Published in final edited form as: *Nature.* ; 476(7361): 446–449. doi:10.1038/nature10305.

A chronological framework for the British Quaternary based on *Bithynia* opercula

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Abstract

Marine and ice-core records show that the Earth has experienced a succession of glacials and interglacials during the Quaternary (last ~2.6 million years), although it is often difficult to correlate fragmentary terrestrial records with specific cycles. Aminostratigraphy is a method potentially able to link terrestrial sequences to the marine isotope stages (MIS) of the deep-sea record^{1,2}. We have used new methods of extraction and analysis of amino acids, preserved within the calcitic opercula of the freshwater gastropod *Bithynia*, to provide the most comprehensive dataset for the British Pleistocene based on a single dating technique. A total of 470 opercula from 74 sites spanning the entire Quaternary are ranked in order of relative age based on the extent of protein degradation, using aspartic acid (Asx), glutamic acid (Glx), serine (Ser), alanine (Ala) and valine (Val). This new aminostratigraphy is consistent with the stratigraphical relations of stratotypes, sites with independent geochronology, biostratigraphy and terrace stratigraphy $^{3-6}$. The method corroborates the existence of four interglacial stages between the Anglian (MIS 12) and the Holocene in the terrestrial succession. It establishes human occupation of Britain in most interglacial stages after MIS 15, but supports the notion of human absence during the Last Interglacial (MIS 5e)⁷. Suspicions that the treeless 'optimum of the Upton Warren interstadial' at Isleworth pre-dates MIS 3 are confirmed. This new aminostratigraphy provides a robust framework against which climatic, biostratigraphical and archaeological models can be tested.

Despite the importance of the terrestrial record for climate models, the difficulties of assigning specific sedimentary sequences to individual climate cycles restricts the use of these data in climate modelling. The British Quaternary is exceptional for the number of

Supplementary Information Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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The authors declare no competing financial interests.

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Author contributions K.E.H.P., M.J.C., R.C.P. and D.H.K. designed the study. R.C.P., D.H.K., T.M., D.R.B., S.A.P., T.S.W. and K.E.H.P. supplied samples. K.E.H.P. undertook the analyses and processed the data. K.E.H.P, M.J.C, T.S.W. and R.C.P. wrote the paper. All authors discussed the results and commented on the manuscript.

recorded sites and their biodiversity, which has fluctuated markedly due to the mid-latitude situation of this ephemeral island. A consensus has emerged from attempted differentiation between interglacials in Britain using river terrace stratigraphy^{6,8} and biostratigraphy³⁻⁵ (Table 1).

This study revisits research pioneered by Miller et al. (predominantly on bivalves)¹ and Bowen et al. (predominantly on gastropod shells)² who used the extent of racemization in the amino acid L-isoleucine (to its diastereomer D-alloisoleucine, yielding an A/I value) in non-marine mollusc shells to build an aminostratigraphy of terrestrial sequences that could be linked to the marine oxygen isotope stratigraphy. Following debate concerning certain correlations, we developed a revised method of extraction and analysis⁹. Shells of freshwater gastropods (*Bithynia* and *Valvata*) from many of the original sites¹⁰ have been re-analysed, confirming much of the A/I stratigraphy. However, it emerged that within-site and within-stage variability increases in shells from older sites. This variability probably results from diagenetic alteration of the biomineral carbonate from aragonite to the more thermodynamically stable calcite^{10,11}.

Our new method has five significant revisions, three of which reduced within-site variability. First, inter-species variation was minimised by analysing only a single genus of freshwater gastropod (Bithynia). Second, variability in amino acid concentration and D/L values was significantly lowered when samples were crushed to 500 µm and exposed to prolonged wet chemical oxidation (48 hours, 12% wt./vol. NaOCl, room temperature), destroying any contamination and leaving a functionally closed-system protein fraction defined as 'intra-crystalline'^{9,12}. Third, the calcitic operculum, which in life closes the aperture of the shell, was analysed instead of the aragonitic shell. Opercula display less within-site variation and greater stability than shells^{10,11}, and show subtle but minimal intraspecific differences in racemization (Supplementary Data 1). Further modifications included the analysis of a range of different amino acids¹³ (rather than only a single measure of racemization), which allows an estimate of 'Intra-crystalline Protein Decomposition' (IcPD). This integrates data from amino acids with differing rates of racemization (Asx >> $Ala > Val \sim Glx$) with the extent of dehydration of serine ([Ser]/[Ala]) to estimate age (Fig. 1). Finally, comparisons were made between free amino acids (FAA), liberated by diagenesis, and the total extent of racemization (THAA), in order to test closed-system conditions¹⁴.

