X.1Micropalaeontological Report (John Whittaker)

X.1.0 Introduction

A total of 15 samples were received from Dr Pope in Dec 2006 at the successful conclusion of the Valdoe Assessment Survey and the Valdoe Contingency Project during that year. In all these comprised some 20% of purposive micropal samples taken for characterisation of local environmental and depositional conditions at the site. The analysis of microfauna has emerged as our most valued analytical tool as it provides for a very rapid and specific overview of sedimentary conditions at specific localities. In addition to the identification of ostracod and foraminifera species, Dr Whittaker provides us with an invaluable overview of sedimentary conditions at the sites in terms of extent of decalcification/weathering, localised presence of pollen, small mammal remains and other organic traces.

Identification of the Ostracod and Foraminifera species themselves has, throughout the course of Boxgrove Project D and the Raised Beach Mapping Project, revolutionise dour understanding of local environmental conditions and variability across the Boxgrove palaeolandscape. This has allowed us to identify the behavioural important character of the Q1/B waterhole sites and determine such vectors as salinity, temperature and depositional energy of particular facies of the Slindon Formation, providing a degree of detail unrivalled by any of our other analytical approaches. While alone Micropal can go a long way to providing a complete overview of sedimentary conditions, when its results are integrated with particle size analysis and sediment chemistry studies detailed sedimentary frameworks can be established which underpin all subsequent detailed analysis. For this reason we begin by assessing the potential for further detailed analysis of microfauna from the Valdoe samples.

x.1.1 Materials and Methodology

15 new samples were (Table 1) sent to Dr Whittaker, nine sample were sent from a single Test Pit (TP5), a location which sat within calcareous sediments and provided a type-section for close interval sampling. A further six samples were sent from promising facies of the Slindon Formation at other locations within the quarry. These samples contributed to Dr Whittaker's previous assessment of material from Valdoe Boreholes 1-4 which were looked at to degree as part of the Raised Beach Mapping Project. Valdoe Borehole 4 in particular was shown to preserve an unprecedentedly detailed microfauna sequence, consideration for undertaking detailed analysis the Borehole in particular in also considered in this assessment report.

Sample	Weight processed	l Unit
Trench 5		
MP1	175g	Unit 3 (Slindon Sands)
MP2	175g	Unit 3 (Slindon Sands; upper)
MP3	320g	Unit 4b (Slindon Silts)
MP4	235g	Unit 4b (Slindon Silts)
MP5	230g	Unit 4c (Palaeosol)
MP6	150g	Unit 6b (Lower Brickearth)
MP7	230g	Unit 6b (Lower Brickearth)
MP8	235g	Unit 6b (Lower Brickearth)
MP9	285g	Unit 6b (Lower Brickearth)
VW Boreholes		

Table 1: Micropal Samples Analysed In the Assessment Phase.				
BH4iv (sands, 8.25-8.50m)	200g	Unit 3 (Slindon Sands)		
BH3ii (sands, 4.45-4.65m)	510g	Unit 3 (Slindon Sands)		
BH2ii (U100i, sands)	175g	Unit 4b (Slindon Silts)*		
BH2i (Brickearth/Unit6, 6.90m)	500g	Unit 6b (Lower Brickearth)		
BH1vi (sands, 11.50-12.00m)	175g	Unit 3 (Slindon Sands		
BH1iv (sands, 10.80-11.00m)	175g	Unit 3 (Slindon Sands)		

Sample assignment to their respective stratigraphical units was provided by Dr M.I. Pope (pers. comm.). However, examination of their microfauna can still provide surprises, as evidenced by BH3ii (sands, 4.45-4.65m) (asterisked *, above), which was initially regarded, based on its lithology, as Unit 3 (Slindon Sands). For further discussion, see below in the Results section.

All the samples were first put into bowls and dried in an oven. Then a teaspoon of sodium carbonate was added (to help remove the clay fraction) and hot water was poured over them. They were left to soak overnight. Each was then washed through a 75 micron sieve, using hot water and the resultant residue returned to the bowl. The residues were returned to the oven again and thoroughly dried, before being placed in labelled plastic bags for storage. Several of the samples broke down to give very little residue and, if more material was available a further wash was therefore made in order to maximise the residue. Before picking, each dry sample was put through a nest of sieves (>500, >250,>150 microns and pan) and a little of each residue at a time sprinkled on a picking tray. A representative number of microfossils were picked out of each sample and transferred to a 32-square 3"x1" faunal slide for future reference. The abundance of each species was estimated semi-quantitatively by experience

and by eye. Other important organic remains were also noted and are listed as it was felt this would be useful for future studies.

x.1.2 Results and interpretation

The results of the 9 samples from Trench 5, first. Foraminifera occur only in MP1 – MP4 (belonging to units 3 and 4b, respectively the Slindon Sands and Slindon Silts) and indicate a very specific assemblage; they represent intertidal flats and show that the upper part of the Slindon Sands (and the Slindon Silts), here, were already a regressive facies. The occurrence of Aubignyna perlucida is interesting. Not only is it a "southern", warm indicator, but where its occurrence is in large numbers within this Raised Beach (e.g. Trumley Copse) and elsewhere, it might indicate a particular or peculiar ecological niche, possible of a brackish nature associated with a stream or streams entering the Boxgrove Embayment. This is of crucial importance in our identification of localised ecological niches and in determining if the Lavant Valley was indeed an active and important component of the Boxgrove Palaeolandscape during MIS13. The Valdoe is located at a point which we predicted might represent the interface between the traditional marine-terrestrial 'sere' of the Boxgrove sequences and possible estuarine components of the localised Lavant Valley. Aubignyna perlucida is, perhaps, our first glimpse of this new environment and one which may go some way to explaining the dense concentration of Palaeolithic surface finds which were made between the Valdoe and the modern Lavant Valley.

There are no ostracods from the Slindon Sands. We now know from experience, however, that ostracods are often subsequently lost due to decalcification (from subaerial erosion) in this type of intertidal environment in the "Boxgrove Raised Beach". Samples MP5 – MP9, on the other hand, are all terrestrial/freshwater (Figure 1). Only Unit 6b, the Lower Brickearth,

contains recognisable organic remains, comprising earthworm granules, slug plates and freshwater ostracods. There were no molluscs. The ostracods were unfortunately unidentifiable to species, but are referable to juvenile candonids. This, nevertheless, seems to indicate that the Brickearth was in part water-laid.

