Middle Pleistocene subsistence in the Azraq Oasis, Jordan: Protein residue and other proxies

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ARTICLE INFO

ABSTRACT

Excavations at Shishan Marsh, a former desert oasis in Azraq, northeast Jordan, reveal a unique ecosystem and provide direct family-specific protein residue evidence of hominin adaptations in an increasingly arid environment approximately 250,000 years ago. Based on lithic, faunal, paleoenvironmental and protein residue data, we conclude that Late Pleistocene hominins were able to subsist in extreme arid environments through a reliance on surprisingly human-like adaptations including a broadened subsistence base, modified tool kit and strategies for predator avoidance and carcass protection.

1. Introduction

Genetic evidence has revealed the complexity of interbreeding and population assimilation that took place across Eurasia between 100,000–45,000 years ago involving modern humans and archaic populations such as the Neanderthals and the Denisovans (e.g., Kuhlwilm et al., 2016; Reich et al., 2010; Simonti et al., 2016). Key to understanding the nature of these interbreeding and assimilation events is learning more about the lives of hominins already subsisting on the landscape when later humans arrived. Modern humans subsisting in extreme environments today often adapt by broadening their foraging strategies to incorporate a wide variety of plants and animals including insects and other arthropods into their diets. Faunal remains from Late Pleistocene sites (130,000–11,000

years ago) in Eurasia show this to be a cumulative trend. A key question in paleoanthropology concerns what strategies earlier hominins followed as they dispersed across a highly variable Eurasian landscape. Here we describe the oldest known family-specific protein residue on stone tools. Using cross-over immuno-electrophoresis (CIEP), seventeen tools from Shishan Marsh 1 (SM-1), a stratified, *in-situ* site in northeast Jordan dating to approximately 250,000 years ago tested positive for rhinoceros (3), duck (3), horse (5), camel (3), and bovine (3) protein residue. Based on lithic, faunal, paleoenvironmental and protein residue data, we conclude that Middle Pleistocene hominins were able to live in extreme environments through a reliance on surprisingly human-like adaptations including a broadened subsistence base, modified tool kit and strategies for predator avoidance and carcass protection.

1.1. SM-1 site: paleoenvironmental context

SM-1 site is a securely dated and stratified site in the Azraq

Oasis, which is located in the center of the endorheic Azraq Basin of Jordan's Eastern Desert (Fig. 1). Historically, the oasis was fed by springs originating from the upper aquifer of the basin, which is recharged in the Jebel Druze basalt massif to the north (El-Naqa, 2010). With a total precipitation in the central basin of 50 mm/year, the environment is arid and able to sustain only Saharo-Arabian vegetation (Al-Eisawi, 1996). The historic spring areas of the oasis, Azraq Druze and Azraq Shishan, have attracted hominin populations for at least 300,000 years, making the oasis one of the richest archaeological and paleontological landscapes in the Middle East. The drying of the marshes in the late 20th century due to considerable pumping of fresh water to supply urban areas permitted the study of deeply buried archaeological layers, which include Lower, Middle, Upper, and Epi-Paleolithic occupations. Moreover, evidence from terraces surrounding the basin and buried lacustrine deposits suggest high lake stands in the past (Abed et al., 2008; Ames and Cordova, 2015; Cordova et al., 2013). The complex stratigraphy of the oasis provides evidence of fluctuations between different depositional contexts, including marsh, playa, lacustrine, deltaic, and aeolian, suggesting extreme environmental changes associated with climatic and hydrological cycles (Abed et al., 2008; Ames and Cordova, 2015; Ames et al., 2014a; Cordova et al., 2013; Jones and Richter, 2011).

In a broader stratigraphic context, the Middle Pleistocene occupations at SM-1 are associated with a transitional environment from a receding lake to marshy ponds formed at the edge of a fan-delta (Fig. 2). Layers 10 and 9 correspond to lake recession with alluvial influx. Marshy ponds with gentle alluvial sediment influx correspond to layers 8, 7c and 7b. Subsequent desiccation is associated with aeolian (sandy silt) deposits represented by Layer 7a.

