The geochronological potential of isoleucine epimerisation in cassowary and megapode eggshells from archaeological sites

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Abstract

Our research demonstrates that the extent of isoleucine epimerisation (A/I) in fragments of avian eggshells provides geochronological information in archaeological contexts. In the archaeological sequence of Hay Cave, northern Queensland, Australia, there is an excellent correspondence between the A/I values of Australian brush-turkey (*Alectura lathami*) eggshells ($n = 99$) and independent geochronological control ($n = 16$ radiocarbon ages including 4 on eggshell calcite). The A/I values identify three phases of deposition during the Holocene at Hay Cave. In contrast to the *Alectura* eggshell A/I values, a poor correspondence was observed between the A/I values of cassowary (*Casuarius*) eggshells from Toé ($n = 35$) and Kria caves ($n = 23$) (Ayamaru Plateau, Papua) and the depths from which the specimens were recovered in these stratified sequences. Given coherent archaeofauna trends and radiocarbon chronologies ($n = 8$ and 2 eggshell calcite radiocarbon ages at Toé and Kria, respectively) with respect to depth, the variable A/I values are not explicable in terms of mixing. Rather, the variability is most likely due to exposure of the eggshells to the high temperatures of campfires. Despite the variability, eggshells with relatively low A/I values amongst specimens recovered from similar depths (and therefore presumably least influenced by high temperatures) exhibit a gradual increase in A/I with respect to depth, as expected in a stratified deposit. From this observation it is suggested that the identification of heated eggshells will increase confidence in geochronological information provided by A/I . These studies illustrate the complications that arise from campfire-induced acceleration of amino acid racemisation and emphasise that although this phenomenon is common, it is not universally encountered in archaeological contexts.

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1. Introduction

The diagenetic reaction, isoleucine epimerisation (analogous to amino acid racemisation), is commonly used as a proxy for geological time in amino acid-bearing fossils such as mollusc shells and fragments of avian eggshells (e.g., Wehmiller

et al., 1995; Miller et al., 1999b). Isoleucine epimerisation is the interconversion of the epimers L-isoleucine (I) and D-alloisoleucine (A), and is expressed as the ratio of these two molecules (A/I). As biologically produced amino acids are almost exclusively L-amino acids, and because the formation of D-alloisoleucine is approximately 30% more common than L-isoleucine formation, A/I progresses from values close to zero in modern specimens towards equilibrium at a value of about $A/I = 1.3$ (Williams and Smith, 1977; Miller and Bringham-Grette, 1989). Unlike geochronological techniques

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based on measuring the extent and effects of radioactive decay, the rate of isoleucine epimerisation is highly sensitive to temperature. Laboratory experiments predict that in warm settings (e.g., mean temperature of 25 °C) a 4 °C temperature increase will double the rate of epimerisation (Brooks et al., 1990).

In the context of African archaeology, the geochronological implications of isoleucine epimerisation have been explored extensively using ostrich (*Struthio*) eggshells (Brooks and Smith, 1987; Brooks et al., 1990; Miller et al., 1991, 1992, 1999a; Henry and Miller, 1992; Miller et al., 1992; Johnson et al., 1997; Kokis et al., 1998). For example, at Equus Cave *A/I* values indicate that *Struthio* eggshells have been extensively mixed throughout the sequence (Johnson et al., 1997), and ages inferred from *A/I* values at Border Cave (Miller et al., 1999a) and Gi (Brooks et al., 1990) exhibit an excellent correspondence with independent numeric ages (Grün and Beaumont, 2001; Bird et al., 2003). Such applications are

strengthened by criteria for the identification of eggshells with an anomalous thermal history produced by exposure to the high temperatures associated with campfire heating events (Miller et al., 1992). In non-archaeological contexts, ages inferred from the *A/I* values in eggshells of Australian birds (the emu *Dromaius*, the extinct giant mihirung *Genyornis*, and water-birds) have proven valuable for examining the changing environments and biogeography of central Australia (Magee and Miller, 1998; Johnson et al., 1999; Miller et al., 1999b).

Given the demonstrated ability to extract reliable geochronological information from eggshell amino acids, we have pursued the possibility of using the extent of isoleucine epimerisation as a proxy for geological time in Australasian archaeological sites. We report here *A/I* values in eggshells from Toé and Kria caves (located near the Ayamaru Lakes, Papua, Indonesia), and Hay Cave (Mitchell-Palmer limestone towers of northern Queensland, Australia) (Fig. 1). Preliminary results on the Ayamaru

Fig. 1. Location maps for the study sites. Maps depict (a) northern Australasia and locations of the study sites, (b) the Bird's Head peninsula, (c) the location of Toé and Kria Caves with respect to the Ayamaru Lakes, and (d) the location of Hay Cave in the Mitchell-Palmer limestone belt of southeast Cape York peninsula.

Lakes eggshells were reported by Pasveer et al. (2002) and Pasveer (2004). The aim of the research is to compare A/I values to independent forms of geochronological control (stratigraphic trends, radiocarbon ages) in order to assess relationships between the reaction extent and time. Combined with a recent report on A/I values in cassowary eggshells from the Aru Islands (Clarke and Miller, 2005), the present research on cassowary and megapode eggshells from Australasian archaeological sites represents a significant increase in the taxonomic and geographic application of eggshell isoleucine epimerisation geochronology.

2. Materials and methods

2.1. Specimen identification

Thick (*ca.* 1 mm) fragments of eggshell attributed to the cassowary (*Casuarius* spp.) were recovered from the Ayamaru Lakes sites. *Casuarius* is a large, flightless, frugivorous bird that inhabits the rainforests of New Guinea, northeastern Australia and neighbouring islands (Rich et al., 1988). Any of the three extant species of *Casuarius* (*C. casuarius*, *C. unappendiculatus* and *C. bennetti*) are potentially represented amongst the eggshells. Thin (*ca.* 0.3–0.4 mm) eggshells were also recovered from the Ayamaru Lakes sites, and from Hay Cave. These thin eggshells are attributed to the megapodes, a family of birds found throughout southeast Asia, Australia, Papua New Guinea and the western Pacific, that are renowned for their use of environmental heat to incubate their eggs (Jones et al., 1995). Megapodes possibly represented amongst the Ayamaru Lakes eggshells include *Megapodius*, *Talegalla* and *Aepyodius* (Jones et al., 1995; Aplin, 1998). The Hay Cave eggshells are attributed to the Australian brush-turkey, *Alectura lathami* (Clarke, 2005). Collections were screened to target large fragments and to avoid discoloured or darkened eggshells as these may have been exposed to high temperatures.

