reassessments of the balance between pelagic production and respiration in lakes (*11*). Our data suggest that the transport of carbon from land to water is an important control on the carbon budget in most lakes.

We can use the frequency distribution of P_{CO_2} to estimate the potential contribution of CO_2 from lakes to the atmosphere. We assume an evasion coefficient of 0.5 m day^{-1} for all lakes, and for undersaturated lakes an enhancement factor of 3 (*12*). Globally, lakes (an area of 2×10^{12} m^2) could contribute CO_2 to the atmosphere in the amount of 0.14×10^{15} g of carbon per year. This flux is slightly less than half as great as the total export of organic plus inorganic carbon from rivers to the sea (*13*), is larger than recent estimates of total organic carbon burial in lake sediments [0.06×10^{15} g (*14*)], and is comparable to organic carbon burial in reservoirs [0.2×10^{15} g (*14*)]. Lakes have longer hydrologic residence times than do flowing waters, which may allow for both the degassing of CO_2 derived from soil respiration and possibly for increased respiration of organic materials derived from the catchment.

REFERENCES AND NOTES

1. G. W. Kling, G. W. Kipphut, M. C. Miller, *Science* **251**, 298 (1991).
2. P. J. Coyne and J. J. Kelley, *Limnol. Oceanogr.* **19**, 928 (1974); J. J. Kelley, *J. Earth. Sci. Nagoya Univ.* **35**, 341 (1987).
3. D. W. Schindler *et al.*, *Science* **177**, 1192 (1972); T.-H. Peng and W. S. Broecker, *Limnol. Oceanogr.* **25**, 789 (1980); S. Kempe, *Mitt. Geol.-Paleontol. Inst. Univ. Hamburg* **52**, 91 (1982); J. W. M. Rudd *et al.*, *Ambio* **22**, 246 (1993).
4. G. W. Kling, G. W. Kipphut, M. C. Miller, *Hydrobiologia* **240**, 23 (1992), and references therein. In all cases adjustments were made for temperature, altitude, and ionic strength. Where complete chemical data were not available, we estimated ionic strength from conductivity [J. N. Butler, *Carbon Dioxide Equilibria and Their Applications* (Lewis, Detroit, 1992)]. Where complete chemical data were available, we also corrected for the effects of specific ion complexes on the activities of both bicarbonate and carbonate, using MINEQL version 2.3 [W. D. Schecher and D. C. McAvoy, *MINEQL⁺ User's Manual* (Procter and Gamble, Oxnard, CA, 1991)] for most data sets and PHREEQ [D. L. Parkhurst, D. C. Thorstenson, L. N. Plummer, *U.S. Geol. Surv. Water Res. Inv. 80-96*, (1990)] for the lakes of the North Temperate Lakes Long Term Ecological Research (LTER) program.
5. R. H. Hesslein, J. W. M. Rudd, C. Kelly, P. Ramlal, K. A. Hallard, in *Air-Water Mass Transfer*, S. C. Wilhelms and J. S. Gulliver, Eds. (American Society of Civil Engineers, Boston, 1991), p. 413.
6. D. H. Landers, W. S. Overton, R. A. Linthurst, D. A. Brakke, *Environ. Sci. Technol.* **22**, 128 (1988); R. A. Linthurst *et al.*, *Environ. Prot. Agency (U.S.) Publ. 600/4/4-86/07A* (1988). In this study a number of redundant chemical measurements were made, and P_{CO_2} can be computed in several ways, all of which lead to the same conclusions. For example, a linear regression of P_{CO_2} calculated from the change in DIC before and after air equilibration against P_{CO_2} calculated from nonequilibrated pH and DIC has a slope of 0.99 ± 0.03 and a correlation coefficient, r^2, of 0.70.
7. During the calculation of P_{CO_2} from pH and ANC, overestimation of true P_{CO_2} may be caused by the presence of noncarbonate ANC. This effect is strongest at low values of ANC; we excluded from our ANC-based calculations samples with ANC below 40 μeq to minimize this effect.
8. We found a strong, unbiased relation between P_{CO_2} directly measured versus P_{CO_2} calculated from pH and DIC. A linear regression of measured versus calculated P_{CO_2} ($N = 330$ samples) has an r^2 of 0.88 and a slope of 1.03 ± 0.01.
9. A. L. Herczeg and R. H. Hesslein, *Geochim. Cosmochim. Acta* **48**, 837 (1984); A. L. Herczeg *et al.*, *Nature* **315**, 133 (1985).
10. J. E. Richey *et al.*, *Science* **202**, 1183 (1978); M. J. Jordan and G. E. Likens, *Verh. Int. Verein. Theoret. Angew. Limnol.* **10**, 994 (1975); D. L. Galat, *Int. Rev. Gesamten Hydrobiol.* **71**, 621 (1986); P. D. Quay *et al.*, *Limnol. Oceanogr.* **31**, 596 (1986).
11. P. A. delGiorgio and R. H. Peters, *Can. J. Fish. Aquat. Sci.* **50**, 282 (1993).
12. Evasion of CO_2 from supersaturated water was calculated as:

