



**Redefining the Age of Clovis: Implications for the
Peopling of the Americas**

Michael R. Waters, *et al.*
Science **315**, 1122 (2007);
DOI: 10.1126/science.1137166

***The following resources related to this article are available online at
www.sciencemag.org (this information is current as of February 23, 2007):***

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/315/5815/1122>

Supporting Online Material can be found at:

<http://www.sciencemag.org/cgi/content/full/315/5815/1122/DC1>

This article **cites 3 articles**, 2 of which can be accessed for free:

<http://www.sciencemag.org/cgi/content/full/315/5815/1122#otherarticles>

This article appears in the following **subject collections**:

Anthropology

<http://www.sciencemag.org/cgi/collection/anthro>

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:

<http://www.sciencemag.org/help/about/permissions.dtl>

ceiving array. A TRM made of eight commercial dipolar antennas is placed in the far field, 10 wavelengths away from the receiving array. (The electronic part of the setup is described in fig. S1.) When antenna 3 sends a short pulse (10 ns), the eight signals received at the TRM are much longer than the initial pulse because of strong reverberation in the chamber (typically 500 ns). An example of the signal received at one of the antennas of the TRM is shown in Fig. 2A. When antenna 4 is used as a source, the signal received at the same antenna in the TRM (shown in Fig. 2B) is considerably different, although sources 3 and 4 are $\lambda/30$ apart. When these signals are time-reversed and transmitted back, the resulting waves converge respectively to antennas 3 and 4, where they recreate pulses as short as the initial ones (Fig. 2, C and D). Measuring the signal received at the other antennas of the receiving array gives access to the spatial focusing around antennas 3 and 4 (Fig. 2E). The two antennas can now be addressed independently, because the focusing spots created around them are much smaller than the wavelength (typically $\lambda/30$). The diffraction limit is overcome, although the focusing points are in the far field of the TRM.

The origin of the diffraction limit, and the way to overcome it, can be revisited by using the time-reversal concept and the Green's function formalism, without the explicit use of the evanescent wave concept (20–22). The time-reversed wave, generated by a closed TRM, which converges to its source, is always followed by a spatially diverging wave due to energy flux conservation. Because the focal spot results from the interference of these two waves, the time-reversed field can always be expressed (for a monochromatic wave) as the imaginary part of the Green's function (22). In a homogeneous medium, the imaginary part of the Green's function oscillates typically on a wavelength scale. To create focal spots much smaller than the wavelength, one introduces subwavelength scatterers in the near field of the source. Therefore, the spatial dependence of the imaginary part of the Green's function is modified to oscillate on scales much smaller than the wavelength.

A promising application of time-reversal subwavelength focusing is telecommunications. One way that has been proposed to increase the data rate of a communication system is to use multiantenna arrays at both transmitter and receiver (23); different bitstreams sent from each antenna of the transmitting array can be decoded at the receiving array under the condition that the medium creates sufficient scattering. It is also generally stated that the spacing between the receiving antennas must be larger than $\lambda/2$ (23). If these two conditions are fulfilled, the global maximum error-free data rate, or “Shannon Capacity,” is at best multiplied by the number of transmitting antennas. Such methods are referred to as MIMO (multiple input–multiple output). However, from a practical perspective,

it is difficult to ensure that the distance between antennas can be made large enough for this requirement to be satisfied. This difficulty is typically encountered when antennas are placed in a laptop and the telecommunication wavelengths are on the centimeter scale (e.g., Bluetooth or Wi-Fi). An illustration of the benefit of time-reversal subwavelength focusing to overcome this difficulty is given in Fig. 3. A three-antenna TRM is used to transmit a color picture to a three-antenna receiving array. The original picture is encoded onto three RGB (red–green–blue) color channels. Each corresponding figure is represented by a bit series giving the gray levels of each pixel on that particular channel. Then the simplest modulation is used (a positive pulse for bit 1, a negative one for bit 0) to create three bitstreams with a data rate of 50 Mbit/s each. The intended global data rate is thus 150 Mbit/s. Time reversal is used to focus each bitstream onto one of the antennas (one antenna for each color) of the receiving array. Then the three bitstreams are decoded and mixed to reconstruct the color image. The communication is performed with two kinds of receiving arrays. The first is “classical”; it consists of three dipolar antennas with a $\lambda/30$ spacing. The second is a microstructured antenna array analogous to the one previously described (Fig. 1). It turns out that the image reconstructed with the classical array is gray-scaled: Its colors are lost. Indeed, subwavelength spaced antennas are strongly coupled, that is, they essentially receive the same signal. Hence, each transmitted pixel is gray because the three different antennas corresponding to the three different color channels receive the same gray levels. However, when the microstructured receiving array is used, each color stream focuses independently at each antenna. Consequently, the relative weights of the RGB components of each pixel are preserved and the image is transmitted

without major losses. This experiment shows that our approach allows one to increase the information transfer rate to a given volume of space.

