FEASIBILITY OF USING PROTEIN RESIDUE ANALYSIS TO DETERMINE MATERIALS PROCESSED WITHIN BEDROCK MILLING FEATURES

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This pilot project tests the feasibility of using crossover immunological electrophoresis (CIEP) analysis to identify plant and animal protein residues on bedrock processing features in order to test long-held assumptions. Positive antiserum-antibody reactions (positive results) were obtained for about 17 percent of the processing surfaces sampled. Both plant and animal protein residues were identified. Thus, it appears feasible to use this analytical technique on bedrock surfaces. Collecting samples in the field presented unanticipated logistical problems that, when addressed in the future, will likely lead to a higher percentage of empirical data.

This is the report of the results of a pilot project designed to test the use of crossover immunological electrophoresis (CIEP) analysis on residues left on bedrock features used in the past to process various materials. The project was to determine (1) if this type of analysis is feasible and (2) if positive reactions to plant and animal materials have the potential to resolve long-standing questions about the uses of bedrock features of various types. The research design called for:

- Identifying one site in each of three environments.
- Collecting 30 matched samples: 10 matched samples (extracts from bedrock milling feature surfaces + soil samples) at each site. Different feature types were to be sampled: mortars, basins, cupules, and slicks.
- Subjecting each set of matched samples to CIEP analysis. CIEP antisera available included 17 faunal antisera and 14 plant antisera

RESEARCH PROBLEMS

Long-held assumptions about the types of materials that were processed on bedrock surfaces within the Colorado Desert and surrounding areas have not been scientifically tested. Certain bedrock features are assumed to be associated with certain types of processing, with certain environments, and with local availability of specific resources. Empirical data, however, are lacking. Many problematic situations exist. For example, why do several types of processing features exist on the same bedrock outcrop? Why are there processing features on bedrock outcrops where there are not obvious resources available today? CIEP has been successfully used on portable processing equipment (e.g., Schneider 2009; Schneider et al. 2006:83-84; Yohe et al. 1991). Could the same type of analysis be used on non-portable processing surfaces?

SPECIFIC RESEARCH QUESTIONS ADDRESSED BY THIS PILOT PROJECT

- Can CIEP be used to determine the types of materials processed on bedrock processing features?
- If CIEP can be used, are the results definitive enough to distinguish introduced substances from naturally occurring substances on bedrock processing features?
- If CIEP can be used, is it possible to document transportation of subsistence items from locales of origin in the Colorado Desert region?
- If CIEP can be used, is it possible to determine if there is a correlation between type of bedrock processing feature and the material being processed?

- If CIEP can be used, how does what we know of Colorado Desert paleoenvironmental change and continuity reflect on results of the analyses?

WHAT IS CIEP?

CIEP is an acronym for crossover (C) immunological (I) electrophoresis (EP). Forensic scientists developed this type of testing for protein residues for criminal investigations, and since then the method has had a number of applications, including archaeological.

CIEP is an antigen-antibody reaction that causes a precipitate to form when a specific antibody is “challenged” by a specific antigen. In most cases, only family-level taxonomic identification is possible at this time. Matched samples extracted from (1) an artifact or feature and (2) a soil sample from the immediate area of the artifact or feature are tested against a series of antisera that have been prepared by various laboratories. An electrical current is applied to the cell plate containing both the introduced sample (an extract from the artifact or feature or soil) and the various prepared antisera. If there is a positive reaction between the prepared antiserum and the introduced antigen (the extract), a precipitate forms, and this is considered a “positive result.”

California State University, Bakersfield Laboratory of Archaeological Sciences (LAS) had available for CIEP analyses antisera from the following protein sources. Positive reactions to any of these might also indicate positive reaction to closely related species (in parentheses).

