individuals of all ages. Two of these bones embedded in the pavement are adult mastodon femora exhibiting cuts and striations that were produced by use of the bones as chopping blocks. Possible stone artifacts embedded in the pavement are under study.

Water-worn bone fragments were also found on an erosional disconformity capping unit I, and these belong to a different faunal assemblage than that of unit I. Mastodon remains were absent. Bones and teeth of horse, glyptodon bones and scutes including an inverted carapace, and tortoise scutes were excavated from this erosional disconformity, as well as a few bones tentatively identified as *Macrauchenia*. No indisputable cultural evidence was found on this horizon. No macrofaunal remains were recovered above the base of unit II.

The lower part of unit II, a reddish sand deposit approximately 50 cm thick, was cemented to a hardness requiring a heavy pick or hammer and chisel for excavation. Paleosol remnants were found with the horse and glyptodon bones at the base of unit II and again near the top of unit II where they are exposed in an undisturbed position beneath a white reduced sand. The upper part of the unit II deposit was apparently drowned and almost all of the A horizon of the soil which had formed on the unit II colluvial sand was completely reduced to a white color to a depth of 10 to 15 cm.

A black organic clay (unit III), about 30 cm in maximum thickness, overlies the white sand. The organic carbon content of the clay yielded six stratigraphically consistent radiocarbon dates between 10,290 ± 90 years B.P. and 9650 ± 80 years B.P. (*6*). The origins of the black clay, which contains unidentified woody plant remains, are uncertain, although ponding must be involved.

Colluvial yellowish-brown sand (unit IV) overlies the black clay to a thickness of 100 to 300 cm. The colluvium contains rolled fragments of cemented red sand, indicating that another paleosol had developed on the slope above the site and had been incorporated into a landslide or mudflow that covered much of the site, thus sealing and preserving the earlier stratigraphic section.

Four radiocarbon dates have been obtained on a wood sample collected in association with the butchered mastodon in unit I. The sample consisted of a concentrated mass of small wood twig fragments which were notably sheared at both ends as if masticated (Fig. 4). The twig fragments were found abundantly and in fresh condition in the saturated sand of unit I in close association with the skeleton of the young mastodon. We hypothesize that the material is derived from the contents of the slain beast's stomach or intestines. The radiocarbon dates obtained on this material are (in years before present): 12,980 ± 85 (SI-3316), 13,000 ± 200 (Birm-802), 13,880 ± 120 (USGS-247), and 14,200 ± 300 (UCLA-2133).

The minimum radiocarbon age of the El Jobo mastodon kill is thus 13,000 years. The field data from Taima-taima demonstrate that a big-game hunting complex of a completely different technological tradition existed in northern Venezuela at least a millennium and a half earlier than the well-known Clovis complex of North America. In the southwestern United States, the radiocarbon age of the Clovis complex has been established at between 11,500 and 11,000 years B.P. (*8*). At present it is widely believed that big game hunters using fluted projectile points of Clovis type were the earliest inhabitants of the Americas. On this premise, a statistical model of wavefront expansion of Clovis big-game hunters from north to south throughout the New World has been presented by Martin (*9*). The minimum dating of 13,000 years B.P. for the El Jobo complex at Taima-taima demonstrates a significant temporal priority over the Clovis complex. The Martin model of the earliest peopling of the New World is thereby refuted by field evidence.

Technologies for the procurement of Pleistocene megafauna must have evolved independently in northern South America and on the North American plains. The Clovis complex and the El Jobo complex must have developed mutually distinctive flaking traditions from simpler technological bases, which had probably entered the New World much earlier. We believe that evidence of the technological traditions ancestral to the various early projectile point technologies of the Americas is to be found by concerted investigation of promising Pleistocene deposits in the New World (*10*).