2.2. Isoleucine epimerisation analyses

To isolate amino acids of the squamatic zone the outer and inner surface of *Casuarius* eggshells were removed by abrasion. Megapode eggshells were cleaned of adhering sediments but were not abraded due to their fragility. Thirty-three percent of the exterior surface of eggshells was removed with 2 N HCl and a fragment weighing between 4 and 14 mg was dissolved in 7 N HCl and heated at 110 °C for 22 h to hydrolyse peptide bonds and obtain free amino acids. The hydrolysed residue was desiccated then re-hydrated with 0.01 N HCl prior to analysis. D-Alloisoleucine and L-isoleucine were separated by liquid chromatography and the output was interpreted using ChemStation software.

The extent of isoleucine epimerisation is the ratio of the relative abundance of D-alloisoleucine to L-isoleucine (A/I) calculated using peak areas. The reported values are averages accompanied by an estimate of analytical uncertainty (± 1 standard deviation (SD)) based on two or more replicates on the same sample preparation: the average uncertainty is ± 0.005 .

A correction factor based on peak width at half peak height was applied to the area data to account for possible errors in the determination of the elution times of the two peaks (especially where A is small), such that:

$$A/I = A_{\text{area}}/I_{\text{area}} \times A_{\text{width}}/I_{\text{width}}$$

Typically, the D-alloisoleucine and L-isoleucine peaks are congruent so the correction factor produces an A/I value only slightly different (within two standard deviations) of that calculated for the more commonly used peak height data. To correct for batch effects the A/I values have been normalised to an internal series of A/I values. These batch effects are discussed in detail elsewhere (Clarke, 2005).

2.3. Radiocarbon age estimation

Eggshells that exhibited low A/I values relative to eggshells recovered from similar depths were selected for radiocarbon dating (although some with apparently elevated A/I values were submitted before this protocol was established). At Toé Cave there is a bias towards eggshells from the base of the sequence because we sought to confirm the Pleistocene antiquity of the occupation of the site (Pasveer et al., 2002). Preparation of eggshell calcite for radiocarbon dating was identical to that for amino acid analyses (except the acid etch was increased to remove 50% of the eggshell calcite). Conventional radiocarbon ages (BP, with 1950 as the base year) are reported corrected for isotopic fractionation and converted to calendar years (cal years BP) using CALIB 4.3 (Stuiver and Reimer, 1993). A southern hemisphere correction was not considered appropriate for the low latitude study sites. Where conventional ages exceeded 18,000 years BP calendar ages were estimated using the calibration curve of Hughen et al. (2004).

3. Results

3.1. Toé Cave

Toé Cave is located inside a low conical hill, situated on a small peninsula that juts into the southern margin of the western basin of the Ayamaru Lakes (1° 15' S; 132° 15' E, Fig. 1c). Johan Jelsma excavated the site in 1995 and reports are provided by Jelsma (1998) and Pasveer (2004). The mean annual temperature of the Ayamaru Lakes region is about 25 °C. The cave has several entrances, one of which was excavated to bedrock. This entrance is *ca.* 3 m wide and 3 m high. The surface in this area is approximately horizontal but about 2.5 m from the entrance it slopes towards the back of the cave. At the horizontal part of the entrance three 1 m² squares were excavated in 5 cm depth intervals, forming a transect between the opposing walls. This excavated area contains a deposit up to 140 cm deep within which two main units were identified. The lower one-third of the deposit is a Pleistocene unit, the upper two-thirds are of Holocene age. The nature of the transition between these two units could not be established. Detailed analyses of the archaeological material and radiocarbon ages have led to

the conclusion that the deposit is essentially intact, albeit with minor local slumping and infill (Pasveer, 2004). Ages obtained on eggshells from the square against the northern wall of the cave (square 2N1E) are in the order expected with respect to depth in the deposit, although three Pleistocene ages with a range greater than *ca.* 15,000 years were found in the same depth interval (Table 1, Fig. 2). However, this is not surprising given the thinness (30 cm) of the Pleistocene unit at this side of the cave. Other radiocarbon ages came from samples in the square located against the southern wall (1S0E) (see Table 1, Fig. 2 and Jelsma, 1998). Three of these ages were obtained on charcoal and are sub-recent and probably reflect contamination or intrusion. The other two were obtained on fragments of bone and eggshell, and although considered reliable, were younger than expected of samples from the depths from which they were obtained and might be the result of local slumping or erosion of the sediment.

The *A/I* values of *Casuaris* and megapode eggshells from Toé Cave are reported in Table 2. In Fig. 2 the *A/I* values are plotted against the depth from which the specimens were recovered in the Toé Cave profile, and in Fig. 3a the frequency distribution of these values is presented. The expected trend of a gradual, well-defined increase in *Casuaris* *A/I* values ($n = 34$) reflecting an increase in eggshell age with respect to depth was not observed. Instead, *A/I* values amongst eggshells recovered from similar depths exhibited a wide range. The greatest range ($0.296 \leq A/I \leq 1.024$) occurred at a depth of approximately 70 cm. A large range of values ($0.420 \leq A/I \leq 0.989$) was also observed amongst eggshells from the base of the excavation (95–100 cm). Age accounts for some of the variability in *A/I* values observed at the base of the sequence: radiocarbon ages for specimens from the base of the excavation ranged from approximately 15,500 to 30,000 cal years BP (Fig. 2).

Of the few ($n = 7$) Toé Cave megapode eggshells analysed many had *A/I* values less than those of *Casuaris* eggshells from similar depths. However, as a whole, the megapode eggshell *A/I* values exhibit the same problematic trends observed in the *Casuaris* eggshell dataset (Fig. 2). Again, the expected trend with respect to depth is replaced by wide scatter amongst *A/I* values of eggshells from similar depths (e.g., $0.131 \leq A/I \leq 1.098$ at approximately 50 cm depth).