Animals:

Alligator (alligator, crocodile)
Bear (black, grizzly, polar, etc.)
Bovine (bison, cow, musk ox)
Camel (all New World and Old World camelids)
Cat (bobcat, cougar, lynx, etc.)
Chicken (chicken, grouse, quail, turkey, other gallinaceous fowl)
Deer (deer, elk, moose, caribou, etc.)
Dog (coyote, dog, wolf)
Guinea pig (beaver, guinea pig, porcupine, squirrel)
Horse (horse, donkey, kiang, etc.)
Rabbit (hare, rabbit, pika)
Rat (all mouse and rat species)
Sheep (bighorn and other sheep)
Swine (pig, possibly javelina)
Top shell (*Tegula*)
Tadpole shrimp (*Triops*, *Lepidurus*)

Plants:

Agavaceae (agave, yucca)
Amaranthaceae (amaranth, pigweed, quelite, etc.)
Asteraceae (rabbitbrush, sagebrush, sunflower, thistle)
Cactaceae (cacti)
Camas (camas, wild hyacinth)
Capparidaceae (beeplant, bladderpod, stinkweed, etc)
Cedar (cedar, cypress, juniper)
Chenopodiaceae (goosefoot, greasewood, pickleweed, saltbush)
Kelp (kelp and possibly algae)
Malvaceae (mallows)
Mesquite (mesquite, palo verde, other legumes)
Piñon (fir, hemlock, pine, spruce)
Poaceae (grasses)
***Quercus* sp.** (oaks)
Walnut (walnut, hickory)

Table 1. Antisera used in this pilot project.

ANTISERUM	SOURCE
Bear	Cappel Research
Cat	“
Chicken	“
Deer	“
Dog	“
Guinea-pig	“
Rabbit	“
Rat	“
Sheep	“
Agave	University of Calgary
Amaranth	“
Aster	“
Cactus	“
Capparid	“
Cedar	“
Chenopod	“
Mesquite	“
Pine	“
Poa	“
Walnut	“

Based on availability and environmental possibilities, LAS selected the antisera in Table 1 to test for protein residues. Each sample collected was tested against nine animal antisera and 11 plant antisera.

SAMPLE SELECTION

It was proposed in the research design that three different environmental situations would be represented in the pilot project. The investigators chose three sites in the Colorado Desert District (Figure 1): the Mine Wash site (CA-SDI-813) in Anza-Borrego Desert State Park (ABDSP) at an elevation of about 1,600 ft. asl (Sampson 1984); Little Blair Valley Morteros site (SDI-2524) in ABDSP at an elevation of about 2,850 ft. asl; and the Los Caballos site (SDI-9538) in Cuyamaca Rancho State Park (CRSP) at an elevation of 4,700 ft. asl (Bruce and Sweet 2004). Within each of these sites, 10 samples of extracts from processing surfaces on bedrock outcrops were collected. For each sample, a matching soil sample was collected. At each site, efforts were made to sample a variety of types of processing features: slicks, basins, mortars, and cupules.

Mine Wash Site (SDI-813)

The Mine Wash site is situated in an open, relatively flat, desert wash environment (Figures 1 and 2). It consists of perhaps 50 or more bedrock outcrops with a variety of processing features; many of the outcrops have more than one type of feature. There is a small rock shelter with cupules on bedrock, visible midden deposits, and a multitude of surface materials. The site was excavated in the 1980s and was found to have a cultural deposit of over 1 m depth. By all indications, only the Late Prehistoric period in the Colorado Desert was represented: from about A.D. 1200 to contact (about A.D. 1775). Vegetation on and surrounding the site consists of creosote, cacti, ocotillo, brittlebush, mallow, occasional agave, chia, annual grasses, and the usual suite of low desert small scrubs. There is a large ironwood tree at the edge of the site. This is a popular site for visitors and school groups.

