Amino acid geochronology

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Abstract

Amino acid geochronology is a well-established dating technique used in a wide range of Quaternary sciences. The primary geochronometer is the extent of racemization of amino acids preserved in a variety of organic remains. As a relative-dating tool, it is used to distinguish and correlate units, identify unconformities, and quantify the distribution of ages within fossil assemblages, among other applications. As a calibrated-dating method, it is used to estimate numerical ages by calibrating the rate of racemization against other dating techniques in a regional context. Where ages are known, the method can also be used for paleothermometry.

Keywords

Amino acid; Aminostratigraphy; Chronostratigraphy; Dating method; Geochronology; Paleothermometry; Quaternary; Racemization

Key points

- Amino acid geochronology is a well-established relative- or calibrated-dating method; it is used to estimate numerical ages and paleotemperatures.
- It is applicable to a wide variety of organic materials and Quaternary depositional settings.
- This article covers basic principles, methods, and applications of the technique.

Introduction

Amino acid geochronology is a biogeochemical dating method used mostly on fossiliferous deposits ranging in age from decades to millions of years. The technique has a wide range of applications related to Quaternary marine and terrestrial stratigraphy, archeology, historical ecology, sea-level history, tectonic geomorphology, glacial geology, and many other topics. The most commonly used media are organic constituents in carbonate biominerals precipitated by bivalves, gastropods, foraminifera, ostracodes, corals, brachiopods, and in avian eggshells. Some studies have also used otoliths, oogonia, echinoderms, bones, teeth, and wood, as well as less biogenic materials, including whole-rock eolianites, speleothems, travertine, tufa and paleosols. The technique is best suited as a relative-dating tool, or as a calibrated-dating method in conjunction with other dating techniques. As a relative-age tool, amino acid geochronology is used to correlate depositional units across exposures or sediment cores with similar post-depositional thermal histories, recognize site-specific temporal unconformities, and to identify reworked skeletal remains, among other targeted applications. This encyclopedia entry provides an overview of the principles, methods, and applications of amino acid geochronology.

General principles

Fossil remains of biogenic minerals contain trace quantities of indigenous organic matter, which can persist for long periods of geologic time. A common environment for the preservation of amino acids is the carbonate skeleton of invertebrates such as mollusks and foraminifera. Organic material is incorporated into carbonate exoskeletons either between the mineral crystals (intercrystalline), where it usually helps determine the form and shape of the mineral phase during precipitation, or within crystals (intracrystalline), where it adds resiliency to the mineral phase. After synthesis by an organism, proteins and their constituent amino acids degrade through a complex series of biogeochemical reactions. Proteins are hydrolyzed into smaller polypeptides, eventually releasing free amino acids. Some amino acids are converted into simpler or secondary amino acids, or they are decomposed into non-amino acid molecules. By measuring the extent to which these chemical changes have progressed, the length of time elapsed since death of an organism can be estimated.

Among the complex network of reactions involved in protein diagenesis, the most reliable for geochronology is amino acid racemization. Racemization is the chemical reaction by which amino acids are slowly and spontaneously interconverted from one molecular arrangement into their alternative enantiomer or mirror-image form (Fig. 1). Almost all living organisms metabolize and generate amino acids exclusively of the L–(*levo*) configuration. Upon death and removal of biologic constraints, the L-amino acids begin to racemize to their D–(*dextro*) configuration. The abundance of D- relative to L- isomeric forms (D/L) of a particular amino acid defines its extent of racemization. Initially, the value is near zero and it increases with time until the rate of formation of D-amino acids is compensated by the reverse reaction, and the value reaches an equilibrium value of 1.0 (racemic equilibrium) when there is an equal abundance of both D- and L- forms.

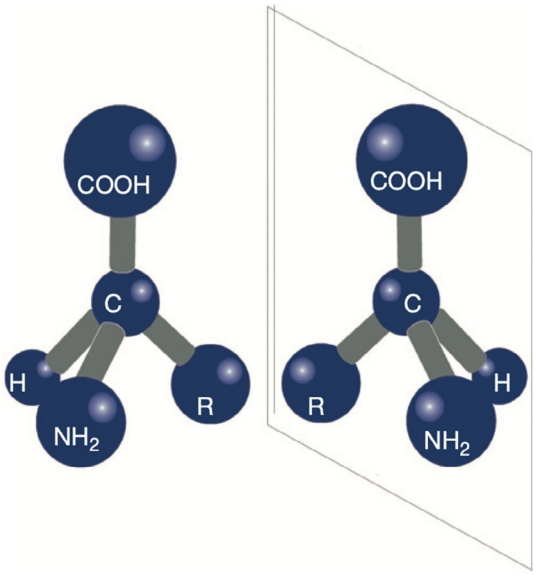

Fig. 1 The structure of a generic amino acid and the mirror-image relation of its two stereoisomers or enantiomers. The reaction that converts one form to the other is known as "racemization." From Miller GH, Kaufman DS, and Clarke SJ (2013) Amino acid dating. In: Elias SA and Mock CJ (eds.) *Encyclopedia of Quaternary Science* (2nd edn). pp. 37–48. Amsterdam: Elsevier.

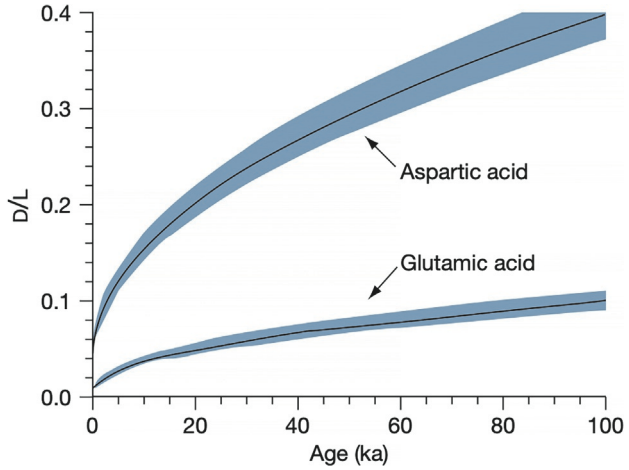

Fig. 2 Relation between time and extent of racemization (D/L) for aspartic acid (Asp) and glutamic acid (Glu) in the ostracode, *Candona*, from deep lakes in the western United States. Ages are estimated by independent evidence. Gray envelopes denote ±1SD uncertainty. From Kaufman DS (2003) Amino acid paleothermometry of Quaternary ostracodes from the Bonneville Basin, Utah. *Quaternary Science Reviews* 22: 899–914.

Most proteins comprise sequences of 10 to 20 amino acids, each with a somewhat different characteristic rate of racemization. For example, aspartic acid is among the fastest racemizing amino acids and is frequently used for relatively young materials or at cold sites, whereas the slower racemization in glutamic acid, leucine, valine, and isoleucine is effective at longer time scales and warm sites. The rate of racemization depends on the ability of R groups to stabilize a carbanion intermediate. The carbanion intermediate is the molecule formed when the hydrogen side chain is abstracted from an amino acid. In an ideal system, upon re-addition of the hydrogen atom to the carbanion intermediate, there is an equal probability of L- and D-amino acid formation. The extent of racemization measured in the total population trends toward a 50:50 mix of the two isomers, reflecting the equal rates of the forward and reverse reactions. The overall rate of racemization in the total amino acid population decreases as the extent of racemization increases, giving rise to the characteristic curvilinear trend produced when D/L values are plotted against linear time (Fig. 2).

Organic diagenesis and its relation to racemization

Chemical dating methods differ from radioactive dating techniques in that their reaction rate depends on environmental conditions, whereas radioactive decay is invariable. The rate of racemization is tied to the entire network of physical, chemical, and biological processes involved in the early diagenesis of organic matter. In most settings temperature is the dominant rate-controlling variable, but other environmental factors are also potentially important in determining the rate of racemization.

Protein diagenesis

Amino acids racemize at different rates depending on their position within the protein or peptide chain (Fig. 3). Peptide bonds that link adjacent amino acids are broken as they undergo hydrolysis and other processes involved with organic diagenesis, including decomposition and leaching. Racemization is generally faster for amino acids in terminally bond positions (especially the N-terminus), and slower for those that are internally bound; amino acids that have been completely released from their peptide chain to form free molecules racemize slowest. The progression of amino acids from bound to free amino acids, mediated by hydrolysis, results in an overall decrease in the overall (apparent) rate at which the D/L value of the entire amino acid population increases with age. This decrease in rate is beyond that which is predicted by a reversable reaction as it progresses toward equilibrium, as described above. The extent of racemization (expressed as D/L, the proportion of the D- and L- isomers) is low in the larger molecular-weight fraction and is higher in the smaller molecular-weight and free amino acid fractions (Fig. 4). Through time, with the progressive attrition of amino acids, the apparent forward rate of racemization in the remaining pool of amino acids can be complicated because molecular weight itself can influence the rate of various diagenesis processes, including microbial influences that operate on organic matter. For example, the preferential removal of free amino acids tends to decrease the D/L of the total population of remaining amino acids because free amino acids are the most highly racemized.

