Chapter 13

Amino acid dating

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INTRODUCTION

A new technique of amino acid racemization (AAR) analysis has been developed over the last 10 years (Penkman 2005; Penkman *et al.* 2007, 2008) that has proved robust and consistently reliable in geo-chronological dating of molluscan remains at the sub-Pleistocene timescale. It has successfully distinguished material to the level of separate marine isotope stages over the last 500,000 years, MIS 1 to MIS13 (Penkman 2010; Penkman *et al.* 2011). This new technique (further explained below) combines a new Reverse-Phase High Pressure Liquid Chromatography method of analysis (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 for multiple amino acids from the chemically-protected protein within the biomineral, enabling both decreased sample sizes and increased reliability of the analysis. The intra-crystalline fraction of the calcitic opercula of the fluvial gastropod *Bithynia tentaculata* has been found to be a particularly robust repository for the original protein, making these opercula the prime (although not the only) source of amino acid data for reliable geochronological determinations.

At the Southfleet Road site other lines of evidence, notably biostratigraphy (Chapters 9, 10 and 11) and geological correlation (Chapter 4), combine to suggest strongly that the interglacial sediments (Phase 6) associated with the elephant skeleton and the dense lithic scatter south of Trench D are attributable to MIS 11, the Hoxnian interglacial. The presence of molluscan remains, including abundant *Bithynia* opercula at certain horizons in the site sequence (Chapter 10), provided the potential to confirm this attribution with the most reliable independent chronometric technique currently available for this time period. Molluscan remains were most abundant in Phase 6b, in the main basal context 40070 of the tufaceous channel sequence and in the overlying contexts 40144 and 40143. Although the more fragile shells of molluscs were absent through the rest of the site sequence, small quantities of the more robust *Bithynia* opercula were also recovered from a number of other deposits (Table 13.1). These include:

1. Context 40078 (Phase 6, though in a part of the deposit more equivalent to context 40158, rather than at the exact horizon of the elephant skeleton);
2. Context 40103 (Phase 6a, in the lowest part of the Phase 6 clay, beneath the tufaceous channel-fill sequence);
3. In the top part of context 40025 (Phase 5), where a few *Bithynia* opercula were recovered from some of the large bulk samples taken for small vertebrates;
4. Context 40062 (Phase 3) where, surprisingly, *Bithynia* opercula were abundant in bulk sample <40042>, taken for small vertebrate assessment, in association with a rich interglacial ostracod fauna (Chapter 11).

Clearly, the possibility of derivation has to be considered for the opercula in these last deposits where they are not in association with other Pleistocene molluscan remains, and this is returned to below when considering the dating results.

A programme of amino acid dating analysis was undertaken that focused on these remains. It had the aims of:

1. Confirming the attribution of Phase 6b to MIS 11;
2. Investigating whether underlying and overlying deposits with *Bithynia* opercula were also attributable to MIS 11, or whether they should be attributed to other MIS stages;
3. Investigating whether it was possible to achieve intra-MIS 11 sub-stage distinction between material from different horizons based on the amino acid dating results;
4. Trying to establish correlations with key horizons from other English MIS 11 sites with significant Palaeolithic archaeological remains (Table 13.2), in conjunction with which the evidence from Southfleet Road is later considered (Chapter 22).

The results of this programme are summarised and discussed below; full details of the methods and individual analyses are also presented as Appendix 9. Analyses were carried out at the University of York amino acid laboratory (NEaar) between 2005 and 2010.

Table 13.1 Southfleet Road samples from which *Bithynia* opercula were picked for amino acid dating
[samples marked * represent material picked from small vertebrate samples]

Phase	*Context*	*Sample <>*	*Figure*	*Sample source*	*Quantity*	*NEaar lab code/s*	*Result code/s*	*Context grouping [Fig. 13.3]*
6	40078 [=40158]	40275*	-	Bulk sample	2	6433-4	SFR-4	6 - 40078
6b	40143	40282/C/0-2	Fig. 13.1d	Mollusc sub-sample from monolith	3	6430-2	SFR-3	6b - 40143
6b	40144	40295/C	Fig. 13.1b	Mollusc sub-sample from bulk sample	3	6435-7	SFR-5	6b - 40144
6b	40144	40333*	-	Bulk sample	3	6438-40	SFR-6	
6b	40070	40315/C	Fig. 13.1b	Mollusc sub-sample from bulk sample	3	6424-26	SFR-1	6b - 40070
"	"	"	"	"	8	6606-13	SFR-1	
6b	40070	40317/C	Fig. 13.1b	Mollusc sub-sample from bulk sample	3	6427-29	SFR-2	
6b	40070	40162*	Fig. 13.1a	Bulk sample	7	2041-7	Eb-A	-
6a	40103	40320*	Fig. 13.1b	Bulk sample	2	6441-2	SFR-7	6a - 40103
5	40025	40286*	Fig. 13.1c	Bulk sample	2	6443-4	SFR-8	5 - 40025
5	40025	40343*	Fig. 13.1c	Bulk sample	2	6445-6	SFR-9	
3	40062	40042*	Fig. 13.1a	Bulk sample	2	6166-7	SFR-10	3 - 40062
"	"	"	"	"	3	6447-9	SFR-10	
"	"	"	"	"	5	6534-38	SFR-10	
"	"	"	"	"	3	6614-16	SFR-10	

Table 13.2 MIS 11 comparator sites contributing to UK amino acid dating framework [based on *Bithynia* opercula]

Site	*Location within site*	*Context, bed*	*Sample <>*	*Sample source*	*No. analysed*	*Identifier for Fig. 13.5*	*Reference*
Hoxne	-	Stratum B2	'(64)' 2-8 mm	BM/AHOB excavations, 2001	4	Ho-B2	West 1956; Ashton *et al.* 2008
Hoxne	-	Stratum B2	'(50)' 2-8 mm	BM/AHOB excavations, 2001	4		
Swanscombe, Barnfield Pit	-	Lower Loam	Uncertain depth in sediment	Sampled by J. Rose, EuroMam 2004 (10-14 May)	6	Sw	Ovey 1964; Kerney 1971; Conway *et al.* 1996
Barnham	Pit 4	Bed 5c	118-128cm	BM excavation, 1993 (BEF 93)	6	Ba	Preece and Penkman 2005; Ashton *et al.* 1998
Hoxne	-	Stratum E	Sq 1,spit 5 (40-50), 'Sample 5'	BM/AHOB excavations, 2000	4	Ho-E	West 1956; Ashton *et al.* 2008
Beeches Pit, West Stow	Cutting 2	Bed 7	-	'1998 series'	4	BP	Preece *et al.* 2007

AMINO ACID DATING: THEORY AND METHODS

A detailed review of the theory and methods behind the new approach to amino acid dating applied here is provided in Appendix 9. In summary, amino acids, the building blocks of proteins that occur within mollusc shells and opercula, occur as two isomers that are chemically identical, but optically different. These isomers are designated as either D or L, depending upon whether they rotate plane-polarised light to the right or left respectively. In living organisms, the amino acids are almost exclusively L and the D/L value approaches zero. The potential application to geochronology arises from the fact that after death, amino acid isomers start to interconvert (or 'racemise'), trending over sufficient time towards parity with the D/L value approaching one. The proportion of D to L amino acids can therefore be taken as a proxy for time passed. Once scaled by an independent framework based on lithostratigraphy, biostratigraphy and other geochronological methods, can be used to estimate the age of a fossil sample, alongside other indications of protein decomposition, such as the degradation of unstable amino acids.

