and were then screened by PCR for acquisition of a higher molecular mass product, *cph1::hisG*. This process of transformation and 5-fluoroorotic acid treatment was repeated to disrupt the second copy of *CPH1*. *cph1/cph1* isolates were screened by PCR for complete loss of the wild-type *CPH1* PCR fragment.
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12 August 1994; accepted 7 October 1994

TECHNICAL COMMENTS

The Entropic Cost of Binding Water to Proteins

In a recent Perspective (*1*) about the entropic cost of binding water molecules to proteins and other macromolecules, Jack D. Dunitz uses thermodynamic data on water, anhydrous salts and their hydrates to set limits on the entropy decrease for transferring a water molecule from liquid water to the macromolecule. The limits set were 0 to 7 cal mol^{-1} K^{-1} with larger entropy decreases corresponding to more tightly held waters. Dunitz also states that thermodynamic data from which these entropy changes can be directly calculated are "nonexistent." Such data do exist and calculations of these entropy changes have been reported in the literature.

Data for calculating dS/dn, the entropy change occuring when a mole of water is transferred from liquid water to solid protein, as a function of n, the moles of water bound per mole of protein, can be obtained from measurements of sorption isotherms of water vapor on solid proteins at several temperatures (*2*). Isotherms of water vapor on proteins generally exhibit hysteresis, but it has been shown that correct entropy calculations can be made even in the absence of isotherm reversibility (*3*). For example, calculations of dS/dn values for water bound to native ovalbumin (*2*) have been made on data from the literature (*4*). The variation of such entropies with n has also been discussed (*5*). Values of $-dS/dn$ for ovalbumin varied from approximately 0 to 12 cal mol^{-1} K^{-1} for absorption isotherms and from approximately 0 to 20 cal mol^{-1} K^{-1} for desorption isotherms. Larger entropy decreases were generally seen at lower values of n. The fact that some of these entropy decreases are greater than the estimated limit of 7 cal mol^{-1} K^{-1} suggests that the binding of more tightly held waters to a protein can cause a decrease in protein entropy as well as a decrease in water entropy. Thus, uptake of water could lead to fewer, or more ordered protein conformations, or both.

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7 July 1994; accepted 3 November 1994

Dating Hominid Sites in Indonesia

C. C. Swisher *et al.* (*1*) recently published two new dates for hominid sites in Java based on $^{40}Ar/^{39}Ar$ laser-incremental heating analyses. They propose mean-weighted ages of 1.81 ± 0.04 million years ago (Ma) for the Mojokerto and 1.66 ± 0.04 Ma for the Sangiran site. On the basis of these dates they draw far-reaching conclusions about the early migration of the ancestor of *Homo erectus* out of Africa as well as an explanation for the absence of the Acheulean stone tool culture in Asia. These new $^{40}Ar/^{39}Ar$ ages are based on hornblende separated from pumice recovered at Sangiran and Mojokerto. However, the geological context of these hornblende samples is not clear, and the new ages are contradicted by a wide range of established data.

A discrepancy of about 0.9 Ma between the $^{40}Ar/^{39}Ar$ ages (1.81 and 1.66 Ma) given by Swisher *et al.* and the existing magnetostratigraphy [which is based on detailed sections of Sangiran (0.97 to 0.73 Ma) and Mojokerto (0.97 Ma) reported by Hyodo *et al.* (*2*) in 1993] is not adequately explained by Swisher *et al.* The Hyodo *et al.* (*2*) magnetostratigraphy, based on a solid lithostratigraphy (*3*), corroborates perfectly with a series of fission track ages (*4*) indicating dates all less than 1.0 Ma. In this light, the geological context of samples which yielded the older dates must be critically reviewed. At the Mojokerto site the pumice was taken from a conglomeratic volcanic sandstone, which invites the interpretation that the pumice was likely reworked and redeposited. Swisher *et al.* state, about the Sangiran sample, that the pumice was handpicked from a volcanic pumice-rich layer. There is inadequate information about the lithostratigraphy and exact stratigraphic position of this sample in the Sangiran section and about the relationship of the volcanic pumice-rich layer to the high number of well-described and recognizable tuff layers in the Sangiran area of which some have fission track data (*3*).