Microbial influence

While amino acids within biominerals are well protected from the environment, no substrate is immune to microbial influence. Microbes are involved in organic diagenesis. They can potentially confound the use of amino acids for geochronology because some

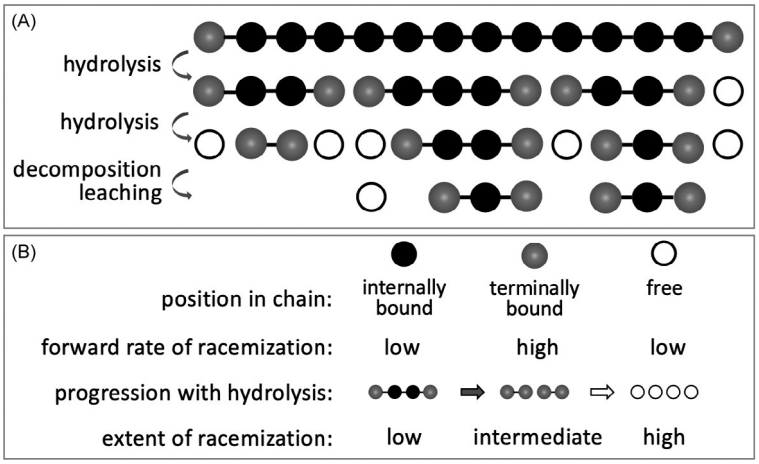

Fig. 3 Schematic showing (A) the breakdown of a peptide chain of amino acids and (B) its relation to racemization. During protein diagenesis, amino acids are separated from neighboring amino acids by hydrolysis, which transfers amino acids from internally-bound to terminally-bound positions, before they are released into the pool of free amino acids. The rate of racemization is highest for terminally bound amino acids. The extent of racemization is highest in the free pool. Diagenetic processes including decomposition and leaching reduce the amino acid population.

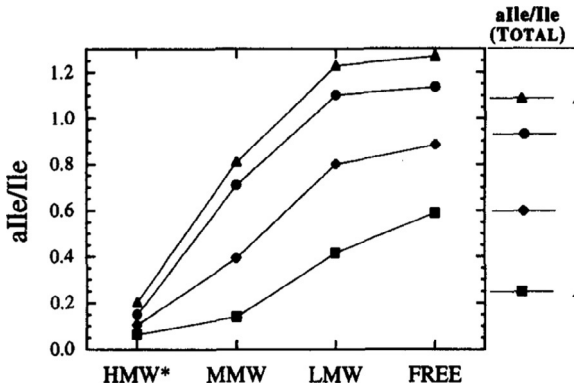

Fig. 4 Extent of racemization/epimerization in isoleucine (alle/Ile) measured in molecular-weight fractions of four late Pleistocene eggshells of the extinct Australian bird, *Genyornis*. alle/Ile is lowest in the high molecular weight fraction (HMW); it increases from the moderate to the low molecular weight fractions (MMW and LMW, respectively), and is highest in the free fraction (FREE) of amino acids. Symbols on the right show the alle/Ile values (y-axis) in the total acid hydrolysate populations of amino acids in the four samples. From Kaufman DS and Miller GH (1995) Isoleucine epimerization and amino acid composition in molecular-weight separations of Pleistocene *Genyornis* eggshell. *Geochimica et Cosmochimica Acta* 59: 2757–2765.

bacteria metabolize and produce D-amino acids. Bacterial cell wall bio-polymers, collectively termed peptidoglycan, contain abundant D-amino acids, especially D-Glu and D-Ala. Indeed, these have been used as biomarkers to track microbial influence. Microbes might also contribute to the decomposition of proteins in ways that accelerate racemization.

Leaching

Pore water that circulates through sediment can physically transport (leach) amino acids from the mineral matrix of fossils. The rate of leaching is controlled by the size of the mobile molecules and their sorption to the mineral matrix, the diameter and complexity of the diffusion pathway, and temperature. Molecules most susceptible to leaching are the low-molecular-weight solutes, especially those that are physically situated between mineral crystals in pathways that lead to the exterior of the biomineral. Because low-molecular-weight residues tend to be more racemized, leaching can decrease the extent of racemization in the total population of amino acids that are left behind in the biomineral. This process thereby adds to the protein decomposition effect by decreasing the overall rate of racemization beyond that which is predicted by a reversable reaction as it progresses toward equilibrium as described above.

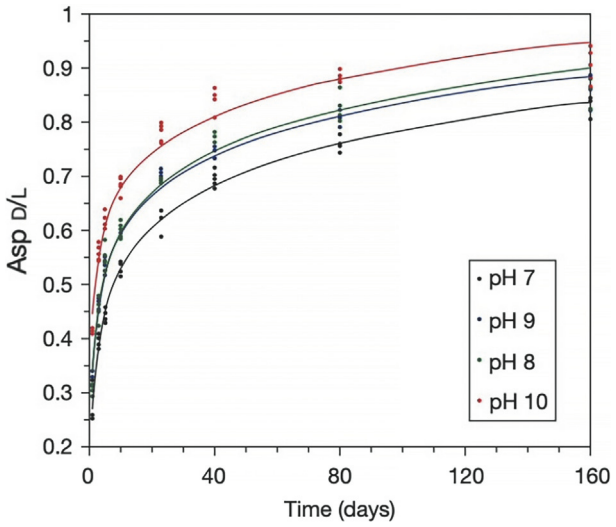

Fig. 5 Extent of racemization (D/L) in aspartic acid (Asp) in *Margaritifera* shell heated in buffered solutions with pH ranging from 7 to 10. Shells were heated at 110 °C for up to 160 h. Rates of racemization increase with increasing pH. Curves are simple logarithmic fits. From Orem CA and Kaufman DS (2011) Effects of basic pH on amino acid racemization and leaching in mollusk shell. *Quaternary Geochronology* 6: 233–245.

Environmental pH

Racemization is catalyzed in basic solutions; biominerals heated in solutions above pH 9 racemize more quickly than those heated at neutral pH (Fig. 5). The proportion of free amino acids in the laboratory-heated shells also increases with increasing pH, as does the rate of leaching. Although carbonate fossils buffer the ambient pH and maintain a local environmental pH of ~8, samples from strongly basic environments likely follow different diagenetic pathways and reaction rates.

Intra- and inter- crystalline amino acids

Protein residues retained within otherwise inorganic crystals of biominerals are least susceptible to leaching and other environmental processes and have been shown to approximate a physically closed system. Isolating and analyzing this fraction of amino acids can improve the reliability of amino acid geochronology. Exposure of powdered biominerals to concentrated sodium hypochlorite (bleach) for 48 h effectively reduces the amino acid content to a residual level. The organic components that are oxidized during the procedure are called the 'inter-crystalline fraction,' and the components that are retained are assumed to comprise the 'intra-crystalline fraction.' In some studies, the variability in D/L values among specimens of a single-age population is reduced when they are pretreated with bleach compared to conventionally analyzed shells. In addition, the correlation between D/L values measured in the free and total hydrolyzed amino acid populations is improved, indicative of increased integrity (Fig. 6). The beneficial effect of the same bleaching pre-treatment on some materials, including freshwater ostracodes and foraminifera, is unclear. For these biominerals, and perhaps others, the inter-crystalline proteins are rapidly lost following burial; therefore, most all of the amino acid content resides within the intra-crystalline fraction.

Taxonomy

The taxonomic effect results from taxon-dependent differences in the relative abundance and structures of the various proteins contained with biominerals. Different proteins generate different peptides, some more refractory than others. Different proteins have different arrangements of amino acids, with variable bonding strengths between adjacent amino acids. These variables influence the rate at which amino acids pass from internally bound to free amino acids, which controls the extent to which they are racemized. In addition, differences in the physical structure and morphology of biominerals might give rise to differences in their susceptibility to leaching and microbial decay, which also influences the extent of racemization in ways that are taxon dependent. Differences in the rate of racemization among taxa can be quantified where they are found co-existing in the same stratigraphic horizon (Fig. 7), or by heating them together in the laboratory to effectively accelerate time. Generally, differences in racemization rates among taxa within a family of organisms are within about 20%, although differences of up to a factor of two have been noted between some genera. Among mollusks, there are significant differences between genera but broadly comparable reaction rates among species of the same genus.