Large bifacial and flake-based stone artifacts are associated with layers 8, 7c and 7b. Not all layers produced pollen and phytoliths, but the archaeological layers indicate a predominance of aquatic vegetation surrounded by desert scrub typical of the hot deserts of the modern Middle East (Fig. S3). Modern desert plants are dominated by dry-adapted and salt-tolerant chenopod scrub (Chenopodiaceae) and sand dune vegetation (Calligonum comosum). The local aquatics are dominated by grasses (Poaceae), rushes (Juncaceae), cattails (Typha spp.), and sedges (Cyperaceae), among others (Table S1). Grass pollen diameters and phytolith cells suggest that the majority of the grasses were reeds (Phragmites australis)

(Tables S1, S2, Fig. S4, Supplementary material). The bioclimatic context of the lithic assemblages more closely resembles modern conditions than those of the Last Glacial Maximum. The high Chenopodiaceae-Artemisia ratio (C/A) (Table S1) suggests that the hominin occupations may have occurred during an interglacial stage or near the transition from an interglacial to glacial stage, or more specifically under warm and dry conditions (see Supplementary material).

The faunal remains recovered to date are poorly preserved, but layers 8, 7b and 7c indicate the presence of gazelle (Gazella sp.), camel (Camelus sp.), wild cattle (Bos cf. primigenius), equids (Equus spp.), an extinct elephant (cf. Elephas), rhinoceros consistent with steppe rhinoceros (Stephanorhinus hemitoechus), probable lion (cf. Panthera leo), and other large carnivores. Steppe rhinoceros, equids, wild cattle, and camel were identified at the nearby Acheulean site C-Spring (Clutton-Brock, 1970, 1989), and steppe rhinoceros, wild cattle, equids, and elephant were identified during previous excavations in the nearly adjacent site of 'Ain Soda (Dirks, 1998; Lister et al., 2013). These taxa are indicators of a dry, open, steppe environment with some shrubs (Bennett and Hoffman, 1999; Davis, 1980; Fortelius et al., 1993). Such animals would be attracted to water margins of the oasis and the associated plant resources, where they may have been ambushed by hominins and/or other predators. The interpretation of 'Ain Soda as a butchery site supports this scenario as one aspect of oasis usage (Lister et al., 2013; Rollefson et al., 1997).

The oldest OSL age estimate at SM-1 is 266 ± 40 kya (Supplementary material), and is assumed to be the minimum date for layer 8 (Fig. 2). Dates from upper layers 7b and 7a, 125 ± 12 kya and 119 ± 40 kya respectively, indicate the minimum age for the burial of the stone tools. However, based on our paleoenvironmental and geomorphic reconstructions, it is unlikely that these minimum dates represent an age for the creation and use of the cultural material. The surface of layer 7a was likely exposed and experienced continuous aeolian reworking for an extended period of time, which would result in a younger date than initial onset of aeolian depositional conditions. Moreover, the base of layer 7b and layer 8 produced pollen, but none was recovered from layer 7a, which suggests that the lithic material is resting in sediments that were relatively quickly buried and in primary context, as pollen is rarely preserved on exposed soil surfaces in warm and arid
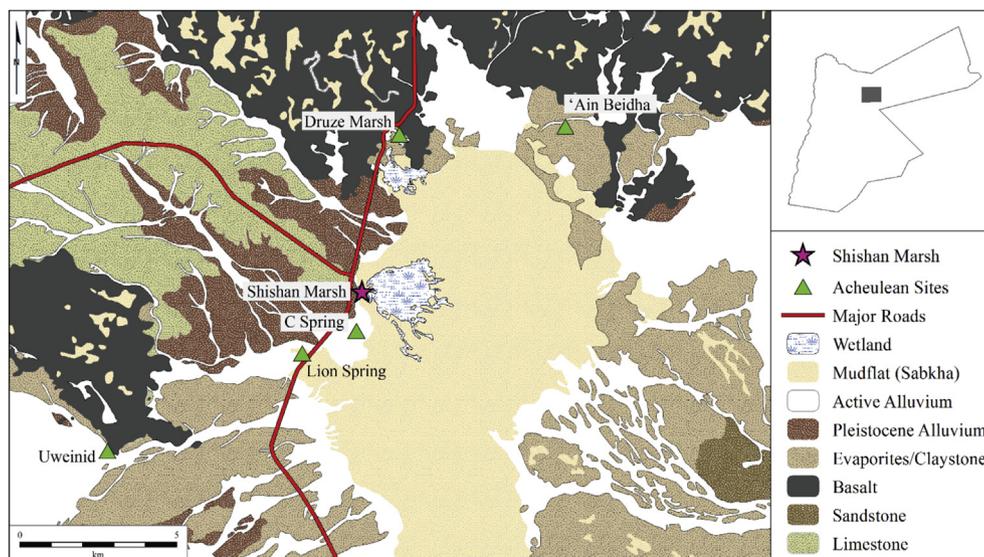