References and Notes

- E. Betzig, J. K. Trautman, *Science* **257**, 189 (1992).
- F. Zenhausern, Y. Martin, H. K. Wickramasinghe, *Science* **269**, 1083 (1995).
- J. B. Pendry, *Phys. Rev. Lett.* **85**, 3966 (2000).
- D. R. Smith, J. B. Pendry, M. C. K. Wiltshire, *Science* **305**, 788 (2004).
- D. R. Smith, *Science* **308**, 502 (2005).
- V. G. Veselago, *Sov. Phys. Usp.* **10**, 509 (1968).
- N. A. Nicorovici, R. C. McPhedran, G. W. Milton, *Phys. Rev. B* **49**, 8479 (1994).
- R. Shelby, D. R. Smith, S. Schultz, *Science* **292**, 77 (2001).
- M. C. K. Wiltshire *et al.*, *Science* **291**, 849 (2001).
- N. Fang, H. Lee, C. Sun, X. Zhang, *Science* **308**, 534 (2005).
- D. O. S. Melville, R. J. Blaikie, *Opt. Exp.* **13**, 2127 (2005).
- Z. Jacob, L. V. Alekseyev, E. Narimanov, *Opt. Exp.* **14**, 8247 (2006).
- T. Taubner *et al.*, *Science* **313**, 1595 (2006).
- M. Fink, *Phys. Today* **50**, 34 (1997).
- C. Draeger, M. Fink, *Phys. Rev. Lett.* **79**, 407 (1997).
- B. E. Henty, D. D. Stancil, *Phys. Rev. Lett.* **93**, 243904 (2004).
- G. Lerosey, J. de Rosny, A. Tourin, A. Derode, M. Fink, *Phys. Rev. Lett.* **92**, 193904 (2004).
- G. Lerosey, J. de Rosny, A. Tourin, A. Derode, M. Fink, *App. Phys. Lett.* **88**, 154101 (2006).
- R. Carminati, J. J. Saenz, J.-J. Greffet, M. Nieto-Vesperinas, *Phys. Rev. A* **62**, 012712 (2000).
- D. Cassereau, M. Fink, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **39**, 579 (1992).
- J. de Rosny, M. Fink, *Phys. Rev. Lett.* **89**, 124301 (2002).
- Further details are available as supporting material on Science Online.
- A. L. Moustakas, H. U. Baranger, L. Balents, A. M. Sengupta, S. H. Simon, *Science* **287**, 287 (2000).
- This work was partially funded by the Agence Nationale de la Recherche under grant ANR-05-BLAN-0054-01.

Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5815/1120/DC1
SOM Text

Fig. S1

References

7 September 2006; accepted 19 December 2006
10.1126/science.1134824

Redefining the Age of Clovis: Implications for the Peopling of the Americas

Michael R. Waters^{1*} and Thomas W. Stafford Jr.²

The Clovis complex is considered to be the oldest unequivocal evidence of humans in the Americas, dating between 11,500 and 10,900 radiocarbon years before the present (¹⁴C yr B.P.). Adjusted ¹⁴C dates and a reevaluation of the existing Clovis date record revise the Clovis time range to 11,050 to 10,800 ¹⁴C yr B.P. In as few as 200 calendar years, Clovis technology originated and spread throughout North America. The revised age range for Clovis overlaps non-Clovis sites in North and South America. This and other evidence imply that humans already lived in the Americas before Clovis.

For nearly 50 years, it has been generally thought that small bands of humans carrying a generalized Upper Paleolithic tool kit entered the Americas around 11,500 radiocarbon years before the present (¹⁴C yr B.P.) and that

these first immigrants traveled southward through the ice-free corridor separating the Laurentide and Cordilleran Ice Sheets (*1*). These people developed the distinctive lithic, bone, and ivory tools of Clovis (2, 3) and then quickly populated

the contiguous United States. Clovis humans and their descendants then rapidly populated Central America and reached southernmost South America by 10,500 ^{14}C yr B.P. (1).

Identifying when the Clovis complex first appeared and knowing the complex's duration is critical to explaining the origin of Clovis, evaluating the Clovis-first model of colonization of the Americas, determining the role of humans in the extinction of late Pleistocene megafauna, and assessing whether people inhabited the Americas before Clovis. We determined a more accurate time span for Clovis by analyzing the revised existing Clovis ^{14}C date record and reporting high-precision accelerator mass spectrometry (AMS) ^{14}C ages from previously dated Clovis sites. Our AMS ^{14}C dates are on culturally specific organic matter—bone, ivory, and seeds—that accelerator mass spectrometers can date accurately (4, 5) to precisions of ± 30 years at 11,000 ^{14}C yr B.P.