Over the last 30 years, various attempts have been made to exploit this natural time-dependent property to allow the D/L values of Pleistocene molluscan remains to be used for Pleistocene dating. It was demonstrated in the 1980s that D/L values correlate sufficiently strongly with independently established dating frameworks for the technique to have value (eg Bowen *et al.* 1989). However, variable results between different species and wide error margins have restricted the reliability of the technique, and compromised the confidence with which it can be applied to deposits that are otherwise undatable. In the new technique applied here, attention is focused on a

variety of different amino acids (and their decay products) which decay at varying rates. In combination these provide a more robust general dating result and greater sensitivity in certain time ranges depending upon which protein/acid cycle is analysed. In addition, analysis is focused on the protected intra-crystalline fraction, which (in contrast to the majority of the organic shell/opercula material) is relatively protected from contamination and external factors during burial (such as temperature changes and aqueous saturation). It therefore provides a closed system from which one can expect D/L values that are more closely aligned to nothing other than the passage of time.

It has been established in the recent work at NEaar, during development of this new technique, that the calcitic opercula of *Bithynia* are a particularly robust repository of protected proteins and amino acids, making these commonly occurring molluscan remains of particular value for dating purposes. A dating framework for the last 500,000 years of the British terrestrial Pleistocene archive has therefore been established on the basis of data derived primarily from opercula of *Bithynia tentaculata* (Penkman *et al.* 2011), and all the analyses discussed in this chapter were carried out on opercula of this species.

Shells selected for amino acid dating analysis were examined under a low powered microscope and any adhering sediment removed. The shell samples were then sonicated and rinsed several times in HPLC-grade water, after which they were crushed to <100μm. They then underwent a bleaching process to remove contaminants and organic materials other than the desired intra-crystalline fraction. This resulted in a tiny amount of dried powder derived from the original opercula, or from an individual operculum, if just one was used for a particular analysis. For each sample, this powder then underwent a series of further chemical treatments, designed to release the various amino acids present, the D and L quantities of which could then be isolated and measured. The details of this process are described in Appendix 9, and the results are discussed below. A key element of the analytical approach is the isolation of both 'Free' and 'Hydrolised' amino acid fractions, the D/L values of which should both give independent dating results and also correlate in closed and uncompromised systems. A lack of correlation between these fractions can therefore be a valuable indicator that a sample has been compromised, and its result is unreliable.

In the analyses conducted on the Southfleet Road material, the extent of racemization (D/L) was established for five amino acids and their decay products (Asparagine/aspartic acid – Asx; Glutamine/Glutamic acid – Glx; Serine – Ser; Alanine – Ala; and Valine – Val), along with the ratio of the concentration of Serine to Alanine ([Ser]/[Ala]), for both the Free and Hydrolised – henceforth, 'Hyd' – fractions. These indicators of protein decomposition have been selected as their peaks are cleanly eluted with baseline separation and they cover a wide range of rates of reaction. It has been demonstrated that with increasing age, the extent of racemization (ie the values of the D/L ratios) will increase, whilst the [Ser]/[Ala] value will decrease due to the decomposition of the unstable serine. Therefore the results from the Southfleet Road analyses can be interpreted in light of the framework now established for the British Pleistocene (Penkman *et al.* 2011).

ANALYSIS PROGRAMME AND SAMPLING LOCATIONS

In total, 208 analyses were carried out, on 52 separate samples (Table 13.1; Fig. 13.1). The only horizon of the site where molluscan remains were abundant was the basal context 40070 of the tufaceous channel-fill (Phase 6b). As soon as this was recognised, a preliminary selection of *Bithynia* opercula from one of the early bulk small vertebrate assessment samples (<40162>) was analysed to verify that material from the site was suitable for AAR analysis. This having proved successful (NEaar 2041-2044), a more comprehensive programme was established, in which *Bithynia* opercula were collected from Phase 3 (context 40062), Phase 5 (context 40025, upper part) and from throughout the Phase 6 sequence (from the base: contexts 40103, 40070, 40144, 40143 and 40078). Following this main phase of analysis, an additional phase took place in which more analyses were carried out on material from Phase 3 (context 40062) and from Phase 6b (context 40070) in order to have sufficient data for comparison of the results between these horizons to be statistically meaningful.

The majority of material analysed was recovered from mollusc or bulk small vertebrate samples, the locations of which were recorded on drawn sections. The Phase 3 material was recovered from bulk sample <40042>, taken from the bottom level of the main east-facing section 40016 early in the excavation (Fig. 13.1a).

The Phase 5 material was recovered from two bulk samples taken from sediment surrounding larger vertebrate remains found within the top 0.2m of context 40025 in the central part of the site, between Trenches B and C, beside the east-west transverse section 40080 (Fig. 13.1c). Bulk sample <40286> was recovered from around the bovid maxilla (Δ.43298 – see Chapter 7), and sample <40343> was recovered from around the single, poor condition elephant tusk (Δ.43788) found near the north end of the tufaceous channel.

The Phase 6 material was all recovered from samples in and around the tufaceous channel, mostly from the two mollusc sampling columns in the longitudinal section 40082 (Fig. 13.1b). The preliminary sample <40162> from which *Bithynia* opercula were collected and analysed came from a bulk small vertebrate assessment sample from one of the early southern exposures of the tufaceous channel in the main east facing section, where it was both thin and heavily contorted (Fig. 13.1a). The later samples (particularly the material from mollusc sub-samples <40315/C> and <40317/C> from Column 2) came from more tightly constrained horizons within the greatest thickness of the main tufaceous channel-fill context 40070 (Fig. 13.1b), so these are