There is agreement between the normal polarity found at the Mojokerto site by Swisher *et al.* (*1*) and that reported by Hyodo *et al.* (*2*) but, on the basis of the $^{40}Ar/^{39}Ar$ age of 1.8 ± 0.04, Swisher *et al.* place this site in the Olduvai event. On the basis of the paleomagnetic properties of the section in Sangiran as well as in Mojokerto, Hyodo *et al.* (*2*) demonstrate that the normal polarity of these sites represent the Jaramillo event, which suggests an age of approximately 0.97 Ma. We see no reason to doubt this paleomagnetic sequence, which is also corroborated by fission track ages (*4*). In addition to the discrepancy of the new $^{40}Ar/^{39}Ar$ ages compared with the paleomagnetic and fission track data, the biostratigraphy of Sangiran and Mojokerto (*5*) contradict the newly proposed ages for these sites. Trinil, which contains the type specimen of *Homo erectus* discovered by Dubois (*6*) and is characterized by Stegodon, is widely considered to have an age of about 1 Ma. The Kedung Brubus fauna, characterized by new arrivals of the Asiatic mainland, like *Elephas*, to which the Mojokerto fauna belongs, is younger (*5*) than the Trinil fauna based on all key biostratigraphic markers (*5*).

The $^{40}Ar/^{39}Ar$ dates of Swisher *et al.* may themselves be "technically correct," but until their geological context is established, it is premature to attach such far reaching conclusions to these new age estimates for the hominid of Java.

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6 April 1994; accepted 2 August 1994

Response: Much of the confusion over the geologic age of Asian *Homo erectus* stems from the lack of high-precision radioisotopic dates directly associated with hominid fossil localities. In contrast with the well-dated fossil hominid record of East Africa, the age of most Asian hominids is derived from their association with certain vertebrate fossils that are considered to be key indexes of geologic time, or from the correlation of fossil-bearing strata with lithologic formations of reported known age, or both. These methods work if the age of the index fossil is well known and if the geologic formation assigned to the accompanying sediments is temporally correlative over broad geographic regions. Unfortunately, this is often not the case. One of the main objectives of the research in our original report (*1*) was to obtain accurate $^{40}Ar/^{39}Ar$ dates on volcanic rocks that are associated directly with hominid sites in Asia, thus avoiding preconceived notions as to the age of correlative lithological units or fossil datums. The two sites we chose (*1*) were those of Mojokerto and Sangiran, Java, which have yielded some of the oldest hominids in Asia. We (*1*) reported mean $^{40}Ar/^{39}Ar$ ages of 1.81 ± 0.04 Ma and 1.66 ± 0.04 Ma, respectively, for these two sites on the basis of incremental laser heating of volcanic hornblende separated from pumice found directly in the reported localities where the fossil hominids were discovered. In contrast, de Vos and Sondaar reiterate a commonly reported view that fossil hominids in Java are no older than 1.0 Ma. The marked difference stems primarily from interpretations of paleomagnetic and fission track studies as recently summarized in Hyodo *et al.* (*2*) and paleontological interpretations as summarized by de Vos (*3*).

At Mojokerto and Sangiran, Hyodo *et al.* (*2*) report a series of westerly deflected paleogeomagnetic directions within a reversed interval that they correlate with the Matuyama Chron. The westerly deflected paleomagnetic directions were considered by Hyodo *et al.* (*2*) to represent a unique long-term (200,000-year) geomagnetic excursion, which was used to correlate the section at Mojokerto with that at Sangiran. At Mojokerto, an interval of normal geomagnetic polarity from which the Mojokerto hominid that was discovered occurs above this excursion and was correlated by Hyodo *et al.* (*2*) with the Jaramillo event, which ranges in age from 0.99 to 1.07 Ma in the most recent Geomagnetic Polarity Time Scale (GPTS) calibrations (*4*).

