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Using genetic evidence to evaluate four palaeoanthropological hypotheses for the timing of Neanderthal and modern human origins

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ABSTRACT

A better understanding of the evolutionary relationship between modern humans and Neanderthals is essential for improving the resolution of hominin phylogenetic hypotheses. Currently, four distinct chronologies for the timing of population divergence are available, ranging from the late Middle Pleistocene to the late Early Pleistocene, each based on different interpretations of hominin taxonomy. Genetic data can present an independent estimate of the evolutionary timescale involved, making it possible to distinguish between these competing models of hominin evolution. We analysed five dated Neanderthal mitochondrial genomes, together with those of 54 modern humans, and inferred a genetic chronology using multiple age calibrations. Our mean date estimates are consistent with a process of genetic divergence within an ancestral population, commencing approximately 410–440 ka. These results suggest that a reappraisal of key elements in the Pleistocene hominin fossil record may now be required.

Introduction

Background

The resolution of evolutionary relationships amongst Middle Pleistocene hominin populations is an important and long-standing problem in the study of human evolution (Howell, 1994; McBrearty and Brooks, 2000; Harvati et al., in press). Central to this debate is the extent to which it is possible to distinguish between different hominin species and to infer ancestral relationships among them from the limited physical evidence of the Pleistocene fossil record. There remain considerable differences in approaches to hominin classification, with some workers preferring to regard Neanderthals as part of a more broadly defined *Homo sapiens* species (e.g., Bräuer, 2008; Wolpoff, 2009), or simply not to apply taxonomic categories at all (e.g., Trinkaus, 2005). However, most palaeoanthropologists accept the validity of a more restricted diagnosis of *H. sapiens* (sometimes known as "Anatomically Modern Humans") and *Homo neanderthalensis*, to refer to evolutionary, rather than biological, species of hominins (Simpson, 1950).

There is much less consensus, however, regarding the diagnosis and origin of the species ancestral to both modern humans and Neanderthals (see e.g., Stringer, 2002; Tattersall and Schwartz, 2006; Rightmire, 2008; Wood and Lonergran, 2008; Hublin, 2009; Harvati et al., in press). The current palaeoanthropological models for the splitting of modern humans and Neanderthals from an ancestral population can be grouped into four broad chronological categories (Fig. 1). These are the late (~250 ka), middle (~400 ka), and early (~600 ka) periods of the Middle Pleistocene, and the late Early Pleistocene (~800 ka). Whilst these differences might appear to be relatively minor within the broader evolutionary context of *Homo*, they have important taxonomic implications for the genus as a whole, and for the origin of our own species.

Formally evaluating the evidence for each of these four models will help to assess the suitability of the *H. sapiens–H. neanderthalensis* species concept overall (Harvati et al., 2004; Trinkaus, 2005; Bräuer, 2008; Wolpoff, 2009). Here, we investigate the potential of genetic data to provide an independent chronology to evaluate the main species diagnoses of *H. sapiens*, *H. neanderthalensis* and the population ancestral to both.

The four models

The late Middle Pleistocene model (Fig. 1a) posits a single ancestral African population of the species, *H. helmei* (~150–300 ka),

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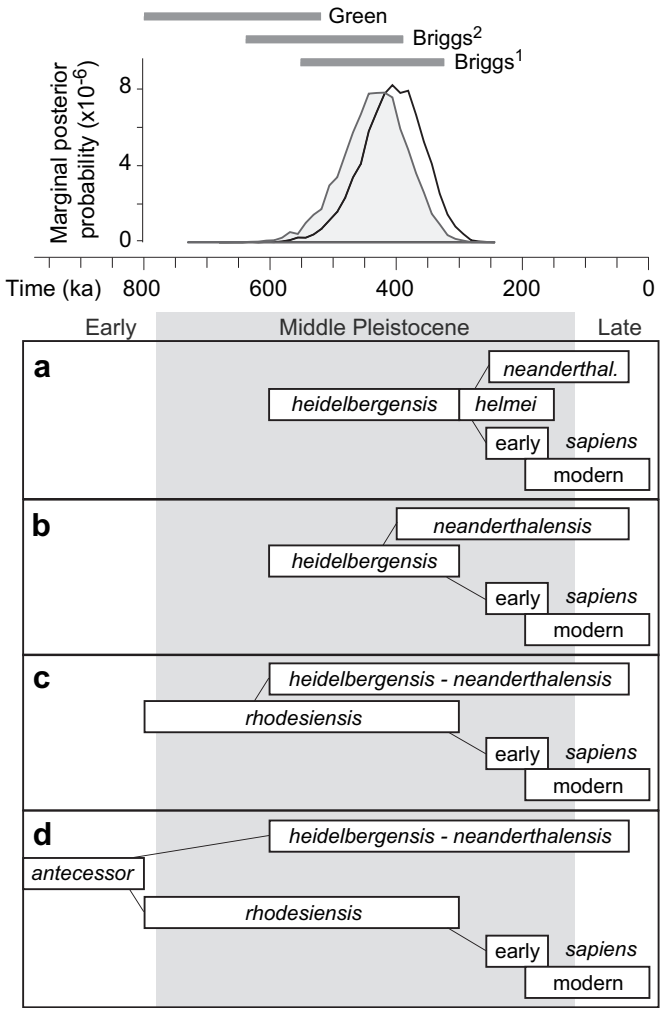

Figure 1. Summary of genetic date estimates in relation to four candidate chronologies for the evolution of Neanderthals and modern humans. The 95% credibility intervals are given for three published estimates of the *H. neanderthalensis–H. sapiens* divergence time, while two estimates from the present study are given as posterior age distributions. Our estimates were obtained using Bayesian phylogenetic analysis of third codon sites from the mitochondrial genomes of 54 modern human, five Neanderthals, one common chimpanzee, and one bonobo. The analyses were calibrated using the radiometric dates of the five Neanderthals, as well as an age calibration for the *Homo–Pan* divergence of either 6.0–7.0 Ma (empty curve with black outline) or 6.5–7.5 Ma (filled curve with grey outline). The four candidate chronologies given in the lower panels are: (a) late Middle Pleistocene; (b) mid-Middle Pleistocene; (c) early Middle Pleistocene; and (d) late Early Pleistocene. Details of these four chronologies are given in the text. All species in these panels are recognised here as members of the genus *Homo*.

based on the Florisbad partial cranium. Dated at ~260 ka (Fig. 2), this taxon is presumed to be associated with the beginnings of a novel lithic technology (Foley and Lahr, 1997; Lahr and Foley, 2001). This Mode 3 hypothesis suggests that both modern humans and Neanderthals first appeared in the archaeological context of Levallois prepared cores and that the Florisbad individual is a member of the population ancestral to both species. In this scenario, therefore, population divergence occurred subsequent to the existence of the Florisbad individual and cannot be earlier than ~260 ka.

The mid-Middle Pleistocene model (Fig. 1b) has a single Eurafrican species, ancestral to *H. neanderthalensis* and *H. sapiens*, containing African fossils such as Broken Hill and Bodo, and European specimens such as Arago and Petralona (Fig. 2), with a possible age range of ~300–650 ka. Stringer (2002) and Rightmire (2008) place the Mauer mandible in this assemblage, thus assigning the species name *Homo heidelbergensis*. However, Hublin (2009) has recently argued that this problematic fossil should be excluded from any current species definitions (but see Mounier et al., 2009, for an alternative view), with precedence instead given to the Broken Hill cranium, thus prioritising the name of *Homo rhodesiensis* for this ancestral species.

The early Middle Pleistocene model (Fig. 1c) is based on the alternative suggestion that *H. heidelbergensis* is not known from Africa, but instead is only present in western Eurasia. Under this scenario, there is a European chronospecies of *H. heidelbergensis- H. neanderthalensis*, which is part of a continuum from the early Middle Pleistocene through to the Late Pleistocene. Defined in this way, the lineage leading to the Neanderthals in Europe includes the extensive Atapuerca Sima de los Huesos (SH) sample (Fig. 2), which already displays clear Neanderthal affinities (Arsuaga et al., 1997; Rosas, 2001), particularly in the dentition (Martinón-Torres et al., 2007). According to the latest dates proposed for the SH material, which argue for a minimum age of ~530 ka ($600^{+\infty}_{-66}$ ka) (Bischoff et al., 2007), the population divergence between this European lineage and that leading to *H. sapiens* likely preceded 600 ka. There is currently no strong argument for extending the ancestral population (represented as *H. rhodesiensis* in Fig. 1c) beyond 650 ka, unless the Tighenif fossil material (Klein, 2009) is included in this species diagnosis.