$$\mathrm{Flux} = D/z[(CO_2)_{aq} - K_h(P_{CO_2})_{air}]$$

where K_h is Henry's constant, D is the temperature-dependent diffusion coefficient, and z is the surface boundary layer. An evasion coefficient (D/z) of 0.5 m day^{-1} would be equivalent to $z = 300$ μm at 22°C (or $z = 200$ μm at 10°C) and is well within published values for lakes. In undersaturated water, atmospheric CO_2 may react chemically with hydroxyl or carbonate ions more rapidly than it would diffuse passively [S. Emerson, *Limnol. Oceanogr.* **20**, 743 (1975)]. We used an enhancement factor of 3 to compute flux into undersaturated waters.

13. M. Meybeck, *Water Air Soil Pollut.* **70**, 443 (1993).
14. P. J. Mullholland and J. W. Elwood, *Tellus* **34**, 490 (1982).
15. G. E. Likens, Ed., *An Ecosystem Approach to Ecology: Mirror Lake and Its Watershed* (Springer-Verlag, New York, 1985).
16. N. F. Caraco *et al.*, *Hydrobiologia* **253**, 280 (1992); *Verh. Int. Verein. Theoret. Angew. Limnol.* **24**, 2985 (1992); N. F. Caraco, unpublished data.
17. References for seasonal data for the 69 lakes are too numerous to list and are available upon request.
18. G. W. Kling, thesis, Duke University (1987).
19. We thank C. Driscoll, K. Postek, C. Bowser, and D. Armstrong for assistance with the computations and M. Pace, G. Likens, and G. Kipphut for thoughtful discussions throughout this work. We thank C. Sandgren, R. Axler, G. Fahnenstiel, C. Driscoll, C. Ellis-Evans, M. Meili, S. Nodvin, and P. del Giorgio for providing published and unpublished data. We thank D. Buso, C. Mason, and E. Morency for making the P_{CO_2} measurements at Mirror Lake and G. Likens, P. Likens, G. Steinhart, R. Miller, P. Raymond, J. Reed, and P. Troell for P_{CO_2} measurements at other sites. Financial support was provided by the National Science Foundation (NSF-DEB 9211775, 9024188, 9317698, 9011660, 9019873). This is a contribution to the Institute of Ecosystem Studies; the Hubbard Brook Ecosystem Study; and the North Temperate Lakes LTER program.

29 April 1994; accepted 27 July 1994

Fossil Evidence for Early Hominid Tool Use

Randall L. Susman

Although several Plio-Pleistocene hominids are found in association with stone and bone tools, it has been generally assumed that at any one time the hominid with the largest brain was the toolmaker. Fossils recovered over the last decade suggest that early hominids subsequent to 2.5 million years ago all might have used tools and occupied "cultural" niches. A test for humanlike precision grasping (the enhanced ability to manipulate tools) is proposed and applied to australopithecines and early *Homo*. The results indicate that tools were likely to have been used by all early hominids at around 2.0 million years ago. The earliest australopithecines, which predate the appearance of stone tools in the archaeological record, do not show signs of advanced precision grasping.

Approximately 2 million years ago (Ma) Africa was home to at least two, and probably more, hominid species (*1*). At least one, but perhaps all, early hominids at this time used implements of bone and stone, but the question of which Plio-Pleistocene hominids engaged in tool behavior (*2*) has been unresolved since the early 1960s (*3*–*5*). Oldowan tools are found throughout Africa beginning around 2.5 to 2.7 Ma. In 1960, the discovery of a partial hand of *Homo habilis* (Olduvai Hominid 7) from Bed I, Olduvai Gorge, prompted the inference that early *Homo* was a toolmaker (*6*) on the basis of anatomical features shared with modern humans. No diagnostic hand fossils of other fossil hominids from East or South Africa were then known. As a result, and because *H. habilis* had a large brain (*7*) and more advanced, humanlike teeth (*8*) and feet (*9*), *H. habilis* was considered to be the principal toolmaker in Bed I times, roughly 2 Ma (*10*).

