

Number of ancestral human species: a molecular perspective

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Summary

Despite the remarkable developments in molecular biology over the past three decades, anthropological genetics has had only limited impact on systematics in human evolution. Genetics offers the opportunity to objectively test taxonomies based on morphology and may be used to supplement conventional approaches to hominid systematics. Our analyses, examining chromosomes and 46 estimates of genetic distance, indicate there may have been only around 4 species on the direct line to modern humans and 5 species in total. This contrasts with current taxonomies recognising up to 23 species.

The genetic proximity of humans and chimpanzees has been used to suggest these species are congeneric. Our analysis of genetic distances between them is consistent with this proposal. It is time that chimpanzees, living humans and all fossil humans be classified in *Homo*. The creation of new genera can no longer be a solution to the complexities of fossil morphologies. Published genetic distances between common chimpanzees and bonobos, along with evidence for interbreeding, suggest they should be assigned to a single species.

The short distance between humans and chimpanzees also places a strict limit on the number of possible evolutionary 'side branches' that might be recognised on the human lineage. All fossil taxa were genetically very close to each other and likely to have been below congeneric genetic distances seen for many mammals.

Our estimates of genetic divergence suggest that periods of around 2 million years are required to produce sufficient genetic distance to represent speciation. Therefore, Neanderthals and so-called *H. erectus* were genetically so close to contemporary *H. sapiens* they were unlikely to have been separate species. Thus, it is likely there was only one species of human (*H. sapiens*) for most of the last 2 million years. We estimate the divergence time of *H. sapiens* from 16 genetic distances to be around 1.7 Ma which is consistent with evidence for the earliest migration out of Africa. These findings call into question the mitochondrial 'African Eve' hypothesis based on a far more recent origin for *H. sapiens* and show that humans did not go through a bottleneck in their recent evolutionary history.

Given the large offset in evolutionary rates of molecules and morphology seen in human evolution, *Homo* species are likely to be characterised by high levels of morphological variation and low levels of genetic variability. Thus, molecular data suggest the limits for intraspecific morphological variation used by many palaeoanthropologists have been set too low. The role of phenotypic plasticity has been greatly underestimated in human evolution.

We call into question the use of mtDNA for studies of human evolution. This DNA is under strong selection, which violates the assumption of selective neutrality. This issue should be addressed by geneticists, including a reassessment of its use for molecular clocks. There is a need for greater cooperation between palaeoanthropologists and anthropological geneticists to better understand human evolution and to bring palaeoanthropology into the mainstream of evolutionary biology.

Introduction

For the past 150 years the systematics of hominid palaeobiology has been based almost exclusively on fossil morphologies. Prior to the 1970s virtually every new

fossil group was assigned a unique taxonomic name (eg, Weidenreich 1946, Broom 1950, Leakey 1963). This practice resulted from a misunderstanding of the purpose of biological classification (Campbell 1969) and in some instances the desire of workers for public recognition and to capture funding for their research. This practice resulted in about 60 formally valid taxa (summarised by Henneberg 1997 – with others in the past five years, see below), although about half are not in current use.

While palaeoanthropology has progressed a great deal since the 1960s, much of the research literature continues to focus on fossil taxonomies. The past decade has seen the description of three new genera and six new species (Leakey et al 1995, 2001, White et al 1995, Bermúdez de Castro et al 1997, Hailie-Selassie 2001, Pickford & Senut 2001), use by some workers of the genera *Paranthropus* and *Praeanthropus* (Groves 1989, Strait et al 1997), calls for the classification of *Homo habilis* in a new genus (Wood & Collard 1999), and acceptance and use by some workers of *H. ergaster* (Wood 1992) and *H. neanderthalensis* (Tattersall 1992). The result is that the present total number of species (on and off the line to living humans) ranges from around 5 to 23 (Tobias 1981a, Groves 1989, Foley 1991, Wood 1994, Leakey et al 1995, 2001, White et al 1995, Johanson & Edgar 1996,

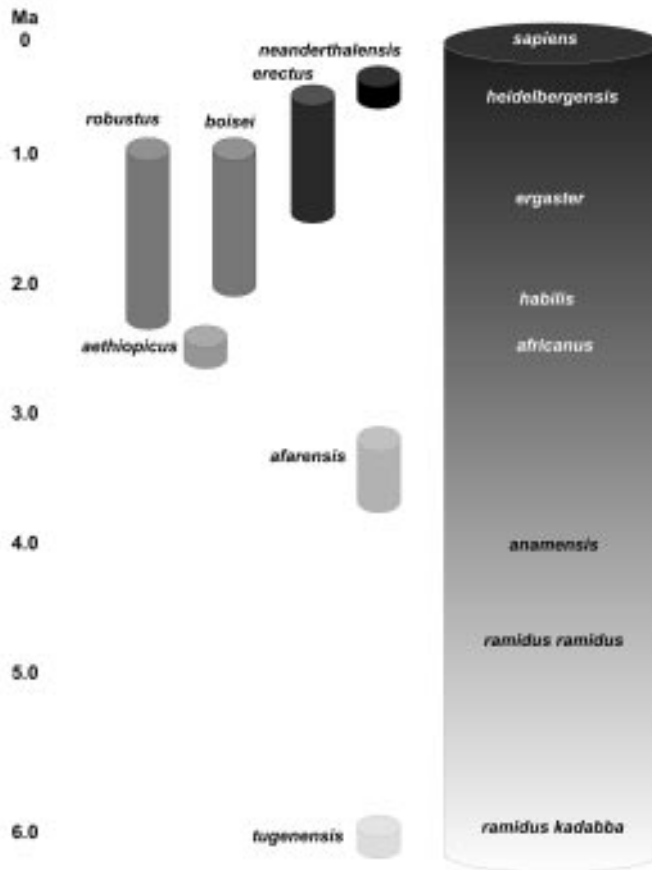


Fig. 1: Current «median» estimate of fossil human species. The column at right indicates species allegedly on the line to living people.

Bermúdez de Castro et al 1997, Asfaw et al 1999, Hailie-Selassie 2001, Pickford & Senut 2001). This bewildering diversity of taxonomies results from the use of different species concepts and the application of a variety of species recognition methods. The wide disagreement has not allowed for a consensus and we determine the median number of taxa in use today to be 14. These species, including seven possibly on the *direct line to modern humans* (DLMH), are shown in figure 1.