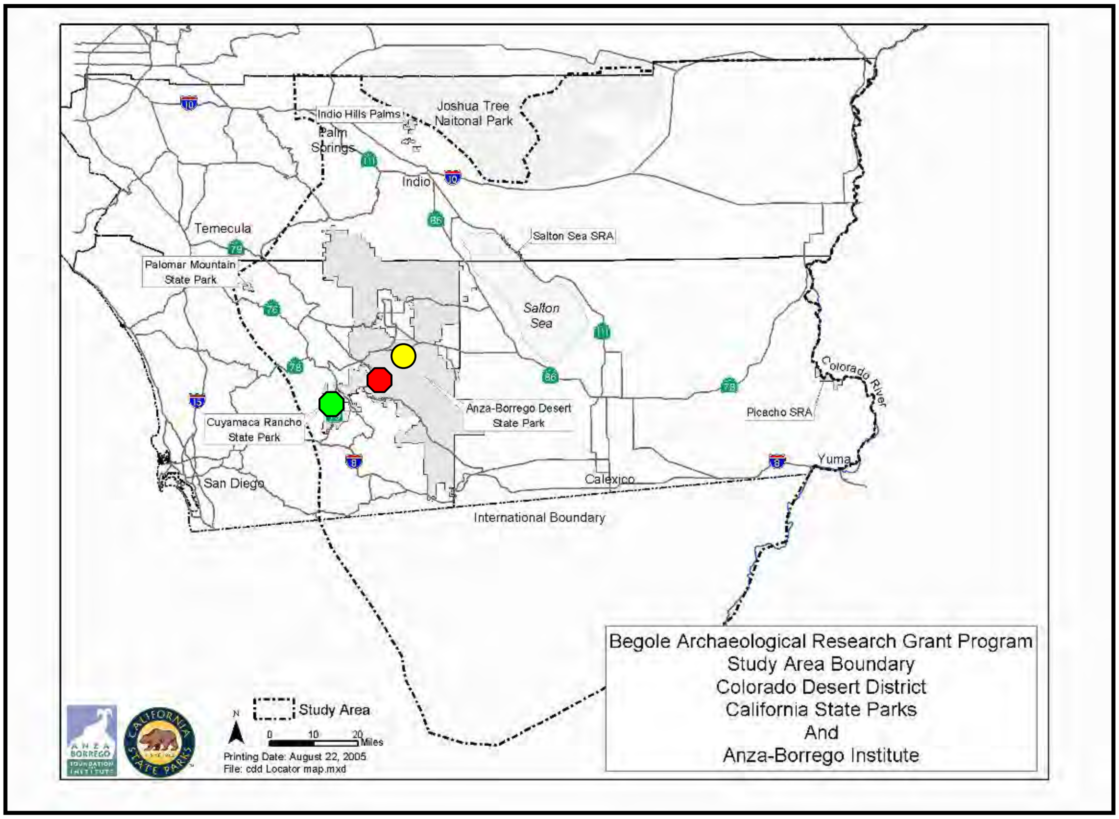


Figure 1. Study area. Yellow dot indicates the location of the Mine Wash site. Red dot indicates the location of the Morteros site. Green dot indicates the location of Los Caballos site.

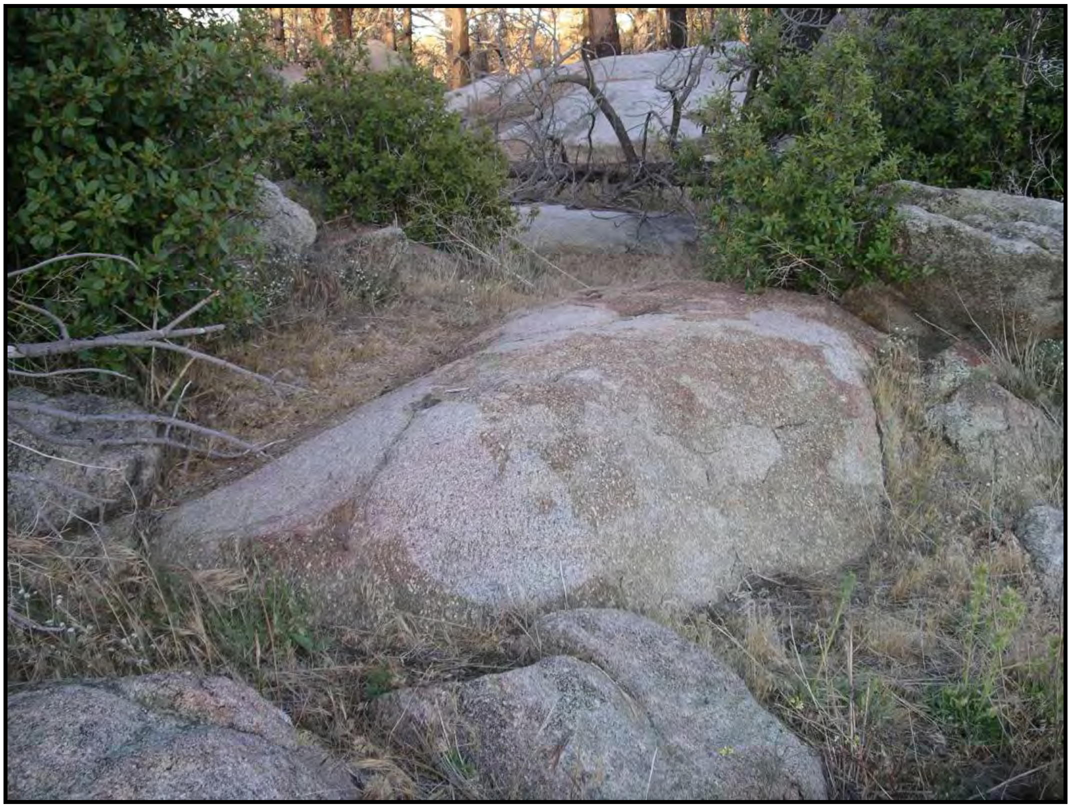
Little Blair Valley Morteros Site (SDI-2524)

The Morteros site in Little Blair Valley is a large site situated along a trail within a small pass between an open area with a remnant playa (to the west) and a lower valley that has a major trail running through it (to the east). Hills rise on either side of the west-east pass (Figures 1 and 2). The site has at least 100 bedrock outcrops with a wide variety of processing surfaces. There are also pictographs and cupules at this site, as well as very frequent roasting pits. The site has never been excavated, but has obvious midden deposits. It is a very popular site for visitors to ABDSP. The only archaeological study of the site has been recording of the surface features by field classes and by volunteers of the Colorado Desert Archaeology Society (CDAS). A nearby site was test excavated as a Master's thesis project (Jacques 2006). Vegetation on and surrounding the site is primarily agave, with creosote, ocotillo, and annual grasses. It has been assumed that most of the bedrock features were used for various types of agave processing. It appears, however, that more than this activity went on at the site. As far as is known from projectile point and bead types found on the surface, the site is Late Prehistoric in chronological context; the site excavated by Jacques (2006), however, had contact-period materials of indigenous manufacture.

Los Caballos (SDI-9538)

Los Caballos is a large village site of Late Prehistoric and contact periods (Figures 1 and 3). It has been identified as the probable ethnohistoric village of *Ah-ha' Kwe-ah-mac* and has been listed on the National Register of Historic Places (Hector 2004). In the 1930s, Malcolm J. Rogers of the San Diego

Figure 2. Collecting samples from a bedrock feature at the Mine Wash site (SDI-813) (above). Sampled bedrock feature and general environment of the Morteros site (SDI-2624; note agave plants (below). Photos by Sam Webb.