Fig. 1. Map of the Azraq Oasis (data digitized from Ibrahim, 1996; Abu Qudaira, 2000).
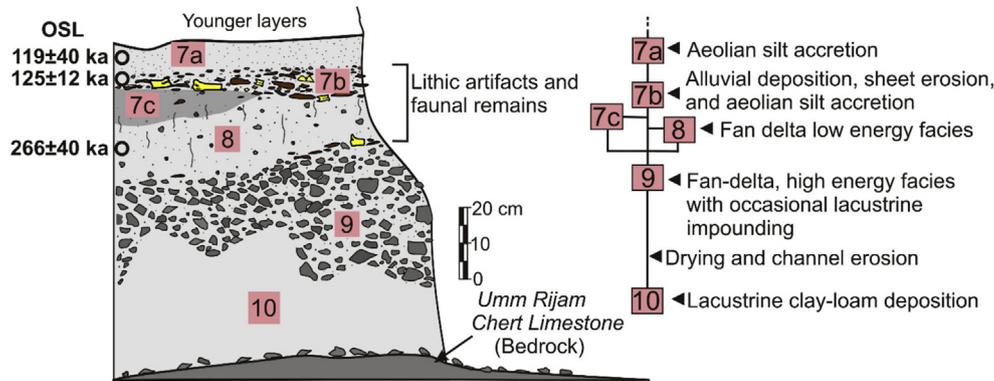
Fig. 2. A generalized stratigraphy associated with Middle Pleistocene occupations at SM-1.

environments (Cordova, 2007). Continuous aeolian reworking of layer 7a may also have resulted in the incorporation of aeolian fine sand and silt into the upper parts of layer 7b, which would produce a younger age estimate and account for the lack of pollen preservation. Together this evidence points to a large gap in the depositional sequence between the production and use of the stone tools and the final deposition of layer 7a, meaning that for now the age of 266 ± 40 kya more closely represents the age of creation and use of the cultural material. Geomorphic and paleoenvironmental evidence from the surrounding region correlates with our interpretation.

A maximum high lake stand in the Azraq basin is reported between 346 and 316 kya (Abed et al., 2008)—when SM-1 would have been under water—while a relatively dry environment due to deposition of evaporative carbonate is suggested by a U-Th age of 220 ± 30 ka (Macumber, 2001) at a nearby site almost at the level of SM-1. Therefore, the derived proxies from SM-1 (pollen and phytoliths, see Supplementary material) suggest a regional transition from an exceptionally wet period to conditions similar to the present or even more arid. The sedimentary facies at SM-1, which are fluviolacustrine, and the aquatic nature of the local vegetation evident in the pollen and phytoliths, suggests that this locality represented an oasis where animals and hominins were brought together in sufficient numbers to leave substantial traces. Overall, the current geomorphic and paleoenvironmental data from SM-1 and the surrounding region indicate that this locale was repeatedly utilized by biface and flake tool-using populations between at least 300–150 ka—conservatively speaking—as minor fluctuations in climatic cycles reorganized the extent and distribution of water resources in the oasis. This age estimate represents the window of time between the highest known lake stand and the desertification of the local landscape. As more dates are obtained and more details of the paleolandscape reconstructed we expect to refine this window for the age of the Middle Pleistocene occupations at SM-1, particularly concerning the younger end of the timeframe.

1.2. SM-1 site: lithic assemblages

Approximately 10,000 artifacts made from local flint nodules and small fluvial clasts from nearby wadi gravels were excavated during three field seasons from 2013 to 2015. Typologically, the archaeological assemblage at SM-1 corresponds to the Late Acheulean of Azraq facies characterized by small to moderately-sized ovate and discoid bifaces and a predominance of flake tools—all with sharp edges with little to no evidence of rolling or post-depositional edge damage (Copeland, 1988). The assemblage differs from a surface collection recovered from 'Ain Soda (Rollefson

et al., 1997) in having fewer cleavers, a moderate use of the Levallois technique and an abundance of small tools (i.e., utilized and/or retouched flakes less than 5 cm in length including scrapers, burins and borers) that were knapped on-site from local fluvial clasts. The condition of the artifacts in conjunction with preliminary analyses of debitage samples and of artifact orientation data suggest that the artifacts were recovered in primary context (Ames et al., 2014b).