The intra-crystalline fraction maintains constant chemical conditions so that the extent of protein degradation can be attributed to the thermal history of the sample. Within the study area differences in thermal history have been minor during the past century and chiefly related to burial depth and the thermal diffusivity of the overburden, mediated by the presence/absence of vegetation and/or snow cover (Supplementary Figure 1 and Discussion). Opercula with similar levels of protein degradation are therefore thought to be of equivalent age, assuming that similarly small thermal gradients existed during past interglacials, when most of the racemization would have occurred.

The consistency of our method has been tested by measuring opercula from British interglacial stratotypes and/or sites with independent geochronology (Table 1; Fig. 1, Supplementary Data 1). All stratotypes yielding *Bithynia* have been analysed, but stratotypes have not been formally defined for all stages¹⁵. Sites with independent geochronology can be used to calibrate IcPD results but, as only 7 of our pre-Holocene sites have associated dates, we have not done this here. Nevertheless, an age-dependent increase in the level of IcPD is seen from the Holocene to the Early Pleistocene, using a combination of fast (e.g. Asx) and slow (e.g. Val) racemizing amino acids to span this range.

Increasing protein decomposition within opercula is also consistent with increasing river terrace elevation (and therefore age) in Quaternary fluvial archives (Table 1 and Supplementary Data 1 and 2, Figure 2). The formation of river terraces is attributed to climatic forcing and uplift, linking aggradation and incision phases with climatically induced changes in sediment and water supply^{6,8,16}. Assemblages of amino acid values from the Thames sequence, the most complete British fluvial archive¹⁷⁻¹⁹, clearly correspond with discrete terrace aggradations (Fig. 2 and Supplementary Data 2). Four interglacials after the Anglian (MIS 12) stage are represented in this system, assigned to MIS 11, 9, 7 and 5e on the basis that each aggradation formed during a complete glacial/interglacial cycle^{6,16}. Although this relationship might not hold for all rivers²⁰, a similar pattern exists between Ala D/L values and terraces in other systems, such as the Severn/Avon²¹, Trent/Witham²² and Nene/Welland²³. Support for these interpretations comes from integrating several lines of evidence (e.g. biostratigraphy⁴⁻⁵ and some of the original A/I data¹⁶), not all of which are wholly independent. However, the ability of the method to differentiate between terrace aggradations (Supplementary Data 2) is not reliant on other data and sites where the age attribution is based fundamentally on amino acid data are not assigned 'consensus MIS ages' in Table 1.

Bithynia is generally rare in cold-stage contexts, although it occurred commonly in the 'Upton Warren interstadial' deposit at Isleworth. This was originally thought to fall within the 'Middle Devensian' (MIS 3) on the basis of a radiocarbon date of ~43 ka BP²⁴. The Isleworth opercula IcPD is consistently higher than from Cassington, a site tentatively correlated with MIS $5a^{25}$, but lower than Last Interglacial opercula. Radiocarbon therefore provides only a minimum age for the Isleworth deposits. Our IcPD data indicate an earlier age and suggest that this new method can potentially be used to distinguish marine isotope sub-stages beyond the limits of radiocarbon dating.

Aminostratigraphic data also provide independent support for biostratigraphic age models developed for the Middle Pleistocene^{5,26,27}. In the early Middle Pleistocene, the water vole *Arvicola* is thought to have replaced its ancestor *Mimomys savini* within a relatively short time over large regions of Europe^{3,27}. This hypothesis gains support from our new data, which show that opercula from sites yielding *Arvicola* show less protein degradation than those containing *M. savini* (Table 1).