The idea that *H. habilis*, and not the australopithecines, was the exclusive toolmaker of the Plio-Pleistocene (*11*) was based on the assumption that the first stone toolmakers had relatively large brains (*5*–*7*). This idea has persisted up to the present (*12*). In this report, I test the assumption that brain size is somehow linked to tool behavior and that only one hominid could be a toolmaker at any one point in time, by an analysis of the functional morphology of ape and human hands and relevant hominid fossils. A major problem in determining which hominids made tools stems from a poor knowledge of which traits in the bones of living animals might be diagnostic of tool behavior. After a discussion of morphological correlates of precision grasping in hu-

---

Department of Anatomical Sciences, School of Medicine, State University at Stony Brook, Stony Brook, NY, 11794-8081, USA.

mans (and their absence in non–toolmaking apes), I apply the test to fossil hominids from Africa, Europe, and the Middle East.

In living hominoids (apes and humans), there are a number of anatomical features that are linked to apelike power grasping and others that are related to humanlike precision grasping (*5, 13*). Apes have hands with, among many other characteristic traits, long, curved fingers with narrow fingertips (*14*), diminutive thumbs (*15, 16*), and elongate pisiform bones in the palm (*17*). Humans and our more advanced hominid ancestors have relatively short, straight fingers with broad fingertips and relatively long, stout thumbs with broad, fleshy tips. Humans have pea-like, rounded pisiform bones in the palm. These and other changes are seen in the adaptive shift from a principally power grasping hand, such as that used by apes in the arboreal setting, to a largely precision grasping, humanlike hand that is adapted for enhanced precision grasping and tool behavior (*18*). Napier established the principle that precision grasp (the acme of which is seen in human hands that engage in tool behavior) could be identified in certain early hominid hands. He recognized that the thumb metacarpal contained critical information about the evolution of tool behavior, but he also noted that thumb bones were lacking in the fossil record 30 years ago (*5*). First metacarpals are well represented in the fossil record at this time, and we can now examine thumb morphology in a comparative-functional analysis to address the question of which Plio-Pleistocene hominids engaged in tool behavior.

I compared the pollical metacarpals of *Australopithecus afarensis*, *Paranthropus robustus*, *Homo erectus*, and *Homo sapiens neanderthalensis* to those of 12 pygmy chimpanzees, 49 common chimpanzees, and 41 modern humans (*19*). A number of morphological and metrical differences in the pollical metacarpals of humans and apes can be related to an emphasis on either precision grasping or power grasping, respectively. Great apes have relatively shorter thumbs than do humans, with metacarpals that are reduced in relative length and diameter. In apes, the pollical metacarpal has a small base and a narrow distal articular surface, or head. When metacarpal head breadth is plotted against metacarpal length in pygmy chimpanzees, common chimpanzees, and humans, the relatively small head of the apes is apparent (Fig. 1A). In the range where apes and humans overlap in length (area between the two hatched lines, Fig. 1A), the head breadth proportions of the pollical metacarpal in apes are consistently less than in humans. Metacarpal head breadth in humans exceeds that of chimpanzees at each given length (for the ANCOVA slope test, $P = 0.072$, and for the intercept test, $P = 0.000$) (Fig. 1A).

In addition to the size of the metacarpal head, a feature that distinguishes pollical metacarpals in humans from those of chimpanzees is the presence of a relatively narrow, parallel-sided shaft in the apes (Fig. 2E). Also, the pollical distal phalanx of apes has a reduced tuft (Fig. 3) (*20*) and, most notably, lacks a ventrobasal depression for the insertion of the flexor pollicis longus muscle, which is lacking in nonhuman primates (*21*).