Clovis technology has strong Old World antecedents, but Clovis-specific traits (e.g., fluted lanceolate projectile points) probably originated in the New World, south of the continental ice sheets (3). Clovis tools and debitage identify and unify archaeological sites over a broad geographic range. Clovis sites and artifacts cluster in North America, especially in the contiguous United States (1). A small number of Clovis artifacts have been recovered from Mexico and possibly as far south as Venezuela (6). Even though Clovis covers a broad geographic range, only 22 Clovis sites in North America have been directly ^{14}C -dated (Fig. 1, Table 1, and table S1). The ^{14}C dates from these sites traditionally place Clovis between 11,500 and 10,900 ^{14}C yr B.P. (1, 7, 8). However, the ^{14}C dates from 11 of these sites are problematic and do not provide accurate or precise chronological information to determine the age of Clovis (5).

Three sites (East Wenatchee, Washington; Blackwater Draw, New Mexico; and Cactus Hill, Virginia) have Clovis diagnostic artifacts but lack precise ages (5). Three sites (Lubbock Lake, Texas; Kanorado, Kansas; and Indian Creek, Montana) fall within the Clovis age range but lack diagnostic Clovis artifacts (5). The site of Sheridan Cave, Ohio, provides only bracketing ages for Clovis artifacts (5). Questions exist about the accuracy of the ^{14}C dates from Aubrey, Texas (5), where diagnostic Clovis artifacts were found. We obtained three dates from the Sheaman site, Wyoming, that averaged $10,305 \pm 15$ ^{14}C yr B.P. These dates indicate that the Clovis context at Sheaman is mixed with younger cultural materials (5). Finally, associations between Clovis artifacts and

^{14}C -dated faunal remains at two sites (Wally's Beach, Canada; and Union Pacific, Colorado) are unresolved (5). Because of these problems, we excluded the dates from these sites in assessing the age of Clovis.

This leaves 11 sites with a total of 43 ^{14}C dates (Table 1 and table S1) (5). These sites have assemblages of Clovis artifacts in secure geological contexts. Existing ages from five sites (Anzick, Montana; Paleo Crossing, Ohio; Lehner, Arizona; Murray Springs, Arizona; and Jake Bluff, Oklahoma) already have high-precision ^{14}C dates on credible materials. We obtained nine new ages from seeds and highly purified bone and ivory collagen for five imprecisely dated sites (Lange-Ferguson, South Dakota; Dent, Colorado; Domebo, Oklahoma; Shawnee-Minisink, Pennsylvania; and Colby, Wyoming) (4, 5). In addition, we obtained five ages on human remains from the Anzick site, Montana (5). We attempted to date samples from Sloth Hole, Florida, but the samples contained no collagen.

These 43 ^{14}C dates place the beginning of Clovis at $\sim 11,050$ ^{14}C yr B.P. (reducing former estimates by 450 ^{14}C years) and its end at $\sim 10,800$ ^{14}C yr B.P. (younger than previous estimates by 100 ^{14}C years). Accurate calendar correlation of ^{14}C ages from the Clovis time period is not currently possible because of correlation uncertainties (9). The Clovis-period segment of the INTCAL04 calibration is based on ^{14}C -dated marine foraminifera and is not accurate for the Clovis time period (10). The most accurate calibration for this time period is provided by a floating European tree-ring chronology that is provisionally anchored to INTCAL04 (11). Using this tentative calibration (11), we estimated that Clovis has a maximum possible date range of 13,250 to 12,800 calendar yr B.P.—a span of 450 calendar years (Fig. 2). By taking the youngest possible calibrated age for the oldest Clovis site and the oldest possible calibrated age for the youngest Clovis site, a minimum range for Clovis is calculated as 13,125 to 12,925 calendar yr B.P.—a span of



Fig. 1. Map showing the location of Clovis and other early sites. The numbers correspond to those found in Table 1. Other sites are 31, Monte Verde, Chile; 32, Nenana Complex sites, Alaska; and 33, Broken Mammoth, Alaska.

¹Departments of Anthropology and Geography, Center for the Study of the First Americans, Texas A&M University, 4352 TAMU, College Station, TX 77843-4352, USA.

²Stafford Research Laboratories, 200 Acadia Avenue, Lafayette, CO 80026, USA.