2. Materials and methods

2.1. Cross-over immunoelectrophoresis

Cross-over immunoelectrophoresis (CIEP) detects residues based on reactions between antibodies and antigens, wherein antibodies are used to detect unknown antigens. CIEP is more sensitive to proteins than other methods of protein residue analysis including enzyme-linked immunosorbent assay, radioimmune assay, and Western blot analysis, being able to detect 10^{-8} g of protein in a 5 μl sample (Culliford, 1964). CIEP has been used to detect protein residues from a variety of fish species on Neolithic tools in Sweden dating to 3500–3900 years ago (Högberg et al., 2009), deer, caribou, bear, and rabbit residues on Paleoindian artifacts dating to approximately 11,200 years ago (Seeman et al., 2008), horse, bison, duck, trout, and mammoth residues on artifacts up to 11,500 years ago (Forgeng, 1998; Loy and Dixon, 1998; Williams, 1990) and most recently deer, bison and gallinaceous fowl (e.g., quail or grouse) on Paleoindian tools from South Carolina (Moore et al., 2016).

While the CIEP technique has been in use in forensic science for half a century, questions have been raised concerning its applicability to archaeological materials due to the uncertainty of the long-term survivability of proteins and the ability of CIEP to identify these protein residues accurately (see discussion in Moore et al., 2016). Although the ancient proteins may not be conserved in their original form, the preservation of linear epitopes allows them to be identified by CIEP and other methods (Abbas et al., 1994). The degree of porosity and surface roughness of the artifact serves to preserve protein residues, as do fissures, within which proteins may be sequestered and buffered against the burial environment. Additionally, the combination of proteins, fatty tissues, and soil particles (as would accumulate on an artifact used in hide scraping, etc.) is resistant to microbes. It is nearly insoluble as well, particularly if the fatty tissues have been modified into adipocere and have taken on calcium ions from either water or a soil with high mineral content (Gill-King, 1997). Experimental data on stone tools confirm this observation. Moore et al. (2016), citing the work of Shanks et al. (2001; see also Shanks et al., 2004), note that,

"experimental studies show that microfractures produced during stone tool manufacture rapidly absorb protein residues due to capillary uptake during tool use (Shanks et al., 2001). The absorption of protein residues below the surface of the artifact likely acts to protect and preserve residues, prevents removal during routine washing of artifacts after recovery, and may explain how residues can be obtained from immunological testing of heavily weathered stone tools. Other debris and residue films may also protect more deeply imbedded proteins by filling in and covering microfractures (Shanks et al., 2001). Thus, residues may be preserved even in regions where acidic sandy soils preclude the likelihood of faunal preservation."

Several factors contribute to identifying ancient proteins successfully, such as the use of high quality antisera, the burial environment, excavation techniques and subsequent handling of the artifacts (Marlar et al., 1995; Shanks et al., 1999). Many early CIEP studies were criticized for relying on custom-produced antisera which were often not tested against a wide range of species (e.g., Shanks et al., 1999). Researchers using CIEP had to experiment in order to find the concentrations in which an antiserum could reliably identify a protein (Shanks et al., 1999). This is no longer the case. Within the last 20 years the production of forensic-quality antisera with specific guidelines as to the concentration of antisera, and rigorous testing by the commercial manufacturers against a large bank of different species has allowed CIEP and other techniques of protein analysis to become much more reliable and replicable. Moreover, to guard against species cross-reactivity, all new antisera that were utilized in the protein residue laboratory for this study were first tested against all other animal sera in the bank (16 species).

2.2. Sample selection

Approximately 7000 of the 10,000 artifacts excavated from SM-1 have been analyzed in detail. Of the 7000 artifacts evaluated, 44 lithic artifacts (scrapers, utilized flakes, Levallois points and bifaces) (Figs. 3—5, S1-S2) made from local flint and excavated from layers 7b, 7c and 8 were chosen for further analysis to identify possible protein residues using CIEP. Artifacts were chosen for this analysis

based on the presence of pronounced use-wear, e.g., microflaking and rounding (Ollé and Vergès, 2014; Wiederhold and Pevny, 2014) ascertained through macroscopic observation as well as low-powered microscopy using both a Meiji EMZ binocular stereoscopic microscope and a Keyence VHX-600 Digital Microscope at the Oregon State University Radiation Center. Photographs were taken with the Keyence microscope. Following Hardy (2004), artifacts were handled minimally and not washed with the exception of the six artifacts chosen for analysis from the 2013 season, which were washed by hand with water without soap or the use of a brush. Based on evidence of use, the location of residue extraction was chosen by one of the authors (DS) and independently verified by Dr. John Fagan, head of the Residue Analysis Laboratory at Archaeological Investigations Northwest.