Over the last 30 years molecular biology has played an increasingly important role in anthropology. Major advances include the application of the genetic clock (eg, Cann et al 1987, Vigilant et al 1991, Wills 1994a,b cited by Tobias 1995, Horai et al 1995, Stoneking 1997), recovery of ancient DNA (Krings et al 1997, 2000, Ovchinnikov et al 2000, Adcock et al 2001a), decoding of the human nuclear genome sequence (International Human Genome Sequencing Consortium, 2001), and sequencing of the complete mitochondrial DNA (mtDNA) of humans and great apes (Horai et al 1995, Arnason et al 1996, Xu & Arnason, 1996). Genetics has been responsible for a major reanalysis of the timing of the earliest human ancestor (Sarich & Wilson 1967) and the removal of the fossil taxon *Ramapithecus* from the human lineage. It has also played a major role in discussions about the emergence of modern humans with important contributions examining nuclear DNA (eg, Nei & Livshits 1989, Cavalli-Sforza et al 1994, Takahata et al 2001, Yu et al 2001), mtDNA (eg, Cann et al 1987, Vigilant et al 1989, Templeton 1993, Stoneking 1997, Adachi & Hasegawa 1995, Horai et al 1995, Maca-Meyer et al 2001) and the Y-chromosome (eg, Lucotte 1989, Spurdle 1992, Semino et al 1996, Poloni et al 1997).

A most important contribution of genetics to human evolutionary studies has been the demonstration of the closeness of humans and chimpanzees. These species share between 98.3% and 99.5% of their DNA (King & Wilson 1975, Goodman et al 1989, 1990, 2001). Specific genetic differences between humans and chimpanzees have also been reported, but apparently only one, the CMP-sialic hydroxylase mutation in humans, results in global biochemical and structural differences between species (Gagneux & Varki 2001).

Traditional methods of taxonomy using morphological features suggest humans and chimpanzees are much more distant than genetic studies (eg, Simpson 1945, Napier & Napier 1985, Conroy 1990). Most palaeoanthropologists classify humans in a separate family or sub-family from the pongids (Andrews & Cronin 1982, Groves 1989). In contrast, genetics indicates that humans and chimpanzees are very close (King & Wilson 1975, Bruce & Ayala 1979, Goodman et al 1989, 1990, Castresana 2001) and might best be classified (with gorillas?) in *Homo* (Castresana 2001, Goodman et al 2001, Watson et al 2001).

The contrast between morphological and genetic taxonomies is stark among some organisms and has led to the observation by geneticists that macromolecules and morphological features can evolve at independent rates. It applies also to humans and chimpanzees (eg, King & Wilson 1975). The importance of this general observation has been recognised and discussed by geneticists and zoologists for several decades (Nei 1978, Bruce & Ayala 1979, Fitch 1982, Collier & O'Brien 1985, Gould 1985, Ridley 1996) but has been almost completely ignored by palaeoanthropologists (but see Eckhardt 1976, 2000, Gallup et al 1977). Similarly, research has also shown that molecules and anatomical structures may evolve at

different rates in different organisms, even within the same family. It has been shown that primates are generally characterised by little molecular but much morphological evolution (Fitch 1982), but within the Felidae, for example, major differences between morphology and genetics have been described between genera, but felid genera are karyotypically stable and morphologically relatively uniform (Collier & O'Brien 1985).

Collard & Wood (2000) have compared phylogenetic trees of craniodental and molecular data for humans, the great apes and a species of Old World monkey. They found the two sets of data produced conflicting cladograms and concluded that «little confidence can be placed in phylogenies generated solely from higher primate craniodental evidence» (2000: 5003). This finding largely reconfirms earlier studies of the decoupling of morphological and molecular evolution. Nei & Kumar (2000) have concluded «the evolutionary change of morphological and physiological characters is so complex that this approach does not produce a clear-cut picture of evolutionary history, and the details of the phylogenetic trees reconstructed have almost always been controversial» (p 3). Collard & Wood neither recognise these facts nor advocate further use of molecular data when answering the question «how can the reliability of human phylogenetic hypotheses be improved?» (2000: 5005).

Molecular characters have advantages over morphological. They offer greater detail in some areas and are potentially more comprehensive as the human genome comprises 3.2 billion base pairs. Molecular methods also provide data that are independent of morphological characters, and although they are subject to the problems of analogy, they are not affected in the same manner (Mayr & Ashlock 1991). DNA is sufficiently complex that analogy may be precluded as a cause of similarity, and sufficiently independent that no simple trigger can cause a large set of apparently intricate similarities (Gould 1985). However, some DNA segments are known to replicate themselves more efficiently than others, resulting in serial homology within the genome (Marks 1992). This provides some limitations at present for phylogenetic interpretation using the two most common laboratory techniques, DNA hybridisation and DNA sequencing.

Rather than determining relationship by the presence or absence of characters, molecular methods permit the determination of distances between taxa (Mayr & Ashlock 1991). The fact that amino acid sequences are subject to constant change is an important feature of molecular data for use in taxonomy. The rate of amino acid substitution has been found to be constant and is known to follow a Poisson distribution (Zuckermandl & Pauling 1962, 1965, Margoliash & Smith 1965, Dayoff 1969). This means that «compared with morphological characters, macromolecules such as protein and DNA show a very simple pattern of evolutionary change, and the amount of change in amino acid sequences of proteins or nucleotide sequences of DNA is roughly proportional to the evolutionary time» (Nei 1978: 343). However, it has been described as «sloppy» clock and its accuracy a matter of resolution (Fitch 1976, 1982, Setoguchi 1995). The clock for protein substitution follows sidereal time, the case with geological dating methods, which means it is a true measure of evolutionary time. However, DNA clocks are affected by generation time and corrections need to be made when comparing organisms with very different life histories (Ridley 1996) (also, see below).

Genetics provides a quantitative and objective approach to taxonomy. It also offers invaluable insights into phylogenetic relationships (Nei & Kumar 2000) and evolutionary histories (Deinard & Kidd 1995). In contrast, it has been demonstrated repeatedly in biology that there is no direct relationship between speciation and adaptation, or a discernible morphological shift, as «speciation emphatically is not simply a passive consequence of accumulated morphological change» (Tattersall 1994: 2). Therefore, morphological differences between groups can be a poor test of reproductive isolation, and thus potentially of limited value for delimiting species.

Marks (1992) has argued that molecular methods and data should not be seen as superseding conventional methods of systematics and «molecular data should be taken as testing hypotheses about phylogeny» (p 250). Here we use molecular data to examine what has hitherto been a morphological problem in human evolution, the broad question of taxonomy. In particular, we consider the use of a variety of genetic data and approaches to address the problem of the number of species, to illustrate current problems with taxonomy in palaeoanthropology and to discuss future areas for critical research.

Materials and methods

Chromosomes

Chromosomal differences may have been, according to some researchers, the most important cause of the development of major differences between humans and chimpanzees (King & Wilson 1975). Chromosomal reconstruction (rearrangement) is likely to have been a powerful force in speciation, given that virtually all of the genetic material in eukaryotes is located on chromosomes (Mayr 1982). Such changes may lead to quantum speciation when they occur as they reduce the fertility of individuals who are heterozygous for the rearrangement (Ayala 1982). Thus, the first stage of speciation may be accomplished through chromosome rearrangement without extensive allelic differentiation.