*To whom correspondence should be addressed. E-mail: mwaters@tamu.edu

200 calendar years. The ages for all Clovis sites overlap within this 200-year period, and this time span probably represents the true range of Clovis. However, the absolute calendar placement of the floating tree-ring record is disputed (12). By an alternative calibration (12), the maximum time range for Clovis is 13,110 to 12,660 calendar yr B.P., and the minimum time range is 12,920 to 12,760 calendar yr B.P. (Fig. 3). Regardless of the exact calendar dates, the 200-year duration for Clovis remains secure because the floating dendrochronological sequence provides calendar-year separations between two ^{14}C -dated sites.

The oldest Clovis sites ($n = 3$ sites) are located in Montana, South Dakota, and Florida; younger Clovis sites are located in the interior ($n = 5$) of the United States and in the Southwest ($n = 2$) and East ($n = 1$). The distribution of dated sites shows no clear indication of north-south or east-west age differences that would indicate movement of people in one direction or another. Instead, Clovis technology seems to have appeared synchronously across the United States at $\sim 11,050$ ^{14}C yr B.P. This pattern of ^{14}C dates is compatible with two contrasting hypotheses.

First, this pattern could support the idea that there was a rapid spread of Clovis people across an empty continent. Demographic models suggest that people exiting the ice-free corridor could have occupied the contiguous United States within 100 years or less (13). Although there is much speculation about a coastal migration of the first Americans from both Asia and Europe (14, 15), the revised date range for Clovis reopens the possibility of a Late Glacial migration through the ice-free corridor that separated the Laurentide and Cordilleran Ice Sheets. People could have easily traveled through the ice-free corridor after $\sim 11,500$ ^{14}C yr B.P. (1)—at least 200 calendar years before the oldest known Clovis date. The biface and blade industry of Nenana (16) was well established at the Broken Mammoth site, Alaska, to $11,770 \pm 210$ ^{14}C yr B.P. (WSU-4351)—at least 300 calendar years before our oldest recalibrated Clovis date. The Nenana lithic assemblage shows strong similarities to the Clovis lithic assemblage (17). It is possible that either Nenana people or others with a biface and blade industry traveled through the corridor, and once south of the ice sheets, they developed the technological hallmarks characteristic of Clovis and spread rapidly across the continent.

An alternative interpretation is that the instantaneous appearance of Clovis across North America represents the rapid spread of Clovis technology through a preexisting but culturally and genetically undefined human population in North America (18). In this case, Clovis technology could have been introduced to this population through a Late Glacial migration of Clovis or Clovis progenitors or developed in situ from a pre-Clovis technology already in the

Americas. Regardless of which hypothesis is correct, our revised chronology indicates that Clovis technology spread rapidly.

Faunal remains associated with dated Clovis sites constrain the timing of the extinction of Proboscideans at the end of the Pleistocene. Mammoths and mastodons were an important source of food and raw materials used to manufacture bone and ivory tools (3), as well as perishable items from soft tissues. Proboscidean remains are associated with seven of the well-dated Clovis sites (Lange-Ferguson, Sloth Hole, Dent, Domebo, Lehner, Murray Springs, and Colby), and the last occurrence of mammoth in the United States is dated at $\sim 10,900$ ^{14}C yr B.P. After this time, Clovis and sites of other complexes (e.g., Goshen and Folsom) contained only bison and other extant species.

The extinction of mammoth and mastodon coincides with the main florescence of Clovis.

Our revised ages for Clovis overlap dates from a number of North American sites that are technologically or culturally not Clovis. The earliest dated sites of the Goshen complex (Mill Iron, Montana; and Hell Gap, Wyoming) (19) overlap the age range of Clovis (Figs. 2 and 3, and Table 1, and table S1). This indicates that Goshen is either coeval with the entire range of Clovis or briefly overlaps the later stages of the Clovis time period. Clovis also overlaps the date for the Arlington Springs human skeleton from Santa Rosa Island, California (Figs. 2 and 3 and Table 1) (20). No artifacts were found with the Arlington Springs human remains, and his cultural affiliation is unknown. The presence of human remains on Santa Rosa

Table 1. Summary of ^{14}C dates from Clovis and Clovis-age sites. Single ^{14}C dates, date ranges, and averaged dates are reported. If multiple ^{14}C dates were available from a single-component site, the dates were averaged with the method in (28). All dates are given at 1σ SD. n , number of dates.

