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Isoleucine Epimerization in *Casuarius* Eggshells from Archaeological Sites in the Aru Islands, Liang Lemdubu and Liang Nabulei Lisa

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Introduction

The epimerization of the amino acid isoleucine in avian eggshells has been used to determine the timing of a variety of events throughout the Late Quaternary. Epimerization is a chemical reaction that interconverts L-isoleucine into its epimer D-alloisoleucine. Geochronological investigations based on isoleucine epimerization in avian eggshells have been used to help assess the timing of the extinction of a member of the Australian megafauna, *Genyornis*, a large flightless bird (Miller et al. 1999a). Isoleucine epimerization in *Genyornis* and water bird eggshells have been used to confine the timing of lacustrine episodes beyond the limit of radiocarbon dating in central Australia (Magee and Miller 1998). Ages derived from isoleucine epimerization in *Struthio* eggshells in African archaeological sites have been important in refining the chronology for the evolution of modern humans (Brooks et al. 1990; Miller et al. 1999b). Recently, the extent of isoleucine epimerization in *Casuarius* eggshells was used to support a radiocarbon chronology attesting to a Pleistocene antiquity for the occupation of Papuan rainforest (Pasveer et al. 2002).

The spatial interaction of eggshell proteins and carbonate produces a microenvironment that is well suited to the preservation of indigenous protein residues over geological time. It is hypothesized that during calcification eggshell proteins form an organic matrix around which crystals precipitate producing a population of intracrystalline proteins (Hincke et al. 1999; *sensu* Sykes et al. 1995). This microenvironment approximates a closed system during diagenesis, thereby restricting the diffusion of protein residues from the eggshell structure (Brooks et al. 1990; Miller et al. 2000). This characteristic makes avian eggshells excellent candidates for biogeochemical analyses in applications such as amino acid racemization-based geochronology and palaeothermometry (e.g. Miller et al. 1997).

The aim of the present study is to evaluate the potential of isoleucine epimerization in *Casuarius* eggshells for age estimation, and to provide an independent age assessment for the Aru

Islands' archaeological sites Liang Nabulei Lisa and Liang Lemdubu (for a location map see Chapters 7 and 9, this volume). It is suggested that the Aru Islands archaeological sites are highly suitable for the application of isoleucine epimerization geochronology for two reasons. First, because the rate of epimerization is temperature-sensitive the reaction proceeds rapidly in warm tropical locations, effectively maximizing the ability of the technique to distinguish specimens of different ages. Second, the effects of diurnal temperature fluctuations are expected to be dampened in cave and rockshelter sites such as Lemdubu and Nabulei Lisa, thereby eliminating problems that are encountered at sites with complex thermal regimes (Pillans 1982).

There are three extant species of cassowary: the Dwarf Cassowary (*Casuarius unappendiculatus*), Bennet's Cassowary (*C. bennetti*), and the Southern Cassowary (*C. casuarius*). All three species inhabit New Guinea but only the subspecies *C. casuarius johnsonii* is found in Australia. *C. casuarius aruensis* is the subspecies that inhabits the Aru Islands at present (Grzimek 1972). The presence of this large flightless frugivore on the Aru Islands is presumably the result of dispersal from New Guinea over a former land bridge, however, it is also possible that the bird was introduced to the archipelago by people. *C. casuarius* stands approximately 1.5–1.7m tall and can weigh over 55kg, with females being slightly larger than males (Crome 1975; Marchant and Higgins 1990). A female will commonly lay a clutch of three or four eggs per season that are incubated by the male. The eggs are approximately 135mm long by 95mm wide, and feature a pea-green 'embossed' surface texture on a pale green background (Beruldsen 1980; Marchant and Higgins 1990).

It is assumed that fragments of *Casuarius* eggshell accumulate in rockshelter and cave deposits as the remnants of meals consumed by people. During historic times the people inhabiting the rainforests of tropical Australasia harvested *Casuarius* eggs as part of their traditional subsistence regimes (Harris 1978; Pernetta 1989). The presence of *Casuarius* eggshell in archaeological deposits throughout New Guinea suggests that this practice has been taking place for thousands of years (Gillieson and Mountain 1982; Jelsma 1998; Pasveer and Aplin 1998; Pernetta 1989).

