

APPENDIX A
PROTEIN RESIDUE ANALYSIS

**PROTEIN RESIDUE ANALYSIS OF A FLUTED
POINT FROM THE BOG SITE, CA-SDI-2506,
SOUTHERN CALIFORNIA**

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INTRODUCTION

A single obsidian fluted point and an associated soil control from the Bog Site, CA-SDI-2506, in the Lost Valley area in northeastern San Diego County, California, were tested for possible protein residues. Although the site has been culturally affiliated with a seasonal camp of the late prehistoric and protohistoric Cupeño People and possibly the Mountain Cahuilla, the fluted point is believed to represent the early Holocene Clovis Culture approximately 10,000 to 12,000 years ago. Obsidian sourcing analysis indicates that the source of the lithic material was Lookout Mountain in the Casa Diablo Complex, about 540 kilometers north of the site. Protein residue analysis was undertaken to determine if proteins were present on the artifact surface that would provide information concerning animals that were hunted with this tool.

METHODS

The artifact submitted for protein residue analysis was tested using an immunologically-based technique referred to as cross over immunoelectrophoresis (CIEP or COE). The method for CIEP is based on forensic work by Culliford (1964; 1971) with changes made by Newman (1989) following the procedure used by the Royal Canadian Mounted Police Serology Laboratory in Toronto, Canada. Further changes were made at Paleo Research Institute following the advice of Dr. Richard Marlar at the Thrombosis Research Laboratory in the Denver VA Medical Center and the University of Colorado Health Sciences Center.

The artifact was washed using 1-2ml of a 0.02M Tris hydrochloride, 0.5M sodium chloride, and 0.5% Triton X-100 solution. The artifact was placed in an ultrasonic bath for 30 minutes, on a rotating mixer for 30 minutes, then in the ultrasonic bath for an additional 30 minutes. Because soils contain compounds such as bacteria and animal feces that can cause false positive results for artifacts buried in the soil, a control sample also was tested. One gram of soil was added to 1 ml of the Tris/NaCl/Triton solution, then refrigerated for several days prior to testing.

The residues extracted from the artifact and the soil control first were tested against pre-immune goat serum (serum from a non-immunized animal) to detect non-specific binding of proteins. Samples testing negative against pre-immune serum then are tested against prepared animal antisera obtained from ICN Pharmaceuticals, Inc. and Sigma Chemical Company, and against antisera raised under the direction of Robert Sargeant in Lompoc, California, and Dr. Richard Marlar. Appropriate positive and negative controls were run for each antiserum. A positive control consists of the blood of an animal for which the antiserum is known to test positively, and a negative control consists of the serum/blood of the animal in which the antiserum was raised, either rabbit or goat.

CIEP is performed using agarose gel as the medium. Two holes are punched in the gel about 5 mm apart. The protein extract from the artifact was placed in the cathodic well and the antiserum is placed in the anodic well. The sample was electrophoresed in Barbital buffer (pH 8.6) for 45 minutes at a voltage of 130v to drive the antigens and antibodies towards each other. Positive reactions appear as a line of precipitation between the two wells. Gels are stained with coomassie blue to make the precipitate line easier to see. Positive reactions were re-tested with dilute antisera to determine between true and false positives. Antisera are diluted to increase specificity of reactions, usually 1:10 or 1:20. Positive reactions obtained after this step are reported.

Identification of animals represented by positive results is usually made to the family level. All mammalian species have serum protein antigenic determinations in common; therefore, some cross reactions will occur between closely and sometimes distantly related animals (Gaensslen 1983:241). For example, bovine antiserum will react with bison blood, and deer antiserum will react with other members of the Cervidae (deer) family, such as elk and moose.

DISCUSSION

CA-SDI-2506 is located near Shingle Spring in the Lost Valley, California, at an elevation of 4600 to 4800 feet. Shingle Spring represents the headwaters of the San Luis Rey Watershed. Vegetation in the area consists of oak (*Quercus*) and pine (*Pinus*) woodlands flanked by mountain chaparral and a riparian environment near the spring. A meadow containing grasses (Poaceae) and sedges (Cyperaceae) is located about one-half kilometer west-southwest of the site. This meadow may have been a Pleistocene lake that has since silted up (George Kline, personal communication, May 28, 2006). Shallow lake and marsh conditions prevailed in southern and western California from about 11,000 to 8,000 B.P. These areas contained varied and abundant plant and animal resources, which in turn would have attracted early hunter-gatherers to the area (Moratto 1984:78; Warren 1963; Weide 1968).

The site originally was described as a late prehistoric Cupeño seasonal camp. A variety of artifacts were found in the Late Prehistoric blackened midden deposits at depths of up to 50 cm below the present ground surface. Sample 4408 consists of an obsidian fluted point recovered at a depth of 100 cm in an otherwise sterile deposit (Table 1). The accompanying soil control (sample 4395) was collected approximately 20 cm west of the point at a depth of 103 cm below the surface. The fluted point is believed to represent the Clovis Culture of the late Pleistocene/early Holocene, about 12,000 to 10,000 years ago. This time period is noted to have experienced warming temperatures, glacial retreat, rising seas, evaporation of pluvial lakes, and major vegetation shifts, and the extinction of the Rancholabrean fauna (Moratto 1984:79).