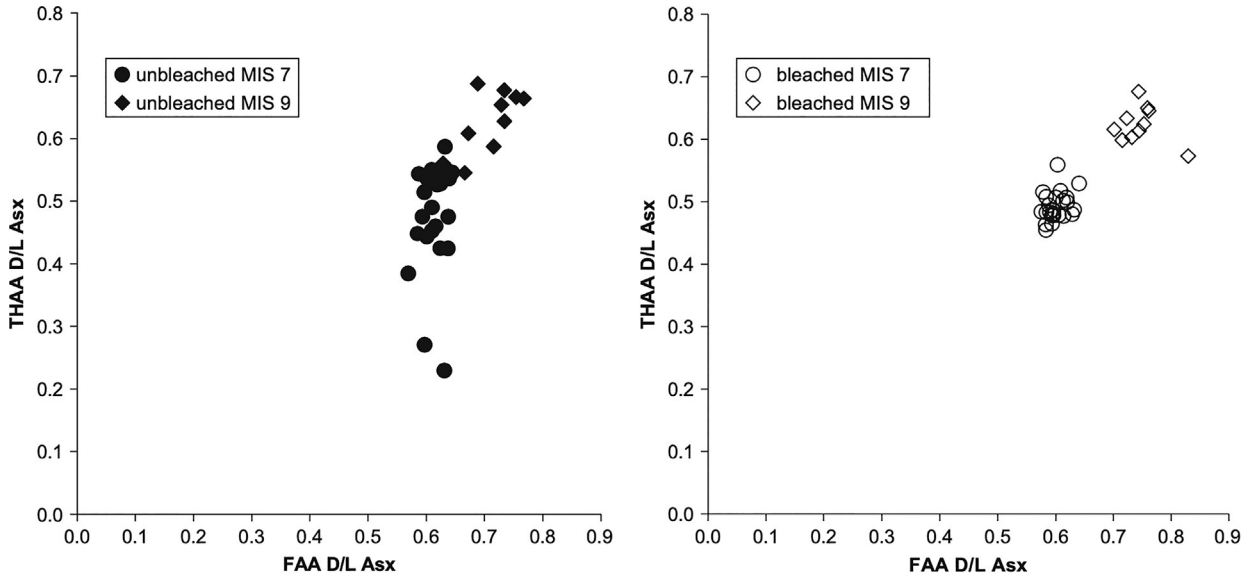

Fig. 6 Extent of racemization (D/L) in aspartic acid plus asparagine (Asx) in the total hydrolysable amino acid (THAA) population versus Asx D/L in the free amino acid (FAA) population of the same shell for unbleached (left) and bleached (right) subsamples of *Bithynia* shells from marine isotope stage 7 and 9 deposits in the United Kingdom. Results of analyses on bleached shells (intracrystalline fraction) yielded greater consistency. From Penkman KEH, Kaufman DS, Maddy D, and Collins MJ (2008) Closed-system behaviour of the intra-crystalline fraction of amino acids in mollusc shells. *Quaternary Geochronology* 3: 2–25.

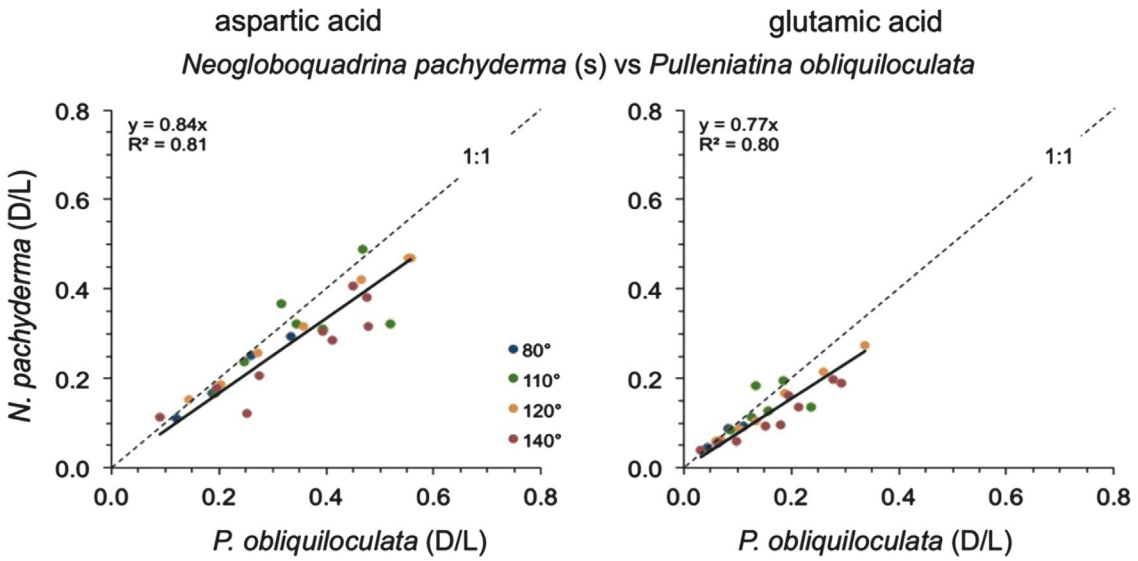

Fig. 7 Extent of racemization (D/L) for aspartic acid and glutamic acid measured in two foraminifera taxa, *Pulleniatina obliquiloculata* and *Neogloboquadrina pachyderma* (*s*) heated together in the laboratory. Samples from core tops were heated at four temperatures to accelerate the rate of racemization. Each data point represents a time-temperature step in the experiment. Modified from Kaufman DS, Cooper K, Behl R, Billups K, Bright J, Gardner K, Hearty P, Jokobbson M, Mendes I, O'Leary M, Polyak L, Rasmussen T, Rosa F, and Schmidt M (2013). Amino acid racemization in mono-specific foraminifera from Quaternary deep-sea sediments. *Quaternary Geochronology* 16: 50–61.

Temperature

Like other chemical reactions, the rate of racemization is controlled by temperature according to an exponential relation. Because of this non-linear relation, a given rise in temperature will increase the reaction rate more than the same decline in temperature will decrease the rate. In this way, the integrated post-depositional temperature (aka, effective diagenetic temperature) is higher than the time-averaged arithmetic mean temperature. These two metrics are equal under isothermal conditions, but increasingly diverge as the amplitude of temperature fluctuations increases. Nonetheless, the effect of temperature has been clearly established in multiple studies in which the current mean annual temperature of collection sites across a region track the extent of racemization for coeval stratigraphic units (Fig. 8).

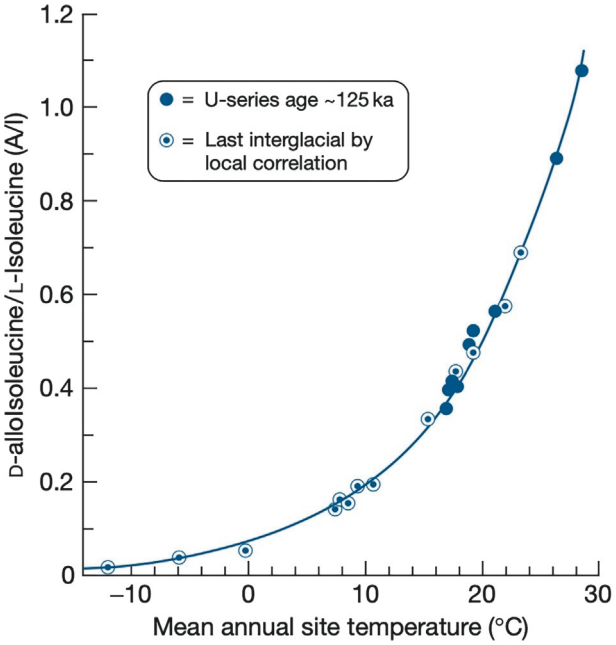

Fig. 8 Extent of racemization/epimerization in isoleucine (A/I) in mollusks from last interglacial (~125 ka) sites across western Europe, ranging from Svalbard and Arctic Russia to the Mediterranean Basin, plotted against current mean annual site temperature. Last interglacial sites are dated by U/Th on corals or correlated with the last interglacial on the basis of diagnostic marine faunal elements. These data show the exponential dependency of racemization rate to site temperature. Unpublished data courtesy G.H. Miller and P.J. Hearty, University of Colorado Boulder.