Site	Date (^{14}C yr B.P.)
<i>Clovis sites (credible ages and Clovis diagnostics)</i>	
1. Lange-Ferguson, SD ($n = 3$)	11,080 \pm 40
2. Sloth Hole, FL ($n = 1$)	11,050 \pm 50
3. Anzick, MT (foreshaft ages) ($n = 2$)	11,040 \pm 35
4. Dent, CO ($n = 3$)	10,990 \pm 25
5. Paleo Crossing, OH ($n = 3$)	10,980 \pm 75
6. Domebo, OK ($n = 1$)	10,960 \pm 30
7. Lehner, AZ ($n = 12$)	10,950 \pm 40
8. Shawnee-Minisink, PA ($n = 5$)	10,935 \pm 15
9. Murray Springs, AZ ($n = 8$)	10,885 \pm 50
10. Colby, WY ($n = 2$)	10,870 \pm 20
11. Jake Bluff, OK ($n = 3$)	10,765 \pm 25
<i>Clovis sites (indirectly dated and Clovis diagnostics)</i>	
12. East Wenatchee, WA ($n = 1$)	<11,125 \pm 130
<i>Clovis-age sites (credible ages but no Clovis diagnostics)</i>	
13. Indian Creek, MT ($n = 1$)	10,980 \pm 110
14. Lubbock Lake, TX ($n = 2$)	11,100 \pm 60
15. Bonneville Estates, NV ($n = 1$)	11,010 \pm 40
16. Kanorado, KS ($n = 2$)	10,980 \pm 40
17. Arlington Springs, CA ($n = 1$)	10,960 \pm 80
<i>Problematic Clovis and Clovis-age sites</i>	
18. Sheriden Cave, OH (above artifacts, $n = 5$)	10,600 \pm 30
Sheriden Cave, OH (below artifacts, $n = 2$)	10,920 \pm 50
19. Blackwater Draw, NM ($n = 3$)	11,300 \pm 235
20. Cactus Hill, VA ($n = 1$)	10,920 \pm 250
21. Wally's Beach, Canada ($n = 4$)	11,350 \pm 80 to 10,980 \pm 80
22. Union Pacific, WY ($n = 1$)	11,280 \pm 350
23. Aubrey, TX ($n = 2$)	11,570 \pm 70
24. Sheaman, WY ($n = 3$)	10,305 \pm 15
<i>Ages from other early sites</i>	
25. Mill Iron, MT (Goshen) ($n = 4$)	10,840 \pm 60
26. Hell Gap, WY (Goshen) ($n = 1$)	10,955 \pm 135
27. Cerro Tres Tetras, Argentina (pre-Fishtail, $n = 5$)	10,935 \pm 35
28. Cuevas Casa del Minero, Argentina (pre-Fishtail, $n = 2$)	10,985 \pm 40
29. Piedra Museo, Argentina (pre-Fishtail, $n = 2$)	10,960 \pm 45
30. Fell's Cave, Chile (Fishtail, $n = 1$)	11,000 \pm 170

Island is unequivocal evidence that water crafts were used during Clovis time and that a Pacific maritime-coastal adaptation was probably contemporaneous with Clovis. At Bonneville Estates Rockshelter, Nevada (21), the earliest date from a series of hearths is coeval with the Clovis time

period and is associated with stone artifacts. Diagnostic artifacts have yet to be found at this level, and it is unknown whether this early horizon is associated with Clovis or stemmed points.

Several sites in South America have yielded ¹⁴C dates that are coeval with Clovis (Figs. 2

and 3). These include the early archaeological horizons at Cerro Tres Tetras, Cueva Casa del Minero, and Piedra Museo, Argentina, and the earliest Fishtail point horizon at Fell's Cave, Chile (Fig. 1, Table 1, and table S1) (8, 22). The actual calendar dates of these South American sites may be slightly more recent because Southern Hemisphere samples have a lower initial ¹⁴C content than contemporaneous samples in the Northern Hemisphere. This latitudinal difference causes Southern Hemisphere terrestrial materials to be 5 to 80 ¹⁴C years older than contemporaneous samples in North America (23). The extent of this interhemisphere offset in ¹⁴C years for the Clovis time period is unknown, but it is probably less than 80 years. Even with an 80-year correction to the ¹⁴C dates from these four sites, Clovis, Fishtail, and other early complexes in the Southern Cone of South America are still contemporaneous.

The presence of non-Clovis sites that are contemporaneous with Clovis in both North and South America implies that Clovis does not represent the earliest occupation of the Americas. It would probably have taken a minimum of 600 to 1000 years for the first Paleoamericans and their descendants to travel by land from the southern limit of the ice-free corridor to Tierra del Fuego (13, 24)—a distance of over 14,000 km (Fig. 1). However, at most 300 to 350 calendar years separate the oldest possible date for Clovis and the youngest possible ages from the well-dated unequivocal sites in southernmost South America (Figs. 2 and 3). The difference is reduced to 200 calendar years, when the minimum date for the beginning of Clovis in North America and the youngest dates for the sites in South America are considered. It is highly improbable that within 200 to 350 calendar years, people entered North America; adapted to biomes ranging from arctic tundra to grasslands, deserts, and rainforests; increased in population; and reached the southern tip of South America within the span of 10 to 18 human generations. This suggests that human populations already existed in the New World before Clovis.

