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Evaluating protein residues on Gainey phase Paleoindian stone tools

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ABSTRACT

Blood protein analysis provides a method for acquiring and interpreting archaeological data bearing on human–animal relationships. The present study makes use of cross-over immunoelectrophoresis (CIEP) and a large sample of stone tools ($N = 130$) from an early Paleoindian site pertaining to the Gainey phase (ca. 11,200 BP) of the Midwestern USA. Results are used to interpret toolkit organization and site structure, and indicate that hafted tools with longer use-life potentialities are more likely to be associated with preserved residues than are tools of a more ad hoc or situational character. Protein residues derived from cervids are the largest category of identified samples, a result consistent with an interpretation that these animals were important first-line resources of Gainey phase populations. The identification of caribou (*Rangifer* sp.) is notable. Interpretations of subsistence importance, however, must be tempered by an appreciation of the possible confounding effects of tool manufacture and maintenance in early Paleoindian contexts.

1. Introduction

Settlement and technological data indicate that the Late Pleistocene societies of Midcontinental North America were oriented towards terrestrial hunting rather than gathering, fishing, or alternative subsistence strategies. To date, more specific inferences on this score have been strongly and negatively affected by poor preservation. Most occupations of the period provide no preserved bone, and in those few cases where bone is present, sample sizes are too small to provide realistic estimates of regional subsistence and/or the cultural relationship between human action and animal bone accumulation (Cannon and Meltzer, 2004). Assuming the basic character of Paleoindian sites in the Midwest will not change in the future, alternative methods for developing inferences on regional foraging practices must be developed. The analysis of protein residues is one such method.

Protein residue analysis has been used in medical, pharmaceutical, industrial, biological, and forensic contexts for nearly a century. It has been incorporated into archaeological investigations for over 25 years, mainly in conjunction with the analysis of stone tools (Loy, 1983). Proteins can provide species-specific signatures; they accumulate on stone tools more quickly than detectable polishes and are likely to identify a broader range of species than is often present in the preserved faunal inventory itself (Shanks et al., 2001: 968; Shanks et al., 2004: 664). Protein residues have been shown to adhere to stone tools for prolonged periods, both experimentally and in archaeological contexts (Hortolà, 2002; Loy and Dixon, 1998; Shanks et al., 2004). Several different diagnostic tests have been developed and utilized in archaeological analyses, notably: cross-over immunoelectrophoresis (CIEP); enzyme-linked immunosorbant assay (ELISA); radioimmune assay (RIA), and Western blot. Each has its advantages and disadvantages (Downs and Lowenstein, 1995; Kooyman et al., 1992; Newman et al., 1996: 679–681; Shanks et al., 2004: 664; Barnard et al., 2007a). The Downs and Lowenstein, 1995 study is perhaps the most widely cited comparative assessment of these methods, despite the small sample sizes for both their controls ($N = 3$) and archaeological cases ($N = 4$). The prospect of false positives due to cross-over reactions, poor preservation, biased sampling, and a lack of antisera to detect proteins from archaeologically important species are all important caveats (Lambert et al., 2000: 401–402.; Loy and Dixon, 1998: 26; Tuross et al., 1996: 289–290; Shanks et al., 1999: 1183–1184, see also Petraglia et al., 1996: 128). As with any analytical procedure, protein residue analysis must be approached critically.

An additional consideration in assessing the viability of protein testing as it pertains to archaeological materials is sample size; the majority of protein residue studies involve a small number of tested tools per site, thus negatively affecting the ability to comment on

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general human–animal relationships. Large samples are useful in identifying patterning in any data set, and protein residue is certainly no exception. The present study focuses specifically on testing 130 tools for preserved protein residues. These tools represent several different classes of hafted and unhafted tools and were recovered from a single Gainey phase Paleoindian site in northeastern Ohio, Nobles Pond (33ST357).

1.1. Nobles Pond

Nobles Pond (33ST357) is a multi-component site located in Stark County, Ohio 16 km north of the southern extent of Wisconsinan glaciation (site datum: 40° 51′29″ N; 81° 29′12″ W). The predominant component is early Paleoindian with diagnostics attributable to the Gainey phase ca. 11,200–10,800 BP (Seeman, 1994; Seeman et al., 1994). Nearly 6000 m^2 have been excavated at the site, and over 55,000 flakes and tools have been recovered, mainly from plow-disturbed contexts. Many of the hafted tools were brought to the site in a depleted condition and show evidence of extreme curation before discard. The scarcity of manufacturing debris and early-stage rejects indicates that few of the hafted tools, including the 94 fluted points and 1765 end scrapers, were made at Nobles Pond. Altogether, the site extends over approximately 90,600 m^2 (8.9 ha).