2.3. Residue extraction

Each lithic artifact to be tested was first examined under magnification to determine the most likely area where protein residue might be preserved. When smaller artifacts were considered, the entire artifact was extracted for analysis. To extract the residue, a 5% ammonia solution was applied to the artifact (Dorrill and Whitehead, 1979; Kind and Cleevy, 1969). Experimentation demonstrated that other solvents are not as effective at lifting protein residue samples (Newman, 1990). A small amount (0.5—1.0 ml) of the ammonia solution was applied to the artifact in a sterile, single-use plastic tray, which was then floated in an ultrasonic bath for at least 10 min. The artifact and tray were then placed on a mechanical rotator for another 10 min. If the artifact was too large for the ultrasonic bath, it was placed on the rotator for 30 min. The extract solution was then drawn off with a micro pipettor utilizing disposable pipette tips and transferred to a microcentrifuge tube. The sample was centrifuged to clarify the sample and then placed in refrigerated storage. Soil samples in association with the tools were also analyzed (see section 3). Approximately 0.5 ml of soil from each sample was placed into a 2 ml microcentrifuge tube and then 0.5 ml of ammonia solution was added to each. The tubes were then sealed and centrifuged for 2 min, after which they were then ready for CIEP analysis.
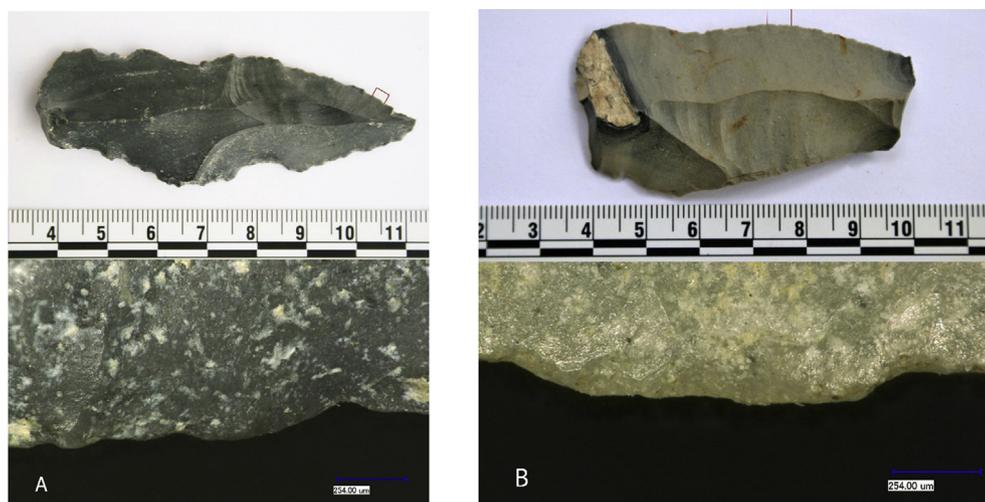
Fig. 3. (A) (top) Artifact SM1-613 is positive for rhinoceros protein. Rectangle indicates location of use-wear photograph and residue extraction (Photo: April Nowell); (bottom) Artifact has unifacial modification along entire right edge and microflaking and rounding use-wear on both lateral margins (magnification 200X) (Photo: Daniel Stueber). (B) (top) Artifact SM1-775 is positive for camel protein. Rectangle indicates location of use-wear photograph and residue extraction (Photo: April Nowell); (bottom) Artifact has use-related microflaking and slight rounding use-wear on both lateral edges (magnification 200X) (Photo: Daniel Stueber).

Fig. 4. (A) (top) Artifact SM1-958 is positive for bovine protein residue. Rectangle indicates location of use-wear photograph and residue extraction (Photo: April Nowell); (bottom) Artifact has well-defined use-related microflaking along both lateral edges (magnification 200X) (Photo: Daniel Stueber). (B) (top) Artifact 2363 is positive for horse. Rectangle indicates location of use-wear photograph and residue extraction (Photo: April Nowell); (bottom) Artifact has microflaking use-wear and rounding on the right, feathered edge. (magnification 200X) (Photo: Daniel Stueber).

Fig. 5. Artifact SM1-2585 is positive for horse protein residue. Rectangle indicates location of use-wear photograph and residue extraction (Photo: April Nowell); (bottom) Artifact has microflaking and some rounding at residue extraction area (magnification 200X) (Photo: Daniel Stueber).