ALAN L. BRYAN
Department of Anthropology, University of Alberta, Edmonton, Canada T6G 2H4
RODOLFO M. CASAMIQUELA
Centro de Investigaciones Científicas, Provincia de Rio Negro, Viedma, Rio Negro, Argentina
JOSÉ M. CRUXENT
Departamento de Antropología, Instituto Venezolano de Investigaciones Científicas, Apartado 1827, Caracas
RUTH GRUHN
Department of Anthropology, University of Alberta
CLAUDIO OCHSENIUS
Centro de Investigaciones del Paleoindio y Cuaternario Sudamericano, Apartado 7482, Coro, Falcón, Venezuela

References and Notes

1. J. M. Cruxent and I. Rouse, *Am. Antiq.* **22**, 172 (1956).
2. J. Royo y Gomez, *Mem. Cong. Geol. Venez.* **2**, 501 (1960); *Soc. Vert. Paleontol. News Bull.* **58**, 31 (1960); J. M. Cruxent, *IVIC (Inst. Venez. Invest. Cient.) Dep. Antropol. Bol. Inf.* **2**, 20 (1961).
3. I. Rouse and J. M. Cruxent, *Am. Antiq.* **28**, 537 (1963).
4. ———, *Venezuelan Archaeology* (Yale Univ. Press, New Haven, Conn., 1963), plate 4.
5. J. M. Cruxent, *Acta Cient. Venez. Sup.* **3**, 3 (1967); *Antiquity* **44**, 223 (1970).
6. M. A. Tamers, *Radiocarbon* **8**, 204 (1966); *ibid.* **11**, 396 (1969); *ibid.* **12**, 509 (1970); *ibid.* **13**, 32 (1971). A. L. Bryan [*Quat. Res. (N.Y.)* **3**, 237 (1973)] has compiled a table summarizing the radiocarbon dates.
7. C. V. Haynes, *Quat. Res. (N.Y.)* **4**, 378 (1974); T. F. Lynch, *ibid.*, p. 356.
8. C. V. Haynes, *Science* **166**, 709 (1969).
9. P. S. Martin, *ibid.* **179**, 969 (1973); J. E. Mosiman and P. S. Martin, *Am. Sci.* **63**, 304 (1975).
10. A conceptual model and testable working hypotheses in support of an early entry of man into America are presented in A. L. Bryan, *Occas. Pap. Dep. Anthropol. Univ. Alberta.* **1**, 306 (1978).
11. We thank the Centro de Investigaciones del Paleoindio y Cuaternario Sudamericano (CIPICS) for sponsoring the 1976 excavations at Taima-taima.

14 December 1977

Soybean Lines Lacking the 120,000-Dalton Seed Lectin

Abstract. *Seeds of 102 lines of* Glycine max *(L.) Merr., the soybean, were screened quantitatively for the presence of the 120,000-dalton soybean lectin. Wide variation in the content of this lectin was noted, and five lines of soybean whose seed totally lacked the lectin were identified. Roots of all five lines were effectively nodulated by several strains of* Rhizobium japonicum, *thus indicating that the 120,000-dalton soybean seed lectin is probably not required for the initiation of soybean-*Rhizobium *symbiosis.*

Approximately 175 million metric tons of atmospheric nitrogen are biologically fixed each year by microorganisms. The fixation of almost half of this amount is the result of symbiosis between legumes and bacteria of the genus *Rhizobium* (*1*). Legume-*Rhizobium* symbioses are markedly species-specific in that a given legume is usually nodulated by only a small group of rhizobial strains. The molecular mechanism whereby leguminous plants differentiate their symbiotic rhi-

