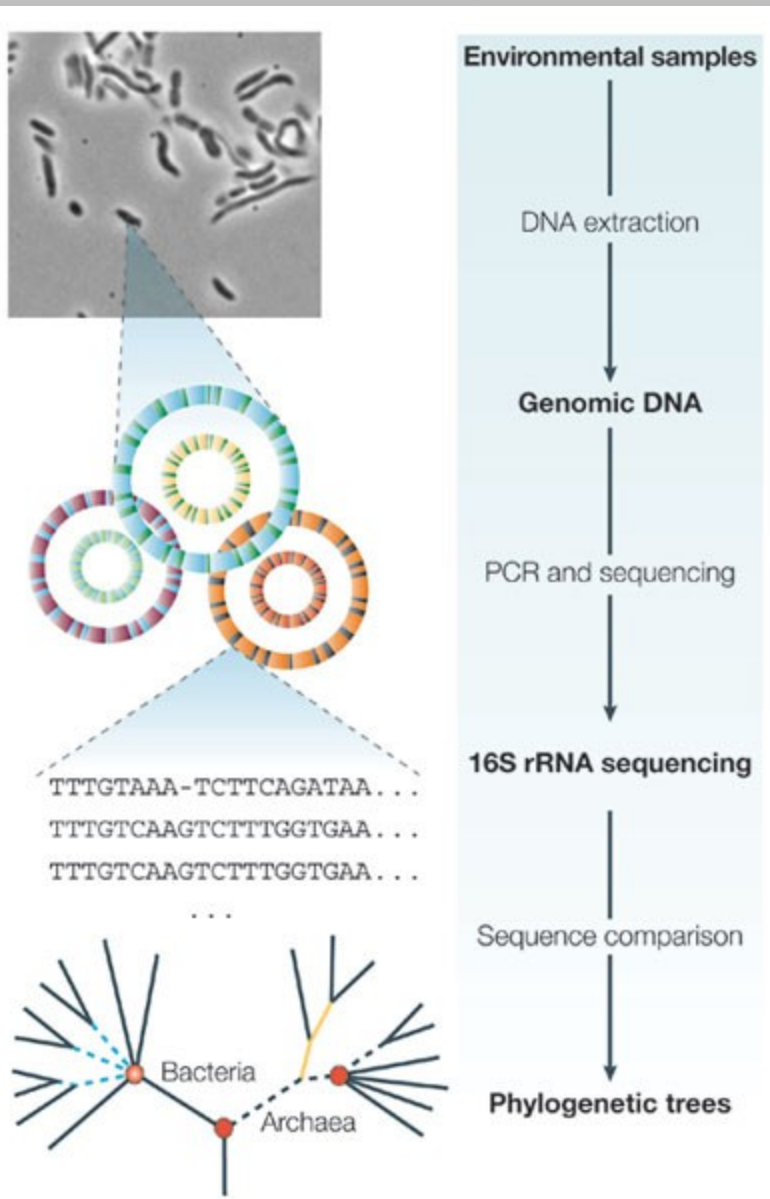
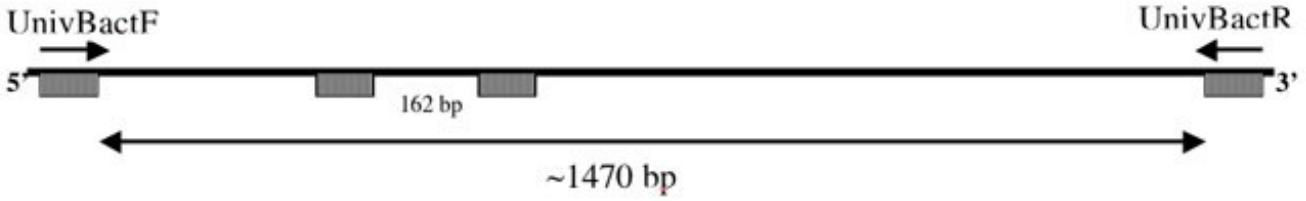


Nature Reviews | Genetics

A Simplified map of the 16S rRNA molecule

Primer:	Primer Sequence:	Primer Length	*E. coli* Position
UnivBactF	5' GAG TTT GAT YMT GGC TC	17-mer	9 - 25
UnivBactR	5' GYT ACC TTG TTA CGA CTT	18-mer	1509 - 1492



= location of primer sequence and its orientation

= area of broad range sequence conservation (sequence is virtually the same in all bacteria)

= area between primers (not including the primer sequences)