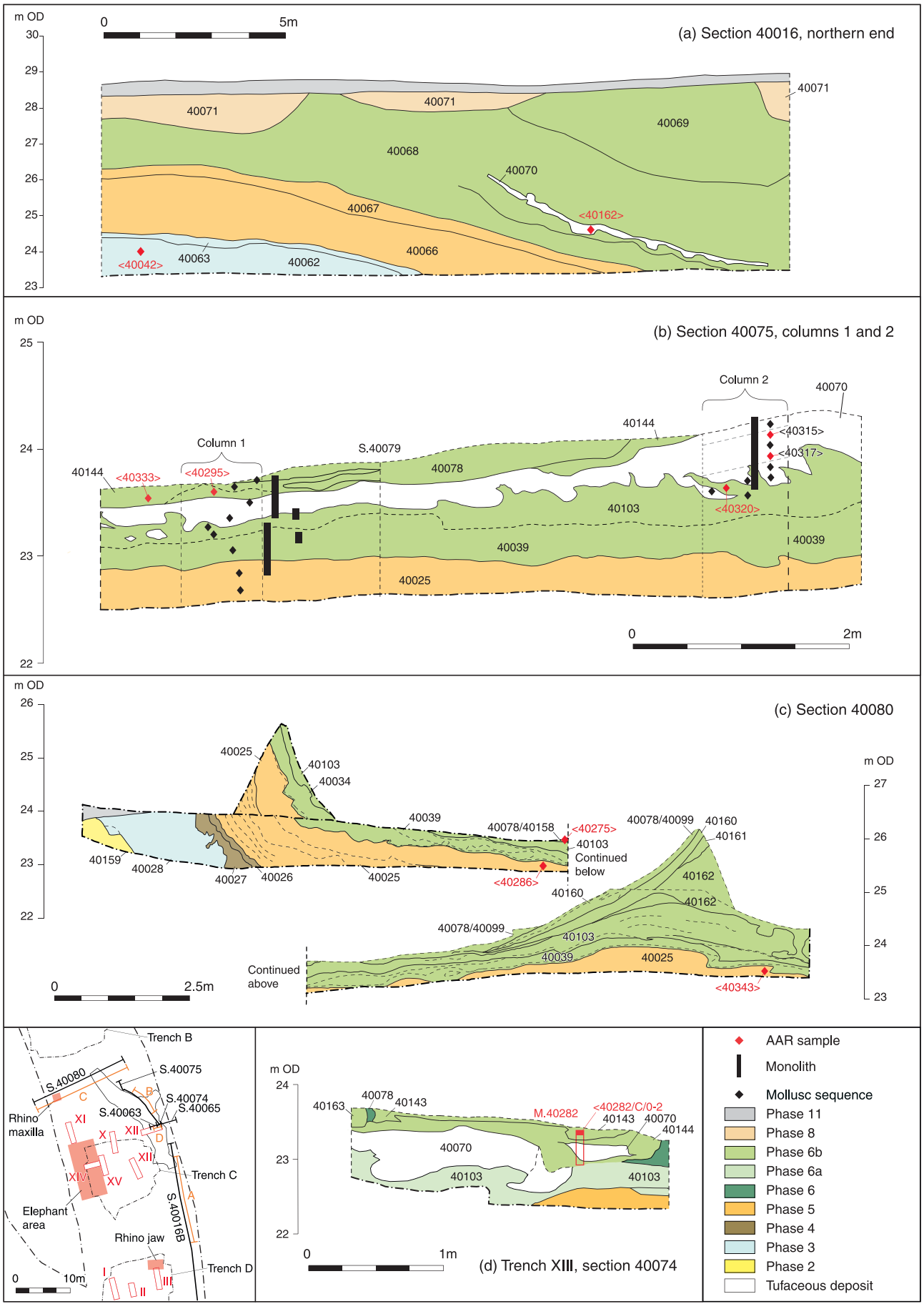

Figure 13.1 Mollusc sampling locations from which *Bithynia* opercula were recovered for amino acid dating: (a) Section 40016, northern end; (b) Section 40075, Columns 1 and 2; (c) Section 40080; (d) Trench XIII, Section 40074

regarded as having marginally higher stratigraphic integrity.

Deposits in and around the tufaceous channel were in places contorted and interdigitated, particularly the thin overlying beds 40143 and 40144. Consequently there is a legitimate question mark over whether the sparse molluscan evidence from these contexts is derived from context 40070, rather than being independent evidence from later phases of deposition. However, the molluscan and small vertebrate analyses of these overlying contexts 40144 and 40143 show continuing local environmental and ecological trends which suggest good integrity of the biological remains (Chapters 7 and 10). Context 40144 was well-defined and reasonably widely distributed, so there is high confidence that the samples and analysed opercula attributed to this context are reliably provenanced. The *Bithynia* opercula analysed from this context came from two samples (Fig. 13.1b): firstly, from molluscan subsample <40295/C> from Column 1 through the tufaceous channel; and secondly, from bulk sample <40333> which was part-sieved on site for small