The occurrence of *Corbicula* (a bivalve) and *Hippopotamus* in the British Pleistocene is mutually exclusive^{5,26}. At British sites securely attributable to the Last Interglacial (MIS 5e) *Corbicula* is absent; conversely, after MIS 12, *Hippopotamus* is only known from the Last Interglacial, and is therefore widely regarded as an 'indicator species' for MIS 5e⁴. Our data support these conclusions, as post-Anglian sites with *Hippopotamus* show less protein breakdown than sites yielding *Corbicula*, with levels of protein degradation consistent with attribution to MIS 5e.

The comparisons above demonstrate the remarkable consistency of our new method with independent lines of evidence. This comprehensive dating framework enables us to explore the British archaeological record. Our data show that human occupation occurred within at least two distinct pre-Anglian stages, the older (Pakefield) associated with *Mimomys* and the younger (Waverley Wood) with *Arvicola*. The conclusion that Waverley Wood is younger than the Cromerian stratotype at West Runton supports the biostratigraphic age model²⁷ and contradicts a conclusion reached in an earlier aminostratigraphical study². Our data can provide age constraints for other archaeological assemblages, enabling attribution to specific marine isotope stages in younger deposits. The development of Levallois technology, characterized by the removal of flakes from specifically prepared cores, is unknown in Britain before MIS 9²⁸. Archaeological evidence from MIS 9 is sparse, but the far better

Nature. Author manuscript; available in PMC 2012 February 25.

record from sites attributed to MIS 7 shows that Levallois industries had become dominant in southern England; our data support this view (Table 1; Fig. 3).

In recent years it has become clear that humans were absent from Britain during the Last Interglacial; earlier claims to the contrary have been shown to be based on misinterpretation of archaeological sites previously thought to be of Last Interglacial age (such as Aveley, Crayford, Grays, Purfleet and Stutton), invariably now assigned to earlier stages²⁹ (Supplementary Data 1). Our results confirm that no British archaeological site can be attributed to the Last Interglacial (Fig. 3), a conclusion consistent with human absence during this stage^{7,29}.

This stratigraphical framework provides a secure basis for relating the British terrestrial British sequence to global Quaternary climate records. This is fundamental to geological and archaeological research but, as importantly, it enables the rich British record to be used to test the ability of climate models to simulate pre-late Quaternary palaeoclimates. This dating technique offers a means of correlating terrestrial with marine and ice-core records, thereby increasing the confidence of model predictions³⁰. Moreover, the calcitic opercula of bithyniid (or similar) gastropods occur commonly in many Quaternary sequences, offering potential for development of regional aminostratigraphies around the world.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank P. Allen, N. Ashton, D. Bain, M. Bates, S. Boreham, D. Bowen, R. Briant, C. Buckingham, J. Clayden, G. R. Coope, A. Cruickshanks, P. Dark, B. Demarchi, M. Greenwood, T. van Kolfschoten, H. Langford, S. Lewis, D. Maddy, R. Markham, D. Mayhew, H. Roe, J. Rose, D. Schreve, R. Scott, K. Scott, C. Stringer, R.Waghorne, M. Warren and F. Wenban-Smith for providing some of the material analysed and general discussion. C. Helmsley and J. Todd released material from the Natural History Museum, London, for destructive analysis. R. Allen provided technical support. G. Peeters gave permission to reproduce images of *Bithynia*. The analyses were funded by English Heritage, NERC and the Wellcome Trust (grant GR076905MA). This is a contribution to the Ancient Human Occupation of Britain (AHOB) project funded by the Leverhulme Trust.

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Nature. Author manuscript; available in PMC 2012 February 25.





Figure 1. Amino acid decomposition at sites of known age

Comparison of racemization in *Bithynia* opercula for FAA aspartic acid (Asx), THAA alanine (Ala) and valine (Val) for all sites with independent geochronology. Y-axis error bars indicate one standard deviation about the mean for the site. X-axis error bars are not shown for the Early Pleistocene and Pliocene samples, as these estimates of age are not based on numerical methods. Note the rapid racemization in Asx at relatively young sites and the plateau beyond ~0.5 Ma; Asx is therefore most valuable for separating sites younger than MIS 9. In contrast, Val racemizes more slowly and provides poorer resolution for younger sites, but is able to differentiate between sites back to the Pliocene. Utilising several amino acids with different rates of degradation therefore enables greater age resolution. The increase in racemization is not linear with time, but slows during cold stages. Inset: shell (a) and operculum (b) of *Bithynia tentaculata*.