2.4. Antisera

In the present study, extractions taken from the working edges of SM-1 artifacts were tested against 8 antisera: rhinoceros, camel, deer, duck, horse, goat, bovine and cat. These antisera react to proteins from the taxa Rhinocerotidae, Camelidae, Cervidae, Anatidae, Equidae, Caprinae, Bovineae, and Felidae respectively. The goat, deer, horse, bovine and cat antisera are forensic grade, manufactured by MP Biomedicals, LLC. The camel antiserum is manufactured by Bethyl Laboratories, Inc., the duck antiserum is produced by Biorbyt Laboratories, Inc., and the rhinoceros antiserum was custom produced for this project, using a goat as a host animal. To increase the likelihood of success, antisera were chosen based on faunal remains found at SM-1 or nearby sites and our assumptions of what animals might be found in a paleomarsh environment. Some antisera were run to eliminate any possible contamination from modern fauna (e.g., goat).

The rhinoceros serum needed to produce the antiserum was obtained from a black rhinoceros in captivity at Cheyenne Mountain Zoo, Colorado Springs, Colorado. A small serum sample obtained from the black rhinoceros was introduced to a host animal, in this case, a goat. The goat formed an immune response, generating IgG antibodies to counteract the rhinoceros serum in its system. After approximately three months, a series of three blood samples spaced 10 days apart were withdrawn from the goat, which was then returned to its herd. Each sample was purified, and tested against reserved rhinoceros serum to determine if the goat had formed antibodies against the rhinoceros serum. After the final test, the anti-rhinoceros IgG within the goat's serum reliably produced a precipitin line when exposed to reserved rhinoceros serum, indicating a usable rhinoceros antiserum had been produced.

Prior to analyzing the artifacts for this project, the rhinoceros antiserum was tested via CIEP against camel, bovine, deer, horse,

sheep, and goat serum to examine the possibility for species cross-reaction, which is standard practice for all forensic grade antisera and an important step in ensuring the reliability of CIEP results. The antiserum-serum interaction did not produce a precipitin line against any of these sera. However, against the horse serum, the non-specific protein reaction was slightly more prominent than the others. No precipitin line was formed, however, indicating that there was no specific reaction between the rhinoceros antiserum and horse serum. The non-specific reaction is likely due to the shared evolutionary history between rhinoceroses and horses as members of Order Perissodactyla. Additionally, no artifacts that tested positive for rhinoceros were also positive for horse, and vice versa. A similar phenomenon is documented by Nollens et al. (2008), who compared bovine serum against baleen whales, beluga, porpoises, orcas, and several species of dolphin, whereby a non-specific protein reaction (slight cross-reactivity) correlated with evolutionary distance within an evolutionary lineage.

2.5. Testing the extracted residue

The testing begins by punching two vertical columns of wells into agarose gels. Approximately 0.5 μl of antiserum is placed into one well, with the same amount of extract from the target artifact in the opposite well. Positive (against the target species serum) and negative controls (against the host animal species) are also run for the specific antiserum on each gel, within separate wells. The positive control is to ensure the antisera is actively interacting with the correct protein during the test in question, and the negative control is to ensure the antisera is reacting appropriately with the host animal species. The positive control tested against the antiserum (e.g., horse antiserum vs. horse serum) should produce a clear precipitin line after staining with Coomassie blue protein stain. The negative control (e.g., horse antiserum vs. goat serum) should produce a diffuse, non-specific reaction after staining, as only the proteins in the sera are stained, and no reaction between the two sera took place.

Once loaded, the gels are placed within the CIEP machine, and an electrical current of 120 V at 10 mA is passed through the gels for 35 min. The antisera are drawn towards the extract wells, a distance of 5 mm. If there is protein present in the extract that corresponds with the specific antiserum used, an antigen-antibody response will take place, which is marked by the formation of a precipitin line. The line is only visible after the gels have been rinsed, blotted, stained, dried, and viewed under magnification from a backlit source. All positive results are re-run on separate gels for confirmation. Sterile equipment and techniques are used to control for contamination. The extracted residue was frozen for future testing, if desired.

3. Results

As noted in Section 2.1., a total of 44 tools out of nearly 7000 stone artifacts from SM-1 were chosen for residue analysis based on the presence of pronounced use-wear, which was evidenced by microflaking and rounding. An initial sample of 6 artifacts was tested against antisera from bovine, camel, goat, and horse with one artifact testing positive for horse protein residue (Table 1). On the strength of these results we carefully chose an additional 38 artifacts to be tested against camel, deer, duck, horse and rhinoceros. In addition, 6 previously frozen extractions obtained from the initial sample of artifacts were tested against deer, duck and rhinoceros antisera, as they were previously run against camel and horse. From this second study, there were 3 positive reactions to rhinoceros, 3 to camel, 3 to duck, and 4 to horse. There were no positive reactions to the deer antiserum. In a third study, 26 of the 38 artifacts from the second study that had yet to test positive were run against bovine antisera. Three artifacts tested positive for bovine antisera. Finally, of the 44 artifacts that were selected for residue analysis 27 had not tested positive against any antisera. These 27 artifacts were run against cat antiserum because of the probable lion (cf. *Panthera leo*) remains uncovered at SM-1. There were no positive reactions.