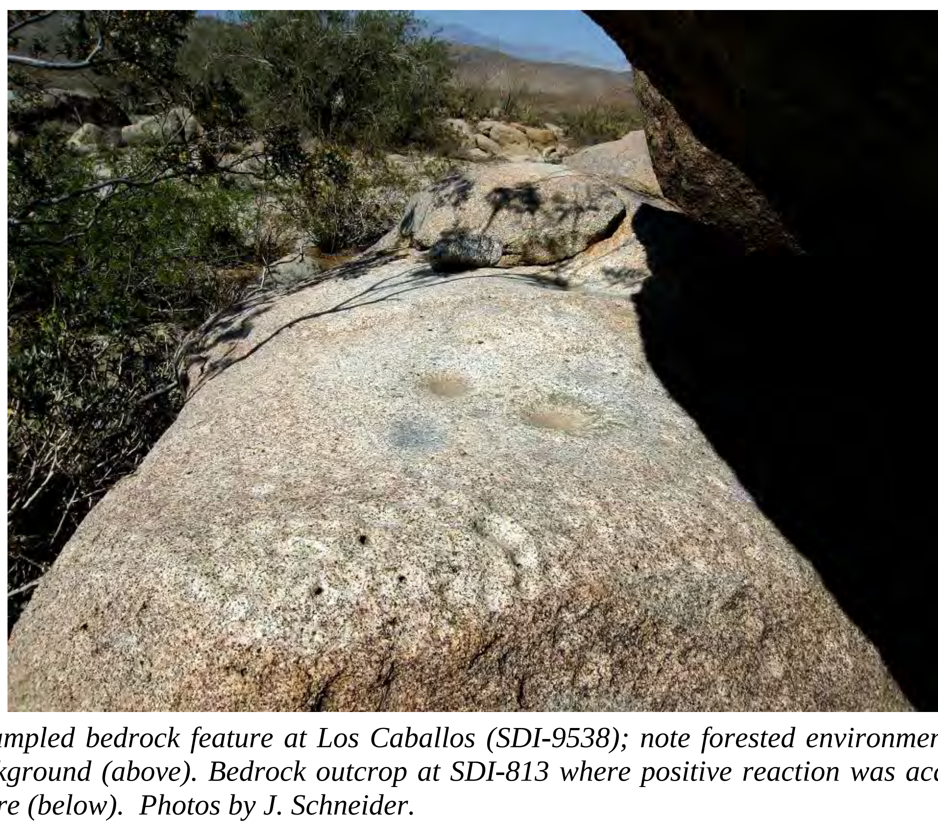


Figure 3. Sampled bedrock feature at Los Caballos (SDI-9538); note forested environment and burned trees in background (above). Bedrock outcrop at SDI-813 where positive reaction was acquired from a cupule feature (below). Photos by J. Schneider.

Museum of Man excavated human remains at this site, but no later excavations have taken place. The site is very large and has numerous bedrock outcrops within what was once an oak forest. Long used as an equestrian campground, Los Caballos is now removed from that use after a devastating fire in 2003 destroyed most of the oaks and covering vegetation, revealing the extent and significance of the site. This site and many others in CRSP are known for a bedrock feature known as the "Cuyamaca oval." This is a moderately deep oval basin that often exists with other types of processing features on bedrock outcrops. It has been assumed that the Cuyamaca ovals at the site are associated with acorn processing.

FIELD METHODS

After each sample bedrock feature was chosen, based on our criteria, the bedrock processing feature was dusted off with a clean brush and then vacuumed with a battery-powered portable vacuum cleaner. The metric attributes of the feature were recorded, the feature location was geocoded, and the feature was photographed.

At each sample site, the following protocol was used, following closely the directions in "Collection Manual for Protein Residue Samples" provided to us by LAS. All materials used to collect the samples were furnished by LAS. The materials included 5 percent ammonium hydroxide solution for extracting the residues from the rock surfaces, pipettes, vials, and wooden stirring sticks. We provided clean surgical gloves for each individual sample, dry ice to immediately freeze samples, and other field equipment.