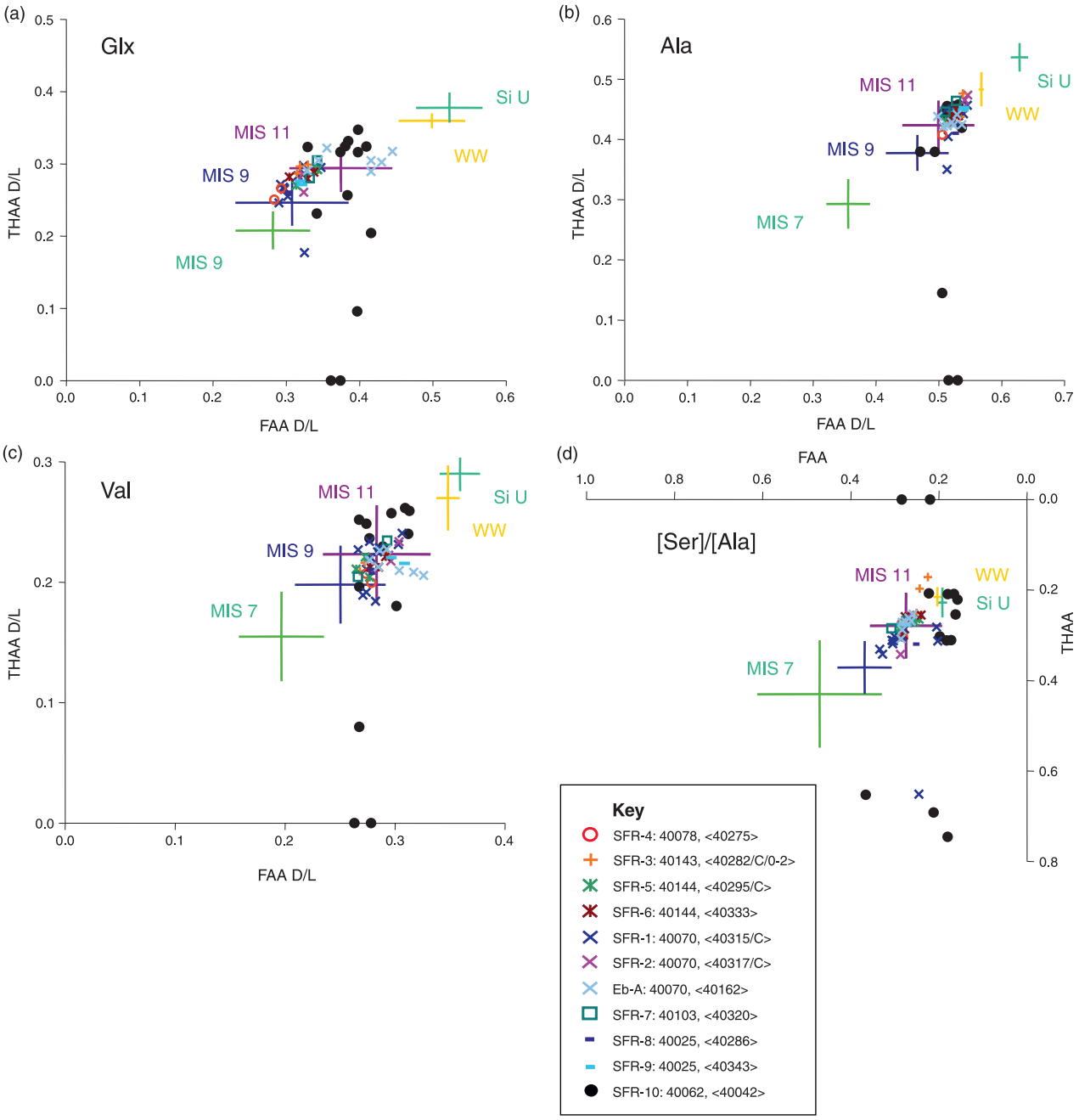

Figure 13.2 D/L Hyd vs D/L Free for *Bithynia* opercula from Southfleet Road, compared with UK Middle Pleistocene framework for: (a) Glutamic Acid/Glutamine Glx; (b) Alanin – Ala; (c) Valine – Val; and (d) [Serine]/[Alanine] – [Ser]/[Ala] [The bars of each cross represent two standard deviations about the mean for data obtained from opercula from sites correlated with MIS 7, MIS 9, MIS 11, Waverley Wood (WW) and Sidestrand '*Unio* bed' (SiU) – see Penkman *et al.* 2011]

vertebrate recovery, but the precise location of which was not recorded

Context 40143, the top level of the tufaceous channel-fill sequence, was very intermittent and often highly contorted, making it difficult to collect large enough samples for mollusc and small vertebrate analysis. It was, however, very distinctive, making it easy to recognise, and likewise leading to high confidence in the provenance of the samples and opercula attributed to it. The best exposures were in Trench XIII, and a subsample for molluscan analysis was taken from monolith <40282> in section 40074 (Fig. 13.1d). Three *Bithynia* opercula were recovered for AAR dating from this monolith.

Finally, two *Bithynia* opercula were recovered from bulk small vertebrate sample <40275>, attributed in the site archive to context 40078, but which came from the very dark, organic-rich Phase 6 deposits in Trench B that were later reattributed as context 40158 (Fig. 13.1c). This sample was originally taken in the hope of recovering insect and/or plant macro-remains, but these being lacking, it was sorted for small vertebrate remains, in course of which two *Bithynia* opercula were recovered, which were then incorporated in the AAR dating program. Despite its similar original context number, this material is therefore not from the same horizon as the elephant skeleton, but can probably be regarded as stratigraphically slightly younger, being from the top part of the Phase 6 sequence.

All processed samples were assigned a NEaar lab code and a shorthand result code (Eb-A and SFR1-10), as shown (Table 13.1), the result code being used in the various graphics and figures used to illustrate the results.

During hydrolysis the vials of two of the samples from context 40062, sample <40042> (SFR-10) cracked, and so no Hyd data is available for these two samples. The material from this sample was in general slightly problematic; the *Bithynia* opercula were particularly friable, with several disintegrating during the initial rinsing step to clean them. As Figure 13.2 shows, besides the two with no Hyd value (plotted on the x-axis, ie with a notional y-axis value of 0), some of the remaining opercula analysed from this horizon also showed much lower than expected THAA D/L values. This is clear evidence of a compromised closed system and an inaccurate dating result for those specimens. The [Ser]/[Ala] plot (Fig. 13.2d) shows three of these Phase 3 samples as clearly compromised, with excessively high values of THAA. One of the opercula from context 40070, <40315/C> (SFR-1) also showed this behaviour. In previous analyses of nearly 500 opercula samples, less than 2% of opercula were compromised in this way, so it is extremely unusual to have so many from one horizon. The friability of the opercula from context 40062 indicates that mineral diagenesis has occurred in at least some of them. This may merely reflect a poor preservational environment; although, if that were the case, it would be puzzling for the smaller and more delicate ostracod fauna to have survived (Chapter 11). This is therefore perhaps an indication that these opercula may have been derived, and one should therefore be wary to argue that their slightly older results (see below) relate to the deposit from which they were recovered. The total data set for these samples is shown in the Free vs Hyd plots below (Fig. 13.2, SFR-10), but the compromised samples were removed from the more detailed bar charts (Fig. 13.3) and the comparative statistical analyses to avoid skewing the data.

SOUTHFLEET ROAD ANALYSES COMPARED WITH THE UK MIDDLE PLEISTOCENE MIS FRAMEWORK

As outlined above, analyses were carried out for five amino acids of differing racemization rates. One of these, Aspartic acid/Asparagine (Asx), is one of the fastest racemising of the amino acids discussed here (due to the fact that it can racemise whilst still peptide bound, Collins *et al.* 1999). This enables good levels of resolution at younger age sites, but decreased resolution beyond MIS 7. The results showed clearly that all the Southfleet Road samples were substantially older than MIS 7 (see Appendix 9), so the results from this particular set of analyses are not presented here.

The data obtained from Glutamic Acid/glutamine (Glx), serine (Ser), alanine (Ala) and valine (Val) are discussed in detail below. If the amino acids were contained within a closed system, the relationship between the Free and the Hyd fractions should be highly correlated, with non-concordance enabling the recognition of compromised samples (Preece and Penkman 2005). The plot of Free versus Hyd data from each sample can also be used as a relative timescale, with younger samples falling towards the bottom left corner of the graph and older samples falling towards the upper right corner, along the line of expected decomposition.