3.2. Kria Cave

Kria Cave (Fig. 1c) was excavated by J.M. Pasveer in 1995 (Pasveer, 1998, 2004). The cave has several entrances, one of which has accumulated over 2 m of sediment. This entrance is *ca.* 3 m high, 6 m wide and 2.5 m deep. Excavation took place in two 1 m² squares using 5 cm depth intervals. The deposit has a largely undisturbed stratigraphic profile, an observation supported by 9 of the 11 radiocarbon ages obtained on samples from the site (two were anomalously young, Fig. 4). Five occupation units were identified; the four lower occupation units differ from each other in terms of quantities of material (mostly stone and bone artefacts and large quantities of faunal remains), while the top unit contains different material and in

Table 1
Radiocarbon ages obtained on the calcite of *Casuaris* and megapode eggshells

Site ^a	Radiocarbon laboratory code ^b	AAL ^c	Taxa	Excavation unit/ find number	Depth (cm)	$\delta^{13}\text{C}$ (‰)	Conventional radiocarbon age (year BP, $\pm 1\sigma$)	Calibrated radiocarbon age (cal year BP, 1 σ range)
Toé Cave (Sq. 1S0E)	AA-32920	8831B	<i>Casuaris</i>	544	120–125	–12.4	10150 $\pm$ 65	11580–12090
Toé Cave (Sq. 2N1E)	AA-32921	8828D	<i>Casuaris</i>	311	25–30	–14.6	5290 $\pm$ 75	5940–6180
	AA-33404	8829A	<i>Casuaris</i>	454	60–65	–15.1	8860 $\pm$ 70	9780–10150
	AA-33405	8830A	<i>Casuaris</i>	524	80–85	–12.4	12870 $\pm$ 110	14940–15730
	AA-33406	8830C	<i>Casuaris</i>	524	80–85	–12.8	12885 $\pm$ 90	15010–15740
	OZF251	9295A	<i>Casuaris</i>	593	95–100	–15.9	13100 $\pm$ 110	15460–16010
	OZF516	9295B	<i>Casuaris</i>	593	95–100	–14.0	20310 $\pm$ 110	23300–23600
	OZG063	9295F	<i>Casuaris</i>	600	105–110	–15.1	25920 $\pm$ 180	29400–30000
Kria Cave	OZF245	9201C	<i>Casuaris</i>	744	65–70	–12.2	5120 $\pm$ 60	5750–5930
	OZF246	9190A	<i>Casuaris</i>	955	130–135	–13.5	6080 $\pm$ 60	6810–7010
Hay Cave	OZF619	9443C	<i>Alectura lathami</i>	3	1–3	–8.5	270 $\pm$ 40	290–420
	OZF620	9435C	<i>Alectura lathami</i>	31	53–56	–7.2	2320 $\pm$ 40	2330–2350
	NSRL-14448	9172H	<i>Alectura lathami</i>	36	63–66	–5.0	5395 $\pm$ 15	6180–6270
	OZG064	–	<i>Alectura lathami</i>	38	67–70	–15.0	2190 $\pm$ 40	2120–2310

^a Radiocarbon ages on materials other than eggshells are reported elsewhere: Toé and Kria Caves, Pasveer et al. (2002); Hay Cave, Rowe et al. (2001).

^b AA, University of Arizona National Science Foundation Accelerator Mass Spectrometry facility; NSRL, INSTAAR Laboratory for AMS Radiocarbon Preparation and Research; OZ, Australian Nuclear Science and Technology Organisation.

^c INSTAAR Amino Acid Laboratory unique identifier.

Fig. 2. Toé Cave (a) radiocarbon ages for excavation squares 1S0E and 2N1E and (b) *Casuaris* and megapode eggshells A/I values for 2N1E. The bone and charcoal radiocarbon ages are those reported by Jelsma (1998). Arrows indicate the direction of expected trends.

much lower quantities. The oldest radiocarbon age from the deepest excavation unit is *ca.* 7700 cal years BP. However, not all of the sediment was excavated and the timing of initial occupation can be extrapolated to *ca.* 9000 years ago. The activities in the cave remained essentially the same over the subsequent *ca.* 4000 years, after which the site appears to have been abandoned. From a radiocarbon date on a pig bone from the uppermost unit it is inferred that occupation had resumed by about 1800 years ago. There are no clear indications that the cave is in use today and it is uncertain when occupation of the site ceased.

Results for the extent of isoleucine epimerisation in eggshells from Kria Cave are presented in Fig. 4 and compiled in Table 3. As observed at Toé Cave, there is much scatter in the *Casuaris* and megapode eggshell A/I values. However, in the Fig. 3b histogram it is observed that there is a tendency towards lower A/I values at Kria Cave than Toé Cave and this is in accord with the radiocarbon chronologies for the respective sites (Figs. 2 and 4). Low *Casuaris* eggshell A/I values ($0.15 \leq A/I \leq 0.25$) are observed across a large span of depths, from about 65 cm to 140 cm depth. From this narrow range of A/I values it appears that much of the sequence was deposited over a short period of time. This inference is supported by radiocarbon ages on eggshells from these depths that suggest that it was deposited over approximately 1000 years (Table 1).