The frequency of chromosomal rearrangements between some species is known to be high, suggesting a general correspondence between the two (Mayr 1982). For example, Bush et al (1977) examined 225 vertebrate genera and found a correlation between the number of species and chromosomal diversity. They also concluded that taxa with higher speciation rates tended to have subdivided population structures. This observation held for mammals, especially primates and horses. However, chromosomal speciation is a complex phenomenon (Marks 1992) and is clearly not universal, even within specific orders of Mammalia. There are examples of species differences between members of karyotypically conservative mammals (eg, felids – Collier & O'Brien 1985), but among primates, subspecies of *Lemur fulvus* vary in their diploid numbers but fully fertile hybrids result from matings (Tattersall 1993). We explore this issue with respect to the human lineage below.

The chromosomal differences between humans and great apes are well known (Tijo & Levan 1956, Young et al 1960, Chiarelli 1962, Hamerton et al 1963, Yunis & Prakash 1982, De Grouchy, 1987, Verma & Luke 1994, McConkey, 1997). Humans possess 46 chromosomes while the great apes each have 48 (Tijo & Levan 1956, Young et al 1960, Chiarelli 1962, Hamerton et al 1963). Chromosomal phy-

logenies have been constructed with rearrangements in chromosomes 2, 7 and 9 supporting the sister status of humans and chimpanzees (Yunis & Prakash 1982). Humans are distinguishable from chimpanzees by the rearrangement of chromosomes 1, 9, 17, and 18, and the fusion between the acrocentrics forming human chromosome 2 (De Grouchy 1987). Similarly, common chimpanzees differ from humans by having seven major non-heterochromatic changes (Yunis & Prakash 1982) and bonobos differ from humans by nine rearrangements (De Grouchy 1987).

Nuclear DNA and mitochondrial DNA

The constant accumulation of mutations provides a measure of evolutionary divergence between paired groups of organisms and genetic distance measures may be used to estimate the degree of difference between pairs of populations (Nei 1978). A variety of different measures exist (for reviews see Kartlin et al 1979, Cavalli-Sforza et al 1994). However, many researchers have preferred Nei's (1972) genetic distance (D), which is «...intended to measure the number of codon substitutions per locus that have occurred after divergence of the two populations under consideration» (Nei 1978: 344). While evolution is clearly a complex process, a relationship has been found between genetic distance and reproductive isolation. That is, reproductive isolation increases with time (ie, genetic distance). The amount of genetic change corresponding to a given level of evolutionary divergence has been shown to follow a general pattern across a wide range of animals (Ayala & Kiger 1980). While there is considerable variation in distances between species, it seems that as a general observation DNA distances between mammals are approximately $D = 0.20$ (Ayala & Kiger 1980, Ayala 1982, Janczewski et al 1990, Johns & Advise 1998). Castresana (2001) has reviewed genetic distances for the cytochrome *b* gene in the mtDNA of 688 commonly recognised mammalian species, from 310 genera in 52 families. He found that modal classes of intrageneric and intergeneric distance distributions were well separated, being 0.10–0.15 and 0.25–0.30, respectively.

In this study speciation rates are calculated using %, D , D_A and mtREV distances derived from sequencing data. These data were taken from: nuclear DNA percentage distances (King & Wilson 1975, Goodman et al 1989, 1990, Starr & McMillan 2001, Takahata et al 2001, Yu et al 2001, Zhao et al 2000); other DNA genetic distances (Bruce & Ayala 1979, Nei & Livshits 1989, Janczewski et al 1990, Nei & Takezaki 1996); mtDNA % distances (Vigilant et al 1989, Xu & Arnason 1996) and distances calculated by the present authors from nucleotide differences in protein coding genes (Horai et al 1995), and other mtDNA distances (Cann et al 1987, Arnason et al 1996) (tables 1 and 2).

We estimated speciation rates for each distance measure separately, where possible, in order to exclude uncertainties deriving from different methods of calculation. Although genetic distances are highly correlated (Kartlin et al 1979), this seemed a sensible approach toward controlling error. Similarly, application of the same distance measure to the same paired organisms by different researchers may lead to differing estimates of distance, resulting from the study of different loci, varying sample sizes and laboratory techniques. We used distances from single

studies as much as possible to further reduce error, but for some data this was not possible (see table 1 footnotes). We preferred distances derived from large numbers of loci as sample size is of paramount importance in determining confidence in results (Cavalli-Sforza et al 1994).

Genetic distances may be used to provide an estimate of the number of species on the DLMH. We first took distances between humans and chimpanzees and

Table 1: Estimated speciation rate/number of species using living humans as a yardstick.

	Interspecific distance ¹⁴	Intraspecific distance	Speciation rate/species number
Chromosomal rearrangements	–	–	3–5
DNA distance			
Total ¹	0.5	0.2	3
Total ²	0.25	0.2	1
Non-coding ²	0.85	0.2	4
Electrophoresis ³	0.156–0.193	0.078	2
2DE «including missing» ⁴	0.043–0.044	0.078	<1
2DE «excluding missing» ⁴	0.034–0.035	0.078	<1
Isozymes ⁴	0.097–0.122	0.078	1–2
Microsatellite ⁵	0.357	0.518	<1
RFLP ⁵	0.232	0.097	2
Protein polymorphism ⁵	0.293	0.062	5
Alu insertion frequency ⁵	0.310	0.219	1
Chromosome 1 ⁶	0.31	0.076	4
Plp ⁷	0.39	0.100	4
Hprt ⁷	0.48	0.050	10
Gkc ⁷	0.33	0.026	13
Pdbal ⁷	0.48	0.100	5
Pdhaf ⁷	0.55	0.175	3
dys44 ⁷	0.45	0.120	4
Zfx ⁷	0.78	0.145	5
Xq13.3 ⁷	0.45	0.050	9
Lpl ⁷	0.75	0.135	6
b-globin ⁷	0.65	0.175	4
Mclr ⁷	0.92	0.130	7
Chromosome 22 ⁸	0.67	0.108	6
Mean ⁹			4
mtDNA distance			
% Synonymous changes ¹⁰	4.1–4.2	0.4	10–11
% Non-synonymous changes ¹⁰	0.8–0.9	0.2	4–5
% Sequence divergence ¹¹	21	2	11
Amino acid ¹²	0.021–0.020	0.05	<1
% Nucleotide ¹³	4.3	2	2
% Amino acid ¹³	2.2	2	1
Mean ⁹			5
Mean (all data) ⁹			4