The relatively small bones of the ape thumb are surmounted by concomitantly reduced soft tissues. Not only do great apes lack a flexor pollicis longus muscle, but they also normally lack both a deep head of the flexor pollicis brevis muscle and the first volar interosseous muscle of Henle (that is, the first palmar interosseous muscle) (Fig. 3). In both chimpanzee species, three developmentally ventral muscles cross the metacarpophalangeal joint in addition to a pollical tendon from the flexor digitorum profundus muscle mass (*22*). Human thumbs have three additional muscles that add strength as well as refined motor control to thumb movements. In humans, six ventrally derived muscles cross the metacarpophalangeal joint (Fig. 3). In humans, there is a separate flexor pollicis longus muscle, a first volar interosseous muscle (of Henle), and a deep head of flexor pollicis brevis (in addition to the adductor pollicis, the flexor pollicis brevis proper, and the abductor pollicis brevis). The presence of enhanced thumb musculature in humans, by the addition of these three muscles, increases the transarticular forces crossing the metacarpophalangeal joint. Expansion of the metacarpal head in humans (Figs. 1A and 2A) reduces stress in the pollical metacarpophalangeal joint that results from the increased forces from the added thumb musculature (*23*).

Applying the ratio of metacarpal head expansion to fossil hominid pollical metacarpals confirms the expectation that toolmakers have expanded metacarpal heads. The hand of *A. afarensis* possesses a narrow, apelike pollical metacarpal (Figs. 1B and 2D). The geological time range that encompasses *A. afarensis* (4.0 to 3.0 Ma) is, likewise, devoid of durable (stone and bone) tools. However, thumbs of later hominids from time ranges, and in some cases sites, associated with stone and bone tools do manifest the toolmaking criterion in their

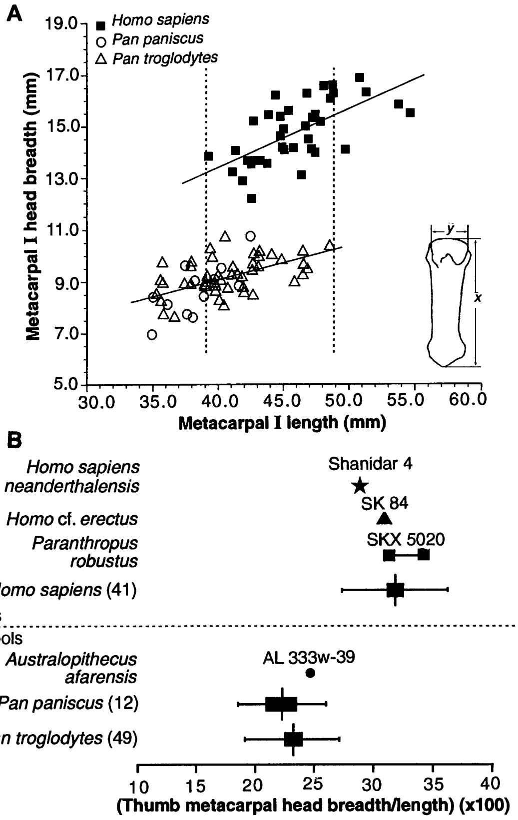
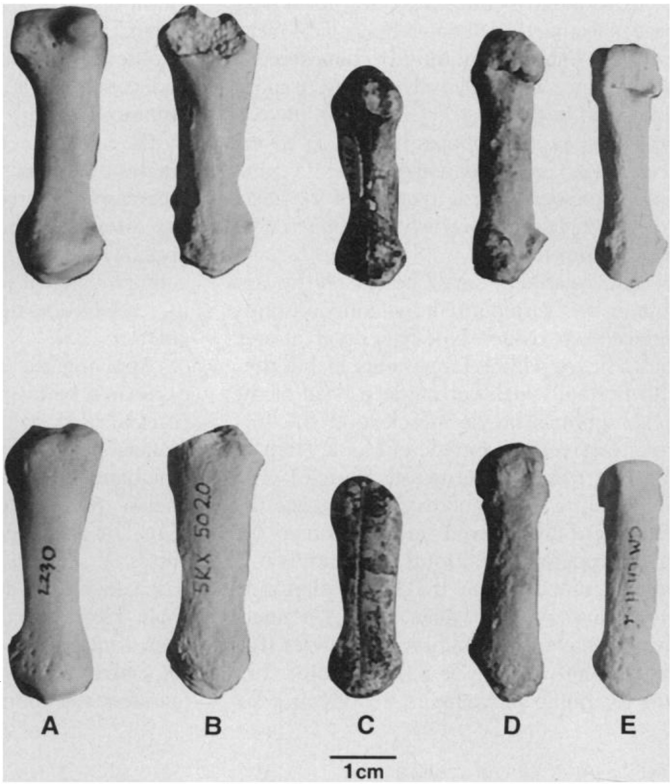