The data from the Southfleet Road samples have been plotted in this way for each of the amino acids (Fig. 13.2), with crosshairs representing the data obtained from other MIS 7, MIS 9, MIS 11 and (probable) MIS 13 sites from the UK with independent geochronology, as listed in Penkman *et al.* (2011). There is unfortunately a lack of comparative *Bithynia* opercula from independently dated MIS 13 sites. The data here are based on analyses of material from two sites: the Unio bed at Sidestrand, Norfolk (shown in Fig. 13.2 as 'Si-U') and Waverley Wood (shown in Fig. 13.2 as 'WW'). At the former interglacial sediments are sealed beneath Anglian glacial till and are also dated to MIS 13 by water vole biostratigraphy, although an MIS 15 date is also possible (Preece *et al.* 2009). The Waverley Wood material is more reliably dated to MIS 13 (Shotton *et al.* 1993), but the opercula come from *Bithynia troschelii* rather than *B. tentaculata*; the racemization rates for these two species are, however, sufficiently comparable to be used interchangeably (Penkman *et al.* 2013). These plots show each Southfleet Road analysis as a separate point, with the colour/shape varying to reflect each separate sample, as indicated on the figure key. The data are thus split to

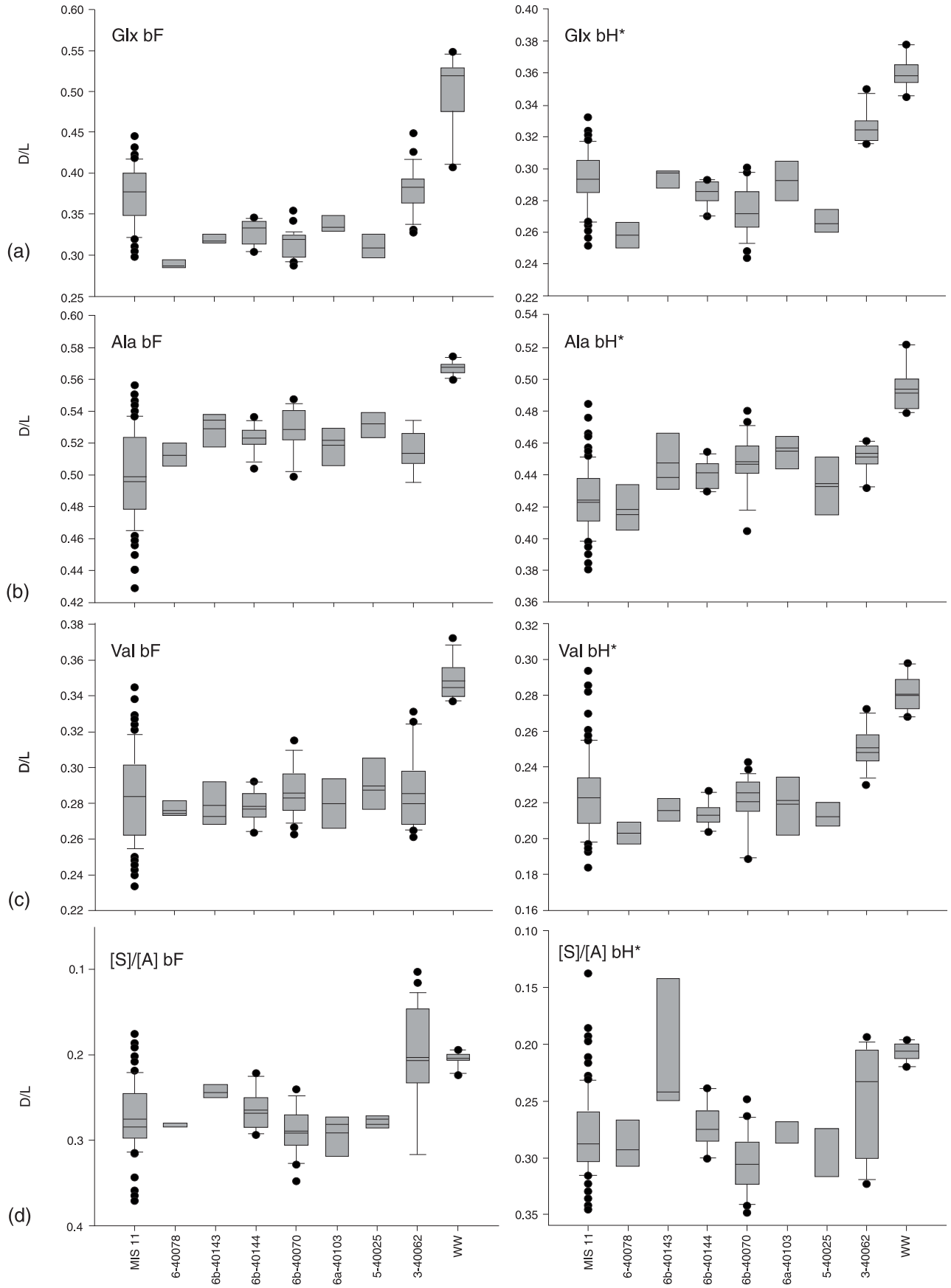

Figure 13.3 Free (left) and Hyd (right) D/L values for uncompromised *Bithynia* opercula from Southfleet Road, plotted/grouped by context in stratigraphic order, and compared with data from MIS 11 and MIS 13 [Waverley Wood (WW)], for: (a) Glutamic Acid/Glutamine Glx; (b) Alanin – Ala; (c) Valine – Val; and (d) [Serine]/[Alanine] – [Ser]/[Ala]

the maximum level of division, since several of these samples are stratigraphically equivalent (as shown in Table 13.1), and could reasonably be lumped together. This diagram, the interpretation of which is discussed in more detail below, establishes a broad correlation of the data with the MIS framework. It clearly shows that most, if not all, of the analyses indicate association with MIS 11, defined here on the basis of comparative material from the sites of Barnham, Hoxne, Beeches Pit, Woodston, Swanscombe, Clacton, Elveden and Dierden's Pit (Penkman *et al.* 2011).

This diagram is complemented by another (Fig. 13.3), which combines data from the same context, as shown in the right-hand column of Table 13.1, in an attempt to investigate whether there is any discernible trend in the amino acid data that correlates with the intra-site stratigraphy. If so, this might indicate that the deposits formed over sufficient time for intra-MIS 11 AAR resolution to be present. In this latter diagram, the Free and Hyd datasets for each analysis within each context group are plotted as box-plots, with top and bottom of the boxed area defined by the 25th and 75th percentiles. Within the box, the solid line indicates the median and the dashed line the mean. Where more than 9 data points are available, the 10th and 90th percentiles can be calculated (shown by lines below and above the boxes respectively). The results of duplicate analyses are included to provide a statistically significant sample size. The outside columns on each box-plot diagram provide overall comparisons with MIS 11 and MIS 13 [Waverley Wood] datasets, and between these, site stratigraphic order descends from left to right.