¹Data of King & Wilson (1975), Starr & McMillan (2001); ²data of Goodman et al (1989, 1990), Starr & McMillan (2001); ³data of Bruce & Ayala (1979), Nei & Livshits (1989); ⁴data of Janczewski et al (1990), Nei & Livshits (1989); ⁵data of Nei & Takezaki (1996); ⁶data of Yu et al (2001); ⁷data of Takahata et al (2001); ⁸data of Zhao et al (2000); ⁹calculated with <1 counted as 1; ¹⁰data of Horai et al (1995); ¹¹data of Vigilant et al (1989); ¹²data of Arnason et al (1996), Cann et al (1987); ¹³Xu & Arnason (1996), Vigilant et al (1989); ¹⁴halved distance between humans and chimpanzees shown (ranges are bonobo and common chimpanzee).

halved them (see figure 2), providing an estimate of the distance since the last common ancestor (LCA) of both humans and chimpanzees (Saitou et al 2000, Takahata et al 2001). We then divided these halved distances by distances between human geographic populations (Africans and Asians) and Sumatran and Bornean orangutans. These distances provided intraspecific yardsticks. This approach allows for the mean speciation rate on the DLMH to be estimated. Some researchers have observed that orangutans exhibit very high levels of intraspecific genetic variation and propose this group be carved into multiple species (Xu & Arnason 1996, Warren et al 2001, see Muir et al 2000 for an alternative view). For us, the fact that geographic populations of orangutan regularly produce viable hybrid offspring establishes them as a single species.

We also divided the estimated distance since the LCA by the distance between common-chimpanzees and bonobos. While some workers regard them as separate species (Johanson 1976, Napier & Napier 1985, Groves 1989, Godefroit 1990, Shea et al 1993), the evidence for specific distinction seems to be finely balanced. They are genetically very similar (Deinard & Kidd 1995, Watson et al 2001), with some common chimpanzee subpopulations more distant from each other than to bonobos (Kaessmann et al 1999). They are also known to interbreed in captivity (Vervaecke & van Elsacker 1992; Curnoe et al in press). We prefer to consider distances between common chimpanzees and bonobos as intraspecific.

Table 2: Estimated speciation rate/number of species using Sumatran versus Bornean orangutan distances and bonobo versus common chimpanzee distances as yardsticks.

	Interspecific distance	Orangutan yardstick		Chimpanzee yardstick	
		Intraspecific distance	Speciation rate/species number	Intraspecific distance	Speciation rate/species number
DNA distance					
Electrophoresis ¹	0.156–0.193	0.130	1	0.103	2
2DE «including missing» ²	0.043–0.044	0.010	4	0.015	3
2DE «excluding missing» ²	0.034–0.035	0.013	3	0.014	2–3
Isozymes ²	0.097–0.122	0.019	5–6	0.072	1–2
Mean			3–4		2–3
mtDNA distance					
% Synonymous changes ³	4.1–4.2	–	–	3.4	1
% Non-synonymous changes ³	0.8–0.9	–	–	0.9	<1–1
Amino-acid distances ⁴	0.021–0.020	0.049	<1	0.021	<1–1
% Nucleotide ⁵	4.3	6.3	<1	3.7	1
% Amino-acid ⁵	2.2	4.7	<1	2.3	<1
Mean⁶			1		1
Mean (all data)⁶			2		1–2

¹Using data of Bruce & Ayala (1979); ²data of Janczewski et al (1990); ³calculated from data of Horai et al (1995); ⁴data of Arnason et al (1996); ⁵data of Xu & Arnason (1996); ⁶Mean calculated with <1 counted as 1.

Studies of intraspecific genomic diversity in humans and great apes have provided contradictory results, depending upon whether nuclear DNA or mtDNA have been examined and the loci studied. At the protein level, humans are more variable than chimpanzees, but the opposite is true for nuclear DNA and mtDNA (Morin et al 1994, Takahata 1995, Gagneux et al 1999, Kaessmann et al 1999, 2001). This means that different data will produce different speciation rates and perhaps also varying estimates of species. We note that intraspecific genetic differences depend not only on the rate of mutation but also on other factors like the rate of migration, level of environmental diversification, mating structure between subpopulations, and the absolute or relative sizes of subpopulations. Without direct knowledge of these events/processes it is not possible to estimate their impacts on intraspecific distances.

This paper represents the application of the «yardstick concept» using genetic data from a group of closely related species (eg, Hull 1993b). While traditionally the «yardstick» has used morphological distance to delimit species, genetic dis-

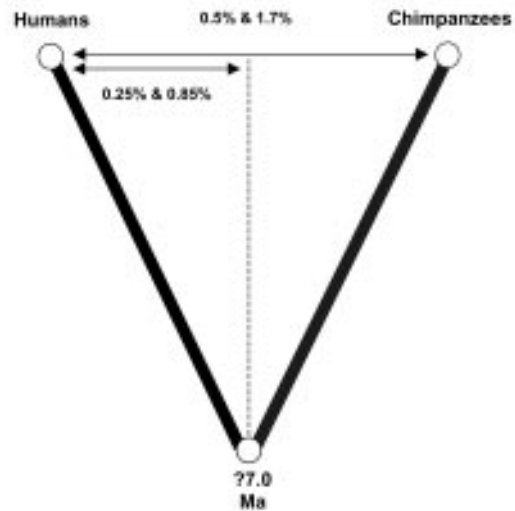


Fig. 2: Genetic differences between humans and chimpanzees, and between humans and the LCA.

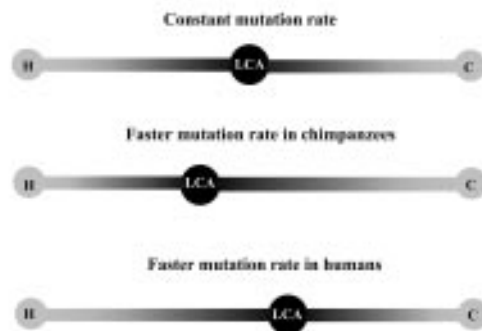


Fig. 3: Impact of different rates of evolution (substitution) between humans and chimpanzees based on genetic distances (Note: H = humans; C = chimpanzees; LCA = last common ancestor).