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Figure 2. THAA vs FAA D/L Ala for individual samples in relation to terrace stratigraphy (a) Idealised transverse sections through the Lower Thames terrace sequence¹⁹ and (b) THAA vs FAA D/L Ala. Data from Thames sites (coloured) are superimposed on the full dataset. Note the concordance of terrace aggradations with the extent of protein degradation. This finding is consistent in all four river systems tested (Table 1, Supplementary Table 2). The relation of FAA:THAA is a useful control on the reliability of the closed-system. For example, opercula distorted following deposition showed levels of racemization of THAA far lower than predicted from FAA¹⁴, suggesting that during demineralization and distortion smaller, more mobile, amino acids were lost whereas larger peptides were retained during re-mineralization. Only ~1% of samples analysed here were compromised, such as the outlier from Sugworth (circled).



Figure 3. THAA vs FAA D/L Ala in relation to the occurrence of archaeology

Data from archaeological sites (coloured) are superimposed on the full dataset. Error bars represent two standard deviations about the mean for each site. Symbols as in Table 1. The earliest Palaeolithic archaeology (Mode 1, i.e. flake tools made with hard-hammers) comes from pre-Anglian contexts yielding *Mimomys savini*. Sites with unequivocal Clactonian (core-and-flake) archaeology all fall within MIS 11. Sites yielding Levallois artefacts form a temporally discrete cluster after MIS 9 but before MIS 5e. Archaeology is unknown at British sites attributed to the Last Interglacial (MIS 5e). Opercula have also been analysed from one Upper Palaeolithic and one Mesolithic site.

Table 1

Intra-crystalline amino acid data from the opercula of *Bithynia* from sites in southern Britain

Sites were chosen because they: (i) are stratotypes (in bold) of various interglacial stages; (ii) have independent geochronology; (iii) occur within a fluvial terrace sequence; or (iv) have relative ages based on biostratigraphy (Supplementary Data 1). Some additional sites, including three key continental Plio-Pleistocene localities, are listed to show how they fit into this general framework. Sites are listed in rank order based on the THAA Ala D/L value, the most useful single measurement covering the timescales under discussion but full interpretation requires consideration of the overall IcPD (Supplementary Data 1). Our data are shown alongside terrace stratigraphy (NW: Nene/Welland; SA: Severn/Avon; Th: Thames; TW: Trent/Witham), occurrences of important biostratigraphic indicator species (? = indirect association), *in situ* archaeology and existing consensus views on correlation with the MIS record. Age attributions reliant on amino acid dating alone are excluded. The occurrence of the water vole *Arvicola* is only shown for pre-Anglian sites (i.e. pre-MIS 12). \blacktriangle archaeology found in overlying sediments; \lor archaeology found in underlying sediments; \times archaeology from the same horizon as the opercula analysed; \blacklozenge indirect association (i.e. archaeology recorded from the site but not this profile). *Bithynia tentaculata* (L.) does not occur