The late Early Pleistocene model (Fig. 1d) has a European hominin (*Homo antecessor*) as the ancestor to a *H. rhodesiensis–H. sapiens* lineage in Africa and a *H. heidelbergensis–H. neanderthalensis* lineage in Europe, suggesting a population divergence soon after 800 ka (Bermúdez de Castro et al., 1997; Arsuaga et al., 1999). There are variants of this model relating to the various Atapuerca fossil samples; for example, new interpretations of the early Elefante–Gran Dolina material assigned to *H. antecessor* (Fig. 2) favour a hypothetical Asian ancestor derived from *Homo erectus*, which is then either replaced or absorbed by a dispersal of *H. heidelbergensis*, also derived from an Asian (*H. erectus*) ancestor (Martinón-Torres et al., 2007; Bermúdez de Castro et al., 2008; Carbonell et al., 2008). However, this interpretation might push the minimum date for the divergence of the lineages leading to modern humans and Neanderthals back to more than one million years (Carbonell et al., 2008).

An independent chronology

When the Pleistocene fossil record, and different analytical approaches applied to it, permits such disparate interpretations, it is not surprising to find secondary lines of evidence employed to augment the positions of the various models. These have included palaeoenvironmental studies (climate) and archaeology (lithics). However, to avoid a tendency towards circularity, it is preferable to have an independent chronology for the divergence between humans and Neanderthals. This would allow the palaeoanthropological evidence to be assessed without any *a priori* diagnosis of an ancestral species, or the linking of particular taxonomic categories to the production of specific techno-complexes (e.g., Lahr and Foley, 2001; Bermúdez de Castro et al., 2008).

The availability of genomic sequence data from modern humans and Neanderthals provides an opportunity to generate an independent estimate of the evolutionary timescale for the divergence between *H. sapiens* and *H. neanderthalensis*. Molecular phylogenetic analysis can be performed to estimate the age of the most recent common ancestor (MRCA) of the two lineages (e.g., Green et al., 2006; Briggs et al., 2009). This can provide a more concrete *terminus ante quem* (maximum bound) because the reconstructed genealogy provides an uninterrupted line of descent back to a time

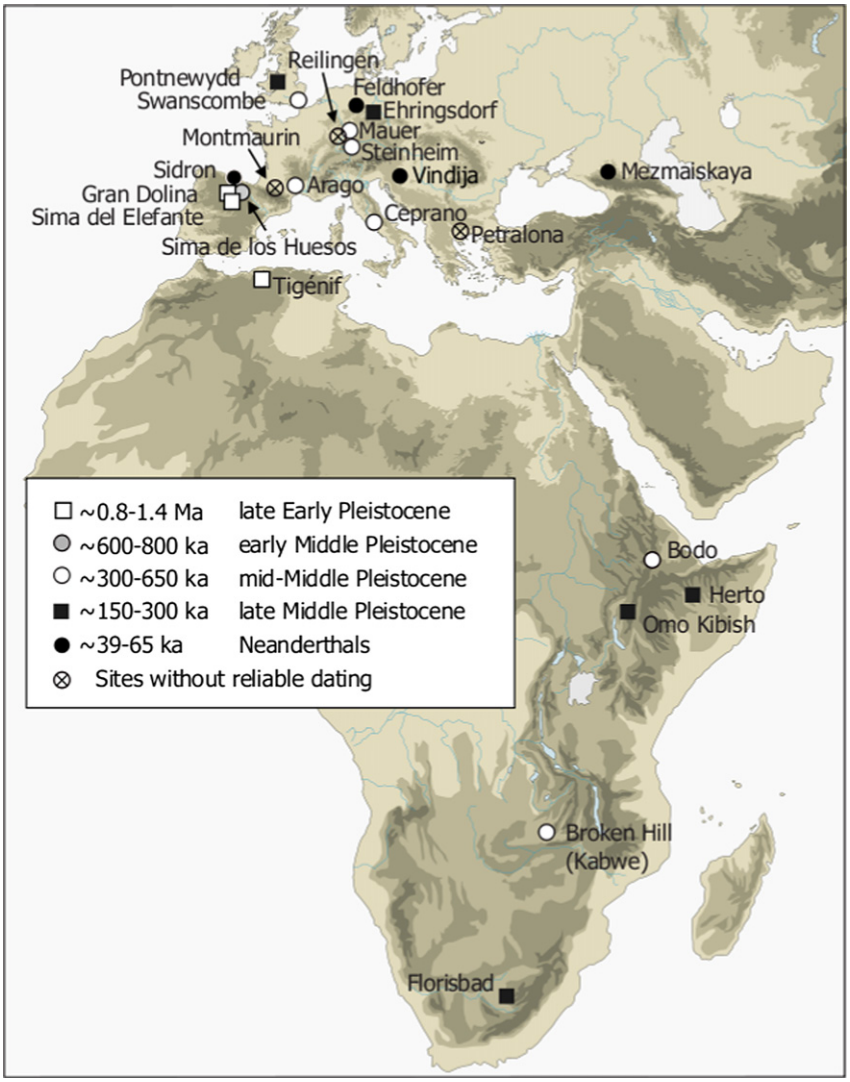

Figure 2. Map displaying approximate geographical locations of European and African Middle and Early Pleistocene fossils referenced in the text. Each site is classified by a symbol for the chronology relating to the four hypotheses for the time of divergence between modern humans and Neanderthals. The location of the archaeological sites relating to the five Neanderthals used in the molecular date estimates are also shown.

preceding the process of population divergence (Fig. 3). In contrast, individuals preserved as fossils may have not necessarily left any descendants. Molecular dating, therefore, has the potential to distinguish between the four palaeoanthropological hypotheses outlined above.

The interpretation of molecular date estimates, however, is itself complicated by several factors. In particular, the choice of data and methodology used to estimate the MRCA can have a significant impact on the results (Endicott et al., 2009). Bayesian phylogenetic methods based on coalescent theory can estimate both divergence times and past demographic parameters directly from genetic data. Whilst there are significant advantages to be gained from using autosomal loci for this type of analysis (Heled and Drummond, 2008, 2010), these types of data are currently available for a single Neanderthal individual only, Vindija 80 (Green et al., 2006; Noonan et al., 2006). Moreover, it is not yet possible to produce a reliable estimate of the Neanderthal branch length using autosomal data, owing to the difficulty of authenticating lineage-specific polymorphisms (Green et al., 2006, 2009; Wall and Kim, 2007; Coop et al., 2008; Brotherton et al., 2010), and the possibility that recombination has occurred between two or more hominin lineages (Eswaran et al., 2005; Plagnol and Wall, 2006; Forhan et al., 2008; Wall et al., 2009).

Mitochondrial DNA (mtDNA) has proved easier to recover and authenticate than autosomal data from Neanderthals (Green et al., 2008; Briggs et al., 2009), largely owing to an increased chance of survival relative to nuclear DNA. In addition, there is a reduced presence of contamination in mtDNA data because of the higher per-nucleotide sequencing coverage that is achieved using both conventional and high-throughput sequencing methods (Ho and Gilbert, 2010). The genes in mtDNA are completely linked as a single locus, however, which can lead to considerable coalescent error if used without additional loci (Nielsen and Beaumont, 2009).