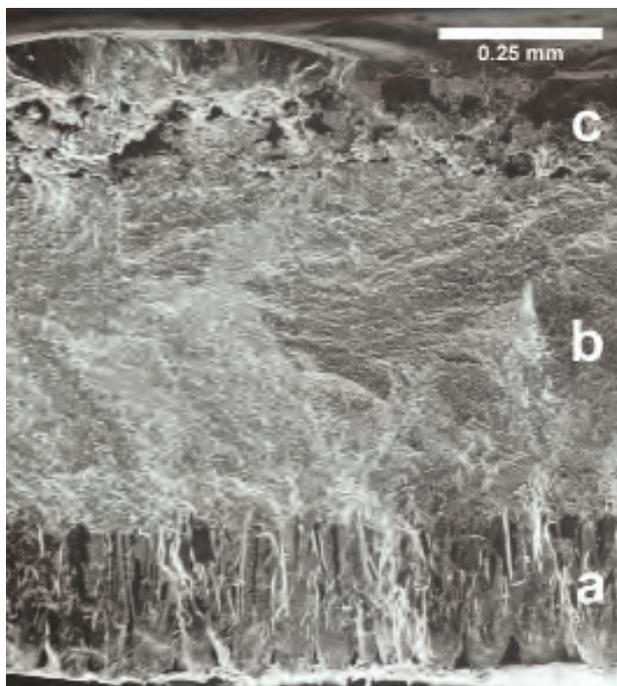


Figure 13.1 Scanning electron microscope image of modern *C. casuarius johnsonii* eggshell. The image is orientated with the outer surface of the eggshell at the top. The eggshell mammillary layers a) squamatic zone b) cavernous outer prismatic layer and c) outer surface are clearly evident

Casuarius eggshell is primarily calcite and consists of four morphological layers: the mammillary layer, squamatic zone, cavernous outer layer, and textured outer surface (Fig. 13.1). Pores interrupt the lateral homogeneity of the eggshell structure. Eggshell membranes present during incubation are not preserved over geological time. The squamatic zone is isolated for biogeochemical analyses because the protein and amino acid composition of ratite eggshells vary between the structural phases (Miller et al. 2000). Consistent analysis of squamatic zone amino acids circumvents the possible introduction of uncertainties relating to intra-sample differences in the extent of isoleucine epimerization.

Materials and Methods

Casuarius eggshells were recovered during excavation from the two Aru Islands cave

sites, Liang Lemdubu and Liang Nabulei Lisa (see O'Connor et al., Chapters 7 and 9, this volume). To examine the relationship between the extent of isoleucine epimerization and time, eight fragments of *Casuarium* eggshells (three from Lemdubu and five from Nabulei Lisa) were submitted for radiocarbon dating. In general, eggshells with the lowest A/I of those excavated from similar depths were submitted for radiocarbon dating. Conventional radiocarbon ages (BP) are reported corrected for isotopic fractionation and converted to calendar years (cal BP) using CALIB 4.3 (Stuiver and Reimer 1993; Stuiver et al. 1998). Where multiple calibrated ages were obtained a unique solution was defined as the median of the 2s range. This method of defining unique calendar ages has been used previously (e.g. Kaufman 2003; Oches et al. 1996).

The methods used to determine the extent of isoleucine epimerization in *Casuarium* eggshells follow those of Miller et al. (2000). To isolate the protein residues of the squamatic layer, the outer surface of samples (including the cavernous layer) was removed with a rotary drill. To remove potential contaminant amino acids from the eggshell surface and within the pores 33% of the calcium carbonate was dissolved with 2N HCl. Cleaned samples were dissolved in vials with 7N HCl (0.02ml/mg), capped under nitrogen gas, and placed in an oven at 110°C for 22 hours to hydrolyze peptide bonds. To determine the extent of isoleucine epimerization in naturally hydrolysed (i.e. free) amino acids, this hydrolysis step was eliminated. The resulting solution was dried in a heating module set at 80°C under a flow of nitrogen gas. Prior to analysis, samples were rehydrated with 0.01N HCl then loaded into an autosampler for high performance ion-exchange liquid chromatographic separation of amino acids. A fluorescence detector identified amino acids after post-column derivatization with o-phthalaldehyde. The extent of isoleucine epimerization is the ratio of the relative abundance of D-alloisoleucine to L-isoleucine (A/I) calculated using peak areas on electronically integrated chromatograms. A correction based on peak width at half peak height was applied to the peak area data to account for possible differences in the geometry of the two peaks such that:

$$A/I = A_{\text{area}}/I_{\text{area}} \times A_{\text{width}}/I_{\text{width}} \quad \text{Eqn. 1}$$

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

To simulate the diagenesis of protein residues at environmental temperatures over thousands of years, fragments of a modern *C. casuarium johnsonii* eggshell from a wildlife park near Cairns, Australia, were heated for a series of discrete time intervals at 143°C. The high temperature enables chemical reactions such as epimerization, peptide bond hydrolysis, and amino acid decomposition to reach the same extent over a period of weeks as would be attained over thousands of years at environmental temperatures. Following the method of Miller et al. (2000) fragments of eggshell weighing approximately 50mg were embedded in two grams of sterilized quartz sand moistened with 0.5ml deionized water, sealed within a pyrex test tube under normal atmosphere, heated at 143°C, then prepared for analysis as described above.