0036-8075/78/0616-1277$00.50/0

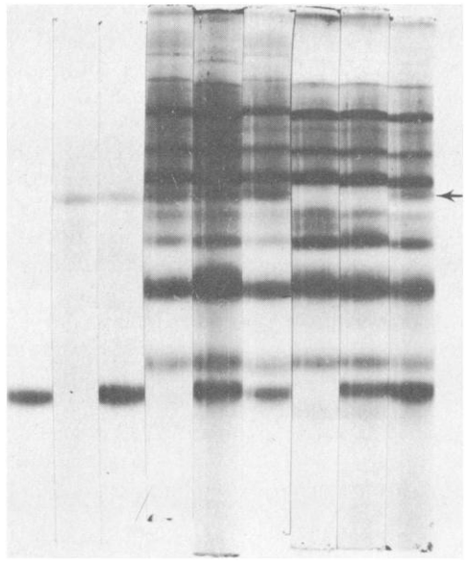

Fig. 1. Protein-stained polyacrylamide gel electrophoresis patterns of soybean extracts, SBL, and lysozyme. Electrophoresis was carried out on 10 percent polyacrylamide gels at 22°C in a glycine–citric acid buffer (*p*H 3.7) for 90 minutes at 3.5 mA per tube. From left to right: 10 μl of lysozyme (1 mg/ml); 35 μl of SBL (1 mg/ml); 35 μl of SBL (1 mg/ml) + 10 μl of lysozyme (1 mg/ml); 50 μl of Amsoy extract; 50 μl of Amsoy extract + 10 μl of lysozyme (1 mg/ml); 50 μl of Amsoy extract + 35 μl of SBL (1 mg/ml) + 10 μl of lysozyme (1 mg/ml); 50 μl of T102 extract; 50 μl of T102 extract + 10 μl of lysozyme (1 mg/ml); and 50 μl of T102 extract + 35 μl of SBL (1 mg/ml) + 10 μl of lysozyme (1 mg/ml). The arrow marks the SBL band at R_F 0.48.

zobia from nonsymbionts is unknown. More than 20 years ago, Mäkelä (*2*) hypothesized, but failed to confirm, that plant lectins may be the molecules responsible for recognition of symbionts. Recent data indicate that the seed lectins of several legumes, including soybean, bind to surface carbohydrates of symbiotic rhizobia, but not to nonsymbionts (*3*). As a result of this striking specificity of a host molecule for symbionts, the lectin recognition hypothesis has been revived and is the subject of considerable investigation.

There have been a number of systematic investigations on the variation in production of lectin among cultivars of a given legume species, but none have been related to recognition of symbionts. Almost 30 years ago, Boyd and Reguera (*4*) reported that seeds of 18 of 62 cultivars of lima bean (*Phaseolus lunatus*) lacked agglutinating lectins. Similarly, Brücher *et al.* (*5*) studied agglutinating lectins in individual seeds of *P. aborigineus*. Although all seeds of some collections (211 of 211 seeds) contained lectins, as few as 23 percent in other collections contained lectins. In addition, the results of a large-scale screening study in genera such as *Canavalia*, *Dolichos*, *Glycine*, and *Pisum* indicate that variation in lectins with cultivar may be widespread in the legumes (*6*).

The soybean, *Glycine max* (L.) Merr., contains a lectin with a molecular weight of about 120,000 and specificity for D-galactose and *N*-acetyl-D-galactosamine (*7*). This lectin, here termed soybean lectin (SBL), is often accompanied by minor isolectins of similar composition and binding specificity (*8*). Fountain and Yang (*9*) recently reported that the major lectin in seeds of the soybean line D68-127 has a molecular weight of 92,000 and may be one of the earlier described isolectins; SBL was present in seeds of D68-127, but as a minor component. A survey of 14 other soybean lines indicated additional variation in isolectin profiles. We report here the results of a successful search for lines of soybean that lack SBL. The availability of such lines in turn allowed us to evaluate the involvement of SBL in soybean-*Rhizobium* symbiosis.

Seeds of 102 soybean lines were powdered and exhaustively defatted with petroleum ether. A portion of the seed meal from each line (1 to 2 g) was extracted for 2 hours with phosphate-buffered saline (PBS) containing tritium-labeled SBL of known specific activity (*10*). Each extract was clarified and subjected to affinity chromatography with a Sepharose affinity adsorbent derivatized with *N*-acylgalactosamine as described previously (*11*). Each column eluant containing SBL was dialyzed, and its specific activity was determined by liquid scintillation spectrometry and protein measurement by the method of Lowry *et al.* (*12*).