The results of the VW boreholes 1-4 are shown in Figure 2. The two samples from BH 1 are both from Unit 3 (Slindon Sands): low diversity foraminifera, but again no ostracods. The ecology is "restricted" and regressive marine. Very large numbers of *Aubignyna perlucida* occurred in sample iv (10.80-11.00m), but the species is absent in the marine Slindon Sand samples in boreholes 2 and 4. However, *Ammonia falsobeccarii*, another well-known warm, marker species of the "Boxgrove Raised Beach" is present, instead.

The real surprise of this Assessment Survey was Borehole 3, where sample ii (4.45-4.65m) was initially attributed, on lithological grounds, to Unit 3 (Slindon Sands). The microfauna, however, is of a distinctly terrestrial nature (Figure 2) and comprises earthworm granules, slug plates, terrestrial molluscs (including *Pupilla*) and small mammal teeth. The teeth belong to shrews and will be reported on separately (S. Parfitt, pers. comm.). These (and the other remains) indicate grassland, and suggest they belong to a local and hitherto unidentified facies of Unit 4b, one which needs now to be understood in detail. This unique environment can be seen alongside the possible evidence for localised estuarine input to mark the Valdoe out as a unique local environment within the Boxgrove palaeolandscape. Such a possibility was factored into the overall project design under Aim X)

x.1.3 Recommendations for further work

There are two recommendations for further work on the microfossils of The Valdoe.

1. Primarily and most necessary would be widening of the scope of prelinary assessment to c.60% of the micropal samples (N=45). Dr Whittaker's turn-round is quick and provides valuable ecological and biostratigraphical information based on the foraminifera and the ostracods (and covering all manner of aquatic environments, whether it be freshwater, brackish or marine), but also listing of all the other organic remains that the samples contain allowing us to tweak the samples list for other specialists. The strength of this is well seen in my analysis of BH 3, sample ii, discussed above where JEW was able to isolate this Unit as being ecologically significant at an early stage in the analytical programme.

2. Secondly it is important that the results of the Valdoe Assessment analysis are fully integrated with information still to be accessed from borholes samples taken during earlier phases of research. In particular the deeper part of the marine, Slindon Sands, as found in the Valdoe Field Borehole 4 (Whittaker, 2004) and the Valdoe Pit, SB5 S4 samples. These assemblages are well preserved, remarkably diverse and contain the best suite of both foraminifera and ostracods, by far, from the "Boxgrove Raised Beach". Whittaker (2004) gives just a small taste of the richness of Valdoe Field BH4.... "Samples from 6.55/6.60m down to 6.95/7.00m, all of which have been examined, in contrast contain a much higher diversity fauna of BOTH foraminifera and ostracods, including the most fragile of specimens and all in the most perfect state of preservation. They also contain many, albeit juvenile and often fragmentary, *Mytilus* shells. Micropalaeontologically, this interval might be referred to as the Hemicythere villosa - Sclerochilus spp. - Leptocythere spp. - Hemicytherura cathrata facies (after the dominant ostracods it contains; a mixture of littoral/sublittoral and inner shelf phytal and benthonic species). It seems to signify a much calmer, low energy environment, following the initial transgressive phase. How long this facies will persevere up-core remains to be seen, but cursory examination of the wet sample residues under the microscope, prior to

drying, suggest that it continues as far as 5.10/5.15m, the limit of processing so far. Interesting, many of these samples also have mollusc shells (albeit fragmentary) in them. Previously, Mollusca have been rarely preserved in the Goodwood-Slindon Raised Beach".So far, about half of the near-100 samples from this borehole have been picked. My plan would be to complete the picking and logging and then to produce something a definitive *Illustrated Key* to the foraminifera and ostracods of The Valdoe, using SEM photography. This standalone publication would contain summaries of the sedimentology, ecology and biostratigraphy of the sequence, but not detailed taxonomy. It would not be aimed primarily either at the archaeologist or the micropalaeontologist *per se*, but would hopefully be useful to both, and moreover, serve as a benchmark for the Late Cromerian microfossils of the British Isles and Europe.

X.2 Assessment of vertebrate remains from Valdoe Pit, West Sussex (Simon Parfitt).

x.2.0 Introduction

Key objectives of the post-excavation analysis are to characterise the environmental and depositional context of the sediment bodies and to understand landscape variation in relation to hominin activity. A subsidiary aim is to make comparisons with the nearby Boxgrove site. To this end, large bulk samples were collected from six test pits at the Valdoe Pit, principally to recover vertebrate remains. A small sub-sample from the various fossiliferous units has been examined to assess their potential for contributing to the resolution of these questions.

x.2.1 Materials and methodology

The samples were collected as part of the Valdoe Project, directed by M. Pope (Institute of Archaeology, London). This was the site of an earlier investigation in 1995, which discovered a small section of marine and terrestrial fossiliferous deposits. No archaeological remains were discovered at the time, and quarrying subsequently destroyed the section. Quaternary deposits at Valdoe Pit are associated with the Goodwood-Slindon Raised Beach. Equivalent sediments at Boxgrove have yielded early Middle Pleistocene human remains, butchered bones and an exceptionally rich archaeological assemblage of international importance.

Fine-grained sediments sealed by chalky gravels are the main focus of the archaeological work at Valdoe as these have been shown to contain *in situ* artefacts. Three bulk samples were taken from Trench 6 during the initial fieldwork stage of the current project while a further three samples were taken from Test Pit 6 (see table x.2). Taken together with samples from Valdoe Test Pit 1, recovered in the late 1990's but yet to be formally analysed or published, 15% of available faunal bulk samples have been assessed for potential.

All of the samples were first air-dried and then washed over a 500 micron sieve, the resulting residues being thoroughly dried before examination under a binocular microscope. A record was made of biological remains seen (vertebrates, molluscs, earthworm granules, etc.) and a representative sample picked for future examination. The residues were sent to Richard Preece (Department of Zoology, University of Cambridge), who will undertake analysis of the molluscan remains.