We applied 1-2 ml of 5 percent ammonium hydroxide to the working surface of each feature and stirred well with a clean wooden applicator stick. We allowed the reagent to remain in contact with the feature surface for 20 minutes. If the reagent was absorbed, we added additional reagent to ensure that there would be sufficient solution to collect. After pre-labeling each of the vials, we removed 1.5 ml of the resulting residue extract solution using a sterile pipette, placed the liquid in the labeled sterile small plastic vial, and sealed the vial. The collected extract solution was frozen immediately in the field using dry ice in a thermal container. All samples remained frozen until received by the LAS.

Matching control soil samples were taken for each sample collected from a bedrock feature. The soil sample was taken from the ground surface to about 2 cm below the surface, as close as possible to the location of the sampled bedrock feature. Efforts were made to take the soil sample beneath an overhang of rock, if this was present at the feature location. Soil samples were also frozen and kept in the same environment as the residue extract solutions until received at the LAS.

Every sample location was geocoded, photographed, and described.

All samples were shipped by overnight express to the LAS; dry ice was used within a thermal shipping container to keep all samples frozen. Each sample was marked with a designation identifying the site and sample number (see Tables 2-4).

RESULTS

Tables 2-4 show the results of the CIEP analyses on the residue extracts and the matching soil samples. The 11 samples taken from the Mine Wash site (SDI-813) yielded only one positive result from Feature 11: for cat (Table 2). Feature 11 was a cupule feature on a multicomponent bedrock outcrop (Figure 3).

At the Little Blair Valley Morteros site (SDI-2524), two positive results were obtained on Feature 8 (Table 3), a cupule in a multicomponent bedrock outcrop (Figure 4). This cupule tested positive for both Capparidaceae and mesquite.

Table 2. Results of CIEP Mine Wash (SDI-813) samples.

Sample	Sample Type	Result
MW1	Bedrock feature	Negative
MW1-S	Soil	Negative
MW2	Bedrock feature	Negative
MW2-S	Soil	Negative
MW3	Bedrock feature	Negative
MW3-S	Soil	Negative
MW3-F	Animal feces in feature	Negative
MW4	Bedrock feature	Negative
MW4-S	Soil	Negative
MW5	Bedrock feature	Negative
MW5-S	Soil	Negative
MW6	Bedrock feature	Negative
MW7	Bedrock feature	Negative
MW6/7-S	Soil	Negative
MW8	Bedrock feature	Negative
MW8-S	Soil	Negative
MW9	Bedrock feature	Negative
MW10	Bedrock feature	Negative
MW11	Bedrock feature	Cat
MW9/10/11-S	Soil	Negative

Table 3. Results of CIEP from Little Blair Valley Morteros site (SDI-2524) samples.

Sample #	Sample Type	Result
BV1	Bedrock feature	Negative
BV1-S	Soil	Dog
BV2	Bedrock feature	Negative
BV2-S	Soil	Negative
BV3	Bedrock feature	Negative
BV3-S	Soil	Negative
BV4	Bedrock feature	Negative
BV4-S	Soil	Negative
BV5	Bedrock feature	Negative
BV5-S	Soil	Negative
BV6	Bedrock feature	Negative
BV6-S	Soil	Negative
BV7	Bedrock feature	Negative
BV7-S	Soil	Negative
BV8	Bedrock feature	Capparidaceae; mesquite
BV8-S	Soil	Rabbit
BV9	Bedrock feature	Negative
BV9-S	Soil	Negative
BV10	Bedrock feature	Negative
BV10-S	Soil	Negative

Table 4. Results of CIEP from Los Caballos (SDI-9538) samples.

Sample #	Sample Type	Results
LC1	Bedrock feature	Rabbit; sheep
LC1-S	Soil	Negative
LC2	Bedrock feature	Negative
LC2-S	Soil	Negative
LC3	Bedrock feature	Negative
LC3-S	Soil	Negative
LC4	Bedrock feature	Rat
LC4-S	Soil	Negative
LC5	Bedrock feature	Negative
LC5-S	Soil	Negative
LC6	Bedrock feature	Negative
LC6-S	Soil	Negative
LC7	Bedrock feature	Negative
LC7-S	Soil	Negative
LC8a	Bedrock feature	Cat
LC8b	Bedrock feature	Negative
LC8-S	Soil	Chicken
LC9	Bedrock feature	Negative
LC10	Bedrock feature	Negative
LC11	Bedrock feature	Negative
LC9/10/11-S	Soil	Negative

Figure 4. Bedrock outcrop and close-up of feature at the Little Blair Valley Morteros site where two positive reactions were obtained from a single cupule feature. Photo by J. Schneider.