In sum, 285 individual tests were run against a total of 8 antisera (i.e., each tool was run against 5 to 8 antisera) resulting in 17 positive reactions (a success rate of 5.96%). There were no cross-reactions (i.e., extractions taken from a tool never reacted with more than one antiserum). All positive reactions were run a second time to confirm the initial result. In one case the second test did not confirm the initial result and was thus discounted. In all other cases (n = 17), a second positive reaction was recorded. Representative soil samples were taken from layers 7b, 7c and 8 and all tested negative against all 8 antisera. It is important to note that if the proteins we detected on the stone tools were the result of soil contamination or nearby decomposing animals then we would expect a much higher success rate than just under 6%, with most or all tools testing positive and many instances of multiple positives per tool. Furthermore, a number of authors (e.g., Dorrill and Whitehead, 1979; Eisele et al., 1995; Fletcher et al., 1984; Sensabaugh et al., 1971a,b; Shanks et al., 2001, 2004; see also Cattaneo et al., 1993) have shown that whole blood does not

Table 1
Positive results of protein residue analysis on lithic tools from SM-1.

Artifact	Layer	Artifact type	Antiserum type positive for	Probable fauna
SM1-0120	7B	Utilized flake	Horse	*Equus* sp.
SM1-188	7B	Levallois flake	Duck	Anatidae (possibly *Anas acuta*, *Anas querquedula*, *Anas crecca* or *Bucephala clangula*)
SM1-0613	7B	Levallois blade	Rhinoceros	*Stephanorhinus hemitoechus*
SM1-0664	7C	Scraper	Horse	*Equus* sp.
SM1-0758	7B	Scraper	Bovine	*Bos primigenius*
SM1-0775	7B	Levallois blade	Camel	*Camelus* sp.
SM1-0885	7B	Levallois point	Camel	*Camelus* sp.
SM1-0958	7B	Utilized flake	Bovine	*Bos primigenius*
SM1-1100	7B	Scraper	Bovine	*Bos primigenius*
SM1-1187	7B	Levallois point	Rhinoceros	*Stephanorhinus hemitoechus*
SM1-1948	7B	Naturally backed knife	Duck	Anatidae (possibly *Anas acuta*, *Anas querquedula*, *Anas crecca* or *Bucephala clangula*)
SM1-2032	7B	Levallois point	Duck	Anatidae (possibly *Anas acuta*, *Anas querquedula*, *Anas crecca* or *Bucephala clangula*)
SM1-2094	7B	Denticulate	Camel	*Camelus* sp.
SM1-2145	7B	Utilized flake	Horse	*Equus* sp.
SM1-2363	7B	Utilized & retouched flake	Horse	*Equus* sp.
SM1-2585	7B	Handaxe	Horse	*Equus* sp.
SM1-2689	8	Handaxe	Rhinoceros	*Stephanorhinus hemitoechus*

preserve well on its own, and therefore if an artifact comes into incidental contact with it or even if it is spilled/dripped onto an artifact after deposition, it is unlikely to leave a lasting signature.

Within the positive tool reactions, all taxa were represented with the exception of cervids, caprines and felids. The positive results for the Family Anatidae most likely represent a species of duck as no geese or swans are yet known from this region at this time (Tyrberg, 1998). The most probable faunal species for each positive result is listed in Table 1 based on identifiable faunal remains from SM-1 or nearby sites.

4. Discussion

The results presented here are the oldest identifiable protein residues in the world and constitute direct evidence of the exploitation of specific taxa in the Levant by Middle Pleistocene hominins. As protein residue analyses become more widely employed in archaeological contexts, previously held suppositions about the preservation of biological evidence may need to be reconsidered (see discussion in Moore et al., 2016). Our data join a growing body of work that demonstrates the survival of biological tissues in identifiable form on Lower and Middle Paleolithic tools. For example, Loy and Hardy (1992) identified red protein cells, collagen, resin and hair fragments on Middle Paleolithic stone tools from Tabun dating to 90 ka, and adipocere and bone residue likely derived from a straight tusked elephant (*Palaeoloxodon antiquus*) have been identified on Acheulean lithic implements from Israel, based on associated faunal remains (Solodenko et al., 2015). Similarly, Rots et al. (2015) have identified collagen and hair fragments on 300,000 year old stone tools from Schöningen in Germany. These types of results in conjunction with studies of plant residue on Paleolithic stone tools, e.g., phytoliths on Acheulean handaxes from Peninj dating to approximately 1.5 mya (Dominguez-Rodrigo et al., 2001) have the potential to greatly enhance our understanding of early hominin lifeways by filling in the gaps in what is normally visible in the archaeological record.