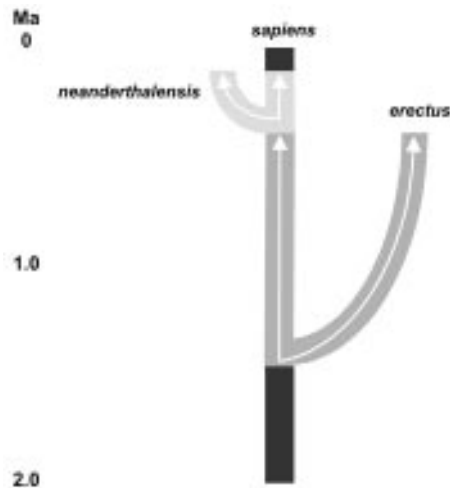


Fig. 4: Genetic differences and genetic distances between synchronous fossil groups were determined by doubling the period of isolation for paired groups, until the extinction of one of them, providing the total time of divergence.

tances are a more reliable indicator of reproductive isolation. The method used here assumes: (1) the genetic divergence between chimpanzees and humans was shared equally, (2) an identical substitution rate in both lineages, and (3) that throughout the DLMH there has been no significant processual variation.

There is of course no direct evidence for the first assumption as the halved distances are between two extant species. If distances between fossil taxa become available it may turn out that those between humans and the LCA may vary from those assumed here.

Regarding the second assumption, it has been suggested that the amino acid substitution rate varies between humans and chimpanzees (Bruce & Ayala 1979). As figure 3 demonstrates, this would have the effect of altering the distance between the LCA and living humans or chimpanzees, depending on which species has the higher substitution rate. We do not regard this to be a significant uncertainty given the closeness of humans and chimpanzees, and the fact that humans and great apes, as a group, share a relatively low substitution rate (Koop et al 1989, Bailey et al 1991, Porter 1997, Page & Goodman 2001).

We believe the third assumption is consistent with the principle of uniformitarianism. That is, there is no reason to believe that evolution acted in accordance with different processes during early or late periods of human evolution or of the chimpanzees. The possible exception to this might be the effect the development of culture has had on the evolution of the human brain (ie, positive feedback loop model – see Tobias 1981b, 1991, Henneberg 1992). Takahata (1995) has proposed a hypothesis of relaxed selective pressure to explain higher protein variability in humans and this seems to be consistent with a positive feedback loop model. Thus, rates of substitution may have altered in recent human evolution.

The approach used here is also consistent with that advocated by some geneticists to understanding the evolution of extant species. As Deinard & Kidd (1995) state: «...genetic data from extant species are the only remaining and available evidence of the ‹last common ancestor.› Data generated on related extant species may serve as the basis for inferences on those species' evolutionary histories» (p 150).

Pleistocene speciation rate

We may also use these data to estimate the possible genetic distance between paired (synchronous) fossil samples, especially when one sample is thought to be on the DLMH. Using the human versus chimpanzee differences described above, and by calculating the mean change in genetic distance per million years, we may estimate the distance between paired (synchronous) species (figure 4). We calculated the period of isolation by taking the time since the paired groups shared an ancestor until the extinction of one of them. This time period was then doubled, providing the total time of divergence of the two «species». We used a date of 7.0 million years (Ma) as the time of divergence of the human lineage from the LCA (after Hailie-Selassie 2001, Pickford & Senut 2001, WoldeGabriel et al 2001).

Possible age of origin for *Homo sapiens*

The mean change in genetic distance (substitution rate) per million years may also be used to estimate the possible age of origin of *H. sapiens*. To do this we used the approach of Cann et al (1987). We used average rates of change (per Ma) for % distance because other measures such as D_A have been shown to not be linear (Nei & Takezaki 1996).

Results and discussion

Chromosomes

If we assume that the five chromosomal changes in humans are each synonymous with speciation, then there may have been a maximum of five species on the DLMH since the shared human-chimpanzee ancestor (table 1). However, there are reasons to suspect that this number may be an overestimate.

Different groups of primate taxa appear to have adopted different chromosome arrangements in the course of their evolution (Dutrillaux 1979) with rearrangements in humans and great apes predominantly pericentric inversions (Dutrillaux cited by De Grouchy 1987). This suggests that chromosomal comparisons are likely to be useful only for assessing speciation rate in human evolution relative to great apes.

Chromosomal changes that lower fertility sufficient to cause reproductive isolation have been argued to cause increase in frequency only through genetic drift in a very small population (Key 1968, Bengtsson & Bodmer 1976). If speciation occurs via such changes it seems most likely to be through the sequential fixation of numerous rearrangements rather than single mutations or single rearrangements (Walsh 1982). For example, Sumatran and Bornean orangutans differ by three chromosomal rearrangements but are known to be fully fertile, and common chimpanzees and bonobos differ by six chromosomal rearrangements, and although some workers regard them as distinct species (see above), they do produce apparently normal hybrid offspring (H. Vervaecke, pers. com.). Most types of rearrangements between orangutan subspecies and between common chimpanzees and bonobos are also seen in humans. This suggests that at least some of the rearrangements in humans might not represent reproductive isolation.

This observation is complicated by the fact that humans appear to possess even greater chromosomal instability than great apes. Humans possess a high level of chromosomal rearrangements, with 1 out of every 120 babies born being abnormal (Hook 1992). The figure rises to about 25% for 10-day old blastocysts (Gardner & Sutherland 1996). We conclude that chromosomal rearrangements were likely to have been important during human evolution, more so than among the great apes, making comparisons with them of limited value. This would seem also to fit with the observation that chromosomal rearrangement is not a general genetic mechanism for speciation, but rather, characterises certain groups (Marks 1992). Humans may be one such group.

Given the chromosomal instability in humans, it seems likely that at least some of the chromosomal rearrangements may have had a significant impact on reproductive isolation when they occurred. The change to 46 chromosomes in humans, and rearrangements leading to human chromosomes 9 and 18 suggest speciation. Rearrangements in chromosome 9 were complicated, with two major shuffling events of genetic material having occurred which may have led to reproductive isolation (Verma & Luke 1994). The pericentric inversion of chromosome 18 may also have had important implications for the phenotype of early humans when it occurred (McConkey 1997). The gene for N-cadherin, located on the central proximal region of human 18q (Wallis et al 1994), has an important role in development and differentiation of the central nervous system (Redies 1995). Thus, the possibility that this gene was at or near one of the breakpoints involved in the inversion of chromosome 18 in a human ancestor may have important implications for human evolution (McConkey 1997). It may also have contributed significantly to reproductive isolation when it occurred.

From the above evidence we conclude that the number of species on the DLMH, as inferred from human chromosome rearrangements, might be around 3 and cannot be more than 5.

Nuclear DNA

Our analyses using 24 genetic distances provide an estimated speciation rate of 1–13 with a mean of 4 for all DNA distances (table 1). Some of the speciation rates in table 1 are <1. This results from the fact that some of the distances between humans and chimpanzees, when halved, are below those between Africans and Asians. They were counted as 1 when estimating mean values.

We interpret the speciation rate to provide an estimate of the number of species on the DLMH. Thus, DNA distances suggest there may have been around 4 species on the DLMH since the LCA of humans and chimpanzees. It should be noted, however, that humans appear to be characterised by lower DNA diversity than all great apes (Deinard & Kidd 1995). This means that human genetic distances might actually overestimate the speciation rate and consequently the number of species on the DLMH.