Fig. 1. (**A**) A plot of metacarpal I length versus metacarpal I head breadth in modern humans (*H. sapiens*), pygmy chimpanzees (*Pan paniscus*), and chimpanzees (*Pan troglodytes*). Humans have broader metacarpal heads than apes, with increased articular surface area. In humans, metacarpophalangeal joint expansion and the presence of increased musculature crossing this joint are linked to an enhanced potential for precision grasping. The human major axis regression equation is breadth = 4.91 + 0.226 (length) with an SE slope of 0.044 and an SE intercept of 2.028. The chimpanzee major axis regression equation is breadth = 3.777 + 0.131 (length) with an SE slope of 0.026 and an SE intercept of 1.071. (**B**) A plot of metacarpal I head ratio in the above human and ape sample, together with a like ratio in fossil hominids. This plot shows that (i) values for African apes and modern humans do not overlap and that (ii) *A. afarensis* (AL 333w-39), an early hominid that predates the occurrence of tools in the fossil record, plots within the 95% fiducial limits of the ape population. Three later hominids found with stone and bones tools, *P. robustus* (SKX 5020), *H. erectus* (SK 84), and *H. s. neanderthalensis* (Shanidar 4), plot near the human mean, outside the 95% fiducial limits of the ape population. A range is presented for SKX 5020 because the fossil has some slight postmortem bone loss on the distal end. The thin vertical bars are sample means, the black boxes are the 95% fiducial limits of the means, and the long bars are the 95% confidence limits of the population. Sample sizes are in parentheses.

pollical metacarpals (*24*). These hominids include *P. robustus* in South Africa (1.8 Ma) (Fig. 2B), *H. erectus* in South Africa (1.8 Ma) (Fig. 2C), and *H. s. neanderthalensis* in Europe and Asia [around 50,000 years ago (not pictured, but see Fig. 1B)].

Fig. 2. Fossil pollical (thumb) metacarpals: (**A**) modern human, (**B**) *P. robustus* (cast of original), (**C**) *H. erectus* (cast of original), (**D**) *A. afarensis*, and (**E**) common chimpanzee (cast). Top row, ventral view; bottom row, dorsal view. See Fig. 1B for specimen numbers.



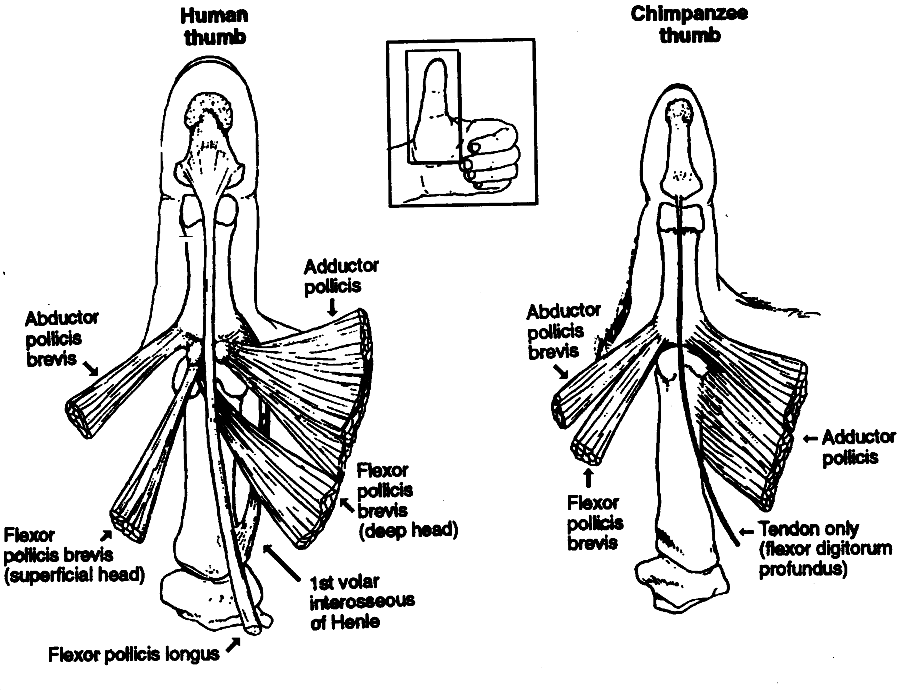


Fig. 3. Ventrally derived muscles crossing the metacarpophalangeal joint of the thumbs in modern human and chimpanzee. Humans have a true flexor pollicis longus (FPL) muscle, a deep head of flexor pollicis brevis muscle, and a first volar interosseous muscle of Henle. Pygmy and common chimpanzees and other primates lack the latter two muscles and have only a tendon that mimics the FPL muscle and lacks a separate muscle belly. The paired sesamoid bones of the human metacarpophalangeal joint provide a bony grove for passage of the FPL tendon. Correlated with the lack of a true FPL, these bones are generally lacking in the metacarpophalangeal joints of chimpanzees, gorillas, and orangutans (*26*).