3.3. Hay Cave

Hay Cave is a large limestone cave located between the Mitchell and Palmer rivers, north Queensland, approximately 140 km west of the coastline, 16°6'S latitude, 144°7'E longitude. The cave entrance is large (10 m deep, 16 m wide and 3 m high) and it was towards the centre where the floor is flat and sediments are soft that excavations were undertaken by Bruno David, Harry Lourandos and Chris Clarkson in 1996. Four juxtaposed 50 cm² squares were excavated, the deepest of which (M30) was excavated in 63 lots of *ca.*

2 cm units (XU) that followed the stratigraphy. Twenty-two distinct stratigraphic units were identified in the 133 cm deep excavation and sediments containing cultural materials were found at all depths. From an AMS radiocarbon age of *ca.* 34,000 cal years BP obtained on a riverine bivalve shell recovered from XU 60 of square M30 (122–125 cm below the surface) (David and Lourandos, unpublished) it is estimated that sediments towards the base of the excavation were deposited about 30,000 years ago. However, assessing the timing of the deposition of the *ca.* 15 cm of sediment immediately below the major stratigraphic break located at *ca.* 60 cm depth was problematic. A radiocarbon age of about 3300 cal years BP (OZD009) was obtained on charcoal recovered close to the stratigraphic break, while an age of *ca.* 13,800 cal years BP was obtained on charcoal from *ca.* 75 cm depth (David and Lourandos, unpublished, Rowe et al., 2001). Thus, it was not clear whether or not this section included sediments deposited during the Early to Mid Holocene, especially considering Early Holocene sediments of many other archaeological sites in north Queensland are either absent or mixed (e.g., David and Chant, 1995). Large numbers of land snail shells and high frequencies of calcium carbonate concretions at *ca.* 60 cm depth indicate the likely presence of materials deposited during the Early Holocene at Hay Cave, as this was a period of heightened humidity and both items signal wetter conditions (Rowe et al., 2001). We thus turned to the A/I values of *Alectura* eggshells to obtain information on the presence of Early to Mid Holocene sediments at Hay Cave.

A total of 99 *Alectura* eggshells excavated from square M30 of Hay Cave were subjected to amino acid analyses (Table 4, Fig. 5). These eggshells primarily come from the upper 75 cm of the sequence: below these depths eggshells were rare or absent (Holden, 1999). A fragment of modern *Alectura* eggshell exhibited an A/I value of 0.021 ± 0.003 . In Fig. 5 it is observed that A/I in the majority of the eggshells analysed occupied a narrow range of values, from

Table 2
Extent of isoleucine epimerisation in *Casuaris* and megapode eggshells from Toé Cave

Square	Find number	Depth (cm)	AAL ^a	A/I ± SD	
				<i>Casuaris</i>	Megapode
1S0E	544	120–125	8831B	0.362 ± 0.008	
2N1E	204	10–15	9277A	0.476 ± 0.018	
	206	15–20	9278A	0.223 ± 0.001	
	206	15–20	9278B	0.328 ± 0.002	
	301	20–25	9279A	0.292 ± 0.012	
	301	20–25	9279B	0.259 ± 0.003	
	311	25–30	8828D	0.237 ± 0.001	
	326	30–35	9296A	0.257 ± 0.008	
	326	30–35	9296B	0.414 ± 0.008	
	326	30–35	9296C	0.264 ± 0.004	
	342	35–40	9280A	0.257 ± 0.007	
	343	35–40	9450A	0.571 ± 0.002	
	361	40–45	9451A	0.622 ± 0.006	
	361	40–45	9451B	0.273 ± 0.002	
	383	45–50	9283A	0.240 ± 0.003	
	383	45–50	9283B	0.268 ± 0.007	
	383	45–50	9284A		0.131 ± 0.001
	384 DIV	45–50	9452A	0.251 ± 0.001	
	384 DIV	45–50	9452B	0.274 ± 0.001	
	414	50–55	9285A		1.098 ± 0.022
	453	60–65	9453A	1.024 ± 0.005	
	454	60–65	8829A	0.384 ± 0.001	
	457	60–65	9454B	0.406 ± 0.002	
	457	60–65	9454C	0.401 ± 0.005	
	484	65–70	9288A		0.241 ± 0.002
	485	65–70	9455A	0.814 ± 0.006	
	485	65–70	9455B	0.296 ± 0.007	
	499	70–75	9289A		0.141 ± 0.002
	500	70–75	9456A	0.398 ± 0.004	
	500	70–75	9456B	0.385 ± 0.001	
	500	70–75	9456C	0.388 ± 0.001	
	516	75–80	9291A		0.992 ± 0.015
	523	80–85	9457A		0.206 ± 0.012
	523	80–85	9457B		0.205 ± 0.003
	524	80–85	8830A	0.501 ± 0.001	
	524	80–85	8830C	0.553 ± 0.001	
	593	95–100	9295A	0.420 ± 0.011	
	593	95–100	9295B	0.514 ± 0.013	
	593	95–100	9295C	0.989 ± 0.006	
	593	95–100	9295D	0.665 ± 0.005	
	593	95–100	9295E	0.813 ± 0.001	
	600	105–110	9295F	0.617 ± 0.007	

^a INSTAAR Amino Acid Laboratory code.

those expected of modern specimens up to $A/I = 0.16$. Only two specimens (2%) exceeded this range. High variation was typically observed at stratigraphic boundaries (reflecting mixing of eggshells of differing ages) and where eggshells with anomalously low A/I values were occasionally recovered from the same excavation unit as eggshells with higher values. In the frequency distribution of these results (Fig. 6) the values fall into three clusters: $0.02 < A/I < 0.05$, $0.05 < A/I < 0.08$, and $0.13 < A/I < 0.16$. These clusters are identified as A, B, and C, respectively. Not incorporated in these three clusters are the A/I values of three eggshells, one of which falls between clusters B and C. The other two have A/I values greater than those observed in cluster C.

Fig. 3. Histogram of (a) Toé Cave and (b) Kria Cave *Casuaris* and megapode eggshell A/I values. Radiocarbon ages obtained on *Casuaris* eggshells are indicated. Because the rate of isoleucine epimerisation is expected to differ between the two taxa the data are superposed.

4. Discussion

4.1. Highly variable *Casuaris* and megapode eggshell A/I values from Toé and Kria caves

The influence of high temperatures associated with campfire heating events is the mechanism most likely to be responsible for the high variability in *Casuaris* and megapode eggshell A/I values amongst specimens excavated from similar depths in the Toé and Kria Cave sequences. At Kria Cave mixing is an

Fig. 4. Kria Cave (a) radiocarbon ages and (b) *Casuarius* and megapode eggshell A/I values. The bone and charcoal radiocarbon ages are those reported by Pasveer and Aplin (1998). Arrows indicate the direction of expected trends.

unsatisfactory explanation for the variability in A/I values because the eggshell calcite radiocarbon ages are in accord with other radiometric dates for the sequences (Fig. 4). Similarly, at Toé Cave the eggshell radiocarbon ages are in accord with the timing of the deposition of the sequence as expected from changes in the archaeofauna assemblage with respect to depth (Jelsma, 1998; Pasveer and Aplin, 1998; Pasveer, 2004).