Early genetic-based studies estimating the most recent common ancestor of modern humans and Neanderthals were restricted to data from the fastest evolving sections of the mtDNA control region (e.g., Krings et al., 1997, 1999; Beerli and Edwards, 2002; Ovchinnikov et al., 2002), which has limited powers of resolution over the timescale involved, due to substitutional saturation and rate variation amongst sites (Endicott and Ho, 2008; Endicott et al., 2009). Studies conducted with whole Neanderthal mtDNA genomes have either suffered from methodological shortcomings

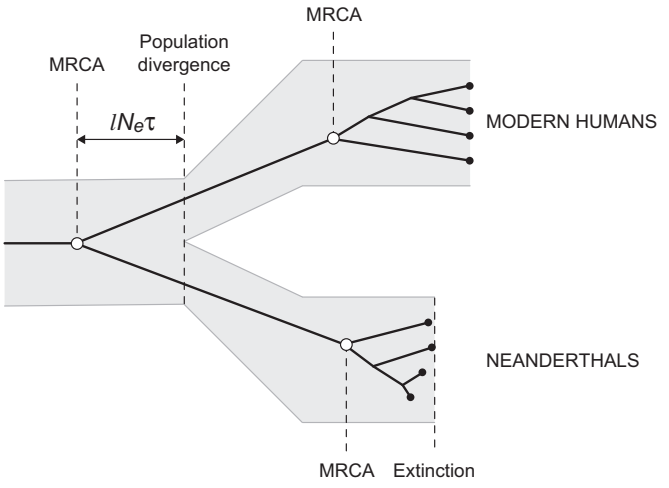

Figure 3. Schematic showing the nature of the disparity between genetic estimates of the time to coalescence between species and the ensuing population divergence. The mean discrepancy is given by $l\,N_e\tau$ years, where l is a locus-specific multiplier, N_e is the ancestral population size, and τ is the generation time. The value of l is 2 for autosomal loci and approximately 0.5 for mitochondrial DNA. The mean time to coalescence *within* the *H. sapiens* and *H. neanderthalensis* mtDNA lineages are also represented on a scale that approximates the values given in Table S1.

(Green et al., 2008) or have not combined all of the available palaeogenomes within a single analysis (Briggs et al., 2009). None of these previous genetic studies used their results to evaluate the competing palaeoanthropological models for Middle and late Early Pleistocene hominin evolution.

Here, we use five dated Neanderthal mtDNA genomes in a Bayesian phylogenetic approach to provide an independent chronology for testing the four main hypotheses proposed for the evolution of *H. sapiens* and *H. neanderthalensis*. This is achieved using multiple sources of information, both within and external to *Homo*, to calibrate the evolutionary substitution rate, and the application of evolutionary models to various data partitions of the mtDNA genome. Our new genetic estimates provide the independent timescale necessary to critically reassess the four palaeoanthropological models for Middle and late Early Pleistocene hominin evolution, together with their associated species diagnoses.

Materials and methods

Molecular date estimates of the *H. sapiens–H. neanderthalensis* divergence have mostly relied on short lengths of sequence data obtained from the rapidly-evolving mitochondrial control region (e.g., Krings et al., 1997, 1999; Ovchinnikov et al., 2002). However, the use of control-region data can lead to an underestimation of the evolutionary rate, owing to excessive mutational saturation (Meyer et al., 1999). The problem of saturation is particularly acute when the evolutionary rate of mtDNA is calibrated using the divergence between *Homo* and *Pan* (Endicott and Ho, 2008). There have been a number of *post hoc* corrections proposed to adjust for saturation in mtDNA sequence data (e.g., Soares et al., 2009). An alternative solution adopted here is to model saturation in the human mtDNA genome directly during the process of parameter estimation, using the characteristics of the data under consideration (Atkinson et al., 2008). This is done in a statistical phylogenetic framework, using explicit models of nucleotide substitution that correct for multiple substitutions and allow for rate variation among sites.

With the publication of whole mtDNA sequences for Neanderthals (Green et al., 2008; Briggs et al., 2009), it is now possible to substantially increase the quantity of data under analysis by including the protein-coding region of the molecule, providing a greater number of informative sites for analysis. Of these data, the third codon positions appear to have the most uniform rate of substitution across sites and are much less subject to the action of purifying selection than the first and second codon positions (Kumar et al., 2005; Kivisild et al., 2006; Endicott et al., 2009; Subramanian et al., 2009). In addition, the average rate of saturation at third codon sites is also much lower than that in the control region, making them a more appropriate data source over an interspecific time-frame (Endicott and Ho, 2008; Subramanian et al., 2009).

We aligned complete mtDNA sequences from 54 modern humans (Andrews et al., 1999; Ingman et al., 2000), five Neanderthals (Green et al., 2008; Briggs et al., 2009), one common chimpanzee (*Pan troglodytes*; GenBank accession number: NC_001643), and one pygmy chimpanzee, or bonobo (*Pan paniscus*; GenBank accession number: NC_001644). The alignment was divided into four partitions: (i) first and second codon sites of protein-coding genes (7232 bp), (ii) third codon sites of protein-coding genes (3616 bp), (iii) control region (1119 bp), and (iv) loop regions of rRNA genes (1443 bp). The remaining portions of the alignment, including rRNA stems, tRNA genes, and intergenic sites, were discarded because of complications in modelling the evolutionary process in these parts of the mtDNA genome (Endicott and Ho, 2008). The *ND6* gene, which is the only protein-coding gene encoded on the mtDNA light strand, was also excluded because of its distinctive base composition and substitution patterns (Kivisild et al., 2006).

Co-estimation of the phylogeny and divergence times was performed using the Bayesian phylogenetic software *BEAST* v1.4.8 (Drummond et al., 2006; Drummond and Rambaut, 2007). Separate coalescent-based priors were specified, with a constant-size model for Neanderthals and an exponential-growth model for modern humans. We chose a constant-size model for Neanderthals because of the small number of sequences (see Ho et al., 2007). A uniform prior was used for the basal branches connecting Neanderthals and modern humans, following the methodology of previous analyses involving data sets of a similar nature (Ho et al., 2008; Briggs et al., 2009; Korsten et al., 2009). Estimates of the posterior distribution were obtained by Markov chain Monte Carlo (MCMC) sampling. Samples from the posterior were drawn every 2000 steps over a total of 20,000,000 MCMC steps, with the first 10% of samples discarded as burn-in. Posterior samples were checked using Tracer v1.4 (Rambaut and Drummond, 2007), indicating convergence to the stationary distribution and sufficient sampling from the posterior. Separate GTR+G models of nucleotide substitution were assigned to each data partition. The data set was analysed in two formats: (i) the concatenated data set comprising all four partitions and (ii) third codon sites only. All of the *BEAST* input files are available as Supplementary Material.

In order to estimate evolutionary divergence times from sequence data, it is necessary to incorporate age estimates from external sources for the purpose of calibration. In the present analysis, we used two independent sources of age calibration. First, we included the fossil-based estimate of the timing of the divergence between *Homo* and *Pan* (Benton and Donoghue, 2007). To account for a degree of uncertainty in the timing of this event, we used prior age distributions rather than fixing it to an errorless point value (Ho and Phillips, 2009). We performed two analyses using different values for this split: (i) normal prior with mean 6.5 Ma and with 95% of the prior density between 6.0 and 7.0 Ma, and (ii) normal prior with mean 7.0 Ma and with 95% of the prior density between 6.5 and 7.5 Ma.

In addition to the *Homo–Pan* calibration, the ages of the five Neanderthal fossils, estimated by radiocarbon and ESR dating, offer

point calibrations at the tips of the tree: Vindija 80, ~38 ka (Serre et al., 2004); Feldhofer 1, ~40 ka (Schmitz et al., 2002); Feldhofer 2, ~39 ka (Schmitz et al., 2002); Sidron 1253, ~39 ka (Lalueza-Fox et al., 2005); and Mezmaiskaya 1, ~65 ka (Skinner et al., 2005) (Fig. 2). The ESR age estimate for Mezmaiskaya 1 and the uncorrected radiocarbon dates for the other four Neanderthal fossils were treated as point estimates, to be compatible with the methodology of Briggs et al. (2009). As such, there is an error associated with the fossil dates, which is not accounted for, but the magnitude of this error is expected to be relatively small compared with the uncertainty associated with the *Homo–Pan* split.

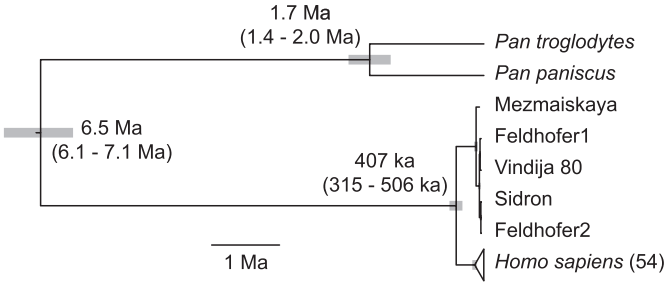

Figure 4. Chronogram estimated from third codon sites of mitochondrial protein-coding genes, based on a human–chimpanzee calibration with a mean of 6.5 Ma (see Table 1 for values using the alternative calibration based on 7.0 Ma). The tree represents the maximum-clade-credibility topology, with mean posterior divergence times. Grey bars denote 95% credibility intervals for estimated divergence times. Note that the subtree of 54 modern humans has been collapsed.