Isoleucine epimerization at high temperature

In Figure 13.2 it is observed that the extent of isoleucine epimerization in *Casuarium* eggshell increases from an initial A/I of 0.025 in modern eggshell towards values in excess of A/I = 1.20.

The pattern of isoleucine epimerization at high temperature in *Casuarinus* eggshell conforms to the model described by Kriausakul and Mitterer (1980) that identifies two phases during which the reaction approximates first-order kinetics. First-order kinetics are observed where the relationship between A/I values transformed using the logarithm term of the integrated rate equation and time is linear. The integrated rate equation relates the extent of isoleucine epimerization to time:

$$\ln [(1 + A/I)/(1 - K'A/I)] = (1 + K') k_1 t + c \quad \text{Eqn. 2}$$

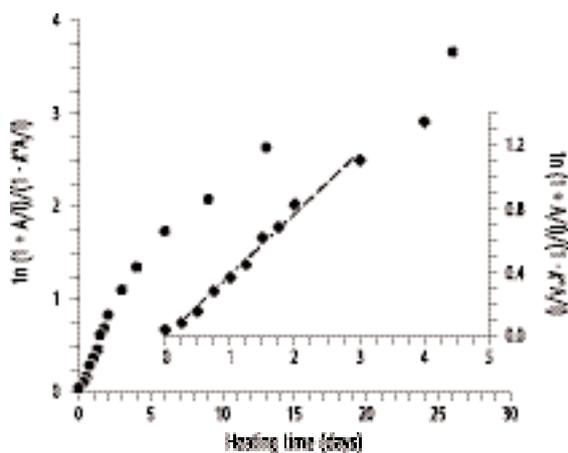


Figure 13.2 The extent of isoleucine epimerization in the hydrolyzate of modern *C. casuarinus johnsonii* eggshell heated at 143°C. A/I values are transformed using the logarithm term of the integrated rate equation. Results obtained over the initial phase of rapid epimerization are shown in the inset. Also highlighted is the range over which the reaction is observed to obey first-order kinetics

where K' is the reciprocal of the equilibrium A/I (0.77), k_1 is the forward reaction rate (years^{-1}), t is time measured in years, and c is a constant derived by solving the left-hand side of the equation for the extent of laboratory-induced epimerization at $t = 0$, obtained by analyzing modern eggshell. Inputting the above-mentioned initial A/I, a value of 0.044 for c is obtained. In *Casuarinus* eggshell there is an initial phase of rapid epimerization (average $k_1 = 66.9 \text{ yr}^{-1}$) separated from a phase of markedly slower epimerization by a transition zone of intermediate reaction rates. The rate of isoleucine epimerization in *Casuarinus* eggshell at 143°C is similar to that observed in *Dromaius* eggshell under the same experimental conditions ($k_1 = 69.3 \text{ yr}^{-1}$) by Miller et al. (2000:Fig. 13–5d). However, the slow non-linear reaction rate observed by these authors in *Dromaius* eggshell at the onset of epimerization is not evident in *Casuarinus* eggshell. These authors provided evidence indicating that this non-linear phase

lasts no more than a decade in warm ($>20^\circ\text{C}$ mean annual temperature) settings and, if it were to be applicable to epimerization in *Casuarinus* eggshell, is negligible at the temporal scale under investigation in this research.

At 143°C the initial phase of isoleucine epimerization in *Casuarinus* eggshells deviates from first-order linear kinetics at an A/I of approximately 0.7. According to Miller et al. (1991) the duration of this phase of first-order kinetics shortens as temperature decreases. It is suggested at environmental temperatures isoleucine epimerization in *Casuarinus* eggshell will deviate from first-order kinetics at an A/I less than 0.7. The integrated rate equation can therefore be used to relate an A/I less than 0.7 to derive age estimates on *Casuarinus* eggshells. The age of eggshells with an A/I approaching or greater than 0.7 will be underestimated.