Within a stratigraphic unit eggshells unaffected or least affected by 'burning' are expected to exhibit the lowest A/I values and the scatter of A/I values between these minima and equilibrium ($A/I = 1.3$) will reflect the effect of high temperature on the rate of epimerisation. The degree to which exposure to campfires inflates the extent of isoleucine epimerisation is also a function of the duration of the heating event, the A/I value at the time of the event, and the number of such heating events encountered. Thus, according to the response of the reaction system to burning, the most coherent geochronological information is expected from eggshells with low A/I values relative to those exhibited by eggshells excavated from similar depths (where mixing is not significant). Indeed, at both Ayamaru Plateau caves the relatively low *Casuarius* eggshell A/I values slowly increase with depth and at both sites the relative age–depth trend is comparable to the respective radiocarbon numeric age–depth trend. For example, amongst eggshells with relatively low A/I values, results of $A/I < 0.2$ are observed at both caves amongst sediments with Holocene radiocarbon ages, and results between $A/I = 0.3$ and $A/I = 0.4$ are observed at Toé Cave where eggshells exhibit Pleistocene radiocarbon ages.

High temperatures associated with campfires will always be a concern when interpreting the biogeochemistry of ancient *Casuarius* and megapode eggshells. This is because, unlike those of other large flightless birds (*Dromaius*, *Struthio* and *Genyornis*) subjected to isoleucine epimerisation analyses, they will almost always be obtained from archaeological sites (megapode nesting mounds thousands of years old have been identified (e.g., Stocker, 1971; Bowman et al., 1999) but eggshell fragments have not been found in them). The A/I values

are not likely to be spurious artefacts of preservation state because the amino acid concentrations indicate that diagenesis has taken place in a regular manner, although like the extent of isoleucine epimerisation, it has been accelerated in some eggshells and not in others (Clarke, 2005). Also supporting the integrity of the amino acid results is the contrast in rates of isoleucine epimerisation between megapode and *Casuarius* eggshells at the Ayamaru Lakes archaeological sites. The tendency for megapode eggshells to exhibit A/I values less than those of *Casuarius* eggshells recovered from similar depths is in accord with laboratory heating experiments (Clarke, 2005) that predict that at 25 °C the rate of isoleucine epimerisation in *Casuarius* eggshells should be approximately half that in megapode eggshells.

The influence of campfire heating events on the extent of amino acid racemisation has previously been noted. Murray-Wallace and Colley (1997) observed elevated Asx D/L values attributable to transient exposure to high temperatures in mollusc shells from a Late Holocene archaeological sequence. Approximately 40% of the land snail shells from Hinds Cave analysed by Goodfriend and Ellis (2000) were deemed unsuitable for isotope analyses due to heating after rejecting samples with unacceptably high A/I values. 'Burnt' *Struthio* eggshells have often been encountered in Pleistocene archaeological sites throughout southern Africa. At the archaeological site of Boomplaas, South Africa, Hare et al. (1993) observed *Struthio* eggshells with an anomalously high extent of racemisation that was attributed to 'burning' on the basis of elevated abundances of amines and ammonia. A little less than half of the *Struthio* eggshells analysed by Miller et al. (1999a) from Border Cave (47%) and Apollo 11 Cave (45%) were identified as exposed to high temperatures by solving simultaneous equations for the extent of leucine hydrolysis and isoleucine epimerisation. Similarly, approximately half of the *Struthio* eggshells from Honey Nest Cave analysed by Miller et al. (1992) were identified as 'burnt' by this method and confirmed by subsequent ¹⁴C dating. 'Burning' therefore seems to be a problem that often complicates the extraction

Table 3
Extent of isoleucine epimerisation in *Casuaris* and megapode eggshells from Kria Cave

Find number	Depth (cm)	AAL ^a	A/I $\pm$ SD	
			<i>Casuaris</i>	Megapode
643/646	15–20	9204A		0.066 $\pm$ 0.002
712	45–50	9203A		0.144 $\pm$ 0.001
723	55–60	9202A		0.728 $\pm$ 0.004
723	55–60	9202C		0.747 $\pm$ 0.040
723	55–60	9202B		0.875 $\pm$ 0.033
723	55–60	9202D		1.122 $\pm$ 0.053
744	65–70	9201A	0.205 $\pm$ 0.010	
744	65–70	9201B	0.172 $\pm$ 0.001	
744	65–70	9201C	0.165 $\pm$ 0.001	
756	70–75	9200A	0.180 $\pm$ 0.011	
766	75–80	9199A	0.191 $\pm$ 0.006	
792	85–90	9198A	0.203 $\pm$ 0.012	
792	85–90	9198B	0.217 $\pm$ 0.002	
792	85–90	9198C	0.252 $\pm$ 0.007	
792	85–90	9198D	0.269 $\pm$ 0.013	
792	85–90	9197A		0.119 $\pm$ 0.013
792	85–90	9197B		0.136 $\pm$ 0.001
792	85–90	9197C		0.122 $\pm$ 0.004
852	105–110	9196A	0.464 $\pm$ 0.003	
865	110–115	9195A	0.232 $\pm$ 0.001	
865	110–115	9195B	0.656 $\pm$ 0.003	
865	110–115	9195C	0.221 $\pm$ 0.008	
865	110–115	9195D	0.299 $\pm$ 0.001	
896	115–120	9193A	0.228 $\pm$ 0.001	
913	120–125	9192A	0.378 $\pm$ 0.015	
913	120–125	9458A		0.181 $\pm$ 0.005
941	125–130	9191A		0.145 $\pm$ 0.002
955	130–135	9190A	0.225 $\pm$ 0.009	
955	130–135	9190B	0.253 $\pm$ 0.004	
955	130–135	9190C	0.275 $\pm$ 0.002	
955	130–135	9190D	0.219 $\pm$ 0.005	
980	130–135	9189A		0.117 $\pm$ 0.008
982	135–140	9188A	0.232 $\pm$ 0.005	
1005	135–140	9187A	0.683 $\pm$ 0.005	
1008	140–145	9186A		0.354 $\pm$ 0.001
1008	140–145	9186B		0.650 $\pm$ 0.008
1035	145–150	9185A	0.415 $\pm$ 0.008	
1084	155–160	9184A		0.921 $\pm$ 0.002
1109	160–165	9183A		0.142 $\pm$ 0.013

^a INSTAAR Amino Acid Laboratory code.

of geochronological information from the amino acid biogeochemistry of fossils in archaeological contexts.