Evaluation of the paleomagnetic data reported in Hyodo *et al.* (*2*) and detailed thermal demagnetization studies on new samples from Mojokerto (*5*) indicate that the westerly deflections directions do not represent a geomagnetic excursion. More likely the westerly deflected directions reported by Hyodo *et al.* (*2*) are a result of strong geomagnetic overprints that were not discriminated by their alternating field demagnetization techniques; an excursion of this magnitude has not been recorded in well-studied rocks elsewhere in the world. The odd westerly directions reported by Hyodo *et al.* (*2*) are most likely a result of an averaging of a reversed polarity direction recorded in magnetite (Matuyama) with a strong normal geomagnetic overprint in goethite, or hematite, or both. Without a unique geomagnetic excursion, there is no independent means of correlating the Mojokerto and Sangiran sections or for that matter any means of unambiguously correlating the short paleomagnetic section at Mojokerto with the GPTS. Consequently, the only independent age control for the normal geomagnetic interval from which the Mojokerto hominid was recovered is the $^{40}Ar/^{39}Ar$ date of 1.8 ± 0.04 Ma reported by us (*1*). This date for normal polarity rocks argues for a correlation with the Olduvai Subchron (*4*) with an age range of 1.77 to 1.95 Ma (*1*, *4*), not the Jaramillo normal event as proposed by Hyodo *et al.* (*2*).

Many of the previously reported fission-track ages for the Sangiran section can now be interpreted as too young, because they disagree with the new $^{40}Ar/^{39}Ar$ dates and numerous dates of the Brunhes-Matuyama boundary (*1*, *2*, *4*). Although there are at least five horizons at Sangiran that have been dated by fission-track methods (*2*, *6*), I know of none on volcanic rocks that are in direct association with fossil hominids S27/31, nor am I aware of any published correlation of tephra associated with S27/31 with rocks dated elsewhere at Sangiran (*6*). The $^{40}Ar/^{39}Ar$ age of 1.66 ± 0.4 Ma for fossil hominids S27/31 reported by my colleagues and I is the only (*1*) date in direct association with the fossil recovery site. This date is consistent with age range estimates for the Pucangan Fm at Sangiran (*6*); however, differences of opinion remain as to the stratigraphic correlation of some of the sections at Sangiran.

The use of proboscideans (for example *Stegodon*, *Elephas*, or *Archidiskodon*) as unique indicators of time as proposed by de Vos (*3*) and Sondaar and de Vos assumes that the age of the earliest appearance of those taxa in Java is accurately known. My opinion is that this is simply not the case. Although de Vos (*3*) and Sondaar and de Vos argue that the first occurrences of these proboscideans in Java are widely known to be no earlier than 1.0 Ma, I would point out that all of these genera are known to occur in Pliocene rocks (older than 1.7 Ma) on mainland Asia as determined by recent paleomagnetic studies (*7*). Consequently, I am not aware of any constraints that prevent occurrences of proboscideans on Java earlier than 1.0 Ma or any restriction that would determine their sequence of arrival. In addition, I know of no record of these genera proboscideans at the Mojokerto site itself as indicated by de Vos and Sondaar that would preclude any age assignment on the basis of faunal correlations. Although proboscideans have been reported from the nearby site of Jetis, I am unconvinced of its faunal association with Mojokerto and I am further unaware of any published stratigraphic correlations of the two sites.

Finally, I agree with de Vos *et al.* that in many ways it is premature to draw too many conclusions from only two dated horizons. My colleagues and I are currently analyzing newly discovered volcanics at Mojokerto, Sangiran, and at sites in West Java—in conjunction with detailed paleomagnetic studies using thermal demagnetization techniques. This ongoing study will no doubt provide additional tests for the $^{40}Ar/^{39}Ar$ ages (*1*) in calibrating the Plio-Pleistocene fossil record of Java.

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4 October 1994; accepted 5 October 1994