It is clear from our present research that hominins in the Shishan Marsh adapted their technologies and broadened their subsistence base to take advantage of a wide range of available prey from rhinoceros to ducks. The protein residue from the ducks is a particularly interesting example. Ducks were likely hunted rather than scavenged because of the low probability of hominins finding scavengeable avian remains. By night, hunting ducks is relatively easy as they are reluctant to leave their nests in the dark. Hominins could have quietly approach the nests procuring the ducks by hand, or they could have thrown a hide at the nests, capturing several ducks at once. Hunting at night near a primary source of water would have been a dangerous endeavor and it is more likely that they hunted ducks by day. In this case, hunting ducks could have been facilitated by nets, hides, boomerangs, slings, throwing sticks or even a well-placed rock. The earliest evidence for the use of nets and boomerangs dates to the Upper Paleolithic (Soffer et al., 2000; Valde-Nowak et al., 1987). However, a Lower Paleolithic throwing stick made from spruce (*Picea* sp.) dating to approximately 300,000 years ago has been identified at Schöningen (Schoch et al., 2015; Thieme, 1997, 2000). A throwing stick is similar to a boomerang but normally longer with a wide, flat profile and when thrown, it spins creating a wide swath in the air. It is a weapon that can predictably take down a moving target and it is well known from Australia where it was used to hunt birds and small mammals. Thieme (2007 cited in Schoch et al., 2015) suggests that it is an ideal weapon for taking down ducks along a lakeshore. The advantage of a throwing stick over a rock is that its wide swath increases the likelihood of hitting one or more ducks each time it is launched.

No matter which strategy hominins followed, hunting ducks is

significantly different from hunting or scavenging larger game animals such as rhinoceros and demonstrates that these hominins were able to adapt to a marginal environment by taking advantage of a wide variety of prey. Such an ability to adapt to localized pockets of concentrated resources throughout a generally resource-poor landscape was likely critical to the success of these late Middle Pleistocene populations, and integral in their ability to disperse across the Eurasian landscape. Furthermore, these hominins must have had highly effective strategies in place for predator avoidance and carcass protection. Given the inherent dangers associated with hunting and/or scavenging at the edge of a watering hole, it is possible that these hominins practiced a very human-like division of labor, one that was highly divergent from non-human primate foraging strategies, and that, based on studies of modern hunter-gatherer societies, may have included specialized task groups (although men and women's specific roles in subsistence activities vary greatly among these societies and likely did in the past as well [Owen, 2005]), task-specific implements and strategies for competing with or avoiding the other predatory species in the area. We will refine this model as our analyses continue.

5. Conclusion

Positive protein residue results on 17 Middle Pleistocene stone tools from an assemblage dating to approximately 250 ka, in conjunction with associated lithic, faunal and other paleoenvironmental data suggest that these Middle Pleistocene hominins were adaptable, opportunistic and capable of exploiting a wide range of fauna—from waterfowl to rhinoceros—in what was likely one of the last humid refugia in the region. Such insights are all the more important as large swaths of inland Arabia, once supposed unoccupied, are now known to have been home to considerable populations of Acheulean tool making hominins (Jennings et al., 2015; Shipton et al., 2014). Occupations near former springs in the El Kowm Basin in central Syria (Hauck, 2011; Le Tensorer et al., 2007) and along the shores of a seasonal lake in the northern Golan Heights (Oron and Goren-Inbar, 2014) further highlight the importance of isolated and ephemeral water resources for these highly mobile Pleistocene hominins. Ultimately, the onset of xeric conditions led to the local disappearance of these hominins. Residue studies, such as the one presented here, in conjunction with a variety of environmental proxies allow us to begin to reconstruct how hominins adapted to and survived in arid regional environments—a critical factor in understanding early human dispersals across Eurasia.

measurements for OSL analyses were carried out by Dr. Eric Benton at Oklahoma State University. This research was fully funded by an Insight Grant (#435-2012-0625) from the Social Sciences and Humanities Research Council of Canada.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2016.07.013.

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