]. Males are also physically larger and more aggressive than females, and so more likely to pose a physical threat [4]. Such physical differences between the sexes may in turn have shaped the cognitive processes involved in detecting threatening behaviour in others. Early detection of an angry facial expression, for example, might reduce the