Results and discussion

Mitochondrial divergence time for modern humans and Neanderthals

Genetic estimates for the time to the most recent common ancestor (MRCA) of modern humans and Neanderthals, obtained using Bayesian phylogenetic analysis of mtDNA sequences, are given in Table 1 and Figs. 1 and 4. The time to the MRCA varies considerably according to the data partition being analysed. Substantially older dates are obtained when using a concatenated data set than those produced by third codon sites only. This is probably due to the amount of saturation and purifying selection acting on the control region, and first and second codon sites, respectively (Endicott and Ho, 2008; Subramanian et al., 2009).

The posterior age distributions for the MRCA of modern humans and Neanderthals, estimated from third codon sites, are plotted in Fig. 1. In each case, the shape of the distribution indicates that the highest probability occurs in the vicinity of the mean. The mean values vary slightly, depending on which external calibration is preferred (6.0–7.0 or 6.5–7.5 Ma), ranging from 407 ka (315–506 ka) to 435 ka (338–538 ka). For two reasons, our dates for the mitochondrial MRCA may be slight overestimates. First, there appears to be a codon-usage bias in protein-coding genes, which might be indicative of mutational bias and/or selection (Duret, 2002; Yang and Nielsen, 2008). Second, third codon sites are linked to first and second codon sites, which are subject to a greater degree of natural selection (Elson et al., 2004; Kivisild et al., 2006; Endicott and Ho, 2008). Whilst there is no evidence that these two forces produce a significant effect amongst humans (Kanaya et al., 2001; Yang and Nielsen, 2008), their existence strengthens the arguments for taking the MRCA date as a maximum bound for the time to population divergence.

Comparison with previous estimates

Comparison between our study and that of Briggs et al. (2009) is facilitated by taking an average from the latter of two separate analyses using third codon sites and external calibrations centred on 6 and 7 Ma (Table 1). This gives a mean time to the mitochondrial MRCA of 475 ka (355–600 ka), compared with 407 ka (315–506 ka) using a calibration of 6–7 Ma and the same methodology in the present study. Some of this 68 ka difference in means is likely due to the reduction in data for the equivalent analysis of Briggs et al. (2009); four Neanderthal genomes (Feldhofer 1, Feldhofer 2, Sidron 1253, and Mezmaiskaya 1), together with 53 of the same 54 human sequences used in the present study, were removed from their alignment whilst estimating the genetic MRCA of both species. To investigate the possibility that the highly divergent Mezmaiskaya genome in our own alignment was not a factor, we repeated the analysis without it, but the results were unaffected (data not shown).

Table 1
Comparison of mtDNA-based date estimates for the time to the most recent common ancestor of Neanderthals and modern humans.

Study	Data partition	Sites	Number of individuals		*Homo–Pan* calibration age	Neanderthal calibrations[a]	Time to most recent common ancestor (ka)	
			H. sapiens	*H. neanderthalensis*			Mean	95% CI[b]
Krings et al. (1997)	HVS1	333	994	1	4.0–5.0	0	620	550–690
Krings et al. (1999)	HVS1+2	600	663	1	4.0–5.0	0	465	317–741
Beerli and Edwards (2002)	HVS1	333	1	1	4.0–5.0	0	710	631–789
Ovchinnikov et al. (2002)	HVS1	333	994	2	4.0–5.0	0	609	365–853
Green et al. (2008)	All sites	16500	10[c]	1	6.0–8.0	0	660	520–800
Briggs et al. (2009)	Third codon sites	3575	1	1	5.5–6.5	1	439	321–553
Briggs et al. (2009)	Third codon sites	3575	1	1	6.5–7.5	1	511	388–641
This study	Third codon sites	3616[d]	54	5	6.0–7.0	5	407	315–506
This study	Third codon sites	3616	54	5	6.5–7.5	5	435	338–538
This study	Protein-coding genes, rRNA loops, control region	13,410	54	5	6.0–7.0	5	565	475–654
This study	Protein-coding genes, rRNA loops, control region	13,410	54	5	6.5–7.5	5	607	510–700

[a] The radiometric dates for the five Neanderthal genomes used as calibrations are given in the main text.
[b] Confidence interval (maximum likelihood) or credibility interval (Bayesian).
[c] This analysis was based on a representative subset of the 54 modern human genomes used in the present study.
[d] The difference in number of third codon sites compared with Briggs et al. (2009) derives from the method of data preparation and has a negligible effect on the outcome of the analyses.

The results from our concatenated data analysis (protein-coding genes, rRNA loops, and control region) confirm that elevated levels of saturation and purifying selection, acting on classes of data other than third codon sites, have a particularly adverse effect on estimates of the MRCA in an interspecific analysis (Endicott and Ho, 2008; Briggs et al., 2009). This is illustrated by our mean ages of the genetic MRCA (565 ka and 607 ka), which are ~40% greater than those obtained using third codon sites only (407 ka and 435 ka). The 607 ka estimate is comparable with a mean value of 660 ka obtained without data partitioning, using the single Vindija 80 Neanderthal genome and a 7 Ma *Homo–Pan* calibration, in the Bayesian analysis of Green et al. (2008) (Table 1). As the study of Green et al. (2008) was based on a representative subset of the same 54 modern human sequences used in the present analyses, the 53 ka disparity in mean dates between the two sets of results likely reflects our usage of five Neanderthal sequences, and the inclusion of tRNA, rRNA stems, and intergenic regions in their analysis.

Dates for the genetic MRCA from four earlier studies (Krings et al., 1997, 1999; Beerli and Edwards, 2002; Ovchinnikov et al., 2002) were estimated using mtDNA control-region data and calibrated by a *Homo–Pan* divergence of 4–5 Ma. The reported means of 465–710 ka (see Table 1) require an upward revision of ~40% to be compatible with the current analyses based on external calibration values of 6.0–7.5 Ma, resulting in estimates for the MRCA of ~600 ka–1 Ma. The significant disparity between these dates and those estimated from third codon sites (Briggs et al., 2009; this study) is predominantly caused by the amount of saturation occurring in the human mtDNA control region over the time-depth of an interspecies calibration (Endicott and Ho, 2008). Consequently, the results of studies based on control-region data (Krings et al., 1997, 1999; Beerli and Edwards, 2002; Ovchinnikov et al., 2002) should be viewed as underestimates of the actual rate of evolution, and should no longer be used to support palaeoanthropological hypotheses for the MRCA of *H. sapiens* and *H. neanderthalensis*.

Population divergence time of modern humans and Neanderthals

In the absence of migration, genetic divergence precedes or coincides with population divergence. The latter can occur in the presence of a population bottleneck, which might be the case for intraspecific colonisation events (e.g., Soodyall *et al.*, 1997; Hey, 2005). Therefore, in order to relate the timing of mitochondrial divergence between *H. sapiens* and *H. neanderthalensis* to the timing of population divergence, it is necessary to consider the relationship between the two events.

Given a constant ancestral population size, divergence at a given genomic locus is expected to precede population divergence by an average of $l\,N_e\tau$ years, where l is a locus-specific multiplier, N_e is the ancestral effective population size, and τ is the generation time (Fig. 3). The value of l will depend on whether the locus is autosomal ($l = 2$) or mitochondrial ($l = 0.5$, assuming that the effective population sizes of males and females are equal). This is because individuals have two copies of autosomal DNA (diploid), compared with only one of mitochondrial DNA (haploid), which is maternally inherited and shared by siblings.