Fossils from deep-marine and deep-lake settings experience more mild temperature fluctuations than in terrestrial settings. The stable thermal environment of deep-sea sites minimizes the often-complicating effect of variable temperature on the long-term rate of racemization. Indeed, some of the earliest research on amino acid geochronology took advantage of the long-term stability of deep-sea settings to investigate the diagenesis of amino acids over geologic time, including the rate of racemization in foraminifera.

The sensitivity of racemization to temperature has been extensively studied in the laboratory by heating various taxa at different temperatures and monitoring the extent of racemization over time (Fig. 9). These data underpin kinetic models used to quantify the relation between D/L, time, and temperature. At 160 °C, a racemic mixture is reached within 10 to 20 h for most taxa. At room

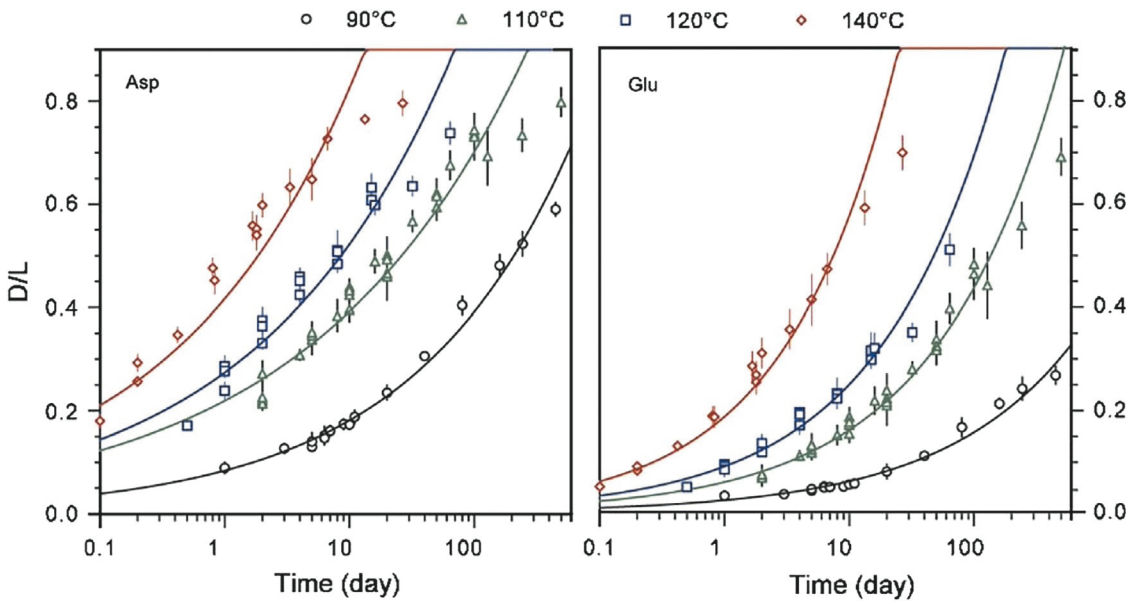

Fig. 9 Relation between the extent of racemization (D/L) in aspartic acid (Asp) and glutamic acid (Glu) in tests of the foraminifera genus, *Pulleniatina*, heated at four temperatures. Curved lines are power functions used to illustrate the trends. Error bars are ±1SD variability among tests heated in a single ampule. From Kaufman DS (2006) Temperature sensitivity of aspartic and glutamic acid racemization in the foraminifera *Pulleniatina*. *Quaternary Geochronology* 1: 188–207.

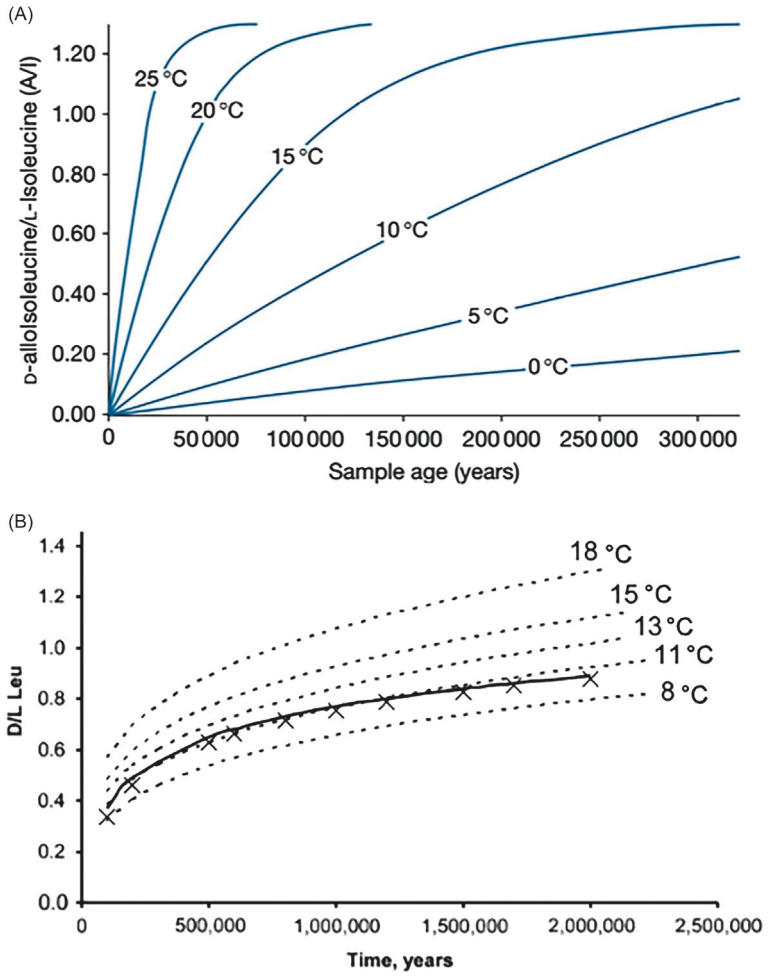

Fig. 10 Relation between the extent of racemization/epimerization and sample age for a range of effective temperatures showing the dependence of the reaction rate on temperature. (A) Isoleucine in eggshells from Australian emu, *Dromaius*. (B) Leucine in mollusk shells from the US Atlantic Coastal Plain; solid line with Xs are measurements on shells with ages estimated using $^{87}Sr/^{86}Sr$. (A) Modified from Miller GH, Hart CP, Roark EB, and Johnson BJ (2000) Isoleucine epimerization in eggshells of the flightless Australian birds, *Genyornis* and *Dromaius*. In: Goodfriend GA, Collins MJ, Fogel ML, Macko SA, and Wehmiller JF (eds.) *Perspectives in Amino Acid and Protein Geochemistry*, pp. 161–181. New York: Oxford University Press. (B) Modified from Wehmiller JF, Harris WB, Boutin BS, and Farrell KM (2012) Calibration of amino acid racemization (AAR) kinetics in United States mid-Atlantic Coastal Plain Quaternary mollusks using $^{87}Sr/^{86}Sr$ analyses: Evaluation of kinetic models and estimation of regional Late Pleistocene temperature history. *Quaternary Geochronology* 7: 21–36.

temperature (22 °C), the reaction is estimated to take several tens of thousands of years for most amino acids. In polar regions (−10 °C), it might take 1 to 2 Myr to reach racemic equilibrium. The useful time range for amino acid geochronology under different ambient temperatures has been modeled for isoleucine (Fig. 10); valine, leucine, and glutamic acid likely have similar reaction rates for a given temperature.

Methods

Field procedures

Because temperature fluctuations are strongest closest to the Earth's surface, collecting samples from the ground should be avoided. Actively retreating and recently formed exposures can be sampled reliably. Paleosols represent extended periods of exposure near the ground surface and should be avoided. At archeological sites, the possibility that mollusk shells and eggshells have been heated during cooking should be considered, especially in cave sites were fires would have been likely. Specimens should not be handled with bare hands, which can readily contaminate surfaces with abundant modern amino acid, nor should specimens be exposed to high temperature. Otherwise, no special handling is needed.