Isoleucine epimerization in archaeological contexts

Casuarinus eggshell A/I values and radiocarbon ages are presented in Tables 13.1 and 13.2, corresponding to the Lemdubu and Nabulei Lisa excavations, respectively. The oldest radiocarbon ages were obtained on eggshells from Spit 19 of Lemdubu Test Pit C. Two separate eggshell

Table 13.1 Liang Lemdubu: radiocarbon ages and A/I values of *Casuarium* eggshells

SPIT	DEPTH (CM) ^a	LAB. CODE	RADIOCARBON AGE ^b	$\delta^{13}\text{C}$ (‰, PDB)	RADIOCARBON AGE ^c	AAL ^d	A/I (\pm 1SD)	
							Hydrolyzate	Free
1	7.8					8553A	0.173 \pm 0.001	0.356 \pm 0.009
1	7.8					8553B	0.115 \pm 0.006	
1	7.8					8553C	1.221 \pm 0.020	1.204 \pm 0.007
1	7.8					8553D	0.728 \pm 0.001	0.998 \pm 0.042
2	12.5	OZF247	2150 \pm 50	-13.5	2310-1990 (2150)	8554A	0.102 \pm 0.004	0.289 \pm 0.025
2	12.5					8554B	0.581 \pm 0.008	1.109 \pm 0.024
4	22.6					8713A	1.239 \pm 0.028	1.180 \pm 0.001
4	22.6					8713B	1.219 \pm 0.023	1.156 \pm 0.014
17	88.3					8557A	0.695 \pm 0.006	1.032 \pm 0.006
19	97.5					8559A		0.734 \pm 0.006
19	97.5	AA-32848	16770 \pm 110	-10.0	20,670-19,320 (19,980)	8559B	0.538 \pm 0.005	0.735 \pm 0.001
19	97.5	OZF248	16850 \pm 120	-11.7	20,770-19,400 (20,070)	8559C	0.523 \pm 0.008	0.719 \pm 0.001
20	102.0					8560A	0.742 \pm 0.002	1.119 \pm 0.001
20	102.0					8560B	0.630 \pm 0.005	1.030 \pm 0.007

a Mean of depths recorded at the four corners of the excavated unit

b Years BP, $\pm 1\sigma$ range

c Calendar years BP, maximum-minimum 2s range (unique age)

d Center for Geochronological Research Amino Acid Laboratory code

Table 13.2 Liang Nabulei Lisa: radiocarbon ages and A/I values of *Casuarium* eggshells

SPIT	DEPTH (CM)	LAB. CODE	RADIOCARBON AGE	$\delta^{13}\text{C}$ (‰, PDB)	RADIOCARBON AGE	AAL	A/I (\pm 1SD)	
							Hydrolyzate	Free
9	24.5					8784A	0.266 \pm 0.001	0.442 \pm 0.003
9	24.5					8784B	0.711 \pm 0.002	0.992 \pm 0.001
11	30.5	OZF249	2530 \pm 60	-13.6	2760-2360 (2710)	8785A	0.151 \pm 0.004	0.336 \pm 0.013
19	50.2					10078A	0.405 \pm 0.002	
25	68.5	OZF250	9310 \pm 80	-16.1	10,730-10,240 (10,490)	8786A	0.384 \pm 0.001	0.621 \pm 0.001
26	71.0	AA-32849	10460 \pm 75	-16.0	12,890-11,950 (12,420)	8787A	0.421 \pm 0.005	
27	72.5					10079A	0.396 \pm 0.001	
28	76.0					10080A	0.520 \pm 0.001	
28	76.0					10081A	0.673 \pm 0.11	
32	86.2	OZF848	10340 \pm 60	-15.9	12,800-11,770 (12,280)	8788A	0.717 \pm 0.002	1.161 \pm 0.007

fragments from this excavation unit had ages of about 20,000 cal BP. Late Holocene age estimates were obtained from both Lemdubu and Nabulei Lisa eggshells. The radiocarbon ages on *Casuarium* eggshell from Nabulei Lisa range from late Pleistocene to late Holocene.

Overall, there is good agreement between the eggshell radiocarbon ages for the Lemdubu and Nabulei Lisa sequences and the respective chronologies of these sites (Figs 13.3, 13.4). At Lemdubu the two similar radiocarbon ages obtained (by different labs) on *Casuarium* eggshells from approximately one metre depth are in close agreement with that of a marine estuarine shell recovered 10cm higher in the profile. The eggshell fragment from the top of the excavation also features a radiocarbon content comparable with charcoal fragments recovered from the same depths. The three Nabulei Lisa eggshells recovered from ca. 65–85cm depth have radiocarbon ages

similar to *Celtis* seeds and marine estuarine shells from the same section of the profile. The eggshell from ca. 90cm depth has the oldest radiocarbon age in the profile and this is explicable in terms of its location towards the base of the sequence. While there are no samples with radiocarbon ages similar to that of the eggshell from ca. 30cm depth this specimen is situated between charcoal and mollusc shells with radiocarbon ages that bracket that of the eggshell.

