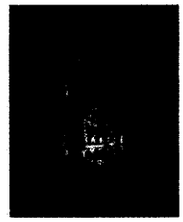
LETTERS

Convincing tests



Independent reviews are said to have concluded that the planned National Ignition Facility would likely achieve "fusion ignition" and be useful for nuclear weapons "stockpile stewardship." "Animal teeth and bones" found along with Neandertal and Cro-Magnon remains are used to assist in the "molecular investigation" of the origins of humankind (right, a Neandertal skull from France). And methods for "precise editing of chromosomal and extrachromosomal sequences" in mouse and other mammalian cells are discussed.

NIF: "Harsh Light" or "Illumination"?

The News & Comment article "A harsh light falls on NIF" by James Glanz and with sidebars by him and Andrew Lawler (18 July, p. 304), is accurate and informative. The article does convey some of the excitement of laboratory fusion ignition and the sense that the National Ignition Facility (NIF) will have broad value for science. However, because of the title and some of the quotes selected early in the article, the sense of controversy is exaggerated. NIF is not a weapon, and its use for nuclear weapon assessment requires scientists to integrate results from laser-based experiments with computations and other experience; thus, judgements on NIF's use in stockpile stewardship may differ. Laboratory ignition has not been achieved and cannot be guaranteed; this can be used to suggest controversy, but many highly respected physicists are willing to bet their careers on ignition with NIF.

Because the mission value and the likelihood of full success of NIF require expert judgements, a number of independent reviews have been conducted since 1990, including those by the JASON group (twice), the Fusion Policy Advisory Committee, the 1990 National Academy of Sciences, and the Inertial Confinement Fusion Advisory Committee (several reports). These and other reviews represent significant effort by qualified scientists to examine the issues discussed in the article. The reviews have examined the issues from every perspective and have established a well-founded view that NIF will have significant value to stockpile stewardship and that ignition is likely. These reviews serve to reduce controversy on these issues, and they deserved more weight in the article.

Accomplishing the goals of NIF is a considerable challenge, as it should be. A better theme for the article could have been, "Illuminating the NIF challenge."

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Neandertal Genetics

The recent discussion regarding Neandertal molecular studies (P. Kahn and A. Gibbons, Research News, 11 July, p. 176) suggests that other Neandertal and Cro-Magnon specimens should be examined for modern human mitochondrial haplotypes to further test multiregionalism (*1*). A Neandertal sequence from another site would also corroborate the initial study (*2*) and provide valuable data about the population structure of this temporally and spatially diverse group. To investigate the possibility that Paleolithic sites other than the Neander valley will be amenable to molecular investigation, we examined remains from four important Neandertal and Cro-Magnon sites in southern Europe, plus control samples of similar or younger age that are known to contain ancient DNA.

Contamination of preserved remains with modern human DNA is a significant problem in ancient DNA research (*3*), seriously complicating Neandertal studies (*2*). We avoided this problem by using relatively unimportant animal teeth and bones associated with Neandertal and Cro-Magnon remains as a proxy to determine the suitability of sites for molecular studies of human material. We analyzed several biochemical parameters indicative of diagenetic change (chemical modification associated with preservation processes) and DNA preservation (*4*, *5*) before using an-

Table 1. Biochemical and histological data from Neandertal, Cro-Magnon, cold-preserved, and Holocene material. PCR reactions performed as described in (*6*) by using primers for 12*S* and control region*; 12*S*, cytochrome b, and control region†; 12*S*‡; and control region§. Histology scores range from 1 (poor) to 5 (good). N/A. experiment not performd.