http://serc.carleton.edu/microbelife/research_methods/genomics/modgen.html

Analiza črevesne flore z DNA-tehnologijo

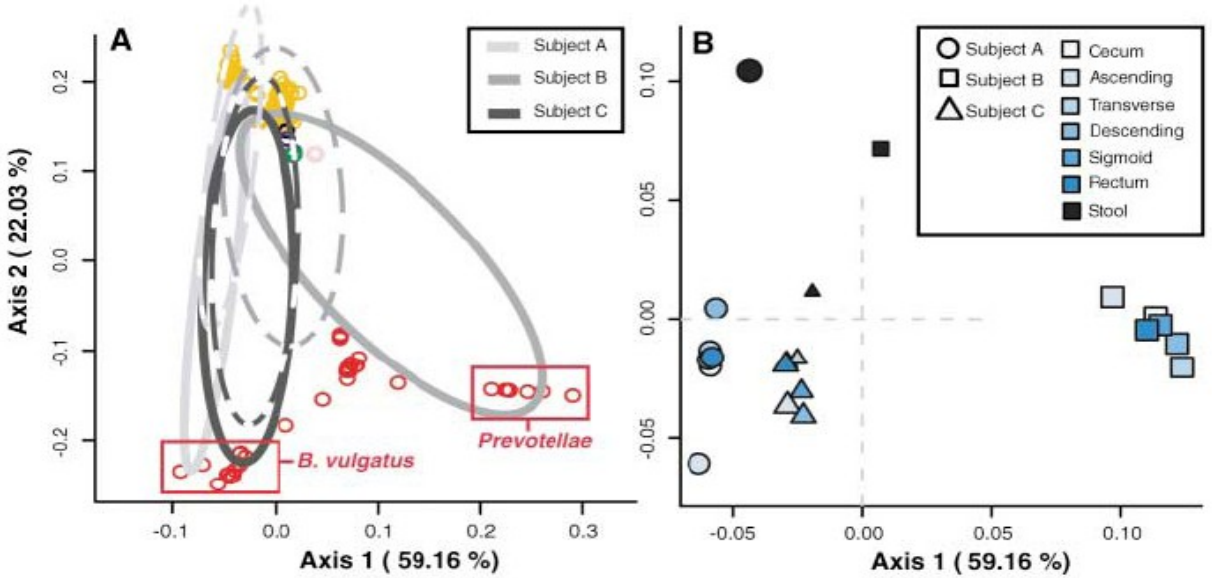


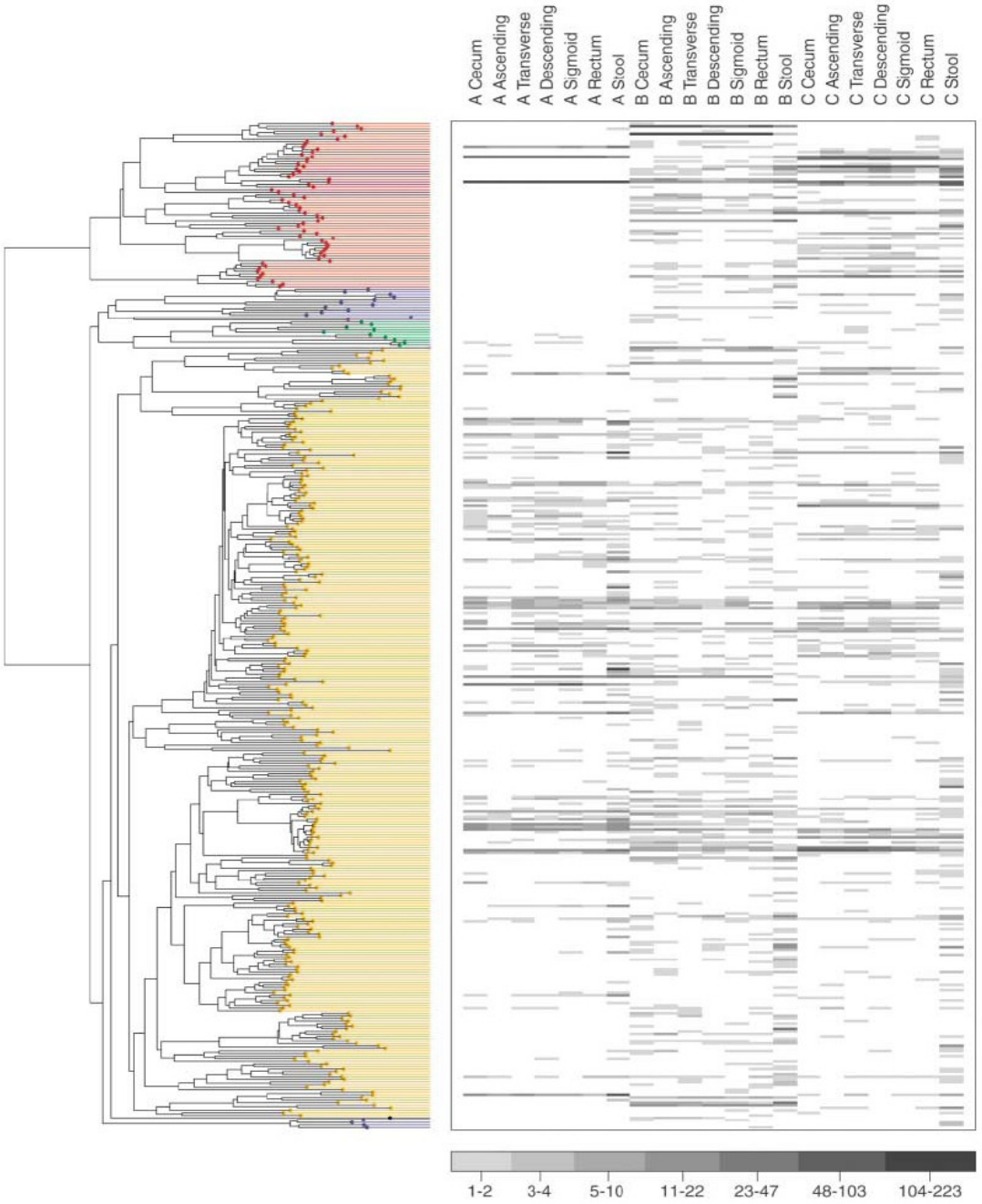
Fig. 3. DPCoA for (**A**) colonic mucosa (solid lines) and stool (dashed lines), (**C**) colonic mucosal sites alone, and (**D**) mucosal sites excluding *Bacteroidetes* phylotypes. Phylotypes are represented as open circles, colored according to phylum as in Fig. 1. Phylotype points are positioned in multidimensional space according to the square root of the distances between them. Ellipses indicate the distribution of phylotypes per sample site, except in (A), where all mucosal sites are represented by one ellipse. Percentages shown along the axes represent the proportion of total Rao dissimilarity captured by that axis. (A) is the best possible two-dimensional representation of the Rao dissimilarities between all samples (*12*). (**B**) is an enlarged view of (A), depicting the centroids of each site-specific ellipse. Subject ellipse distributions remain distinct after stool phylotypes (C) and *Bacteroidetes* phylotypes (D) are excluded from the analysis.

Diversity of the Human Intestinal Microbial Flora

SCIENCE VOL 308 10 JUNE 2005

The human endogenous intestinal microflora is an essential "organ" in providing nourishment, regulating epithelial development, and instructing innate immunity; yet, surprisingly, basic features remain poorly described. We examined 13,355 prokaryotic ribosomal RNA gene sequences from multiple colonic mucosal sites and feces of healthy subjects to improve our understanding of gut microbial diversity. A majority of the bacterial sequences corresponded to uncultivated species and novel microorganisms. We discovered significant intersubject variability and differences between stool and mucosa community composition. Characterization of this immensely diverse ecosystem is the first step in elucidating its role in health and disease.

Fig. 1. Number of sequences per phylotype for each sample. The *y* axis is a neighbor-joining phylogenetic tree containing one representative of each of the 395 phylotypes from this study; each row is a different phylotype. The phyla (*Bacteroidetes*, *non-Alphaproteobacteria*, unclassified near *Cyanobacteria*, *Actinobacteria*, *Firmicutes*, *Fusobacteria*, and *Alphaproteinobacteria*, ordered top to bottom) are color coded as in Fig. 3 and fig. S1. Each column is labeled by subject (A, B, C) and anatomical site. For each phylotype, the clone abundance is indicated by a grayscale value.



Filotip: filogenetska kategorija – organizmi (običajno prokarionti) z neko določeno filogenetsko značilnostjo / podobnostjo genoma glede na ostale filotipe, pri čemer kriterij za uvrstitev v nek filotip določimo po lastni presoji (npr. 97- odstotna identičnost); gre za evolucijsko, ne pa klasično taksonomsko kategorijo.

Do 2010 so zbrali podatke za >45.000 rRNA bakterijske flore in jih razvrstili v 1800 rodov (>90 % identičnost) s ~16.000 filotipi na ravni vrste (>97 % identičnost) in 36.000 na ravni seva (>99 % ident.).