The aim of this assessment is to determine the range of remains present and to assess their abundance and state of preservation. In addition, the archive from a preliminary

investigation undertaken at the pit in the late 1990's (Valdoe Test Pit 1), was also undertaken.

x.2.3 Results

Trench		VTP (6	VTP 5		
Sample	1	2	3	а	b	c
Unit	4b	4c	5b	6	5a	4c
Sample weight (kg)	26	30	22	29	29	34
Small mammal	А	Р	Р		А	Р
Large mammal	Р				Р	
Terrestrial molluscs	А	А				
Slug plates	Р	Р				
Earthworm granules	А					
A – abundant; P - present						
Table x.2: Presence of mammalian fauna						

Table x.2 (below) summarises the biological remains found.

A range of calcareous fossils was noted. Of particular interest is the abundance of molluscan remains in Sample 1. The preservation of the shells is excellent, but in the overlying sample 2, molluscs are not in such large numbers and they are frequently fragmentary. Sample 1 also yielded a diverse assemblage of small vertebrate remains, including those of amphibians and small mammals, together with fragments of large mammal bone. Finally, abundant earthworm granules may indicate that this horizon is a buried soil. The abundance of fossil

material is lower in Sample 2 and 3, but again a range of fossils are present, which would merit detailed study. In addition to the small mammal remains from the bulk samples, John Whittaker recovered six fragmentary shrew teeth from a 0.5kg micropaleontology sample (BH3ii, 4.4.5-4.65m). This highlights the variation in abundance of small vertebrate remains at the site. One factor that may account for the variation in abundance of bones and shells is post-depositional loss through decalcification (see Recommendations, below).

In terms of preservation and abundance of calcareous fossils, the Valdoe 4u and 5b units appear to be comparable with equivalent horizons at Boxgrove. However, the addition of a rich molluscan remains from unit 4b at Valdoe is exceptional given that terrestrial mollusca are rare and poorly preserved at Boxgrove. Taken together, therefore, these remains are likely to have great ecological significance. This find also highlights the great variation in preservational conditions of deposits associated with the Goodwood-Slindon Raised Beach from decalcified deposits devoid of fossils, to calcareous sediments with a range of calcareous fossils and rare waterlogged sediments that contain plant macrofossils and beetles. Environmental evidence of such antiquity that can be related to Palaeolithic occupation is extremely rare. The importance of the Valdoe site lies in the information it may yield on landscape ecology and ecological preferences of early Middle Pleistocene humans. Further work on the samples is strongly recommended and full advantage should be made of the opportunity to process further samples from the site.

x.2.4 Note on the Valdoe Test Pit 1 archive

The archive from this investigation is already housed in the Department of Palaeontology at the Natural History Museum, London. There is a comprehensive photographic record (colour, black and white negatives and prints, 35mm colour transparencies), a drawn section showing location of samples with comments on the lithology, sieve residues and monoliths. The biological remains, including ostracods, foraminifera, molluscs and small vertebrates, have been identified and curated.

Small vertebrates include the extinct shrew *Sorex runtonensis*, a small mole *Talpa minor*, an extinct vole *Pliomys episcopalis*, water vole *Arvicola cantiana terrestris* and an indeterminate small vole *Microtus* sp. A number of these taxa are key elements of the small mammal fauna from Boxgrove, including species that went extinct at the end of the early Middle Pleistocene. The small mammals provide confirmation that the deposits are indeed early Middle Pleistocene in age.

x.2.4 Recommendations

Analysis should focus on the main archaeological horizon (Unit 4c) with the processing of bulk sampled sediment from each of the six test pits. It is recommended that the sediment from three 1x1 m squares (estimated at 150-300 kg) is processed from each of the six test pit. This will provide a sufficiently large sample to characterise the small vertebrates from the individual areas. The sieving will be undertaken in Sussex by GS, and residues will be sorted to 0.5 mm mesh size. The fine fractions will be sorted with the aid of a low-power binocular microscope to ensure total recovery of the smallest identifiable bones and teeth. In addition, samples from Unit 5a and 5b should be analysed to provide information on environmental change through the sequence. Two samples from each of these units have been collected for analysis. The 'brickearth' (Unit 6) sample processed during the assessment was barren. Further processing of 'brickearth' samples for small vertebrates is not warranted. At Boxgrove, a similar approach provided important information on local landscape variation.

For example, the presence of trees can be identified from high concentrations of small mammal bones deposited by birds of prey at roost sites and ponds and areas prone to flooding were identified from high concentrations of fish bones representing natural death assemblages.

Taphonomic analysis of small mammal remains will use methods developed by Peter Andrews (1990, *Owls Caves and Fossils*. London, Natural History Museum). Work on taxonomic identification will utilise collections in the Natural History Museum. Calcium carbonate content and sediment pH should be measured for each of the Unit 4c samples to assess the extent of post-depositional decalcification.

The report will focus on environmental change through the sequence and comparison of the Unit 4c vertebrate assemblages across the site. Comparisons will be made with assemblages from the same unit at Boxgrove and the significance of the vertebrate assemblages for understanding local landscape variation will be highlighted.

The database and specimens will be archived in the Department of Palaeontology, Natural History Museum, London. A specimen register will be deposited with the specimens to facilitate accession numbering.

x.3 Pollen Analysis (Phil Gibbard & Sylvia Peglar)

x.3.0 Introduction

Pollen has been isolated from sediments forming part of the Slindon Formation before. In 1986 Scaife reported on three sample taken from early excavation at Boxgrove (Roberts 1986). These were low-yield samples each requiring 30g of sediment to undertake the analysis. The sediments themselves were characterised as being highly mineralgenic and weathered however some statements on local vegetation conditions were possible. Sample 1m from the Upper Slinon Silts (Unit 4c) was characterised by arboreal pollen dominated by *pinus* with *picea* and *abies* also present. There were less herbaceous pollen but it was noted that the brackish sedimentary conditions would favour the preservation of conifers, a small amount of Oak and beach was present. More material was recovered form the Unit 5a sample and here again pine dominated but to a lesser extent as *picea*, which is usually underrepresented reached levels of 23%). Pollen was also recovered from an early interglacial organic layer at Slindon as part of the RBMP survey, yet to be reported on this should an opne landscape with some spruce typical on Middle Pleistocene early glacial landscapes (Gibbard pers Comm). In general however our knowledge of vegetation conditions within the Boxgrove Palaeolandscape is poor and fragmentary and so the early identification of pollen in sub-samples taken from the Valdoe Pit (Whittaker pers com) made it imperative that potential for pollen analysis at the site was undertaken