4.2. Coherent trends in Hay Cave eggshell A/I values

In the frequency distribution of the 99 Hay Cave A/I values (Fig. 6) there are two eggshells with atypically high A/I values. A relatively high A/I value (AAL9171A, A/I = 0.333) was exhibited by an eggshell recovered near the top of the excavation, amongst eggshells where A/I < 0.04. The other relatively high A/I value (AAL10086A, A/I = 0.218) is also anomalous relative to the values observed in eggshells recovered from similar depths (Fig. 5). Therefore, like the Toé and Kria Cave results, the Hay Cave dataset appears to contain eggshells with anomalously warm thermal histories.

The three clusters of A/I values identified in the Hay Cave results exhibit an excellent but imperfect correlation with the

stratigraphic units in this sequence. Of the A/I values that do not conform to the depths that host the majority of eggshells of their respective cluster, all but one was recovered below the typical depth. While bioturbation is a potential explanation for the location of eggshells lower in the Hay Cave profile than expected, the likelihood of this process is reduced by the fragility of the thin megapode eggshells which makes them unlikely to withstand disturbance. Excavation error (such as fragments dropping from the walls of the excavation) in dark, cramped conditions is considered a more likely explanation for the occurrence of young eggshells low in the sequence. This is despite the implementation of precautions to protect the excavated surface from contamination between sessions of excavation.

The radiocarbon chronology indicates that the upper 80 cm of the sequence was deposited during one of three time periods, the terminal Pleistocene, 2400–3400 cal years BP, and 300–900 cal years BP. Similarly, the *Alectura* eggshell A/I results indicate that the upper 80 cm can be divided into the clusters, A, B and C. That clusters A and B correspond with the two younger phases identifiable by the radiocarbon chronology is supported by paired amino acid–eggshell calcite radiocarbon ages (Table 1, Fig. 6) and the observation that the depth the clusters occupy correspond with the depths from which the radiocarbon samples of David and Lourandos (unpublished) and Rowe et al. (2001) were recovered, with the lower A/I values corresponding to the younger radiocarbon ages (Fig. 5).

Although the age of clusters A and B may be unambiguous, the age of eggshells in cluster C is unclear for two reasons. First, there is not a paired amino acid–radiocarbon analysis for an eggshell from cluster C. Second, defining the age of the eggshells on stratigraphic grounds is complicated by the occurrence of the cluster at a major stratigraphic break (Fig. 5). The likely age for the cluster is greater than 3500 cal years BP and perhaps younger than the next oldest radiocarbon date, ca. 14,000 cal years BP (OZD943).

It is unlikely that the A/I values of cluster C eggshells are an artefact of exposure to a campfire heating event for several reasons. First, the fragments were not discoloured as might be expected of eggshells exposed to high temperatures. Second, the extent of isoleucine epimerisation is low (A/I < 0.16) and only slightly higher than those of cluster B: if heating has taken place the effect has been subtle. Third, the range in A/I values is small (0.03 A/I units) and this is opposite to the effect expected of exposure to high temperatures. If the rate of isoleucine epimerisation had accelerated due to exposure to high temperatures we would expect greater variability due to the heterogeneity of temperatures within a campfire and variability in the length of time these heat sources act upon the fragments. Therefore we are confident the A/I values of cluster C primarily relate to the age of the eggshell fragments.

4.3. Assessing the age of cluster C from A/I values

In the absence of independent geochronological control for cluster C, estimation of the rate of isoleucine epimerisation in

Table 4
Extent of isoleucine epimerisation in *Alectura lathamii* eggshells from Hay Cave

Excavation unit	Mean depth (cm)	AAL ^a	SID ^b	A/I ± SD
2	1.3	9178	A B C D E	0.029 ± 0.006 0.024 ± 0.001 0.023 ± 0.001 0.023 ± 0.005 0.021 ± 0.004
3	3.1	9443	A B C D E	0.025 ± 0.002 0.025 ± 0.004 0.021 ± 0.008 0.021 ± 0.003 0.020 ± 0.004
5	6.2	9177	A	0.333 ± 0.006
7	10.2	9442	A B C D E	0.025 ± 0.006 0.033 ± 0.001 0.045 ± 0.002 0.026 ± 0.001 0.028 ± 0.002
11	14.6	9176	A	0.029 ± 0.002
13A	18.4	9441	A B C D E	0.038 ± 0.009 0.027 ± 0.001 0.025 ± 0.002 0.025 ± 0.001 0.025 ± 0.001
14	19.5	9175	A B C D E	0.032 ± 0.001 0.032 ± 0.004 0.025 ± 0.002 0.029 ± 0.003 0.026 ± 0.001
17	26.0	9440	A B C D E	0.026 ± 0.001 0.032 ± 0.002 0.030 ± 0.004 0.031 ± 0.001 0.037 ± 0.004
18	27.4	9439	A	0.041 ± 0.011
21	33.1	9438	A B C D E	0.031 ± 0.003 0.033 ± 0.008 0.030 ± 0.005 0.032 ± 0.003 0.035 ± 0.008
22	35.6	9437	A B C D E	0.029 ± 0.002 0.037 ± 0.006 0.031 ± 0.004 0.033 ± 0.004 0.039 ± 0.003
24	40.2	9174	A B C D E	0.028 ± 0.001 0.030 ± 0.002 0.037 ± 0.002 0.040 ± 0.002 0.056 ± 0.001
25	42.3	9436	A B C D E	0.042 ± 0.005 0.031 ± 0.006 0.039 ± 0.007 0.043 ± 0.008 0.037 ± 0.005
26	43.9	10082	A B	0.030 ± 0.002 0.053 ± 0.002