V 2 letih sledenja se flora posameznika ni bistveno spreminjala. Kar 98 % mikrobov je mogoče razvrstiti v 4 taksonomske skupine, ostala 2 % pa sta zelo raznolika.

Črevesni mikrobiom

(vsi genomi mikrobne združbe)

- taksonomska kompleksnost
- ekološka dinamičnost
- vpliva na razvoj in delovanje črevesja
- 10E14 bakterij
- človek kot ‚nadorganizem‘

- analize z metagenomskim pristopom
- analize preko sekvenciranja genov za 16 S rRNA (~1,5 kb)

Mikrobna flora dojenčkov je bistveno drugačna kot pri odraslih, a po 1. letu življenja postane že zelo podobna, kot jo imajo odrasli. Pri novorojencih so razlike med posamezniki zelo velike, kompleksnost pa je majhna.

Sestava ni bila odvisna od vrste poroda (naravno ali carski rez) in načina hranjenja (materino mleko ali ne).

Table 1. Top 15 species with the high ratio of adult and infant gut-enriched genes

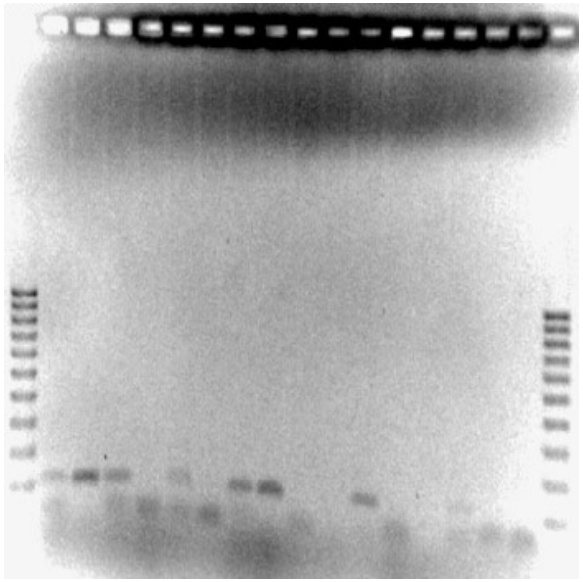
Species having higher ratio of adult gut-enriched genes		Species having higher ratio of infant gut-enriched genes	
Ratio	Species	Ratio	Species
0.152	*Bacteroides ovatus*	0.102	*Bifidobacterium longum* NCC2705
0.148	*Bacteroides* WH2	0.098	*Clostridium ramosum* JCM1298
0.144	*Bacteroides* sp. A01	0.093	*Bifidobacterium catenulatum* JCM1194
0.143	*Bacteroides vulgatus*	0.092	*Clostridium clostridioforme* JCM1291
0.141	*Bacteroides thetaiotaomicron* 3731	0.087	*Collinsella aerofaciens*
0.137	*Bacteroides thetaiotaomicron* VPI-5482	0.082	*Lactobacillus johnsonii* NCC 533
0.136	*Bacteroides thetaiotaomicron* 7330	0.081	*Ruminococcus gnavus*
0.130	*Bacteroides uniformis*	0.081	*Enterococcus faecalis* V583
0.128	*Bacteroides caccae*	0.081	*Lactobacillus acidophilus* NCFM
0.126	*Eubacterium ventriosum*	0.079	*Dorea longicatena*
0.125	*Ruminococcus gnavus*	0.077	*Listeria monocytogenes* EGD-e
0.123	*Dorea longicatena*	0.076	*Lactobacillus plantarum* WCFS1
0.121	*Bacteroides* sp. A03	0.073	*Streptococcus agalactiae* A909
0.121	*Ruminococcus torques*	0.072	*Streptococcus pneumoniae* TIGR4
0.121	*Bacteroides fragilis* NCTC 9343	0.072	*Streptococcus pneumoniae* R6

DNA Research **16**, 1–12, (2009)

Uporaba DNA-tehnologije v arheologiji

Vzorci stare DNA so pogosto razgrajeni do take mere, da analize sploh niso mogoče. Razen tega prihaja do kemičnih sprememb nukleotidov, ki preprečujejo natančno analizo in zmanjšujejo natančnost encimskih reakcij. Najbolj stabilna je mitohondrijska DNA.

Poskušali so pomnožiti kratke fragmente DNA iz vzorca kosti kennewiškega človeka (konzerviran v blatu ~9000 let), analizirali pa so tudi DNA iz želodca Ötzija (~5000 let) in s tem ugotovili, s čim se je prehranjeval (srne, divje koze, različne zelnate rastline).



http://www.cr.nps.gov/aad/kennewick/kaestle.htm

The Seattle Times

Proc Natl Acad Sci U S A. 2002 October 1; 99(20): 12594–12599.

Ötzi's last meals: DNA analysis of the intestinal content of the Neolithic glacier mummy from the Alps

Franco Rollo*, Massimo Ubaldi, Luca Ermini, and Isolina Marota

Laboratorio di Archeo-Antropologia molecolare/DNA antico, Università di Camerino, I-62032 Camerino, Italy

Table 1. Taxonomic identification of the consensus sequences for the 18S rRNA, rbcL, and 12 rRNA gene clones from the Iceman's intestinal content

Consensus	No. of clones	Type of sequence	Taxonomic identification*								Base similarity
			Kingdom	Phylum	Class	Order	Family	Tribe†	Genus	Species	
A	12	18S rRNA	Fungi	Basidiomycota	—	—	—		—	—	107/107
B	25		Fungi	Basidiomycota	Urediniomycetes	—	—		—	—	115/119
C	4		Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Triticeae	—	—	117/117
D	13		Viridiplantae	Streptophyta	Coniferopsida	Coniferales	Pinaceae	—	Pinus	—	116/118
E	8		Fungi	Ascomycota	—	—	—		—	—	116/117
F	8		Fungi	Basidiomycota	Urediniomycetes	—	—		—	—	118/119
G	5		Viridiplantae	Streptophyta	Eudicotyledons	—	—	—	—	—	112/118
CR18S022	1		Viridiplantae	Pteridophyta	Filicopsida	Filicales	—	—	—	—	116/119
I18S001	1		Fungi	Basidiomycota	Heterobasidiomycetes	—	—		—	—	114/117
H	15	rbcL	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	—	—	—	48/48
I	12		Viridiplantae	Streptophyta	Eudicotyledons	—	—	—	—	—	50/50
J	8		Viridiplantae	Streptophyta	Liliopsida	Poales	—	—	—	—	50/50
K	9	12S rRNA	Metazoa	Chordata	Mammalia	Artiodactyla	Cervidae		*Cervus*	*elaphus*	76/76
L	2		Metazoa	Chordata	Mammalia	Artiodactyla	Bovidae		*Capra*	*ibex*	76/76

*The degree of confidence of the identification was checked by the construction of the corresponding phylogenetic trees as shown in Figs. 2–5. It can be observed that the accuracy of the 18S rDNA identification varies widely among the different kingdoms and classes, the highest being reached by the Coniferopsida (Viridiplantae) and the lowest with the Fungi.