Sample selection – material type

Specimen size is typically not a limiting factor in amino acid analyses because only a few milligrams of biomineral are needed. Fragments can be used but should be indefinable to the generic level to account for taxonomic effects. Analysis and interpretation of D/L values typically focus on one or a few of the most commonly occurring genera. The most suitable taxa are those in which measured D/L values are uniform across a specimen and are reproducible among individuals of the same age. These taxa provide higher-resolution geochronological information than those that exhibit more variability. Many reliable taxa have already been identified by previous studies. Where multiple species are recovered in association, intergeneric differences in racemization rates provide an internal check on the reliability of D/L values. Generally, well preserved specimens yield less-variable results compared with chalky, abraded and fragment materials.

Sample selection – between-specimen variability

Different individual specimens within a collection (multiple specimens from one stratigraphic horizon) will inevitably yield somewhat different D/L values. Some of the variability can be attributed to analytical reproducibility, and some might reflect a heterogenous thermal histories. The variability can also reflect true differences in the ages of individuals in a collection, which is the primary focus of demographic, taphonomic, and time-averaging studies. For studies aimed at estimating the best age of a collection assumed to represent a single population, at least 5 and preferably 10 individuals of one taxon should be analyzed separately and the results averaged to obtain a robust D/L value with realistic uncertainties. For studies that rely on microfossils, several individuals of one taxon may need to be combined to attain a sufficient quantity for analysis. Granular materials (e.g., whole-rock eolianite) are treated similarly. At least five multi-specimen (multi-grain) subsamples should be analyzed individually to calculate a mean D/L value.

Sample selection – within-specimen variability

Amino acid composition and D/L values can vary spatially within individual specimens. This reflects primary differences in protein composition associated with anatomical positions and mineral ultrastructure, and can result from physical heterogeneities, including growth layering, that can influence the extent of leaching and other diagenetic processes from place to place. To minimize this effect, either whole specimens are analyzed, or they are subsampled to consistently isolate a specific location within each specimen. Typically, the thickest and most dense section is selected, where diffusional loss of low molecular weight polypeptides and free amino acids is minimized. In some cases, individual specimens are subsampled to generate multiple D/L values that are pooled mathematically or used to check for intra-specimen reproducibility.

Preparation procedures

Considering the huge variety of natural materials used for amino acid geochronology, there is no one-size-fits-all preparation procedure. The overall goal is to remove non-indigenous surface or infilling materials and to isolate the inner portion of a specimen, which has been least exposed to the external environment. For relatively large specimens, this typically involves an initial mechanical cleaning followed by dissolution of the outer surface in dilute acid. Microfossils are typically treated by gentle sonication followed by soaking in peroxide. For studies that rely on the intra-crystalline amino acid fraction, the specimen is powdered and soaked in bleach. All cleaned specimens are dissolved in acid and undergo standard laboratory hydrolysis to break peptide bonds. This generates a solution of free amino acids, known as the total hydrolysable amino acid (THAA) population. In some studies, analyzing the amino acids that comprise the naturally free amino acid (FAA) pool can be useful as a cross check on the integrity of the results. To measure the FAA, specimens are dissolved but not hydrolyzed, prior to analysis. For very cold or very young sites, D/L in the free amino acid fraction can be a more reliable geochronometer than the THAA population.

Analytical procedures

The separation, detection, and quantification of the D- and L-amino acid isomers is accomplished by either gas or liquid chromatography. Isoleucine can be separated from its secondary, non-biological diastereomer allo-isoleucine, by conventional ion-exchange chromatography. This method, along with gas chromatography, was commonly used for amino acid geochronology prior to around 2000. Since then, reverse-phase liquid chromatography has been more commonly used to separate enantiomers of multiple amino acids with high sensitivity (Fig. 11). Interlaboratory comparative standards (ILC), now available through the Lamont-Doherty Earth Observatory, are routinely analyzed by amino acid geochronology laboratories as an independent check on instrumental accuracy.

Data analysis and curation

Amino acid analysis generates a large quantity of data including concentrations of multiple amino acids and their enantiomeric pairs, often for multiple specimens or subsamples within a collection, and sometimes for both THAA and FAA fractions. Multiple

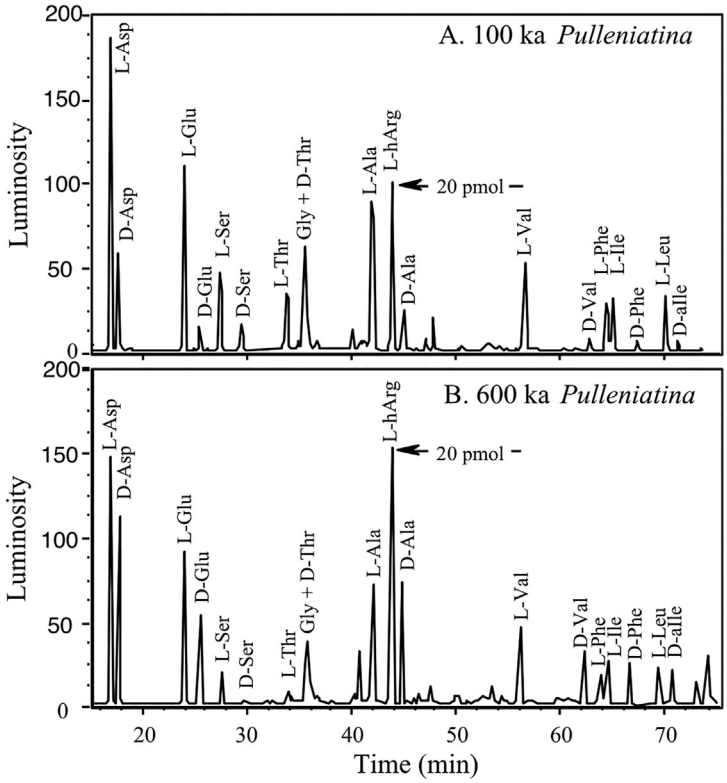

Fig. 11 Reverse-phase liquid chromatography chromatograms of single individuals of the large planktonic foraminifer, *Pulleniatina,* from marine cores recovered off northeastern Australia, with approximate ages of (A) 100 ka and (B) 600 ka. From Hearty PJ, O'Leary MJ, Kaufman DS, Page M, and Bright J (2004) Amino acid geochronology of individual foraminifer (Pulleniatina obliquiloculata) tests, north Queensland margin, Australia: A new approach to correlating and dating Quaternary tropical marine sediment cores. *Paleoceanography* 19: PA4022.

screening criteria have been developed to systematically reject data from this multi-variate array. For example, the relative concentration of serine, a labile amino acid that is present only in low concentrations in fossils, has been used widely as an indicator of contamination by young amino acids. The extent to which racemization (D/L) covaries between different amino acids (Fig. 12), or between the THAA and FAA fractions, has been used to identify aberrant results. These might indicate excessive open-system behavior or pronounced microbial activity. Many recent studies have curated and transferred the full suite of amino

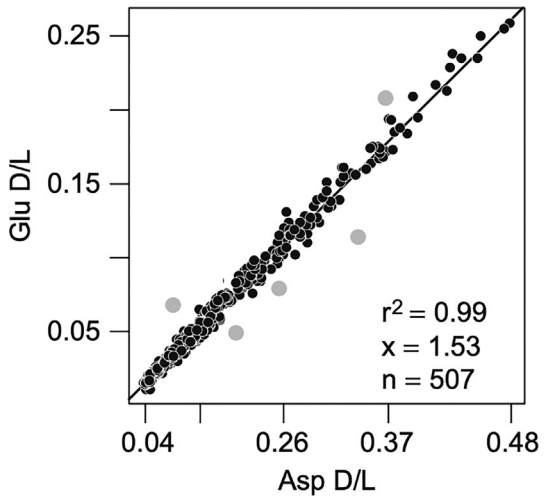

Fig. 12 Relation between the extent of racemization (D/L) in aspartic acid (Asp) and glutamic acid (Glu) in individual Holocene *Tellina* shells from Great Barrier Reef. The line is an exponential fit to the data; gray symbols are considered outliers because they fall outside of the 0.997 quantile range. The best-fit regression, r^2, best-fit Asp D/L exponent (x), and sample size (n) are indicated. Modified from Kosnik MA and Kaufman DS (2008) Identifying outliers and assessing the accuracy of amino acid racemization measurements for geochronology: II. Data screening. *Quaternary Geochronology* 3: 328–341.

acid data, along with tracking of the data editing, to a publicly accessible data repository which is housed by the US National Oceanic and Atmospheric Administration (NOAA) World Data Service for Paleoclimatology.