Table 4 (continued)

Excavation unit	Mean depth (cm)	AAL ^a	SID ^b	A/I ± SD
27	46.0	10083	A B	0.057 ± 0.004 0.039 ± 0.001
28	47.2	10084	A B	0.053 ± 0.002 0.054 ± 0.001
29	50.5	9173	A B C D E	0.069 ± 0.013 0.061 ± 0.001 0.056 ± 0.004 0.056 ± 0.003 0.022 ± 0.001
31	55.9	9435	A B C D E	0.054 ± 0.001 0.059 ± 0.001 0.054 ± 0.007 0.056 ± 0.004 0.053 ± 0.007
32	57.5	9434	A B C D E	0.056 ± 0.003 0.058 ± 0.007 0.056 ± 0.003 0.071 ± 0.001 0.036 ± 0.009
33	59.5	10085	A B C	0.071 ± 0.001 0.058 ± 0.003 0.062 ± 0.002
34	61.8	10086	A B C	0.218 ± 0.004 0.056 ± 0.001 0.059 ± 0.001
35	63.2	10087	A B C D	0.073 ± 0.003 0.134 ± 0.006 0.067 ± 0.003 0.091 ± 0.003
36	65.7	9172	A B C D E F G H	0.066 ± 0.002 0.132 ± 0.005 0.054 ± 0.008 0.063 ± 0.004 0.156 ± 0.003 0.143 ± 0.001 0.064 ± 0.001 0.144 ± 0.001
37	66.8	9433	A	0.146 ± 0.007
38	70.0	10088	A B	0.152 ± 0.007 0.037 ± 0.006
39	71.6	9171	A	0.031 ± 0.001
41	77.4	9170	A	0.054 ± 0.009
42	80.1	9432	A	0.067 ± 0.001
62	127.7	9431	A	0.064 ± 0.002

^a INSTAAR Amino Acid Laboratory code.

^b Unique AAL identifier.

Alectura eggshells provides a means of assessing the age of the specimens comprising this group of A/I values. This approach provides an opportunity to assess the ability of isoleucine epimerisation in avian eggshell to yield numeric geochronological control and is an inexpensive alternative to radiocarbon dating. There are two radiocarbon-dated eggshells whose A/I values could be used to calibrate the epimerisation rate (i.e., OZF619 and OZF620). However, one of these (OZF619) is

Fig. 5. Hay Cave (a) radiocarbon ages and (b) *Alectura* eggshell *A/I* values plotted with respect to depth in the sequence. The approximate locations of stratigraphic breaks are indicated. Charcoal radiocarbon ages are those of David and Lourandos (unpublished) and reported by Rowe et al. (2001).

unsuitable because the *A/I* it exhibits is indistinguishable from that of modern eggshell: this is most likely due to the combined effects of analytical uncertainty and the slow rate of isoleucine epimerisation in *Alectura* eggshell. The other radiocarbon-dated eggshell has an *in situ* rate constant of $k_1 = 1.37 \times 10^{-5} \text{ year}^{-1}$, calculated by inputting the calendar age (plus 50 years) and *A/I* of this specimen into the integrated rate equation:

$$\ln(1 + A/I)/(1 - K'A/I) = tk_1(1 + K') + c \quad (1)$$

where K' is the reciprocal of the equilibrium $A/I = 1/1.30 = 0.77$, t is the time measured in years, and c is a constant obtained by solving the left-hand side of the equation using the *A/I* of a modern specimen (i.e., $t = 0$). Solving the integrated

rate equation for the mean *A/I* of cluster C yields an age estimate centred on 8870 calendar years. This mean value, $A/I = 0.144$, falls within the range that isoleucine epimerisation in *Alectura* eggshells was observed to obey first-order kinetics at high temperatures (up to approximately $A/I = 0.6$, Clarke, 2005), supporting the use of the integrated rate equation for age estimation.

Assessing the uncertainty associated with the age estimate for cluster C is difficult given the single acceptable calibration point for the reaction rate and poor understanding of the thermal history of the site. An attempt to quantify this uncertainty is presented in Table 5. To combine the uncertainty arising from the amino acid and radiocarbon measurements, these sources of error were algebraically propagated through the integrated rate equation using methods described by Taylor (1982). By this method, the age of cluster C has an associated uncertainty of approximately 1%, but this is based on the assumption that the eggshells of unknown age have experienced a thermal history equivalent to that experienced by the calibration sample. Assuming that the thermal histories of the calibration sample and eggshells of unknown age agree to within $\pm 0.5^\circ\text{C}$, and using an E_a of $30.8 \text{ kcal mol}^{-1}$ as a representative value for modelling the temperature sensitivity of the rate of isoleucine epimerisation in *Alectura* eggshells (Clarke, 2005), the uncertainty in the age estimate increases to $+9.1, -8.4\%$ (Table 5). For the eggshells with *A/I* values in cluster C, this corresponds to an age and asymmetric error term of $8870 + 810, -740$ years. Because the uncertainty associated with this age increases from about 1% to 9% when the $\pm 0.5^\circ\text{C}$ temperature uncertainty is incorporated (Table 5), and because the size of the uncertainty associated with temperature is an indeterminate source of error, the age calculation is necessarily considered an estimate. This error range seems to be a reasonable approximation, however, because the range of *A/I* values in cluster C is similar to those observed in clusters

Fig. 6. Histogram of Hay Cave *Alectura* eggshell *A/I* values. Radiocarbon ages paired with *A/I* values are indicated. Three clusters (A, B and C) of *A/I* values are identified.