Site	Age (kyr)	Taxa	Common name and sample	Aa racemization (D/L Asp, D/L Ala)	Aa (ppm)	% N	Histology (1–5)	Amplifiable DNA (bp)
			Neandertal					
Zafarraya, Spain	33 (*7*)	*Capra pyrenaica*	Goat tooth	0.10, 0.04	2,750	0.07	1	No*
	〃	〃	Goat tooth	0.12, 0.04	3,400	0.25	1	No*
Krapina, Croatia	113 (*8*)	*Ursus spelaeus*	Bear tooth	0.16, N/A	900	0.18	N/A	No†
	〃	〃	Bear bone	0.17, 0.04	450	0.09	1	No†
	130 (*8*)	*Dicerorhinus mercki*	Rhino tooth	0.18, 0.03	700	0.36	N/A	No†
	〃	〃	Rhino bone	N/A, N/A	N/A	0.61	1	No†
	〃	*Bison priscus*	Bison tooth	0.13, 0.02	7,600	0.54	1	No†
La Chaise, Suard, France	120–200 (*12*)	Neandertal	Neandertal bone	0.53, N/A	15	0.05	1	N/A
	〃	〃	Neandertal tooth	0.20, 0.05	700	0.15	1	N/A
	〃	*Equus* sp.	Horse tooth	0.23, 0.07	80	0.02	1	No‡
			Cro-Magnon					
Nerja, Spain	18 (*13*)	Cro-Magnon	*H. sapiens* tooth	0.36, 0.48	170	N/A	N/A	No§
	7–18 (*13*)	*Aves* sp.	Bird bone	0.14, 0.04	9,200	0.29	1	No‡
	〃	*Aves* sp.	Bird bone	0.11, 0.34	17,200	0.21	1	No‡
			Holocene/permafrost					
Barcelona, Spain	2	Modern human	H. sapiens tooth	0.10, 0.05	33,000	3.50	4	D-loop (120)
Otago, New Zealand	1–3 (*14*)	*Euryapteryx geranoides*	Bird bone	0.07, 0.02	79,000	3.80	5	12*S*, cyt b (300)
Canterbury, New Zealand	3.5 (*14*)	*Emeus crassus*	Bird bone	0.06, 0.02	57,000	4.32	5	12*S*, cyt b (300)
Fairbanks, Alaska	27 (*5, 9*)	*Equus hemionus*	Horse bone	0.07, 0.01	68,000	4.80	5	16S (140)
Shandrin, Siberia	35–40 (*5, 9*)	*Mammuthus primigenius*	Mammoth tissue	0.06, 0.01	98,000	N/A	5	16S (200)