For 97 lines of *G. max*, the amount of SBL ranged from 2.5 to 12.2 mg per gram of defatted seed meal. At $\alpha = .05$, there were no significant correlations between lectin and protein content or lectin and oil content in the seed. As expected, there was a significant negative correlation between oil and protein content. In 7.5 percent acidic gels (*13*), SBL from each of the 97 lines electrophoresed as a single band that corresponded to that of the 120,000-dalton SBL. The remaining five lines (Columbia, Norredo, Sooty, T102, and Wilson-5) lacked any detectable endogenous SBL. Recovery of added tritium-labeled SBL from these five lines was from 38 to 68 percent (mean, 51 percent), a range of variation which is well within that of the other 97 lines (20 to 83 percent; mean, 50 percent). Therefore, the failure to recover endogenous SBL from the five lines was not due to inactivation of the lectin during extraction.

Several additional tests were performed to determine if even trace amounts of lectins could be detected in the seeds of the five soybean lines that lacked SBL. The first was hemagglutination, which had a detection limit of 1 μg of purified SBL per milliliter. Seed meal from each of the five lines and from 20 randomly selected control lines was extracted with PBS (50 mg of meal per milliliter of PBS). After clarification and dialysis, the hemagglutination titer of each extract with trypsinized type O erythrocytes was determined (*14*). Whereas titers of the extracts from the control lines varied from 8 to 128, the erythrocytes were not agglutinated by extracts of the five lines at any dilution.

Proteins in extracts of the seeds of the five soybean lines and the 20 controls were next labeled with fluorescein isothiocyanate and evaluated for binding to cells of eight strains of *Rhizobium* (*15*). The binding of extracts from each of the 20 control lines to rhizobial cells was identical to that of purified SBL, but extracts of each of the five soybean lines failed to bind to cells of any of the rhizobia strains.

In a third test, protein extracts from seeds were electrophoresed in polyacrylamide disk gels (*16*) and the presence of a band migrating coincident with authentic SBL was noted at R_F 0.48 (R_F is the mobility relative to the lysozyme protein band in a 10 percent polyacrylamide gel cathodic system in which a glycine–citric acid buffer, *p*H 3.7, is used) (Fig. 1). The gels of the five lines without SBL did not have the protein band associated with the lectin, while all other lines tested had the SBL protein band.

None of the five lines that lacked SBL are grown commercially in the United States. Columbia is a line introduced into the United States from China in 1908, Norredo is of unknown origin, and Sooty was selected from the line Cloud, which contains SBL. Line T102 is closely related to Wilson-5, a subline of Wilson (contains SBL) which was introduced into the United States from northeast China around 1910.

The capacity of the five soybean lines lacking SBL, as well as the SBL-containing line Harosoy 63, to be nodulated by

R. japonicum (strains 3I1b138, 505W, and 61A72) was evaluated by use of greenhouse-grown plants potted in washed sand. Each *Rhizobium* strain produced numerous healthy-appearing pink nodules on all six soybean lines within 3 weeks of inoculation. Thus soybean lines whose seeds lack SBL retain their ability to be nodulated by symbiotic *R. japonicum*.

The remaining unanswered question for symbiosis is whether lines of *G. max* whose seed lack SBL are also without the lectin in their roots. Seedling roots of the line Beeson contain small amounts of SBL, but the lectin decreases to an undetectable level when the cotyledons abscise, about 2 weeks after planting (*11*). The origin of the root SBL in seedlings is uncertain, but it probably represents mobilized reserve SBL that had been stored in the cotyledons. If the genes controlling the absence of SBL in seeds of the five lines are structural, it follows that SBL is not involved in recognition of rhizobial symbionts by *G. max*. If, on the other hand, the genes are regulatory genes that control the levels of SBL in soybean tissues, it is plausible that SBL could be present in the roots of the five lines. Both possibilities, as they relate to soybean-*Rhizobium* symbiosis, should be examined.