Considering Fig. 13.2, the first plot (Fig. 13.2a) shows data for Glutamic Acid/glutamine (Glx), which is one of the slower racemising amino acids discussed here. This makes it less useful for distinguishing younger Pleistocene material, but the low levels of racemization do help discriminate between material of Middle Pleistocene age. The Glx D/L values from Southfleet Road show values within the range of those expected from sites of MIS 9 and MIS 11 age. The plot also shows four sample points from Phase 3 (SFR-10 – context 40062, sample <40042>) and one point from Phase 6b (SFR-1 – context 40070, sample <40315/C) where the fall away from the expected line of correlation between the Free and the Hyd data indicates compromised samples. The results of these are therefore unreliable. Apart from the compromised data, two datasets (SFR-10, from sample <40042>, Phase 3; and Eb-A, from sample <40162>, Phase 6b) cluster in a slightly older part of the plot. Whilst this potentially makes sense for the Phase 3 material, it is anomalous for the other group, which should be the same age as other material from context 40070 (samples SFR-1 and SFR-2).

The second plot (Fig. 13.2b) shows data for Alanine (Ala). This is a hydrophobic amino acid, whose concentration is partly contributed from the decomposition of other amino acids (notably serine). Ala racemises at an intermediate rate, so is one of the most useful amino acids for distinguishing samples at Middle-Late Pleistocene timescales. The Ala data shows a tight clustering of data, consistent with a correlation with MIS 11 and clearly enabling discrimination from both sites of MIS 9 age and the two comparator sites of probable MIS 13 age. Aside from the three data points where the very low values of the Hyd dataset (THAA D/L) clearly show compromised results, the two samples from SFR-10 and that from SFR-1 which fall within the MIS 9 cluster show clear evidence in the other amino acids of being compromised, so can be disregarded. These leaves a tight cluster of uncompromised data points corresponding with the crosshair for the comparative MIS 11 dataset.

The third plot (Fig. 13.2c) shows data for Valine, which has extremely low rates of racemization. As the concentration of Val is quite low, the difficulty of measuring the D/L accurately results in higher variability. It does however still prove useful for age discrimination within material of Middle Pleistocene age. The D/L values for Val in the Free and the Hyd fractions again support the other amino acid data, centring on the crosshair for MIS 11. There is also, however, again a preferential clustering of the Phase 3 dataset (SFR-10) in the slightly older part of the plot, although still by no means approaching the comparative MIS 13 values; this is discussed further below.

The fourth plot (Fig. 13.2c) shows data for the ratio of the concentrations of serine and alanine, which provides an extremely useful tool for age estimation. Serine is a very unstable amino acid, and it can degrade via dehydration into alanine (Bada *et al.* 1978). As the protein within a sample breaks down, the concentration of serine will decrease with an increase in the concentration of alanine, thus the [Ser]/[Ala] value will decrease with increasing time. In order to ease the interpretation, the axes are plotted in reverse, so that the age-related direction of increase in protein degradation is the same as for the racemization plots. The [Ser]/[Ala] of the Southfleet Road samples are again consistent with an age in MIS 11, but the level of discrimination is not particularly high. As with the other plots, the Phase 3 dataset (SFR-10) is grouped in the slightly older part of the plot, although with a high proportion of compromised data. Also in the slightly older part of the plot, curiously, are two data points from SFR-3 (Phase 6b, context 40143) which on stratigraphic grounds should be amongst the youngest material.

In combination, the data presented above provide robust confirmation that Phases 5 and 6 of the site sequence were laid down in MIS 11. They also suggest that Phase 3 of the site sequence was probably also laid down in MIS 11.However, the consistent clustering of dataset SFR-10 from Phase 3 as slightly older within MIS 11 raises the possibility that the AAR data are demonstrating intra-MIS 11 distinction within the aggradational sequence. In order to investigate intra-sequence variability in more detail, the Free and Hyd data are here shown as box-plots (Fig. 13.3), as described above, with material grouped by context (but omitting material from sample <40162>, where the

tufaceous pocket sampled was relatively thin and contorted, near the edge of the tufaceous channel). As before, there are four datasets, one for each of the three amino acids Glx (Fig. 13.3a), Ala (Fig. 13.3b) and Val (Fig. 13.3c), and a fourth showing [Ser]/[Ala] (Fig. 13.3d). And for each of these fours datasets there is a separate set of plots for the Free and Hyd D/L values. These data show several consistent trends.

Firstly, the group of material from Phase 3 (SFR-10) appears (statistically significantly at the 10% level in the box plot for Glx, Fig. 13.3a) slightly older than the other material from the elephant site in half of the analyses – Glx (Free and Hyd); Val (Hyd); and [Ser]/[Ala] (Free). This group of material is nonetheless clearly aligned with other data from MIS 11 rather than MIS 13 in the three individual amino acid analyses (Fig. 13.3a-c). In the fourth analysis [Ser]/[Ala] (Fig. 13.3d), it appears better aligned with the MIS 13 comparator Waverley Wood for the Free dataset, and the situation is similar for the Hyd dataset, although the wide variability in the sample dataset make the separation from MIS 11 less clear-cut. The top of Phase 3 is marked by a zone of decalcification (context 40063), perhaps reflecting a depositional hiatus between the top of Phase 3 and the base of Phase 5, although there is no evidence of a major depositional unconformity. Any depositional hiatus is likely to be minor and of minimal chrono-stratigraphic significance, considering the interglacial attribution of Phases 3 and 5, and the lack of any intervening evidence for climatic deterioration.

Secondly, there is almost complete consistency for the results from Phase 5 (context 40025), Phase 6a (context 40103) and Phase 6b, within the tufaceous channel (contexts 40070, 40144 and 40143). In all these datasets, (apart from one: [S]/[A] Hyd – where the results for context 40143 appear anomalously old), these groups are indistinguishable both from each other, and from the MIS 11 comparator dataset, confirming the MIS 11 attribution throughout this stretch of the site sequence.

Thirdly and finally, at the top of the sequence, the data from the very dark organic rich upper part of the clay (context 40158, sample SFR-4) appears very slightly younger in several of the analyses: Glx, Free and Hyd (Fig. 13.3a); Ala, Hyd (Fig. 13.3b); and Val, Hyd (Fig. 13.3c). The number of data points is, however, too low for this result to be statistically significant.

In general, the data consistently show an MIS 11 date for Phases 5 and 6, and there is also a suggestion of a recognisably earlier MIS 11 date for Phase 3. This latter result is worthy of some discussion, because to-date AAR dating has not achieved sub-stage precision. It should be remembered that this result was not consistently replicated in all the datasets. There was, for instance, no distinction represented in the Free or the Hyd data from Alanine (Fig. 13.3b), which would normally be one of the most useful for distinguishing between different MIS stages within the Middle Pleistocene. It should also be remembered that there was a high degree of compromised chemical systems in the opercula from Phase 3 and that many of them were in poor condition, perhaps indicating preservational and post-depositional problems that might have affected the results. No other molluscan remains were recorded in association with the Phase 3 opercula (see Chapter 10), suggesting conditions for preservation were not ideal. On the other hand, they were associated with a rich interglacial ostracod fauna that was undoubtedly an *in situ* autochthonous assemblage (see Chapter 11). This fauna might be expected to be less resistant to decay than any larger molluscs, which are, however, entirely lacking apart from the (quite abundant) *Bithynia* opercula.