Figure 5. Feature 1 at Los Caballos tested positive for rabbit and sheep; photo by B. Bruce (above left). Feature 4 at Los Caballos tested positive for rat; photo by J. Schneider (above right). Feature 8 at Los Caballos tested positive for cat; photo by J. Schneider (below).

At Los Caballos (SDI-9538), four positive results were obtained on three features (Table 4): Feature 1, a round basin, tested positive for sheep and rabbit (Figure 5). Feature 4, a cupule, tested positive for rat; Feature 8a, a large and deep mortar, tested positive for cat.

Positive reactions were obtained for seven residues: two plant proteins and five animal proteins. The Mine Wash and Little Blair Valley Morteros site features in ABDSP had fewer positive results: one feature at Mine Wash had one positive result; one feature at Little Blair Valley Morteros had two positive results from one feature. Los Caballos, the higher-altitude site at CRSP, had the best results, with four positive results from three features.

DISCUSSION

No positive results were obtained from any bedrock slicks or “Cuyamaca ovals.”

All positive results were obtained from cup-like features of various sizes: a deep mortar yielded a positive result for cat (bobcat, mountain lion, lynx). Cupules yielded positive results for Capparidaceae (bladderpod, stinkweed, beeplant), mesquite, and rat. A deep basin yielded positive results for rabbit and sheep. No positive results were obtained for agave, grasses, or other plants, in spite of the fact that previous protein residue analyses on portable milling equipment had produced positive reactions to these plants (Schneider 2009).

None of the samples obtained were tested against oak antiserum due to the fact that the LAS had run out of the oak antiserum at the time when the samples were run. This seriously compromised the results of the pilot project in that we would have expected that oak/acorn would be one of the most common resources processed, especially at CRSP.

It seems worthwhile to discuss here the problematic aspects of the fieldwork, in general. Collecting samples for CIEP in the field presented unanticipated problems. These included several aspects of keeping the ammonium hydroxide solution in contact with the use surface, the tendency for granitic outcrops to absorb the extraction solution, and evaporation of the solution due to aridity and wind in the desert environment.

Keeping the Extraction Solution on the Use Surface

We found it extremely difficult to find a flat surface such as a slick or a “rub” that was not at some angle. If we applied the 5 percent ammonium hydroxide to a surface that appeared to be level, we soon found that this was not so—the solution ran off the surface. This was the most difficult type of bedrock processing surface with which to work. Perhaps in future work, it might be possible to create some kind of dam or containing apparatus to keep the solution in contact with the use surface for a sufficient time. Having a carpenter’s level in the field might be helpful.

Absorption of the Extract Solution

All the surfaces we dealt with in this pilot project were of granitic materials. The open pores and weathering spaces within the surface of the bedrock made it porous, and in almost all cases, the first application of the 5 percent ammonium hydroxide solution was almost completely absorbed. This necessitated adding more solution, and sometimes adding more solution twice. It seemed that after the first absorption round, the solution was less likely to be absorbed.

Evaporation of the Extract Solution

Although summer was avoided for the fieldwork, the aridity of the desert and the wind made it difficult to keep the 5 percent ammonium hydroxide solution in place on a bedrock feature for the required 20 minutes. In the montane environment, this was much less of a problem. We finally learned that we could add the solution and then shade and cover the feature; this would keep the extracting solution from disappearing into the atmosphere. In future work, we will provide better technology to address this problem.