Figure 13.3 shows the extent of isoleucine epimerization in *Casuarium* eggshells versus excavation depth at Lemdubu. The *Casuarium* eggshells analyzed form a bimodal distribution within the profile because they are concentrated in the upper 25cm and between 85cm and 100cm depth. The range of A/I values within the less deeply buried samples is approximately 1.150. The range of A/I values within the group of eggshells excavated from greater depth is approximately 0.220. As expected of such highly variable A/I values, there appears to be little correlation between the radiocarbon chronology for Lemdubu and the amino acid results.

Figure 13.4 shows the extent of isoleucine epimerization in *Casuarium* eggshells versus excavation depth at Nabulei Lisa. There is not a well-defined increase in the extent of isoleucine

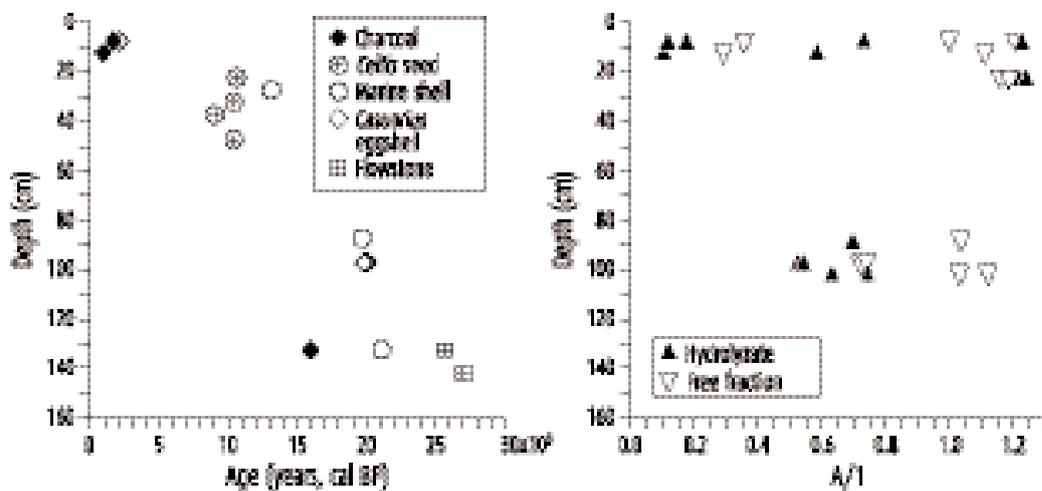


Figure 13.3 Liang Lemdubu: comparison of numeric age control and *Casuarium* eggshell A/I values. Values for the extent of isoleucine epimerization in both the hydrolysate and free amino acids are shown. Radiocarbon ages on eggshells are listed in Table 13.2 while ages on other sample types are from O'Connor et al. (Chapter 9, this volume)

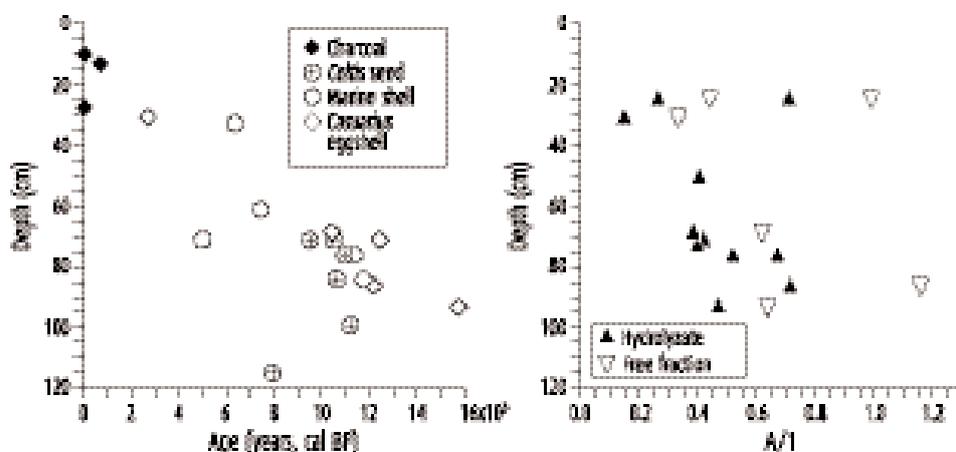


Figure 13.4 Liang Nabulei Lisa: comparison of radiocarbon ages and *Casuarium* eggshell A/I values. Values for the extent of isoleucine epimerization in both the hydrolysate and free amino acids are shown. Radiocarbon ages on eggshells are listed in Table 13.2 while ages on other sample types are from O'Connor et al. (Chapter 7, this volume)

epimerization with respect to depth. The range in A/I amongst eggshells excavated from similar depths is as high as 0.560 (units 9 and 11) and as low as 0.037 (units 25 to 27). Despite the noise the A/I values in both the hydrolyzate and free amino acids bear some resemblance to the radiocarbon chronology. The A/I values from ca. 70–100cm depth tend to be greater than those from ca. 20–30cm depth, and these depth intervals have terminal Pleistocene and Holocene radiocarbon ages, respectively. At both Lemdubu and Nabulei Lisa free amino acid A/I values essentially mirror the results obtained for the hydrolyzate.