Estimates derived from multilocus autosomal DNA for the long-term effective population size (N_e) of modern humans vary between 1000 and 10,000 individuals, with a trend towards lower estimates when demographic history typical of human colonisations is taken into account (Takahata et al., 1995; Voight et al., 2005; Liu et al., 2006; Tenesa et al., 2007). For the exercise of estimating the time of population divergence between *H. sapiens* and *H. neanderthalensis* from mtDNA, we adopt an intermediate value of 5000. A mean generation time of 25 years is assumed here for females (Fenner, 2005). The formula above gives a difference from the mean for the mitochondrial MRCA of ~62.5 ka. This equates to a mean time for population divergence of ~345–373 ka (6.5–7.5 Ma and 6.0–7.0 Ma *Homo–Pan* calibrations, respectively) under the constant-size demographic scenario considered here. The effect of substituting either 20 or 30 year generation times is relatively small, adding or subtracting ~15 ka to these values. If the ancestral effective population size has been overestimated (e.g., if a significant bottleneck accompanied the process of speciation), then the time of population divergence would probably be closer to the time of mtDNA divergence (i.e., the MRCA). Similarly, the date estimates for population divergence will be somewhat biased if the ancestral population had been expanding or declining.

Estimates made using autosomal data from Vindija 80 produced a time to the MRCA of ~700 ka and population divergence ~370–325 ka (Noonan et al., 2006; Wall and Kim, 2007). Using the same demographic assumptions (constant population size and equal numbers of males and females), the interval between these two periods should be approximately four times that obtained using mtDNA (see above and Fig. 3). Using our estimate of ~62.5 ka for the difference between the mtDNA MRCA and population divergence, adjusted for the larger value of N_e used in the autosomal studies (10,000), provides an equivalent estimate for the MRCA of ~800 ka. Taking into account the stochastic variance associated with dates obtained from a single Neanderthal individual, these estimates from mitochondrial and autosomal DNA are in relatively good agreement with each other.

However, strong caveats are necessary concerning genetic estimates for population divergence, including the fact that the effective population size is itself linked to the mean generation time. It should also be noted that the variances of the date estimates for population divergence are very large. For mtDNA, which represents a single locus, the variance of the estimated MRCA age is given by the square of the effective female population size.

The most reliable inference for palaeoanthropology, therefore, is that population divergence must have accompanied, or occurred more recently than, genetic divergence (i.e., the MRCA). Consequently, to reassess the four palaeoanthropological hypotheses, we rely only on our date estimates for the most recent genetic ancestor of both populations as an upper boundary for the process of population divergence (Figs. 1 and 3). We adopt a conservative approach by using the combined 95% confidence intervals of our two analyses, reflecting a possible time to coalescence for the lineages leading to *Homo* and *Pan* between 6.0 and 7.5 Ma.

Reassessing the four models for population divergence

Our 95% credible intervals for the MRCA of modern humans and Neanderthals, ranging from 315 to 538 ka, rule out a Eurasian hominin ancestral to both modern humans and Neanderthals in Europe at 1 Ma to 800 ka, because the MRCA considerably *postdates* the known time range of the fossils assigned to *Homo antecessor* (Fig. 1d). As the MRCA cannot occur after population divergence, our estimate should be taken as a *terminus ante quem* for the commencement of these separate evolutionary trajectories.

The late Middle Pleistocene model (Foley and Lahr, 1997; Lahr and Foley, 2001) is already doubtful on palaeoanthropological grounds alone, because of the presence of Levallois flake technology in Europe and Africa by MIS 9 (~300–327 ka) (White and Ashton, 2003; Tryon et al., 2005) and the existence of derived Neanderthal features in fossils from Swanscombe, Steinheim, and Sima de los Huesos (Stringer, 2002; Hublin, 2009) (Fig. 2). All of these fossils seemingly predate the proposed timescale for *Homo helmei,* the dates of which (~150–300 ka) fall outside of our 95% credible intervals (315–538 ka) for the genetic MRCA (Fig. 1a).

The early Middle Pleistocene model requires an MRCA *prior* to the earliest fossil remains of this period attributed to a population that was clearly part of the Neanderthal lineage. This model, therefore, has the Sima de los Huesos (SH) material from Atapuerca acting as a *terminus post quem*, because the SH sample already displays derived Neanderthal features (Arsuaga et al., 1997; Rosas, 2001; Martinón-Torres et al., 2007). The preferred date of 600 ka (minimum ~530 ka) claimed for all of the SH material (Bischoff et al., 2007) places these fossils outside of our 95% credible intervals for the genetic MRCA (Fig. 1c).

Therefore, our genetic date estimates are inconsistent with the late Early Pleistocene, early Middle Pleistocene, and late Middle Pleistocene models for the divergence between *H. sapiens* and *H. neanderthalensis* (Fig. 1d, c, and a, respectively). Rejection of these three models leaves just the mid-Middle Pleistocene model for the time of divergence between modern humans and Neanderthals. Here, our 95% credible intervals for the MRCA (315–538 ka) fall squarely within the proposed dates from palaeoanthropology (~300–650 ka) (Fig. 1b).

We note that the mean values for the ensuing population divergence, obtained using a female generation time of 20–30 years (383–322 ka), are also within the dates for the mid-Middle Pleistocene model and are compatible with those estimated from autosomal DNA (370–325 ka) (Wall and Kim, 2007). Our estimates are also consistent with dates derived from analysis of neutral morphological characters in both species, 182–592 ka (mean 373 ka) (Weaver et al., 2008). However, we rely solely on our genetic analyses to place a mean upper bound on the population divergence between modern humans and Neanderthals at ~410–440 ka.

Implications for palaeoanthropology

If the three alternative hypotheses are discounted, the evolutionary history of hominins in both Eurasia and Africa during the Middle Pleistocene and late Early Pleistocene becomes somewhat clearer, but a reappraisal of the Sima de los Huesos (SH) fossils is required. These are central to the definition of *H. heidelbergensis–H. neanderthalensis* as a European species continuum, yet they display many more Neanderthal characteristics than do apparently later European fossils, such as Mauer (Street et al., 2006), Arago (Lumley et al., 1984), and Ceprano (Muttoni et al., 2009) (Fig. 2). These features do not sit easily with other aspects of the fossil record (Stringer, 2002; Hublin, 2009; Harvati et al., in press) and are not compatible with population divergence commencing, from our estimates, no earlier than ~538 ka.

The dating of the SH material is the key to this issue, but is dependent on the sample essentially being in a primary context and sealed by dated speleothem, thus providing a minimum age of more than 530 ka (Bischoff et al., 2007). Alternative scenarios, however, suggest that the SH fossils are the result of fluvial/mudflow redeposition (Andrews and Fernandez-Jalvo, 1997; Fernandez-Jalvo and Andrews, 2003). Considering these taphonomic doubts concerning the mode of deposition of the sample, it may be that previous dates for the SH sample of ~200–400 ka (Arsuaga et al., 1997; Bischoff et al., 2003) were in fact more appropriate than the current $600^{+\infty}_{-66}$ ka (Bischoff et al., 2007). This would place the SH material closer in age to comparable European fossils, such as Swanscombe (Stringer and Hublin, 1999), Steinheim and Ehringsdorf (Street et al., 2006), and Pontnewydd (Green, 1984) (Fig. 2).

Reclassifying the SH material as an early form of *H. neanderthalensis* on the basis of its derived Neanderthal features, and dating it to no earlier than 400 ka, would also remove most of the data supporting a European chronospecies of *H. heidelbergensis–H. neanderthalensis*. This would open up the possibility of a less inclusive definition for the species ancestral to modern humans and Neanderthals (Frieß, 2003; Tattersall and Schwartz, 2006; Harvati et al., in press). The younger age (~460 ka) now assigned to the Ceprano cranium (Muttoni et al., 2009) might also suggest greater complexity in the European hominin sequence. This fossil may represent a particularly primitive example of *H. heidelbergensis*, or it may indicate that distinct lineages co-existed in the European Middle Pleistocene, just as they did in the late Pleistocene. Given the uncertain dating of key finds, such as Petralona (Stringer, 1983; Harvati et al., 2009), Montmaurin (Hublin, 1998), and Reilingen (Street et al., 2006) (and perhaps SH), this is certainly a possibility, just as it is in Africa.

If the *H. sapiens–H. neanderthalensis* population divergence is constrained by a lower bound of ~300 ka, then the morphological diversity of the African late Middle Pleistocene hominin fossil record (Frieß, 2003; Stringer, 2006; Gunz et al., 2009) is brought into greater focus. This transitional phase lasts until the first well-dated and relatively complete modern *H. sapiens* in Africa, represented by Herto (~150–161 ka) and Omo Kibish (~98–192 ka) (Millard, 2008) (Fig. 2). In this context, the alternative use of the name *H. helmei* (Stringer, 1996; McBrearty and Brooks, 2000), or that of *H. rhodesiensis* (Hublin, 2009), to refer to the pre-modern phase of *H. sapiens* in Africa, no longer seems appropriate. This is due to the possibility of multiple lineages of hominins co-existing within Africa prior to the Late Pleistocene (Stringer, 2002; Tattersall, 2009). Yet another possibility, previously proposed by one of us (Stringer, 2002), is to utilise the term archaic *H. sapiens* for the African fossils of this period, although it is recognised that this rather unsatisfactory label has also been used in many different ways in palaeoanthropology.