There is an emerging archaeological record that supports a pre-Clovis human occupation of the Americas. Stone tools and butchered mammoth remains dating to ~12,500 ¹⁴C yr B.P. have been found at the Schaefer and Hebior sites in Wisconsin (25, 26). Older butchered mammoth remains dating to ~13,500 ¹⁴C yr B.P. have been recovered from the Mud Lake site, Wisconsin (25, 26). In South America, humans appear to have been present at 12,500 ¹⁴C yr B.P. at Monte Verde, Chile (27). The archaeological data now show that Clovis does not represent the earliest inhabitants of the Americas and that a new model is needed to explain the peopling of the Americas.

References and Notes

1. C. V. Haynes Jr., in *Paleoamerican Origins: Beyond Clovis*, R. Bonnichsen, B. T. Lepper, D. Stanford,

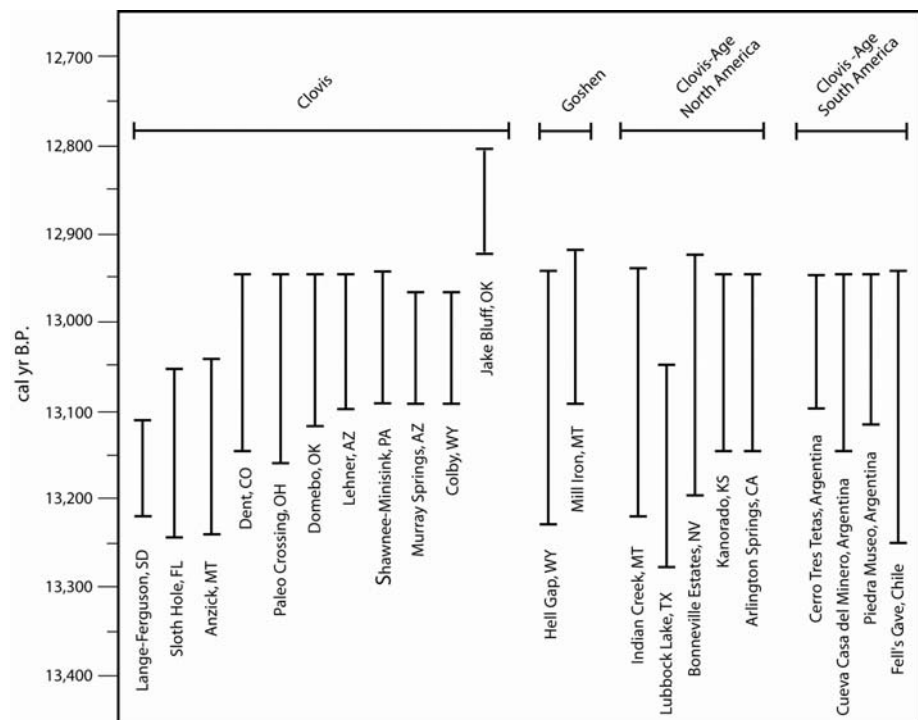


Fig. 2. Calendar-year age ranges for Clovis and other early sites based on the European dendrochronological calibration (11) at 1σ SD.