x.3.1 Materials and Methodology

8 samples taken from a range of sedimentary Units across the Valdoe palaeolandscape were submitted for assessment for their pollen and spore content, these represent 155 of purposive pollen samples collected during the project. Of these two samples contained pollen: VWBH3 Unit 6 and TP9 Unit 4c

x.3.2 Results

The pollen assemblage is dominated by herbaceous taxa (60% of total land pollen and spores (TLP)), indicative of dry grassland with grasses (Poaceae), birds-foot trefoil (*Lotus*), dandelion-type (*Taraxacum*-type), yarrow-type (*Achillea*-type), buttercups (*Ranunculus*

acris-type), sorrels (*Rumex acetosa*-type), umbellifers (Umbelliferae) and nettles (*Urtica*). There is also 37% TLP of arboreal and shrub pollen, the most abundant taxon (15%) being oak (*Quercus*), but with several other taxa including birch (*Betula*), alder (*Alnus*), maple (*Acer*), yew (*Taxus*), hazel (*Corylus*), willow (*Salix*), pine (*Pinus*), and spruce (*Picea*).

Such an assemblage is characteristic of a temperate period in the mid-Pleistocene - spruce is present but there are no exotic taxa such as hemlock-spruce (*Tsuga*) or wingnut (*Pterocarya*) which are found in early Pleistocene assemblages.

The presence of spruce suggests that this sediment comes from later in an interglacial (temperate) period when the climate was cooler than in the full interglacial.

There is no pollen of obligate aquatic or telmatic taxa present, and thus no evidence for wet or damp ground close by.

The pollen was well preserved and indeterminable pollen and spores low (7% TLP + sum indet.)

From this limited pollen assemblage, we can suggest that, at the time the sediment was laid down, the local vegetation was a dry grassland, with some mixed woodland, including oak, some distance away from the site.

x.3.3 Recommendations

x.4 Molluscan Analysis (Richard Preece)

x.4.0 Introduction

Studies of molluscan fauna have been undertaken twice at Boxgrove (Bates 1986: Preece and bates 1999). These studies identified the remnants of an originally rich fauan which has undfortuantely been differentially prererved across the Boxgrove quarries due to decalcification. Nonetheless, the fauna from Boxgrove was able to charcterise a range of open and wooded environments indicating both wet open grassland through the developments of Units 4b, 4c and 5a; warm conditions during the formation of the birckearth (Unit 6b) although these species may be derived and the presence of shade demanding species close to cliff, perhaps being introduced form the coniferous woodland indicated for the chalk hinterland of the Boxgrove palaeosols.

Thus despite the patchy preservational conditions at Boxgrove it occupies an important place in Eiropean malacological studies, in offering the only truly terrestrial fauna for the early Middle Pleistocene (Preece and Bates 1999). It is therefore exciting that we can now add Valdoe as a second example, yet here the preservational considiotns appear to be much better and offer the chance of a more complete and detailed insight into mollusc faunas for this key period.

x.3.1 Materials and Methodology

Washed residues from 12 samples covering Units 4b, 4c and 5b from Trench 6 and Trench 5 were passed to R.C. Preece for preliminary assessment. No quantitative analyses were undertaken. The following taxa were recovered:

	Unit 4b	Unit 4c
Aquatic species		
Lymnaea truncatula	-	+
Pisidium sp.	+	-
Terrestrial species		
Carychium cf. minimum	+	+
Succineidae	+	+

<i>Cochlicopa</i> sp.	+	+	
Vertigo sp.	+	+	
Pupilla muscorum	+	-	
Vallonia costata	+	+	
Vallonia pulchella	+	+	
Punctum pygmaeum	+	+	
Vitrinidae (2 spp.)	+	+	
Vitrea contracta	+	+	
Nesovitrea hammonis	+	+	
Aegopinella nitidula	+	+	
<i>Oxychilus</i> sp.	+	-	
Retinella (Lyrodiscus) skertchlyi	+	+	
Deroceras/Limax	+	+	
Euconulus fulvus agg.	+	+	
Clausiliidae	+	+	
Trichia hispida	+	+	
Arianta arbustorum	+	+	
Earthworm granules	+	+	
Marine shells			
Mytilus edulis	+	-	
<i>Spisula</i> sp.	+	-	
Cerastoderma sp.	+	-	
- -			

x.4.2Results

The preservation of shells from this site was *far* better than at the main site at Eartham Quarry, Boxgrove, 6 km to the east, where decalcification had been a particular problem. Indeed, virtually all the material composed of aragonite (i.e. most non-marine shells) has been lost from several of the units at Boxgrove. At Valdoe Quarry, the shells are still fragmentary but the surface detail has survived in many cases. It should eventually be possible to identify some of the clausiliids to species, since diagnostic apertures occur here (they were completely absent at Boxgrove).

The molluscan assemblages from unit 4b and 4c were similar, both dominated by a somewhat restricted fauna of land snails composed of at least 20 species; unit 5b proved to be

barren. A damp, rather open habitat is indicated, although some shade-demanding taxa are present. The molluscan fauna at Valdoe Quarry is broadly similar to that reported from Boxgrove (Preece & Bates, 1999) but differs in two important respects. First, *Spermodea lamellata* a species of dense woodland, was not present at Valdoe Quarry. Second, *Retinella (Lyrodiscus) skertchlyi*, absent at Boxgrove, here forms a significant component of the molluscan assemblage. This is an extremely important discovery. First, it is of considerable zoogeographical importance because members of this subgenus are now confined to the Canary Islands (Rousseau & Puisségur, 1990). Second, being extinct, it has some biostratigraphical significance. Hitherto, Pleistocene occurrences of *Lyrodiscus* in NW Europe are usually found in tufa deposits yielding molluscan assemblages from closed forest environments – forming the so-called *Lyrodiscus* Biome (Rousseau et al., 1992). These assemblages are thought to characterize forest environments of the Hoxnian (MIS 11). At Valdoe Quarry *Lyrodiscus* occurs with species typical of more open habitats. Its occurrence at Valdoe Quarry demonstrates that Middle Pleistocene records of *Lyrodiscus* can no longer be taken to indicate a MIS 11 age, especially those records from more open habitats.

x.4.3 Recommendations

It is therefore recommended that further work is undertaken to characterise in detail the molluscan fauna of the Valdoe. It should be possible, through close interval sampling of three sediment monoliths, to determine changes in habitat conditions across the transition from open lagoon edge sediments, through terrestrial landsurfaces to the development of apparent cold stage silts (Unit 6). In addition picked residues from the analysis of small mammal fauns and ostracod/forma processing can also be passed on for isolation of molluscan fauna. It is suggested time be allocated to examine up to ten productive residues of this nature in addition to the close sampling of the sediment monoliths.