The considerable variation in the speciation rate/species number is not surprising given the variety of gene frequency data and wide range of loci covered, range of sample sizes and laboratory techniques employed. However, the majority of distances (18) provide an estimate of 5 species or less, with the remaining 6 ranging from 6 to 13 species.

The speciation rate/number of species may also be estimated using intraspecific distances between subspecies of orangutan, and the bonobo versus common chimpanzee (table 2). Using orangutan distances provides an estimated speciation rate of 1–6 (species) with means of 3 and 4 (species) on the DLMH. Using bonobo versus common chimpanzee distances provides an estimated speciation rate of 1–3 (species), with means of 2 and 3, on the DLMH. These estimates are consistent with most produced from human intraspecific data. The estimated mean in table 1 falls within the range of means from orangutan and chimpanzee distances.

Mitochondrial DNA

Using the same approach for 6 mtDNA distances provides estimated speciation rates/number of species of 1 to 11 (species) on the DLMH (table 1). The mean speciation rate using mtDNA distances is 5 species. The number of species may also be estimated using intraspecific mtDNA distances between subspecies of orangutan, and the bonobo and common chimpanzee (table 2). Using orangutan and bonobo versus common chimpanzee distances provides a mean estimate of 1 species on the DLMH. Some of the speciation rates in table 2 are <1. This results from the fact that some of the mtDNA distances between humans and chimpanzees, when halved, are below those between orangutan subspecies, and common chimpanzees versus bonobos.

There is an important problem with mtDNA that appears to have been ignored by anthropological geneticists. It has been shown that mtDNA rearrangements and point mutations are directly linked to more than 110 neurological disorders in humans (Wallace et al 1995, Servidei 2001). In other words, human mtDNA is under intense natural selection. This renders invalid the assumptions of selective neutrality and constant rate of substitution essential for many genetic analyses.

We conclude, therefore, that until the functions of the mtDNA genome are better understood, the inferences that may be made for human evolution using this molecule are limited. This applies to the mtDNA data used herein and applies to studies of human evolution using mtDNA molecular clocks to provide a divergence time for modern humans (eg, Cann et al 1987, Vigilant et al 1989, 1991, Adachi & Hasegawa 1995, Horai et al 1995, Stoneking 1997, Maca-Meyer et al 2001).

Pleistocene speciation rate

Table 3 presents estimates of 16 genetic distances, and table 4 provides estimates of 8 distances, between paired Pleistocene species. They also provide the average rate of change (per Ma) for each distance used to calculate these paired distances. We excluded mtDNA distances (see above).

So-called *Homo neanderthalensis* allegedly disappeared about 0.02 Ma and shared an ancestor with *H. sapiens* around 0.5 Ma, a longevity of 0.48 million years and thus a total divergence time of 0.96 million years. (Note: we use *H. sapiens* here for the form on the DLMH for this part of the Pleistocene following species estimates presented in tables 1 and 2). We estimate the mean distance between *H. sapiens* and «terminal» *H. neanderthalensis* from 16 distances to be around 0.08%. This is a very small distance and is less than half the estimated genetic difference

between living sub-Saharan Africans and Eurasians (Starr & McMillan 2001). The mean of 8 genetic distances between *H. sapiens* and *H. neanderthalensis* is 0.026–0.027. This is equivalent to the genetic distance between Papua New Guineans and Thais or Na Dene and Indonesians (Cavalli-Sforza et al 1994).

Ancient DNA might provide a basis for checking our estimates. Samples of mtDNA have been extracted from Neanderthal (Feldhofer) remains (Krings et al 1997) and possible Neanderthals (Krings et al 2000, Ovchinnikov et al 2000). The Feldhofer sample differed from a human standard by under 10% (Adcock et al 2001b), and this is much greater than our estimates derived from nuclear DNA. Given that mtDNA is under intense selection in living humans the significance of this research is problematic and most likely provides an unreliable comparison for our data.

Homo sapiens and so-called *H. erectus* living about 0.3 Ma, about the time *H. erectus* is alleged to have disappeared, may have shared an ancestor around 1.5 Ma (a total divergence time of 2.4 million years). The distance between them as determined from the mean of 16 distances may have been around 0.19%. This is about equivalent to the estimated genetic difference between living sub-Saharan Africans and Eurasians of 0.2% (Starr & McMillan 2001). The mean of 8 other genetic distances between *H. sapiens* and *H. erectus* is 0.065–0.068. This overlaps the range of distances for living humans, with the lower estimate identical to the distance between «Bantu» and «Eskimo» (Cavalli-Sforza et al 1994).

Table 3: Estimated average rate of change of DNA (%) distances and estimated DNA distances (%) between paired Pleistocene species.

Distance (%) ¹	Avg. rate (per Ma) ²	Distance		
		<i>H. sapiens</i> v. <i>H. neanderthalensis</i> ³	<i>H. sapiens</i> v. <i>H. erectus</i> ⁴	<i>H. sapiens</i> v. <i>H. boisei</i> ⁵
Total	0.070	0.070	0.170	0.280
Total	0.040	0.040	0.100	0.160
Non-coding	0.120	0.120	0.290	0.480
Chromosome 1	0.040	0.040	0.110	0.180
<i>Plp</i>	0.055	0.053	0.132	0.220
<i>Hprt</i>	0.069	0.066	0.166	0.276
<i>Gkc</i>	0.047	0.045	0.113	0.188
<i>Pdhal</i>	0.069	0.066	0.166	0.276
<i>Pdhal</i>	0.079	0.076	0.190	0.316
<i>dys44</i>	0.064	0.061	0.154	0.256
<i>Zfx</i>	0.111	0.107	0.266	0.444
<i>Xq13.3</i>	0.064	0.061	0.154	0.257
<i>Lpl</i>	0.107	0.103	0.257	0.428
<i>b-globin</i>	0.093	0.089	0.223	0.372
<i>Mclr</i>	0.131	0.126	0.314	0.524
Chromosome 22	0.100	0.096	0.240	0.400
Mean	0.079	0.076	0.190	0.316

¹Using data from Table 1; ²using human-chimpanzee divergence time of 7.0 Ma; ³estimated using total divergence time of 0.96 million years; ⁴estimated using total divergence time of 2.4 million years; ⁵estimated using total divergence time of 4.0 million years.

Homo sapiens and so-called ‹robust australopithecines› living about 1 Ma, about the time this form becomes extinct, may have shared an ancestor around 3 Ma (a total divergence time of 4 million years). The distance between them as determined from the mean of 16 distances may have been around 0.32%. This is about 160% of the estimated genetic difference between living sub-Saharan Africans and Eurasians (Starr & McMillan 2001). The mean of 8 other genetic distances between the two forms is 0.109–0.113, about 170% of the largest distance among living humans (Cavalli-Sforza et al 1994).