†Used exclusively for the Viridiplantae kingdom.

How did they get there? Hypothetical routes along the Indian Ocean coastline that could have been taken by early humans emigrating out of Africa. The oldest human traces outside of Africa and the Levant are at Lake Mungo in Australia (>46,000 years old) and in the Niah Cave of Borneo (>45,000 years ago). New mtDNA data, from Malaysians and aboriginal Andaman islanders, suggest that human settlements appeared along the Indian Ocean coastline 60,000 years ago (*7*, *8*).

Did Early Humans Go North or South?

Peter Forster and Shuichi Matsumura

SCIENCE VOL 308 13 MAY 2005

Single, Rapid Coastal Settlement of Asia Revealed by Analysis of Complete Mitochondrial Genomes

SCIENCE VOL 308 13 MAY 2005

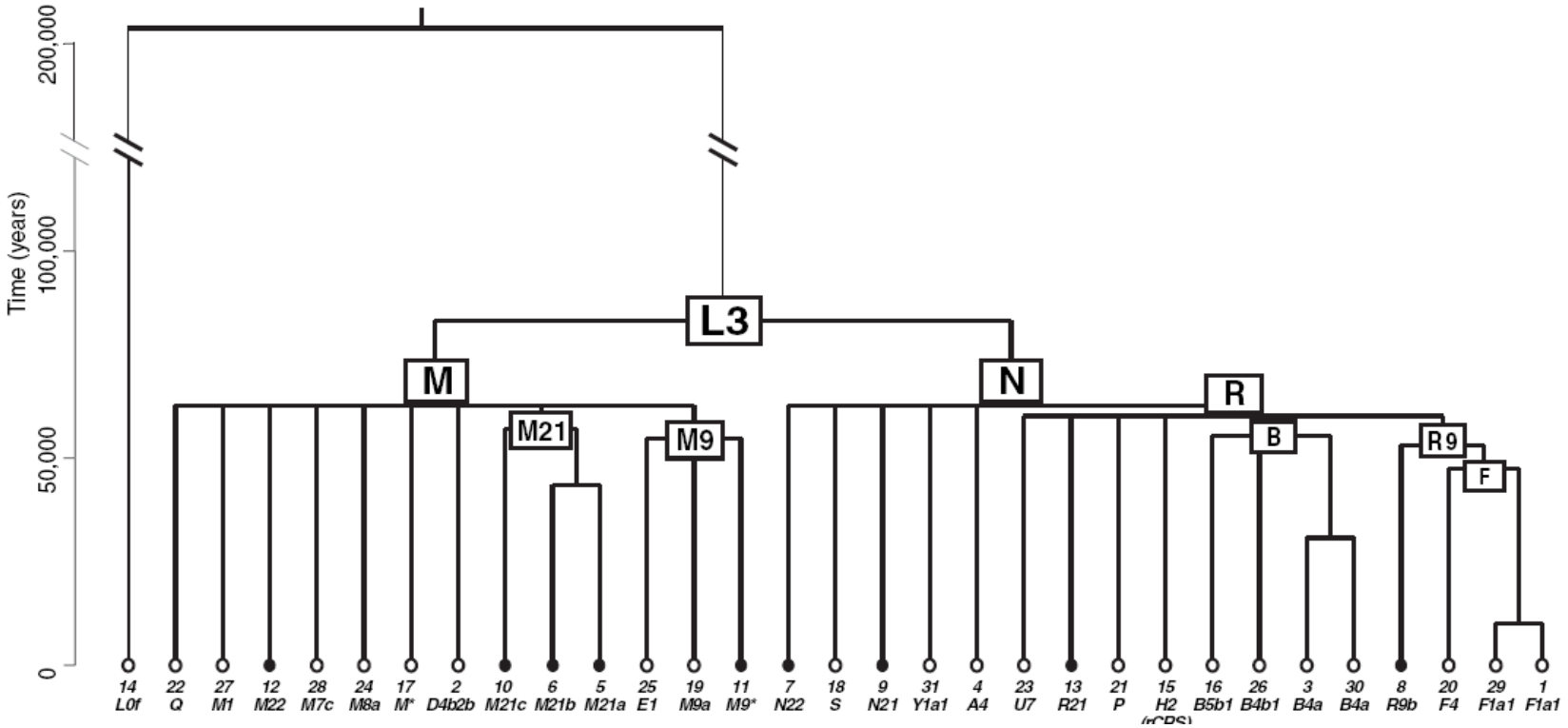


Fig. 1. Reconstructed phylogeny of 31 mtDNA coding region sequences. mtDNAs from the Malay Peninsula are indicated by solid circles at the tips of the tree.

Reading Ancient DNA the Community Way

SCIENCE VOL 308 3 JUNE 2005

"One of the key issues is disentan-
gling the evolu-
grizzlies seem puny. But
giants, and before the last
bered throughout Europe
on berries, honey,
ing time in ca
silized b
cl
pa
humans: Any DN
from the researchers
be quite apparent.
Once Noonan sequer
samples from the bear fos
any sequences that resem
dog genomes are about 9

Gentle giant. Ancient-DNA sequencing strategy has passed its first test on lost cave bears.

Genomic Sequencing of Pleistocene Cave Bears

James P. Noonan,[1,2] Michael Hofreiter,[3] Doug Smith,[1] James R. Priest,[2] Nadin Rohland,[3] Gernot Rabeder,[4] Johannes Krause,[3] J. Chris Detter,[1,5] Svante Pääbo,[3] Edward M. Rubin[1,2*]

SCIENCE VOL 309 22 JULY 2005

- analiza genoma jamskega medveda *Ursus spaeleus*
- starost kosti ~40.000 let (čas neandertalcev)
- genomska zaporedja so bila večinoma mikrobna
- predpostavljajo, da so dobili 27 000 nt medvedove DNA, ki je bila 98 % identična DNA današnjih medvedov
- pristop omogoča analizo genoma nendaertalca