In situ reaction rates calculated by solving the integrated rate equation for k_1 using *Casuarium* eggshell A/I values and radiocarbon ages are presented in Figure 13.5. The majority of values fall within the range $2.5 \times 10^{-5} < k_1 < 4.6 \times 10^{-5} \text{ yr}^{-1}$ but there is an outlier for which $k_1 = 6.0 \times 10^{-5} \text{ yr}^{-1}$. Amongst the majority of the k_1 values there

appears to be a systematic negative correlation between the reaction rate and eggshell age. Although this could be a statistical artefact of the small dataset it potentially represents depression of the reaction rate by cooler temperatures during the Pleistocene. Further work is necessary to gauge the temperature sensitivity of the isoleucine epimerization in *Casuarium* eggshells in order to explore the implications of the observed trends in terms of palaeotemperatures.

Discussion

Ancient *Casuarium* eggshells, unlike those of several other large flightless birds (*Dromaius*, *Struthio*, and *Genyornis*) studied for isoleucine epimerization geochronology, have only been obtained from archaeological sites. *Casuarium* nests are thin mattresses of vegetative matter (Beruldsen 1980) that are not constructed in settings conducive to the preservation of eggshells over geological time. The association with archaeological sites means that heating in or near hearths will always pose a potential problem to studies of isoleucine epimerization in *Casuarium* eggshell. Such transient heating events are problematic because they accelerate the rate of isoleucine epimerization to produce elevated and highly variable A/I values (Ellis et al. 1996; Goodfriend and Ellis 2000; Miller et al. 1992; Murray-Wallace and Colley 1997).

Exposure of *Casuarium* eggshells to high temperature is a likely explanation for the wide range of A/I values observed within excavation units and the absence of a well-defined down-profile increase in A/I at Lemdubu and Nabulei Lisa. For example, several eggshells from the upper excavation units of Lemdubu feature A/I values of approximately 1.2, close to the value expected at equilibrium ($A/I = 1.3$, Williams and Smith 1977). The simplest explanation for such high A/I values is an anomalously high integrated thermal history such as that produced by short-term exposure to the high temperatures of hearth flames or coals. Mixing within a site could bring older samples (high A/I) upward through the profile to be recovered amongst younger specimens (low A/I), while downward mixing would have the opposite effect. In the absence of objective criteria for the identification of exposure to high temperature such processes cannot be ruled out.

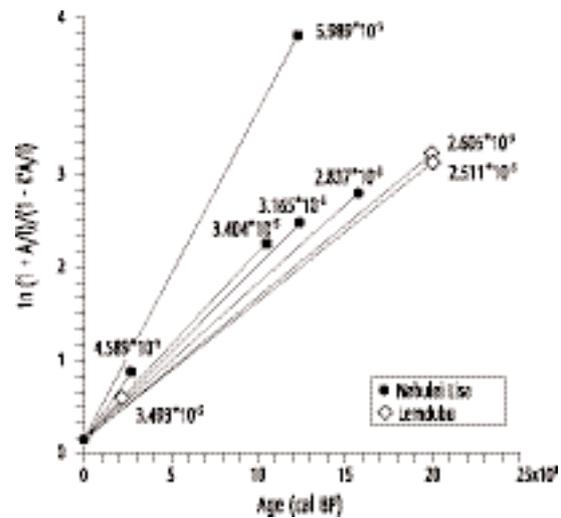


Figure 13.5 Liang Lemdubu and Liang Nabulei Lisa: *In situ* rate constants for isoleucine epimerization in *Casuarium* eggshells. A/I values are transformed using the logarithm term of the integrated rate equation. Rate constants (k_1 , yr^{-1}) are indicated next to their corresponding data points

Overall, however, the radiocarbon ages and sharply defined boundaries that mark changes through time in other cultural materials suggest a high degree of stratigraphic integrity, at least at Lemdubu.