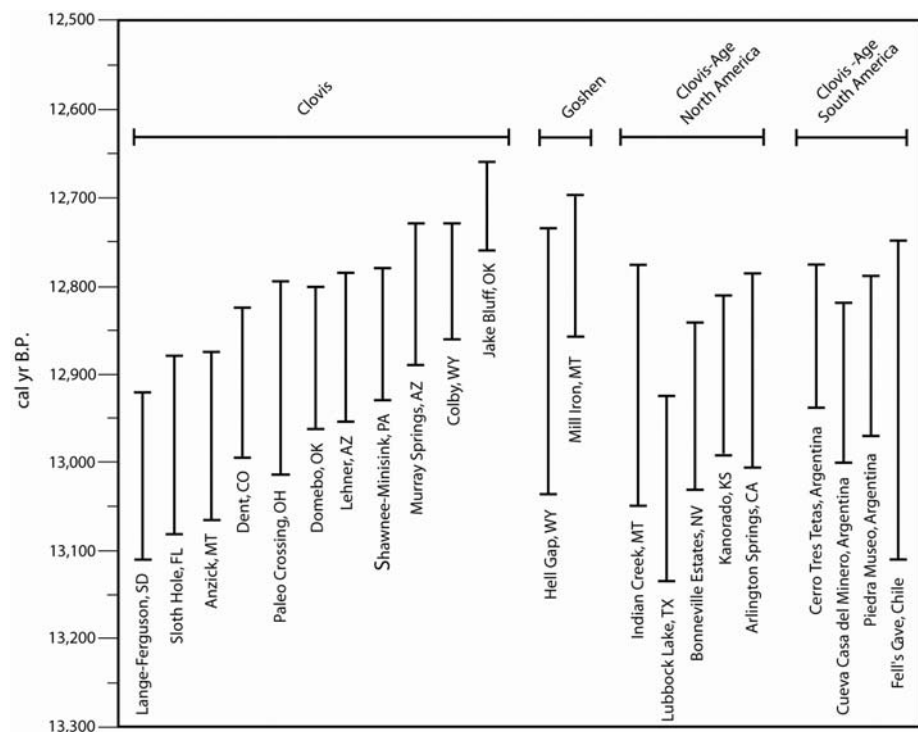


Fig. 3. Calendar-year age ranges for Clovis and other early sites based on the Fairbanks calibration (12) at 1σ SD.

- M. R. Waters, Eds. (Center for the Study of the First Americans, College Station, TX, 2005), pp. 113–132.
2. M. B. Collins, *Clovis Blade Technology* (Univ. of Texas Press, Austin, TX, 1999).
 3. K. B. Tankersley, in *The Settlement of the American Continents: A Multidisciplinary Approach to Human Biogeography*, C. M. Barton, G. A. Clark, D. R. Yesner, G. A. Pearson, Eds. (Univ. of Arizona Press, Tucson, AZ, 2004), pp. 49–63.
 4. T. W. Stafford Jr., P. E. Hare, L. Currie, A. J. T. Jull, D. J. Donahue, *J. Archaeol. Sci.* **18**, 35 (1991).
 5. Materials and methods are available as supporting material on Science Online.
 6. G. A. Pearson, in *The Settlement of the American Continents: A Multidisciplinary Approach to Human Biogeography*, C. M. Barton, G. A. Clark, D. R. Yesner, G. A. Pearson, Eds. (Univ. of Arizona Press, Tucson, AZ, 2004), pp. 85–102.
 7. A. C. Roosevelt, J. Douglas, L. Brown, in *The First Americans: The Pleistocene Colonization of the New World*, no. 27 of *Memoirs of the California Academy of Sciences*, N. G. Jablonski, Ed. (California Academy of Sciences, San Francisco, CA, 2002), pp. 159–235.
 8. C. V. Haynes Jr., in *Radiocarbon After Four Decades: An Interdisciplinary Perspective*, R. E. Taylor, A. Long, R. S. Kra, Eds. (Springer-Verlag, New York, 1992), pp. 355–374.
 9. S. J. Fiedel, *Am. Antiq.* **64-1**, 95 (1999).
 10. S. Bondevik, J. Mangerud, H. H. Birks, S. Gulliksen, P. Reimer, *Science* **312**, 1514 (2006).
 11. B. Kromer et al., *Radiocarbon* **46-3**, 1203 (2004).
 12. R. G. Fairbanks et al., *Quat. Sci. Rev.* **24**, 1781 (2005).
 13. S. J. Fiedel, *J. Archaeol. Res.* **8-1**, 39 (2000).
 14. C. A. S. Mandryk, H. Josenhans, D. W. Fedje, R. W. Mathewes, *Quat. Sci. Rev.* **20**, 301 (2001).
 15. B. Bradley, D. Stanford, *World Archaeol.* **36**, 459 (2004).
 16. T. D. Hamilton, T. Goebel, in *Ice Age Peoples of North America: Environments, Origins, and Adaptations of the First Americans*, R. Bonnichsen, K. L. Turnmire, Eds. (Oregon State Univ. Press, Corvallis, OR, 1999), pp. 156–199.
 17. J. F. Hoffecker, W. R. Powers, T. Goebel, *Science* **259**, 46 (1993).
 18. R. Bonnichsen, in *Clovis: Origins and Adaptations*, R. Bonnichsen, K. L. Turnmire, Eds. (Oregon State Univ. Press, Corvallis, OR, 1991), pp. 309–329.
 19. G. C. Frison, Ed., *The Mill Iron Site* (Univ. of New Mexico Press, Albuquerque, NM, 1996).
 20. J. R. Johnson, T. W. Stafford Jr., H. O. Aje, D. P. Morris, in *Proceedings of the Fifth California Islands Symposium* (U.S. Department of the Interior, Minerals Management Service, Pacific Outer Continental Shelf Region, Washington, DC, 23 March to 1 April 1999), pp. 541–544.
 21. T. Goebel, B. Hockett, K. Graf, D. Rhode, paper presented at the 30th Great Basin Anthropological Conference, Las Vegas, NV, 19 to 21 October 2006.
 22. L. Miotto, M. C. Saleme, *Quat. Int.* **109-110**, 95 (2003).
 23. M. Barbetti et al., *Nucl. Instrum. Methods* **223-224B**, 366 (2004).
 24. D. G. Anderson, J. C. Gillam, *Am. Antiq.* **65-1**, 43 (2000).
 25. D. F. Overstreet, in *Paleoamerican Origins: Beyond Clovis*, R. Bonnichsen, B. T. Lepper, D. Stanford, M. R. Waters, Eds. (Center for the Study of the First Americans, College Station, TX, 2005), pp. 183–195.
 26. D. J. Joyce, *Quat. Int.* **142-143**, 44 (2006).
 27. T. D. Dillehay, Ed., *Monte Verde: A Late Pleistocene Settlement in Chile: Volume 2: The Archaeological Context and Interpretation* (Smithsonian Institution Press, Washington, DC, 1997).
 28. G. K. Ward, S. R. Wilson, *Archaeometry* **20**, 19 (1978).
 29. We thank A. Hannus, C. V. Haynes, J. Gingerich, G. Frison, and A. Hemmings for providing samples for dating; M. Payn and the North Star Archaeological Research Program established by J. Cramer and R. Cramer for providing funding; P. Reimer and J. Southon for providing advice on ¹⁴C calibration; D. Carlson, T. Goebel, J. Southon, S. Forman, and three anonymous reviewers for offering useful comments to improve this paper; and L. Lind, C. Pevny, J. Halligan, and P. Johnson for helping in the preparation of the text and illustrations.

Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5815/1122/DC1
Materials and Methods
SOM Text
Table S1
References

3 November 2006; accepted 12 January 2007
10.1126/science.1137166

Quantitative Phylogenetic Assessment of Microbial Communities in Diverse Environments

C. von Mering,^{1*} P. Hugenholtz,² J. Raes,¹ S. G. Tringe,² T. Doerks,¹ L. J. Jensen,¹ N. Ward,³ P. Bork^{1†}

The taxonomic composition of environmental communities is an important indicator of their ecology and function. We used a set of protein-coding marker genes, extracted from large-scale environmental shotgun sequencing data, to provide a more direct, quantitative, and accurate picture of community composition than that provided by traditional ribosomal RNA–based approaches depending on the polymerase chain reaction. Mapping marker genes from four diverse environmental data sets onto a reference species phylogeny shows that certain communities evolve faster than others. The method also enables determination of preferred habitats for entire microbial clades and provides evidence that such habitat preferences are often remarkably stable over time.

Microorganisms are estimated to make up more than one-third of Earth's biomass (1). They play essential roles in the cycling of nutrients, interact intimately with animals and plants, and directly influence Earth's climate. Yet our molecular and physiological knowledge of microbes remains surprisingly fragmentary, largely because most naturally

occurring microbes cannot be cultivated in the laboratory (2).

For characterizing this “unseen majority” of cellular life, the first step is to provide a taxonomic census of microbes in their environments (3–6). This is usually achieved by cloning and sequencing their ribosomal RNA (rRNA) genes (most notably the 16S/18S small subunit rRNA). This approach has been extremely successful in revealing the overwhelming diversity of microbial life (7), but it also has some limitations due to quantitative errors: The polymerase chain reaction (PCR) step introduces amplification bias, and it generates chimeric and otherwise erroneous molecules that hamper phylogenetic analysis (8, 9).

Shotgun sequencing of community DNA (“metagenomics”) provides a more direct and unbiased access to uncultured organisms (10–13): No PCR amplification step is involved, and because no specific primers or sequence anchors are needed, even very unusual organisms can be captured by this technique. Although current metagenomics data are still not entirely free of quantitative distortions (mostly due to sample preparation), remaining biases are bound to diminish further with the optimization of yield and reproducibility of DNA extraction protocols (14–16).

To make use of metagenomics data for taxonomic profiling, we analyzed 31 protein-coding marker genes previously shown to provide sufficient information for phylogenetic analysis [they are universal, occur only once per genome, and are rarely transferred horizontally (17)]. We extracted these marker genes from metagenomics sequence data (9), aligned them to a set of hand-curated reference proteins, and used maximum likelihood to map each sequence to an externally provided phylogeny of completely sequenced organisms [tree of life; we used the tree from (17), although any reference tree can be used as long as the marker genes have been sequenced for all its taxa]. Our procedure provides branch length information and confidence ranges for each placement (18) (Fig. 1), allowing statements such as “This unknown sequence evolves relatively fast, is from a proteobacterium (95% confidence), and more specifically, probably from a novel clade related to the Campylobacterales (65% confidence).” The procedure weighs the number of informative residues that are found on each sequence fragment, then adjusts the spread and confidence of its placement

¹European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany. ²DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598, USA. ³The Institute for Genomic Research, Rockville, MD 20850, USA.

*Present address: University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.

†To whom correspondence should be addressed. E-mail: peer.bork@embl.de