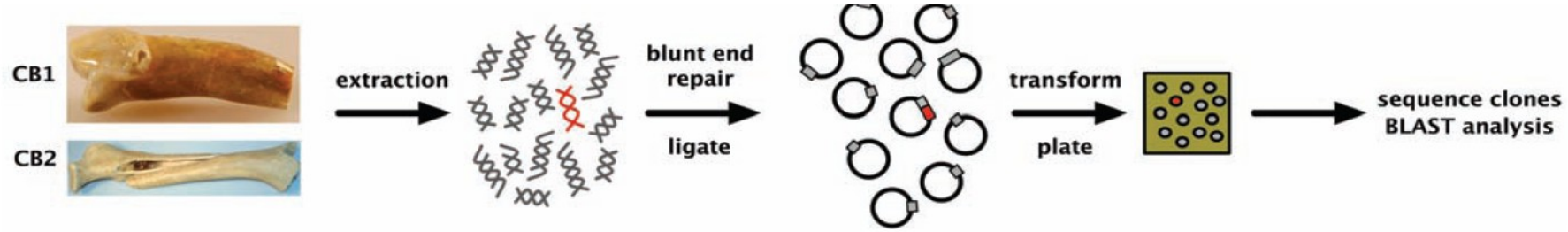
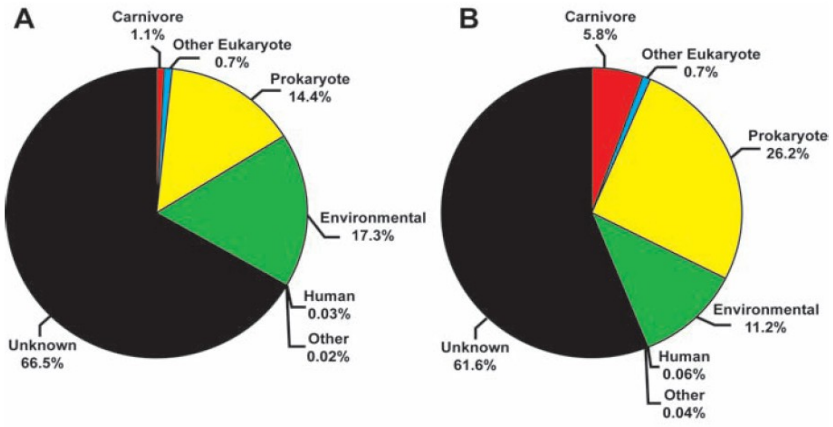


Fig. 1. Schematic illustration of the ancient DNA extraction and library construction process. Extracts prepared from a cave bear tooth (library CB1) and a bone (library CB2) contain cave bear DNA (red) and a mixture of DNA from other organisms (gray).



Sequencing the nuclear genome of the extinct woolly mammoth

Webb Miller[1], Daniela I. Drautz[1], Aakrosh Ratan[1], Barbara Pusey[1], Ji Qi[1], Arthur M. Lesk[1], Lynn P. Tomsho[1], Michael D. Packard[1], Fangqing Zhao[1], Andrei Sher[2]‡, Alexei Tikhonov[3], Brian Raney[4], Nick Patterson[5], Kerstin Lindblad-Toh[5], Eric S. Lander[5], James R. Knight[6], Gerard P. Irzyk[6], Karin M. Fredrikson[7], Timothy T. Harkins[7], Sharon Sheridan[7], Tom Pringle[8] & Stephan C. Schuster[1]

In 1994, two independent groups extracted DNA from several Pleistocene epoch mammoths and noted differences among individual specimens[1,2]. Subsequently, DNA sequences have been published for a number of extinct species. However, such ancient DNA is often fragmented and damaged[3], and studies to date have typically focused on short mitochondrial sequences, never yielding more than a fraction of a per cent of any nuclear genome. Here we describe 4.17 billion bases (Gb) of sequence from several mammoth specimens, 3.3 billion (80%) of which are from the woolly mammoth (*Mammuthus primigenius*) genome and thus comprise an extensive set of genome-wide sequence from an extinct species. Our data support earlier reports[4] that elephantid genomes exceed 4 Gb. The estimated divergence rate between mammoth and African elephant is half of that between human and chimpanzee. The observed number of nucleotide differences between two particular mammoths was approximately one-eighth of that between one of them and the African elephant, corresponding to a separation between the mammoths of 1.5–2.0 Myr. The estimated probability that orthologous elephant and mammoth amino acids differ is 0.002, corresponding to about one residue per protein. Differences were discovered between mammoth and African elephant in amino-acid positions that are otherwise invariant over several billion years of combined mammalian evolution. This study shows that nuclear genome sequencing of extinct species can reveal population differences not evident from the fossil record, and perhaps even discover genetic factors that affect extinction.

Table 1 | Basic statistics on the mammoth genome sequence

Sequenced bases	4.168 Gb
Sequencer runs (Roche GS-FLX and GS20)	77
Sequenced reads	32,619,456
Average read length	128 bp
Bases that align to *L. africana*	3.3 Gb
Sequence coverage for M4's mitochondrial genome	4,430-fold
Total error rate based on mitochondrial genome	35 per 10,000 bp
Substitutions from DNA damage	6 per 10,000 bp
Substitutions from sequencing error	8 per 10,000 bp
Insertions/deletions from sequencing error	21 per 10,000 bp
Total error neglecting indels	14 per 10,000 bp (0.14%)
Estimated genome size	4.7 Gb
Estimated nucleotide identity of M4 to African elephant	99.41%
Estimated amino-acid identity of M4 to African elephant	99.78%

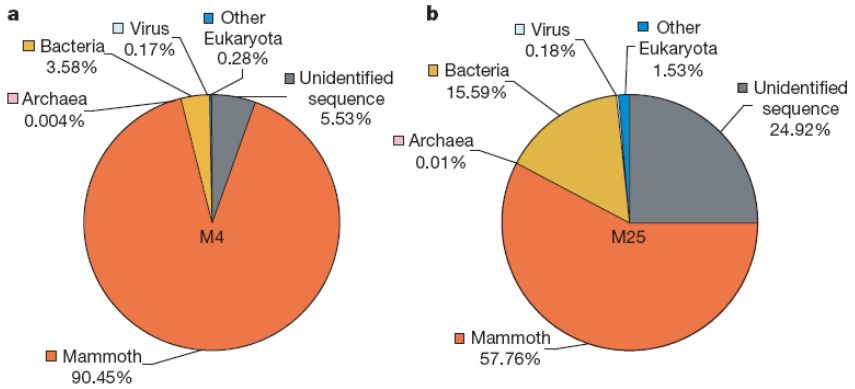


Figure 1 | Species composition of metagenomic DNA extracted from mammoth hair. **a**, **b**, Pie charts for the M4 (**a**) and M25 (**b**) data sets show the percentage of sequencing reads assigned to taxa for mammoth, Archaea, Bacteria, virus, as well as the two unspecified categories 'other Eukaryota' and 'unidentified sequence'. The taxon distribution exemplifies the variability of the endogenous DNA content of ancient specimens.