Applications

Relative ages

A simple application of D/L values is to treat them as relative-age indices. Within a limited geographic area, where the thermal history is nearly uniform, D/L values can be used directly to construct a regional relative chronostratigraphic framework. This application, termed 'amino stratigraphy,' is independent of assumptions regarding the rate of racemization, so that differences in D/L values can be interpreted exclusively as differences in relative age; specimens with high D/L values are older than those with lower values. Groupings of specimens with similar D/L values (called 'amino zones') represent intervals of sediment accumulation within a depositional sequence and gaps between them represent intervals of non-deposition. Amino zones can be used to correlate sedimentary units across sediment cores and terrestrial exposures (Figs. 13 and 14).

Age-population distributions

By analyzing multiple individuals from a single stratigraphic horizon, D/L values can be used to quantify the age-population structure. A unimodal distribution of D/L values reflects a single-age population deposited contemporaneously with the enclosing sediments, whereas a multimodal distribution indicates the presence of a mixed-age population. For example, analyses of individual tests of the foraminifera, *Lamellodiscorbis dimidiatus*, collected from emergent nearshore barrier bars reveals the extent to which they are reworked over multiple interglacial periods (Fig. 15). A large number of studies published in the past 20 years used D/L values of individual shells calibrated with radiocarbon to quantify the age-population distribution of various taxa in near-surface Holocene marine deposits. This approach is being superseded by low-cost alternatives for radiocarbon dating.

Calibrated ages

In this mode, D/L values are used to interpolate between, or extrapolate beyond, the known ages of independently dated stratigraphic layers. These ages are provided by any of multiple dating methods including radiometric techniques, other calibrated techniques including marine Sr isotopes, or correlated ages based on paleomagnetism and biostratigraphy. The D/L value and corresponding age are fit to a mathematical function that describes the rate of racemization for a given taxon and region. This calibrated age equation can then be applied to other specimens whose D/L values have been measured, but whose ages are unknown (Fig. 16). Because the rate of racemization in natural materials over long periods does not often follow simple kinetics of a first-order reversable reaction over the full range of D/L values, and because post-depositional temperatures fluctuate, the D/L versus age data that are used to calibrate the overall apparent rate of racemization are commonly fit using different mathematical functions to identify the one that follows the trend in the data most closely. Code is available for a Bayesian procedure to calculate goodness-of-fit statistics and to model the distribution of the residuals (prediction uncertainty) using multiple mathematical functions (Fig. 17). These alternative calibration functions include two that fit the empirical data using basic power functions (apparent parabolic kinetics and simple power kinetics), one that is constrained to reach a D/L value of 1.0 asymptotically (constrained power kinetics), and one that is underpinned by first-order reversible kinetics while allowing for changes in the reaction rate (time-dependent kinetics).

Uncalibrated ages

In this mode, the effects of time and temperature on the extent of amino acid racemization are determined in modern shells subjected to high-temperature laboratory experiments, and in Holocene samples whose ages are known from ^{14}C analysis and whose temperature history can be inferred based on historical climate data. This relation, together with a model of racemization kinetics, is used to calculate the age of a sample if its D/L value and temperature history are known. In practice, some combination of calibrated and uncalibrated approaches are typically employed: a kinetic model is used to estimate the age of an undated sample by extrapolating beyond a calibration point, or a calibration curve developed for one region is applied to another using a kinetic model to adjust the reaction rate for reasonable differences in site temperature.

Short of generating numerical ages, amino acid geochronology can provide reasonable constraints on the likely age range of a sample. For example, a Holocene rate constant can be calculated using radiocarbon-dated fossils from the last 10,000 years. This rate constant can then be applied to D/L values measured in older, late Pleistocene fossils from the same area. Because temperatures during the Holocene were generally warmer than during the late Pleistocene, the age calculated using the Holocene rate constant provides a minimum-limiting age on the late Pleistocene sample. In some cases, the late Pleistocene temperature depression can be determined using independently dated glacial-age and earliest Holocene samples, which in turn can be used to constrain the time and/or temperature difference, needed to explain a D/L values.

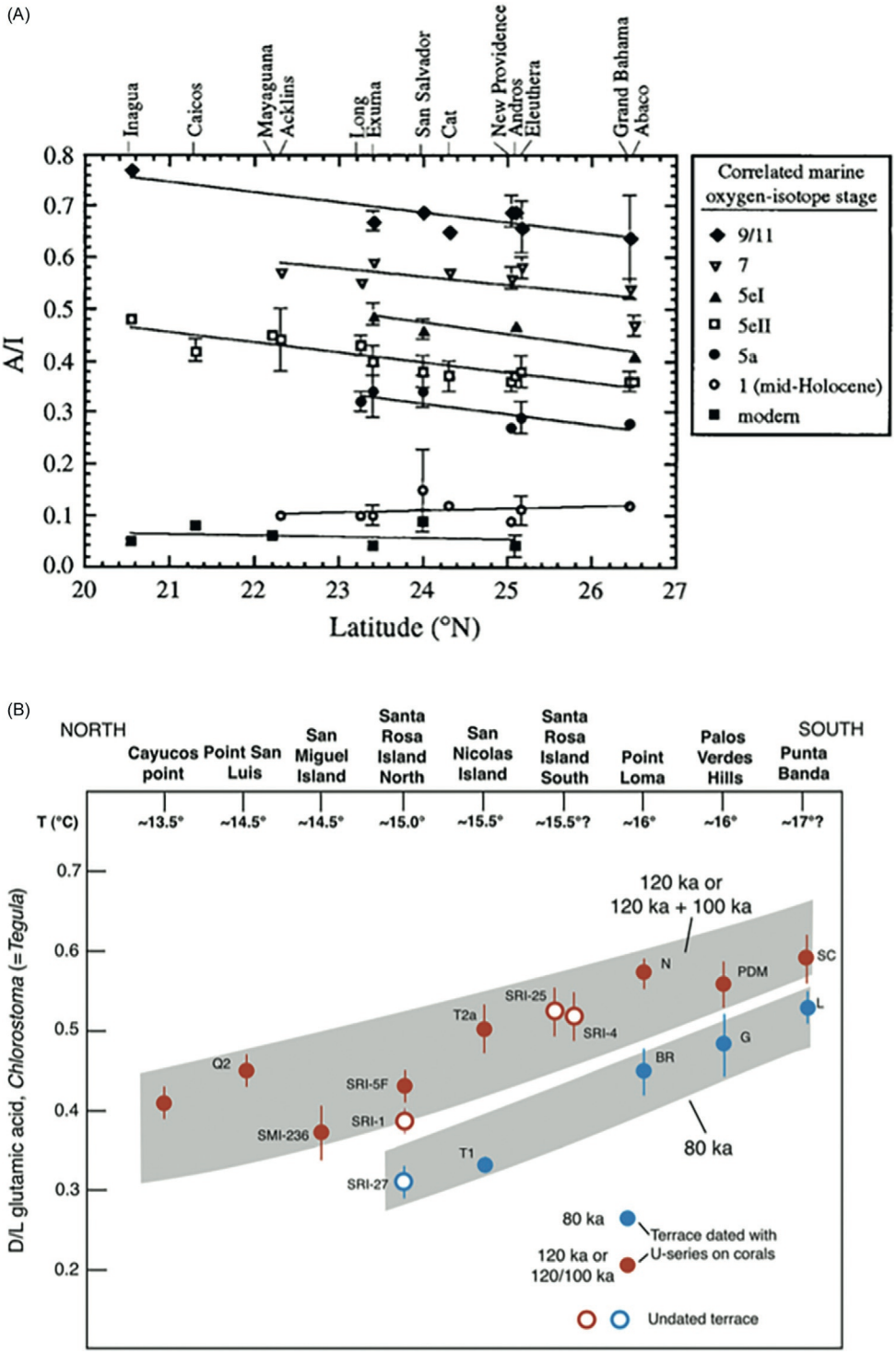

Fig. 13 Extent of racemization/epimerization in biocarbonates along latitudinal transects were temperatures increase southward. (A) Isoleucine (A/I) in whole-rock samples from across the Bahamian archipelago. Lines and symbols show the regional correlation of stratigraphic units and their assigned marine isotope stages and substages. (B) Glutamic acid (D/L) in *Chlorostoma* shells from dated (filled circles) and undated (open circles) marine terraces on the California and Baja California coast. Gray bands indicate correlation between fossil localities of the same age based on U-series dating of corals. Terrace name abbreviations: SMI = San Miguel Island, SRI = Santa Rosa Island N = Nestor, BR = Bird Rock, PDM = Paseo del Mar, G = Gaffey, SC = Sea Cave, L = Lighthouse terrace. Errors bars in both panels are ±1SD based on multiple samples from each unit. (A) From Hearty PJ and Kaufman DS (2000) Whole-rock aminostratigraphy and Quaternary sea-level history of the Bahamas. *Quaternary Research* 54: 163–173. (B) From Muhs DR, Simmons KR, Schumann RR., Groves LT, DeVogel SB, Minor SA, and Laurel D (2014) Coastal tectonics on the eastern margin of the Pacific Rim: Late Quaternary sea-level history and uplift rates, Channel Islands National Park, California, USA. *Quaternary Science Reviews* 105: 209–238.