Given the distinctive morphology of precision grasping thumbs, it should now be possible to detect the capability for refined, humanlike precision grasping (and its correlate, tool behavior) in fossil hominid species of the Plio-Pleistocene that are represented by pollical metacarpals (*25*). The morphological criteria for toolmaking proposed by Napier in the early 1960s, including, among others, a fully opposable thumb with increased relative thumb length and broad distal phalanges of the thumb and fingers, are extended by this study. While Napier's work has stood well the test of time, the application of his test has been limited (i) because it relies on fossils that are rare and delicate (for example, manual distal phalanges) and (ii) because it has been limited to individual hominid specimens that are represented by associated hand bones (necessary to calculate thumb and finger proportions). The test I propose relies on a single thumb element and one that is well represented in the fossil record. Prudent application of the proposed diagnosis should help resolve the question of which Plio-Pleistocene hominids were responsible for the earliest tool assemblages of the Oldowan industrial complex.

REFERENCES AND NOTES

1. B. Wood, *Nature* **355**, 783 (1992).
2. Tool behavior refers to tool use and toolmaking, following B. Beck, *Animal Tool Behavior* (Garland STMP, New York, 1980), p. 4.
3. K. P. Oakley, *Man the Tool-Maker* (Univ. of Chicago Press, Chicago, 1961), p. 111.
4. J. R. Napier, *Nature* **196**, 409 (1962).
5. Napier studied the functional morphology of primate hands and the relation between structure and function. He observed that certain features of the human hand were correlated with an enhanced precision grasp. Napier noted that the presence or absence of these features in primates (including fossil hominids) indicated, "...within limits, the capacity of those hands in the cultural realm of tool-using and toolmaking" [J. R. Napier, *Sci. Am.* **205**, 56 (December 1962)].
6. P. V. Tobias, *S. Afr. Archeol. Bull.* **20**, 167 (1965).
7. L. S. B. Leakey, P. V. Tobias, J. R. Napier, *Nature* **202**, 7 (1964); P. V. Tobias, *ibid.*, p. 3.
8. P. V. Tobias, *Curr. Anthropol.* **6**, 391 (1966).
9. M. H. Day and J. R. Napier, *Nature* **201**, 969 (1964).
10. L. S. B. Leakey initially proposed that *Australopithecus (Zinjanthropus) boisei* made the tools found in Bed I at Olduvai [L. S. B. Leakey, *ibid.* **184**, 491 (1959)]. He later equivocated [*ibid.* **191**, 417 (1961)] when *H. habilis* was recovered a few months later. Finally, Leakey argued that *H. habilis* was the more likely toolmaker in Bed I [Leakey *et al.*, in (*7*)].
11. "Australopithecine" refers to the earliest hominids beginning with *A. afarensis* at around 4.0 Ma and including later *A. africanus*, *P. robustus* in South Africa, and *P. boisei* in East Africa. The latter two species are commonly referred to as the "robust" australopithecines because of their well-developed chewing apparatus, including massive jaws and expansive post-canine dentition.
12. R. Potts, *Early Hominid Activities at Olduvai* (Aldine De Gruyter, New York, 1988), p. 3; K. D. Schick and N. Toth, *Making Silent Stones Speak* (Simon & Schuster, New York, 1993), pp. 102–104.
13. J. R. Napier, *J. Bone J. Surg.* **38B**, 902 (1956).
14. R. L. Susman and N. Creel, *Am. J. Phys. Anthropol.* **50**, 311 (1979).
15. M. F. Ashley-Montague, *ibid.* **15**, 291 (1931).
16. A. H. Schultz, *Q. Rev. Biol.* **2**, 259 (1936).
17. M. Spinner, *Kaplan's Functional and Surgical Anat-*

omy of the Hand (Lippincott, Philadelphia, ed. 3, 1984), p. 159.