STEVEN P. PULL
STEVEN G. PUEPPKE
Department of Biology,
University of Missouri,
St. Louis 63121

THEODORE HYMOWITZ
JAMES H. ORF
Department of Agronomy,
University of Illinois,
Urbana 61801

References and Notes

1. R. W. F. Hardy and U. D. Havelka, *Science* **188**, 633 (1975).
2. O. Mäkelä, *Ann. Med. Exp. Biol. Fenn.* **35** (Suppl. 11) (1957).
3. B. B. Bohlool and E. L. Schmidt, *Science* **185**, 269 (1974); R. B. Dazzo and D. H. Hubbell, *Appl. Microbiol.* **30**, 1017 (1975); T. V. Bhuvaneswari, S. G. Pueppke, W. D. Bauer, *Plant Physiol.* **60**, 486 (1977).
4. W. C. Boyd and R. M. Reguera, *J. Immunol.* **62**, 333 (1949).
5. O. Brücher, M. Wecksler, A. Levy, A. Palozzo, W. G. Jaffé, *Phytochemistry* **8**, 1739 (1969).
6. W. C. Boyd, E. Waszczenko-Zacharczenko, S. M. Goldwasser, *Transfusion* **1**, 374 (1961).
7. H. Lis, N. Sharon, E. Katchalski, *J. Biol. Chem.* **241**, 684 (1966); R. Lotan, H. W. Siegelman, H. Lis, N. Sharon, *ibid.* **249**, 1219 (1974); S. Wada, M. J. Pallinsch, I. E. Liener, *ibid.* **233**, 395 (1958).
8. H. Lis, C. Fridman, N. Sharon, E. Katchalski, *Arch. Biochem. Biophys.* **117**, 301 (1966).
9. D. W. Fountain and W. Yang, *Biochim. Biophys. Acta* **492**, 176 (1977).
10. The SBL was labeled with [^{3}H]$NaBH_4$ by the oxidation-reduction procedure of R. Lotan *et al.* [*J. Biol. Chem.* **250**, 1955 (1975)] and purified by affinity chromatography. Typically, 180 μg of [^{3}H]SBL (specific activity, 10^6 cpm/mg) was diluted into each seed extract.
11. S. G. Pueppke, K. Keegstra, A. L. Ferguson, W. D. Bauer, *Plant Physiol.*, in press.
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
13. R. A. Reisfeld, O. J. Lewis, D. E. Williams, *Nature (London)* **195**, 281 (1962).
14. Each extract was diluted in twofold increments to a final dilution of 1:2048 in (i) PBS and (ii) in PBS containing 5 m*M* *N*-acetyl-D-galactosamine. Portions of each dilution (25 μl) were transferred to wells in a microtiter plate (Abbott Laboratories), and 25 μl of a 3 percent suspension of trypsinized type O human erythrocytes were added to each well. The following titers were recorded for these lines of *G. max* after 3 hours at room temperature: Ada, 32; Amsoy, 32; Chippewa, 64; Columbia, 0; Early White Eyebrow, 8; Fabulin, 32; Flambeau, 32; Funk Delicious, 64; Kanro, 32; Kent, 64; Kim, 16; Kura, 64; Manitoba Brown, 64; Norredo, 0; Peking, 64; Sato, 64; Scott, 64; Sooty, 0; T102, 0; Wea, 64; Wilson, 128; Wilson-5, 0; Wilson-6, 64; Wisconsin Black, 32; and Yellow Marvel, 16. There was no agglutination in any well containing *N*-acetylgalactosamine.
15. Purified SBL and dialyzed protein extracts were labeled with fluorescein isothiocyanate by the method of S. Udenfriend [*Fluorescence Assay in Biology and Medicine* (Academic Press, New York, 1962), p. 221]. Details of the culture method for rhizobia and the assay procedure are in T. V. Bhuvaneswari *et al.* [*Plant Physiol.* **60**, 486 (1977)]. *Rhizobium japonicum* (strains 3I1b38, 3I1b138, and 3I1b143) bound both fluorescent-labeled SBL and the 20 fluorescent-labeled control extracts, but did not bind fluorescent-labeled extracts of the five lines. *Rhizobium japonicum* (strains 505W and 61A72) and *Rhizobium* sp. (strains 3G4b10 and 3G4b19) did not bind SBL or any extract.
16. For the procedure for protein extraction from soybean seed and for staining and destaining methods, see T. Hymowitz and H. H. Hadley [*Crop Sci.* **12**, 197 (1972)]. The 10 percent polyacrylamide gels were made by using the catalyst system of E. M. Jordan and S. Raymond [*Anal. Biochem.* **27**, 205 (1969)] and a gel buffer of 0.25*M* tris adjusted to *p*H 2.4 with citric acid. The electrode buffer was 0.37*M* glycine adjusted to *p*H 3.7 with citric acid. The lysozyme (muramidase) was purchased from Sigma Chemical Company.
17. Portions of this work were supported by the Illinois Agricultural Experiment Station, a faculty research grant to S.G.P. from the University of Missouri-St. Louis, and a grant to T.H. by the Illinois Crop Improvement Association.