The marked heterogeneity of African hominins in the late Middle Pleistocene contrasts with the early appearance of derived Neanderthal characteristics in European fossils of this period (Hublin, 2009; Harvati et al., in press). The apparent asymmetry in patterns of morphological evolution between the two species is all the more striking because the times to genetic coalescence *within* each species estimated in our analyses are quite similar; ~113–117 ka (86–149 ka) for *H. neanderthalensis*, and ~134–143 ka (99–182 ka) for *H. sapiens* (Table S1). These relatively recent dates suggest substantial genetic drift occurred in both species, perhaps during the glaciation of MIS 6 (~130–191 ka). A better understanding of what these intraspecific mtDNA coalescence times represent, together with the detection of previous evolutionary bottlenecks, will only be achieved through the analysis of high quality, multilocus, autosomal data sets (Heled and Drummond, 2008, 2010; Nielsen and Beaumont, 2009). The production of this type of data from the majority of Neanderthal fossils remains a significant technical challenge (Green et al., 2009; Brotherton et al., 2010).

Conclusion

Our genetic estimates for the MRCA of *H. neanderthalensis* and *H. sapiens* support the concept of a widely-dispersed ancestral species during the middle part of the Middle Pleistocene, which split into at least two descendent populations prior to the late Middle Pleistocene, perhaps driven by the global climatic severity of MIS 12, ~480–425 ka (Hublin, 1998, 2009). Whether the fossils currently placed in the *H. heidelbergensis* hypodigm actually represent members of this common ancestral population cannot be determined from the analyses presented here, but those fossils are much more likely candidates, on both morphological and chronological grounds, than more ancient specimens in Europe, or younger ones in Africa.

The impending publication of the complete nuclear genome sequence of Neanderthals will allow further insights into the evolution and relationship of these two species, but the current evidence from mtDNA is consistent with the palaeoanthropological interpretation that later Middle Pleistocene lineages north and south of the Mediterranean gave rise to Neanderthals and modern humans, respectively (Stringer, 2002; Harvati et al., in press). Our results, however, cannot discount the possibility of some level of subsequent gene flow between these distinct human lineages. Differentiation within populations may have been primarily the result of accretional drift (Weaver et al., 2007), selection-driven change or some combination of these and other processes (Weaver, 2009), but the signal from mtDNA is unequivocal in its support for a separation of the *H. neanderthalensis* and *H. sapiens* lineages commencing during the mid-Middle Pleistocene.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.jhevol.2010.04.005.

References

Andrews, P., Fernandez-Jalvo, Y., 1997. Surface modifications of the Sima de los Huesos fossil humans. J. Hum. Evol. 33, 191–217.
Andrews, R.M., Kubacka, I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M., Howell, N., 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat. Genet. 23, 147.
Arsuaga, J.L., Martínez, I., Gracia, A., Lorenzo, C., 1997. The Sima de los Huesos crania (Sierra de Atapuerca, Spain). J. Hum. Evol. 33, 219–281.
Arsuaga, J.L., Martínez, I., Lorenzo, C., Gracia, A., 1999. The human cranial remains from Gran Dolina Lower Pleistocene site (Sierra de Atapuerca, Spain). J. Hum. Evol. 37, 431–457.
Atkinson, Q.D., Gray, R.D., Drummond, A.J., 2008. mtDNA variation predicts population size in humans and reveals a major Southern Asian chapter in human prehistory. Mol. Biol. Evol. 25, 468–474.
Beerli, P., Edwards, S.V., 2002. When did Neanderthals and modern humans diverge? Evol. Anthropol. 1, 60–63.
Benton, M.J., Donoghue, P.C., 2007. Palaeontological evidence to date the tree of life. Mol. Biol. Evol. 24, 26–53.
Bermúdez de Castro, J.M., Arsuaga, J.L., Carbonell, E., Rosas, A., Martínez, I., Mosquera, M., 1997. A Hominid from the Lower Pleistocene of Atapuerca, Spain: possible ancestor to Neandertals and modern humans. Science 276, 1392–1396.
Bermúdez de Castro, J.M., Pérez-González, A., Martinón-Torres, M., Gómez-Robles, A., Rosell, J., Prado, L., Sarmiento, S., Carbonell, E., 2008. A new early Pleistocene hominin mandible from Atapuerca-TD6, Spain. J. Hum. Evol. 55, 729–735.
Bischoff, J.L., Shamp, D.D., Aramburu, A., Arsuaga, J.L., Carbonell, E., Bermúdez de Castro, J.M., 2003. The Sima de los Huesos hominids date to beyond U/Th equilibrium (>350 kyr) and perhaps to 400–500 kyr: new radiometric dates. J. Archaeol. Sci. 30, 275–280.
Bischoff, J.L., Williams, R.W., Rosenbauer, R.J., Aramburu, A., Arsuaga, J.L., Garcia, N., Cuenca-Bescos, G., 2007. High-resolution U-series dates from the Sima de los Huesos hominids yields 600+/+∞−66 kyr: implications for the evolution of the early Neanderthal lineage. J. Archaeol. Sci. 34, 763–770.
Bräuer, G., 2008. The origin of modern anatomy: by speciation or intraspecific evolution? Evol. Anthropol. 17, 22–37.
Briggs, A.W., Good, J.M., Green, R.E., Krause, J., Maricic, T., Stenzel, U., Lalueza-Fox, C., Rudan, P., Brajković, D., Kućan, Ž., Gušic, I., Schmitz, R., Doronichev, V.B., Golonvanova, L.V., de la Rasilla, M., Fortea, J., Rosas, A., Pääbo, S., 2009. Targeted retrieval and analysis of five Neandertal mtDNA genomes. Science 325, 318–321.