18. The terms power and precision grip have been redefined over the years [see C. Long, in *The Hand*, R. Tubiana, Ed. (Saunders, Philadelphia, 1981), pp. 427–440; M. Marzke and M. S. Shackley, *J. Hum. Evol.* **15**, 439 (1986)]. Long noted that "dextrous manipulation of objects by the thumb and fingers, or precision handling, has relatively few variations" (above, p. 436). These variations involve the thumb and different combinations of the four fingers in two, three, four, or five jawed grips. All precision handling, however, involves the thumb. Marzke and Shackley expanded on the definitions of Napier and Long and described a further subset of precision handling that in some cases involves the thumb as a buttress rather than as a fully opposing structure. The precision grasping adaptations described here and that are correlated with early bone and stone tool use reflect the enhanced role of the thumb and its musculature as part of a multi-jawed chuck. The more primitive precision handling that does not depend on a well-developed thumb, and where the base of the thumb serves as a buttress, is often observed in chimpanzee tool behavior such as the hammering of hard nuts in the Tai Forest of Ivory Coast. Thus, the more general concept of precision grasping of Napier (*13*) or precision handling (Long, above) would seem adequate to explain behavioral correlates of thumb morphology in apes and humans, and in fossil hominids.
19. Pygmy chimpanzees are from the Tervuren Museum and Department of Anatomical Sciences, State University of New York at Stony Brook. Common chimpanzees are from the American Museum of Natural History (AMNH), the Smithsonian Institution, and the Cleveland Museum of Natural History (CMNH). Gorillas are from AMNH and CMNH. Modern humans are drawn from the Todd Collection at CMNH. Fossil hominids include the following: AL 333w-39 (*A. afarensis* from Hadar), SKX 5020 (*P. robustus* from Swartkrans), SK 84 (*H. erectus* from Swartkrans), and Shanidar 4 (*H. s. neanderthalensis* from Shanidar Cave, Iraq). All fossil hominid measurements, except Shanidar 4, were taken from original specimens by the author. Measurements of Shanidar 4 were reported by E. Trinkaus, *The Shanidar Neandertals* (Academic Press, New York, 1983).
20. R. L. Susman, *Science* **240**, 781 (1988).
21. In apes there is a tendon from the common deep finger flexor mass [flexor digitorum profundus (FDP)] that mimics the tendon of the flexor pollicis longus in humans. This tendon does not represent a separate muscle to the thumb in apes. In lesser apes (gibbons and siamang) there is a muscle belly, but it is functionally coupled with the FDP. Our electromyography experiments on an adult female gibbon (*Hylobates lar*) did not elicit flexion of the thumb separate from flexion of the fingers, as is the case in humans. In a sample of 90 great apes (96% of orangutans, 72% of gorillas, 52% of chimpanzees), the flexor pollicis longus muscle was "rudimentary and functionless" [W. L. Straus, *Q. Rev. Biol.* **17**, 228 (1942)].
22. Ape hands reveal a contrasting morphology to those of humans. In apes, the fingers (digits II to V) rather than the thumb (digit I) have an enhanced musculature. Apes have a variable series of contrahentes muscles, often to digits IV and V and sometimes to digit II. Humans and apes all have a contrahentes to the thumb, called the adductor pollicis (see Fig. 3).
23. Stress (S) = Force (F)/Area (A). In the present case, stress is increased by an increase in the transarticular compressive forces (F) due to the addition of three muscles crossing the metacarpophalangeal joint. The effect of increasing force is mitigated by expanding the area (A) of the joint over which muscle forces are concentrated. Humans and other hominids that co-occur with stone and bone tools such as SKX 5020 (*P. robustus*), SK 84 (*H. erectus*), and Shanidar 4 (*H. s. neanderthalensis*) have an increased area, A, presumably because they have added one or all of the complement of human thumb muscles. AL 333w-39 (*A. afarensis*), which predates the appearance of stone tools, does not display metacarpophalangeal joint expansion and most likely lacked a "human" complement of thumb muscles.
24. The oldest stone tools associated with reliable radiometric dates are from East Africa, members E and F of the Shungura formation of the lower Omo Valley, and at Hadar, from the Kada Hadar member along the Gona River in Ethiopia. These sites are dated at around 2.5 Ma [H. Roche and J.-J. Tiercelin, *C. R. Acad. Sci. Paris* **284**, 1871 (1977); J. W. K. Harris, *Afr. Archeol. Rev.* **1**, 3 (1983); F. C. Howell, P. Haesaerts, J. de Heinzelin, *J. Hum. Evol.* **16**, 665 (1987)].
25. The question of whether refined precision grasping is indeed related to tool behavior and not simply small-object feeding is settled by the fact that *Theropithecus gelada*, the quintessential non–tool-using small object feeder [C. J. Jolly, *Man* **5**, 6 (1970)] lacks a hominid-like thumb.
26. J.-M. Le Minor, *Gegenbaurs Morphol. Jahrb.* **134**, 693 (1988).