	1			Ala	D/L	vai	D/L			Bios	strat.	Archaeology						_
Site number (SI Fig. 1)	Site	<i>Bithynia</i> species	Number analysed	nean	standard deviation	nean	standard deviation	ndependent geochronology	Terrace stratigraphy	Hippopotamus/Corbicula	4 <i>rvicola</i> (pre-Anglian) / <i>Mimomys</i>	Mesolithic	Upper Palaeolithic	evallois	Acheulian	Clactonian	Jndiagnostic	Consensus MIS
1	Acle (modern)	le	3	0.04	0.00	0.02	0.00	•				-		_				
1	Acle (modern)	te	2	0.04	0.00	0.02	0.00											
2	Enfield Lock	te	4	0.05	0.01	0.03	0.01											1
3	Quidenham Mere, 250-260cm	te	4	0.05	0.01	0.03	0.00											1
3	Quidenham Mere, 640-650cm	te	3	0.05	0.01	0.03	0.00											1
4	Newby Wiske	te	3	0.06	0.01	0.04	0.00	•										1
5	Aston-upon-Trent	te	4	0.06	0.00	0.04	0.00	•										1
6	Star Carr, 245-250cm	te	2	0.07	0.01	0.04	0.00	•				٥						1
7	Sproughton	te	4	0.09	0.00	0.05	0.00	•										2
6	Star Carr, 524-528cm	te	3	0.10	0.02	0.04	0.00	•										2
8	Cassington	te	4	0.12	0.01	0.07	0.00	•	Th									5c-4
9	Isleworth	te	4	0.14	0.01	0.07	0.01											
10	East Mersea (R. site)	te	2	0.16	0.02	0.08	0.01			н								5e
11	Coston	te	3	0.17	0.00	0.08	0.00			н								5e
12	Махеу	te	4	0.17	0.03	0.08	0.02		NW									
13	Woolpack Farm	te	1	0.17	0.00	0.09	0.00											
14	Jaywick Sands	te	4	0.17	0.01	0.09	0.01											
15	Saham Toney, 92	te	4	0.17	0.00	0.10	0.01											
16	Itteringham	te	1	0.18	0.00	0.09	0.01											
17	Tattershall Castle	te	4	0.18	0.01	0.09	0.01	•	TW									5e
18	Bobbitshole	te	3	0.18	0.01	0.10	0.00											5e
19	Shropham	te	4	0.18	0.01	0.09	0.01			(H)								5e
20	Trafalgar Square	te	6	0.19	0.01	0.10	0.01		Th	н								5e
15	Saham Toney, 94	te	2	0.19	0.00	0.11	0.01											
21	Bardon Quarry, Area 3	te	3	0.19	0.02	0.09	0.01		TW									
15	Saham Toney, 93	te	4	0.19	0.01	0.10	0.01											
16	Itteringham, Bed d	te	4	0.19	0.01	0.10	0.00											
22	Eckington	te	4	0.20	0.01	0.10	0.01		SA	н								5e
23	Cropthorne New Inn	te	4	0.20	0.01	0.09	0.00			н								5e
21	Bardon Quarry, Area 2	te	4	0.21	0.03	0.11	0.02											
24	Funtham's Lane East	te	25	0.22	0.01	0.12	0.01		NW	С								
25	Stanton Harcourt	te	4	0.23	0.01	0.11	0.00			(C)							\$	7
26	Crayford	te	4	0.24	0.00	0.12	0.01			С				0				7
27	Barnwell	te	2	0.24	0.01	0.12	0.01			С								
28	Ailstone-on-Stour	te	4	0.24	0.01	0.12	0.01		SA	С								
29	Histon Road	te	2	0.24	0.01	0.12	0.01			(C)								
30	Strensham	te	4	0.25	0.02	0.12	0.02		SA									
31	Stutton	te	4	0.25	0.01	0.14	0.01			С				٥				
32	Coronation Farm	te	1	0.25	0.00	0.13	0.00		τw									7
33	Somersham	te	4	0.25	0.01	0.12	0.00			С				\$				
34	Norton Bottoms	te	15	0.25	0.01	0.13	0.01		τw	С								7
35	Block Fen	te	4	0.25	0.02	0.13	0.01			(C)	1		1	1	1		1	1
33	Somersham	tr	4	0.26	0.01	0.13	0.01			c				0		+		
			-	0.20	0.01	0.10	0.01							Ť				

throughout the British Pleistocene, so where necessary we have used other species of *Bithynia* (b/t = B. *bavelensis/tentaculata*; le = B. *leachii*; te = B. *tentaculata*; tr = B. *troschelii*).

		1	1			1	1	1			1		1		h	6	
36	Ebbsfleet, U. loam, L. part	te	2	0.26	0.01	0.14	0.01							ŧ			
37	Brough Nature, A	utho	r ma	nuscri	0.01	ailabl	$e_{10}^{0.01}P$	MC	2ð¥2	Feb	uary	25.					7
38	Ilford (Uphall Pit)	te	2	0.27	0.01	0.14	0.02		Th	С	1						7
39	Maidenhall	te	2	0.28	0.02	0.15	0.00							٥			
40	West Thurrock (Lion Pit), 4	te	25	0.29	0.01	0.15	0.01		Th	с				ŧ			7
40	West Thurrock (Lion Pit), 2000	te	3	0.29	0.01	0.16	0.00		Th	с				ţ			7