The identified presence of *Lyrodiscus* fauna will ensure that any publication deriving from this work, in addition to providing a profile of the Valdoe site, will make an important contribution to Quaternary biostratigraphy.

x.5 Amino Acid Racimization: Kirsty Penkman

x.5.0 Introduction

Amino acid analyses were undertaken at the York Laboratory (NEaar) from one trench within the Valdoe Pit. This involves isolating the intra-crystalline protein fraction of mollusc shells, in this case the slug plates from Derocerax/Limax. This report assesses the potential for age estimation of the Valdoe material using amino acid racemization (AAR).

A new technique of amino acid analysis has been developed for geochronological purposes (Penkman, 2005; Penkman *et al.*, submitted, a), combining a new Reverse-Phase High Pressure Liquid Chromatography method of analysis (Kaufman & Manley, 1998) with the isolation of an "intra-crystalline" fraction of amino acids by bleach treatment (Sykes *et al.*, 1995). This combination of techniques results in the analysis of D/L values of multiple amino acids from the chemically-protected protein within the biomineral; enabling both decreased sample sizes and increased reliability of the analysis. Amino acid data obtained from the intra-crystalline fraction of the calcitic *Bithynia* opercula has been found to be a particularly robust repository for the original protein, for which an excellent and growing database of protein degradation data has recently been assembled (Penkman, 2005). It is proposed that other calcitic materials may also retain their intra-crystalline fraction of protein more successfully than aragonitic shells. As no opercula have yet been found in the deposits at Valdoe, calcitic slug plates have been focused on in this study.

Amino acids, the building blocks of proteins, occur as two isomers that are chemically identical, but optically different. These isomers were designated as either D (dextro-rotary)

or L (laevo-rotary) depending upon whether they rotate plane polarised light to the right or left respectively (Fig. 1). In living organisms the amino acids in protein are almost exclusively L and the D/L value approaches $zero^1$. The potential application to geochronology arises from the fact that after death amino acid isomers start to interconvert. This process is commonly termed racemization. In time the D/L value approaches one. The proportion of D to L amino acids is therefore an estimate of the extent of protein degradation, and if this is assumed to be predictable over time can be used to estimate age. Other indications of protein decomposition, such as the degradation of unstable amino acids, can also be used to estimate the age of a sample.



Figure x.x: L- and D- amino acid structure

Mechanisms of racemization

The rate of racemization is governed by a variety of factors, most of which have been studied in detail only for free amino acids. North East amino acid racemization (NEaar) analyse the intra-crystalline amino acid fraction and in this way, within a closed environment in which

¹ D-amino acids are synthesised by some organisms; they are found free in invertebrate body fluids where they play a role in osmoregulation and can occur peptide bound in bacterial peptidoglycan, where part of their function is resistance to proteases.

other factors (water content, concentration of cations, pH) are constant, the extent of racemization is a function of time and temperature. Over a small geographical area, such as that represented in this study, it can be assumed that the integrated temperature histories are effectively the same. Any differences in the extent of decomposition of protein within the sample are therefore age-dependent.

The organic matter existing within individual crystals (intra-crystalline fraction) is believed to be a more reliable substrate for analysis than the whole shell (Sykes *et al.*, 1995; Penkman, 2005; Penkman *et al.*, submitted, a). The initial bleaching step in the recovery of the intra-crystalline fraction removes both secondary contamination and the organic matrix of the shell. This organic matrix degrades and leaches at an unpredictable rate over time, leading to variation in the concentration and D/L of the amino acids. Thus, as appears to be the case in ostrich eggshell (Miller *et al.*, 2000), the D/L values of amino acids in the intracrystalline fraction of shells have been analysed; in the case of ostrich eggshell no bleaching step was used. The molluscan racemisation data reported therefore contrasts with previous work that examined D/L values from whole mollusc shells containing both intra- and intercrystalline material.

This isolation of the intra-crystalline fraction is believed to provide a closed system repository for the amino acids during the burial history of the shell. Only the amino acids within this fraction are protected from the action of external rate-affecting factors (except temperature), contamination by exogenous amino acids and leaching. Amino acids within the whole shell are not protected and can be leached out into the environment. Figure 2 shows a schematic of the intra-crystalline fraction with respect to the whole shell. The low level of Free amino acids observed in the inter-crystalline fraction of unbleached samples (Penkman, 2005) indicates that these have been lost through diagenesis, and as these tend to be more

highly racemised than the Total fraction, this loss would lead to a lower than expected D/L for the Total fraction of the whole shell.



Figure x.x: Schematic of intra-crystalline amino acids entrapped within carbonate crystallites. Unlike the proteins of the organic matrix between the crystallites, which leach from the shell with time, in a closed "intra-crystalline" system the amino acids are entrapped. Thus the relationship between the DL ratios of different amino acids and between free (non-protein bound) and total (both free and originally protein-bound amino acids, released by acid hydrolysis) amino acids is predictable. Analysis of the whole shell would result in lower than expected D/L for the total fraction, due to the loss of the more highly racemised frees.

Once a closed system inside mollusc shells has been isolated, then the kinetics of protein decomposition are much simpler to predict. In this laboratory the concept of age estimation using the extent of overall Intra-crystalline Protein Degradation (IcPD) has been devised, which links the hydrolysis, racemisation and decomposition of all the amino acids isolated by this method. The concept behind the IcPD is to combine multiple information from a single sample to derive an overall measure of the extent of diagenesis of the protein in that fossil. Similar ideas have been used before, although not in such a comprehensive way. Divergence

from the normal in a plot of A/I vs Gly/Ala is thought to indicate leaching in molluscs (Murray-Wallace and Kimber, 1987). Kaufman (2000) used ratios of Asx to Glx to screen out samples with any unusual values.

Traditionally AAR studies targeted a single amino acid racemisation reaction, that of L-isoleucine to D-alloisoleucine (A/I), due to the technical ease of separation and its slow rate of racemisation. The approach used in this study diverges from this, as dates are derived from the analysis of multiple amino acids. Whilst racemisation rates differ between individual amino acids, they should be highly correlated in a closed system. By linking together different amino acids, and then linking this to a temperature driven model of decay, which includes hydrolysis, racemisation and degradation, the extent of protein degradation can be derived. The pattern of decomposition appears to be different between mollusc genera, requiring separate models for each genus or species studied.

If a closed system is isolated, it should be possible to predict the relationship between geological time and IcPD increase, using not just racemisation but other measures of protein decomposition, such as total and relative concentrations. It follows from the innovations above that, assuming sampling is from an idealised closed system, the pattern of protein decomposition governs the observed racemisation of (a) free amino acids and (b) the total system, (c) the percentage of free amino acids and (d) the total concentration of amino acids.

This model can also be used as a method of assessing the internal reliability of each biomineral used and to determine how closely these substrates approximate to a closed system. Subsequently palaeotemperature information can be included and estimates made of the link between degradation and absolute age in environments with fluctuating temperatures. If an accurate temperature model is used, then age estimates can be derived directly from the IcPD data, although the results presented here do not incorporate any palaeotemperature information and are presented simply as a relative dating tool.

x.2.2 Materials and Method

Molluscan samples were supplied by Richard Preece (University of Cambridge). Amino acid racemization (AAR) analyses were undertaken on four individual Derocerax/Limax slug plates from a single horizon: Valdoe Pit, Trench 6, Unit 4b (NEaar 4156-4159).

Shells were examined under a low powered microscope and any adhering sediment removed. The shell samples were then sonicated and rinsed several times in HPLC-grade water. The shells were then crushed to $<100 \ \mu m$. Only bleached samples were analysed. 50 μ l of 12% solution of sodium hypochlorite at room temperature was added to each milligram of powdered sample and the caps retightened. The powders were bleached for 48 hours with a shake at 24 hours. The bleach was pipetted off and the powders were then rinsed five times in HPLC-grade water and a final rinse in HPLC-grade methanol (MeOH) to destroy any residual oxidant by reaction with the MeOH. The bulk of the MeOH was pipetted off and the remainder left to evaporate to dryness. Protein bound amino acids are released by adding an excess of 7 M HCl to the bleached powder and hydrolysing at 110°C for 24 hours (H*). 20 µl per milligram of sample of 7 M Hydrochloric Acid (HCl) was added to each Hydrolysis ("Hyd", H*) sample in sterile 2 ml glass vials, were flushed with nitrogen for 20 seconds to prevent oxidation of the amino acids, and were then placed in an oven at 110°C for 24 hours. After 10 minutes in the oven, the caps of the 2 ml vials were re-tightened to prevent the samples drying out. After 24 hours, the samples were dried in a centrifugal evaporator overnight.

Free amino-acid samples ("Free", F) were demineralised in cold 2 M HCl, which dissolves the carbonate but minimises the hydrolysis of peptide bonds, and then dried in the centrifugal evaporator overnight. When completely dry, samples were rehydrated with 20 μ l of Rehydration Fluid: a solution containing 0.01 mM HCl, 0.01 mM L-homo arginine internal standard, and 0.77 mM sodium azide at a pH of 2. Each vial was vortexed for 20 seconds to

ensure complete dissolution, and checked visually for undissolved particles. Approximately 13.5 µl of rehydrated sample was then placed in a sterile, labelled 2 ml autosampler vial containing a glass insert, capped and then placed on the autosampler tray of the HPLC. For each set of sub-samples a blank vial was included at each stage to account for any background interference from the bleach, acid, or rehydration fluid added to the samples. Amino acid enantiomers were separated by Reverse Phase High Pressure Liquid Chromatography (RP-HPLC). NEaar uses the method of Kaufman and Manley (1998) using an automated RP-HPLC system. This method achieves separation and detection of L and D isomers in the sub- picomole range. Samples (2 µl) were derivitised with 2.2 µl ophthaldialdehyde and thiol N-isobutyryl-L-cysteine automatically prior to injection. The resulting diastereomeric derivatives were then separated on Hypersil C₁₈ BDS column (sphere d. 5 µm; 250 x 3 mm) using a linear gradient of a sodium acetate buffer (23 mM sodium acetate, 1.3 mM Na₂EDTA; pH6), methanol, and acetonitrile on an integrated HP1100 liquid chromatograph (Hewlett-Packard, USA). Individual amino-acids are separated on a non-polar stationary phase according to their varied retention times: a function of their mass, structure, and hydrophobicity. A fluorescence detector is used to determine the concentrations of each amino-acid and record them as separate peaks on a chromatogram. A gradient elution programme was used to keep the retention time to below 120 minutes. The fluorescence intensity of derivitised amino acids was measured (Ex = 230 nm, Em = 445 nm) in each sample and normalised to the internal standard. All samples and blank extracts that had been subjected to identical preparation procedures were run in triplicate. Quantification of individual amino acids was achieved by comparison with the standard amino acid mixture.

External standards containing a variety of D- and L- amino acids, allowing calibration with the analyte samples, were analyzed at the beginning and end of every run, and one standard was analyzed every ten samples. Blanks were randomly interspersed

amongst the standards. The L and D isomers of 10 amino acids were routinely analysed. During preparative hydrolysis both asparagine and glutamine undergo rapid irreversible deamination to aspartic acid and glutamic acid respectively (Hill, 1965). It is therefore not possible to distinguish between the acidic amino acids and their derivatives and they are reported together as Asx and Glx.

X.5.3 Results and Discussion

In total 8 analyses were conducted, all of which were on bleached samples. As previously observed, bleaching reduced the yields of amino acids and also increased reproducibility. The intra-crystalline protein concentration within the individual slug plates were high enough to enable individual samples to be analysed for both Free and Hydrolysed amino acids. The extent of racemization in four amino acids (D/L of Asx, Glx, Ala and Val) are reported for both the Free and Hyd fractions (Appendix 4). These indicators of protein decomposition have been selected as their peaks are cleanly eluted with baseline separation and they cover a wide range of rates of reaction. It is expected that with increasing age, the extent of racemization (D/L) will increase. Data from slug plates from two other sites are presented for comparison: Aveley, a site in the Thames valley correlated with MIS 7 (Bowen *et al.*, 1989; Bridgland, 1994); and West Runton, the type site of the Cromerian.

The data obtained from Asx, Glx, alanine (Ala) and valine (Val) is discussed in detail below. If the amino acids were contained within a closed system, the relationship between the Free and the Hyd fractions should be highly correlated, with non-concordance enabling the recognition of compromised samples (Preece & Penkman, 2005). No slug plates in this study showed this non-concordance. The pattern of protein degradation with time is different for the slug plates than for the opercula of *Bithynia* sp. Amino acid racemisation is governed by the original protein sequence and conformation. Whilst developing the research into closed-system protein degradation it became clear that the reaction rates were species-specific, even in the intracrystalline fraction (Penkman *et al.*, submitted, a). This necessitates the comparison of amino acid data only within a single species, meaning that the slug plate data cannot be directly compared to amino acid data obtained from another biomineral.

Aspartic acid / Asparagine (Asx)

Asx is one of the fastest racemizing of the amino acids discussed here (due to the fact that it can racemize whilst still peptide bound; Collins *et al.*, 1999). This enables good levels of resolution at younger age sites, but decreased resolution beyond MIS 7.

The values of Asx D/L for the Free and Hyd samples for Valdoe are significantly higher than Aveley. There is overlap between the Asx D/L data from Valdoe and West Runton, with Valdoe having slightly lower ratios in the Free fraction. Discrimination between sites of this age is difficult with this amino acid due to the plateauing of the increase in D/L at these timescales, but the data indicates that Valdoe is slightly younger than West Runton.



Figure X.X: D/L values of Asx for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) Derocerax/Limax slug plates from Aveley, West Runton and Valdoe. For each site, the base of the box indicates the 25th percentile. Within the box, the solid line plots the median. The top of the box indicates the 75th percentile. The results of each duplicate analysis are included in order to provide a statistically significant sample size. Note different scales on the y-axes.

Glutamic Acid/Glutamine (Glx)

Glx is one of the slower racemizing amino acids discussed here and so the level of resolution from young sites is less than that seen with faster racemizing amino acids such as Asx. It is noteworthy that Glx has a slightly unusual pattern of racemization in the free form, due to the formation of a lactam (see Walton, 1998). This results in difficulties in measuring Glx in the Free form, as the lactam cannot be derivitized and is therefore unavailable for analysis.

The D/L values from Valdoe are significantly higher than those from Aveley. Interestingly the pattern in D/L values seen in the other amino acids, with the Valdoe samples tending to have lower ratios than at West Runton, is not observed with Glx. On their own this would indicate that Valdoe is perhaps slightly older than West Runton. However, this interpretation is not supported from the data from the other amino acids. No studies into the degree of natural variability within samples have yet been undertaken on slug plates, and it is possible that the higher D/L values obtained at Valdoe are not significant. Further studies need to be undertaken to resolve this issue.



Figure X.X: D/L values of Glx for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) Derocerax/Limax slug plates from Aveley, West Runton and Valdoe. For a full description of this figure see the legend for Figure 3.

Alanine

Alanine (Ala) is a hydrophobic amino acid, whose concentration is partly contributed from the decomposition of other amino acids (notably Serine). Ala racemises at an intermediate rate, so is one of the amino acids that may help distinguish samples at these timescales. In both the Free and Hydrolysed fractions, the D/L of the Valdoe samples is significantly greater than that of Aveley, and slightly lower than that of West Runton, indicating an intermediate age, but closer in age to West Runton.



Figure X.X: D/L values of Ala for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) Derocerax/Limax slug plates from Aveley, West Runton and Valdoe. For a full description of this figure see the legend for Figure 3.

Valine (Val)

Valine has extremely low rates of racemisation, and so is one of the more useful amino acids for age discrimination within material of this age.

The Free and Hyd D/L values of the Valdoe samples again show a significant increase in those from Aveley. Slightly lower values are obtained in the Free fraction to those from West Runton, with no significant difference between these two sites in the Hydrolysed fraction. It should be noted that the spread of data within the Hydrolysed fraction of the Valdoe samples is large. The Val data therefore indicates an age for Valdoe similar to but possibly slightly younger than West Runton.



Figure X.X: D/L values of Val for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) Derocerax/Limax slug plates from Aveley, West Runton and Valdoe. For a full description of this figure see the legend for Figure 3.

x.5.4 Recommendations

Little work has been undertaken into the protein degradation within slug plates, as they have only recently been identified as potentially providing a consistent closed system protein fraction over Quaternary timescales (Penkman, 2005). Although there are aragonitic shells within the Valdoe material, they were not targeted in this assessment phase. Based on studies on opercula, it has been proposed that calcitic material provide a more robust repository than aragonitic shells for the original protein and its breakdown products, enabling increased resolution within the Cromerian (Penkman *et al.*, submitted, b). The only calcitic material available for amino acid analysis from Valdoe were slug plates, and unfortunately samples from only two other sites have been previously analysed. However, the data from Valdoe can be usefully compared with data from these two sites, and a relative stratigraphy developed. The amino acid data from the slug plates from Valdoe indicate that the site is significantly older than that of Aveley, a site correlated with MIS 7, and similar in age but slightly younger than West Runton. This is consistent with other age estimates for the horizon. Further studies are being undertaken on the natural variability of the slug plates and extending to further sites; this will aid the interpretation of this preliminary dataset.

This preliminary study presents data to show that amino acids can be recovered from the intra-crystalline fraction from Cromerian-age slug-plates, and that the D/L data obtained from this protein allow relative age estimations to be made.

The further study of material from the Valdoe Pit would be an important contribution to the development of an amino acid chronology based on the new techniques, not only by helping to narrow down the age of this important site, but also enabling the extension and correlation of the methodology from fluvial and lacustrine environments using *Bithynia* opercula to sites with a more land-based fauna. It is therefore proposed to undertake further analyses on molluscan shells from this site. Slug plates have proved to be a useful tool; earthworm granules, which are also calcitic and occur at this site, will also be exploited. Databases are already being developed for several of the gastropod shells present at Valdoe (e.g. *Lymnaea*, *Pupilla*, *Trichia* and *Cepaea*) so it would also be useful to analyse these shells, although caution should be taken with data from gastropod shells of this age (Penkman *et al.*, submitted, b).