Brotherton, P., Sanchez, J., Cooper, A., Endicott, P., 2010. Preferential high-resolution amplification of damaged and degraded endogenous DNA from ancient hominin samples. Nucleic Acids Res. 38 (2), e7.
Carbonell, E., Bermúdez de Castro, J.M., Parés, J.M., Pérez-González, A., Cuenca-Bescós, G., Ollé, A., Mosquera, M., Huguet, R., van der Made, J., Rosas, A., Sala, R., Vallverdú, J., García, N., Granger, D.E., Martinón-Torres, M., Rodríguez, X.P., Stock, G.M., Vergès, J.M., Allué, E., Burjachs, F., Cáceres, I., Canals, A., Benito, A., Díez, C., Lozano, M., Mateos, A., Navazo, M., Rodríguez, J., Rosell, J., Arsuaga, J.L., 2008. The first Hominin of Europe. Nature 452, 465–469.
Coop, G., Bullaughey, K., Luca, F., Przeworski, M., 2008. The timing of selection at the human FOXP2 gene. Mol. Biol. Evol. 25, 1257–1259.
Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88.
Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Duret, L., 2002. Evolution of synonymous codon usage in metazoans. Curr. Opin. Genet. Dev. 12, 640–649.
Endicott, P., Ho, S.Y.W., 2008. A Bayesian evaluation of human mitochondrial substitution rates. Am. J. Hum. Genet. 82, 1–8.
Endicott, P., Ho, S.Y.W., Metspalu, M., Stringer, C.B., 2009. Evaluating the mitochondrial timescale of human evolution. Trends Ecol. Evol. 24, 515–521.
Elson, J.L., Turnbull, D.M., Howell, N., 2004. Comparative genomics and the evolution of human mitochondrial DNA: assessing the effects of selection. Am. J. Hum. Genet. 74, 229–238.
Eswaran, V., Harpending, H., Rogers, A.R., 2005. Genomics refutes an exclusively African origin of humans. J. Hum. Evol. 49, 1–18.
Fenner, J.N., 2005. Cross-cultural estimation of the human generation interval for use in genetics-based population divergence studies. Am. J. Phys. Anthropol. 128, 415–423.
Fernandez-Jalvo, Y., Andrews, P., 2003. Experimental effects of water abrasion on bone fragments. J. Taphon. 1, 147–163.
Foley, R.B., Lahr, M.M., 1997. Mode 3 technologies and the evolution of modern humans. Cambridge Archaeol. J. 7, 3–36.
Forhan, G., Martiel, J-L., Blum, M.G.B., 2008. A deterministic model of admixture and genetic introgression: The case of Neanderthal and Cro-Magnon. Mathematical Biosciences 216, 71–76.
Frieß, M., 2003. An application of the relative warp analysis to problems in human palaeontology – with notes on raw data quality. Image Anal. Sterol. 22, 63–72.
Green, H.S. (Ed.), 1984. Pontnewydd Cave, a Lower Palaeolithic Hominid Site in Wales: First Report. National Museum of Wales, Cardiff.
Green, R.E., Krause, J., Ptak, S.E., Briggs, A.W., Ronan, M.T., Simons, J.F., Du, L., Egholm, M., Rothberg, J.M., Paunovic, M., Pääbo, S., 2006. Analysis of one million base pairs of Neanderthal DNA. Nature 444, 330–336.
Green, R.E., Malaspinas, A.-S., Krause, J., Briggs, A.W., Johnson, P.L.F., Uhler, C., Meyer, M., Good, J.M., Maricic, T., Stenzel, U., Prüfer, K., Siebauer, M., Burbano, H. A., Ronan, M., Rothberg, J.M., Egholm, M., Rudan, P., Brajković, D., Kućan, Ž., Gušić, I., Wikström, M., Laakkonen, L., Kelso, J., Slatkin, M., Pääbo, S., 2008. A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing. Cell 134, 416–426.
Green, R.E., Briggs, A.W., Krause, J., Prüfer, K., Burbano, H.A., Siebauer, M., Lachmann, M., Pääbo, S., 2009. The Neandertal genome and ancient DNA authenticity. EMBO 2009, 1–9.
Gunz, P., Bookstein, F., Mitteroecker, P., Stadlmayr, A., Seidler, H., Weber, G.W., 2009. Early modern human diversity suggests subdivided population structure and a complex out-of-Africa scenario. Proc. Natl. Acad. Sci. U.S.A. 106, 6094–6098.
Harvati, K., Frost, S.P., McNulty, K.P., 2004. Neanderthal taxonomy reconsidered: implications of 3D primate models of intra- and inter-specific differences. Proc. Natl. Acad. Sci. U.S.A. 101, 1147–1152.
Harvati, K., Panagopoulou, E., Runnels, C., 2009. The Palaeoanthropology of Greece. Evol. Anthropol. 18, 131–143.
Harvati, K., Hublin, J.-J., Gunz, P. Evolution of Middle-Late Pleistocene human cranio-facial form: A 3-D approach. J. Hum. Evol., in press.
Heled, J., Drummond, A.J., 2008. Bayesian inference of population size history from multiple loci. BMC Evol. Biol. 8, 289.
Heled, J., Drummond, A.J., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27, 570–580.
Hey, J., 2005. On the number of New World founders: a population genetic portrait of the peopling of the Americas. PLoS Biol. 3, e193.
Ho, S.Y.W., Phillips, M.J., 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. Syst. Biol. 58, 367–380.
Ho, S.Y.W., Gilbert, M.T.P., 2010. Ancient mitogenomics. Mitochondrion 10, 1–11.
Ho, S.Y.W., Kolokotronis, S.O., Allaby, R.G., 2007. Elevated substitution rates estimated from ancient DNA sequences. Biol. Lett. 3, 702–705.
Ho, S.Y.W., Larson, G., Edwards, C.J., Heupink, T.H., Lakin, K.E., Holland, P.W.H., Shapiro, B., 2008. Correlating Bayesian date estimates with climatic events and domestication using a bovine case study. Biol. Lett. 4, 370–374.
Howell, F.C., 1994. A chronostratigraphic and taxonomic framework of the origins of modern humans. In: Nitecki, M.H., Nitecki, D.V. (Eds.), Origins of Anatomically Modern Humans. Plenum Press, New York, pp. 253–319.
Hublin, J.J., 1998. Climatic changes, paleogeography, and the evolution of the Neandertals. In: Akazawa, T., Aoki, K., Bar-Yosef, O. (Eds.), Neandertals and Modern Humans in Western Asia. Plenum Press, New York, pp. 295–310.
Hublin, J.J., 2009. The origin of Neandertals. Proc. Natl. Acad. Sci. U.S.A. 106, 16022–16027.