Given the observation that living humans have relatively low genetic diversity, it is useful to compare estimated distances between paired fossil groups with those of other species and genera. As discussed above, as a general observation DNA distances between congeneric mammals are about $D = 0.20$. Castresana (2001) has reported intrageneric and intergeneric distance modes of 0.10–0.15 and 0.25–0.30 for cytochrome *b*. Johns and Avise (1998) have reported a general agreement with distances from these genes with those derived from allozymes (see Avise & Aquadro 1982), suggesting the modes reported by Castresana (2001) are applicable across a range of genetic data.

Distances calculated here for *H. neanderthalensis* versus *H. sapiens* and for *H. erectus* versus *H. sapiens* are around one-third and two-thirds, respectively, of the mammalian intrageneric mode. This finding is of relevance to determining whether these fossil taxa might actually be separate species, as the distances between them are too small to support specific distinction. This is consistent also with the finding that mammals with large body masses have, as a group, been oversplit taxonomically (Castresana 2001). Distances calculated between *H. sapiens* and ‹robust australopithecines› fall within the mammalian intrageneric mode suggesting they might reasonably be considered congeneric species.

Table 4: Estimated average rate of change of other DNA distances and estimated DNA distances between paired Pleistocene species.

Distance ¹	Avg. rate (per Ma) ²	Distance		
		<i>H. sapiens</i> v. <i>H. neanderthalensis</i> ³	<i>H. sapiens</i> v. <i>H. erectus</i> ⁴	<i>H. sapiens</i> v. <i>H. boisei</i> ⁵
Electrophoresis	0.022–0.028	0.021–0.027	0.053–0.067	0.088–0.112
2DE «including missing»	0.006	0.006	0.014	0.024
2DE «excluding missing»	0.005	0.005	0.012	0.02
Isozymes	0.014–0.017	0.013–0.016	0.034–0.041	0.056–0.068
Microsatellite	0.051	0.049	0.122	0.204
RFLP	0.033	0.032	0.079	0.132
Protein polymorphism	0.042	0.040	0.101	0.168
<i>Alu</i> insertion frequency	0.044	0.042	0.106	0.176
Mean	0.027–0.028	0.026–0.027	0.065–0.068	0.109–0.113

¹Using data from Table 1; ²using human-chimpanzee divergence time of 7.0 Ma; ³estimated using total divergence time of 0.96 million years; ⁴estimated using total divergence time of 2.4 million years; ⁵estimated using total divergence time of 4.0 million years.

Age of origin for *Homo sapiens*

Sixteen distances were used to estimate the time of origin of *H. sapiens* (table 5). The mean rate of substitution of 0.079 per Ma is virtually indistinguishable from the genetic distance between Africans and Asians ($D = 0.078$ – Nei & Livshits 1989). Estimates of the age of origin for *H. sapiens* have a wide range of 0.55 to 5.0 Ma. However, 12 estimates range between 0.99 and 3.0 Ma, with a further 9 falling between 1.0 and 2.0 Ma. The mean of all 16 estimates is 1.71 Ma. These results are similar to those reported recently by Yu et al (2001) who studied a ~10-kb region on chromosome 1, and Zhao et al (2000) who studied a 10-kb region on chromosome 22, and determined the age of origin for *H. sapiens* to be well over 1 Ma. Our mean estimate is consistent with the age for the first migration out of Africa as suggested by the Dmanisi remains (Gabunia & Vekua 1995).

These estimates contrast starkly with others for the origin of modern humans using mtDNA of between 59,000 years and 300,000 years ago (Cann et al 1987, Vigilant et al 1989, Stoneking 1997, Adachi & Hasegawa 1995, Horai et al 1995, Maca-Meyer et al 2001). The difference is easily explained and derives directly from the fact we used (halved) distances between humans and chimpanzees (ie, estimated distance from LCA to living humans) to calculate the average rate of substitution, and an age for the LCA of 7.0 Ma. In contrast, Cann et al (1987) used mtDNA substitution rates of 2% and 4% derived from a range of vertebrates to determine the age of origin for modern humans of 142,500–285,000 year ago. Other researchers have used an age for the LCA much less than 7.0 Ma and this has also contributed to higher average substitution rates and hence the younger estimates of origin attributed to *H. sapiens* than those presented here.

Table 5: Estimated time of origin of *Homo sapiens*.

Distance (%)	Avg. rate (per Ma) ¹	Living human distance (LHD) ²	Avg. rate/LHD	Age of origin (Ma)
Total	0.070	0.200	0.35	2.86
Total	0.040	0.200	0.20	5.00
Non-coding	0.120	0.200	0.60	1.67
Chromosome 1	0.040	0.076	0.53	1.89
<i>Plp</i>	0.055	0.100	0.55	1.82
<i>Hprt</i>	0.069	0.050	1.38	0.72
<i>Gkc</i>	0.047	0.026	1.81	0.55
<i>Pdhal</i>	0.069	0.100	0.69	1.45
<i>Pdhal</i>	0.079	0.175	0.45	2.22
<i>dys44</i>	0.064	0.120	0.53	1.89
<i>Zfx</i>	0.111	0.145	0.77	1.30
<i>Xq13.3</i>	0.064	0.050	1.28	0.78
<i>Lpl</i>	0.107	0.135	0.79	1.27
<i>b-globin</i>	0.093	0.175	0.53	1.89
<i>Mclr</i>	0.131	0.130	1.01	0.99
Chromosome 22	0.100	0.108	0.93	1.08
Mean ($n = 16$)	0.079	0.124	0.775	1.71
Mean ($n = 12$)	0.087	0.139	0.644	1.69

¹Data from Table 3; ²data from «Intraspecific distance» column of Table 1 (see footnotes of Table 1 for references).

Finally, our results also suggest there is no evidence for a bottleneck during recent human evolution, rather, that much of the genetic heritage of living humans is very ancient (see also Yu et al 2001).

Taxonomy of humans and chimpanzees

Some geneticists have suggested humans and chimpanzees should be classified (along with gorillas?) in *Homo* on the basis of the apparently short genetic distance between them (King & Wilson 1975, Goodman et al 1989, 1990, 2001, Castresana 2001, Watson et al 2001). We calculated the mean of 13 DNA distances (%) between humans and chimpanzees (Chromosome 1, *Plp*, *Hprt*, *Gkc*, *Pdhal*, *Pdhal*, *dys44*, *Zfx*, *xq13.3*, *Lp1*, *g-globin*, *Mclr*, Chromosome 22 data in table 1, multiplied by 2). The value of 1.1% is consistent with other estimates of around 1% (King & Wilson 1975) and 98.3% and 99.5% for non-coding and coding DNA respectively (Goodman et al 1989, 1990, 2001). This reinforces the view that humans and chimpanzees are genetically very close and should be considered congeneric. We note that Fujiyama et al (2002) have recently detected previously unknown differences between humans and chimpanzees on chromosome 21.