ADDRESSING THE RESEARCH QUESTIONS

The original Research Questions that we posed are addressed below.

- Can CIEP be used to determine the types of materials processed on bedrock surfaces: *Yes*

  Our success rate for positive reactions in this pilot project was about 17 percent. It is likely that with some preparation to address some of the logistical and physical problems we encountered in the field, a higher percentage of positive reactions may be obtained. It is interesting that the only positive reactions were obtained in concave features that would have held the reagent solution in contact with the use surface for a longer period of time with less addition of reagent and little run-off.

- If CIEP can be used, are the results definitive enough to distinguish introduced substances from naturally occurring substances: *Yes*

  The matched samples (processing surface and soil samples) did not produce the same positive results in any case.

- If CIEP can be used, is it possible to document transportation of subsistence items from locales of origin to locales of processing: *Yes*

  We obtained positive reactions from both plant and animal materials. Although our data did not support transport of desert plants into the mountains or mountain plants into the desert, we feel confident that additional data will make it possible to document transportation. The family level of identification might provide us with enough data to determine if plants (e.g., acorn and agave), in particular, could have been transported. In another study of portable processing equipment, we were able to determine that agave proteins were present on tools at a site in CRSP (Schneider 2009).

- If CIEP can be used, is it possible to determine if there is a correlation between type of bedrock processing feature and the material being processed? *Unknown at the present time.*

  We have insufficient data to address this question, mainly because of the unanticipated logistical problems in the field (see above).

- If CIEP can be used, how does what we know of Colorado Desert paleoenvironmental change and continuity reflect on results of the analyses? *Unknown, pending further data.*

  Again, we have insufficient data to address this question at this time.

CONCLUSIONS

This pilot project has demonstrated that protein residues can be identified on bedrock processing features. About 17 percent of the trial features in this study produced at least one positive result. More samples need to be taken in one environment and within one site in order to further pursue this line of research and challenge some of the assumptions that generations of archaeologists have made. Further investigation is needed to solve logistical field problems and to determine why no positive results were obtained from relatively flat surfaces. We anticipate that, with the availability of oak antiserum, we will be able to obtain more positive reactions from features in our region. The lack of acorn antiserum has seriously compromised the findings of this pilot project.

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REFERENCES CITED

Bruce, Bonnie, and Melvin Sweet

2004 Site record for Los Caballos (CA-SDI-9538). On file at the Begole Archeological Research Center, Colorado Desert District Headquarters, Borrego Springs, California.

Hector, Susan

2004 National Register nomination for *Ah-ha' Kwe-ah-mac* (CA-SDI-9538). On file at the South Coastal Information Center, San Diego State University, San Diego.

Jacques, Steven Paul

2006 Kumeyaay Site Analysis in Blair Valley, Anza Borrego Desert State Park, Southern California. Unpublished Master's thesis, Department of Anthropology, California State University, Fullerton.

Sampson, Michael

1984 *Test Excavations at CA-SDI-813, Mine Canyon, Anza-Borrego Desert State Park*. Report on file at the Begole Archeological Research Center, Colorado Desert District Headquarters, Borrego Springs, California.

Schneider, Joan S.

2009 *Archaeological Documentation and Data Recovery at the Stacked Stone Site (CA-SDI-17666): A Site Incorporating Stone Architecture*. Report on file at the Begole Archeological Research Center, Colorado Desert District Headquarters, Borrego Springs, California.

Schneider, Joan S., Karen K. Swope, and Claude N. Warren

2006 *Archaeological Investigations at the Desert Queen Ranch, Joshua Tree National Park: Report of the University of Nevada, Las Vegas Archaeological Field Schools, 1997 and 1998*. Report on file at the Cultural Resources Branch, Joshua Tree National Park, Twentynine Palms, California.

Yohe, Robert M., Margaret Newman, and Joan S. Schneider

1991 Immunological Identification of Small-Mammal Proteins on Aboriginal Milling Equipment. *American Antiquity* 56:659-666.