Ingman, M., Kaessmann, H., Pääbo, S., Gyllensten, U., 2000. Mitochondrial genome variation and the origin of modern humans. Nature 408, 708–713.
Kanaya, S., Yamada, Y., Kinouchi, M., Yoshihiro, K., Ikemura, T., 2001. Codon usage and tRNA genes in Eukaryotes: correlation of codon usage diversity and translation efficiency and with CG-dinucleotide usage as assessed by multivariate analysis. J. Mol. Evol. 53, 290–298.
Kivisild, T., Shen, P., Wall, D.P., Do, B., Sung, R., Davis, K., Passarino, G., Underhill, P.A., Scharfe, C., Torroni, A., Scozzari, R., Modiano, D., Coppa, A., de Knijff, P., Feldman, M., Cavalli-Sforza, L.L., Oefner, P.J., 2006. The role of selection in the evolution of human mitochondrial genomes. Genetics 172, 373–387.
Klein, R.G., 2009. The Human Career. University of Chicago Press, Chicago.
Korsten, M., Ho, S.Y.W., Davison, J., Pähn, B., Vulle, E., Roht, M., Tumanov, I.L., Kojola, I., Andersone-Lilly, Z., Ozolins, J., Pilot, M., Mertzanis, Y., Giannakopoulos, A., Vorobiev, A.A., Markov, N.I., Saveljev, A.P., Lyapunova, E.A., Abramov, A.V., Männil, P., Valdmann, H., Pazetnov, S.V., Pazetnov, V.S., Rõkov, A. M., Saarma, U., 2009. Sudden expansion of a single brown bear maternal lineage across northern continental Eurasia after the last ice age: a general demographic model for mammals? Mol. Ecol. 18, 1963–1979.
Krings, M., Stone, A., Schmitz, R.W., Krainitzki, H., Stoneking, M., Pääbo, S., 1997. Neandertal DNA sequences and the origin of modern humans. Cell 90, 19–30.
Krings, M., Geisert, H., Schmitz, R.W., Krainitzki, H., Pääbo, S., 1999. DNA sequence of the mitochondrial hypervariable region II from the Neandertal type specimen. Proc. Natl. Acad. Sci. U.S.A. 96, 5581–5585.
Kumar, S., Filipski, A., Swarna, V., Walker, A., Hedges, S.B., 2005. Placing confidence limits on the molecular age of the human–chimpanzee divergence. Proc. Natl. Acad. Sci. U.S.A. 102, 18842–18847.
Lahr, M.M., Foley, R.A., 2001. Mode 3, *Homo helmei*, and the pattern of human evolution in the Middle Pleistocene. In: Barham, L., Robson-Brown, K. (Eds.), Human Roots: Africa and Asia in the Middle Pleistocene. Western Academic & Specialist Press, Bristol, pp. 23–29.
Lalueza-Fox, C., Sampietro, M.L., Caramelli, D., Puder, Y., Lari, M., Calafell, F., Marínez,-Maza, C., Bastir, M., Fortea, J., de la Rasilla, M., Bertranpetit, J., Rosas, A., 2005. Neandertal evolutionary genetics: mitochondrial DNA from the Iberian Peninsula. Mol. Biol. Evol. 22, 1077–1081.
Liu, H., Prugnolle, F., Manica, A., Balloux, F., 2006. A geographically explicit genetic model of worldwide human-settlement history. Am. J. Hum. Genet. 79, 230–237.
Lumley, H.de., Fournier, A., Park, Y.C., Yokoyama, Y., Demouy, A., 1984. L'industrie du Pleistocène inférieur de la grotte du Vallonet Roquebrune-Cap-Martin, Alpes-Maritime. L'Anthropologie 92, 501–614.
Martinón-Torres, M., Bermúdez de Castro, J.M., Gómez-Robles, A., Arsuaga, J.L., Carbonell, E., Lordkipanidze, D., Manzi, G., Margvelashvili, A., 2007. Dental evidence on the hominin dispersals during the Pleistocene. Proc. Natl. Acad. Sci. U.S.A. 104, 13279–13282.
McBrearty, S., Brooks, A.S., 2000. The revolution that wasn't: a new interpretation of the origin of modern human behaviour. J. Hum. Evol. 39, 453–563.
Meyer, S., Weiss, G., von Haeseler, A., 1999. Pattern of nucleotide substitution and rate heterogeneity in the hypervariable regions I and II of human mtDNA. Genetics 152, 1103–1110.
Millard, A.R., 2008. A critique of the chronometric evidence for hominid fossils: I. Africa and the Near East 500–50 ka. J. Hum. Evol. 54, 848–874.
Muttoni, G., Scardia, G., Kent, D.V., Swisher, C.C., Manzi, G., 2009. Pleistocene magnetochronology of early hominin sites at Ceprano and Fontana Ranuccio, Italy. Earth Planet. Sci. Lett. 286, 255–268.
Mounier, A., Marchal, F., Condemi, S., 2009. Is *Homo heidelbergensis* a distinct species? New insight on the Mauer mandible. J. Hum. Evol. 56, 219–246.
Nielsen, R., Beaumont, M.A., 2009. Statistical inferences in phylogeography. Mol. Ecol. 18, 1034–1047.
Noonan, J., Coop, G., Kudaravalli, S., Smith, D., Krause, J., Alessi, J., Chen, F., Platt, D., Pääbo, S., Pritchard, J., Rubin, E., 2006. Sequencing and analysis of Neandertal genomic DNA. Science 314, 1113–1118.
Ovchinnikov, I.V., Götherström, A., Romanova, G.P., Kharitonov, V.M., Lidén, K., Goodwin, W., 2002. Molecular analysis of Neandertal DNA from the northern Caucasus. Nature 404, 490–493.
Plagnol, V., Wall, J.D., 2006. Possible ancestral structure in human populations. PLoS Genet. 2, e105.
Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. University of Oxford.
Rightmire, G.P., 2008. *Homo* in the Middle Pleistocene: hypodigms, variation and species recognition. Evol. Anthropol. 17, 8–21.
Rosas, A., 2001. Occurrence of Neanderthal features in mandibles from the Atapuerca-SH site. Am. J. Phys. Anthropol. 114, 74–91.
Schmitz, R.W., Serre, D., Bonani, G., Feine, S., Hillgruber, F., Krainitzki, H., Pääbo, S., 2002. The Neandertal type site revisited: interdisciplinary investigations of skeletal remains from the Neander Valley, Germany. Proc. Natl. Acad. Sci. U.S.A. 99, 13342–13347.
Serre, D., Langaney, A., Chech, M., Teschler-Nicola, M., Paunovic, M., Mennecier, P., Hofreiter, M., Possnert, G., Pääbo, S., 2004. No evidence of Neandertal mtDNA contribution to Early Modern Humans. PLoS Biol. 2, e257.
Simpson, G.G., 1950. Some principles of historical biology bearing on human origins. Cold Spring Harbour Symp. Quant. Biol. 15, 55–66.
Skinner, A.R., Blackwell, B.A.B., Martin, S., Ortega, A., Blickstein, J.I.B., Goloovanova, L.V., 2005. ESR dating at Mezmaiskaya cave Russia. Appl. Radiat. Isot. 62, 219–224.
Soares, P., Ermini, L., Thomson, N., Mormina, M., Rito, T., Rohl, A., Salas, A., Oppenheimer, S., Macaulay, V., Richards, M.B., 2009. Correcting for purifying selection: an improved human mitochondrial molecular clock. Am. J. Hum. Genet. 84, 1–20.
Soodyall, H., Jenkins, T., Mukherjee, A., Du Toit, E., Roberts, D.F., Stoneking, M., 1997. The founding mitochondrial DNA lineages of Tristan da Cunha. Am. J. Phys. Anthropol. 104, 157–166.
Street, M., Terberger, T., Orschiedt, J., 2006. A critical review of the German Paleolithic hominin record. J. Hum. Evol. 51, 551–579.
Stringer, C.B., 1983. Some further notes on the morphology and dating of the Petralona hominid. J. Hum. Evol. 12, 731–742.
Stringer, C.B., 1996. Current issues in modern human origins. In: Meikle, W.E., Howell, F.C., Jablonski, N. (Eds.), Contemporary Issues in Human Evolution. Mem. Calif. Acad. Sci., vol. 21, pp. 115–134.
Stringer, C.B., 2002. Modern human origins – progress and prospects. Phil. Trans. R. Soc. B 357, 563–579.
Stringer, C.B., 2006. The origins of modern humans 1984–2004. In: Soodyall, H. (Ed.), The Prehistory of Africa. Jonathan Ball, Johannesburg, pp. 10–20.
Stringer, C.B., Hublin, J.-J., 1999. New age estimates for the Swanscombe hominid, and their significance for human evolution. J. Hum. Evol. 37, 873–877.
Subramanian, S., Denver, D.R., Millar, C.D., Heupink, T., Aschrafi, A., Emslie, S.D., Baroni, C., Lambert, D.M., 2009. High mitogenomic evolutionary rates and time dependency. Trends Genet. 25, 482–486.
Takahata, N., Satta, Y., Klein, J., 1995. Divergence time and population size in the lineage leading to modern humans. Theor. Popul. Biol. 48, 198–221.
Tattersall, I., 2009. Human origins: out of Africa. Proc. Natl. Acad. Sci. U.S.A. 106, 16018–16021.
Tattersall, I., Schwartz, J.H., 2006. The distinctiveness and systematic context of *Homo neanderthalensis*. In: Harvati, K., Harrison, T. (Eds.), Neanderthals Revisited: New Approaches and Perspectives. Springer, Berlin, pp. 9–22.
Tenesa, A., Navarro, P., Hayes, B.J., Duffy, D.L., Clarke, G.M., Goddard, M.E., Visscher, P. M., 2007. Recent human effective population size estimated from linkage disequilibrium. Genome Res. 17, 520–526.
Trinkaus, E., 2005. Early modern humans. Annu. Rev. Anthropol. 34, 207–230.
Tryon, C.A., McBrearty, S., Texier, P.-J., 2005. Levallois lithic technology from the Kapthurin Formation, Kenya: Acheulian origin and Middle Stone Age diversity. Afr. Archaeol. Rev. 22, 199–229.
Voight, B.F., Adams, A.M., Frisse, L.A., Qian, Y., Hudson, R.R., Di Rienzo, A., 2005. Interrogating multiple aspects of variation in a full resequencing data set to infer human population size changes. Proc. Natl. Acad. Sci. U.S.A. 102, 18508–18513.
Wall, J.D., Kim, S.K., 2007. Inconsistencies in Neandertal genomic DNA sequences. PLoS Genet. 3, e175.
Wall, J.D., Lohmueller, K.E., Plagnol, V., 2009. Detecting ancient admixture and estimating demographic parameters in multiple human populations. Mol. Biol. Evol. 26, 1823–1827.
Weaver, T.D., Roseman, C.C., Stringer, C.B., 2007. Were Neandertal and modern human cranial differences produced by natural selection or genetic drift? J. Hum. Evol. 53, 135–145.
Weaver, T.D., Roseman, C.C., Stringer, C.B., 2008. Close correspondence between quantitative-and molecular-genetic divergence times for Neanderthals and modern humans. Proc. Natl. Acad. Sci. U.S.A. 105, 4645–4649.
Weaver, T.D., 2009. The meaning of Neandertal skeletal morphology. Proc. Natl. Acad. Sci. U.S.A. 106, 16028–16033.
White, M., Ashton, N., 2003. Palaeolithic core technology and the origins of the Levallois method in northwestern Europe. Curr. Anthropol. 44, 598–609.
Wolpoff, M., 2009. How Neandertals inform human variation. Am. J. Phys. Anthropol. 139, 91–102.
Wood, B., Lonergran, N., 2008. The hominin fossil record: taxa, grades and clades. J. Anat. 212, 354–376.
Yang, Z., Nielsen, R., 2008. Mutation-selection models of codon substitution and their use to estimate selective strengths on codon usage. Mol. Biol. Evol. 25, 568–579.