Table 5

Calculations used to estimate the age of *Alectura* eggshells from Hay Cave based on isoleucine epimerisation

Calculation	$t \pm \text{SD}$ (years) (% error)	$A/I \pm \text{SD}$ (% error)	k_1 (years ⁻¹) (% error)
<i>In situ</i> calibration of the rate of isoleucine epimerisation	2390 $\pm$ 40 (1.7) ^a	0.054 $\pm$ 0.007 (13.0)	1.37 $\times 10^{-5} \pm 1.64 \times 10^{-7}$ (1.2) ^b
Calculating the age and associated random errors of eggshells with cluster C <i>A/I</i> values	8865 $\pm$ 80 (0.9) ^b	0.144 $\pm$ 0.013 (9.0) ^c	
Estimating the uncertainty of a 0.5 °C error in temperature			(+9.1, -8.4) ^d
Rounding after accounting for temperature uncertainty	8870 + 810, -740 (+9.1, -8.4) ^c		

^a Fifty years was added to equate the duration of epimerisation with the duration of radiocarbon decay, and the analytical uncertainty (1 σ) was used to estimate the standard deviation.

^b Error term estimated by algebraically propagating errors through the integrated rate equation.

^c Error term estimated from the square root of the sum of errors squared.

^d Error term estimated from the slope of the Arrhenius equation, $\ln(k_1) = -E_a/RT$, using the E_a for isoleucine epimerisation in *Alectura* eggshell calculated by Clarke (2005), 30.8 kcal mol⁻¹, where R is the gas constant (= 0.0019872 kcal mol⁻¹) and T is temperature in Kelvin.

A and B, and each of these younger clusters span about 1000 years (Figs. 5 and 6).

The age estimate for cluster C is in accord with the radiocarbon chronology for the sequence (i.e., it does not suggest stratigraphic inversion or mixing) and is in accord with the possibility that the Hay Cave sequence includes sediments deposited during the Early Holocene (Rowe et al., 2001). However, to assess the isoleucine epimerisation age estimate an additional *Alectura* eggshell from cluster C (AAL9172H) was submitted for radiocarbon dating. This eggshell has a calibrated radiocarbon age of approximately 6200 cal years BP (NSRL-14448, Table 1). This is about 2700 years or 30% less than the isoleucine epimerisation age estimate. It therefore appears that the calculated *in situ* rate constant is too low for cluster C eggshells. The reason for this is not clear but could be explained by an effective diagenetic temperature for the cluster C eggshells 2 °C warmer than the 2400 year old calibration sample, perhaps resulting from *ca.* 3000 years of shallow burial prior to the deposition of sediments associated with cluster B. It is concluded that the cluster C eggshells represent occupation of Hay Cave in the Mid Holocene, and, in accord with the findings of previous researchers (Miller and Hare, 1980; Wehmiller and Miller, 2000), uncertainty associated with use of isoleucine epimerisation to estimate numeric ages is inherently greater than the use of *A/I* values to infer relative ages, especially when extrapolating outside the range of calibration points.

Less than 5% of the *Alectura* eggshells subjected to amino acid analysis yielded high *A/I* values due to an anomalously high effective diagenetic temperature produced by exposure to campfire heating events. This increases confidence in the use of *A/I* to estimate sample age, and is in sharp contrast to the greater abundance of *Casuaris* eggshells with anomalously high *A/I* values in the archaeological sites of the Ayamaru Plateau and Aru Islands. The results reported by Clarke and Miller (2005) on *Casuaris* eggshells from the Aru Islands are significant because they reiterate the trends seen in the Ayamaru Plateau *A/I* values. From good correlations between relatively low *A/I* values (presumably ‘unburnt’) and radiocarbon ages, the Aru Islands results also indicate that *Casuaris*

eggshell amino acids can provide geochronological information, but confidence in *A/I* as an independent form of age control was undermined by the large range in values observed in eggshells from similar depths (Clarke and Miller, 2005).

The low incidence of ‘burnt’ megapode eggshells may reflect the methods used to prepare *Alectura* eggs for consumption. Exposure to high temperatures expands the contents of eggs, inducing cracks and risking the loss of their nutritious contents. Several methods of cooking megapode eggs are known and some of these minimise the exposure of the egg to high temperatures. For example, Lumholtz, writing in 1889, observed Australian Aborigines pierce fresh megapode eggs, place them in hot ashes and retrieve the contents by dipping a make-shift brush through the hole, a cooking method that would expose only one half of the eggshell to high temperatures (Lumholtz, 1980). Similarly, Pernetta (1989) observed the cooking of megapode eggs in ashes but also by emptying their contents into bamboo tubes or wrapping them in banana leaves to be cooked, methods that do not necessitate the exposure of eggshell to high temperatures. *Casuaris* eggs, on the other hand, may not have been treated so carefully as their robust eggshell is more likely to resist cracking. The *Alectura* eggshell amino acid results provide further evidence that anomalous thermal histories in archaeological sites can produce elevated *A/I* values. However, the low incidence of unusually high *A/I* values at Hay Cave indicates that this is not necessarily a disabling problem when seeking to refine site chronology with amino acid biogeochemistry.

5. Conclusions

As observed in *Struthio* eggshells and other sample types in which the extent of amino acid racemisation or isoleucine epimerisation has been investigated to generate geochronological control for archaeological sequences, interpretation of the *A/I* values of *Casuaris* and megapode eggshells from Ayamaru Plateau archaeological sites in terms of sample age is complicated by their exposure to the high temperatures associated with campfire heating events. Nevertheless, the tendency towards relatively low *A/I* values in Kria Cave eggshells

compared to values observed in Toé Cave specimens is in accord with radiocarbon ages indicating that the former sequence was deposited during the Holocene, and that the latter site has been accumulating since the Pleistocene. The most coherent geochronological information was obtained from eggshells with low *A/I* values relative to those exhibited by eggshells excavated from similar depths: amongst such samples *Casuarinus* eggshells with values of *A/I* < 0.2 were observed at both caves amongst sediments deposited during the Holocene, and results where $0.3 < A/I < 0.4$ were observed amongst Pleistocene Toé Cave eggshells.

Examination of the extent of isoleucine epimerisation in *Alectura* eggshells has helped determine the timing of the deposition of the Hay Cave archaeological site. The *A/I* values grouped into two Late Holocene clusters, one dated from ca. 2000 to 3500 cal years BP, the other from ca. 1000 cal years BP to the present, and another cluster upon which a Mid Holocene radiocarbon age was obtained. Although an attempt to estimate a numeric age from *A/I* values was problematic the eggshell amino acid results are nonetheless important for defining the temporal nature of the Holocene archaeological record at Hay Cave.

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