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Research paper

Sub-centennial resolution amino acid geochronology for the freshwater mussel *Lampsilis* for the last 2000 years

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ABSTRACT

We present an amino acid racemization-based age calibration with sub-centennial resolution from historical specimens of several species of the freshwater mussel genus, *Lampsilis*, from multiple river basins in the eastern United States. Valve samples that were bleached to isolate the intra-crystalline fraction of amino acids resulted in more consistent amino acid concentration and D/L values amongst duplicate sub-samples than those subjected to standard sample preparation techniques; therefore bleached samples are preferable for this geochronological application. The relation between D/L and age was determined for historical specimens housed in museums, allowing for the age determination of valves stored at room temperature for up to 150 years. We derived the Arrhenius parameters for the racemization of aspartic acid and glutamic acid by combining the results of heating experiments using live-collected specimens with data from museum specimens. This allows the determination of age for undated samples that lived during the last 2000 years and that experienced a range of post-depositional thermal histories. The procedure provides an accurate and low-cost geochronological tool for studies in biology, geology, environmental science, and conservation biology and paleobiology.

1. Introduction

The shells of freshwater mussels, as with other invertebrates whose skeletons grow by accretion, serve as records of changing environmental conditions, commonly through the filter of their own biological processes (Rhoads and Lutz, 1980). In many cases it is possible to produce a relative history of environmental change recorded in the skeletons through sclerochronological analyses. These techniques combined with accurate dating provide a powerful method for reconstructing histories of both "natural" and anthropogenic environmental change. Indeed this is a major goal of conservation paleobiology: the integration of techniques common in paleobiology and the geosciences in order to address modern conservation issues in a way that is minimally invasive to living populations, typically by studying the skeletal elements of dead organisms (e.g., Kowalewski et al., 2000; Jackson et al., 2001; