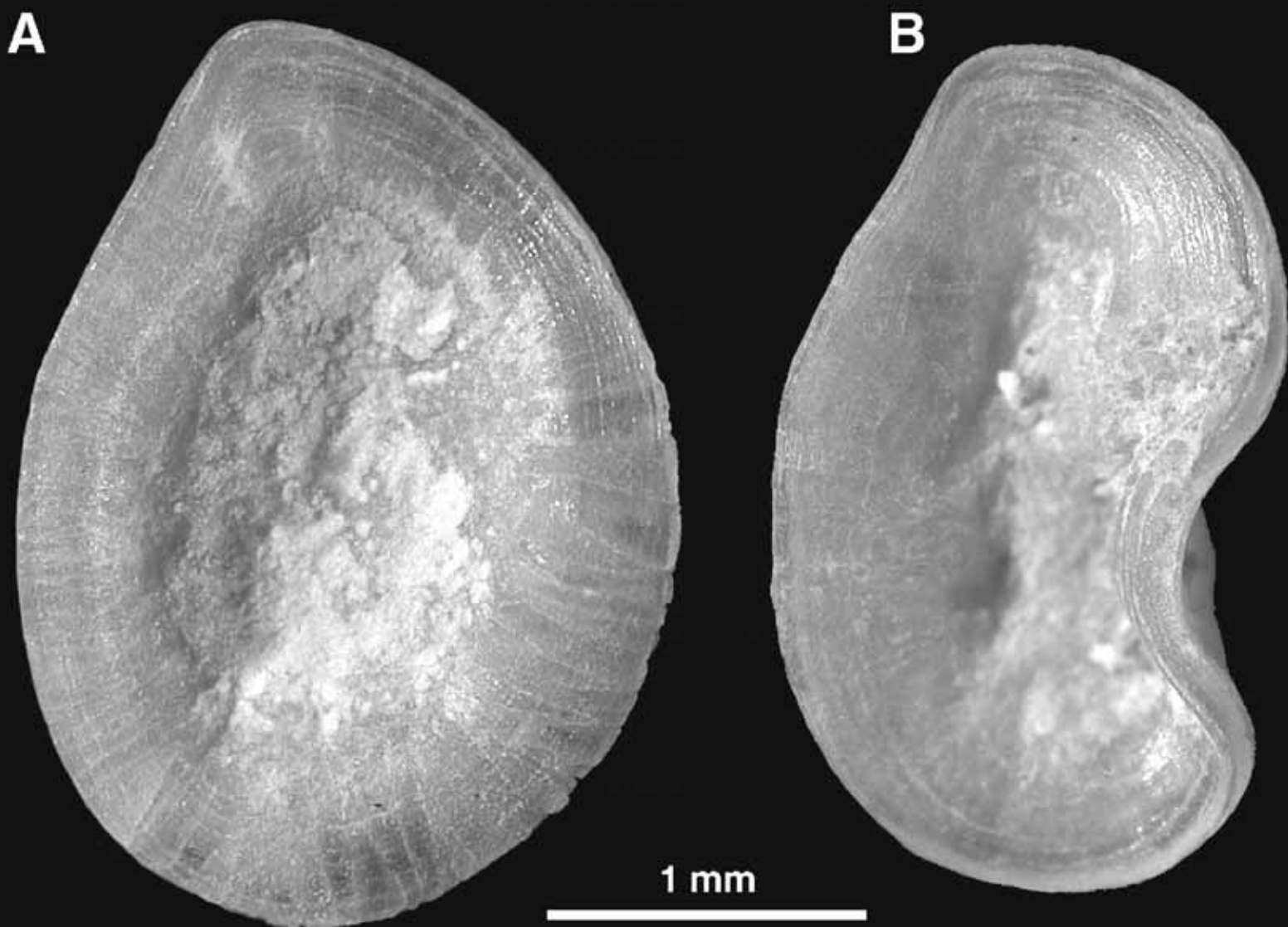# BRITISH AGGREGATES

AN IMPROVED CHRONOLOGY USING AMINO ACID RACEMIZATION AND DEGRADATION OF INTRACRYSTALLINE AMINO ACIDS (IcPD)

SCIENTIFIC DATING REPORT

Kirsty Penkman, Matthew Collins, David Keen, and Richard Preece

ARCHAEOLOGICAL SCIENCE

ENGLISH HERITAGE

BRITISH AGGREGATES

AN IMPROVED CHRONOLOGY USING AMINO ACID RACEMIZATION AND DEGRADATION OF INTRACRYSTALLINE AMINO ACIDS (IcPD)

Kirsty Penkman, Matthew Collins, David Keen, and Richard Preece

SUMMARY

The project assessed the utility of a novel method of amino acid racemization analysis (Intra-crystalline Protein Degradation, or IcPD, analysis of intracrystalline proteins) and interpretation on the calcitic opercula of the gastropod *Bithynia*. The new technique combines a Reverse-Phase High Pressure Liquid Chromatography method (Kaufman and Manley 1998) with the isolation of an 'intra-crystalline' fraction of amino acids by bleach treatment (Sykes *et al* 1995), resulting in the analysis of D/L values of multiple amino acids from the chemically protected organic matter within the biomineral, enabling both decreased sample sizes and increased reliability. This study has shown that the intra-crystalline fraction within calcitic fossils, such as the opercula of the freshwater prosobranch *Bithynia*, provides tighter clustering of amino acid data than obtainable from shells composed of aragonite (Penkman 2005).

The study shows that, using the IcPD approach, it is possible to provide relative age estimates of different aggregate deposits in England, and to relate these ages to the marine oxygen isotope record, so that it is possible to link aggregates to specific phases of the British Quaternary, beyond that which is possible using radiocarbon dating. The work has enabled us to identify the earliest human occupation in northern Europe (Parfitt *et al* 2005).

CONTRIBUTORS

Kirsty Penkman, Matthew Collins, David Keen, and Richard Preece

ARCHIVE LOCATION

NE-aar, BioArch, Departments of Biology, Archaeology and Chemistry, Biology S Block, PO Box 373, University of York, YO10 5YW

DATE OF INVESTIGATION

2004–7

CONTACT DETAILS

Kirsty Penkman, Matthew Collins
NE-aar, BioArch, Departments of Biology, Archaeology and Chemistry, Biology S Block, PO Box 373, University of York, YO10 5YW
kp9@york.ac.uk

David Keen† formerly University of Birmingham

Richard Preece
University Museum of Zoology Cambridge, Downing Street, Cambridge, CB2 3EJ

CONTENTS

1. INTRODUCTION

The project design intended to assess the utility of a novel method of amino acid racemization analysis (Intra-crystalline Protein Degradation, or IcPD, analysis of intracrystalline proteins) and interpretation on a novel, but widespread, material, the calcitic opercula of the gastropod *Bithynia*.

The new technique of amino acid analysis developed combines a Reverse-Phase High Pressure Liquid Chromatography method (Kaufman and 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 organic matter within the biomineral, enabling both decreased sample sizes and increased reliability. This study has shown that the intra-crystalline fraction within calcitic fossils, such as the opercula of the freshwater prosobranch *Bithynia*, provides tighter clustering of amino acid data than obtainable from shells composed of aragonite (Penkman 2005).

The proposal tested the IcPD approach to date aggregate deposits. It is concluded that it is possible using this technique to provide relative age estimates of different aggregate deposits and furthermore to relate the age to the marine oxygen isotope record, so that it is possible to link aggregates to specific phases of the British Quaternary. The method has been used to provide a framework of age estimation beyond that which is possible using radiocarbon dating. The work has enabled us to identify the earliest human occupation in northern Europe (Parfitt *et al* 2005).

2. BACKGROUND

Amino acid racemization (AAR), along with optically stimulated luminescence (OSL), is the principal method for dating Quaternary aggregate deposits beyond the range of radiocarbon dating. Of these approaches, AAR is more rapid and has a wider time-range, but the method suffers a number of disadvantages and in recent years has been largely overlooked as a tool for dating aggregates. We believe that the method is particularly suitable for these deposits, particularly as analyses can be conducted post-hoc on collected (unheated) samples. This technique can then be used to help inform the chronology of past, present and future sites in the British Quaternary.

2.1 Amino acids and proteins

In brief, the technique measures the extent of protein decomposition within archaeological and geological samples. Proteins are built from the sequential condensation of amino acids (20 different types of which are coded for in the genes of all living organisms). They are simple molecules based on a tetrahedral carbon atom attached to two functional groups, the amino group and the carboxyl group, a hydrogen atom and a side chain (R) which determines the type of amino acid.   All the amino acids, except glycine, have a chiral centre and therefore can exist in different forms known as stereoisomers. Most amino acids have only one chiral centre, leading to two stereoisomers (an L-amino acid and a D-amino acid; Fig 2.1) and an equilibrium D/L value of 1. However in protein biosynthesis D-amino acids are discriminated against by >1:104, so in living organisms only the L-amino acid is present. As the protein decomposes, the amino acids are hydrolysed and undergo isomerization (racemization) reactions that ultimately form an equilibrium mixture of chiral forms.
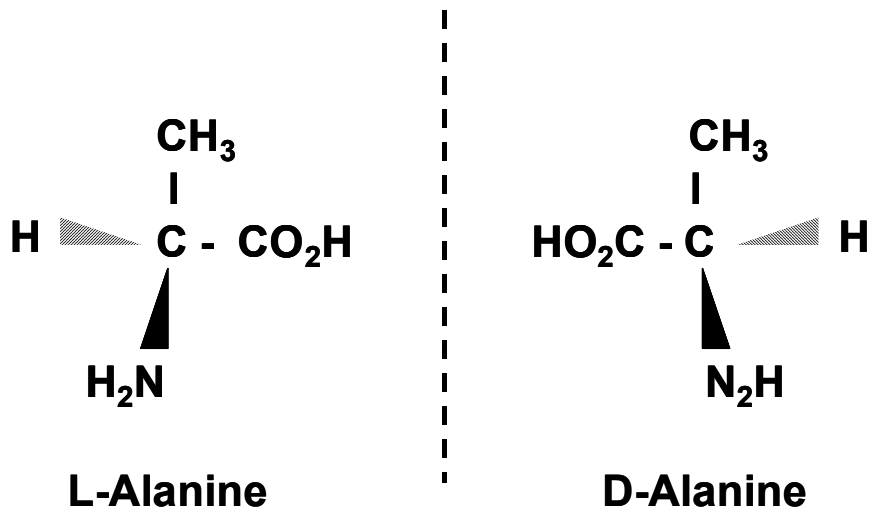
Figure 2.1: L- and D- amino acid structure

2.2 Amino acid geochronology

Amino acids were first reported in fossils by Abelson (1954). He suggested the possibility of using the kinetics of the degradation of amino acids as the basis for a dating method (Abelson 1955). In 1967 Hare and Abelson measured the extent of racemization of amino acids extracted from modern and sub-fossil *Mercenaria mercenaria* shells (edible clam). They found that the total amount of amino acids present in shell decreased with the age of the shell. The amino acids in recent shell were all in the L configuration, and over time the amount of D configuration amino acid increased (Hare and Abelson 1967). The AAR method of dating has been used and developed over 35 years, but is still not widely adopted and is subject to vigorous debate (Johnson and Miller 1997; Hare *et al* 1997; Rutter and Blackwell 1995; Murray-Wallace 1993).

In comparison with direct and indirect radiogenic based methods (such as ^{14}C and luminescence), which operate at the atomic scale, racemization is a molecular-scale process and the rate is influenced by both intrinsic and extrinsic factors. Intrinsic factors include:
- the side chain group
- the ease of hydrolysis of particular peptide pairs
- the ability and ease of undergoing secondary reactions (eg formation of succinimide / diketopiperazine / lactam).

Extrinsic factors include (Rutter and Blackwell 1995):
- temperature
- pressure
- solvent
- catalytic effects
- steric effects.

We have argued (Collins and Riley 2000) that if an intra-crystalline protein fraction could be isolated, the constant environment within the biomineral would mean that the only confounding variables would be temperature and protein sequence (and the latter may be sufficiently conservative not to matter). Using the intra-crystalline fraction would, we argued, enhance the consistency within measurements taken from any one species at any one site.

2.3 Species effect

The rate of racemization is found to differ between genera in foraminifera and molluscs (King and Hare 1972; Miller and Hare 1975; King and Neville 1977; Wehmiller 1980; Miller and Andrews 1983). In molluscs, significant differences in the racemization rates of most of the amino acids occur at and above the taxonomic level of family, and are also sometimes seen between sub-families (LaJoie *et al* 1980a; Kimber and Milnes 1984). Andrews *et al* (1985) used the differences in the abundance of amino acids within the shells of 14 genera of both Bivalvia and Gastropoda to distinguish them. It has been suggested that the species effect is a reflection of the different bonding strengths of the amino acids in specific proteins within the shell, or differences in the stability of the proteins in the crystalline matrices (Kimber and Griffin 1987; Miller and Brigham-Grette 1989).

This species effect limits the use of amino acid racemization for geochronological purposes to studies of particular species. Samples can only be directly compared to other samples of the same species, so an aminostratigraphic framework must be developed for each species studied. It was hoped that the isolation of the intra-crystalline fraction would reduce the species effect. Sadly, this does not appear to be the case (*see* Section 3.2).

2.4 Aminostratigraphy of the UK Quaternary

The rate of racemization is temperature dependent; therefore in order to directly compare values between sites, they must have experienced similar temperature history. With careful sampling and analysis it is possible to take account of regional temperature differences, and 'aminozones' have been correlated for thousands of kilometres by calibrating for differences in latitude, diagenetic or modern mean annual temperature (Clark *et al* 1989; Hearty 1986; Hearty *et al* 1986; Kennedy *et al* 1982; Miller and Mangerud 1985; Murray-Wallace *et al* 1988b). In this respect Southern Britain is an attractive area to study as regional variation within this area is assumed to be small.

The first use of racemization geochronology on terrestrial molluscs in the United Kingdom was by Miller *et al* (1979), who used aminostratigraphy to date Pleistocene terrestrial sites in England. Early methods of chemical separation, using Ion-Exchange liquid chromatography, were able to separate the enantiomers of one amino acid found in proteins, L-isoleucine (L-Ile, I), from its most stable diastereoisomer alloisoleucine (D-aile, A), and it is this amino acid pair that has been used in all previous studies within the UK. Andrews *et al* (1979) used isoleucine in limpets and bivalves in England and Wales to build up an aminostratigraphy for marine terraces.

In 1989, Bowen *et al* proposed an aminostratigraphy of the UK, which correlated terrestrial deposits with the detailed climatic records being revealed by analysis of oxygen isotopes in deep marine cores (leading to the recognition of the Marine oxygen Isotope Stages, MIS). These aminozones have been further refined in subsequent studies and an example is given in Table 2.1.

Table 2.1: Aminostratigraphy of valley systems in southern Britain.
Bold: Amino zone type localities and lithostratigraphic units with D-Aile/ L-Ile ratios characteristic of these amino zones (Table 18-2 of Bowen 2000)

MIS	δ¹⁸O age (ka) min	max	A/I 1999	SD	n	aminozone	Severn Valley & Bristol area	Avon Valley	Lower Thames	Eastern England valleys
5e	114	127	0.10	0.01	11	**Trafalgar Square**		New Inn Bed	Trafalgar Square Member	Tattershall Bed; Ravenstone Member; Bobbitshole Formation
6	127	186					Ridgacre Formation; Kidderminster Member	Cropthorne Member	Mucking & Taplow Gravel	Stoke Goldington Member; Thorpe Member
7	186	242	0.17	0.01	14	**Strensham**	Strensham Member	Ailstone Bed	Aveley, Crayford,; Ilford: Uphall	Stutton Formation; Kirkby Red; Hartigan's Pit Member
8	242	301					Bushley Green Member	Pershore Member	Corbets Tey & Basal; Mucking Gravel	
9	301	312	0.26	0.02	9	**Hoxne**	Hill House Bed	Frog Member	Ilford: Cauliflower; Purfleet; Belhus Park	Hoxne Formation; Woodston Reds; March Formation
10	312	364					Spring Hill Member		Orsett Heath & basal; Corbets Tey gravel	
11	364	426	0.30	0.02	34	Swanscombe			Swanscombe, Ingress; Vale & Little Thurrock; & Sugworth	Barnham & Elveden Formations; West Stow Formation; Nar Member
12	427	474					Wootridge Member	Wolston Formation	Orsett Heath, basal gravel; & Hornchurch till	
13	474	528	0.35	0.01	9	West Runton				
15	568	621	0.38	0.03	15	**Waverley Wood**	**Yew Tree Formation**	**Waverley Wood Member**		
16	621	659					Kenn Formation		Northern Drift	

Whilst many of the amino acid ratios have been supported by other lines of evidence, at some sites, notably those that are believed to be older than MIS 7, the ratios have proved problematic. The ratios obtained on the thick-shelled *Corbicula* spp. in particular have not been in concordance with either other amino acid ratios obtained on other species from the same horizon, or with the age estimates obtained by other geomorphologic or stratigraphic information. For example, Miller *et al* (1979) and Bowen *et al* (1989) both obtained A/I ratios from Purfleet that were inconsistent with the stratigraphical and geomorphological position of the deposits (Bridgland 1994; Schreve *et al* 2002).

Similar controversy surrounds the dating of early hominin sites (eg Boxgrove and Hoxne). For example, at Boxgrove shells from the layer beneath the hominin tibia gave a date estimation consistent with OSL dates (E Rhodes pers comm), but 100ka younger than estimates derived from the mammalian biostratigraphy and geomorphology (Bowen and Sykes 1994; Roberts *et al* 1994; Roberts and Parfitt 1997).

In a recent paper, McCarroll (2002) questioned the ability of amino acid racemization to identify isotope stages based on data from studies of marine terraces, from which published data on individual AAR ratios were available (Bowen *et al* 1985) and concluded that the techniques needs to be applied with due regard to the natural variability of amino acid ratios. 'All the measured ratios should be presented, not just those considered to represent *in situ* material' (McCarroll 2002, 651). He argued that 'The [amino]stratigraphical framework presented for the British Pleistocene may overestimate both the precision and the accuracy of the technique and needs to be applied with caution' (McCarroll 2002, 651).

2.5 Closed vs open systems

The higher variability within an open system (such as mollusc shells) arises because protein decomposition and amino acid racemization rates can be influenced by external factors, such as environmental conditions. Furthermore, as the proteins (predominantly a gel-like silk) break down, small peptide fragments and free amino acids may leach out of the shell. These molecules tend to be the most highly racemized, and so their loss artificially depresses the D/L value measured. The possible loss of original amino acids from the shell and the presence of other amino acids from an external source, at unknown points in time during its burial history, make the rate of the racemization, and hence the age of the sample, incalculable. The consistent results obtained from ostrich shells (Miller *et al* 2000) are believed to arise because this is a closed system, and as a consequence, neither external influences, nor the rate of leaching will affect the rate of the reaction.

The significance of a reduction in the natural variability can be seen if estimates of the increase in IcPD (broadly equivalent to A/I) with age are plotted (Fig 5.12). The difficulty of discriminating beyond MIS 7 is partly due to the smaller differences between isotope stages before this time. Further confounding problems in older samples will include diagenetic alteration or dissolution. Dissolution will act preferentially on aragonite rather than calcite as the former is thermodynamically more unstable under burial conditions.

2.6 A closed system from bleached shell powders

We have used bleach to treat shell powders in an attempt to isolate a closed system, originally proposed by Sykes *et al* (1995) and further extended (Penkman 2005; Penkman *et al* 2008). This closed system appears to give significant benefits over conventional AAR analysis. However, bleaching leads to a drop of up to 90% in amino acid content and therefore this method has only proved possible with the advent of high sensitivity chiral analysis.

3. PROJECT RATIONALE

3.1 An improved chronology of sites of aggregate extraction

The decision to initially focus on opercula arose from promising preliminary data at Purfleet and Hoxne, in which opercula solved a problem of a large spread in the shell data, probably due to the extent of diagenetic alteration of the aragonitic shells. Despite the refinements of using multiple amino acids and the Intra-crystalline Protein Degradation (IcPD) model, the intra-crystalline system within gastropod shells appeared to fail in samples older than MIS 9 (Penkman 2005; Penkman *et al* 2007a).

Therefore this project set out to:
- develop IcPD models for *Bithynia* opercula using D/L values from the principal amino acids, along with serine and threonine decomposition rates.
- improve the age estimates of previous ALSF projects and other Quaternary sediments using the refined IcPD model, correlating results with other geochronology where possible.
- promote a wider awareness of the IcPD approach to both the archaeological community as well as the wider aggregates industry.
- aid in the interpretation and management of aggregate extraction from terrestrial, and ultimately marine, environments.

3.2 Gastropod results

We have conducted in excess of 5,000 analyses of extracts of modern and fossil shells. These have included analyses for two ALSF-funded AAR studies of molluscs from Lynford and a range of sites on the Sussex-Hampshire Coastal Corridor.

The majority of the research has been conducted upon three species, two freshwater gastropods, *Bithynia tentaculata* and *Valvata piscinalis*, and the freshwater bivalve *Corbicula fluminalis*. We have observed an increase in reproducibility following bleaching, consistent with the view that analysis of a closed system improves the reliability of AAR. In the case of shells, despite the fact that the IcPD value is not entirely equivalent to A/I values used by Bowen *et al* (1989), the ordering of sites is very similar. However, even when the IcPD approach is used on bleached shells, problematic sites, such as Purfleet, remain problematic (Penkman 2005).

When we investigated these data, we identified a number of problems:

1. Inter-species variability. Comparing two prosobranch gastropods, *Valvata* and *Bithynia*, reveals that the intra-crystalline fraction retains a species effect (Fig 3.1). The intra-crystalline proteins extracted from the two different species are sufficiently different to alter the rates of protein breakdown. This finding is *contra* Collins and Riley (2000) who expressed the hope that the acidic protein entrapped within crystallites would be more highly conserved and therefore would not display such a large species effect. We conclude that it is necessary to develop aminostratigraphic frameworks using a single species.

2. There is a tendency for the range of IcPD values of gastropods to increase with age of the site. It is possible that the mineral phase of the biomineral is increasingly altered in older samples, leading to the variable loss of degraded peptides and free amino acids from these samples. Therefore, despite the preparation of an intra-crystalline fraction, a reliable closed system has not been isolated in material > 300ka.
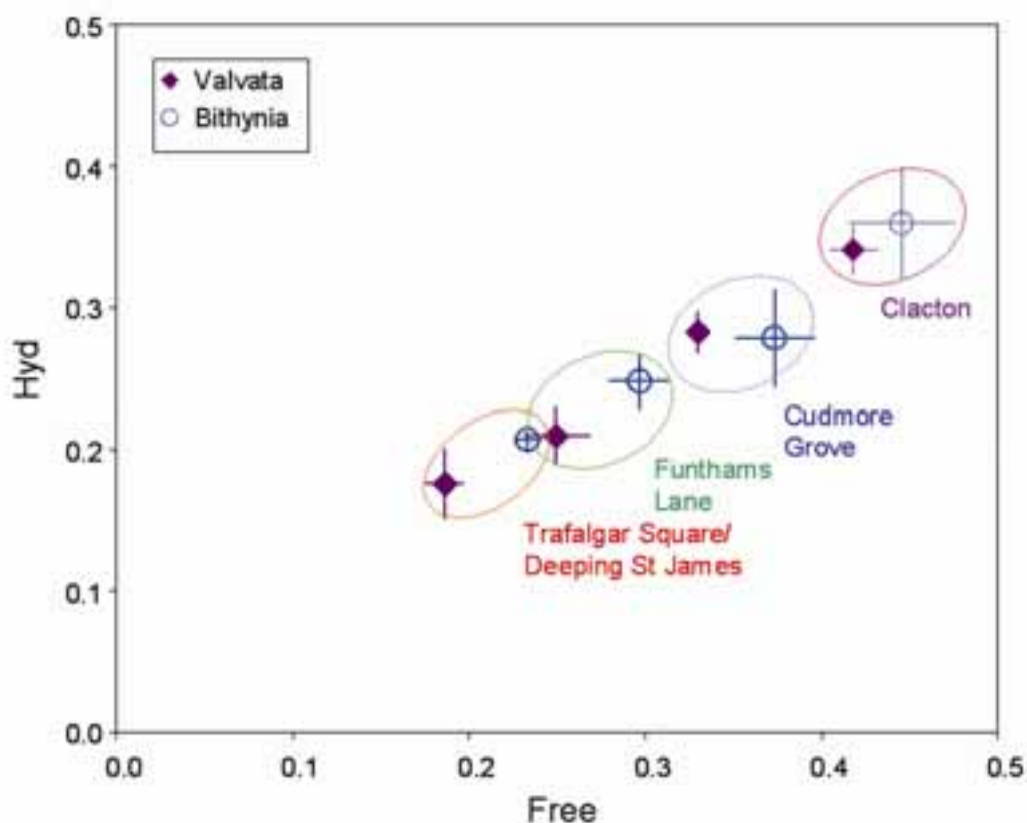
Figure 3.1: Plot of IcPD hydrolyzed vs IcPD free mean values, with 1 standard deviation, for shells of Bithynia tentaculata *and* Valvata piscinalis
Notice that for an individual species, there is good separation of the sites (putatively correlated with MIS stages 5e, 7, 9, and 11), but the rates of protein breakdown are different between the species, making them not directly comparable.

Calcium carbonate commonly exists in two forms, calcite and aragonite. The crystal form of gastropod shells is aragonite, a polymorph of calcite with a rhombohedral crystal structure. Unlike in calcite, the carbonate ions do not lie in a single plane pointing in the same direction, but in two planes that point in opposite directions, destroying the trigonal symmetry of the calcite crystal. The metastable aragonite is converted to the more stable calcite form by inversion, dissolution, and reprecipitation or replacement (Land 1967). Diagenetic changes in the mineral phase could have implications for the preservation of the intra-crystalline fraction within the mollusc shell. The calcitic structure is less susceptible to diagenetic mineral alteration, therefore it is likely that the results obtained from calcitic biominerals will be more consistent over longer time spans than those obtained from aragonitic minerals.

In order to estimate the extent of the problem, the natural variability of D/L values in individual bleached *Bithynia tentaculata* and *Valvata piscinalis* shells from a single horizon at Funthams Lane, were compared. Whilst the bleached intra-crystalline amino acid fraction was found to be significantly more consistent than unbleached shells (Penkman 2005; Penkman *et al* 2008), variation in the relative degrees of racemization of different amino acids were observed, both between the two species and within shells of the same species from the site (Fig 3.2). This means that whilst assignment to MIS 7 was possible, with no evidence of reworking, the position within the stage could not be determined.
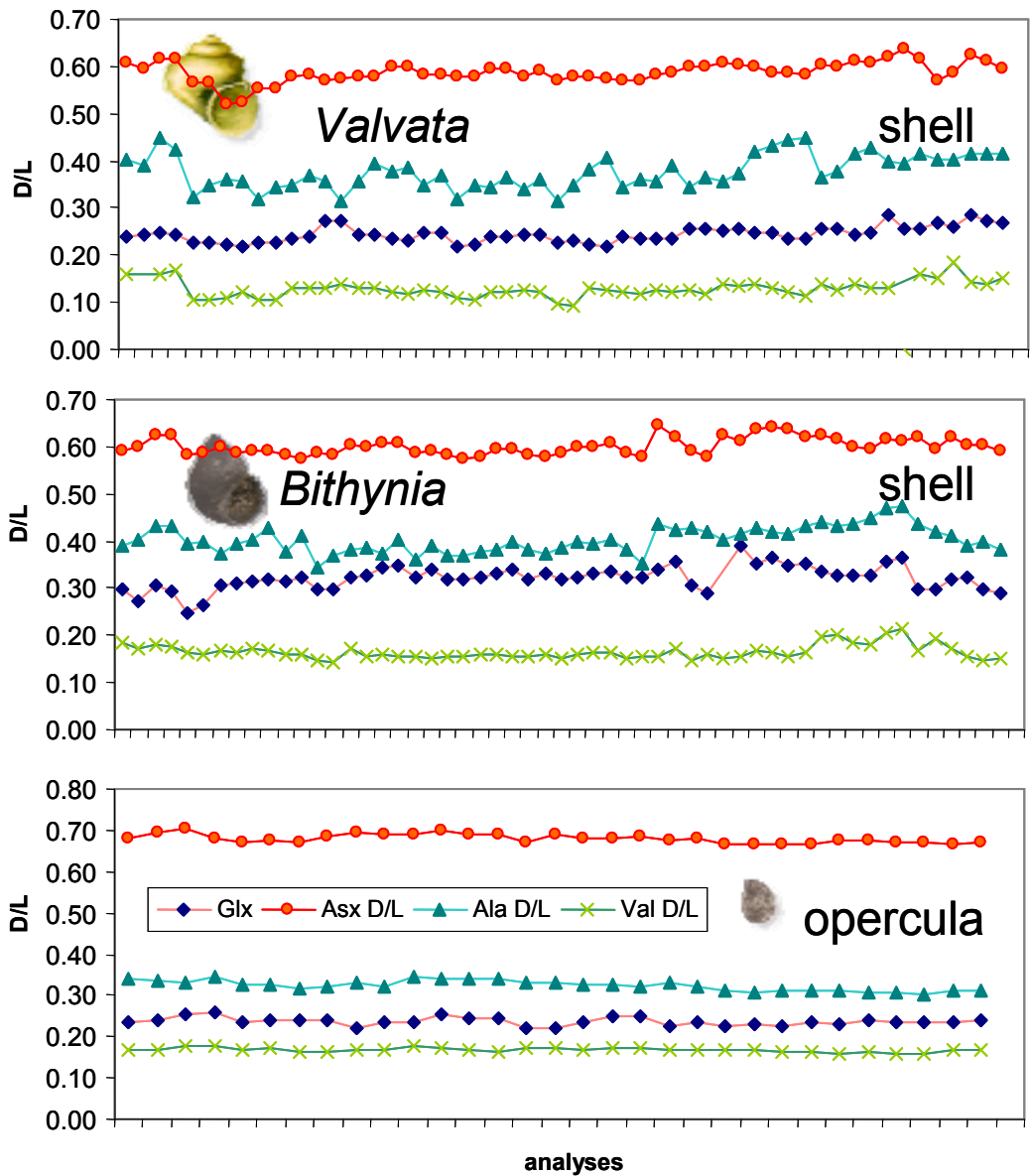
Figure 3.2: Comparison of D/L values from free amino acids in bleached powders of Valvata *and*
Bithynia *shells (above) and* Bithynia *opercula (below).*
Note the variation in relative rates of amino acids between the three samples (such as the difference
between Glx and Ala) and the much greater consistency in the measurements obtained from opercula.
Each of the measurements is from a single shell or operculum. Variation in hydrolysed samples was also
smaller for the opercula

To our great surprise and delight, individual (calcitic) opercula of one of the gastropods, *Bithynia*,
present in the same horizon, produced much more consistent data (Fig 3.2, bottom). This result
was obtained between the submission of the pre-proposal and writing the full proposal for this
ALSF project. We therefore switched the focus of the ALSF proposal exclusively to the analysis
of opercula. In this report, except where discussing pilot data, the study reports values from
opercula. These calcitic biominerals are robust and often found in abundance in Quaternary
sediments. Despite the small size and weight of a single operculum (~ 1mg), it is possible to
conduct both free and hydrolysed analyses.

From our small pilot study, it seemed to be clear that *Bithynia* opercula enabled both better

resolution in younger sites as well as the potential to go much further back in time than possible with gastropod shells. We therefore decided in this project to focus our attention exclusively on this material.

4. MATERIALS AND METHODS

4.1 Sample collection

A full list of mollusc samples is given in Table 4.1. All material was collected and identified by David Keen, Richard Preece, and Kirsty Penkman. Samples were mainly collected from unheated sediments. Previously sieved material is largely unusable as this may have been oven dried at unknown temperatures for unknown amounts of time. Racemization is accelerated by heating, and oven drying will compromise analyses. Care was therefore taken to only sample collections that were known not to have been heated (but see the Bobbitshole results, *below*).
Bithynia has a distinctive operculum (Fig 4.1). This calcite operculum is more stable than the aragonitic shell and tends to be better preserved, often occurring in very large numbers by selective winnowing. There is a danger, due to the robust nature of the opercula, that some samples may be reworked from older beds. Therefore we analysed as many samples as possible from each site, to determine if there is a bimodal distribution of AAR values. Remarkably, there is no evidence of reworking in this study (eg Fig 3.2).

Table 4.1: List of samples and sites (see Fig 4.2)

Genus	species	material	site	n	specific location
Bithynia	*tentaculata*	operculum	Acle, modern	1	1995 (modern)
Bithynia	*leachii*	operculum	Acle, modern	3	TG4/2/01, modern
Bithynia	*tentaculata*	operculum	Aveley	4	Purfleet Rd, adj pumping station, 0.25–0.33m
Bithynia	*tentaculata*	operculum	Barling	4	
Bithynia	*tentaculata*	operculum	Barnham	8	BEF 92, Sample 91, distorted
Bithynia	*troschelii*	operculum	Barnham	4	BEF92, Area II Spit 6 (82)
Bithynia	*tentaculata*	operculum	Barnham	6	BEF93, Pit 4 128–118cm
Bithynia	*tentaculata*	operculum	Beeches Pit	4	Bed 7, Cutting 2 (98 series)
Bithynia	*conica*	operculum	Bembridge Limestone	2	Prospect Quarry, Thorley, Eocene
Bithynia	*tentaculata*	operculum	Bobbitshole	3	Bed 5. 1952, Belstead, Ipswich
Bithynia	*tentaculata*	operculum	Bobbitshole	3	spoil heap
Bithynia	*tentaculata*	operculum	Cassington	4	4B, top 10cm
Bithynia	*tentaculata*	operculum	Clacton	4	Jaywick Beach
Bithynia	*tentaculata*	operculum	Clacton	4	Trafalgar Road, deep piling and bottom levels from freshwater bed
Bithynia	*tentaculata*	operculum	Coston	3	Ip IIb, 15–25cm
Bithynia	*tentaculata*	operculum	Crayford	4	Norris's Pit, 1952
Bithynia	*tentaculata*	operculum	Cropthorne New Inn	4	
Bithynia	*tentaculata*	operculum	Cudmore Grove	4	
Bithynia	*tentaculata*	operculum	Dierden's Pit	4	Ingress Vale
Bithynia	*troschelii*	operculum	Dierden's Pit	2	Ingress Vale
Bithynia	*tentaculata*	operculum	East Mersea	2	Restaurant site, Sample 3
Bithynia	*tentaculata*	operculum	Ebbsfleet	7	ARC 342 WO2 (40070) Sample <40162>
Bithynia	*tentaculata*	operculum	Ebbsfleet	4	EV 1994 A.TP1.b (15)
Bithynia	*tentaculata*	operculum	Eckington	4	D
Bithynia	*tentaculata*	operculum	Elveden	5	ELV 95 (5)–(4)

Bithynia tentaculata	operculum	Elveden	2	ELV 96 Sample 36, 60–70
Bithynia tentaculata	operculum	Enfield Lock	4	3 (10–15cm)
Bithynia tentaculata	operculum	Froghall	4	amino samples
Bithynia tentaculata	operculum	Funthams Lane	2	Danielle's section
Bithynia tentaculata	operculum	Grays	2	from upper premolar of S. kirchbirgensis
Bithynia tentaculata	operculum	Hackney	2	7
Bithynia tentaculata	operculum	Harnham	2	2004
Bithynia tentaculata	operculum	Hoxne	4	Hoxne 2000, Sq1, Spit 5 (40-50) Sample 4
Bithynia tentaculata	operculum	Hoxne	4	64
Bithynia tentaculata	operculum	Hoxne	4	50
Bithynia tentaculata	operculum	Ilford	2	Uphall
Bithynia tentaculata	operculum	Isleworth	4	Middle Devensian
Bithynia tentaculata	operculum	Lion Pit	2	08.07.00
Bithynia tentaculata	operculum	Lion Pit	5	Sample 6, Section 5
Bithynia troschelii	operculum	Pakefield	3	laminated organic channel fill
Bithynia troschelii	operculum	Pakefield	4	Unio Bed, Sample 36
Bithynia tentaculata	operculum	Quidenham Mere	4	2005 core, 250–260cm
Bithynia tentaculata	operculum	Quidenham Mere	3	2005 core, 640–650cm
Bithynia tentaculata	operculum	Shropham	4	
Bithynia tentaculata	operculum	Somersham	4	Sample SBK 3 19/10/90
Bithynia troschelii	operculum	Somersham	4	Sample SBK 3 19/10/90
Bithynia tentaculata	operculum	Stanton Harcourt	4	1021A
Bithynia tentaculata	operculum	Star Carr	2	245–250cm
Bithynia tentaculata	operculum	Star Carr	3	524–528cm
Bithynia tentaculata	operculum	Swanscombe	4	Lower Loam
Bithynia tentaculata	operculum	Tattershall Castle	4	IpIIb, sample (76K)
Bithynia tentaculata	operculum	Trafalgar Square	6	New Zealand House Foundation 1957
Bithynia troschelii	operculum	Waverley Wood	6	Bulk 1
Bithynia troschelii	operculum	West Runton	10	Freshwater Bed D

Figure 4.1: Bithynia tentaculata *with distinctive calcitic operculum, which in life closes the shell aperture © www.bioimages.org.uk*

Figure 4.2: Map showing distribution of key sites mentioned in text

4.2 Sample preparation

Opercula were analysed as two separate fractions for analysis: bleached Free (bF) and bleached Hydrolysed (bH).

Cleaning

Samples are examined under a low-powered microscope and any adhering sediment removed, then sonicated and rinsed several times in HPLC-grade water. The opercula are left to air-dry overnight.

Bleaching

The dried opercula are crushed and 50µl of 12% NaOCl (BDH) added per mg of carbonate. The sample is then shaken, left for 24 hours, reshaken (to ensure that all the sample was in contact with the bleach), and left for a further 24 hours. The NaOCl is then pipetted off, the sample rinsed with H_2O, centrifuged, and rinsed with water again. This is repeated 5 times. Then HPLC-grade methanol (BDH) is added to the samples to be oxidised by any remaining bleach, left for a few minutes, centrifuged, and pipetted off. The bleached samples are then left to dry overnight in air, and the dried opercula split and weighed out accurately into two sterile 3ml vials to form the bF and bH samples.

Demineralization

Free subsamples are demineralised in 2M HCl (Aristar), 10μl per mg of CaCO3, and dried overnight in a centrifugal evaporator.

Hydrolysis

The Hydrolysis samples (bH) have 20μl 7M HCl added per mg of sample. The vials are then flushed with nitrogen and placed in an oven at 110°C for 24 hours. The caps are retightened after ten minutes. Once taken out of the oven, the samples are then dried under vacuum in a centrifugal evaporator.

Rehydration

For analysis on the RP-HPLC, the samples were originally rehydrated with 20μl rehydration fluid, which was then diluted down if necessary. The rehydration fluid contains a non-protein amino acid, L-homo-arginine, at a concentration of 0.01mM, which elutes with base-line separation approximately 50 minutes into the run time. This is used as an internal standard to quantify the concentrations of the amino acids in the sample. L-homo-arginine is similar to the compounds of interest, but does not occur naturally in the sample, co-elute with any other amino acid, and is stable, so it is an excellent internal spike to use.

4.3 Sample analysis

Reversed Phase HPLC

The analysis of the amino acids is performed by Reverse Phase High Pressure Liquid Chromatography, adapted from Kaufman and Manley (1998) using an Agilent 1100 HPLC. The technique enables multiple amino acids to be measured, giving multiple D/L values. Nine pairs of amino acids are routinely analysed with a base-line resolution in 90 minutes. This means that the history of the sample is no longer reliant on just one of the amino acids but on a suite (and has lead to the development of the IcPD). The way in which the amino acids vary with respect to one another is predictable and so any abnormal behaviour in the system can be picked up. It also enables the identification of bacterial contamination, due to the characteristic signature of high concentrations of D-Ala for example. By optimising the pre-column derivatisation the method has compound detectability in the picomole range. This increased sensitivity allows the analysis of smaller concentrations than conventional HPLC, which enables very small sample sizes to be analysed.

Derivatisation

Samples are derivatised using 2.2μl per 2μl of sample solution of OPA/IBLC (260mM n-Iso-L-butyryl L-cysteine (IBLC), 170mM o-phthaldialdehyde (OPA) in 1M potassium borate buffer, adjusted to pH 10.4 with potassium hydroxide pellets).

Separation

Samples are separated on a 250 × 3mm, 5μm Hypersil BDS (Base Deactivated Silica) (Batch # 5/120/4772) containing aliphatic octadecyl groups (C18) bound to a 300Å pore diameter silica gel at 25°C using a gradient elution of three solvents: sodium acetate buffer (solvent A; 23mM sodium acetate tri-hydrate, 1.5mM sodium azide, 1.3μM EDTA, adjusted to pH 6.00 ±0.01 with 10% acetic acid and sodium hydroxide), methanol (solvent C), and acetonitrile (solvent D). The

initial solvent conditions (95% A and 5% C) are maintained isocratically at 0.56ml/min for 35 minutes, then grading to 50% C and 2% D. Prior to the injection of the next sample, the column is then flushed with 95% C and D for 15 minutes, followed by equilibration of 95% A and 5% C for 5 minutes. 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.

Detection

Amino acids were detected at picomole sensitivity using fluorescence detection. The detector uses a Xenon-arc flash lamp at a frequency of 55Hz, with a 280nm cut-off filter, an excitation wavelength of 230nm and emission wavelength of 445nm. The fluorescing derivative absorbs light of 230nm and emits light at 445nm, which is detected by a photomultiplier.

4.4 Sample interpretation

Data handling

Integration is performed automatically using the Chemstation software. The D-amino acid is typically lower than that of the L-amino acids, and so the magnitude of the error is highly dependent on the D-peak obtained. Although the vast majority of peaks are integrated automatically, all traces are examined visually and occasionally the peaks are integrated manually if the automatic integration had appeared to have judged incorrectly. All the raw data are extracted and stored using specialist software developed at NEaar and stored in Excel spreadsheets, backed up each day on the Biology department network.

Intra-crystalline Protein Decomposition (IcPD) estimate

The basic premise of the IcPD model is that (i) racemization analysis can only be predicted in a closed system (ii) that bleaching shell powders provides a closed system, and (iii) amino acid racemization is driven by protein decomposition.

Modelling was conducted using standard Excel and ModelMaker software, used previously on ALSF and other projects. We are reporting output from version 2.0 of the model. However we are working on futher refinements, notably improved resolution of younger samples. Models used the best resolved amino acids pairs (Asx, Glx, alanine (Ala), valine (Val) and serine (Ser); Fig 4.3). In later versions 3.0+1 the ratio of Ser/Ala is also used, but this is not used for version 2.0. The basic approach is to normalise the differing ratios to an amino acid; in the case of version 2.0 of the model this is glutamic acid. These normalised values are averaged and the standard deviation of these values enables us to reveal compromised samples / analyses (Fig 4.4). The closed system futher enables comparison of the IcPD values of the total and free amino acids from a single shell. If the shell is operating as a closed system, these should be highly correlated. Failure to demonstrate correlation between these two IcPD values leads to rejection of the data from the shell as unreliable.

The development of this IcPD approach has been made possible by a series of refinements made

---

[1] The current version of IcPD that the lab is working on, IcPD v3.0, uses loadings and more amino acids, but this again requires further refinement. The following (loadings in brackets) Glx (3) Asx (1–4 depending upon DL ratio) Ala (2) Val (2) Phe (1) [Ser] (1, 2 for free) [Thr] (1, 2 for free) are used in IcPD v3.0, but v 3.1 begins to scale these for the extent of protein degradation. We have used the simple v 2.0 for reporting in the current study.

within this study, enabling a greatly increased level of resolution, accuracy and precision. These include:

Improved analyses

Recent advances in analytical methods (i) improve sensitivity (ii) increase the number of amino acids separated , and (iii) use automated chemical treatment to improve reproducibility: all enabling a far more efficient throughput of samples, each of which is much smaller (eg ~ 1mg) than was previously possible.
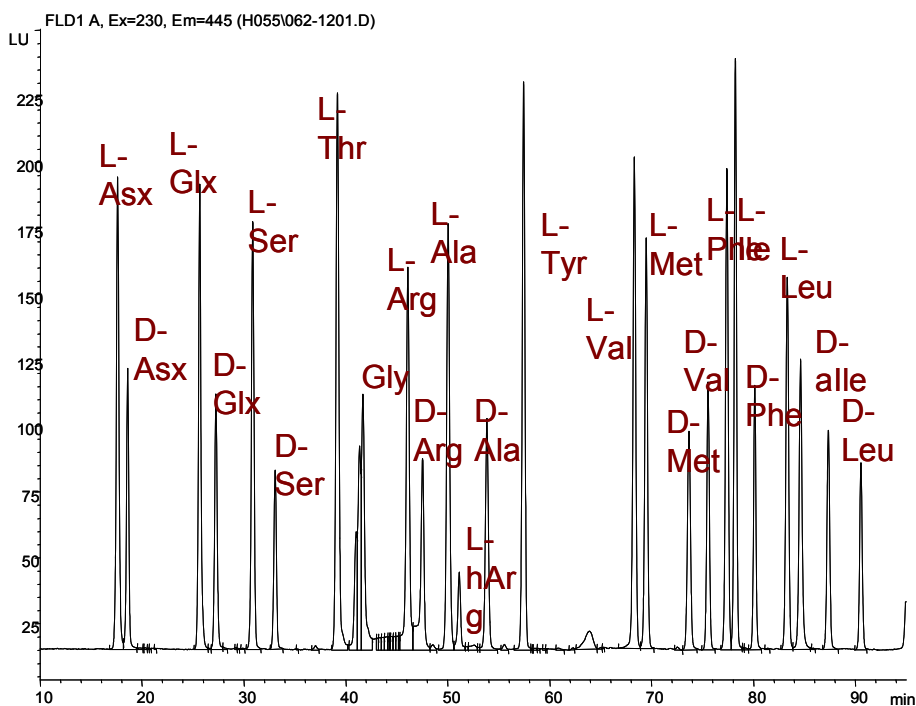
Figure 4.3: Separation of chiral amino acids by rHPLC
(using a modified method of Kaufman and Manley 1998)

Isolation of the intra-crystalline fraction

The isolation of the intra-crystalline fraction yields more reproducible data, firstly by removing both potential secondary contamination and the organic matrix of the shell (that separates the carbonate crystallites); this matrix degrades and leaches over time. Secondly, closed system behaviour is theoretically predictable, enabling us to develop a number of quality control criteria based upon expected patterns of D/L values of different amino acids and the patterns of D/L values of the free and total amino acids. The free amino acids have broken free of the peptide chain over time and can be analysed directly. The remaining amino acids are released by strong acid treatment, yielding a second (total/ hydrolysed) fraction.

Improved error detection (part 1)

The ability to obtain multiple D/L values enables comparison of these different ratios with predicted patterns developed from a combination of laboratory heating experiments and modelling of protein decomposition. Our approach (the Intra-crystalline Protein Degradation model, IcPD) provides a single value (with error) which predicts the overall state of racemization in each fraction. This enables us to reveal compromised samples / analyses (Fig 4.4).
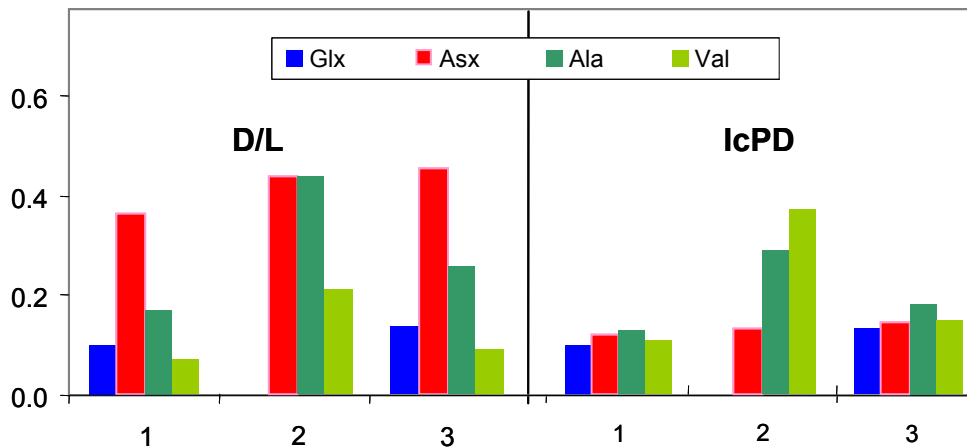
Figure 4.4: Comparison of D/L and IcPD values for different shells to illustrate the IcPD concept. Each D/L value is corrected to a Glx equivalent (right panel). The average and standard deviation of these IcPD values is reported as the IcPD value for the (in this case total) value of the shell. Shells 2 and 3 are rejected because the standard deviation of the IcPD values is > 15%

Improved error detection (part 2)

The closed system enables comparison of the IcPD values of the total and free amino acids from a single shell. If the shell is operating as a closed system, these should be highly correlated (Penkman 2005; Preece and Penkman, 2005; Penkman *et al* 2008). Failure to demonstrate correlation between these two IcPD values leads to rejection of the data from the shell as unreliable.

5. RESULTS AND DISCUSSION

All the sites were analysed blind with no prior knowledge of their age or stratigraphy. The Hyd vs Free IcPD graphs (Fig 5.1) show a strong positive correlation, enabling the sites to be presented graphically in relative stratigraphic order, falling along a line with older samples having higher values of IcPD (Fig 5.2). Samples that fall off the line are likely to be compromised, and no age estimation can be made from their protein decomposition. These samples, circled in red (Fig 5.1), show non-concordance with the expected Free: Hyd relationship and so are rejected from the dataset. Only two samples from over 200 were rejected, a success rate of 99%. Compromised samples are discussed in Section 5.5.

5.1 Thames comparison

Debate over the existence of two or more interglacials since the Anglian has been widespread, and research has focused on the Thames fluvial deposits in an attempt to solve this question. Gibbard (1985; 1994) proposed that only two interglacials were represented in the Thames terraces after the Anglian diversion: the Hoxnian (correlated with MIS 11), such as at the site at Swanscombe, and the Ipswichian (correlated with MIS 5e) represented at the Trafalgar Square deposits. Bridgland (1994), on the other hand, identified four interglacials in the same post-diversion sequence.

It is argued that the terrace deposits are direct records of climate change, with sequences of incision and aggradation leading to the formation of the terraces and then their preservation in the stratigraphical sequence due to uplift (Maddy 1997). In the Lower Thames four terrace sequences, with the interglacials corresponding to MIS 11, 9, 7, and 5e, have been proposed on

the basis of the lithostratigraphic sequence of the terrace sediments (Fig 5.3) (Bridgland 1994). This conclusion is supported to some extent by the original amino acid A/I data and by molluscan and vertebrate biostratigraphy (Preece 1995; Schreve 1997; 2001). The molluscan fossils within the terrace interglacial deposits allow the testing of the multiple-interglacial theory by amino acid geochronology, and correlations to be made with the bio- and litho-stratigraphical studies. The Thames terrace sequence therefore provides an ideal testing ground for the development of the new amino acid approach.
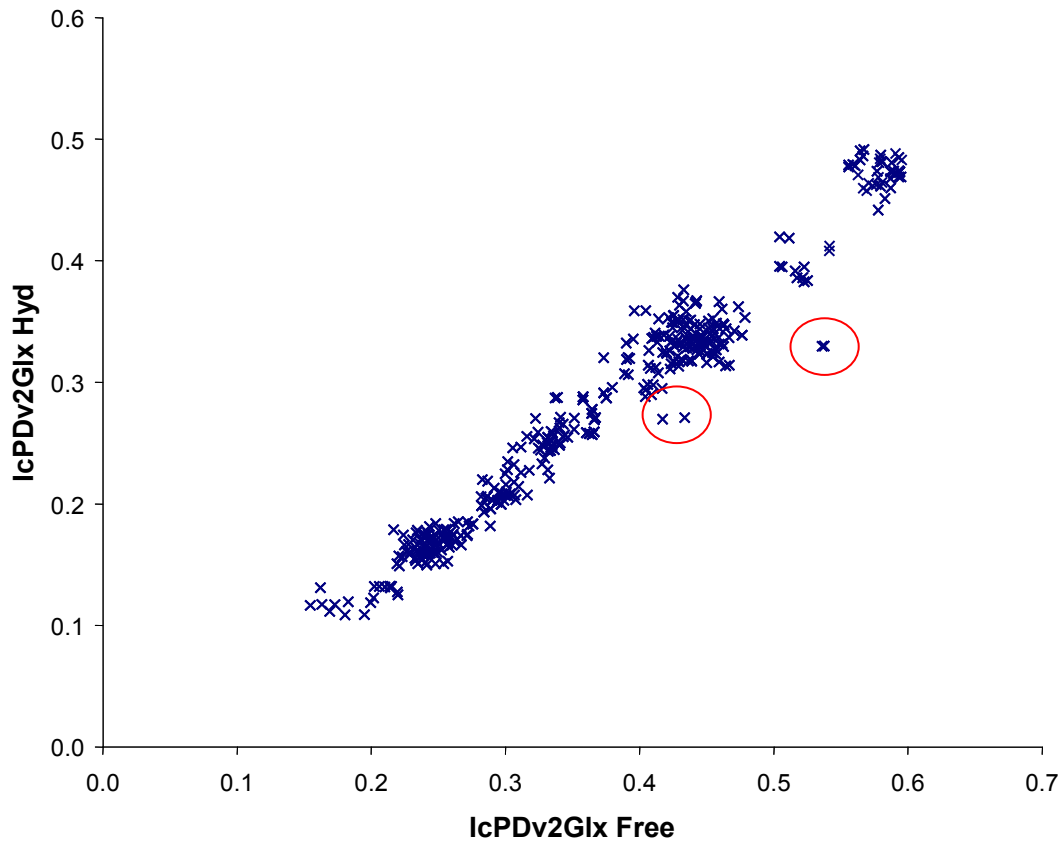
Figure 5.1: IcPD Hydrolysed vs IcPD Free for individual opercula samples
The strong positive correlation observed in the Hyd vs Free IcPD graphs and the clustering of the datapoints indicate that the extent of protein degradation can be correlated with the age of the sites. This enables the construction of an aminostratigraphic framework for the opercula, with the mean and standard deviation of the IcPD results from the sites in question reported. It is therefore important to interpret the data in conjunction with the Hyd vs Free IcPD graphs shown in Figure 5.2.

If only two interglacials are present in the post-diversion Thames record, then the amino acid data would cluster into two groups, representing the Hoxnian and Ipswichian deposits. However, the data from the Upper and Lower Thames cluster into at least five discrete groups (Fig 5.4). The samples from the Upper Thames site at Cassington cluster together and lie at D/L values lower than that of Trafalgar Square, correlated with the Ipswichian and therefore MIS 5e. Above Trafalgar Square, but separated from it, lie the D/L values of Aveley and the Lion Pit Tramway Cutting. The terrace model of the Lower Thames places the interglacial deposits at Aveley and Lion Pit within the Mucking Formation, the third of four terraces within the Post-Anglian succession (Bridgland 1994) and is therefore correlated with MIS 7. Above this cluster lie the sites of Grays and Purfleet, separable from the Aveley cluster. Above these datapoints lies the amino acid data from the Lower Thames site of Swanscombe.
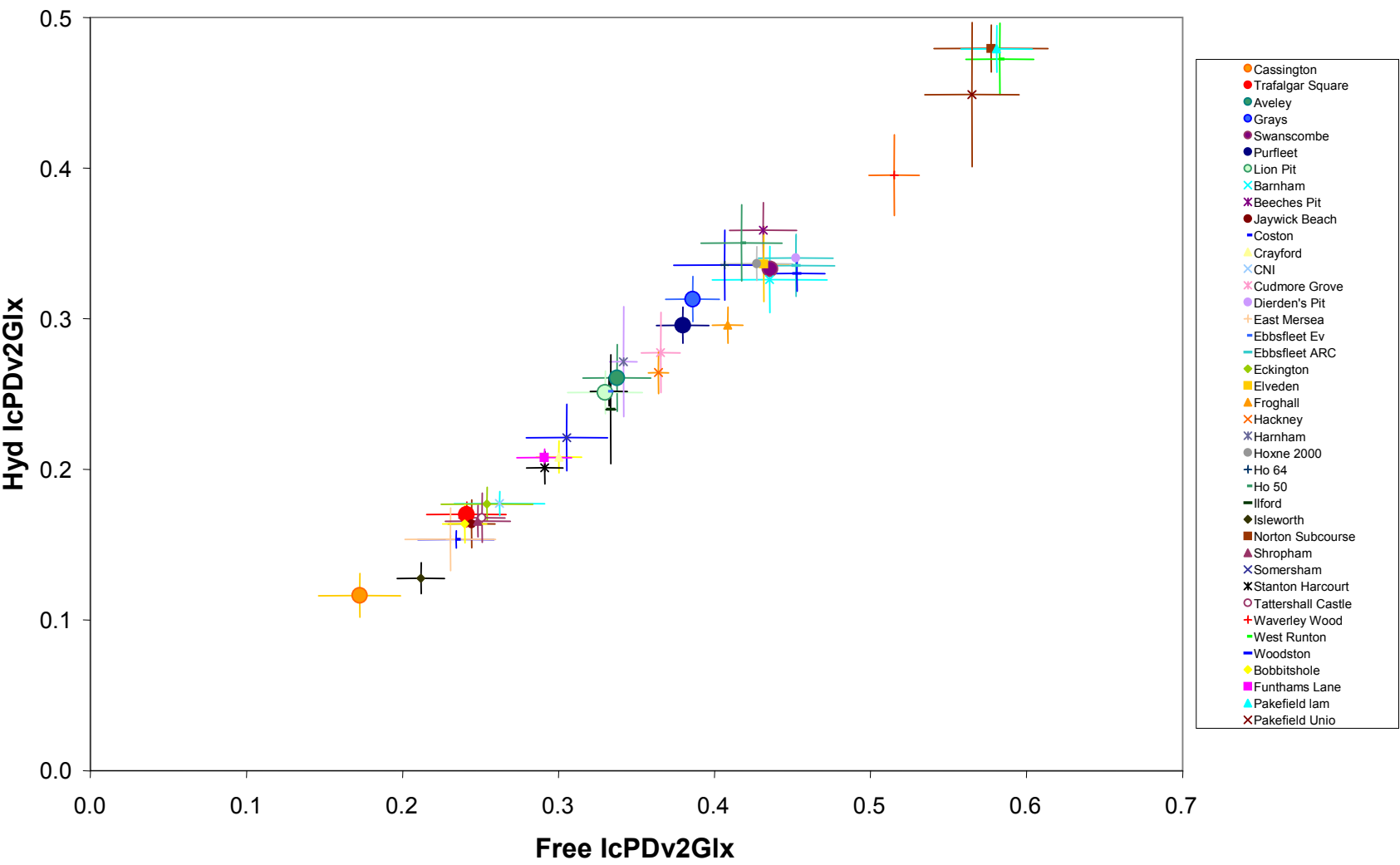
Figure 5.2: IcPD Hyd vs IcPD Free, site by site. Error bars are two standard deviations about the mean of multiple samples. The Thames sites are shown as filled circles, and form the basis of the correlation to marine oxygen isotope stages (*see* Section 5.1)
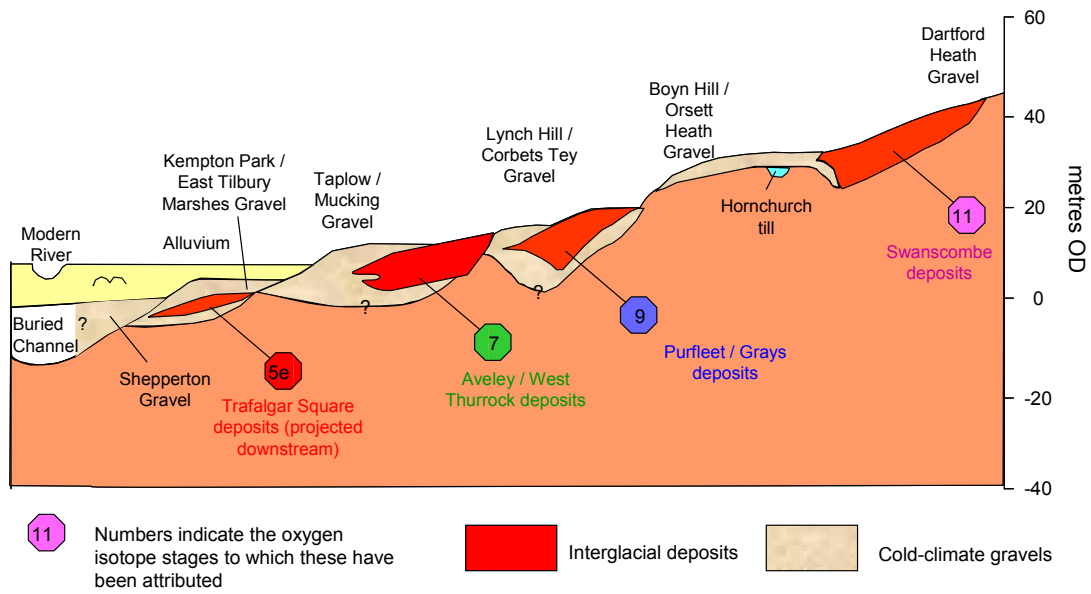
Figure 5.3: Lower Thames terrace staircase (after Bridgland 1994)
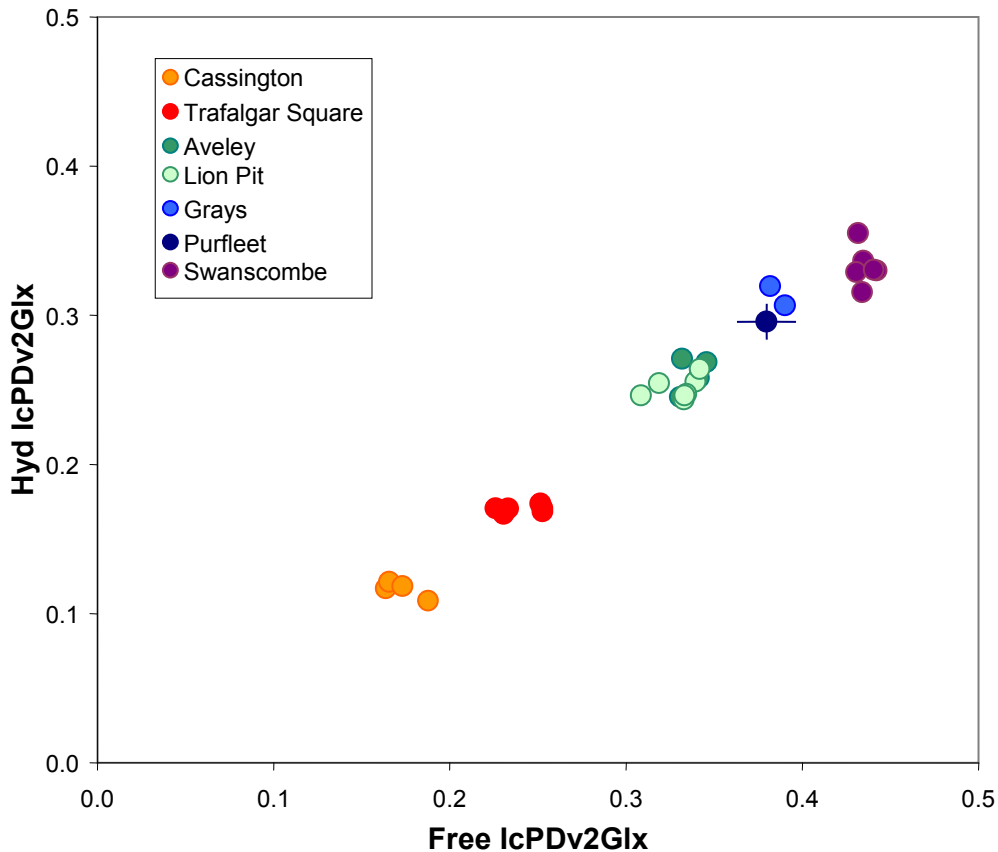
Figure 5.4: Hyd IcPD vs Free IcPD for the Thames aminostratigraphic sequence
Compare with Figure 5.3. Each point represents the overall extent of intra-crystalline protein decomposition from an individual *Bithynia tentaculata* opercula sample. The data from Purfleet were obtained on 23 individual *Bithynia tentaculata* opercula, analysed as part of a NERC-funded project, and therefore the mean data are presented here for comparison, with error bars representing two standard deviations

The racemization data support both the stratigraphic position of the terraces and their relationship determined by the biostratigraphy of their faunal differences. Regardless of whether these separated clusters represent complete isotope stages, substages, or a smaller timespan, they can clearly be discriminated from each other. The predictions of the temperature model combined with the geomorphological information and faunal data all indicate that the Cassington and Trafalgar Square deposits represent substages within MIS 5, 5a, and 5e respectively, whilst the Aveley cluster correlates with MIS 7, Purfleet with MIS 9, and Swanscombe with MIS 11. Therefore these data supports a multiple-interglacial scenario for the Thames Terraces, rather than the two-interglacial model proposed by Gibbard (1994).

This initial test of the applicability of this new technique indicates that it is possible to discriminate between these Thames sites, proposed to have been deposited in different interglacials by terrace- and bio-stratigraphy. Further analyses were then undertaken on a range of sites from the UK, the details of which are reported below. The amino acid data for each site are compared to the aminostratigraphic framework developed for the Thames Terraces.

5.2 Details of sites

Aveley (NGR TQ 552808)

The Aveley Member stratotype is at Sandy Lane Quarry, now infilled. The sediments, consisting of gravel, sand, silt and detritus mud, rest on the bedrock or on earlier Pleistocene sediments, and include important palaeontology (Hollin 1977; Gibbard 1994; Bridgland 1994). The deposits occur as discontinuous spreads, correlated by A/I of 0.148 ±0.016 (7) with West Thurrock, Crayford (0.17 ±0.02 (12)), Ilford (Uphall) and with MIS 7 (Bowen *et al* 1989; Bridgland 1994). The pollen biostratigraphy led Gibbard (1994) to correlate the site with MIS 5e.

The deposits at Aveley were originally assigned to the Ipswichian (West 1969), but their situation below a higher terrace level than that of the Trafalgar Square sediments, and the widely different mammalian assemblages between the deposits, led Sutcliffe (1975) to suggest their assignment to a different interglacial. The absence of hippopotamus and the presence of horse and woolly mammoth (*Mammuthus primigenius*) in pollen zone Ip IIb, the converse of factors that are usually diagnostic of correlation with the Ipswichian (Stuart 1976) suggested a correlation with an alternative interglacial for the site. Along with the differences in deposit heights, the differences in mammalian faunas led to the identification of a post-Hoxnian, pre-Ipswichian temperate interval (Sutcliffe 1975).

The terrace succession model of Bridgland (1994, but see Gibbard 1994 for an alternative view) places the interglacial sediments within the Mucking Formation, the third of four terraces of the Lower Thames thought to post-date the Anglian. The molluscan biostratigraphy (Preece 1995; Keen 2001) and coeleopteran biostratigraphy (Coope 2001) also support the allocation to MIS 7 (Shotton 1983). Bowen *et al* (1989) obtained A/I of 0.148 ±0.016 (7) from *Bithynia tentaculata* and 0.172 from Lymnaea, indicative of a correlation with MIS 7. A dramatic change in the mammalian fauna in the sequence, with two assemblage zones identified of warm stage fauna, has been proposed to indicate an intervening period of cooler conditions, leading to a reconnection to the European mainland due to a drop in sea level (Schreve 2001b). Three warm peaks within MIS 7, designated 7a, c, and e, are observed in the SPECMAP curve (Imbrie *et al* 1984), with the deepest trough, 7d, separating the first and second peaks, 7e and 7c. However, there is no direct evidence for cooling at this site, although various hiatuses are present.

The amino acid samples were all taken from the shelly base of the sequence under the same terrace, with at least 5m of sediment above. The samples should be closely similar in age. The molluscan assemblages from Aveley indicate relatively quiet conditions, with a large number of 'ditch' and occasional 'slum' species (Sparks 1961; Cooper 1972). The fauna is predominantly freshwater, with brackish-water taxa transported by tidal activity (Holyoak 1983). The presence of *Corbicula fluminalis* may also indicate an age older than the Last Interglacial (Ellis 1978; cf Meijer and Preece 2000).

Four *Bithynia tentaculata* opercula were analysed from Aveley, and results are presented in Fig 5.4. The protein from the Aveley opercula is clearly more degraded than that from Trafalgar Square, but not as degraded as that from Purfleet. This is consistent with a correlation with MIS 7, and it is likely that these samples from Aveley were deposited in early-mid MIS 7 (Fig 5.2).

Barling (NGR TQ 935899)

Fossiliferous interglacial deposits were formerly exposed in the floor of an active gravel pit near Barling, Essex, on the northern side of the Thames estuary. These filled a channel cut into London Clay bedrock and were overlain by the Barling Gravel, a Thames-Medway deposit equivalent to the Lynch Hill and Corbets Tey Gravels of the Middle and Lower Thames, respectively (Bridgland *et al* 2001). The channel sediments yielded diverse molluscan and ostracod assemblages, both implying fully interglacial conditions and a slight brackish influence.

The age of the interglacial deposits is inferential. The geological context suggests a late Middle Pleistocene interglacial, part of the post-diversion Thames system and therefore clearly post-Anglian. This conclusion is supported by A/I values on *Corbicula* and *Valvata* but the small number of samples and scatter in the data precludes more detailed correlations. The correlation of the overlying Barling Gravel with the Lynch Hill/Corbets Tey aggradation of the Thames Valley constrains the age of the Barling interglacial to MIS 11 or 9. The presence of *Corbicula fluminalis* and Pisidium clessini confirms a pre-Ipswichian (Marine oxygen Isotope Substage 5e) age and their occurrence in the early part of the interglacial cycle at Barling precludes correlation with MIS 11, as these taxa occur only later in that interglacial at sites such as Swanscombe and Clacton. Thus, by a process of elimination, an MIS 9 date would seem probable (Bridgland *et al* 2001).

Four *Bithynia tentaculata* opercula were analysed from Barling. These samples came from the bulk samples taken from the channel deposits, exposed in 1983 (Bridgland *et al* 2001). The protein is more degraded than that from the MIS 9 sites of Cudmore Grove and Purfleet, but slightly less degraded than that from Swanscombe and significantly less than that from Clacton. This indicates that the opercula from Barling derive from deposits laid down in early MIS 9 or late MIS 11. As the environmental information from the site indicates that it was deposited early in an interglacial, the amino acids point to an age of early in MIS 9.

Barnham (NGR TL 875787)

The East Farm Pit at Barnham lies in a dry valley, with chalk rising to the south forming the watershed with the Lark Valley, and separated from the present Little Ouse River to the north by another low chalk ridge (Ashton *et al* 1998). Incised into the chalk along the axis of the dry valley is a deep (at least 19.5m) channel, which is filled with glaciofluvial sand and gravel and chalky till. The till forms part of the regionally extensive Lowestoft formation, which is believed to have been deposited during the Anglian Stage (MIS 12) (Bowen 1999). The upper surface of the till is also channelled. This feature reaches a maximum depth of 13.5m and is filled with fine-grained sediments. The sediments filling the channel indicate still or slow-flowing water, with mainly laminated silt and clay and a number of persistent bedded sand facies.

The site at Barnham exhibits the same stratigraphy and Lower Palaeolithic archaeology as at Elveden, with a kettle hole in the surface of chalky boulder clay similar to that at Hoxne, West Stow, Hitchin, and Copford. The Anglian cold-stage deposits (MIS 12, Bowen *et al* 1986) lie at the base of the sequence and if no major unconformity is present then the overlying interglacial sediments can be attributed to the warm stage following this glacial (Ashton *et al* 2000). The composition of the mammal fauna indicates a correlation with the Lower Gravel and Lower Loam from Swanscombe, the Woodston Beds, Beeches Pit, and Hitchin. A/I ratios on *Bithynia tentaculata* 0.31 and 0.29, and *Valvata piscinalis* of 0.26 and 0.28 are similar to those from Swanscombe, Clacton and Beeches Pit (Bowen 1998), also indicating a MIS 11 age. However, thermoluminescence dates from burnt flint have yielded younger MIS 9 dates (Ashton *et al* 1998). All the opercula samples analysed in this study should be of the same age, as they are both from the infilled channel, filled up in an interglacial after the Anglian. As the kettle hole filled up there is a slight change in sediments and environment from an aquatic into semi-terrestrial into terrestrial. It is likely that the depression began to fill at the end of the glacial and into the temperate phase. The archaeologically-rich horizons are decalcified, but these channel margins can be related to the thick fossiliferous centre of the channel fill sequence. Freshwater mollusca indicating slow flowing water (Seddon 1998) dominate in the laminated shelly clay, brown-grey clay, and shelly clay.

Four *Bithynia tentaculata* opercula were analysed from Barnham BEF93 Pit 4 128–118cm, eight distorted *Bithynia tentaculata* opercula were analysed from Barnham BEF 92 Sample 91, and four *Bithynia troschelii* opercula were analysed from Barnham BEF92 Area II Spit 6 (82). The comparison between the distorted and pristine opercula is discussed in more detail in Section 5.5, and in Preece and Penkman (2005). The comparison between the *B. tentaculata* and *B. troschelii* data is discussed in Section 5.4.

The protein in the samples from Barnham is more degraded than that from MIS 9 sites such as Cudmore Grove and Purfleet, and at a similar state of breakdown as that from Swanscombe. This supports a correlation with MIS 11.

Beeches Pit (NGR TL 798719)

Beeches Pit is a nineteenth-century brick pit at West Stow, Suffolk that has yielded both warm and cold stage faunas from the Middle Pleistocene. Glacial deposits of till and outwash gravels, believed to be part of the Anglian Lowestoft Formation, fill a subglacial channel cut into chalk. Above the glacial deposits a series of interglacial sediments is found, which contain limnic, tufaceous, and colluvial silts. These sediments have no pollen, but are rich in shells, ostracods, and vertebrate fauna.

Refitting examples of Lower Palaeolithic Acheulian flint artefacts have been found, together with hearths. The tufa contains an extremely rich molluscan fauna dominated by land snails from a woodland environment and is the type locality for *Retinella (Lyrodiscus) skertchlyi*, a land snail that belongs to a sub-genus now restricted to the Canary Islands (Kerney 1976; Preece *et al* 1991). The molluscan and vertebrate faunas suggest correlation with the Hoxnian (MIS 11). Uranium-series dating has yielded ages of ~455ka, while thermoluminescence on burnt flint give a mean range of 414 ±30ka BP (Preece *et al* 2007).

Four *Bithynia tentaculata* opercula were analysed from Beeches Pit, Bed 7, Cutting 2 (98 series). The protein in these samples is more degraded than that from Cudmore Grove and Purfleet, with similar levels of degradation as found at Swanscombe. This supports a correlation with MIS 11.

Bembridge Limestone (NGR SZ 384866)

Two *Bithynia conica* opercula were analysed from the Bembridge Limestone at Prospect Quarry,

Thorley, Isle of Wight. This quarry works a freshwater limestone dating from the Late Eocene (Curry *et al* 1978). The results of these analyses are discussed in Section 5.9.

Bobbitshole (NGR TM 148414)

The freshwater interglacial deposits at Bobbitshole, Ipswich, have been designated as the stratotype for the Ipswichian interglacial, correlated with the continental Eemian (Last) interglacial (West 1957; Mitchell *et al* 1973). The interglacial deposists occupy a lake basin in a valley cut into a chalky boulder clay. The lacustrine sediments, up to c. 3.5m, consist of initially silt, probably of aeolian origin, then clay-mud and finally clay. The interglacial sediments are sealed unconformably by sandy gravel, likely to have been deposited under cold conditions. The succession of pollen zones, indicating the earlier part of an interglacial succession, allowed correlation with the Eemian. The molluscan fauna is reported in Sparks (1957). A/I values of 0.09 ±0.14 (n=4) on *Bithynia* and 0.1 ±0.014 (n=2) on Lymnaea from Beds B & C at Bobbitshole also indicated correlation with MIS 5e (Bowen *et al* 1989).

Three *Bithynia tentaculata* opercula were analysed from Bobbitshole, Bed 5 1952, Belstead, Ipswich, from the Natural History Museum collection. A further three *Bithynia tentaculata* opercula were analysed from a spoil heap (labelled as Bobbitshole, Ipswich). Whilst these samples cannot be related to the published stratigraphy, it is certain that they were recovered *in situ* from the interglacial deposits.

The Bobbitshole data are discussed in detail in Section 5.3. However the data from the second batch of samples, obtained from the spoil heap, showed similar levels of protein decomposition to the opercula from Trafalgar Square. This supports the correlation of Trafalgar Square to the Ipswichian.

Cassington (NGR SP 482 107)

The deposits underlying the Floodplain Terrace (Northmoor member) at Cassington, in the Upper Thames consist of basal, fine-grained lithofacies overlain by coarser gravel lithofacies. These sediments are thought to represent a change in the fluvial environment from a low-energy meandering system to a high-energy braided system, associated with a deteriorating climate signal (Maddy *et al* 1998).

There is a divergence of opinion in the age of the site between a late MIS 5 age or MIS 4, based on mammal biostratigraphy (Currant and Jacobi 2001). The evidence from the section indicates decreasing temperatures during the period when the deposits were laid down. The basal samples are almost interglacial in their warmth, but those in the gravel are much colder. Whether this is the climatic breakdown from 5e, 5c, or 5a, or some part of 4 is difficult to prove from palaeontology in the current state of knowledge. OSL analyses resulted in dates of between 80–138ka (Maddy *et al* 1998).

The shells obtained came from the main fossil horizon, in the temperate zone. A/I analyses were originally undertaken on 6 *Bithynia tentaculata* and 5 *Valvata piscinalis* from association B (sample M1), base of sediments were analysed using method of Bowen *et al* 1985 (Maddy *et al* 1998). *Bithynia tentaculata* was found to have a mean A/I of 0.081, σ = 0.009, *Valvata piscinalis* a mean A/I of 0.136, σ = 0.009. As *Valvata piscinalis* appear to epimerise at slow rates (Miller and Mangerud 1985; Bowen *et al* 1985; 1989) then the *Valvata piscinalis* ratios indicate an age earlier rather than later in the Ipswichian. The *Bithynia tentaculata* results are younger, and intermediate between those of 0.135 (early MIS 5e) and 0.066 correlated with Upton Warren (MIS 5a), although Upton Warren has also been postulated as correlating with MIS 3. These results therefore correlated the basal sediments to MIS 5.

Four *Bithynia tentaculata* opercula were analysed from Cassington, 4B, top 10cm. The samples have much lower levels of protein degradation than that found in Trafalgar Square. The opercula were also more degraded than the late-glacial samples from Star Carr (*see* Section 5.3). Unfortunately, due to a lack of samples from this part of the Quaternary with which to make direct comparisons, it is not possible to tell from the amino acid analyses whether the site is MIS 5a in age, or slightly younger (MIS 4). With further samples from younger sites this should be resolved. However, the ratios observed along with the other stratigraphical information and the OSL dating from the site are consistent with a Marine oxygen Isotope Substage 5a correlation.

Clacton (NGR TM 146128)

The remarkable preservation of what is believed to be a wooden spear from Clacton, found in 1911, is one of the most significant archaeological finds from sediments of this age, believed to be Hoxnian (Oakley *et al* 1977). This point, made of yew, has been interpreted as the end of a spear, made by Neanderthals, similar to the spruce spears discovered in Germany (Thieme 2000). The complex series of channels at Clacton-on-Sea are filled with silt, sand, organic sediment, and basal gravel and sand (Bridgland *et al* 1999). The fossil assemblage is Hoxnian.

The initial sample analysed for A/I from Clacton was a single *Corbicula fluminalis* valve, from the Natural History Museum collection, which yielded unexpectedly low values, originally correlated with the Ipswichian (Miller *et al* 1979; Table 1), although equivalent ratios were subsequently correlated with MIS 7 (Bowen *et al* 1989). However, two shells of the marine bivalve *Macoma balthica* also analysed from Clacton gave higher ratios (Miller *et al* 1979). Five gastropod shells gave A/I values consistent with a correlation with the Swanscombe Member and MIS 11 (0.3 ±0.001 (n=2) on *Trichia* and 0.3 ±0.002 (n=3) on *Cepaea*, Bowen *et al* 1989). Channels of different ages are present at Clacton (Warren 1955), but museum labels rarely specify the precise channel, which may account for the scatter of ages from this 'site'.

The first material from Clacton analysed in the present study comes from samples correlated with the Lower Freshwater Bed at West Cliff, attributed to MIS 11 (Bridgland *et al* 1999). Analyses were performed on four *Bithynia tentaculata* opercula from Trafalgar Road, deep piling and bottom levels from freshwater bed. The amino acid composition in the Clacton samples shows a slightly greater degree of decomposition than that from Swanscombe, but less than that from Waverley Wood, therefore consistent with a correlation with MIS 11.

Four *Bithynia tentaculata* opercula have also been analysed from Jaywick Beach. Inspection of the faunal lists in Warren (1955) suggested that some of the channels present were of different ages. The fauna from Channel 4, of which this material from Jaywick Beach is provenanced, was so different that it was suspected that it might not even belong to same interglacial as the Hoxnian channels. The protein in the samples from Jaywick Beach are significantly less degraded than that from Trafalgar Road, Clacton. The extent of IcPD is similar to that observed in samples from Trafalgar Square, therefore indicating a correlation with the Ipswichian, MIS 5e. This supports the idea that the channels at Clacton represent multi-age deposits.

Coston (NGR TG 062065)

The interglacial site at Coston lies in a gravel pit near Wymondham, Norfolk (Lightwing 1982). The sediments consist of a 4m organic layer, with the shells analysed collected from the upper 0.5m. The gravel at the base of the sediments lies underneath grey silts and semi-lacustrine deposits. Above this lies detritus mud (attributable to Ip IIb), that formed in a more energetic environment. Thick deposits of Devensian gravel overlie the interglacial sequence. The presence of *Hippopotamus* suggests correlation with the Ipswichian (MIS 5e).

Three *Bithynia tentaculata* opercula were analysed from Ip IIb, 15–25cm. The protein in these samples is at a similar state of decomposition as that found in Trafalgar Square, although with slightly lower values in the Hyd fraction. This is consistent with a correlation with the Ipswichian, and therefore MIS 5e.

Crayford (NGR TQ 514768)

The stratigraphy of Crayford is complex and covers a range of different deposits, both warm and cold stage, in a series of gravel pits (Kennard 1944). The gravel underneath the interglacial sediments descends to well below OD and is thought to represent cold-stage deposits. The overlying interglacial brickearth can be divided into lower fluviatile and upper colluvial elements, and extends to ~ 11m OD. The brickearth has yielded important faunal assemblages, with the *Corbicula* Bed indicative of fully interglacial conditions. Whilst the provenance of the material obtained from the Natural History Museum is not detailed, it is likely that the opercula came from the *Corbicula* Bed, as *Bithynia tentaculata* is thermophilous. Palaeolithic artefacts were recovered from the gravel, between the gravel and the brickearth, and at several levels within the brickearth. Levalloisian working floors were reported from the base of the brickearth in two of the pits, both yielding conjoinable material. Cold-stage material is also present above the interglacial deposits. The deposits have been correlated with Phase 3 of the Mucking Formation, and have yielded A/I values of 0.170 ±0.007 (n=3) from *Bithynia*, 0.187 ±0.007 (n=3) from *Corbicula* and 0.185 ±0.018 (n=9) from *Valvata* shells (Bowen *et al* 1989), correlated with MIS 7.

Four *Bithynia tentaculata* opercula were analysed from Crayford, Norris's Pit, 1952. These samples were obtained from the collections at the Natural History Museum. The IcPD values are greater than that from Ipswichian sites such as Trafalgar Square, but less than that from Aveley. This indicates an age range correlating with late MIS 6 to early-mid MIS 7.

Cropthorne New Inn (NGR SO 997443)

The stratotype of the New Inn Member at Cropthorne New Inn, Worcestershire, consists of c. 2.5m of fluvially deposited sands and gravels, from the base of Avon Terrace 3 (Tomlinson 1925; Maddy *et al* 1991). The site yielded a *Hippopotamus* fauna, so has been correlated with the Ipswichian.

Four *Bithynia tentaculata* opercula were analysed from Cropthorne New Inn. Due to probems with the initial preparation, three of the opercula had a second Free subsample prepared (NEaar 2734–6, CNIBto1–3). The extent of protein decomposition in these samples is similar to that from Trafalgar Square and significantly less than that from Aveley. This indicates a correlation with the Ipswichian (MIS 5e), supporting the biostratigraphy.

Cudmore Grove (NGR TM 064143)

The Cudmore Grove channel deposits are exposed on the foreshore of Mersea Island at Cudmore Grove. The main deposit, containing Middle Pleistocene sediments, was originally associated with the main Thames-Medway system and therefore correlated with the Clacton Channel deposits of the Hoxnian (MIS 11). However, on the basis of vertebrate evidence they are now thought to have accumulated in a subsequent interglacial, probably MIS 9 (Schreve 2001b). The cliff and foreshore exposures of a large fossiliferous channel-fill and overlying terrace gravels clearly post-date the Anglian. The interglacial deposits extend to -11m OD and are sandwiched between two layers of gravel. The 4m-deep Mersea Island Gravel which lies above the interglacial sediments has been postulated to be a product of the post-diversion Thames-Medway system (Bridgland 1994). The interglacial deposits are mostly estuarine silts and detritus,

but a bed of organic-rich material occurs at the level of the current beach, which is rich in vertebrate remains and shells in the lower part (including freshwater taxa). A second channel deposit at the Restaurant Site (*see* East Mersea) contains *Hippopotamus* and is therefore probably of Ipswichian age.

Four *Bithynia tentaculata* opercula were analysed from Cudmore Grove, from Bed 4, beneath the gravels (Roe 1995). The protein in these samples is slightly more degraded than that from Aveley, and significantly less degraded than that from Swanscombe. The lcPD values are slightly lower than those observed from Purfleet, which is consistent with an age within the later part of MIS 9, as indicated by the pollen recording deposition during a late temperate substage of an interglacial.

Dierden's Pit (Ingress Vale) (NGR TQ 595748)

This site is part of the complex of sites near Swanscombe, less than 1km north-west of Barnfield pit. The shelly gravels there have yielded the so-called 'Rhenish fauna' including *Theodoxus danubialis*, *Viviparus diluvianus*, and *Valvata naticina*, which allow their correlation with the Middle Gravels of the Barnfield sequence, the horizon containing the female hominin skull (Kerney 1971). The site has yielded abundant archaeology, including handaxe thinning flakes. A/I values of 0.298 (n=1) on *Corbicula* and 0.3 ±0.016 (n=10) on *Valvata* from the gravel were consistent with a correlation with Swanscombe and therefore MIS 11 (Bowen *et al* 1989). The samples used in this study, obtained from the Natural History Museum, were excavated in 1975.

Four *Bithynia tentaculata* opercula and two *B. troschelii* opercula were analysed from Dierden's Pit, Ingress Vale. One of the *B. tentaculata* samples, NEaar 2649, DPBto4, had very low concentrations in the Free fraction, and the data from this individual should be treated with caution. All the samples show levels of protein decomposition similar to that from Swanscombe and Clacton, therefore supporting a correlation with MIS 11.

East Mersea Restaurant site (NGR TM 053136)

The second of the two channel deposits at Cudmore Grove (*above*), of different age, has less extensive exposures, and is essentially a fossiliferous gravel, buried by modern beach sand (Bridgland *et al* 1995). The deposits are probably of Ipswichian age, since they contain the remains of *Hippopotamus*.

Two *Bithynia tentaculata* opercula were analysed from East Mersea, Restaurant site, Sample 3. Surprisingly there is quite a large difference between the data from each individual, with one opercula having very similar values to opercula from Trafalgar Square, and one having slightly lower Glx and Val D/L values. However, the younger sample (NEaar 2408, EMRBto2) does have greater protein decomposition than found in opercula from Cassington. Four more opercula samples were analysed as part of the ALSF-funded Medway project, and each of these opercula yielded values consistent with a correlation with the Ipswichian (MIS 5e) (Penkman *et al* 2007b).

Ebbsfleet (NGR TQ 615735)

Excavations at Southfleet Road, Ebbsfleet, Kent, as part of the construction of the Channel Tunnel Rail Link, have yielded a complex sequence of fossiliferous Middle Pleistocene sediments. These deposits include an incomplete skeleton of the straight-tusked elephant *Palaeoloxodon antiquus*, in close association with cores, flake, and notched flake tools, appearing to reflect *in situ* flake production and butchery (Wenban-Smith *et al* 2006).

Seven *Bithynia tentaculata* opercula were analysed from Ebbsfleet, ARC 342 WO2 (40070) Sample <40162> and four *Bithynia tentaculata* opercula were analysed from Ebbsfleet, EV 1994

A.TP1.b (15).

The samples from EV 1994 A.TP1.b (15) had lcPD values that clustered with the data obtained from Aveley, therefore indicating a correlation with early-mid MIS 7. The opercula from ARC 342 WO2 (40070) Sample <40162> had quite a large spread in the data, with Samples 1 and 6 possibly forming outliers in the dataset. However, they all show levels of protein decomposition similar to that from Swanscombe and Clacton, therefore supporting a correlation with MIS 11.

Eckington (NGR SO 919417)

The stratotype of the Eckington Bed is the Eckington Railway Cutting in Worcestershire. It comprises *c.* 1m of sandy gravel, which rests on the Lower Lias Clay at 23.3m OD. The deposits yield a temperate molluscan fauna, accompanying a *Hippopotamus* fauna. The material underlies the Third Terrace of the Avon and is correlated with the Ipswichian (Keen and Bridgland 1986; Maddy *et al* 1991).

Four *Bithynia tentaculata* opercula were analysed from Eckington, D, with the Frees of NEaar 2730–3, EcBto1–3 repeated. The opercula data from sample EcBto3i have inconsistent replicates and should be treated with caution. The levels of protein decomposition are very similar to that from Trafalgar Square, correlating this site with MIS 5e.

Elveden (NGR TL 809804)

The site at Elveden in Suffolk consists of over 4m of grey clays and 3.5m of brown sandy clays found in a channel cut into the chalky till. The molluscan and vertebrate fauna indicate temperate climate conditions. The archaeology includes both a biface assemblage and a core and flake assemblage, as at Barnham (Ashton *et al* 2005).

Five *Bithynia tentaculata* opercula were analysed from Elveden, ELV 95 (5)–(4) and two B. *tentaculata* opercula were analysed from Elveden, ELV 96 Sample 36, 60–70. All the samples from Elveden showed very similar levels of protein decomposition to that of Swanscombe, and greater levels than that seen from Purfleet. This indicates a correlation with MIS 11.

Enfield Lock (NGR TQ 370980)

Enfield Lock is a highly fossiliferous site dated to the late-glacial and the Holocene in Middlesex. Palynological and other palaeoecological data from late glacial sediments indicate an environment dominated by sedges with dwarf birch and arctic willow (Chambers *et al* 1996). The sediments indicate floodplain pond formation during the transition of the River Lea to a meandering form at 9,500 BP. This pond was infilled by 6,500 BP, after the development of Chara marl and then deposition of peat and silt. The opercula analysed are taken from just below a radiocarbon date of 6620 ±50 BP (UB-3329; Chambers *et al* 1996).

Four *Bithynia tentaculata* opercula were analysed from Enfield Lock, 3 (10–15cm). The lcPD model has not been developed fully for samples < 60ka, and so the data from this site are discussed in Section 5.3.

Froghall (NGR SP 415734)

The organic deposits at Froghall Pit in Warwickshire are cut into the Anglian glacial succession, the Wolston Clay and Dunsmore Gravel, so must be post-Anglian. Situated 1km from the Wolston type site, its stratigraphy has been caught up in the debate over the age (and classification) of the Wolstonian. The site sits on the interfluve between the Avon and Leam valleys, with the river

channel in a hollow over the Anglian till. The two gravel sequences are separated by an organic mud. The pollen biostratigraphy suggests a Hoxnian affinity, although is not a strict Hoxnian assemblage. The insect, molluscan, ostracod, and poor vertebrate assemblages at this site do not allow discrimination between an MIS 11 or an MIS 9 age.

Amino acid analyses were undertaken (Keen *et al* 1997) on *Bithynia tentaculata* and *Valvata piscinalis* from the channel sediments immediately overlying the Anglian glacial deposits. A/I on *Bithynia tentaculata* of 0.24 ±0.023 (n=16) and 0.23 ±0.011 (n=7) on *Valvata piscinalis* and indicate correlation with MIS 9 (Keen *et al* 1997). The ratios have been suggested to indicate that the latter part of the Anglian glaciation in this area occurs during MIS 10 (Keen 1999), or that there is an unconformity present, with no accumulation of sediment in MIS 11 or 10, and infilling only occurring in MIS 9.   There is an erosional contact between the fluvial sequence and the underlying deposits, so an MIS 9 association is plausible. There is a question of the timing of the Anglian in the Midlands, as to whether it occurred in MIS 10, or that the Anglian glaciation in the Midlands is composite, and occurred in MIS 12 and 10.

Four *Bithynia tentaculata* opercula were analysed from Froghall amino samples. The extent of protein decomposition in the opercula is significantly greater than that observed in samples from Cudmore Grove, but only slightly greater than that from Purfleet and Grays. The protein is less degraded than that from Swanscombe. This therefore indicates an age consistent with late MIS 11 or early MIS 9.

Funtham's Lane G (NGR TL 238979)

The gravel pit at Funtham's Lane, Whittlesey, near Peterborough, has yielded an interesting range of warm and cold-climate sediments throughout the pit, likely to represent more than one interglacial/glacial (Bridgland *et al* 1991). One section of the site has produced some important molluscan fauna, with *Corbicula* and *Theodoxus danubialis*, along with *Bradybena*, which has not been observed before. The molluscan and insect evidence indicate the basal deposits are of freshwater deposition, and correlate with MIS 11. The appearance of the *Theodoxus danubialis* is particularly significant, as this has been used as one of the indicator species of a Rhenish fauna, indicating a link to the Rhine in the Hoxnian interglacials (Kerney 1971). The molluscan fauna is most similar to that of the MIS 11 deposits at Swanscombe. The pollen indicates that the sediments are from late within the interglacial.

The Funtham's Lane succession also has a bearing on the age of the Woodston Beds, which lie only 8km to the north-west. The molluscan assemblages are different, with the Woodston Beds assemblage indicative of a strong marine transgression into the inner Wash Basin. Woodston and Funtham's Lane can only be from the same oxygen isotope stage if there were two transgressions within one stage, which is not possible to determine due to lack of resolution. The key point is that all the assemblages examined are from fluvial faunas, which tend to be homogenised by river activity, so it is very difficult to get isolated faunas different from those of other catchments of the same age.

As the site lies directly in the path of any likely ice advance, but does not appear to have been overridden by ice, the age of the site has consequences for the glaciations after it. If they are of MIS 11 age, then it is unlikely that the area was glaciated in MIS 10.

The samples were collected from subunits 3a and 3b in section G, in the proximity of logs G1 and G2, from the basal sediments above the Oxford Clay. The 25 bleached *Bithynia tentaculata* opercula results from Funtham's Lane analysed as part of a NERC-funded project are higher than those from Trafalgar Square and lower than those from Cudmore Grove. The D/L values are lower than those from Aveley and the Lion Pit Tramway Cutting, which could mean that the

samples are from a different isotope sub-stage within the aminozone.

The amino acid data from Funtham's Lane therefore strongly point to a correlation with MIS 7, possibly late MIS 7. This contrasts with the only dating evidence available, that from the molluscan fauna, which indicates a correlation with MIS 11. The presence of Theodoxus danubialis in the sediments, a key member of the 'Rhenish' fauna (Kerney 1971), has been used to make a correlation with Swanscombe and therefore MIS 11. There is a degree of molluscan faunal development in the Middle Pleistocene (Keen 2001) and the fauna at Funtham's Lane is unlike and more archaic-looking than any of the other MIS 7 sites yet seen. MIS 7 sites are reasonably widespread, and it is thought that most of the stages within the interglacial are represented in the UK record. However, the amino acid data point clearly to a late MIS 7 age, with a large number of analyses performed on 3 substrates. This age supports the marine evidence of relationship of the site to the Woodston Beds, dated in this study to MIS 11 (*below*).

The molluscan biostratigraphical interpretation is however under debate. *Bradybena fruticum*, a land snail unknown from MIS 11 sites but present in later interglacials, has been found at Funtham's Lane. *Theodoxus danubialis* has very specific habitat requirements, which may explain why it has not been found in MIS 7 deposits before. It has been found in only two UK sites, in the upper levels of Swanscombe and at East Hyde, Tillingham (Roe and Preece 1995), which both contain sediments late in the interglacial. If Funtham's Lane is late MIS 7, then this site could represent a time period within this isotope stage that has not been seen before in the UK record. The mammalian fauna at other MIS 7 sites such as Aveley indicate a reconnection to the European continent with a corresponding change in fauna during this stage.

Two more *Bithynia tentaculata* opercula were analysed from Funtham's Lane, Danielle's section, and the extent of protein degradation is very similar to the other Funtham's Lane samples, being greater than that from Trafalgar Square and less than that from Aveley. These samples therefore also indicate an age in mid-late MIS 7. At this site it is therefore possible that the amino acid racemization evidence has led to the recognition of a time period within MIS 7 that has not been previously identified within the UK record.

Grays (NGR TQ 6078)

Fossiliferous brickearths, along with Palaeolithic artefacts, were exposed during the nineteenth century in brick pits at Grays, Essex. The sediments were originally associated with the 'Middle Terrace' of the Lower Thames, mapped as the Taplow Gravel by the British Geological Survey, but are now correlated with the Lynch Hill Formation of the Middle Thames as Corbet Tey Gravel deposits (Bridgland 1994). These deposits are no longer accessible, but a great deal of research documented in the literature has been carried out on the site. The abundant molluscan and vertebrate fauna indicates a correlation with sediments older than the Ipswichian, but younger than the Hoxnian. The link with the interglacial sediments at Purfleet indicates a correlation with MIS 9.

Two *Bithynia tentaculata* opercula were analysed from Grays, Essex, from upper premolar of *S. kirchbirgensis*. The level of protein decomposition in these opercula is greater than that from Aveley and less than that from Swanscombe, therefore consistent with a correlation with MIS 9.

Hackney (NGR TQ 34528587)

The deposits at Hackney Downs consist of gravel and sand, resting on the Highbury or Leytonstone Members (Bowen 1999) or on bedrock (Gibbard 1994). Hackney is believed to be a site of MIS 9 age. It is a rich Palaeolithic site with a preserved working floor. The deposits are believed to have been laid down by a tributary of the Thames, the River Lea. Fossil insects which

today live in Spain and southern France indicate a summer temperature of up to 3°C warmer than today (Green *et al* 2006).

Ten molluscan subsamples were taken in the excavations and the amino acid analyses were performed from the shells in sample 7. Two *Bithynia tentaculata* opercula were analysed from Hackney, 7. The opercula show slightly greater levels of protein decomposition than that from Aveley, with similar levels as that from Cudmore Grove. This indicates an age late within late MIS 9 or early MIS 7.

Harnham (NGR SU 1520 2785)

A Late Middle Pleistocene handaxe manufacturing site was discovered recently at Harnham, in the Avon Valley, south of Salisbury, Wiltshire (Bates *et al* in prep). The site lies within the Hampshire Basin, on an interfluve between the Avon Valley and the Ebble Valley. A series of coarse flint gravels overlie the chalk bedrock at about 72m OD. These basal gravels are overlain by a series of sand units, containing molluscan and mammalian fossil material. Flint artefacts were recovered from within, and resting on, the sand. The surface is sealed by solifluction deposits, from which a cluster of re-fitting artefacts were excavated. Substantial numbers of artefacts were also found in the chalk gravels overlying the sand. Bone exhibiting marks consistent with human modification were also found.

Two *Bithynia tentaculata* opercula were analysed from Harnham, 2004. The protein decomposition in these two samples shows quite a difference, although both have values greater than that from Aveley and less than that from Cudmore Grove. This indicates an age between late MIS 9 and early MIS 7.

Hoxne (NGR TM 175767)

The site at Hoxne in Suffolk is the type site of the Hoxnian Interglacial (Mitchell *et al* 1973), situated in the Oakley Park brick pit. It is an extremely important Middle Pleistocene site, combining both archaeological artefacts and extraordinary fossil records to produce one of the rare hominin sites in a primary context. Up to 15m of fluviatile sands, silts, and gravels, containing Palaeolithic artefacts, are present, overlying interglacial peats and lacustrine clays that lie directly above the Lowestoft till. Pollen biostratigraphy suggests an immediately post-Anglian age, but A/I values correlated it with MIS 9 (Bowen *et al* 1989).

The first study of the site was published in 1800 (Frere), with follow-up work in the nineteenth and twentieth centuries (Evans *et al* 1896; West 1956; Singer *et al* 1993). The sequence at Hoxne is Anglian till, with a kettle hole in the surface of the till filled with lacustrine clays. The lake beds contain pollen that are believed to span the majority of the interglacial (West 1956), with the Late Glacial (Ho I) represented at stratum F, Early Temperate (Ho II) at stratum E, Late Temperate (Ho III) in the peat of stratum D, and the Early Glacial (Ho IV) at stratum C. Stratum C has been described by West as the 'Arctic bed', containing leaves of dwarf birch and dwarf willow. The same cold climate fauna has been observed at Beeches Pit and at Quinton, but whether this represents a full glacial cycle or just a short deterioration in climate is not known. The same pollen sequence is represented in the sediments at Marks Tey, although no cold episode is recorded in this sequence.

Above the lake sediments lie fluvial, colluvial, and solifluction deposits, whose stratigraphy is less clearly established, named as Strata B and A (West) and Beds 1–9 (Singer *et al* 1993). They are interpreted by Singer *et al* to date from the end of the Hoxnian interglacial through to the early part of the following cold stage.

The archaeological deposits have been divided into the Lower and Upper Industries, with the Lower Industry post-dating the Arctic Bed of Stratum C and the fluvial sediments of Bed 4. The Upper Industry lies stratigraphically above the Lower Industry, but a newly-identified channel appears to have been recut several times downslope from south to north, the two industries may be broadly contemporary. The industries are therefore thought to date from the significant cold event represented in Stratum C. If Stratum C is a cold event within the Hoxnian or a full glacial episode, it has implications for the age of the archaeology.

The lake at Hoxne is believed to have flooded a hollow left by the melting ice. However, the lake beds may not be a kettle hole formation. Several lake basins (meres) in East Anglia can be shown not to have been formed directly after the last glaciation, with infill of modern or Holocene sediments. They have been proposed to be due to the solution of bedrock chalk underneath the Anglian till cover (Bennett *et al* 1991).

The insect fauna are indicative of summer temperatures similar to the present day. The oak forest surrounding this lake, and a number of other East Anglian sites of the same proposed age, appears to have undergone a widespread forest fire during the warmest part of the interglacial, as indicated by a deep layer of charcoal. This charcoal layer is at the same horizon as a layer with abundant stone tools, leading to proposals of humans either causing the fire or their exploitation of a natural event.

On the basis of aminostratigraphy, Bowen *et al* (1989) placed the Hoxnian interglacial in MIS 9, but assigned the deposits at Swanscombe, also thought to be Hoxnian (Oakley 1957), to MIS 11. The A/I results on *Valvata piscinalis* gave a mean of 0.261 ±0.01, lower than the 0.3 ±0.015 proposed for the MIS 11 age such as at Swanscombe. It is possible that if MIS 10 was a weak cold stage then the two temperate stages separated by it could have similar biotic signatures (Dowling and Coxon 2001). The original U-series/ESR data from two teeth from Stratum C supported the correlation with MIS 9 (Schwarcz and Grün 1993). However both these age correlations seem to run counter to evidence from mammalian stratigraphy that provides a link between Hoxne and Swanscombe (Schreve 1997). New U-series/ESR calculations from the two teeth originally analysed from Stratum C resulted in a mean age of 404 $\pm^{33}_{42}$ ka and they were therefore correlated with MIS 11 (Grün and Schwarcz 2000).

There is an odd pattern of data from the gastropod shells from Hoxne, with the free amino acid D/Ls being lower than would be expected within the closed system. If the bleaching isolates a closed system, then the IcPD Total should not be higher than expected for a given IcPD Free. One explanation for this result is that the shells had undergone recrystallisation at some point in their burial history, and therefore the original intra-crystalline fraction had been compromised, but a second closed system had reformed. This suggests that the system has been opened at some point in the burial history of the sample, possibly due to recrystallization of the shells from aragonite to calcite. Some of the first opercula to be analysed for amino acids were therefore selected from Hoxne, to determine if the mineral phase could be a factor in the problematic results.

Four *Bithynia tentaculata* opercula were analysed from Hoxne 2000, Sq1, Spit 5 (40–50) Sample 4. Four *Bithynia tentaculata* opercula were analysed from Hoxne 64 and four *Bithynia tentaculata* opercula were analysed from Hoxne 50. All the samples show levels of protein degradation greater than that seen in samples from Purfleet, and similar to that from Swanscombe. This therefore indicates correlation with MIS 11. However, it is possible that Hoxne 64 is slightly younger, with levels of protein breakdown less than that observed in the other Hoxne samples. This sample comes from above the others, from a layer above an indication of climatic deterioration. Whether the amino acid ratios allow us to determine if the colder-stage deposit is within MIS 11, or represents MIS 10, requires further study and is currently under investigation.

It is possible that at this site some diagenetic alteration has affected the gastropod samples, leading to the unusual pattern of scattered data. This diagenesis does not appear to have affected the *Bithynia* opercula however, enabling the site to be correlated with MIS 11, in agreement with the biostratigraphy and the original definition of the Hoxnian interglacial as being immediately post-Anglian.

Ilford (NGR TQ 447865–446868)

The sediments at Ilford, Essex, consist of interglacial organic sediments, underlying a brickearth rich in mammalian fauna (West *et al* 1964). Originally correlated with the Ipswichian, the differences in mammal assemblages from that of Trafalgar Square were hypothesised to be due to their being of different ages (Sutcliffe 1964), and was one of the key localities which initially provided evidence for a post-Hoxnian, pre-Ipswichian interglacial in the British record. The Uphall pit at Ilford, from which a large proportion of the mammalian and molluscan fauna from Ilford were recovered, exploited lower-level deposits of Mucking Formation, whereas the Cauliflower pit exploited the higher Corbets Tey formation. Uphall pit yielded a rich molluscan fauna. It has been proposed that the sediments at Uphall pit are older than those at Cauliflower pit (Bridgland 1994). The mammalian fauna has been attributed to the Sandy Lane MAZ of MIS 7 (Schreve 2001b). A/I values on *Bithynia* 0.23 ±0.02 (n=5) from the shelly bed led Bowen *et al* (1989) to correlate Ilford with MIS 7.

Two *Bithynia tentaculata* opercula were analysed from Ilford, Essex, Uphall. The extent of protein breakdown in the samples is similar to that found at Aveley, greater than that from Trafalgar Square and less than that from Cudmore Grove and Purfleet. This indicates that the Ilford samples are of similar age to that of Aveley and Lion Pit, correlating with early-mid MIS 7.

Isleworth (NGR TQ 158746)

An organic silt horizon was exposed in Willments Pit, Isleworth, Middlesex, in the Upper Flooplain terrace of the Thames (Coope and Angus 1975). The terrace includes deposits accumulated in the Ipswichian interglacial, as well as during the Last Glacial period, the Devensian. The samples were obtained from the organic deposits associated with the Last Glaciation. The even bedding of the organic silts indicates that they were deposited in quiet water, although the silt horizon near the base of the sequence is separated from the bulk of the gravel by an erosion level. The sands and gravels overlying the organic deposits are likely to have been deposited in a high-energy environment, and contain ice-casts. The Isleworth assemblage is therefore dated to a period before the phase of maximum cold in the latter part of the Last Glaciation. Coarse plant debris was analysed for 14C using β-counting, which yielded a date of 43,140 +1520/-1280 BP (Birm-319), although this is now thought to represent a minimum age for the deposits.

The insect fauna indicates that the sediments can be correlated with the Upton Warren interstadial, a treeless episode within the last cold stage. The faunal remains at Isleworth were spectacular, including rich thermophilous beetles, indicating a treeless environment which was borne out by the pollen. However, this is the only cold-stage deposit yet found that has yielded *Bithynia* sp, apart from the late glacial deposits at Star Carr.

Four *Bithynia tentaculata* opercula were analysed from Isleworth, Middle Devensian. The protein in these opercula samples is significantly less degraded than from Ipswichian sites such as Trafalgar Square, but slightly more degraded than opercula protein from Cassington.

Lion Pit (NGR TQ 598783)

The Lion Pit tramway cutting at West Thurrock, Essex, lies in the Mucking formation of the Lower Thames, which is the downstream equivalent of the Taplow Formation of the Middle Thames (Bridgland 1994). The deposits consist of fluvial gravel and sand, up to 9m in thickness, resting on the bedrock at 4m OD (Gibbard 1994). The Middle Palaeolithic Levallois assemblage lies on the basal gravel of terrace sediments thought to have been deposited between MIS 8–6. The interglacial sediments overlying the gravel are likely to be part of the Aveley Silts and Sands member and therefore attributed to MIS 7 by terrace lithostratigraphy and biostratigraphy (Schreve *et al* 2004). The mammal assemblage belongs to the Sandy Lane MAZ, thought to be the later part of the interglacial (Schreve 2001a).

Two *Bithynia tentaculata* opercula were analysed from Lion Pit Tramway Cutting 08.07.00 and 5 *Bithynia tentaculata* opercula were analysed from Lion Pit, Sample 6, Section 5. The protein in these opercula samples is at a similar state of breakdown as that from Aveley, therefore consistent with a correlation with early-mid MIS 7.

Modern

The Holocene shells obtained from Stratford-upon-Avon come from a river cliff of the modern Avon near the racecourse. Although the shells are not modern, their age must lie between 6500 BP, the first appearance of *Theodoxus* in Britain since the Pleistocene, but predate 1820, the first introduction of the bivalve *Dreissana polymorpha* in the Avon catchment, which do not appear in the sediment. The lack of terrestrial species indicates an age after the main woodland clearances in the Midlands, thought to be during the Neolithic or Iron Age. The probable age of the samples is late medieval or younger, therefore late Holocene. The sediment provides an abundant supply of late Holocene but pre-industrial samples of *Bithynia tentaculata*, their opercula and *Valvata piscinalis*, which can be used as a baseline comparison for the older Pleistocene amino acid compositions of interest.

The modern samples from Acle (Norfolk) are all less than 100 years old. Both *Bithynia tentaculata* and *B. leachii* opercula were analysed from Acle – *B. leachii* is thought by some to be a closely related species to *B. troschelii*, hence its use in this study as a modern comparison. The different species are contrasted in Section 5.4.

The modern *Bithynia* opercula both had very low concentrations of Free amino acids, and low D/L values in the Hyd fraction. The IcPD model has not been developed fully for samples < 60ka, and so the data from this site are presented in Figure 5.5.

Norton Subcourse (NGR TM 403994)

The interglacial sediments at Norton Subcourse are found in an active gravel quarry in Norfolk, about 15km inland (Lewis *et al* 2004b). The site lies within the Neogene Crag basin, containing Pliocene to early Middle Pleistocene sediments of the Norwich and Wroxham Crags and the Cromer Forest Bed Formations. This mix of marine, estuarine, and freshwater sediments was laid down at the western margin of the southern North Sea basin. The organic sediments of interest are sandwiched between the Wroxham Crag Formation and the Ingham Formation. The Ingham Formation consists of Pre-Anglian fluvial sediments, part of the extinct Bytham River system. These fluvial sediments lie under the regionally extensive glaciogenic sediments of the North Sea Drift and Lowestoft formations, thought to correspond to the Anglian glaciation in MIS 12. These organic sediments underlying the Ingham Formation have yielded remains of articulated *Hippopotamus*, along with *Equus altidens* and *Mimomys savini*, which indicates an age of no younger than the early part of the Cromerian Complex (Lewis *et al* 2004b). The mollusc remains

are very sparse, due to diagenetic decalcification of the aragonitic shells, although the calcite *Bithynia* opercula are relatively common.

The site is important due to the biostratigraphical constraint on the fluvial sediments of the Bytham River and Ingham Formation. The Bytham River terraces in the area contain very early British Palaeolithic archaeology at the sites of High Lodge and Warren Hill, so the dating of this site could have implications for the ages of these Palaeolithic sites (Lewis *et al* 2004b). The presence of hippopotamus at both this site and at Pakefield, where recent discoveries have included human artefacts, could be significant. In the basal sediments of the site there has been a hint of a magnetic reversal, although the palaeomagnetic data are not conclusive.

Four *Bithynia troschelii* opercula were analysed from Norton Subcourse, 17. Although the D/L of Glx and Ser were found to be higher in two of the samples, NEaar 1992-3bF, the protein in the Norton Subcourse samples are of similar states of protein degradation as the samples from West Runton, although there are indications that they may be slightly more degraded. Further work on expanding the chromatography in order to cleanly elute some of the more hydrophobic and slower-racemizing amino acids is being undertaken to try to improve the resolution in these Cromerian sites.

Pakefield (NGR TM 537 888)

Sections along this stretch of coast have been obscured for many years. They were originally studied by Blake (1877), who described an extensive outcrop of the 'Rootlet Bed'. West's (1980) re-investigation suggested that the 'Rootlet Bed' formed during the early temperate substage of the Cromerian and this was underlain by laminated silts and clays belonging to the Pastonian and pre-Pastonian a. A bed of reddish-brown sand occurs in places below the 'Rootlet Bed' and overlies these earlier units. Many large vertebrate fossils have been recovered from the 'Rootlet Bed' including *Megaloceros verticornis* and *Hippopotamus*, as well as *Mimomys savini* (Stuart and Lister 2001). The molluscan fauna from the 'Unio Bed' includes *Valvata goldfussiana* (Preece 2001). The plants *Trapa natans* (water chestnut) and *Salvinia natans* (floating water fern), which indicate that the summers were warmer than now, are found at both Corton and Pakefield, but not at West Runton. This palaeobotany indicates warmer summer temperatures making Pakefield distinct from West Runton, with its indications of cooler summer temperatures from insects and Mollusca. An age in the early part of the Cromerian Complex seems likely. The discovery of flake artefacts in the interglacial channel infill were the first unequivocal evidence for human presence north of the Alps (Parfitt *et al* 2005). The deposits were dated to the early part of the Brunhes Chron by event and lithostratigraphy, palaeomagnetism, biostratigraphy, and the amino acid results obtained within this study.

Three *Bithynia troschelii* opercula were analysed from Pakefield, laminated organic channel fill and four *Bithynia troschelii* opercula were analysed from Pakefield, Unio Bed, Sample 36. All the opercula were significantly older (95% confidence) than those analysed from Waverley Wood, but they could not be statistically separated from opercula from the type Cromerian West Runton Freshwater Bed. One sample from the Unio Bed (NEaar 2166) seems to be slightly younger than the others, resulting in the large error bars in Figure 5.2. It is clear that the samples are significantly older than opercula from sites previously thought to represent the earliest hominin occupation of the UK. Further work is being undertaken to try to refine these Cromerian timescales.

Purfleet (NGR TQ 568785)

Two quarries on the northern bank of the Thames at Purfleet (Bluelands Pit, NGR TQ 560784, and Greenlands Pit) have uncovered highly fossiliferous gravel, sand, and intertidal silt exposures of up to 5m in depth (Hollin 1977). The Purfleet Member is recognised as an independent unit by Bridgland (1994), correlated with MIS 9 and supported by A/I ratios (Bowen *et al* 1995), but

Gibbard (1994) places it within the Aveley member. A rich shell bed of fine-grained aggradation containing many articulated bivalves of all sizes from *Unio* to *Pisidium* in life position indicates that reworking is limited within the site, although it cannot be ruled out in large rivers. The presence of *Corbicula* indicates that the site is older than 5e (Ipswichian). The site is also rich in vertebrate remains and Palaeolithic artefacts in the layers above the shell bed. These bifaces are found in the gravels overlying the fluvial sediments, and are likely to be Neanderthal in origin. The molluscs indicate river temperatures warmer than today. The vertebrate bones indicate a forest fauna including elephant, rhinoceros, deer, and notably barbary ape, the European population today found only in Gibraltar. The lack of brackish water fossils shows that the river estuary must have been far downstream, with the sea estimated to have been 90km further to the north-east than today (Schreve *et al* 2002).

There appears to be a tidal influence in the sediments overlying the gravel. A correlation with MIS 9 has been made indicated by the lithology, terrace stratigraphy, and biostratigraphy (Schreve *et al* 2002). However, amino acid ratios from this site have always been problematic. While the possibility of reworking seems unlikely, the amino acid ratios have often been found to indicate correlation with much older isotope stages. However, reprecipitated carbonate is a characteristic feature of both the Greenlands and Bluelands Pit. Both sites contain calcium carbonate concretions (sand dogs), which could account for the discrepancies in the amino acid signature. The shells analysed are from the main shell bed in Greenlands Pit. A series of stratified samples numbered 1–8, were taken, with the majority of the shells analysed for amino acid racemization coming from section 6, near the top of the shell bed (Schreve *et al* 2002). 25 *Bithynia tentaculata* opercula were analysed from Purfleet 6, and 25 *Bithynia tentaculata* opercula were analysed from Purfleet 1 as part of the NERC-funded project. The data from Purfleet 1 are presented here for comparison, being more degraded than that from Aveley and less degraded than that from Swanscombe (Fig 5.4). This is consistent with an age correlation with MIS 9.

The *Bithynia tentaculata* opercula again enable firm correlation of a site which has proved difficult to date with other materials. Isolation of the intra-crystalline fraction does not significantly improve the scatter of data from the *Valvata piscinalis*, *Bithynia tentaculata*, or *Corbicula fluminalis* shells, but the bleached opercula provide a tight dataset consistent with the biostratigraphy and the position of the site within the terrace sequence. The difficulty in obtaining consistent ratios from the mollusc shells may be due to mineral diagenetic alteration; there is reprecipitated carbonate evident throughout the site. The aragonitic gastropod shells may be more susceptible to this alteration, but the calcitic structure of the opercula appears to be significantly more robust.

Quidenham Mere (NGR TM 040875)

A thick lacustrine sequence covering the late glacial and Holocene occurs at Quidenham Mere (Horne 1999). The marginal sediments are highly calcareous marls rich in shells and ostracods. Detailed palynological studies have been undertaken on cores taken from the margins and centre of the lake. The chronology of the sequence rests largely on the pollen zonation, although two radiocarbon dates have been obtained.

Four *Bithynia tentaculata* opercula were analysed from Quidenham Mere, 2005 core, 250–260cm and three *Bithynia tentaculata* opercula were analysed from Quidenham Mere, 2005 core, 640–650cm.

The IcPD model has not been developed fully for samples < 60ka, and so the data from this site are discussed in Section 5.3 and presented in Figure 5.5.

Shropham (NGR TM 005938)

The late Pleistocene sediments at Shropham in Norfolk were uncovered in a gravel pit. Amphibians and reptiles were identified in small pockets within coarse sediments of Devensian glacial age at the top of the site, as well as from finer underlying sediments of Ipswichian (MIS 5e) age. The Devensian glacial age fauna includes the only record of a snake from any of the British Pleistocene cold stages, the grass snake *Natrix natrix*. The only other species found was the cold-tolerant common frog *Rana temporaria* (Holman and Claydon 1990).

Within the Ipswichian sediments at least six amphibian species and four reptilian species were identified, including four exotic species. The high percentage of exotic species (40%), including the occurrence of moor frog (*Rana arvalis*), a water frog (*Rana* sp. indet), the European pond tortoise (*Emys orbicularis*) and the water snake *Natrix maura* or *tessellata*, correlates with the percentage of exotic species of other Ipswichian faunas in the UK, which total 45.5%. These exotic herpetological species are found in the west or southwest of continental Europe currently, which indicates warmer conditions during this interglacial than is found in the UK today. The fauna are indicative of a well-vegetated pool bordered by grassy meadows and shrubby or wooded habitats. The presently endangered natterjack toad *Bufo calamita* appears to have been more widespread in Britain during the Pleistocene interglacials. The shells analysed were found in the Ipswichian sediments.

Four *Bithynia tentaculata* opercula were analysed from Shropham. The protein decomposition is similar to that at Trafalgar Square, consistent with a correlation with the Ipswichian (MIS 5e).

Somersham (NGR TL 375798)

The gravel quarries at Somersham, Cambridgeshire, contain Pleistocene channel deposits of the River Great Ouse, with interglacial sediments sandwiched between cold stage sediments (West *et al* 1999). The earlier interglacial sediments indicate temperate freshwater and marine-influenced organic sediments. These warm-stage deposits were originally correlated with the Ipswichian on the basis of pollen analysis. However, the deposits contain *Corbicula fluminalis*, suggesting that the *Corbicula*-bearing sediments were not deposited during the Last Interglacial (eg Keen 1990). A/I analyses undertaken on *Corbicula* sampled at the umbo, centre, and edge yielded results similar to that obtained from Stutton and Wretton, but whether this meant correlation with MIS 5e or MIS 7 was under debate (West *et al* 1999).

Four *Bithynia tentaculata* opercula and four *B. troschelii* opercula were analysed from Somersham, Sample SBK 3 19/10/90. These samples are likely to have been reworked (see West *et al* 1994) and so may be older than the associated deposit. The data from the *B. tentaculata* and *B. troschelii* shells are compared in Section 5.4 below. The extent of protein decomposition in the samples is significantly greater than that from Trafalgar Square, but less than that from Aveley and Lion Pit. This indicates that the opercula samples are of mid-late MIS 7 age, and if reworking has occured this constrains the age of the site to younger than MIS 7.

Stanton Harcourt (NGR SP 414051)

The sediments that were uncovered in a quarry at Stanton Harcourt form part of an extensive gravel terrace of the Upper Thames. The terrace stretches along the west side of the river for several miles between Northmoor and Eynsham, extending north to Cassington and towards the southwest to Stanlake. The ancient river channel deposits have yielded a rich range of animal and plant remains (Buckingham *et al* 1996). The large mammal fauna in particular make this site important, with mammoth, elephant, horse, bison, bear, lion, and hyaena represented. The mammoth remains are spectacular, with at least 50 tusks found, the largest 3m in length. The

mammoths are significantly smaller than those found from British cold-stage deposits (*Mammuthus primigenius* or *trogontherii*) and are referred to as the Ilford-type mammoth. The remains of a warm climate (straight-tusked) elephant *Palaeoloxodon antiquus* with the hip, leg, and vertebra bones almost connected indicate that the animal died at the spot where they were found, as very little post-depositional transport could have occurred (Scott 2001). Over 50 species of molluscs, along with 90 species of insects, the remains of fish, and small vertebrates have been discovered. It is not only the animal fauna that is outstandingly preserved, as shown by the excellent condition of the roots and branches of oak, alder, and willow trees, sometimes of over 2m in length. The tree fauna, including oak, hornbeam, alder, and hazel, indicate fully temperate conditions. The river appears to have been bordered by grasslands, with herds of grazing animals, and neighbouring forests. A wide range of archaeological material has been found, with 24 stone tools uncovered, making this site particularly important.

The site has been identified as MIS 7 (Briggs *et al* 1985; Bridgland 1994; Buckingham *et al* 1996) and was suggested as one of the recommended type localities for this interglacial (Bowen *et al* 1989), now defined at Strensham (Bowen 1999). ESR and U-series dates on a mammoth tooth from the site indicate an age of at least 147ka in age, therefore predating the last interglacial, with the recent uptake ESR dates indicating correlation with MIS 7 (Zhou *et al* 1997). The faunal content of the basal levels suggests an age in the later part of MIS 7 (7a), but it has also been suggested that these could relate to MIS 5. Amino acid racemization could prove useful to distinguish the depositional units. The A/I ratios of 0.154 ±0.007 (n=3) for *Bithynia tentaculata*, 0.153 ±0.014 (n=6) for *Lymnaea*, 0.163 ±0.016 (n=9) for *Corbicula* and 0.174 ±0.014 (n=8) for *Cepaea* in Bowen *et al* (1989) support correlation with MIS 7.

The opercula are from the channels in the Oxford Clay below the base of the Summertown-Radley Terrace of the Thames. The stratigraphy is as yet unpublished, but the samples are from Kate Scott's excavations in the late 1980s.

Four *Bithynia tentaculata* opercula were analysed from Stanton Harcourt, 1021A. The extent of protein decomposition in the samples is significantly greater than that from Trafalgar Square, but less than that from Aveley and Lion Pit, similar to that from the Somersham samples. This indicates that the site correlates with mid-late MIS 7.

Star Carr (NGR TA 028810)

Star Carr is an important Mesolithic site in the Vale of Pickering, Yorkshire, first excavated in 1949, after its discovery in 1947 during the clearing of a field drain. An early Mesolithic encampment yielded evidence of environment management, sophisticated carpentry skills, and adult red deer skulls with antlers, deliberately worked and postulated to be head-dresses. The site included a brushwood platform, sited on the edge of the former lake.

Occupation was likely to have been seasonal, between the spring and autumn. A detailed pollen and sedimentary study was carried out in the new trenches dug as part of the investigations in the 1980s. The samples analysed in this study came from lake marls described by Preece in Mellars (1998).

Two *Bithynia tentaculata* opercula were analysed from Star Carr, 245–250cm and 3 *Bithynia tentaculata* opercula were analysed from Star Carr, 524–528cm. The IcPD model has not been developed fully for samples < 60ka, and so the data from this site are discussed in Section 5.3 and presented in Figure 5.5.

Swanscombe (NGR TQ 597743)

The disused gravel pit, Barnfield Pit (TQ 595745), at Swanscombe has yielded the remarkable remains of a hominin skull, more remarkably because the three pieces that fit perfectly together were found at different times, the third 20 years after the first (Wymer 1964). The skull is thought to be that of an archaic Neanderthal woman. Both fluvial and non-fluvial sediments have included Palaeolithic artefacts and faunal remains (Conway *et al* 1996). Straight-tusked elephant, rhinoceros, wild boar, giant, fallow and roe deer, extinct giant beaver, and lion remains have been found. A major change in the molluscan fauna occurs between the Lower Loam and the Middle Gravel, where the 'Rhenish fauna' first appears (Kerney 1971). The vertebrate assemblages indicate correlation with the Hoxnian. A/Is of 0.3 ±0.017 (n=34) suggests correlation with MIS 11. The lithostratigraphy is detailed in Bridgland *et al* (1985), Bridgland (1994), and Gibbard (1994).

Six *Bithynia tentaculata* opercula were analysed from Swanscombe, Lower Loam. Whilst the glutamic acid in the samples shows quite high levels of variability, particularly in NEaar 1450 and 3058-9, overall the protein in the Swanscombe samples is more degraded than that from Purfleet and Grays, and less degraded than that from Waverley Wood. This is consistent with a correlation with MIS 11.

Tattershall Castle (NGR TF 210570)

Interglacial organic sediments, with a rich faunal assemblage, occur at gravel pits near Tattershall Castle. These sediments are cut into the Wragby till and are beneath thick Devensian gravels, in which large ice-wedge casts have formed (Holyoak and Preece 1985). The deposits are found at a lower elevation than the deposits at nearby Tattershall Thorpe, and also have evidence of brackish molluscs, which are not present in the higher deposits. The presence of *Corbicula* in the higher deposits at Tattershall Thorpe, and the lack of *Corbicula* at Tattershall Castle, was one indication that the sediments at Tattershall Castle were Ipswichian, and those at Tattershall Thorpe older. U-series disequilibrium dates suggest and age between 75–115ka BP, whilst a single thermoluminesence date yielded an age of 114 ±16ka BP. A/I analyses on *Cepaea nemoralis* gave values of 0.094 ±0.004 (n=3) and on *Valvata piscinalis* yielded values of 0.127 (n=1) (Bowen *et al* 1989).

Four *Bithynia tentaculata* opercula were analysed from Tattershall Castle, Lincs, IpIIb, sample (76K). The protein in the opercula is at similar levels of degradation to that of Trafalgar Square, consistent with an Ipswichian correlation (MIS 5e).

Trafalgar Square (NGR TQ 300804)

The stratotype of the Trafalgar Square Member is at Canada House, Trafalgar Square (Bowen 1999). The thick layer of interglacial deposits, of up to 12m of gravel, sand, silt, and detritus mud uncovered under Trafalgar Square are part of an aggradational terrace of the Thames. During foundation work in 1957–8 under Uganda House and New Zealand House (where these amino acid samples come from) excellent exposures of the interglacial deposits were uncovered. The base of the sediments lies on Tertiary London Clay between -0.5m and -3.3m OD, with the surface of the sediments at 7.4m OD. This 'Upper Floodplain Terrace' comprises a basal gravel overlain by fine grained interglacial sediments. Above this, over a much wider area, lies the Kempton Park gravel, a cold-climate sand and gravel (Bridgland 1994, but alternative view in Lewis *et al* 2004a). The deposits occur as discontinuous spreads and are correlated with the Ipswichian MIS 5e (Gibbard 1985). A/I of 0.11 ±0.005 (n=5) for *Bithynia tentaculata*, 0.113 ±0.005 (n=3) for *Trichia hispida* and 0.094 ±0.004 (n=4) for *Cepaea* (Bowen *et al* 1989) have been obtained, correlating with MIS 5e.

The vertebrate evidence of hippopotamus, lion, and straight-tusked elephant indicated correlation with the last interglacial, the Ipswichian (MIS 5e) which was supported by the molluscan (Preece 1999), beetle (Coope unpubl), and pollen (Franks 1960) biostratigraphy. *Corbicula* is conspicuously absent in the Trafalgar Square deposits, which is clearly not the result of failure in collection (Preece 1995). The environmental conditions indicated by the sediment would be favourable for this species, so its absence most likely results from the deposits at Trafalgar Square being of different age to those at Aveley and Purfleet.

Six *Bithynia tentaculata* opercula were analysed from Trafalgar Square, New Zealand House Foundation 1957. The protein is more degraded than that from Cassington, and less degraded than that from Aveley. The ratios observed along with the other stratigraphical information from the site are consistent with an Ipswichian correlation. The Ipswichian site of Bacon Hole has been U-series dated to 124 ±5.4ka (Stringer *et al* 1986) and correlated with the Eemian stratotype in the Netherlands (Bowen and Sykes 1988). The Ipswichian and therefore the Trafalgar Square aminozone is correlated with marine oxygen isotope substage 5e.

Waverley Wood (NGR SP 365715)

The Waverley Wood quarry in Warwickshire contains a channel complex underlying the Baginton-Lillington Gravel (the Baginton Formation, deposited in cooling conditions during the early Anglian) (Shotton *et al* 1993). The quarry contains the deposits of the Baginton River, a major river destroyed in the Anglian (Shotton 1953; Maddy 1999), but originally flowing from Stratford upon Avon to the North Sea in Norfolk (Rose 1987). The sands and gravels are low in fossil remains, except at the base, where straight-tusked elephant remains have been buried by sands from the developing braided river.

Underneath the sands, four separate stages of channel fill were identified, all mainly occurring in a cool temperate climate, with abundant fossil material. Evidence for a cold, continental climatic episode at the top of Channel 2 suggests the deposition of the entire complex during a fluctuating climate at the end of a temperate stage. These Arctic conditions are indicated by the presence of the beetle *Helophorus obscurrellus* (Coope 2006). The interglacial sediments record evidence of boreal forest. The small vertebrate and molluscan faunas indicate an age no later than the latter part of the Cromerian Complex (Shotton *et al* 1993), with the presence of *Arvicola* being particularly important. The presence of mole, which cannot live in areas of deeply frozen ground, provides an excellent environmental indicator. The occurrence of *Unio crassus*, a temperate freshwater bivalve, with the land snail *Discus ruderatus* indicates a continental climate.

Hominin artefacts were recovered from two levels in the channels. A single struck quartzite flake was found *in situ* in the base of the sandy bed of Channel 1 (chronologically earlier than Channel 2). Other tools (four handaxes, a cobble tool, scraper, and second flake) found by quarry workers were probably derived from river-side contexts. Three of the handaxes are made from andesitic tuff, whose nearest source is in North Wales and the Lake District (Shotton and Wymer 1998). No andesitic flakes were found at Waverley Wood and it is also thought that these tools were never used. Although they are reworked, their discovery supports the view of Cook *et al* (1991) of the production of high quality ovate handaxes in the Lower Palaeolithic at High Lodge, Mildenhall. Further archaeological material has recently been recovered *in situ*, providing for the first time, firm evidence of their geological provenance (Keen *et al* 2006).

The mollusc samples come from Channel 3, whilst the biostratigraphically critical *Arvicola* come from Channel 4 (Shotton *et al* 1993). Amino acid racemization on molluscs from Channel 2 indicate a Cromerian age. A/I of 0.381 ±0.027 (n=12) on *Bithynia troschelii* and 0.381 ±0.025 (n=3) on *Trichia hispida* indicate a pre-Cromerian age as greater than that at West Runton, and

have been correlated with MIS 15 (Bowen *et al* 1989).

Six *Bithynia troschelii* opercula were analysed from Waverley Wood, Bulk 1. NEaar 3319 was bleached in a glass vial rather than a plastic eppendorf, which seems to affect the D/L Glx in the Hydrolysed fraction, and so this value of Glx is not reported in the dataset.

The protein is significantly more degraded than that from Swanscombe and Clacton, but less degraded than that from West Runton. However, the closed system of protein of one of the Waverley Wood opercula appears to have been compromised. If the amino acids were contained within a closed system, the relationship between the Free and the Hydrolysed fractions should be highly correlated, with non-concordance enabling the recognition of compromised samples (Preece and Penkman, 2005). One of the opercula from Waverley Wood, 2036bH*, showed this non-concordance and so was rejected from the dataset.

The new ratios observed, along with the other stratigraphical information from the site are consistent with an age greater than the Anglian, and therefore a correlation within the Cromerian Complex. However, this positioning of the site within the Cromerian Complex using the new data conflicts with the original A/I ratios, where West Runton was found to have lower ratios than Waverley Wood. However, it does support the biostratigraphical data, with the presence of *Arvicola* at this site indicating an age younger than West Runton (Penkman *et al* in press).

West Runton (NGR TG 182432)

The interglacial sediments exposed in the cliffs here have been defined as the stratotype of the Cromerian Stage. It has yielded the articulate skeleton of an early form of mammoth, *Mammuthus trogontherii*, the largest fossil ever recovered from the Forest Bed. It was discovered on 13 December 1990, and two phases of excavation were required to recover all the bones. In 1992 the excavation recovered all the bones that could be reached without unsafe tunnelling, which amounted to about a quarter of the skeleton. The second excavation in 1995 removed thousands of tons of overlying sediment with a dragline.

The original work by Clement Reid (1882; 1890) classified the pre-glacial sequence into two major units, the Weybourne Crag and the younger Cromer Forest-bed series. The Cromer Forest bed was then further subdivided into three units: the Lower Freshwater Bed, the 'Forest Bed', which was an estuarine environment, and the Upper Freshwater bed. Based on the stratigraphy and the macrofossil evidence, fluctuation in climate from temperate to arctic conditions and major changes from marine to non-marine environments were noted. West (1980) has shown that Reid's 'Upper Freshwater Bed' is composite and includes not only interglacial organic sediments but also underlying marls belonging to the preceding Beestonian Stage. Pollen analysis has revealed that the organic sediments cover only the first half of the stage (CrI-II) and these, together with the underlying marls, constitute the 'West Runton Freshwater Bed' (West 1980).

West (1980) conducted a re-investigation of the sections using pollen analysis and was able to demonstrate that two distinct temperate stages, the Pastonian and the Cromerian, and one cold stage, the Beestonian, were represented in the newly-defined Cromer Forest-bed Formation (CF-bF). Pre-Pastonian cold stages were also recognized at the base of the sequence. The succession was not continuous, with large gaps. Recent work has shown that the Pastonian is Early Pleistocene and is separated from the Cromerian by a huge interval in time, represented in the Netherlands by the Menapian, Bavelian, Eburonian, and Waalian (Gibbard *et al* 1991).

The West Runton Freshwater Bed (WRFB) is extremely rich in non-marine molluscs and vertebrates, mainly in the interglacial organic sediments but also in the Late Beestonian marls. The

richness of the molluscan fauna is very distinctive (Preece 2001), with a lack of strong thermophiles. There is no water chestnut (*Trapa natans*) present in the plant record, thought to be a 'southern species', and no *Corbicula fluminalis* in the molluscan record. The vertebrate assemblage includes *Mimomys* with rooted teeth, a biostratigraphic indicator of an early Cromerian age (Preece and Parfitt 2000). The upper levels of the WRFB are decalcified, and likely to be truncated.

The original A/I analyses yielded A/I values of 0.38 (n=1) from *Bithynia*, 0.346 ±0.031 (n=5) on Pisidium and 0.348 ±0.001 (n=9) on *Valvata*, although it was noted that problematic results giving younger ratios were also obtained from this site (Bowen *et al* 1989). These results placed the site within the Cromerian, but younger than the site of Waverley Wood. However, these data conflicted with the molluscan and mammalian biostratigraphy (Preece and Parfitt 2000). Ten *Bithynia troschelii* opercula were analysed from West Runton Freshwater Bed D. NEaar 3317–8 were bleached in a glass vial rather than a plastic eppendorf, which seems to affect the D/L Glx in the Hydrolysed fraction, and so this value of Glx is not reported in the dataset. The bleached *Bithynia troschelii* opercula results from the Freshwater Bed are higher than those from Waverley Wood, therefore consistent with a pre-Anglian assignment, older than that represented by the site of Waverley Wood.

These results do not support the original A/I aminostratigraphy, which yielded ratios from West Runton younger than those at Waverley Wood. However, the new opercula data are supported by the molluscan and mammalian biostratigraphy. It is possible that the calcitic opercula provide a more robust repository for the original protein and its breakdown products, enabling increased resolution within the Cromerian (Penkman *et al* in press).

Woodston (NGR TL 189956)

The Quaternary sediments at the Hick's Pit No 1 brickyard in the Nene Valley consist of a 3m sequence of fluvial silts and sand (Horton *et al* 1992). The plant, beetle, vertebrate, and molluscan fauna indicate that the fluvial sediments accumulated under interglacial conditions. The pollen suggests correlation with biozone Ho IIc of the Hoxnian. They form part of the Woodston Beds, which outcrop in an arc to the south and west of Peterborough. The abundant insect, molluscan and plant fossils indicate their correlation with the 'Hoxnian' interglacial in the Middle Pleistocene. The mammalian biostratigraphy indicates an age in MIS 11. Hicks Pit has produced one species of vole that apparently becomes locally extinct in Britain after MIS 11 so this is the most likely date for these deposits (Schreve 2001b). Pollen analysis suggests correlation with Ho IIc of the Hoxnian. However, amino-acid ratios have suggested correlation with MIS 9 (Horton *et al* 1992). A/I from *Cepaea* of 0.248 ±0.095 (n=2), *Trichia hispida* of 0.24 ±0.023 (n=2), and *Bithynia tentaculata* of 0.248 ±0.29 (n=2) indicate correlation with MIS 9.

The age of the Woodston Beds is linked closely with that of the Funtham's Lane interglacial deposits, as they are geographically very close. At 11.7m OD there are brackish mollusca and marine ostracods, suggesting marine transgression to this elevation. The two deposits can only be of the same age if two marine transgressions occurred within the same isotope stage. However, MIS 11 has several substages and a complex sea-level history so it is not necessarily expected that all sites in the local area would show the same pattern, as it is very unlikely that they would be exactly contemporaneous. The possibility of glaciation of the Wash Basin in MIS 10 may also affect the age of the Woodston Beds.

Four *Bithynia tentaculata* opercula were analysed from Woodston, Bulk B. Bulk B is a grab sample from a fossiliferous lens which contained well preserved molluscs detailed in Horton *et al* (1992). The protein is more degraded than that from Purfleet, and of similar levels of breakdown as that from Swanscombe and Clacton. This indicates that the site can be correlated with MIS 11.

Over and above the age estimations, the results from several samples had important implications for amino acid geochronology in general and are discussed in detail below.

5.3 Late Quaternary samples

The current IcPD model is not particularly effective at discriminating between sites at young ages. The strength of the model lies in combining multiple amino acid data to provide a compound value representing the overall state of protein degradation. However, the low levels of racemization observed in several of the amino acids in young material result in their incorporation into the model less useful, and in some cases their inclusion masks important information from the faster-racemising amino acids.

This part of the IcPD model is undergoing more work, but the relative ages of younger sites can still be determined by focusing on two amino acids in particular, Asx and Ser. Asx is one of the fastest racemizing of the amino acids discussed here, partly due to the fact that it can racemize whilst still peptide bound (Collins *et al* 1999). Serine is one of the most unstable amino acids, with fast rates of racemization and decomposition. It is therefore very useful for discriminating between sites at younger timescales as in this study. These amino acids therefore provide useful relative age information for these young sites (Fig 5.5).
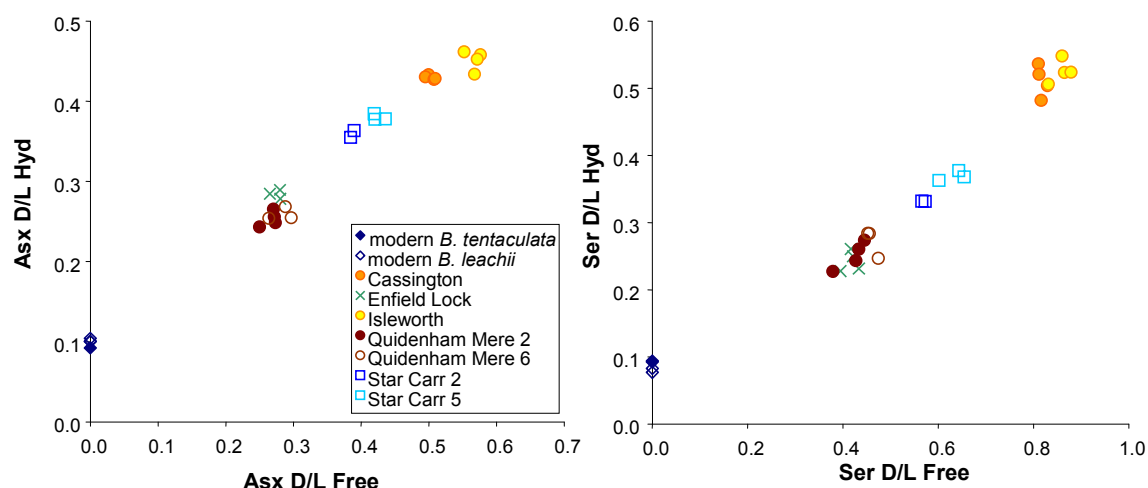

Figure 5.5: Plots of Hyd vs Free for D/L Asx (left) and D/L Ser (right), for sites younger than the Ipswichian (MIS 5e)

Bithynia tentaculata opercula have been analysed from two depths from a core taken in 2005 at Quidenham Mere (250–260 cm and 640–650 cm); from Enfield Lock (3, 10–15cm) and from two horizons from Star Carr (245–250 cm and 524–528 cm).

The samples from Quidenham Mere come from a core adjacent to one that has been radiocarbon dated. Sample 2 comes from the 250–260 cm level and has an inferred age of 5000 BP, based on original pollen assemblage zones (Horne 1999). Sample 6 comes from the 640–650 cm level and is harder to date, but is older than sample 2 and younger than 9250 BP.
The opercula from Enfield Lock come from just below the radiocarbon date of 6620 ±50 BP (UB-3329; Chambers *et al* 1996).

The samples from Star Carr come from the pollen core taken from the new palaeoecological investigations undertaken by Petra Dark (Mellars 1998). Sample 2 comes from the 245–250 cm level from marl immediately below the switch from marl to organic lake mud. The base of the

mud has been dated to 7640 ±85 BP (OxA-4042), with the opercula likely to be only marginally earlier, unless there is a hiatus. Sample 5 comes from the 524–528 cm level, representing a late-glacial interstadial woodland recession/cooling phase, estimated to have been deposited between *c.* 11,300–11,400 BP (Mellars 1998). The Asx D/L and Ser D/L data exhibit very similar patterns, with very low D/L values in the Hyd fraction in the modern samples. The concentration of Free amino acids in the modern samples are too low to determine the D/L values.

At higher ratios lie the Quidenham Mere and Enfield Lock samples. Although the Quidenham Mere 640–650 cm samples tend to have higher ratios than those from 250–260 cm, their ratios do overlap. The Enfield Lock samples appear to be of similar age.

The next oldest samples are those from Star Carr 245–250cm, which are separable from opercula from 524–528 cm and represent ratios from the late-glacial. The data from Cassington and Isleworth are also presented here, with significantly higher ratios of Ser and Asx. The extent of racemization and decomposition in the other amino acids in samples of this age have reached values sufficient for the use in the IcPD model (Fig 5.2).

The relative ordering of the sites is therefore consistent with the radiocarbon dates obtained and stratigraphical information for these samples. The racemization of Asx and Ser therefore provides a useful technique for discriminating samples of different ages within the Holocene, but work is ongoing to incorporate this information into the IcPD model.

West Runton vs Clacton

The decrease in natural variability afforded by the opercula, as compared to the shell, provides the possibility of an increase in age resolution using this calcitic biomineral. This was studied in detail at two sites in particular, that of the pre-Anglian site of West Runton and the post-Anglian site of Clacton. This study was submitted to *Quaternary International* for consideration in a special issue dedicated to the West Runton elephant (Penkman *et al* in press).

Bobbitshole samples

As chemical reactions, amino acid racemization and protein decomposition are affected not only by time but also by temperature. In preparing soil material for molluscan analysis, it was common practice to wet sieve the sediment and then dry it out in an oven. This practice would therefore increase the extent of protein degradation within molluscan samples, making any age derived from the amino acids a maximum age estimation. Since the advent of AAR dating in the 1970s, molluscs for amino acid analysis have been routinely picked from sediment that has not been dried out at high temperatures. However, as part of this research, archive material for some sites was obtained from the Natural History Museum. One of these sites was Bobbitshole, the type-site of the Ipswichian interglacial and therefore a site key to the testing of this new method of amino acid geochronology. Three individual *Bithynia tentaculata* opercula were analysed from the NHM collection and each gave widely varying extents of protein decomposition (Fig 5.6).
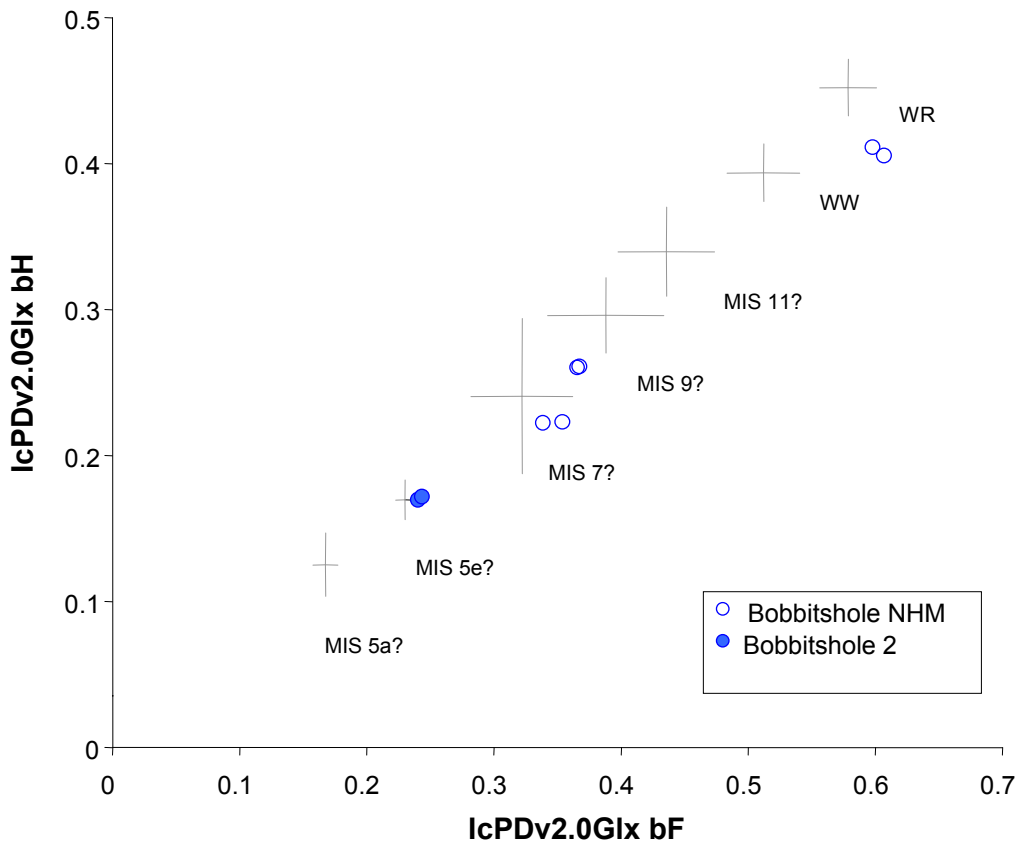
Figure 5.6: IcPD Hydrolysed vs IcPD Free for archive Bobbitshole samples from the Natural History Museum (NHM; ○) and the second batch of Bobbitshole samples taken from the spoil heap (2; ●)

The mean data and two standard deviations for opercula correlated with MIS 5a, 5e, 7, 9, and 11, and data from Waverley Wood (WW) and West Runton (WR) are included for comparison

The variation in the extent of protein decomposition observed in these samples is far greater than that seen from any other site. It is possible that this is a true measure of the age of the individual opercula, making it possible that the site is of MIS 7 age, and that reworking has occurred of MIS 9 and Cromerian material into the deposit. However, as these samples were collected in 1952, it is also possible that they had been heated during the sorting process.

A second batch of samples that had been collected from interglacial material from a spoil heap during the 1950s excavations, and had been known to have been unheated, was analysed. These three individuals all had ratios consistent with a MIS 5e age. It is therefore likely that the age of the deposit does correlate with MIS 5e and that the NHM samples had been heated after excavation. It is fortunate that the archived opercula appear to have been heated for different time periods or temperatures, possibly in different batches, as this enabled the possibility of heating to be recognised. It is possible that the pattern of protein decomposition artificially induced at high temperatures, as opposed to that during burial at low temperatures, has a characteristic pattern that will enable heated samples to be excluded from amino acid results; this is undergoing further study.

5.4 *Bithynia* species differences

All the analysed specimens were members of the family Bithyniidae, which secrete shells made of aragonite and opercula composed of calcite. The opercula are concentric in *Bithynia s.s.* and paucispiral in other taxa.   Most analyses used the opercula of either *Bithynia* (*Bithynia*) *tentaculata* (Linnaeus 1758), which has an elongate operculum with a pointed apex, or *Bithynia* (*Bithynia*) *troschelii* (Paasch 1842), which has a more rounded operculum (Meijer 1985). There is some uncertainty concerning the use of the name *B. troschelii*. Some authors regard it as merely a subspecies of *B. leachii* (Sheppard 1823), whereas others consider it a distinct species. The name *Bithynia inflata* (Hansén 1845) has often been used for this taxon, especially in early English literature, whereas some continental authors think that *Bithynia (Codiella) transsilvanica* (E A Bielz 1853) is the correct name (Falkner *et al* 2001).

The only other species used in this study is '*Bithynia conica*' (C Prévost 1821), from the Eocene limestone, Isle of Wight. It was therefore important to determine if the two main *Bithynia* species used in this study, *B. tentaculata* and *B. troschelii*, had significantly different protein compositions. Paired comparisons were made with samples from the same horizon from modern, MIS 7 (Somersham) and MIS 11 (Barnham and Dierden's Pit) sites (Fig 5.7).
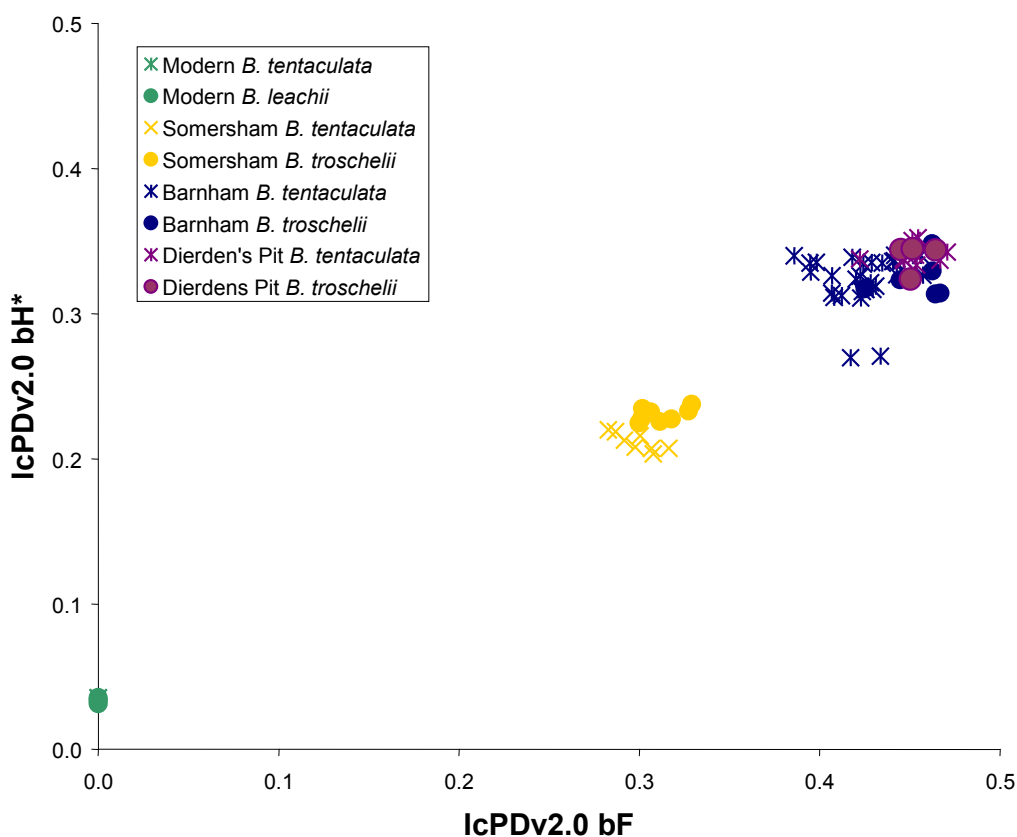

Figure 5.7: IcPD Hydrolysed vs IcPD Free for modern, MIS 7 and MIS 11 Bithynia tentaculata, B. leachii, and B. troschelii.
The Barnham dataset includes all the distorted opercula results (*see below*)

Overall, the *Bithynia troschelii* opercula tend to have slightly higher D/L values than that obtained on *B. tentaculata* opercula from the same horizon, tested statistically using two-tailed *t*-tests. In the samples from Somersham, Hyd D/Ls of Asx, Glx, Ser and Val all are slightly higher, although the D/L of Ala is slightly lower. In the Free fraction only the Ala D/L is higher. However, the differences in the means are not large, all being less than 0.07. It is in the [Ser]/[Ala] values that

the largest difference is observed, with the *B. troschelii* samples having significantly lower ratios. In the Barnham dataset, the increase in D/L for the *B. troschelii* samples is observed in the Free fractions, with Asx, Glx and Ser D/L all having higher values, although again the differences are small, none more than 0.05. In the Hyd fraction, only Ser is increased in the *B. troschelii*, whilst Ala is increased in the *B. tentaculata* samples. The largest differences are observed in the [Ser]/[Ala] values, where *B. troschelii* has value around 0.09 lower.

At Dierden's Pit, only the Free Asx and Ser D/Ls have higher values in the *B. troschelii*, with lower values than the *B. tentaculata* in the Hyd Ala D/L. Again however the [Ser]/[Ala] values of the *B. troschelii* are systematically lower.

The composition of the protein within the *B. troschelii* opercula differs slightly to that of the *B. tentaculata* opercula, with slightly lower concentrations, most marked in Asx, although the differences are less in the MIS 11 samples. This indicates that the protein composition is different in the opercula from the same genera, and that the way the protein breaks down is also slightly different. However, the differences between the two species is not as severe as that observed in gastropod of different genera (eg Fig 3.1). The effect on the overall Intra-crystalline Protein Decomposition value is little (Fig 5.7), and so the data from these opercula from the same genera can be compared directly. It should be noted, however, that the extent of protein degradation in the *B. troschelii* opercula tends to be slightly greater than that from *B. tentaculata* opercula.

5.5 Distorted opercula

The Lower Palaeolithic site at East Farm, Barnham, Suffolk provides a rich assemblage of non-marine molluscs. Palaeontological evidence suggests attribution to the Hoxnian Stage, broadly equivalent to part of Marine oxygen Isotope Stage (MIS) 11.

However, the molluscan assemblages from certain levels at Barnham had clearly suffered considerable post-mortem sorting and diagenesis. Not only were there huge differences in the representation of *Bithynia* shells and their opercula, but the apertures of some shells showed evidence of significant recrystallization. Moreover, some of the opercula were slightly folded or even crumpled, indicating that they must once have been soft and deformable rather than hard and brittle (Fig 5.8). Such diagenetic effects are likely to have compromised their suitability for amino acid dating. New amino acid analyses were therefore undertaken on the intra-crystalline component of both pristine and distorted opercula. These results were published in Preece and Penkman (2005) in the *Proceedings of the Geologists' Association*.
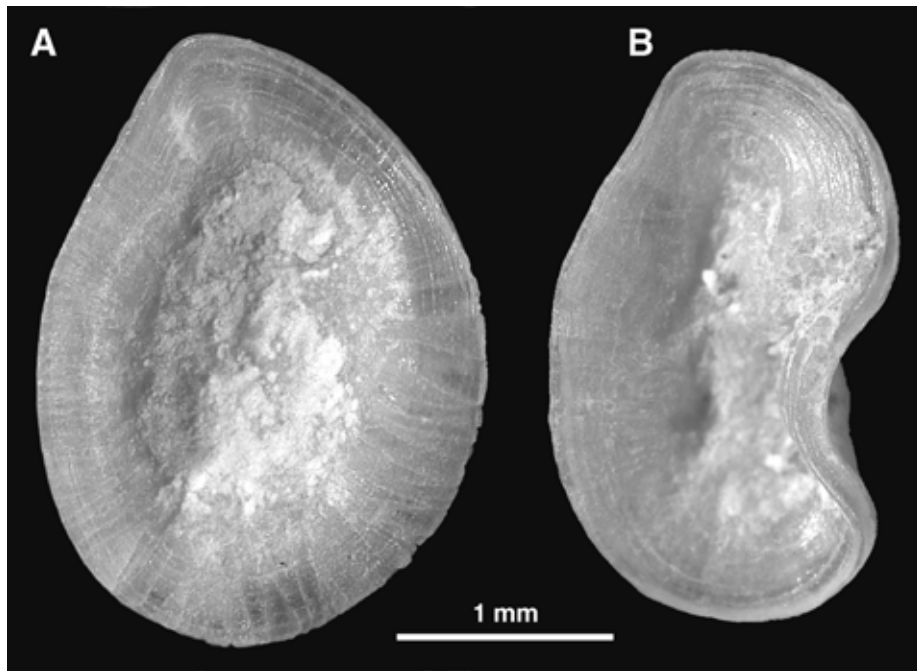
Figure 5.8: A. Undeformed operculum of Bithynia tentaculata *with translucent edge (possibly resulting from 'splitting' or partial dissolution. B. Crumpled operculum that must have been deformed while soft, highlighting the extent of post-depositional diagenesis*

Figure 5.9: Correlation between Hydrolysed and Free IcPD for bleached opercula from Barnham (●) compared with the values obtained from other UK Pleistocene sites.
The IcPDv2.0 values from opercula at Barnham are consistent, with the exception of NEaar 1676, which shows a Free to Hyd pattern significantly different to those of the other opercula of this age

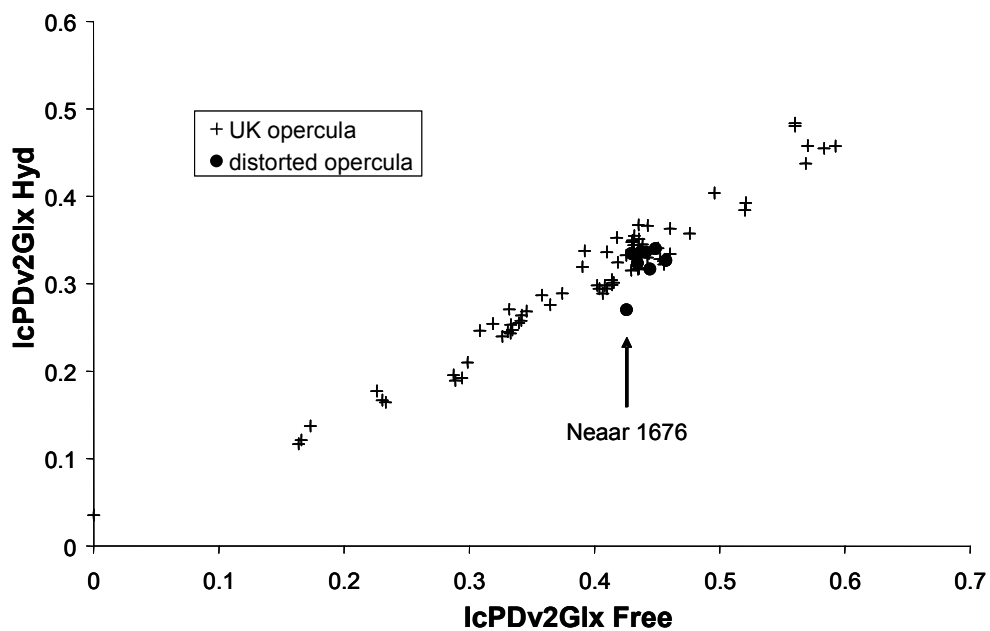The key finding from this piece of research was the ability to recognise a compromised sample by the non-concordance of the Free to Hyd relationship. This tight correlation therefore provides a useful test for recognising a closed system, and also enabled the rejection of one of the Waverley Wood samples (NEaar 2036bH*) due to similar non-concordance (Fig 5.1).

5.6 Clustering and discrimination

One criticism of previous aminostratigraphic interpretations has concerned where boundaries are drawn within a dataset to discriminate between samples of different ages (McCarroll 2002). It is important that the technique is applied with due regard to the natural variability of amino-acid ratios and that all ratios obtained are reported, without subjective filtering.

An indication of the extent of overlap between proposed interglacials can be demonstrated by a frequency distribution graph (Fig 5.10). In this case the Free and Hydrolysed datasets are presented, with putative interglacial MIS correlations. There is a small degree of overlap between the MIS clusters. This might be expected, as not only is there a degree of natural variability within the samples, but the rate of the racemization reaction is extremely slow within cold stages. This results in difficulties in discrimination between samples that were formed towards the end of one interglacial and those that occur in sediments from the early part of the next interglacial.
It is therefore important to analyse multiple samples from a single horizon, in order to account for the natural variability. The plot of Hyd vs Free IcPD also increases the ability to discriminate between sites. The aminostratigraphic framework in Figure 5.2 is, we believe, the optimal way in which to present the data, as this clearly shows the relationships between the sites and the confidence in which sites can be correlated with marine oxygen isotope stages.

5.7 Deriving an age estimate?

If a closed system is isolated and a plausible temperature history is modelled, it should be possible to predict the relationship between geological time and IcPD increase. In order to convert the IcPD to an absolute age estimation, we have begun to investigate a temperature modelling approach. The model uses estimates for the temperature dependence of key reactions (hydrolysis, racemization, and decomposition; Fig 5.11) and is driven by a temperature model. Various different models (representing different ideas of the major factors driving decomposition; *see* Collins and Riley 2000) are used, only one is illustrated here, a closed system in which water content is restricted and thus peptide bond hydrolysis is treated as a second order reaction.

The temperature history of southern England was estimated using a present day mean annual air temperature as the intercept, ice core and deep sea records for the pattern and timing of variation (modelled by Bintanja *et al* 2005), and coleopteran Mutual Climate Range (MCR) estimates of temperature range. The results are shown in Figure 5.12.

We are aware that there are significant problems with this approach. Borehole temperatures show that the $\delta^{18}O$ paleothermometer breaks down during glacials and Antarctic ice core isotopes (used back to 800ka) are some distance from the UK. Furthermore this model estimates suggest lower temperatures in the Cromerian than would be anticipated from biostratigraphic evidence, and therefore should be treated with caution.
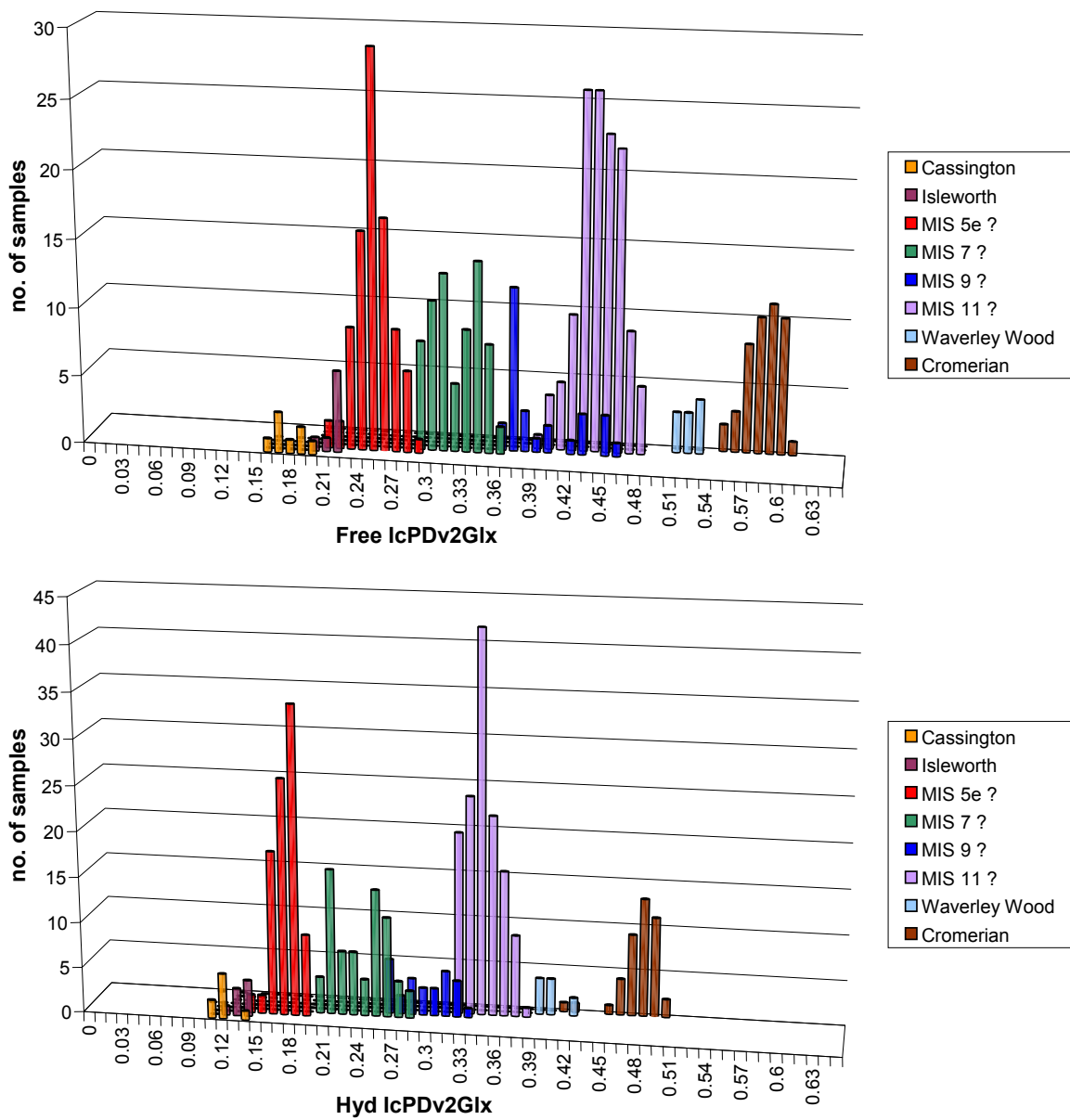
Figure 5.10: (top) Free IcPD frequency distribution (bottom) Hydrolysed IcPD frequency distribution
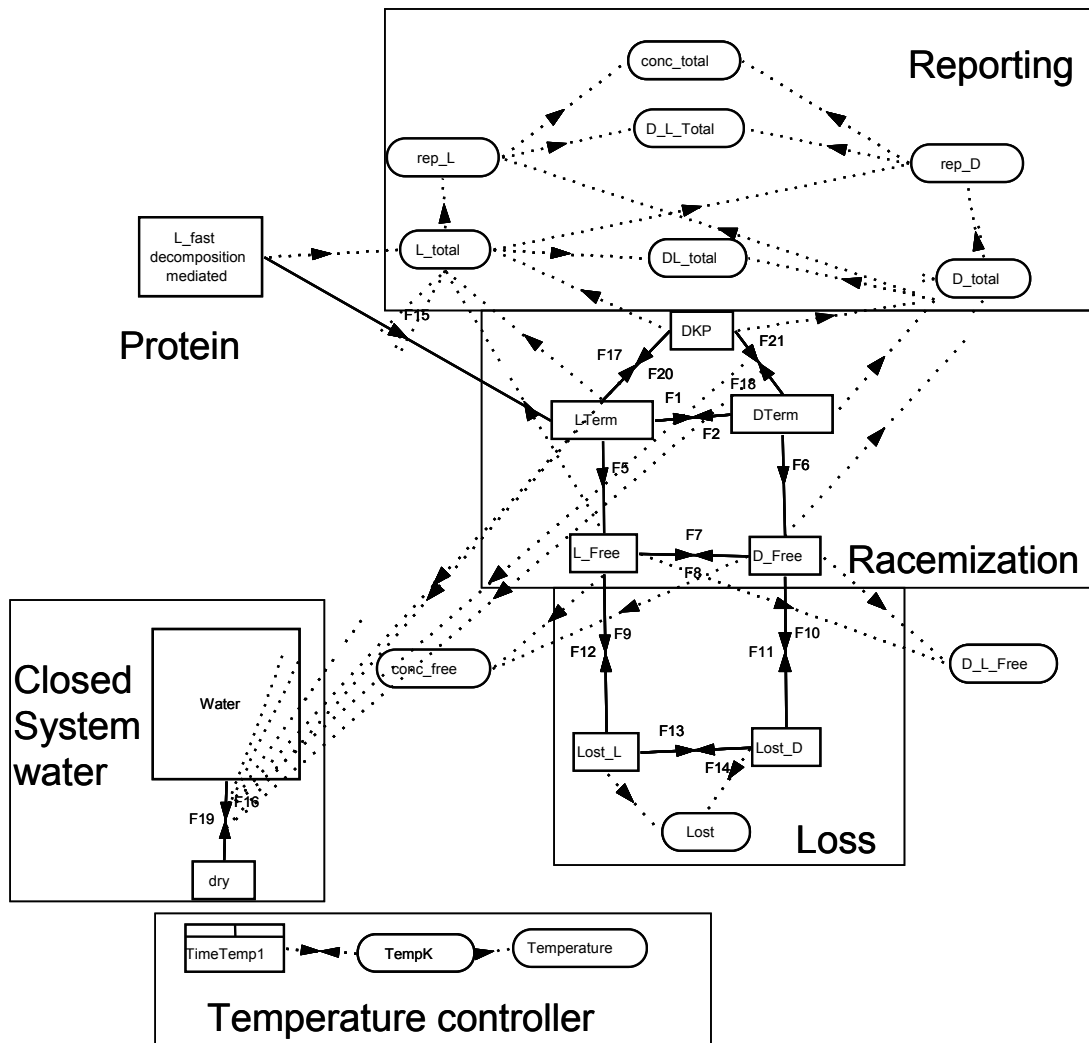
Figure 5.11: Basic structure of the IcPD model.

This illustrated version of the model assumes that the closed system has restricted water, and each water molecule is then taken from the available pool during hydrolysis or released back into the pool during condensation. All reactions are treated as temperature-dependent and have different activation energies, estimated from published literature and our own experimental data

Using the observed standard deviations we calculated a normally distributed dataset which was then draped over the temperature model to produce a probablility distribution of the age. The results of this approach are illustrated for two sites, Funthams Lane (MIS 7) and Hoxne (MIS 11). The envelope is an underestimate of the age error as it assumes that the thermal history model is correct and does not account for any errors within it.

Note that in the case of Hoxne (Fig 5.13; purple) the distribution of data is narrow, albeit slightly wider for the 2001 sample (which has a larger standard deviation). The narrow distribution at Hoxne is because the model / temperature history combination used in this analysis places the sample in the middle of the warmest part of this stage. Conversely, in the Funthams Lane data (Fig 5.13; green) there is a small probability that the samples are from early MIS 5, but on the balance of probability it falls late in MIS 7.
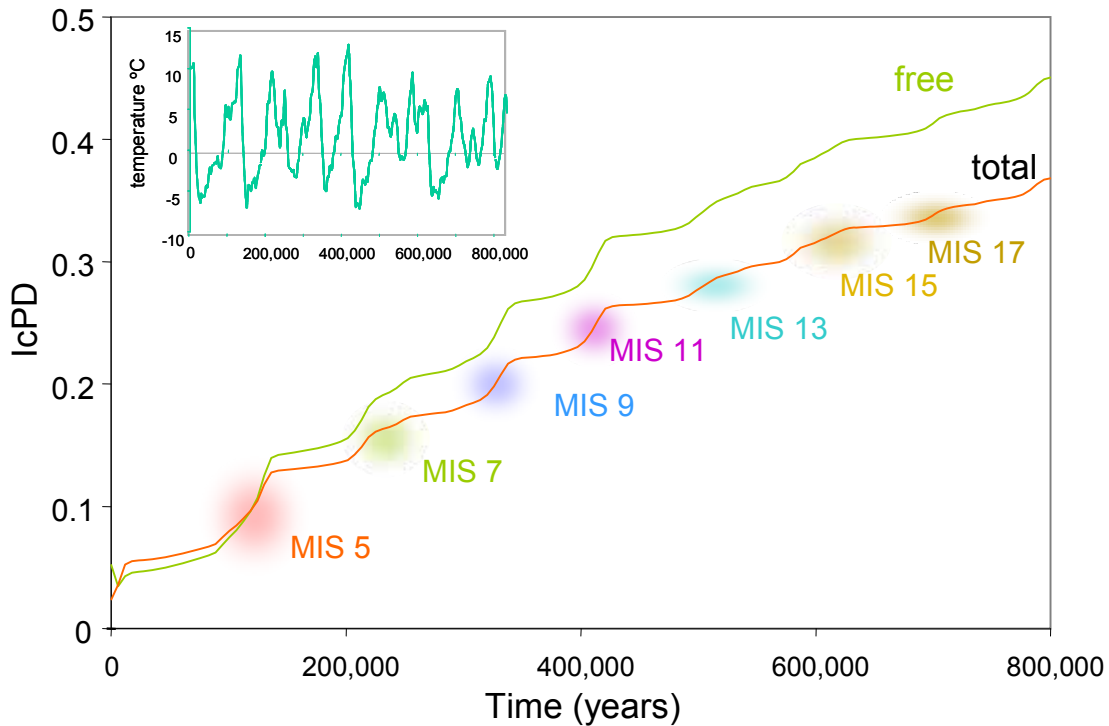
Figure 5.12: Example of output from IcPD model for the Free (green) and Total (Hydrolysed; red) fractions.

MIS correlations are shown on the total fraction only for simplicity. The temperature model used to drive the reactions is shown as in inset. Note the stepped nature of the increase in racemization with age (caused by the much faster rates of racemization in warm than in cold stages. The ability to discriminate between isotope stages declines with time, firstly because of the tendency for the reaction to slow, and secondly because up until approximately 800ka the periodicity of the climate change was governed by the obliquity cycle of the earth, due to variations in its axial tilt, leading to a 41ka cycle. After this time the eccentricity of the earth's orbit became the main forcing mechanism, creating a stronger 100ka cycle

If a site falls within the middle of a warm stage the age error is (relatively) small. However if the sites lies at the beginning or end of a stage, the lack of racemization during the intervening cold period means that there is a probablility that the age may fall in either the intervening cold stage or the 'adjacent' warm stage.

We envisage that for end users the optimal situation would be for data to be provided by a laboratory along with software that would allow them to explore the meaning of this data by running such a probability density model using different temperature scenarios, in a manner analogous to ^{14}C calibration curves.

This will eventually result in a means of deriving age estimates directly from IcPD values. However, the absolute date derived from this model will of course be a function of the accuracy of the temperature record used. It is clear that the temperature records currently available do not capture the true temperatures experienced in the terrestrial environment, as evidenced by the fossil fauna. Therefore the data are presented in this study solely in the form of an aminostratigraphic framework.
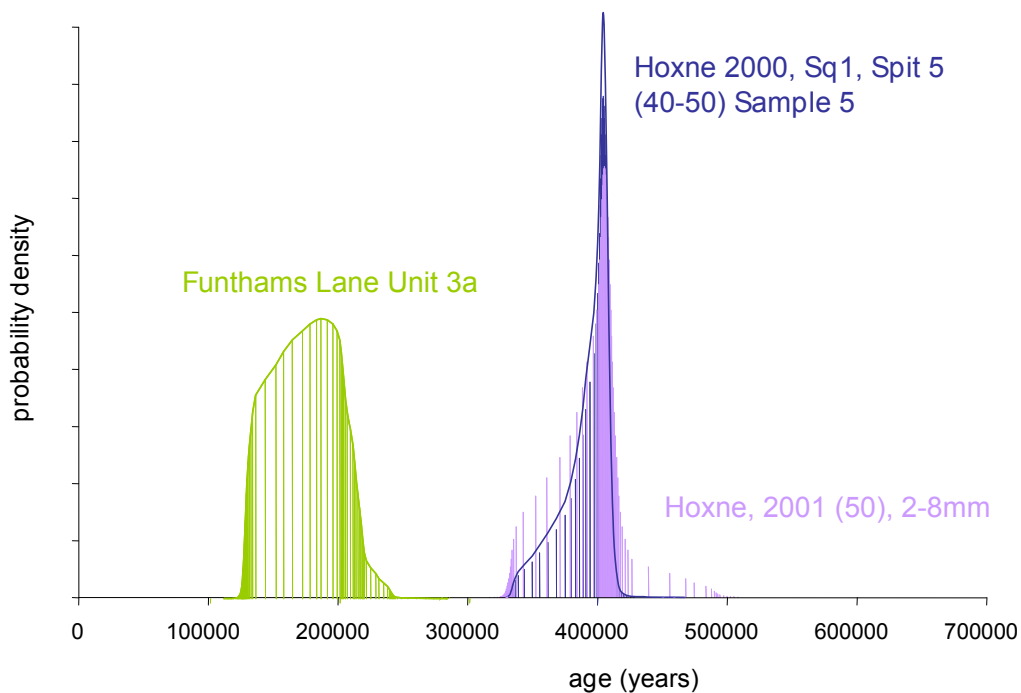
Figure 5.13: A first attempt of the probability density distribution of three samples (for more discussion see text)

5.8 Statistical reporting of data

At present the greatest obstacle to reporting the data for an end user is our difficulty in finding a method to report the sum of errors within our overall estimate of Total and Free IcPD. With this is compounded the problem of deriving age estimates – because the latter are dependent upon temperature models. Each of these problems is being considered and strategies for resolving them are presented.

The problem of compounded errors appears to be complex due to the large number of different pieces of data obtained from a single operculum. The value of these different pieces of data to the overall estimate of IcPD varies (a) because some of the measurements are inherently more reliable than others and (b) because as protein decomposition progresses, the relative value of different pieces of data changes. In the former case, using internal standards and comparison of replicate data, the problem can be resolved (although the values are different for the free and total measurements). In the latter case fast racemizing amino acids (eg Asx) give greater discrimination than slow racemizing amino acids (eg Glx) in young samples, but as the fast racemizers reach equilibrium, it is the slow racemizers which provide the resolution.
We have used three student projects to explore these issues (one in collaboration with Caitlin Buck, University of Sheffield) but now believe that a more sustained piece of work is required to find an optimal solution to this apparently trivial problem.

5.9 Eocene samples

One of the most exciting outcomes of the project was the ability to isolate a closed intra-crystalline fraction of protein from samples within the Eocene. Two opercula of *Bithynia conica* were analysed from the Bembridge Limestone, dating from the late Eocene of the Isle of Wight (about 33 million years old; Curry *et al* 1978). The amino acids obtained from the intra-crystalline fraction were racemic, as would be expected from samples of this age and therefore indicating that the amino acids were indeed original. Of most interest were the relative abundances of the

amino acids – with the more unstable amino acids such as aspartic acid and serine under-represented and the more stable amino acids (Gly, Ala) dominating the protein content. This is exactly what would be expected if the protein was original.

The concentration of the amino acids was extremely low in these samples, with almost no aspartic acid present, by far the most concentrated amino acid in modern *Bithynia* opercula. The amino acid composition differed greatly from that found in modern and Quaternary samples (Fig 5.14). The levels of aspartic acid, serine, and phenylalanine were only just above that of the background level. However alanine was the most abundant amino acid, with the majority present in the Free form. As the most stable amino acid, the longevity of alanine in the burial environment compared to the other amino acids is expected. This therefore supports the hypothesis that these amino acids isolated in the bleached fraction are original and have not been contaminated by exogenous amino acids. The concentration of glutamic acid was about a quarter of that of alanine, with only about 15% in the Free fraction, probably due to lactam formation.
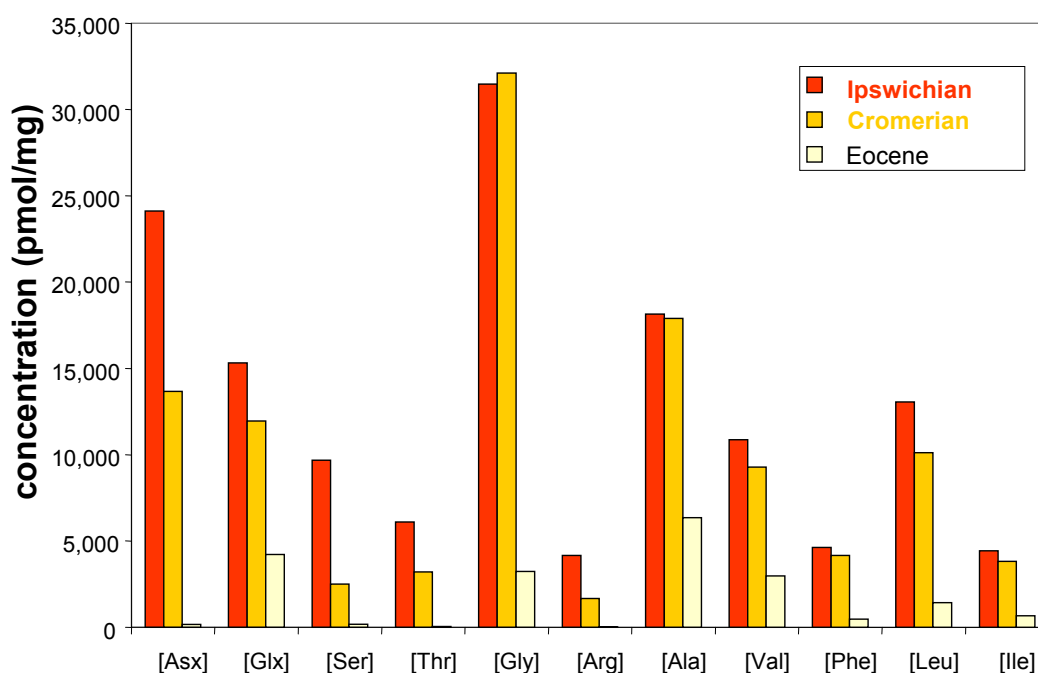
Figure 5.14: Average concentrations of amino acids in Quaternary (Ipswichian and Cromerian) and Eocene samples

It is therefore clear that this closed system of protein can be isolated from samples as far back as the Eocene. The pattern of amino acid composition opens up the potential for the use of amino acid breakdown as a chronological tool, well beyond the range of the racemization reactions. Although the level of resolution at these timescales will not be on the order of that obtainable through both racemization and degradation, the potential of using this technique on samples of this great antiquity is extremely exciting.

6. CONCLUSIONS

A number of methodological advances were developed during this study. The Reverse Phase–High Pressure Liquid Chromatography methodology enabled the resolution of multiple amino acid D/L values. Bleach treatment of the opercula powders removed contaminants and the degradable organic matrix. Bleaching experiments conducted upon unheated and powders of

modern opercula revealed the isolation of an intra-crystalline protein fraction, comprising ~10% of the total protein concentration. Leaching experiments failed to identify significant loss of the intra-crystalline fraction into the environment. The racemization and decomposition kinetics of the proteins in the intra-crystalline fraction was compared to that of the whole shell and the supernatant water. Racemization of free amino acids was used for comparison to the opercula protein kinetics.

The isolation of the intra-crystalline fraction allowed the development of the Intra-crystalline Protein Degradation (IcPD) approach, enabling the multiple amino acid data to be combined and highlighting a strong correlation between the extent of deterioration / racemization of Free and Total amino acids within this fraction. This IcPD approach both ensured the analysis of only the original amino acids, and allowed the identification of bacterial contamination and post-depositional recrystallization (Preece and Penkman 2005).

The difference in amino acid ratios from different species within the same site indicates the importance of developing an aminostratigraphic framework using single species. This difference in rate could be due to differences in the shell ultrastructure or in the different amino acid compositions of the proteins within the biomineral. The rates of reaction have been shown to be dependent on the individual proteins, so it is unsurprising that a difference in observed racemization rate is seen in the different species. However, within a single species the protein and structural composition should be identical, and therefore the fraction retained within the intra-crystalline fraction should be directly comparable.

With the isolation of a closed system, the extent of protein decomposition should be predictable, leading to the development of a relative dating technique, enabling the direct comparison of samples that have experienced the same thermal history. The racemization rates of amino acids would be expected to be strongly influenced by the temperature fluctuations of the environment, decreasing during cold stages and increasing in warm stages. The 'shoehorning' of UK glacial and interglacial sediments into marine oxygen isotope stages has been seductive, but it is proposed in this study that there is strong evidence for the indentification of MIS 5, 7, 9, 11, and post-12 deposits within the UK based on protein decomposition.

6.1 Significance of the *Bithynia* opercula

From previous work undertaken at NEaar, there appeared to be a greater smearing of the D/L values from older sites in the bleached gastropods, resulting in a less clear separation between the proposed isotope stage 'pigeonholes' past MIS 9. Although this could be due to reworking of the samples, it is possible that the potential recrystallization of the aragonitic shell plays a significant role in the integrity of the crystal structure of the shell as samples get older. Whilst the Cromerian gastropods analysed, *Valvata piscinalis* and *Bithynia* shells from West Runton, did show separation from the Clacton aminozone cluster, correlated with MIS 11, despite this, the separation between the West Runton and MIS 11 cluster was relatively small compared to the large separation seen with the *Bithynia* opercula, which indicates that the resolution within the opercula is be greater (Penkman *et al* 2007a; in press).

The preliminary results from the *Bithynia* opercula led to their targeting in this study. A total of 59 UK Quaternary sites have been studied using individual opercula from the gastropod *Bithynia*. Free and Total IcPD values were obtained from bleached samples. The importance of distinguishing between the Free and Total amino acids has been highlighted and it is recommended that these two analyses are undertaken routinely on subsamples of individual shells. The cross-correlation of these two analyses enables the identification of compromised samples (Preece and Penkman 2005). The bleached intra-crystalline data from the opercula generally support the original amino acid framework developed for the UK (Hughes 1987; Bowen *et al*

1989; Bowen 1999; 2000), although significantly the sites that do not confirm the original A/I assignments are those where the original amino acid age estimates proved controversial. It appears that the majority of leaching in fossil gastropods occurs relatively early in the burial history of the shell. This could result in the original analysis of the whole shell actually measuring the amino acids within the intra-crystalline fraction, without bleach pre-treatment. However, the results do differ at certain key sites.

The site at Purfleet, believed to correspond to MIS 9, has yielded shells with inconsistent A/I values (Miller *et al* 1979; Bowen *et al* 1989). Bleaching of gastropods from Purfleet did not significantly improve the scatter of data. However, the problematic high and variable ratios obtained from this site on unbleached and bleached *Corbicula* (Bowen *et al* 1995) and bleached shells in Penkman (2005) appear to have been overcome by the utilisation of the calcitic *Bithynia tentaculata* opercula (Penkman *et al* 2007). These opercula results correlate the Purfleet deposits with those of Grays and Cudmore Grove and therefore to MIS 9.

The type site of the Hoxnian interglacial at Hoxne in Suffolk has been the subject of debate since the publication of ESR and AAR data indicating a correlation with MIS 9 as opposed to an immediately post-Anglian interglacial age (Grün *et al* 1988; Bowen *et al* 1989). However, the mammalian fauna (Schreve 1997) and revised U-series/ESR age calculations (Grün and Schwarcz 2000) both indicate a MIS 11 correlation. The analyses on bleached gastropods were not able to resolve this, but as can be seen in Figure 5.2, the bleached opercula tightly cluster with other MIS 11 sites such as Clacton and Swanscombe, which is supported by the mammalian biostratigraphy. The pre-Anglian site at Waverley Wood has yielded hand-axes, thus making it one of the earliest archaeological sites in the UK and its dating especially significant. A/I ratios on Trichia indicated an age older than that of the Cromerian type site of West Runton, but the presence of Arvicola, the descendant of Mimomys savini (found at West Runton), has fuelled debate over their relative ages (Bowen *et al* 1989; Preece and Parfitt 2000). The *Bithynia troschelii* opercula results from Waverley Wood and West Runton indicate that the site at Waverley Wood is the younger, which supports the mammalian and molluscan biostratigraphy (Penkman *et al* in press).

The isolation of the intra-crystalline fraction, correlation of the Free and Hyd IcPD values and the use of calcitic opercula has greatly strengthened amino acid racemization as a stratigraphic tool. With these modifications, it can now be used with far greater confidence to determine the ages of Quaternary sites. It is clear that the *Bithynia* opercula have been particularly important in enabling the correlation of the amino acid ratios with marine oxygen isotope stages, especially at the sites which produced problematic D/L values of mollusc shells.

The clustering of the sites (Figs 5.1, 5.2, and 5.4) appears to support previous stratigraphic assignments based on methods such as biostratigraphy, geomorphology, and terrace stratigraphy. The consistency of measurements further suggests that sub-stages can be discriminated at least to MIS 7 (Fig 5.2). Without the added resolution of the opercula, this sub-stage separation would not be possible.

Unfortunately, at a number of high-profile sites (Hoxne, Waverley Wood, West Runton) earlier results from AAR do now appear to require revision. It is notable that even in these cases the problems with amino acid racemization dating was either already identified (Purfleet) or appeared to be the result of diagenetic modification, as observed for the shells from Hoxne. Furthermore, at sites such as Hoxne, new cuttings are revealing a more complex stratigraphy with intervening cold stages between fossiliferous sequences. The only site where there is clear disparity between this work and the earlier studies is in Cromerian materials, and at these sites it is only by using the more diagenetically stable calcitic opercula that it is possible to provide sufficient discrimination to distinguish these sites.

The data suggest that this calcite structure is less susceptible to diagenesis than aragonitic biominerals, allowing the excellent preservation of the intra-crystalline closed system of amino acids. Analysis of this fraction offers a simple and cheap geochronological tool for correlation of terrestrial sediments with marine oxygen isotope stages and substages.

This study has shown that the IcPD of opercula can be used to resolve not only stages but sub-stages within the Quaternary, and the separation of individual temperate events within the Cromerian Complex. Further work following on from this project has found this coherent calcite intra-crystalline system allows the development of an IcPD chronology to at least the Pliocene (> 2.5Ma).

The utilisation of these robust biominerals has given increased resolution over the last 0.5Ma and pushed back the range of this technique in Europe far further than expected. Whilst the limits of dating using solely racemization is reached within the Pliocene in the areas studied, the isolation of an intact closed-system of amino acids from Miocene and Eocene opercula opens up a world of other amino acid degradation reactions which can be used for dating. The utility of the overlooked calcitic opercula has been clearly demonstrated and it is believed that this biomineral holds the key to the future of amino acid geochronology.

7. REFERENCES

Abelson, P H, 1954 Amino acids in fossils, *Science*, **119**, 576

Abelson, P H, 1955 Organic constituents of fossils, *Carnegie Inst Washington Yearbook*, **54**, 107–9

Andrews, J T, Bowen, D Q, and Kidson, C, 1979 Amino acid ratios and the correlation of raised beach deposits in south-west England and Wales, *Nature*, **281**, 256–8

Andrews, J T, Davies, K H, Davies, D C, and Miller, G H, 1985 Generic identification of fragmentary Quaternary mollusks by amino acid chromatography – a tool for Quaternary and paleontological research, *Geol J*, **20**, 1–20

Ashton, N, Lewis, S G and Parfitt, S (eds), 1998 *Excavations at the Lower Palaeolithic Site at East Farm, Barnham, Suffolk 1989–1994*, Brit Mus Occas Pap, 125

Ashton, N M, Lewis, S G, and Parfitt, S A, 2000 East Farm, Barnham, Suffolk, in *The Quaternary of Norfolk and Suffolk: Field Guide* (eds S G Lewis, C A Whiteman, and R C Preece), London (Quat Res Ass), 165–75

Ashton, N, Lewis, S, Parfitt, S, Candy, I, Keen, D, Kemp, R, Penkman, K, Thomas, G, and Whittaker, J, 2005 Excavations at the Lower Palaeolithic site at Elveden, Suffolk, UK, *Proc Prehist Soc*, **71**, 1–61

Bates, M R, Wenban-Smith, F F, Bridgland, D R, Collins, M J, Keen, D H, Parfitt, S A, Penkman, K E H, Rhodes, E and Whittaker, J, in prep Late Middle Pleistocene human occupation and palaeoenvironmental reconstruction at Harnham Salisbury (UK): Interim results

Bennet, K D, Peglar, S M, and Sharpe, M J, 1991 Holocene lake sediments in central East Anglia, in *Central East Anglia and the Fen Basin* (eds S G Lewis, C A Whiteman, and D R Bridgland), London (Quat Res Ass), 111–18

Bintanja R, van de Wal R S W, and Oerlemans J, 2005 Modelled atmospheric temperatures and global sea levels over the past million years, *Nature*, **437** (7055), 125–8

Blake, J H, 1877 On the age of the mammalian Rootlet Bed at Kessingland, *Geol Magazine*, **4**, 298–300

Bowen, D Q, 1998 Aminostratigraphy and amino acid geochronology, in *Excavations at the Lower Palaeolithic Site at East Farm, Barnham, Suffolk 1989–1994* (eds N Ashton, S Lewis, and S Parfitt), Brit Mus Occas Pap, 125, 173–4

Bowen, D Q, 1999 *A Revised Correlation of Quaternary Deposits in the British Isles*, Geol Soc London Special Rep, 23

Bowen, D Q, 2000 Revised aminostratigraphy for land-sea correlations from the northeastern North Atlantic margin, in *Perspectives in Amino Acid and Protein Geochemistry* (eds G A Goodfriend, M J Collins, M L Fogel, S A Macko, and J F Wehmiller), Oxford (Oxford Univ Press), 253–62

Bowen, D Q, and Sykes G A, 1988 Correlation of marine events and glaciations on the Northeast Atlantic Margin, *Phil Trans Roy Soc London, B*, **318**, 619–35

Bowen, D Q, and Sykes, G A, 1994 How old is Boxgrove Man, *Nature*, **371**, 751

Bowen, D Q, Sykes, G A, Reeves, A, Miller, G H, Andrews, J T, Brew, J S, and Hare, P E, 1985 Amino acid geochronology of raised beaches in south west Britain, *Quat Sci Rev*, **4**, 279–318

Bowen, D Q, Rose, J, McCabe, A M, and Sutherland, D, 1986 Correlations of Quaternary glaciations in England, Ireland, Scotland and Wales, *Quat Sci Rev*, **5**, 299–340

Bowen, D Q, Hughes, S, Sykes, G A, and Miller, G H, 1989 Land-sea correlations in the Pleistocene based on isoleucine epimerization in non-marine mollusks, *Nature*, **340**, 49–51

Bowen, D Q, Sykes, G A, Maddy, D, Bridgland, D R, and Lewis, S G, 1995 Aminostratigraphy and amino-acid geochronology of English lowland valleys: the lower Thames in context, in *The Quaternary of the Lower Reaches of the Thames: Field Guide* (eds D R Bridgland, P A Allen, B A Haggart), London (Quat Res Ass), 61–3

Bridgland, D R, 1994 Quaternary of the Thames, *Geol Conserv Rev Ser*, **7**

Bridgland, D R, Gibbard, P L, Harding, P, Kemp, R A, and Southgate, G, 1985 New information and results from recent excavations at Barnfield Pit, Swanscombe, *Quat Newslett*, **46**, 25–38

Bridgland, D R, Keen, D H and Davey, N D W, 1991 The Pleistocene sequence in the Peterborough district: possible correlations with the deep-sea oxygen isotope record, in *Central East Anglia and the Fen Basin* (eds S G Lewis, C A Whiteman, and D R Bridgland), London (Quat Res Ass), 209–12

Bridgland, D R, Currant, A P, and Preece, R C, 1995 East Mersea Restaurant Site, in *The Quaternary of the Lower Reaches of the Thames: Field Guide* (eds D R Bridgland, P A Allen, B A Haggart), London (Quat Res Ass), 271–4

Bridgland, D R, Field, M H, Holmes, J A, McNabb, J, Preece, R C, Selby, I, Wymer, J J, Boreham, S, Irving, B G, Parfitt, S A, and Stuart, A J, 1999 Middle Pleistocene interglacial Thames-Medway deposits at Clacton-on-Sea, England: reconsideration of the biostratigraphical and environmental context of the type Clactonian Palaeolithic industry, *Quat Sci Rev*, **18**, 109–46

Bridgland, D R, Preece, R C, Roe, H M, Tipping, R M, Coope, G R, Field, M H, Robinson, J E, Schreve, D C, and Crowe, K, 2001 Middle Pleistocene interglacial deposits at Barling, Essex, England: evidence for a longer chronology for the Thames terrace sequence, *J Quat Sci*, **16**, 813–40

Briggs, D J, Gilbertson, D D, Goudie, A S, Osborne, P J, Osmaston, H A, Pettit, M E, Shotton, F W, and Stuart, A J, 1975 New interglacial site at Sugworth, *Nature*, **257**, 477–9

Buckingham, C M, Roe, D A, and Scott, K, 1996 A preliminary report on the Stanton Harcourt channel deposits, (Oxfordshire, England): geological context, vertebrate remains and Palaeolithic stone artefacts, *J Quat Sci*, **11** (5), 397–415

Chambers, F, Mighall, T and Keen, D H, 1996 A Holocene pollen and molluscan record from Enfield Lock, Middlesex, UK, *Proc Geol Ass*, **107** (1), 1–17

Clark, P U, Barnes, D K, McCoy, W D, Miller, B B, and Nelson, A R, 1989 Quaternary aminostratigraphy of Mississippi Valley loess, *Geol Soc America Bull*, **101**, 918–26

Collins M J, and Riley M S, 2000 Amino acid racemization in biominerals, the impact of protein degradation and loss, in *Perspectives in Amino Acid and Protein Geochemistry* (eds G A Goodfriend, M J Collins, M L Fogel, S A Macko, and J F Wehmiller), Oxford (Oxford Univ Press), 120–42

Collins, M J, Waite, E R, and van Duin, E C T, 1999 Predicting protein decomposition: the case of aspartic-acid racemization kinetics, *Phil Trans Roy Soc London, B*, **354** (1379), 51–64

Conway, B W, McNabb, J, and Ashton, N (eds), 1996 *Excavations at Barnfield Pit, Swanscombe, 1968–1972*, Brit Mus Occas Pap, 94

Cook, J, Ashton, N, Coope, G R, Hunt, C O, Lewis, S G, and Rose, J, 1991 High Lodge, Mildenhall, Suffolk, in *Central East Anglia and the Fen Basin* (eds S G Lewis, C A Whiteman, and D R Bridgland), London (Quat Res Ass), 59–69

Coope, G R, 2001 Biostratigraphic distinction of interglacial coleopteran assemblages from

Southern Britain attributed to oxygen isotope stages 5e and 7, *Quat Sci Rev*, **20** (16-17), 1717–22

Coope, G R, 2006 Insect faunas associated with Palaeolithic industries from five sites of pre-Anglian age in central England, *Quat Sci Rev*, **25**, 1738–54

Coope, G R, and Angus, R B, 1975 An ecological study of a temperate interlude in the middle of the Last Glaciation based on coleopteran from Isleworth, Middlesex, *J Animal Ecol*, **44**, 365–91

Cooper, J, 1972 Last interglacial (Ipswichian) non-marine mollusca from Aveley, Essex, *Essex Naturalist*, **33**, 9–14

Currant, A, and Jacobi, R, 2001 A formal mammalian biostratigraphy for the Late Pleistocene of Britain, *Quat Sci Rev*, **20**, 1707–16

Curry, D, Adams, C G, Boulter, M C, Dilley, F C, Eames, F E, Funnell, B M, and Wells, M K, 1978 *A Correlation of Tertiary Rocks in the British Isles*, Geol Soc London Special Rep, 12

Dowling, L A, and Coxon, P, 2001 Current understanding of Pleistocene temperate stages in Ireland, *Quat Sci Rev*, **20**, 1631–42

Ellis, A E, 1978 *British Freshwater Bivalve Mollusca*, Linnean Soc London (Academic Press), Synopses of the British Fauna (New Series), 11

Evans, J, Morse, E, Reid, C, Ridley, E P, and Ridley H N, 1896 The relation of Palaeolithic man to the glacial epoch, *Rep Brit Ass*, Liverpool, 400–16

Falkner, G, Bank, R A, and von Proschwitz, T, 2001 Check-list of the non-marine molluscan species-group taxa of the states of Northern, Atlantic and Central Europe (CLECOM I), *Heldia*, **4**, 1–76

Franks, J W, 1960 Interglacial deposits at Trafalgar Square, London, *New Phytol*, **59**, 145–52

Frere, J, 1800 Account of flint weapons discovered at Hoxne in Suffolk, in a letter to the Rev John Brand, secretary, *Archaeologia*, **13**, 203–5

Gibbard, P L, 1985 *The Pleistocene History of the Middle Thames Valley*, Cambridge (Cambridge Univ Press)

Gibbard, P L, 1994 *Pleistocene History of the Lower Thames Valley*, Cambridge (Cambridge Univ Press)

Gibbard, P L, West, R G, Zagwijn, W H, Balson, P S, Burger, A W, Funnell, B M, Jefferey, D H, De Jong, J, Van Kolfschoten, T, Lister, A M, Meijer, T, Norton, P E P, Preece, R C, Rose, J, Stuart, A J, Whiteman, C A, and Zalaseiwicz, J A, 1991 Early and Late Pleistocene correlations in the southern North Sea basin, *Quat Sci Rev*, **10**, 23–52

Green, C P, Branch, N P, Coope, G R, Field, M H, Keen, D H, Wells, J M, Schwenninger, J-L, Preece, R C, Schreve, D C, Canti, M G, and Gleed-Owen, C P, 2006 Marine Isotope Stage 9 environments of fluvial deposits at Hackney, north London, UK, *Quat Sci Rev*, **25**, 89–113

Grün, R and Schwarcz, H P, 2000 Revised open system U-series/ESR age calculations for teeth from Stratum C at the Hoxnian Interglacial type locality, England, *Quat Sci Rev*, **19**, 1151–4

Grün, R, Schwarcz, H P and Chadham, J, 1988 ESR dating of tooth enamel: Couple correction for U-uptake and U-series disequilibrium, *Nuclear Tracks and Radiation Measurements*, **14**, 237–41

Hare, P E, and Abelson, P H, 1967 Racemization of amino acids in fossil shells, *Carnegie Inst Washington Yearbook*, **66**, 526–8

Hare, P E, von Endt, D W, and Kokis, J E, 1997 Protein and amino acid diagenesis dating, in *Chronometric Dating in Archaeology* (eds R E Taylor and M J Aitken), 1st edn, New York (Plenum Press), Advances in Archaeological and Museum Science, 2, 261–96

Hearty, P J, 1986 An inventory of Last Interglacial (sensu lato) age deposits from the Mediterrenean Basin: a study of isoleucine epimerization and U-series dating, *Zeitschrift für Geomorphologie*, **62**, 51–69

Hearty, P J, Miller, G H, Stearns, C E, and Szabo, B J, 1986 Aminostratography of Quaternary shorelines in the Mediterrenean Basin, *Geol Soc America Bull*, **97**, 850–8

Hill, R L, 1965 Hydrolysis of proteins, *Advances in Protein Chem*, **20**, 37–107

Hollin, J T, 1977 Thames interglacial sites, Ipswichian sea levels and Antarctic ice surges, *Boreas*, **6**, 33–52

Holman, J A, and Clayden, J D, 1990 A Late Pleistocene interglacial herpetofauna near Shropham, Norfolk, *Brit Herpetol Soc Bull*, **31**, 31–5

Holyoak, D T, 1983 A Late Pleistocene interglacial flora and molluscan fauna from Thatcham, Berkshire, with notes on mollusca from interglacial deposits at Aveley, Essex, *Geol Magazine*, **120**, 823–9

Holyoak D T, and Preece R C, 1985 Late Pleistocene Interglacial Deposits at Tattershall, Lincolnshire, *Phil Trans Roy Soc London, B*, **311**, 193–236

Horne, D C, 1999 Biostratigraphy and palaeolimnology of Late-glacial and Holocene lake marls at Quidenham Mere, Norfolk, unpubl PhD thesis, Univ Cambridge

Horton, A, Keen, D H, Field, M H, Robinson, J E, Coope, G R, Currant, A P, Graham, D K, Green, C P, and Phillips, L M, 1992 The Hoxnian Interglacial deposits at Woodston, Peterborough, *Phil Trans Roy Soc London, B*, **338**, 131–64

Hughes, S A, 1987 The aminostratigraphy of British Quaternary non-marine deposits, unpubl PhD thesis, Univ Wales

Imbrie J, Shackleton, N J, Pisias, N G, Morley, J J, Prell, W L, Martinson, D G, Hays J D, Macintyre, A, and Mix, A C, 1984 The orbital theory of Pleistocene climate: support from a revised chronology of the marine $\delta^{18}O$ record, in *Milankovitch and Climate, Part 1* (ed A Berger), Reidel, Hingham MA 269–305

Johnson, B J, and Miller, G H, 1997 Archaeological applications of amino acid racemization, *Archaeometry*, **39**(2), 265–87

Kaufman D S, and Manley, W F, 1998 A new procedure for determining D/L amino acid ratios in fossils using Reverse Phase Liquid Chromatography, *Quat Sci Rev*, **17**, 987–1000

Keen, D H, 1990 Significance of the record provided by Pleistocene fluvial deposits and their included molluscan faunas for palaeoenvironmental reconstruction and stratigraphy: case studies from the English Midlands, *Palaeogeogr, Palaeoclimatol, Palaeoecol*, **80**, 25–34

Keen, D H, 1999 The chronology of Middle Pleistocene ('Wolstonian') events in the English Midlands, in *Late Cenozoic Environments and Hominid Evolution: a tribute to Bill Bishop* (eds P Andrews and P Banham), London (Geol Soc), 159–68

Keen, D H, 2001 Towards a late Middle Pleistocene non-marine molluscan biostratigraphy for the British Isles, *Quat Sci Rev*, **20**, 1657–66

Keen, D H, and Bridgland, D R, 1986 An interglacial fauna from Avon No 3 terrace at Eckington, Worcestershire, *Proc Geol Ass*, **97** (3), 303–7

Keen, D H, Coope, G R, Jones, R L, Field, M H, Griffiths, H I, Lewis, S G, and Bowen, D Q, 1997 Middle Pleistocene deposits at Frog Hall Pit, Stretton-on-Dunsmore, Warwickshire, English Midlands, and their implication for the age of the type-Wolstonian, *J Quat Sci*, **12**, 183–208

Keen, D H, Hardaker, T, and Land, A T O, 2006 A Lower Palaeolithic industry from the

Cromerian (MIS 13) Baginton Formation of Waverley Wood and Wood Farm Pits, Bubbenhall, Warwickshire, UK, *J Quat Sci*, **21**, 457–70

Kennard, A S, 1944 The Crayford brickearths, *Proc Geol Ass*, **55**, 121–69

Kennedy, G L, Lajoie, K R, and Wehmiller, J F, 1982 Aminostratigraphy and faunal correlations of Late Quaternary marine terraces, Pacific Coast, USA, *Nature*, **289**, 162–4

Kimber, R W L, and Griffin, C V, 1987 Further evidence of the complexity of the racemization process in fossil shells with implications for amino acid racemisation dating, *Geochimica et Cosmochimica Acta*, **51**, 839–46

Kimber, R W L, and Milnes, A R, 1984 The extent of racemization of amino acids in Holocene and Pleistocene marine mollusks in southern South Australia – preliminary data on a time framework for calcrete formation, *Australian J Earth Sci*, **31**, 279–86

Kerney, M P, 1971 Interglacial deposits at Barnfield Pit, Swanscombe, and their molluscan fauna, *J Geol Soc London*, **127**, 69–93

Kerney, M P, 1976 Mollusca from an interglacial tufa in East Anglia, with the description of a new species of Lyrodiscus pilsry (Gastropoda: Zonditidae), *J Conchol*, **29**, 47–50

King, K, and Hare, P E, 1972 Amino acid composition of the test as a taxonomic chacter for living and fossil foraminifera, *Micropalaeontol*, **18**, 285–93

King Jr, K, and Neville, C, 1977 Isoleucine epimerisation for dating marine sediments: importance of analysing monospecific foraminiferal samples, *Science*, **195**, 1333–35

Lajoie, K R, Peterson, E, and Gerow, B A, 1980 Amino acid bone dating: a feasibility study, South San Francisco Bay region, California, in *Biogeochemistry of Amino Acids* (eds P E Hare, T C Hoering, and K King Jr), New York (John Wiley and Sons), 477–89

Land, L S, 1967 Diagenesis of skeletal carbonates, *J Sedimentary Petrol*, **37**, 914–30

Lewis, S G, Maddy, D, and Glenday, S, 2004a The Thames valley sediment conveyor: fluvial system development over the last two interglacial-glacial cycles, *Quaternaire*, **15**, 17–28

Lewis, S G, Parfitt, S A, Preece, R C, Sinclair, J, Coope, G R, Field, M H, Maher, B A, Scaife, R G, and Whittaker, J E, 2004b Age and palaeoenvironmental setting of the Pleistocene vertebrate fauna at Norton Subcourse, Norfolk, in *The Quaternary Mammals of Southern and Eastern England: Field Guide* (ed DC Schreve), London (Quat Res Ass), 4–14

Lightwing, J, 1982 Vertebrates from a new site at Coston, Norfolk, *Bull Geol Soc Norfolk*, **33**, 73

Maddy, D, 1997 Uplift-driven valley incision and river terrace formation in southern England, *J Quat Sci*, **12** (6), 539–45

Maddy, D, 1999 English Midlands, in *A Correlation of Quaternary Deposits in the British Isles* (ed D Q Bowen), Geol Soc London Special Rep, **23**, 28–44

Maddy, D, Keen, D H, Bridgland, D R, and Green, C P, 1991 A revised model for the Pleistocene development of the River Avon, Warwickshire, *J Geol Soc London*, **148** (3), 473–84

Maddy, D, Lewis, S G, Scaife, R G, Bowen, D Q, Coope, G R, Green, C P, Hardaker, T, Keen, D H, Rees-Jones, J, Parfitt, S, and Scott, K, 1998 The Upper Pleistocene deposits at Cassington, near Oxford, England, *J Quat Sci*, **13**, 205–31

McCarroll, D, 2002 Amino-acid geochronology and the British Pleistocene: secure stratigraphical framework or a case of circular reasoning?, *J Quat Sci*, **17**, 647–51

Meijer, T, 1985 The pre-Weichselian non-marine molluscan fauna from Maastricht-Belvédère (Southern Limburg, The Netherlands), *Mededelingen Rijks Geologische Dienst*, **39**, 75–103.

Meijer, T, and Preece, R C, 2000 A review of the occurrence of *Corbicula* in the Pleistocene of North-West Europe, *Geologie en Mijnbouw-Netherlands J Geosci*, **79**, 241–55

Mellars, P A, 1998 *Star Carr in context: new archaeological and palaeoecological investigations at the early Mesolithic site of Star Carr, North Yorkshire*, Cambridge, McDonald Inst Archaeol Res Monogr

Miller, G H, and Hare, P E, 1975 Use of amino acid reactions in some Arctic marine shells: stratigraphic and geochronological indicators, *Carnegie Inst Washington Yearbook*, **74**, 612–17

Miller, G H, and Andrews, J T, 1983 Characterization of molluscan taxa by their amino acid composition: a potential phylogenetic index, *Geol Soc America Abstracts*, **15**, 644

Miller, G H, and Mangerud, J, 1985 Aminostratigraphy of European marine interglacial deposits, *Quat Sci Rev*, **4**, 215–78

Miller, G H, and Brigham-Grette, J, 1989 Amino acid geochronology: resolution and precision in carbonate fossils, *Quat Int*, **1**, 111–28

Miller, G H, Hollin, J T, and Andrews, J T, 1979 Aminostratigraphy of UK Pleistocene deposits, *Nature*, **281** (5732), 539–43

Miller, G H, Hart, C P, Roark, E B, and Johnson, B J, 2000 Isoleucine epimerization in eggshells of the flightless Australian birds *Genyornis* and *Dromaius*, in *Perspectives in Amino Acid and Protein Geochemistry* (eds G A Goodfriend, M J Collins, M L Fogel, S A Macko, and J F Wehmiller), Oxford (Oxford Univ Press), 161–81

Mitchell, G F, Penny, L F, Shotton, F W, and West, R G, 1973 *A Correlation of Quaternary Deposits in the British Isles*, Geol Soc London Special Rep, 4

Murray-Wallace, C V, 1993 A review of the application of the amino acid racemisation reaction to archaeological dating, *Artefact*, **16**, 19–26

Murray-Wallace, C V, Belpeiro, A P, Kimber, R W L, and Gostin, A V, 1988 Aminostratigraphy of the Last Interglacial in Southern Australia, *Search*, **19**, 33–6

Oakley, K P, 1957 Stratigraphical age of the Swanscombe skull, *Amer J Phys Anthropol*, **15** (2), 253–60

Oakley, K P, Andrews, P, Keeley, L H, and Clark, J D, 1977 A reappraisal of the Clacton spearpoint, *Proc Prehist Soc*, **43**,13–30

Parfitt, S A, Barendregt, R W, Breda, M, Candy, I, Collins, M J, Coope, G R, Durbidge, P, Field, M H, Lee, J R, Lister, A M, Mutch, R, Penkman, K E H, Preece, R C, Rose, J, Stringer, C B, Symmons, R, Whittaker, J E, Wymer, J J, and Stuart, A J, 2005 The earliest humans in Northern Europe: artefacts from the Cromer Forest-bed Formation at Pakefield, Suffolk, UK, *Nature*, **438**, 1008–12

Penkman, K E H, 2005 Amino acid geochronology: a closed system approach to test and refine the UK model, unpubl PhD thesis, Univ Newcastle

Penkman, K E H, Preece, R C, Keen, D H, Maddy, D, Schreve, D C and Collins, M, 2007a Amino acids from the intra-crystalline fraction of mollusc shells: Applications to geochronology, *Quat Sci Rev*, **26**, 2958–69

Penkman, K, Collins, M, and McGrory, S, 2007b *Medway Valley Palaeolithic Project: Amino Acid Racemization Analysis*, EH Res Dep Rep Ser, **73/2007**

Penkman, K E H, Kaufman, D S, Maddy, D and Collins, M J, 2008 Closed-system behaviour of the intra-crystalline fraction of amino acids in mollusc shells, *Quat Geochronol*, **3**, 2-25

Penkman K E H, Preece, R C, Keen, D H, and Collins, M J, in press Amino acid geochronology of the type Cromerian of West Runton, Norfolk, UK, *Quat Int*

Preece, R C, 1995 Mollusca from interglacial sediments at three critical sites in the Lower Thames, in *The Quaternary of the Lower Reaches of the Thames: Field Guide* (eds D R Bridgland, P A Allen, and B A Haggart), London (Quat Res Ass), 53–62

Preece, R C, 1999 Mollusca from Last interglacial fluvial deposits of the River Thames at Trafalgar Square, London, *J Quat Sci*, **14** (1), 77–89

Preece, R C, 2001 Molluscan evidence for differentiation of interglacials within the 'Cromerian Complex', *Quat Sci Rev*, **20** (16-17), 1643–56

Preece, R C, and Parfitt, S A, 2000 The Cromer Forest-bed formation: new thoughts on an old problem, in *The Quaternary of Norfolk and Suffolk: Field Guide* (eds S G Lewis, C A Whiteman, and R C Preece), London (Quat Res Ass), 1–27

Preece, R C, and Penkman, K E H, 2005 Molluscan assemblages and amino acid dating of the Lower Palaeolithic site at East Farm, Barnham, Suffolk, *Proc Geol Ass*, 116, 363–77

Preece, R C, Lewis, S G, Wymer, J J, Bridgland, D R, and Parfitt, S, 1991 Beeches Pit, West Stow, Suffolk, in *Central East Anglia and the Fen Basin* (eds S G Lewis, C A Whiteman, and D R Bridgland), London (Quat Res Ass), 94–104

Preece, R C, Bridgland, D R, Lewis, S G, Parfitt, S A, and Griffiths, H I, 2000 Beeches Pit, West Stow, Suffolk, in *The Quaternary of Norfolk and Suffolk: Field Guide* (eds S G Lewis, C A Whiteman, and R C Preece), London (Quat Res Ass), 185–95

Preece, R C, Parfitt, S A, Bridgland, D R, Lewis, S G, Rowe, P J, Atkinson, T C, Candy, I, Debenham, N C, Penkman, K E H, Rhodes, E J, Schwenninger, J-L, Griffiths, H I, Whittaker, J E, and Gleed-Owen, C, 2007 Terrestrial environments during MIS 11: evidence from the Palaeolithic site at West Stow, Suffolk, UK, *Quat Sci Rev*, **26**, 1236-1300

Reid, C, 1882 *The geology of the country around Cromer*, Memoirs Geol Survey England and Wales

Reid, C, 1890 *The Pliocene deposits of Britain*, Memoirs Geol Survey England and Wales

Roberts, M B, and Parfitt, S A, 1997 *Boxgrove: A Middle Pleistocene Hominid Site at ARC Eartham Quarry, Boxgrove, West Sussex, UK*, London (Engl Heritage)

Roberts, M B, Stringer, C B, and Parfitt, S A, 1994 A hominid tibia from Middle Pleistocene sediments at Boxgrove, UK, *Nature*, **369**, 311–13

Roe, H M, and Preece, R C, 1995 A new discovery of the Middle Pleistocene 'Rhenish' fauna in Essex, *J Conchol*, **35**, 272–3

Roe, H M, 1995 The Cudmore Grove Channel Site, in *The Quaternary of the Lower Reaches of the Thames: Field Guide* (eds D R Bridgland, P A Allen, and B A Haggart), London (Quat Res Ass), London 258–69

Rose, J, 1987 Status of the Wolstonian Glaciation in the British Quaternary, *Quat Newslett*, **53**, 1–9

Rutter, N W, and Blackwell, B, 1995 Amino acid racemization dating, in *Dating methods for Quaternary deposits* (eds N W Rutter and N R Catto), St John's, Newfoundland (Geol Assoc Canada), 125–64

Schreve, D C, 1997 Mammalian biostratigraphy of the later Middle Pleistocene in Britain, unpubl PhD thesis, Univ London

Schreve, D C, 2001a Mammalian evidence from Middle Pleistocene fluvial sequences for complex environmental change at the oxygen isotope substage level, *Quat Int*, **79**, 65–74

Schreve, D C, 2001b Differentiation of the British late Middle Pleistocene interglacials: the

evidence from mammalian biostratigraphy, *Quat Sci Rev*, **20**, 1693–1705

Schreve, D C, Bridgland, D R, Allen, P, Blackford, J J, Gleed-Owen, C P, Griffiths, H I, Keen, D H, and White, M J, 2002 Sedimentology, palaeontology and archaeology of late Middle Pleistocene River Thames terrace deposits at Purfleet, Essex, UK, *Quat Sci Rev*, **21**, 1423–64

Schwarcz, H P, and Grün, R, 1993 Electron Spin Resonance (ESR) dating of the Lower Industry, In Singer R, Gladfelter, B G and Wymer, J J (eds), *The Lower Palaeolithic site at Hoxne, England*, Chicago (Univ Chicago Press), 210–11

Scott, K, 2001 Late Middle Pleistocene Mammoths and Elephants of the Thames Valley, Oxfordshire, in *The World of Elephants - International Congress, Rome* (eds G Cavaretta, P Gioia, M Mussi, M R Palomba), Rome (Consiglio Nazionale delle Richerche-Roma), 247–54

Seddon, M B, 1998 Mollusca from the Lower Palaeolithic Site at Barnham, in *Excavations at the Lower Palaeolithic Site at East Farm, Barnham, Suffolk 1989–1994* (eds N Ashton, S Lewis, and S Parfitt), Brit Mus Occas Pap, 125, 173–74

Shotton, F W, 1953 Pleistocene deposits of the area between Coventry, Rugby and Leamington and their bearing upon the topographic development of the Midlands, *Phil Trans Roy Soc London, B*, **237**, 209–60

Shotton, F W, 1983 Interglacials after the Hoxnian in Britain, in *Quaternary glaciations in the northern hemisphere* (eds A Billard, O Conchon, and F W Shotton), UNESCO International Geological Correlation Programme, Project 73/1/24, Report 9, Paris, 109–15 (reproduced in *Quat Newslett*, **39**, 20–5)

Shotton, F W and Wymer, J J, 1998 Handaxes of andesitic tuff from beneath the standard Wolstonian succession in Warwickshire, *Lithics*, **10**, 1–7

Shotton, F W, Keen, D H, Coope, G R, Currant, A P, Gibbard, P L, Aalto, M, Peglar, S M, and Robinson, J E, 1993, The Middle Pleistocene deposits of Waverley Wood Pit, Warwickshire, England, *J Quat Sci*, **8**, 293–325

Singer, R, Gladfelter, B G, and Wymer, J J, 1993 *The Lower Palaeolithic Site at Hoxne, England*, Chicago (Univ Chicago Press)

Sparks, B W, 1957 The non-marine Mollusca of the interglacial deposits at Bobbitshole, Ipswich, *Phil Trans Roy Soc London, B*, **241**, 33

Sparks, B W, 1961 The ecological interpretation of Quaternary non-marine Mollusca, *Proc Linnean Soc London*, **172**, 71–80

Stringer, C B, Currant, A P, Schwarcz, H P, and Collcutt, S N, 1986 Age of Pleistocene faunas from Bacon Hole, Wales, *Nature*, **320**, 59–62

Stuart, A J, 1976 The history of the mammal fauna during the Ipswichian/Last Interglacial in England, *Phil Trans Roy Soc London, B*, **276**, 221–50

Stuart, A J, and Lister, A M, 2001 The mammalian faunas of Pakefield/ Kessingland and Corton, Suffolk, UK: evidence for a new temperate episode in the British early Middle Pleistocene, *Quat Sci Rev*, **20**, 1677–92

Sutcliffe, A J, 1964 The mammalian fauna, in *The Swanscombe Skull* (ed C D Ovey), Occas Pap Roy Anthropol Inst, 20, 85–111

Sutcliffe, A J, 1975 A hazard in the interpretation of glacial-interglacial sequences, *Quat Newslett*, **17**, 1–3

Sykes, G A, Collins, M J, and Walton, D I, 1995 The significance of a geochemically isolated intracrystalline organic fraction within biominerals, *Organic Geochem*, **23** (11/12), 1039–65

Thieme, H, 2000 Lower Paleolithic hunting weapons from Schöningen, Germany – the oldest spears in the World, *Acta Anthropologica Sinica*, 19, S136–S143

Tomlinson, M E, 1925 River terraces of the lower valley of the Warwickshire Avon, *Quarterly J Geol Soc London*, 81, 137–163

Warren, S H, 1955 The Clacton (Essex) Channel deposits, *Quarterly J Geol Soc London*, 111, 283–387

Wehmiller, J F, 1980 Intergeneric differences in apparent racemization kinetics in molluscs and foraminifera: Implications for models of diagenetic racemization, in *Biogeochemistry of Amino Acids* (eds P E Hare, T C Hoering, and K King Jr), New York (John Wiley and Sons), 341–55

Wenban-Smith, F F, Allen, P, Bates, M R, Parfitt, S A, Preece R C, Stewart J R, Turner, C, and Whittaker, J E, 2006 The Clactonian elephant butchery site at Southfleet Road, Ebbsfleet, UK, *J Quat Sci*, 21, 471–84

West, R G, 1956 The Quaternary deposits at Hoxne, Suffolk, *Phil Trans Roy Soc London, B*, 239, 265–356

West, R G, 1969 Pollen analysis from interglacial deposits at Aveley and Grays, Essex, *Proc Geol Ass*, 80, 271–82

West, R G, 1980 *The pre-glacial Pleistocene of the Norfolk and Suffolk coasts*, Cambridge (Cambridge Univ Press)

West R G, Lambert, C A, Sparks, B W, 1964 Interglacial deposits at Ilford, Essex, *Phil Trans Roy Soc London, B*, 247, 185–212

West R G, Knudsen K-L, Kenney, D N, Preece, R C, and Robinson, J E, 1994 Palaeontology and taphonomy of Late Quaternary fossil assemblages at Somersham, Cambridgeshire, England, and the problem of reworking, *J Quat Sci*, 9, 357–66

West R G, Andrew, R, Catt, J A, Hart, C P, Hollin, J T, Knudsen, K-L, Miller, G F, Penney, D N, Pettit, M E, Preece, R C, Switsur, V R, Whiteman, C A, and Zhou, L P, 1999 Late and Middle Pleistocene deposits at Somersham, Cambridgeshire, UK: A model for reconstructing fluvial/estuarine depositional environments, *Quat Sci Rev*, 18, 1247–1314

West, R G, 1957 Interglacial deposits at Bobbitshole, Ipswich, *Phil Trans Roy Soc London, B*, 241, 33–44

Wymer, J J, 1964 Excavations at Barnfield Pit, 1955–1960, in *The Swanscombe Skull* (ed C D Ovey), Occas Pap Roy Anthropol Inst, 20, 19–60

Zhou, L P, McDermott, F, Rhodes, E J, Marseglia, E A, and Mellars, P A, 1997 ESR and mass-spectrometric uranium-series dating studies of a mammoth tooth from Stanton Harcourt, Oxfordshire, England, *Quat Sci Rev*, 16, 445–54

ENGLISH HERITAGE RESEARCH DEPARTMENT

English Heritage undertakes and commissions research into the historic environment, and the issues that affect its condition and survival, in order to provide the understanding necessary for informed policy and decision making, for sustainable management, and to promote the widest access, appreciation and enjoyment of our heritage.

The Research Department provides English Heritage with this capacity in the fields of buildings history, archaeology, and landscape history. It brings together seven teams with complementary investigative and analytical skills to provide integrated research expertise across the range of the historic environment. These are:

* *Aerial Survey and Investigation*
* *Archaeological Projects (excavation)*
* *Archaeological Science*
* *Archaeological Survey and Investigation (landscape analysis)*
* *Architectural Investigation*
* *Imaging, Graphics and Survey (including measured and metric survey, and photography)*
* *Survey of London*

The Research Department undertakes a wide range of investigative and analytical projects, and provides quality assurance and management support for externally-commissioned research. We aim for innovative work of the highest quality which will set agendas and standards for the historic environment sector. In support of this, and to build capacity and promote best practice in the sector, we also publish guidance and provide advice and training. We support outreach and education activities and build these in to our projects and programmes wherever possible.

We make the results of our work available through the Research Department Report Series, and through journal publications and monographs. Our publication Research News, which appears three times a year, aims to keep our partners within and outside English Heritage up-to-date with our projects and activities. A full list of Research Department Reports, with abstracts and information on how to obtain copies, may be found on www.english-heritage. org.uk/researchreports

For further information visit www.english-heritage.org.uk

ENGLISH HERITAGE