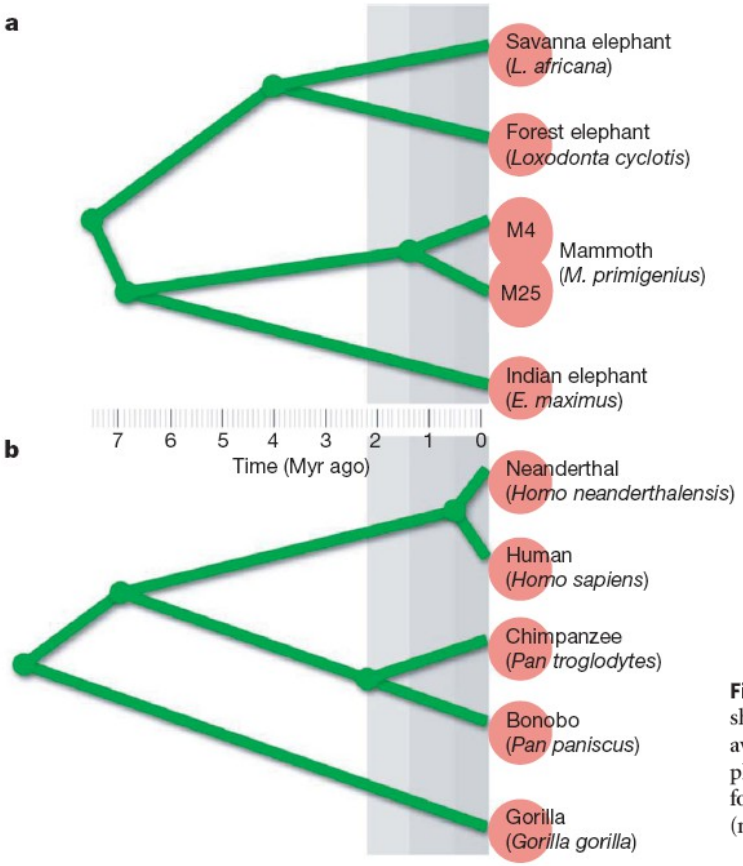


Figure 3 | Comparison of phylogenies. **a**, Elephantids; **b**, hominoids. We show estimated divergence times, that is, times to the common ancestor averaged across autosomes (see Methods). Red circles at the leaves of the phylogenetic tree indicate discernable species. This distinction was not made for the two clades of mammoth (M4 and M25) based on the fossil record (merged red circles).

DNA from a 38,000-year-old Neandertal is revitalizing the once-moribund field of ancient DNA, and it promises a fresh perspective on how we differ from our closest relatives

The Dawn of Stone Age Genomics

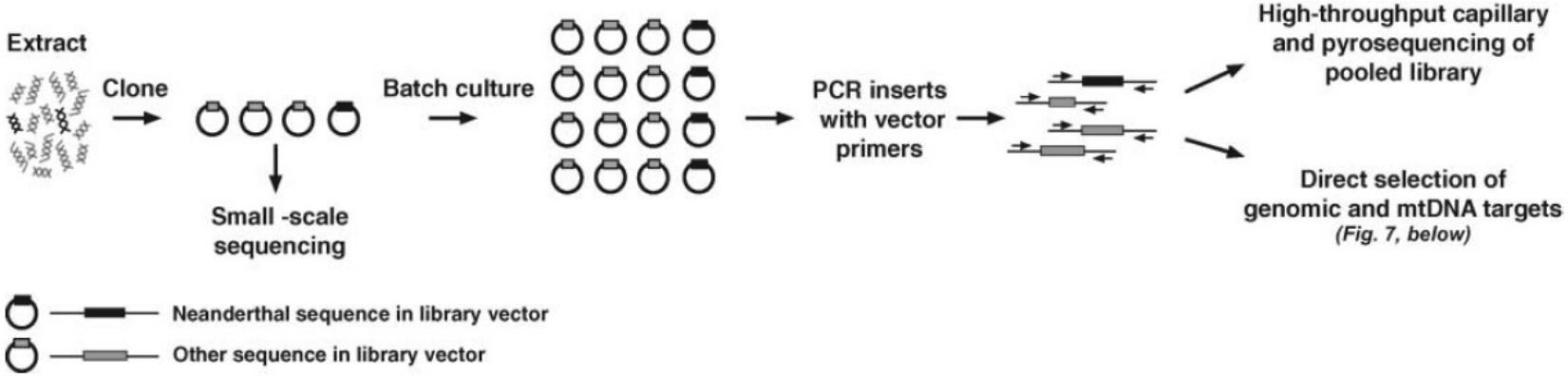
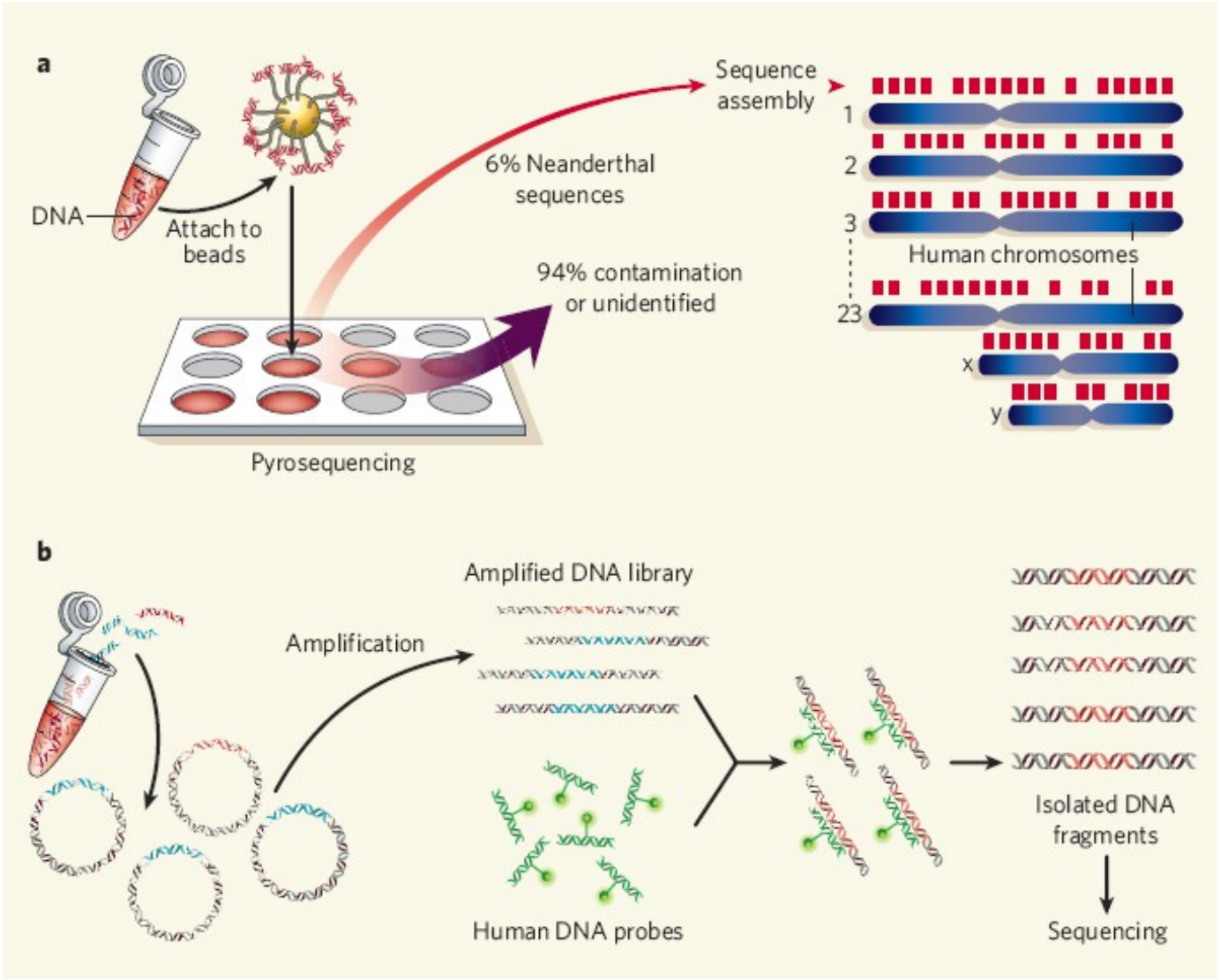