Comparing distances for a range of mammals (see Johns & Avise 1998, Castresana 2001) with those between common chimpanzees and bonobos (table 2) suggests they should be classified as a single species.

Conclusions

Analyses of chromosomal rearrangements, genetic distances between humans and chimpanzees and genetic variation within humans and great apes, have implications for systematics in palaeoanthropology:

1. Current taxonomies, based on morphology alone, have overestimated the number of fossil species. Research presented here indicates there may have been around 4 species on the direct line to modern humans (see figure 5). This is

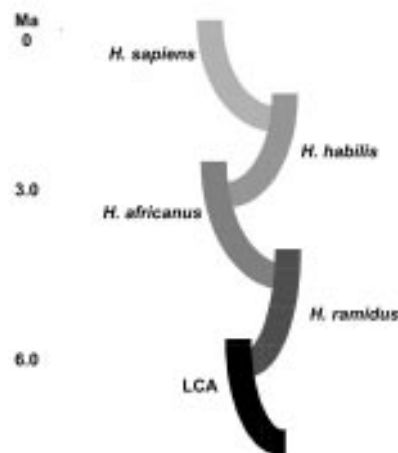


Fig. 5: Possible systematic arrangement for the direct line to modern humans (ignoring possible extinct side-branches) based on genetic analyses. Note: new species «bud off» leading to the formation a single species which may be synchronic with its parent.

consistent with the «extreme» morphological position taken by Tobias (1981a) who recognised only five species. We are aware that there is major debate over species recognition concepts and the mechanics of speciation. While one end of the spectrum prefers anagenetic speciation to account for human evolution (Brace 1967, 1995, Henneberg & Thackeray 1995) we find cladogenesis consistent with the biological species concept for the present and the past (eg, Ghiselin 1974, Eldredge 1989, Krishtalka 1993, Hull 1993a, Mayr 1993). Human evolution is best viewed as a bush, but one with far fewer branches than many workers currently accept.

2. After a minimum of 6 million years of evolution by two lineages (humans and chimpanzees), and thus a minimum of 12 million years of separate evolution, their living descendents are now only about 1% different genetically. This strongly suggests that humans and chimpanzees are congeneric (Goodman et al 1989, 1990, 2001, Castresana 2001, Watson et al 2001). Our analysis of a further published 13 DNA distances reconfirms this proximity (1.1% difference). This has major implications for the number of genera used in human evolution – there can be only one! The continuing preference by some workers for up to 8 human genera, with the belief that genera are an acceptable solution to the complexities of morphological variation (eg, Strait et al 1997, Wood & Collard 1999, Leakey et al 2001), is no longer tenable. It is time that chimpanzees and living humans (and thus all fossil humans) be classified in *Homo*.

3. Published genetic distances between common chimpanzees and bonobos, along with evidence for interbreeding (Vervaecke & van Elsacker 1992, Curnoe et al in press), indicates they should be assigned to a single species.

4. The findings of this research also apply to possible evolutionary «side branches». The fact that humans and chimpanzees are sister species means that no two fossil human species may be genetically more distant than humans and the LCA. This makes all fossil taxa very close to each other and below congeneric genetic distances seen for many mammals (see Ayala & Kiger 1980, Ayala 1982, Janczewski et al 1990, Johns & Avise 1998, Castresana 2001).

5. Our estimates of genetic divergence suggest that periods of around 2 million years are required to produce sufficient genetic distances to represent speciation between fossil populations. Neanderthals and so-called *H. erectus* are unlikely to have been separate (genetically disjunct) species. A single «robust» species may be recognised, therefore, the four «robust» species currently recognised should be pooled into a single taxon (*H. robustus*).

6. The short genetic distance between fossil taxa suggests an important approach to identifying species using non-genetic criteria. As a general observation, paired species with a genetic distance $D \leq 0.5$ retain their reproductive isolation through prezygotic barriers (see Ridley 1996). Researchers who prefer to examine only morphology for systematic purposes might direct their efforts towards attempting to identify such barriers. They might include factors like ecological, habitat or seasonal isolation (Dobzansky 1970).

7. Given the large offset in evolutionary rates of molecules and morphology seen in human evolution, which is in fact a feature common to the primates (Fitch 1982), fossil human species are characterised by high levels of morphological variation and low levels of genetic variability. Thus, molecular data suggest the limits set

for intraspecific morphological variation in palaeoanthropology have been set too low (see also Henneberg & Thackeray 1995). Many of the species recognised currently are likely to sample adaptations within a small number of species across space and through time. These adaptations appear to have occurred with very little genetic change. Thus, the role of phenotypic plasticity is likely to have been greatly underestimated in human evolution.

8. We call into question the use of mtDNA for studies of human evolution. It has been shown that this genome is under strong selection, which negates the assumption of neutrality of many studies. This issue should be addressed as a matter of urgency by geneticists, including assessment of its impact on hypotheses about modern human origins.

9. We estimate the divergence time of *H. sapiens* from 16 genetic distances to be around 1.7 Ma. This is consistent with evidence for the earliest migration out of Africa as suggested by the Dmanisi remains (Gabunia & Vekua 1995). Our lower estimated rate of substitution was derived from the estimated distance between the human-chimpanzee LCA and living humans. Our estimates suggest that the widely cited dates of Cann et al (1987) have dramatically overestimated this rate, perhaps by a factor of 25–50, and as a consequence, underestimated the time of origin for *H. sapiens* by a factor of 6–12.

10. Finally, we conclude there is no evidence for a bottleneck in recent human evolution (see also Yu et al 2001).

Our study demonstrates an important use for molecular data as an approach to testing hypotheses about human systematics. It also opens up new avenues for research about humans and our closest relatives. Morphology provides the basis for understanding adaptations, selective pressures and many of the processes involved in phenotypic change. Genetics provides a powerful and objective basis for testing existing fossil taxonomies and for refining them in order that they better reflect the evolutionary history of living humans and their ancestors.

Clearly, there is a need for greater cooperation between palaeoanthropologists and anthropological geneticists. This is fundamental to improving our understanding of these critical issues, to advance the study of human evolution and to bring palaeoanthropology closer to the mainstream of evolutionary biology.

Acknowledgements

We wish to thank colleagues who commented on our research during two seminars held in 2001 at the Australian National University, Simon Easteal and two anonymous referees. We acknowledge Robert Eckhardt whose published research inspired ours.

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