On balance, it appears possible that the opercula from Phase 3 have been dated by AAR to an earlier part of MIS 11 than Phases 5 and 6. However, the evidence that the opercula were poorly preserved and often chemically compromised slightly undermines the reliability of this result. The lack of preservation of associated mollusc shells, despite the presence of smaller and more delicate ostracods, also suggests that the opercula may have been reworked. This indicates that, even if they are dating to an earlier part of MIS 11, or perhaps a late MIS 13 or an intra-MIS 12 interstadial, this dating does not relate to deposition of Phase 3. Phase 3 is therefore probably of very similar age to Phases 5 and 6, supporting the zoological and geological evidence for climatic and depositional continuity through Phases 3 to 6. Further investigation is, however, required to try and establish whether preservational factors are having an influence, and whether the result is replicable with larger datasets from deep sequences at other MIS 11 and MIS 12–13 comparator sites.

PHASE 6B ANALYSES (TUFACEOUS CHANNEL, CONTEXT 40070) COMPARED WITH KEY MIS 11 HORIZONS

Having confirmed that Phase 6, which contained the elephant skeleton and the lithic concentration south of Trench D, can be securely dated by AAR to MIS 11, and investigated the AAR dating of the site sequence more generally, the final stage of the AAR dating programme was to investigate how the dating results from the site compared with a number of other significant MIS 11 comparators, particularly those that also have important archaeological remains (Table 13.2). Two more charts were plotted, analogous to those used to investigate the intra-site data. The first of these (Fig. 13.4) shows scatter plots of Free vs Hydrolised D/L values for the same datasets as discussed above, in other words (a) Glx; (b) Ala; (c) Val; and (d) [Ser]/[Ala], for the most heavily analysed sample (sample <40315/C>, SFR-1) from the middle of context 40070 in the tufaceous channel, alongside datasets from: Barnfield Pit, Swanscombe (Lower Loam); Barnham (Bed 5c); Hoxne (Stratum E); Hoxne (Stratum B2); and Beeches Pit, West Stow (Cutting 2, Bed 7). The dataset from Phase 3 of the elephant site (sample <40042>, SFR-10) was also included, to continue investigation of whether this could be related to other specific sites and/or a

specific part of MIS 11. The second of these shows separate box-plots for Free and Hydrolised data separately (Fig. 13.5), for the same set of comparator sites, and the same range of racemization data. These data fail to show any significant inter-site groupings or correlations, and this is probably currently beyond the precision of the technique for the Middle Pleistocene, although something to be aspired to in future research. The scatter-plots (Fig. 13.4) and the histograms (Fig. 13.5) all show that, for almost all the datasets, the elephant site Phase 6b data are indistinguishable from any of the MIS 11 comparator sites, particularly for Val and [Ser]/[Ala] (Fig. 13.4c, d; Fig. 13.5c, d). There are occasional minor and statistically insignificant deviations from this similarity, but these do not correspond conisistently with known stratigraphic order or postulated relative dating (ie the Glx (Hyd) values for Stratum B2 at Hoxne are slightly greater than those for the lower Stratum E) and there is no consistency in which particular site/horizon appears slightly older or younger. The data from Barnham have marginally lower D/L values (Free and Hyd) for Val and [Ser]/[Ala] but are conversely marginally higher for Free Ala and Glx. Likewise, the data from the Lower Loam at Swanscombe have marginally lower D/L values than the Phase 6b data for Ala (Free and Hyd) but are otherwise marginally higher than the other MIS 11 comparator sites.