The relationship between the extent of isoleucine epimerization in the hydrolyzate and free amino acids of *Casuarinus* eggshells is in accord with the idea that the excellent preservation of amino acids in avian eggshells promotes the systematic diagenesis of these molecules. The A/I values observed in the hydrolyzate of *Casuarinus* eggshells from Lemdubu and Nabulei Lisa are plotted against the corresponding A/I values of free amino acids in Figure 13.6. There is a linear relationship between these two sets of A/I values where the reaction extent amongst free amino acids is less than A/I = 0.8. Across this range the A/I values in free amino acids are approximately 30% higher than that observed in the hydrolyzate. This systematic trend is explicable in terms of the retention of both high and low molecular weight protein residues. Typically, amino acids in low molecular weight residues (e.g. free amino acids) are preferentially lost from the fossil matrix. This is significant because (as apparent in Fig. 13.6) these amino acids have a characteristically high extent of isoleucine epimerization. If the retention of the low molecular weight residues of *Casuarinus* eggshells during diagenesis is variable then their contribution to the A/I value of the hydrolyzate would be variable, thereby introducing noise into the relationship between the extent of isoleucine epimerization in free amino acids and the hydrolyzate.

While amino acid diagenesis in *Casuarinus* eggshells may take place in a systematic fashion where A/I values are low, the correlation between A/I values in the hydrolyzate and free amino acids deteriorates where A/I > 0.8 in the latter fraction. Because the eggshells that feature anomalous results in Figure 13.6 also exhibit anomalously high A/I values in the contexts of Figures 13.3 and 13.4, confidence in ascribing their spurious nature to the effects of campfire heating events is increased. However, it is not clear from the available data what geochemical mechanisms lead to the deterioration of the relationship between hydrolyzate and free amino acid A/I values. Normally, the rate of epimerization in free amino acids is governed by the epimerization of amino acids at the terminal positions of polypeptides and their subsequent release from the chain by hydrolysis. Perhaps the rate of epimerization in the free pool is particularly responsive to the high temperatures of campfire heating events, forcing these A/I values higher than would normally be attained by the hydrolytic release of terminal amino acids.

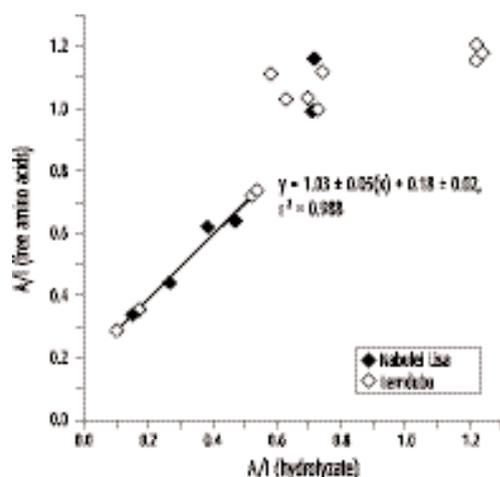


Figure 13.6 Relationship between the extent of isoleucine epimerization in the hydrolyzate and free amino acids of *Casuarinus* eggshells. Results for the linear regression where A/I < 0.8 amongst free amino acids are presented

Like their influence on the extent of isoleucine epimerization, it may be difficult to account for the effect of short duration, high temperature events on the radiocarbon content of eggshells. As observed in other biominerals (e.g. Haas and Banewicz 1980), high temperatures can release carbon dioxide from carbonates such as eggshell calcite and produce residual calcium oxide. Calcium oxide will react with CO₂ and water producing secondary carbonate and calcium hydroxide, respectively. The exchange of CO₂ between the biomineral and the environment promoted by the formation of secondary carbonates is of concern because it will introduce ¹⁴C that is not contemporaneous with the radiocarbon content of the indigenous calcite. However, it is unlikely that eggshells

featuring a large amount of calcium hydroxide will be recovered from geological sequences because the formation of this mineral destroys a specimen's mechanical integrity (SJC pers. observ.). Also of concern are the observations of Williams (1981) and Vogel et al. (2001) of radiocarbon age estimates on modern ratite eggshells that suggest the samples are hundreds of years old (see also Higham 1994). These results contrast with those of Miller et al. (1999a) that demonstrate the radiocarbon activity of modern *Dromaius* eggshells to be equivalent to that of the contemporaneous atmosphere. As the results of these studies fail to demonstrate the existence of a consistent ratite eggshell radiocarbon anomaly, and in the absence of similar research on *Casuarius* eggshell, a correction has not been applied to the radiocarbon ages presented here. The resulting uncertainty is negligible in terms of the temporal scale of environmental and cultural change under investigation in the Aru Islands archaeological sites.