7 December 1977

Subpicosecond Spectroscopy of Bacteriorhodopsin

Abstract. *Subpicosecond pulses have been used to study the ultrafast dynamics of the photochemistry of bacteriorhodopsin. An optically induced absorption that appears in about 1.0 picosecond at physiological temperatures has been resolved in time. The data can be interpreted in terms of the photochemical formation of bathobacteriorhodopsin and provide support for an excitation mechanism involving molecular rearrangement in the protein induced by electron redistribution in the chromophore.*

Newly developed techniques for the generation and utilization of subpicosecond optical pulses (*1*) have been applied to the study of systems ranging from relatively simple organic molecules (*2–4*) to complex proteins (*5*). We have employed these techniques to study the ultrafast photochemistry of bacteriorhodopsin at physiological temperatures. Bacteriorhodopsin is of interest because of its similarities to visual pigment rhodopsins and its biological role as an energy converter. Time-resolved studies play an important role in distinguishing between different types of molecular transformations involved in its photochemistry.

Bacteriorhodopsin develops in the plasma membrane of *Halobacterium halobium* when this bacterium is grown in light and at low oxygen tension (*6*). It acts as a light-driven proton pump (*7*), converting light energy into chemical energy and subsequently into a proton gradient across a cell membrane. This gradient is then used to generate adenosine triphosphate, in agreement with the Mitchell hypothesis (*8*).

The chromophore in both rhodopsin and bacteriorhodopsin is retinal, complexed to an ϵ-amino group of the lysine residue (*6*) through a protonated Schiff base linkage (*9*). It has been shown that light absorption in both these pigments produces a new species, called the batho intermediate, which has an absorption maximum ~ 40 to 45 nm to the red of that of the rhodopsin from which it is produced. The batho intermediate then thermally relaxes to a species with an absorption that is once again similar to that of the parent pigment. These similarities may indicate that the primary mechanism of light excitation is the same in both rhodopsin and bacteriorhodopsin.

Figure 1 shows the photochemical cycle of light-adapted bacteriorhodopsin as outlined by other workers (*10*). We are interested in the dynamics of the primary step: bacteriorhodopsin $bR_{570} \rightarrow$ bathobacteriorhodopsin K_{610} at physiological temperatures. Previous studies (*11, 12*) indicated very rapid ($\leq$ 6 psec) formation of the batho species at higher energy (*13*). The results (*11, 12*) were limited in resolution by the duration of the excitation and probing pulses. In the work reported here we were able to resolve the formation time of K_{610} by using subpicosecond optical pulses.

The experimental apparatus we used has been described (*1, 4*). The pulse train consists of subpicosecond optical pulses at 615 mm, near the absorption maximum of K_{610}, from a passively modelocked continuous-wave dye laser. The pulses are obtained by acousto-optic dumping from the laser resonator at a repetition rate of 10^5 per second. After compression with a grating pair, the puls-

0036-8075/78/0616-1279$00.50/0