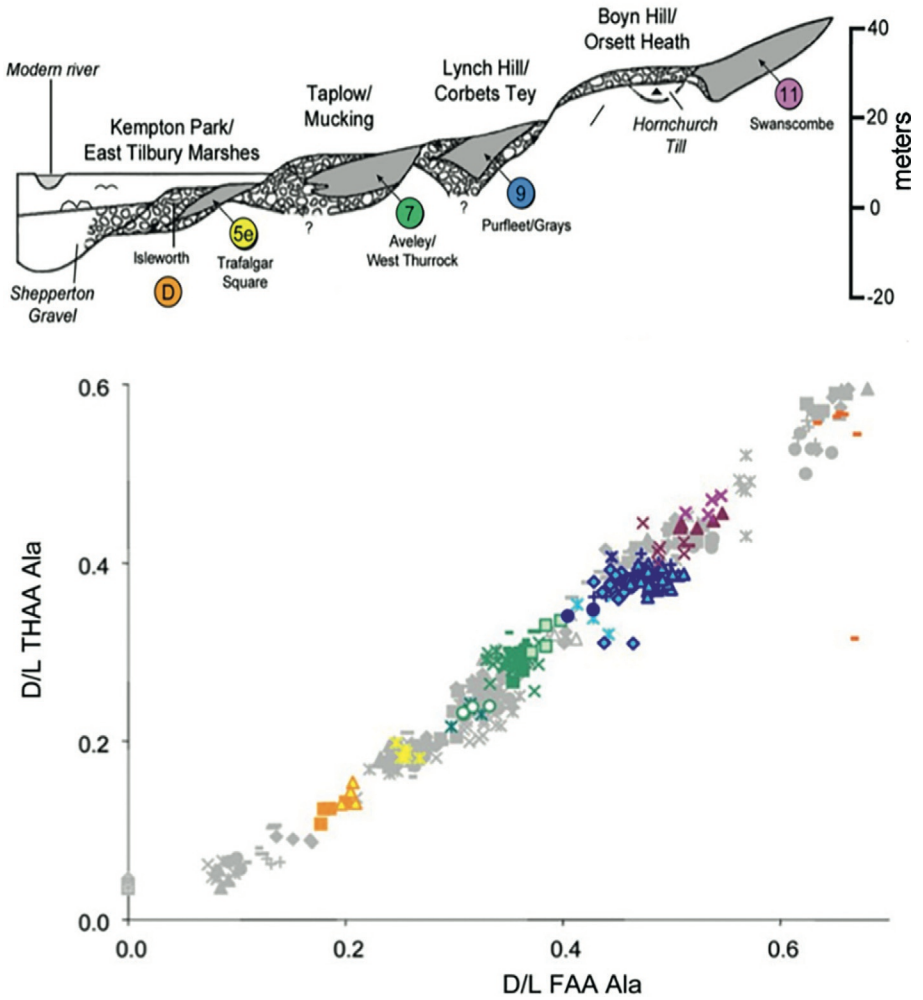

Fig. 14 Idealized transverse section through the terrace sequence along the Lower Thames (upper) and the extent of racemization (D/L) in alanine (Ala) in *Bithynia* opercula (lower). The plot show the covariance of D/L values in the free amino acid (FAA) and total hydrolysable amino acid (THAA) populations. Higher terraces are older and their opercula show higher D/L values. Data from various Lower Tames terraces (colored symbols) are superposed on the full dataset from the region (gray symbols). From Penkman KE, Preece RC, Bridgland DR, Keen DH, Meijer T, Parfitt SA, White TS, and Collins MJ (2013) An aminostratigraphy for the British Quaternary based on *Bithynia* opercula. *Quaternary Science Reviews* 61: 111–134.

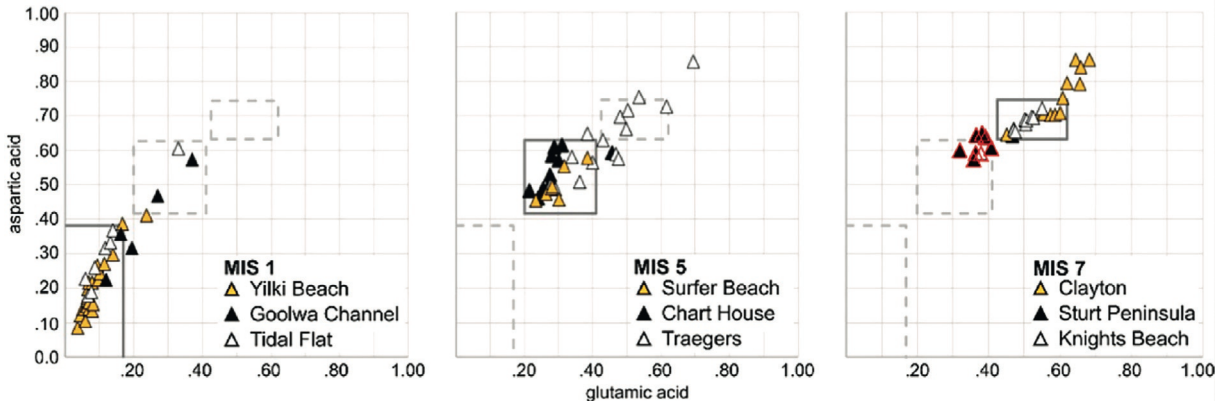

Fig. 15 Extent of racemization (D/L) in aspartic acid and glutamic acid of individual foraminifera tests (*Lamellodiscorbis dimidiatus*) from modern beach and Pleistocene barrier sediments, South Australia. Gray boxes enclose data representing three interglacial marine isotope stages. Individuals from previous interglacials are represented in subsequent deposits. From Ryan DD, Lachlan TJ, Murray-Wallace CV, and Price DM (2020) The utility of single foraminifera amino acid racemization analysis for the relative dating of Quaternary beach barriers and identification of reworked sediment. *Quaternary Geochronology* 60: 101103.

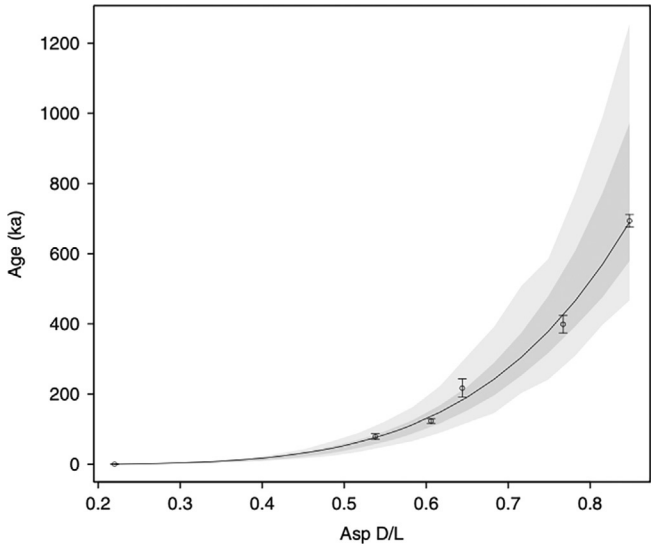

Fig. 16 Extent of racemization (D/L) in aspartic acid (Asp) in *Tawera* shells from uplifted marine deposits in New Zealand. Ages are from luminescence and correlations with marine oxygen isotope stages. The curve (best-fitting simple power-law kinetics model and lognormal distribution) provides a calibrated age equation that can be used to date other shells in the region. Dark shading shows the 95% confidence intervals for mean age, and light shading represents 95% prediction intervals. From Oakley DOS, Kaufman DS, Gardner TW, Fisher DM, and Vander Leest RA (2017) Quaternary marine terrace chronology, North Canterbury, New Zealand using amino acid racemization and infrared stimulated luminescence. *Quaternary Research* 87: 151–167.