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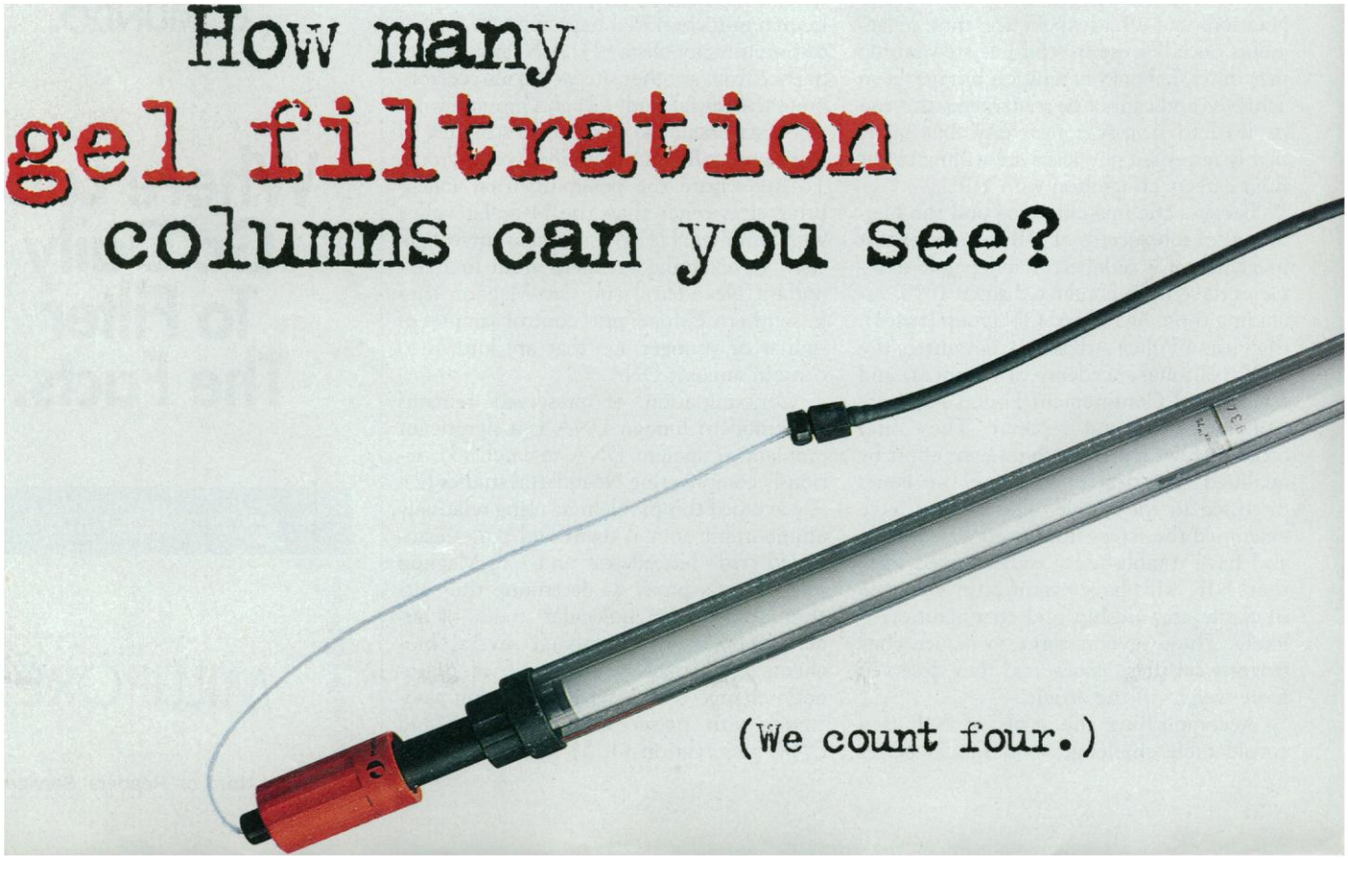

cient DNA techniques and the contamination-prone polymerase chain reaction (PCR) to amplify preserved DNA (*6*). PCR primers were designed to match faunal, but not human, sequences, removing the problem of modern human contamination.

We obtained faunal and human samples from three key Neandertal sites. Zafarraya, in southern Spain, is one of the youngest sites known (33,000 years old) (*7*), the Krapina collection from Croatia is the most extensive (110,000 to 130,000 years old) (*8*), and La Chaise is a classic Bordeaux site in France. Table 1 presents the results from analyses of Neandertal and Cro-Magnon sites, as well as from permafrost and Holocene controls. Samples from the Paleolithic sites are characterized by poor overall amino acid (Aa), nitrogen (N), and histological preservation and moderate to highly racemized aspartic acid (Asp) and alanine (Ala) residues. These values suggest extensive diagenetic modification (*4*, *5*); correspondingly, we were not able to amplify authentic DNA from any of these specimens. In contrast, the permafrost and Holocene samples appear to have little diagenetic modification, and amplifications of short mitochondrial DNA (mtDNA) sequences can be reproducibly obtained from this material (*5*, *9*).

The biochemical data indicate that material from these Neandertal sites, and even relatively young Mediterranean deposits, exhibit signs of considerable diagenetic change and appear unsuitable for DNA analysis. Therefore, preserved Neandertal DNA is likely to be rare, and the DNA in the type specimen may result from its unique preservation conditions. The Neander valley is one of the northernmost Neandertal sites known, and it is not far south of the limit of maximum glaciation during the late Pleistocene. Consequently, the site is likely to have experienced cold or periglacial conditions for the majority of the time since the skeleton was deposited. This is important because low temperatures are known to assist DNA preservation over long time periods (*10*), as shown by the data from the permafrost samples. In contrast, the Paleolithic sites in this study are from areas with elevated mean temperatures and, like other prehistoric remains in the Levant (*11*), show large amounts of diagenetic alteration.