Field investigations at Nobles Pond resulted in the recognition of 14 distinct loci of Paleoindian lithic debris largely resulting from the curation and resharpening of unifacial tools. These concentrations are situated on a series of slight ridges on a glacial outwash terrace and immediately southeast of a large pond (Fig. 1). Plow disturbance has affected the shape and size of the individual clusters, most notably by elongating them somewhat in a north–south perspective, the predominant direction of plowing as identified from plow scars and aerial photographs. Analysis of raw material distributions and refitted specimens indicate that six of these roughly 10 × 15 m concentrations were organized in a semi-circle and were probably contemporaneous. Our current interpretation is that these loci were strongly redundant with regard to human activities on the land. For the South Field area which contains four of these loci, 45% of the refits are less than 5 m apart and 76% are less than 10 m apart ($N = 530$). As expected, there is some elongation in the dominate direction of plowing between refit pieces (EW mean = 3.70 m; NS; mean = 6.35 m; $N = 530$), but not enough to negatively affect cluster integrity. Refits in other portions of the site are predicted to show similar relationships, but analyses of these patterns are still in progress. The over 970 refits from the site represent one of the most successful refitting programs for an eastern North American Paleoindian site, and carry considerable utility for the study of manufacture, use, tool curation and discard patterns. Overall, the closest structural comparisons of the Nobles Pond site appear to be to Shoop (Witthoft, 1952) and Bull Brook (Grimes et al., 1984) to the east.

The purpose of the present protein residue investigation is to better characterize the functionality of end scrapers and fluted points, the two most common classes of hafted tools at the site and components of a common early Paleoindian toolkit. Regarding the functional link between humans and animals, it can be assumed that most fluted points were used to kill and butcher game and that at least some end scrapers were used to process the hides of these animals. The incidence, location, and species-association of protein residues with these tool classes, therefore, should bear a direct relationship to the link between point and scraper: the greater the functional dependence between these classes, the greater should be the similarity with regard to any protein residues present. To date, most protein residue analyses have focused strictly (and descriptively) on the identification of species and their implications for prey selection. The present study makes use of a large sample of tools with an emphasis on toolkit organization and site structure.

2. Methods

One hundred and thirty tools from the Nobles Pond assemblage were selected for protein residue analysis, emphasizing those tools deemed most likely to be: (1) in direct contact with blood, specifically end scrapers and fluted points; (2) untouched and unwashed; (3) not heat damaged; and (4) associated with specific Paleoindian loci. The breakdown by tool type is: 17 fluted points; 96 end scrapers; 10 side scrapers; 2 flakeshavers; 2 *outre passé* flakes; 1 perforator; 1 single-edged knife; and 1 graver. Of the 130 tools in the study, 115 were untouched and unwashed (115/130 = 88%). The remaining 15 were washed in tap water only (15/130 = 12%). The majority of the tools selected for testing showed no evidence of being heated (120/130 = 92%) and it has been shown that protein residues heated to temperatures greater than 100 °C will not give positive reactions (Newman et al., 1996: 678). Heat alteration of flint or chert typically takes place at temperatures above 250 °C (Luedtke, 1992: 92). Regarding spatial distribution, tools were selected from seven contexts, specifically: (1) nine tools from A Block; (2) one tool from B Block; (3) 32 tools from C Block; (4) seven tools from D Block; (5) five tools from E Block; (6) 28 tools from F Block; and (7) 48 tools from elsewhere on the site and unassociated with any lithic concentration. Fifty-six soil samples directly associated with submitted stone tools also were analyzed as controls. The South Field Block at the site, that area that thus far has seen the most extensive analytical attention, was not sampled in the present investigation; the South Field Block was the earliest area excavated at Nobles Pond and all materials were washed, catalogued, analyzed, and extensively handled before the present study was initiated.

The testing method used in this analysis is cross-over immunoelectrophoresis (CIEP). The specific substances tested for in CIEP are immunoglobulins, or antibodies, a group of glycoproteins present in the serum and tissue fluids of all mammals. Initially, each tool was placed in a plastic dish with 0.5 ml ammonium hydroxide (5%) and sonicated for 5 min. This has been shown to be an effective extractant for old and denatured bloodstains and does not interfere with subsequent testing (Dorrill and Whitehead, 1979). Both dish and contexts were then placed in a rotating mixer for 30 min. The resulting solution was transferred into a sterile plastic vial and refrigerated below 0 °C prior to testing. In the case of soil tests, 2 ml of Tris buffer (pH 8.0) was added to 1 g of each soil sample, mixed well and allowed to extract for 24 h on a shaking mixer at 4 °C. The resultant supernatant fluids were removed, refrigerated below 0 °C, and subsequently tested together with samples obtained from the lithics. About 3 μl of each ammonia solution was then transferred into an agarose gel in a Shandon electrophoresis chamber containing a barbital buffer with a pH of 8.6 and paired to a second well with 3 μl of antiserum. The application of an AC current of 100 V was applied to the gel for 45 min causing the test sample and the antiserum to migrate, with antigens towards the anode and antibodies (antiserum) towards the cathode. As they come into contact and if the test sample contains protein corresponding to the species antiserum against which it is being tested, an antigen–antibody reaction occurs which results in the formation of an extended lattice as the protein precipitates. Appropriate positive and negative controls, prepared in 5% ammonia solution were run with each gel. These were: positive, proteins of species being tested for; and negative, proteins of the species in which the antiserum was raised. After the test run was completed the gel was pressed and dried. The dry gel was immersed in a Coomassie Blue R250 stain for 3 min and then destained in a solution of ethanol, distilled water and acetic acid (5:5:1, v/v) until the background was clear and any positive