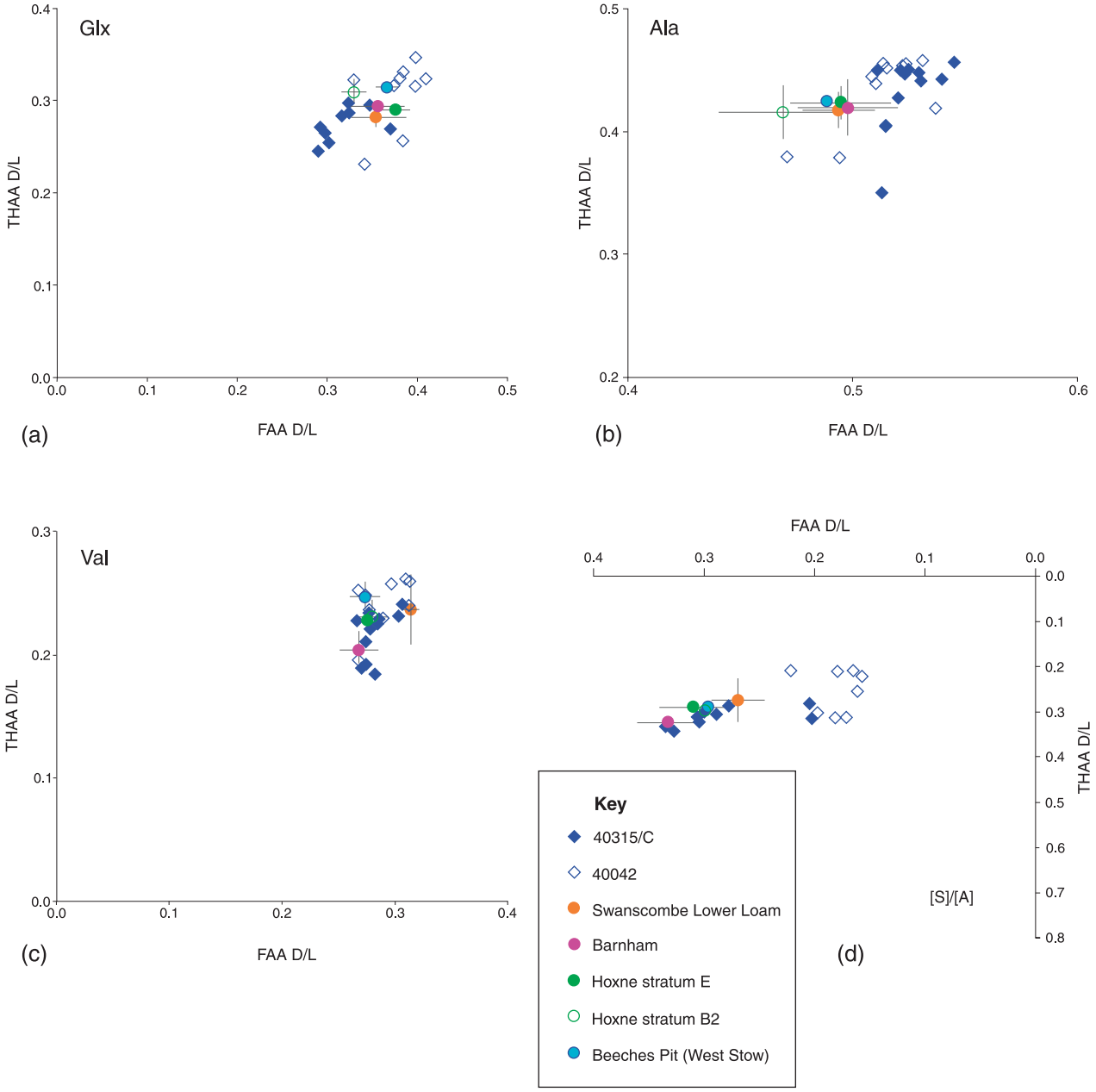

Figure 13.4 D/L Hyd vs D/L Free for uncompromised *Bithynia* opercula from Southfleet Road, Phase 6b tufaceous channel (sample <40315C>, context 40070, SFR-1) and Phase 3 (sample <40042>, context 40062, SFR-10) compared with key MIS 11 horizons for: (a) Glutamic Acid/Glutamine Glx; (b) Alanin –Ala; (c) Valine – Val; and (d) [Serine]/[Alanine] – [Ser]/[Ala] [See Table 13.2 for MIS 11 comparator horizons]

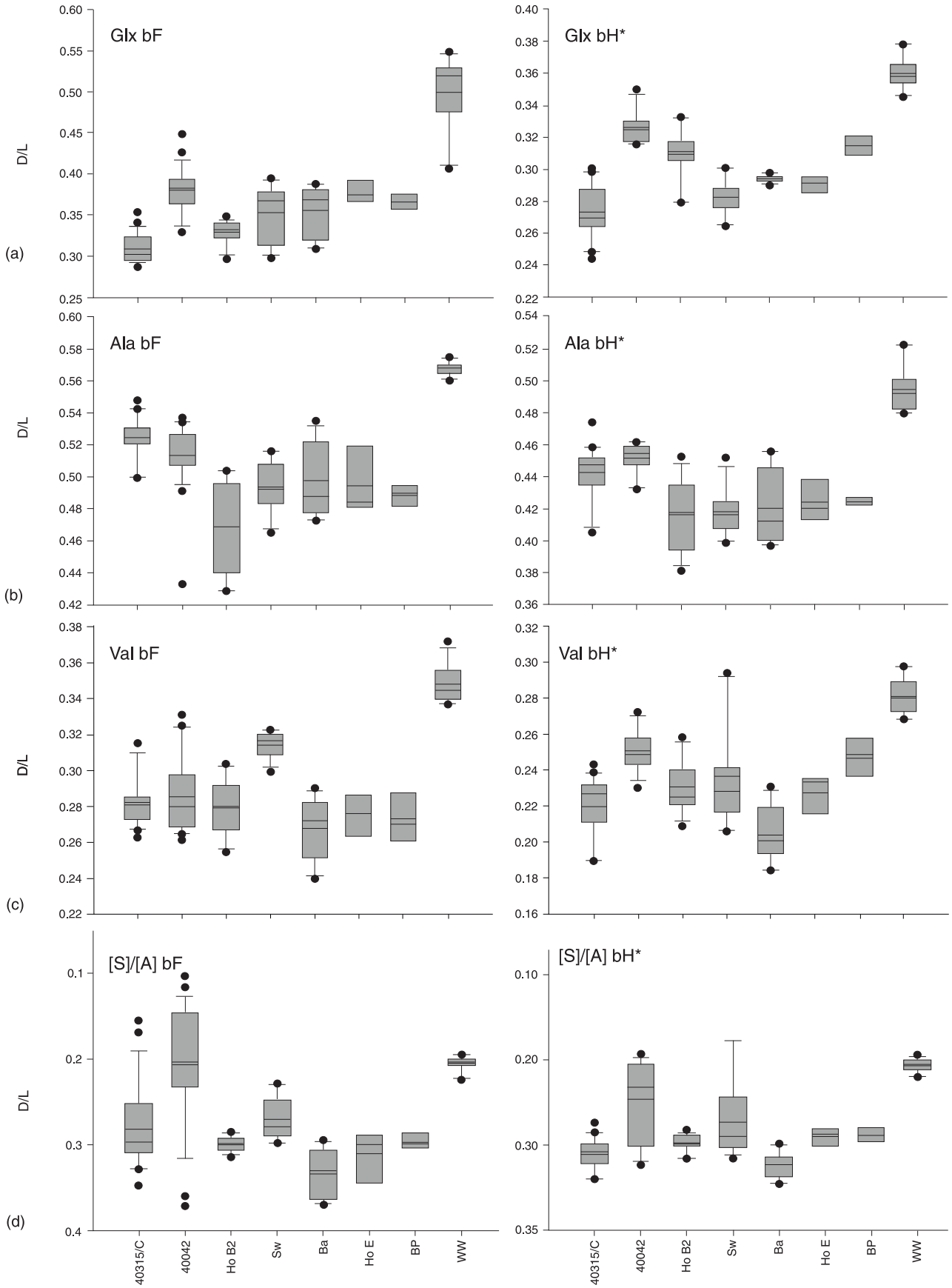

Figure 13.5 Free (left) and Hyd (right) D/L for uncompromised *Bithynia* opercula from Southfleet Road, Phase 6b tufaceous channel (sample <40315/C>, context 40070, SFR-1) and Phase 3 (sample 40042, context 40062, SFR-10) compared with key MIS 11 horizons and Waverley Wood (WW) for: (a) Glutamic Acid/Glutamine Glx; (b) Alanin – Ala; (c) Valine – Val; and (d) [Serine]/[Alanine] – [Ser]/[Ala] [See Table 13.2 for MIS 11 comparator horizons]

DISCUSSION AND CONCLUSIONS

The amino acid dating programme has convincingly confirmed the attribution of Phase 6 of the site sequence to MIS 11. Although the evidence from Phase 5 is much sparser, this too seems securely provenanced and reliably dated to MIS 11. Below this, the data from Phase 3, from where abundant opercula were recovered, appear to cluster slightly earlier within MIS 11. There are, however, possible indications of reworking and an unusually high proportion of chemically compromised specimens in the material from Phase 3, which is generally in poor condition. These factors may be affecting the results, and it is suggested that, if the dating is nonetheless correct, the dated opercula are reworked from an earlier horizon, rather than the Phase 3 deposits having formed in a significantly earlier part of MIS 11 to Phases 5 and 6.

Comparisons with other MIS 11 sites were investigated, particularly those with significant archaeological horizons, namely: Swanscombe, Hoxne, Barnham and Beeches Pit. However, no consistent pattern or groupings emerged from the data that allowed any more detailed inter-site correlation of specific archaeological horizons. This appears to currently be beyond the precision of the AAR technique, but is certainly an area for further future investigation.