These findings suggest that pre-Holocene hominid material from most southern European and northern African areas will be unsuitable for molecular analyses. Most Neandertal specimens are therefore unlikely to contain amplifiable DNA, and should only be subjected to destructive analysis after associated faunal remains are investigated. Accordingly, further tests of the multiregionalist hypothesis using Neandertal DNA will be difficult at best.

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Kahn and Gibbons discuss the findings of a team of molecular biologists led by Svante Pääbo—the sequencing of a tiny fragment of mtDNA from the upper arm bone of the original Neandertal skeleton, recovered from a limestone quarry in Germany in 1856. While this is an astounding technical breakthrough (it is difficult to recover DNA from ancient bone) and an important result, what it means with respect to modern human origins, and the Neandertals' relationship to us, remains open to question.

Molecular clock models are full of problematic assumptions. Leaving aside differences of opinion about the rate of base pair substitutions, how to calibrate a molecular clock (*1*), and whether or not mtDNA mutations are neutral (*2*), the fact that the Neandertal sequence (and it should be kept in mind that there is—to date—only one) differs from those of modern humans does not resolve the question of whether or not "moderns" and "Neandertals" were different species. This is the primary conclusion of the research, according to the Pääbo team (*3*). Continuity advocates (people who think that Neandertals contributed genetically to people of western Eurasian ancestry) argue that differences were only subspecific (*4*). Harvard geneticist Maryellen Ruvolo is quoted as saying that the genetic variation between the modern and the Neandertal sequences is within the range of other single species of primates and that there is in fact no genetic "yardstick" that might allow us to define a species. A more convincing test of the implications of the Pääbo data would be to sequence mtDNA from an unambiguously modern early European (the suggested candidate, Cro-Magnon, has not been dated) or, better yet, from alleged archaic and modern human fossils from the Israeli cave sites of Skhul and Qafzeh (supposedly "modern") and Kebara and Tabun (supposedly "Neandertals"). If the Israeli fossils all show differences with modern humans of the same order of magnitude of those between Neandertals and moderns, that would be compelling evidence that *all* Upper Pleistocene hominids diverge from moderns by about the same amount (evidence for continuity) and that the distinction between Neandertals and moderns in the Levant, at least, is without foundation.

That researchers cannot distinguish a "Neandertal" from a "modern human" might seem surprising to some, but there is little consensus on what these terms mean. Arizona State University researcher Catherine Willermet reexamined the criteria used to classify and describe Neandertal and early modern human skulls by continuity and replacement advocates and found that only 11% of the variables were common to both groups of workers (*5*). In short, continuity and replacement advocates are using different sets of variables to assess differences and similarities and are defining differently those variables held in common.

A single DNA sequence from a single, undated skeleton can be interpreted in many different ways. If we are to come up with a satisfactory explanation for our origins, it should reconcile pattern searches in archaeology and in human paleontology, as well as in molecular biology. Those who would argue that Neandertals became extinct without issue should show how it could have occurred without leaving traces of disjunction in the archaeological record and in fossils themselves.

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Mouse Engineering

Eliot Marshall's article "The mouse that prompted a roar" (News, 4 July, p. 24) discusses some of the constraints faced by researchers using the Cre-*loxP* site-specific recombination system. In fact, there are alternatives to the Cre-*loxP* system that can be used in mice.

The FLP site-specific recombinase system will also mediate precise editing of chromosomal and extrachromosomal sequences in mammalian cells (*1*) and in mice (*2*). Although the relative efficiencies of FLP and Cre in specific murine tissues remain to be determined, FLP works well in many tissues of transgenic mice. Several groups have found that FLP recombines chromosomal targets in murine embryonic stem cells less efficiently than does Cre, but it may be that relative efficiencies will be found to be different for other cell types or tissues.

The Salk Institute has obtained allowed claims for some applications of FLP in mammalian systems and will disseminate FLP-related research materials to the academic community under a simple Materials Transfer Agreement. It will not attempt to regulate or impede the transfer of FLP-related materials among researchers, and also offers nonexclusive commercial licenses under reasonable terms.

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