One of the major advantages of amino acid racemization as a geochronological tool is the ability to determine the timing of events beyond the limits of radiocarbon dating (ca. 50,000 years BP). The epimerization of isoleucine in *Casuarius* eggshells is no exception to this observation. However, the warm temperatures of the bird's tropical habitat and the range of A/I values over which the reaction was observed to obey reversible first-order kinetics, impose limits to this application. Using the mean of the rate constants in Figure 13.5 (excluding the anomalously high data point) as an example, isoleucine epimerization in *Casuarius* eggshell ceases to obey reversible first-order kinetics after approximately 21,000 years. Under these conditions, in eggshells older than 21,000 years A/I will be greater than 0.7 and the integrated rate equation will systematically underestimate specimen age. This problem may be reconciled by the mathematical transformation of amino acid data. For example, Murray-Wallace and Kimber (1993), and Hearty and Kaufman (2000) observed that the D/L values of field samples could be accurately related to time by describing the decrease in the reaction rate as a parabolic function of time (Mitterer and Kriausakul 1989).

Setting uncertainties in deriving numeric ages aside, isoleucine epimerization in *Casuarius* eggshells will provide a reliable index of relative age for samples with similar thermal histories. Furthermore, when supported by a radiocarbon chronology, the A/I values can be used to estimate numeric age. The results from the Aru Islands combined with those from the Ayamaru Plateau, Papua (Pasveer et al. 2002; Clarke and Miller unpublished data) constitute a substantial set of paired *Casuarius* eggshell A/I values and radiocarbon ages spanning the last 30,000 years (Fig. 13.7). Importantly for studies of long-term environmental and cultural change, the Holocene can be distinguished from the Pleistocene at an A/I of approximately 0.3. Late Holocene eggshells can be distinguished from those of the early Holocene, and an A/I of approximately 0.15 is expected of mid-Holocene eggshells. From the oldest eggshells in the dataset, although limited by few data points and the variability in those available, an A/I greater than 0.5 may provide a useful means of identifying eggshells greater than 20,000 years old. In

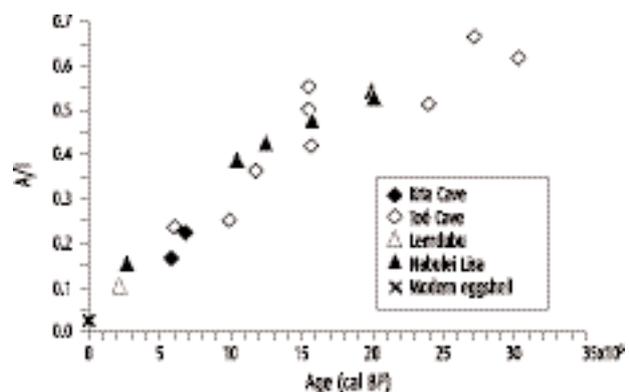


Figure 13.7 Extent of isoleucine epimerization in radiocarbon dated *Casuarius* eggshells from archaeological sites in the Aru Islands (Liang Lemdubu and Liang Nabulei Lisa) and the Ayamaru Plateau, Papua (Kria and Toé Caves). Data points represent mean A/I values and calibrated radiocarbon ages. An anomalously high data point from Liang Nabulei Lisa has been omitted from the plot. Ayamaru Plateau data from Pasveer et al. (2002) and Clarke and Miller (unpublished data)

summary, the combined Aru Islands and Ayamaru Plateau datasets offer a simple index for ascribing an age to a *Casuarius* eggshell based on its A/I value; a method applicable to other lowland archaeological sites occupied over the last 30,000 years where this sample type is encountered. It should be noted that in both relative and numeric chronologic applications the geochronological use of A/I will be most confident in archaeological sites where *Casuarius* eggshells are abundant. Multiple analyses permits rigorous assessment of within-excavation unit variability and identification of the expected down-profile increase in A/I, two trends that aid in the identification of anomalous A/I values resulting from effects such as exposure to campfire heating events.

Conclusions

Eggshells of *Casuarius* are well preserved in archaeological sites on the Aru Islands. Laboratory experiments indicate isoleucine epimerization within this eggshell type follows linear first-order kinetics to an A/I of 0.7, permitting interpretation of the extent of the reaction in terms of numeric age. The eggshells are also suitable for radiocarbon dating. A close correlation between paired A/I values and ages has been demonstrated. However, isoleucine epimerization shows substantial variability in many excavation units and this is attributable to transient heating by hearths. By selecting the eggshells with the lowest A/I values from each unit it is possible to reduce the likelihood of extracting erroneous inferences from amino acids influenced by such transient heating events. Radiocarbon ages and A/I values from *Casuarius* eggshells indicate initial occupation of Liang Lemdubu and Liang Nabulei Lisa during the late Pleistocene and further occupation during the Holocene. Combined with previous work on isoleucine epimerization in *Casuarius* eggshells, the results from the Aru Islands archaeological sites provide a basis for which an approximate age can be assigned to a *Casuarius* eggshell based on its A/I value in future work.

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