Fig. 1. Generation of ancient metagenomic library DNAs for direct selection and pyrosequencing.

Table 1. Amount of unique Neanderthal sequence obtained from library NE1 by Sanger sequencing of individual clones, as well as Sanger sequencing and pyrosequencing of clones in batch culture. n.a., not applicable.

	Individual clones	Batch culture	
Sequencing chemistry	Sanger	Sanger	Pyrosequencing
Reads	9984	19,200	1,474,910
Average insert	134 bp	196 bp	n.a.
Average BLAST hit	52 bp	52 bp	48 bp
Unique loci	131	69	1126
Total unique hominid sequence	6845 bp	4103 bp	54,302 bp



NATURE|Vol 444|16 November 2006

A Draft Sequence of the Neandertal Genome

Richard E. Green,[1]*†‡ Johannes Krause,[1]†§ Adrian W. Briggs,[1]†§ Tomislav Maricic,[1]†§ Udo Stenzel,[1]†§ Martin Kircher,[1]†§ Nick Patterson,[2]†§ Heng Li,[2]† Weiwei Zhai,[3]†|| Markus Hsi-Yang Fritz,[4]† Nancy F. Hansen,[5]† Eric Y. Durand,[3]† Anna-Sapfo Malaspinas,[3]† Jeffrey D. Jensen,[6]† Tomas Marques-Bonet,[7,13]† Can Alkan,[7]† Kay Prüfer,[1]† Matthias Meyer,[1]† Hernán A. Burbano,[1]† Jeffrey M. Good,[1,8]† Rigo Schultz,[1] Ayinuer Aximu-Petri,[1] Anne Butthof,[1] Barbara Höber,[1] Barbara Höffner,[1] Madlen Siegemund,[1] Antje Weihmann,[1] Chad Nusbaum,[2] Eric S. Lander,[2] Carsten Russ,[2] Nathaniel Novod,[2] Jason Affourtit,[9] Michael Egholm,[9] Christine Verna,[21] Pavao Rudan,[10] Dejana Brajkovic,[11] Željko Kucan,[10] Ivan Gušic,[10] Vladimir B. Doronichev,[12] Liubov V. Golovanova,[12] Carles Lalueza-Fox,[13] Marco de la Rasilla,[14] Javier Fortea,[14]¶ Antonio Rosas,[15] Ralf W. Schmitz,[16,17] Philip L. F. Johnson,[18]† Evan E. Eichler,[7]† Daniel Falush,[19]† Ewan Birney,[4]† James C. Mullikin,[5]† Montgomery Slatkin,[3]† Rasmus Nielsen,[3]† Janet Kelso,[1]† Michael Lachmann,[1]† David Reich,[2,20]*† Svante Pääbo[1]*†

Neandertals, the closest evolutionary relatives of present-day humans, lived in large parts of Europe and western Asia before disappearing 30,000 years ago. We present a draft sequence of the Neandertal genome composed of more than 4 billion nucleotides from three individuals. Comparisons of the Neandertal genome to the genomes of five present-day humans from different parts of the world identify a number of genomic regions that may have been affected by positive selection in ancestral modern humans, including genes involved in metabolism and in cognitive and skeletal development. We show that Neandertals shared more genetic variants with present-day humans in Eurasia than with present-day humans in sub-Saharan Africa, suggesting that gene flow from Neandertals into the ancestors of non-Africans occurred before the divergence of Eurasian groups from each other.

SCIENCE VOL 328 7 MAY 2010

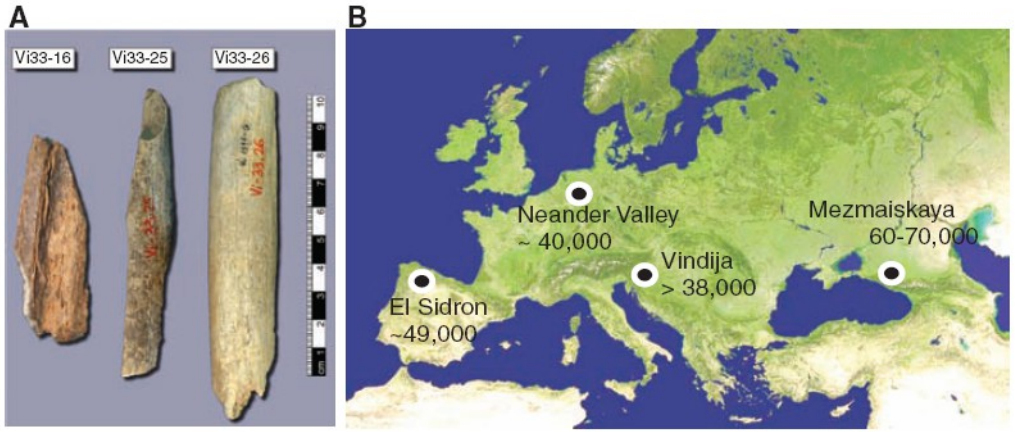


Fig. 1. Samples and sites from which DNA was retrieved. (**A**) The three bones from Vindija from which Neandertal DNA was sequenced. (**B**) Map showing the four archaeological sites from which bones were used and their approximate dates (years B.P.).