The fluted point and the soil control were tested against the various antisera listed in Table 2. Sample 4408 tested positive to deer antiserum (Table 3), suggesting use of the point to hunt a member of the Cervidae (deer family). Soil control sample 4395 yielded a negative result to deer antiserum, indicating that the positive result for the artifact is not due to soil contamination. Members of the Cervidae in North America include deer (*Odocoileus* sp.), elk (*Cervus elaphus/canadensis*), moose (*alces*), and caribou (*Rangifer* sp.). Moose and caribou are found mainly in Canada, although moose can be found in the northeastern United States and southwest through the Rocky Mountains to northeastern Utah and northwestern Colorado. Elk are found primarily in the Rocky Mountain region and along the Pacific Northwest coast, with great numbers found in Colorado, Wyoming, Montana, and Washington. Tule elk were once found in the California Central Valley Lower Sonoran zone, and Canadian elk can be found in the California Transition zone (Morratto 1984:23-24). Elk are reported to have once ranged through most of the United States and Canada. Prior to European settlement, elk are noted to have been among the most common and widely distributed wild ungulates in North America, but their numbers decreased due to hunting and reduction in habitat from settlement and farming. Mule deer (*Odocoileus hemionus*) are found primarily in the western portion of North America, from southern Yukon and Mackenzie south through the western United States, extending east to Wisconsin and west Texas. Prehistoric ranges may have extended further east. White-tailed deer (*Odocoileus virginianus*) currently is the most widespread and numerous member of the Cervidae. White-tailed deer are found throughout most of the United States in a variety of habitats, except for northern Arizona, southwest Colorado, northwest New Mexico, and most of California, Nevada and Utah, as well as in southern Canada, through Central America, and into northern South America

SUMMARY AND CONCLUSIONS

Protein residue analysis was conducted on a fluted projectile point found at site CA-SDI-2506 in Lost Valley, San Diego County, California. This point is believed to be associated with the Clovis Culture. The point yielded a positive result to deer antiserum, suggesting that it was used to hunt a member of the deer family. A negative result to deer antiserum for the soil control indicates that the positive result was not due to soil contamination.

TABLE 1
PROVENIENCE DATA FOR SAMPLES FROM SITE CA-SDI-2506

Sample No.	Feature No.	Depth (cmbs)	Provenience/Description	Analysis
4408		100	20S 6W; Obsidian fluted point	Protein residue
4395		103	20S 6W; Soil control from the west wall of unit, 20 cm west of fluted point	Protein residue

TABLE 2
LIST OF ANTISERA USED IN TESTING ARTIFACT AND SOIL CONTROL FROM SITE CA-SDI-2506

ANTISERA	SOURCE	POSSIBLE RESULTS
ANIMALS:		
Bear	ICN Pharmaceuticals, Inc.	Black bear, Brown bear, Grizzly, Polar bear
Bison	Prepared under the direction of Dr. Richard Marlar at the University of Colorado Health Sciences Center	Bison, Domestic bovids
Bovine	Sigma Chemical Company	Domestic bovids, Bison
Camel	ICN Pharmaceuticals, Inc.	Camel, Llama, Prehistoric camelids
Cat	Sigma Chemical Company	Domestic cat, Mountain lion, Bobcat, Lynx, other wild cat species
Chicken	Sigma Chemical Company	Domestic chicken, Partridge, Quail, Grouse, Ptarmigan, Pheasant
Deer	ICN Pharmaceuticals, Inc.	White tail deer, Mule deer, Elk, Moose, Caribou
Dog	Sigma Chemical Company	Domestic dog, Coyote, Wolf, Fox
Elephant	Robert Sargeant	Elephant, Mammoth

ANTISERA	SOURCE	POSSIBLE RESULTS
Goat	Sigma Chemical Company	Pronghorn, Mountain goat, Domestic goat
Horse	ICN Pharmaceuticals, Inc.	Horse, Donkey, Zebra, Extinct species of wild horse
Human	ICN Pharmaceuticals, Inc.	Human
Rabbit	Sigma Chemical Company	Rabbit, Jackrabbit (hare)
Sheep	ICN Pharmaceuticals, Inc.	Domestic sheep, Bighorn sheep
Turkey	Sigma Chemical Company	Domestic turkey, Wild turkey, Ducks
FISH:		
Striped bass	Robert Sargeant	Perciformes order (Spiny-rayed or percoid fish)

TABLE 3
 POSITIVE PROTEIN RESIDUE RESULTS FOR SAMPLES
 FROM SITE CA-SDI-2506

Sample No.	Description	Positive Result (Antiserum Type)	Possible Animal(s) Represented
4408	Obsidian fluted point	Deer	Cervidae (Deer family)

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