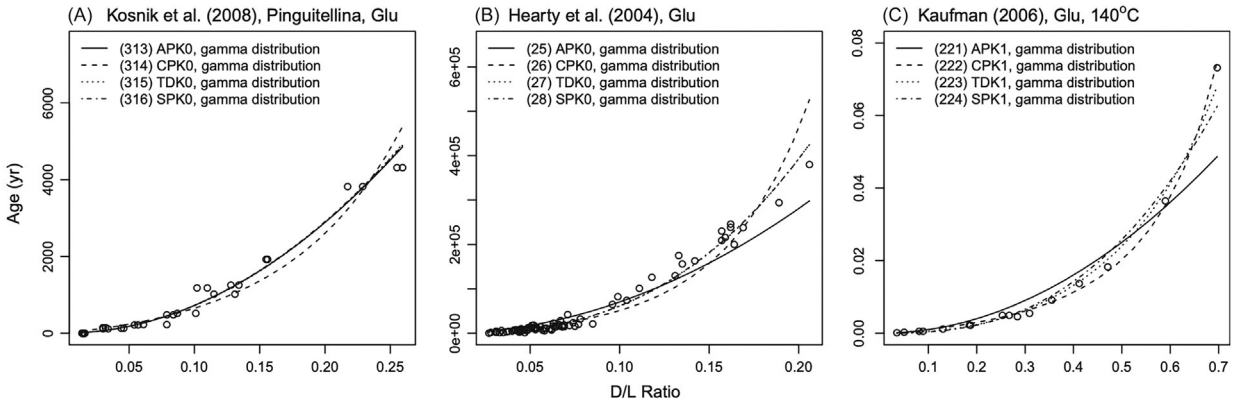

Fig. 17 Comparisons of different calibration functions fit to three example datasets. In each case, the age (or laboratory heating duration) is known independently and the extent of racemization (D/L) in glutamic acid (Glu) is measured. The data are fit with four alternative functions to determine the best-fit calibrated-age equation, including: apparent parabolic kinetics (APK), constrained power kinetics (CPK), time dependent kinetics (TDK), and simple power kinetics (SPK). (A) Example data set with an age versus D/L relation best-fit by APK but is also well described by SPK and TDK. (B) Example of an age versus D/L relation well fit by SPK and TDK, but APK and CPK fail to capture the relation. (C) Example of an age versus D/L relation with high D/L values. Modified from Allen AP, Kosnik MA, and Kaufman DS (2013) Characterizing the dynamics of amino acid racemization using time-dependent reaction kinetics: A Bayesian approach to fitting age-calibration models. *Quaternary Geochronology* 18: 63–77.

Paleo thermometry

Because the extent of amino acid racemization is dependent on both temperature and time, the average post-depositional temperature (effective diagenetic temperature) can be determined from the D/L value if the sample age is known independently. By integrating the entire post-depositional temperature history of a deposit, amino acids record long-term climate changes, as distinct from the geologically instantaneous paleoenvironmental evidence contained within the deposits. For example, the temperature change from Pleistocene to Holocene in semiarid Australia was quantified using radiocarbon-dated emu eggshells (Fig. 18). The change in slope in the D/L versus age trend at around 16 ka reflects at least 7 °C of warming into the Holocene. In another example, D/L values of ostracodes collected from independently dated shorelines of pluvial Lake Bonneville were used to constrain the paleotemperatures of intervals separating the formation of each shoreline level (Fig. 19).

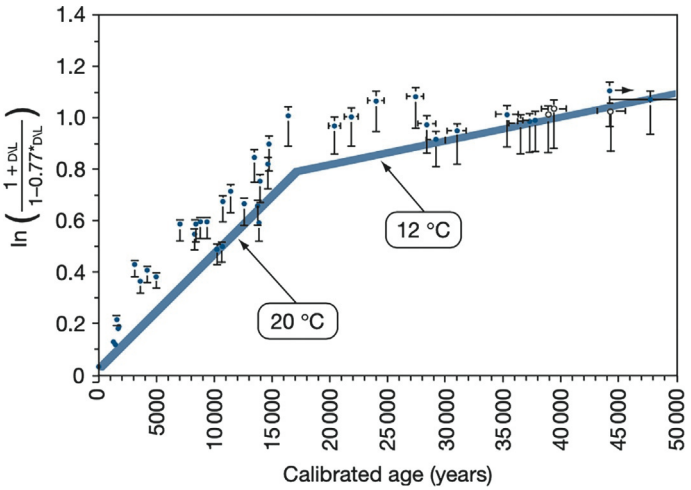

Fig. 18 Extent of racemization/epimerization in isoleucine (A/I) in its linearized form based on principles of first-order reversible kinetics, plotted against corresponding calibrated radiocarbon ages for 43 samples from central Australia. Asymmetric vertical error bars incorporate the possibility of shallow burial. Horizontal error bars are ±1SD dating errors where the uncertainty exceeds the diameter of the plotted point. Regression lines, with slopes that are directly proportional to temperature, pass through the generally lowest A/I. The lower slope for samples older than 16 ka indicates much lower Pleistocene temperatures in central Australia than during the Holocene. From Miller GH, Magee JW, Jull AJT (1997) Low-latitude glacial cooling in the Southern Hemisphere from amino acid racemization in emu eggshells. *Nature* 385: 241–244.

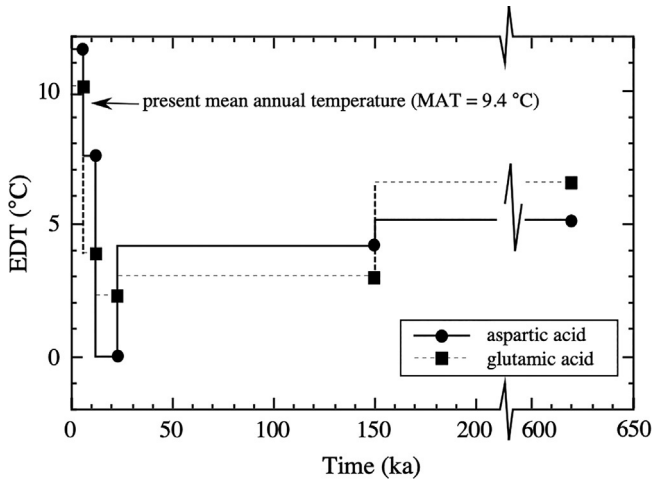

Fig. 19 Effective diagenetic temperature (EDT) based on the extent of racemization in aspartic acid and glutamic acids in the lacustrine ostracode, *Candona*, collected from well-dated shorelines of the southern Bonneville Basin. From Kaufman DS (2003) Amino acid paleothermometry of Quaternary ostracodes from the Bonneville Basin, Utah. *Quaternary Science Reviews* 22: 899–914.

Summary

Amino acid geochronology is a well-established, chronostratigraphic tool that can be used in a wide range of Quaternary sciences. The technique is versatile and requires relatively little specialized analytical instrumentation. It has been applied to deep-sea and coastal marine deposits, terrestrial deposits, and archeological sites. The most successful applications rely on D/L values as relative-age indices that are independent of assumptions regarding post-depositional temperatures. As a relative-age tool, D/L values provide a basis for identifying unconformities in stratigraphic sequences, resolving mixed-age populations, and evaluating stratigraphic correlations among disjunct exposures or sediment cores. D/L values, combined with independent paleoenvironmental evidence, can be used to constrain the reasonable time/temperature range of a stratigraphic unit. Where the ages of units are known from independent determinations, D/L values can be used to evaluate the overall temperature history of a deposit.

Further reading

Major works that present the principles and applications of amino acid geochronology, with references to the primary literature, include books dedicated to the topic (Hare et al., 1980; Goodfriend et al., 2000; Demarchi, 2020), chapters in books (Wehmiller, 1990, 1993; Rutter and Blackwell, 1995; Wehmiller and Miller, 2000; Blackwell, 2002), an edited journal volume (Penkman and Kaufman, 2013), and several entries in the Encyclopedia of Scientific Dating Methods (Rink and Thompson, 2014), including an overview by Demarchi and Collins (2014). Other reviews include those of Schroeder and Bada (1976), Miller and Brigham-Grette (1989), Johnson and Miller (1997), and Bravenec et al. (2018).

See also: Fission-Track Dating; K/Ar and $^{40}Ar/^{39}Ar$ Dating; Overview of dating techniques; U-series dating

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Relevant websites

https://www.ncei.noaa.gov/pub/data/paleo/aar/—National Centers of Environmental Information, NOAA Paleoclimatology, Amino Acid Racemization Database.