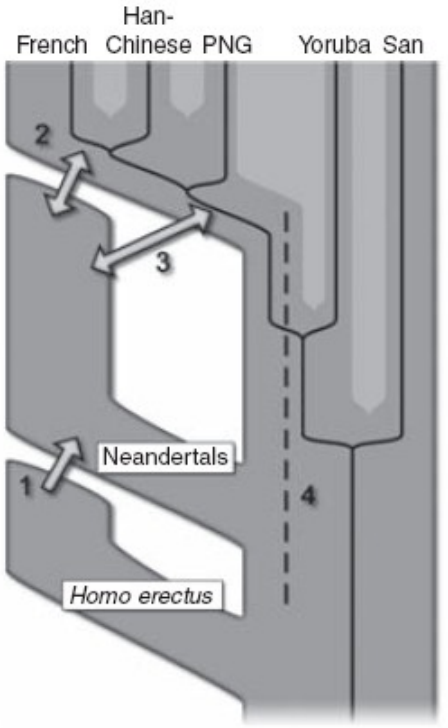
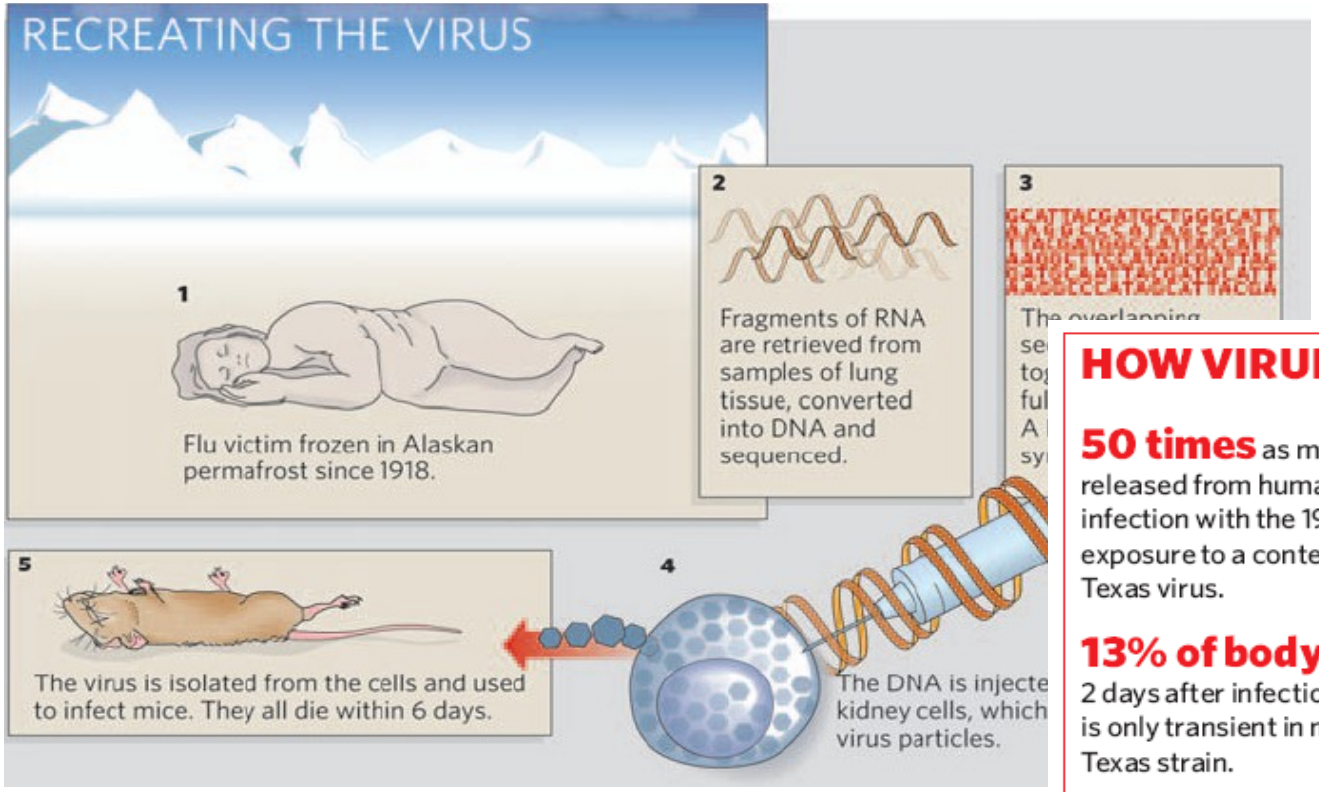


Fig. 6. Four possible scenarios of genetic mixture involving Neandertals. Scenario 1 represents gene flow into Neandertal from other archaic hominins, here collectively referred to as *Homo erectus*. This would manifest itself as segments of the Neandertal genome with unexpectedly high divergence from present-day humans. Scenario 2 represents gene flow between late Neandertals and early modern humans in Europe and/or western Asia. We see no evidence of this because Neandertals are equally distantly related to all non-Africans. However, such gene flow may have taken place without leaving traces in the present-day gene pool. Scenario 3 represents gene flow between Neandertals and the ancestors of all non-Africans. This is the most parsimonious explanation of our observation. Although we detect gene flow only from Neandertals into modern humans, gene flow in the reverse direction may also have occurred. Scenario 4 represents old substructure in Africa that persisted from the origin of Neandertals until the ancestors of non-Africans left Africa. This scenario is also compatible with the current data.

The 1918 flu virus is resurrected

nature
Vol 437|6 October 2005



HOW VIRULENT IS 1918 FLU?

50 times as many virus particles are released from human lung cells a day after infection with the 1918 virus as are released after exposure to a contemporary strain called the Texas virus.

13% of body weight is lost by mice 2 days after infection with 1918 flu; weight loss is only transient in mice infected with the Texas strain.

39,000 times more virus particles are found in mouse lung tissue 4 days after infection with 1918 flu than are found with the Texas virus.

All mice died within 6 days of infection with 1918 flu; none died from the Texas strain.

Characterization of the 1918 influenza virus polymerase genes

Jeffery K. Taubenberger[1], Ann H. Reid[1]†, Raina M. Lourens[1]†, Ruixue Wang[1], Guozhong Jin[1] & Thomas G. Fanning[1]

Vol 437|6 October 2005|doi:10.1038/nature04230

Table 1 | Amino acid residues distinguishing human and avian influenza polymerases

Gene	Residue no.	Avian	1918	Human H1N1	Human H2N2	Human H3N2	Classical swine	Equine
PB2	199	A	S	S	S	S	S	A
PB2	475	L	M	M	M	M	M	L
PB2	567	D	N	N	N	N	D	D
PB2	627	E	K	K	K	K	K	E
PB2	702	K	R	R*	R	R	R	K
PB1	375	N/S/T†	S	S	S	S	S	S
PA	55	D	N	N	N	N	N	N
PA	100	V	A	A	A	A	V	A
PA	382	E	D	D	D	D	D	E
PA	552	T	S	S	S	S	S	T

*All human H1N1 PB2 sequences have an Arg residue at position 702, except that two out of three A/PR/8/34 sequences have a Lys residue.
†The majority of avian sequences have an Asn residue at position 375 of PB1, 18% have a Ser residue, 13% a Thr residue.