Thus, Amino acid analysis of the intra-crystalline fraction of slug-plates has been successfully undertaken on individual samples. The extent of protein breakdown within these slug-plates allows the development of an aminostratigraphy using these biominerals. The extent of protein degradation within samples from Valdoe is significantly greater than that from the MIS 7 site of Aveley, as expected. The amino acid data indicates that Valdoe is closer in age to West Runton, but likely to be slightly younger. It is hoped that further studies will enable an increased level of resolution using this, and other, calcitic materials.

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X.6 Lithic Technology

x.6.0 Introduction

From an early stage in the VAS it was established that in-situ archaeology in the form of spread of knapping debris was present both within the Valdoe Quarry footprint and within the immediate hinterland of the pit. In all seven test pits produced evidence for in-situ archaeology, reaching a maximum density of five artefacts >20mm per m² in VTP5. Artefacts were found in-situ within four distinct stratigraphic units. (Unit 4b. Unit 4c, Unit 5a and Unit 6b) covering environments from fully temperate interglacial lagoon edge to early cold stage brickearths. The presence of in-situ archaeology alone at the Valdoe was hugely significant, it established beyond doubt that the zone of human activity, demonstrated at Boxgrove to preserve evidence for early human hunting and carcass processing activities extends across the wider extent of the Westbourne-Arundel Raised Beach. Artefactual material in the form of biface finds from ploughsoils (e.g. Valdoe, Lavant) or spreads of knapping debitage within Head Deposits (e.g. Slindon) had certainly suggested a wider distribution for human activities indicated at Boxgrove (Woodcock 1981; Pope 2001) but the material from the VAS represents the first to be excavated from in-situ palaeolandsurfaces outside of Boxgrove.

x.6.1 Materials and Methodology.

The test pit excavations undertaken during the VAS followed a methodology derived from the Boxgrove project B rescue excavation (Robert et al 1997). Fine-grained sediments (Units 6b, 4c, 5a, 4b) were excavated by hand in a series of 20mm spits and each artefact >200mm was recorded in terms for x,y,z position and angle/orientation of rest. All spoil was collected as bulk samples. In addition a single trench square was bulked in its entirety to collect all artefactual material as well as intact and undamaged organic material for environmental analysis. The detailed recording would allow aspect of human behaviour and site formation be reconstructed using techniques developed during the course of analysis in GTP17 and Q1/B (Pope 2002; Roberts and Pope forthcoming). Debitage taken from the bulk sampled material could then be sieved and graded to develop size-class profile, a technique demonstrated in earlier analysis at Boxgrove and at other Palaeolithic sites to be a useful measure of assemblage integrity (Schick 1987; Sahoumi 1999; Pope 2002). For the purposes of assessment the assemblages from two test pits (TP5 and TP9) were subject to a taphonomic overview. They were chosen because each had demonstrated evidence for in-situ human activity yet while one was situated outside the Valdoe Pit in the woods to the immediate north, the other was located to south within the centre of Extraction Area 2. For each pit only the assemblage from the main Unit 4c palaeosol was considered and debitage from bulk samples of the sedimentary unit were processed for micrdebitage analysis.

x.6.3 Results.

TP5 produced an overall assemblage of 33 artefcats >20mm. of these 22 were recovered from the Unit 4c Palaeosol, extrapolating to an artfact density for this landsurface of 5.5 artefacts per m², a level commensurate with average for the Boxgrove test pits (Pope 2002; Figure x). Technologically these flakes were broadly similar, each representing late stage thinning debitage from the manufacture of bifaces. These characteristic flakes were soft-hammer, and manufactured on fine-grained flint identical to those exploited from the cliff face at the main Boxgrove sites. In one part of the trench a cluster of five of these flakes closely spaced together suggested a short, single resharpening event. Among these flakes was a single tranchet sharpening flake, preserving the tip of the original biface (figure x).

Figure x a shows a taphonomic breakdown for the TP5 material indicating intact compositional profile commensurate with experimental observations and no preferred



angle of orientation. While no substitute for a detailed taphonomic study these two indicators do provide a rapid overview of assemblage sates and here suggest a pristine, in-situ signature.

The assessment of material from Test Pit 9 shows a similar picture. Here, two hard hammer flakes form the earlier stages of handaxe production were found alongside 15 typical handaxe sharpening/thinning flakes. The hard hammer flakes were the only two to be found in-situ as part of the VAS and suggest a localised episode of early stage reduction at distance form the cliff, an activity often associated with primary butchery sites at Boxgrove. Again, the taphonomic picture shown in figure xb indicates an in-situ artefact profile with microdebitage counts conforming to experimental norms and no preferred orientation for artefacts.

x.6.4 Summary and Recommendations

The assessment of artefactual material from TP5 and TP9 has confirmed the general impression gained during the excavation of the Valdoe tets pits : namely that they contain a low-level in-situ human signature comparable in quality of preservation to scatters excavated at Boxgrove. No complete reduction sequence were encountered and most knapping spreads seem to relate to the late stage finishing and shaping of existent tools, perhaps in association with butchery. These assemblages offer the potential to study the highly mobile hunting and tool using behaviours of the Boxgrove hominins in an 'off-site' context matching a range of signatures identified by other Palaeolithic researchers including Isaac (1981) and Roebroek's (1991) which recognises the importance of inter-site signatures in reconstructing early human landuse, mobility patterns and food procurement behaviour. The taphonomic studies have confirmed the un-situ nature of the scatters and suggest their suitability to more detailed site-formation studies and modelling of behavioural human traits.

It is now essential that a more taphonomic study is rolled out to each of the 9 test pits which produced artefactual material. For each assemblage a full technological overview should be undertaken accompanied by:

1. Sieving of contextualising bulk samples for microdebitage recovery (c.50kg sample per unit should suffice).

2. Production of microdebitage curves and mapping of variation in size class across size.

3. Statistical assessment of artefact orientation and development so f taphonomic models encompasses sedimentary context.

4. Direct comparison of artefactual signatures with other Middle Pleistocene locales both with the Boxgrove Palaeolandscape and within the Acheulean Europe.

The latter analysis would take the form of a literature review to work towards a synthesis of the evidence for inter-site activity and form the basis of a discussion paper on this aspect of human behaviour separate from the main Valdoe report.

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