27. I thank W. Jungers, D. Krause, S. Leigh, J. Stern, and K. Steudel for their comments and help; L. Betti-Nash for the illustrations; and C. K. Brain and B. Asfaw for permission to study early hominid fossils. Supported by NSF grants BNS 870687 and SBR 9209004.

2 May 1994; accepted 22 June 1994

Requirement of Transcription Factor PU.1 in the Development of Multiple Hematopoietic Lineages

Edward W. Scott, M. Celeste Simon, John Anastasi, Harinder Singh*

The transcription factor PU.1 is a hematopoietic-specific member of the *ets* family. Mice carrying a mutation in the *PU.1* locus were generated by gene targeting. Homozygous mutant embryos died at a late gestational stage. Mutant embryos produced normal numbers of megakaryocytes and erythroid progenitors, but some showed an impairment of erythroblast maturation. An invariant consequence of the mutation was a multilineage defect in the generation of progenitors for B and T lymphocytes, monocytes, and granulocytes. Thus, the developmental programs of lymphoid and myeloid lineages require a common genetic function likely acting at the level of a multipotential progenitor.

Hematopoiesis is a regulated developmental cascade that generates at least eight distinct lineages that differentiate into the mature cell types of the blood (*1*). The process is initiated by a self-renewing, pluripotential stem cell that generates a hierarchical array of developmental intermediates consisting of multipotent and monopotent progenitor cells. Most mature blood cell types have short life-spans and little or no proliferative capacity—therefore, they are continuously regenerated.

The transcription factor PU.1 is the product of the *PU.1-Spi1-Sfpi1* proto-oncogene (*2–4*). The *PU.1* gene is expressed specifically in hematopoietic tissues, particularly in the monocytic and B lymphoid lineages (*2, 5, 6*). Numerous presumptive PU.1 target genes have been identified in these lineages (*7–11*). Therefore, PU.1 has been suggested to control the differentiation of B lymphocytes and monocytes. Overexpression of PU.1 in erythroblasts is sufficient for their immortalization (*12*). Binding sites for PU.1 are present in intron 2 of the mouse β-major (β^M)–globin gene (*5*), in a region of altered chromatin structure in erythroid cells. Thus, PU.1 may also be required for the development of erythroid progenitors during hematopoiesis.

To genetically analyze the functions of PU.1, we engineered mice carrying a mutant allele using gene targeting in embryonic stem cells. The structure of the *PU.1* gene is depicted in Fig. 1A. Exon 5 encodes the DNA binding domain (*2*). The targeting vector (pES-PUT) was designed to delete this coding segment and to replace it with the positive selectable marker *PGK::neo* (Fig. 1A). To enrich for homologous recombination events, we used the negative selectable marker *PGK::TK* (*13*). The targeting construct was assembled with isogenic DNA segments (*14*). The *neo* gene was flanked by approximately 2 and 12 kb of *PU.1* homologous sequence on its 5′ and 3′ ends, respectively (Fig. 1A). Targeted CCE.1 embryonic stem (ES) cell clones were used to generate mice heterozygous for the *PU.1* mutation (*15*).

At 7 weeks of age, *PU.1* heterozygotes were mated to generate *PU.1* homozygous mutant progeny. Three-week-old pups were genotyped by Southern (DNA) blot analysis. Out of 31 progeny, 10 were wild type and 21 were heterozygotes. The absence of homozygous mutant progeny was highly significant ($P > 99.9\%$) and indicated that *PU.1* is an essential gene, disruption of which results in prenatal lethality. Embryos

E. W. Scott and H. Singh, Department of Molecular Genetics and Cell Biology and Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637, USA.
M. C. Simon, Department of Medicine and Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637, USA.
J. Anastasi, Department of Pathology, University of Chicago, Chicago, IL 60637, USA.

---

*To whom correspondence should be addressed.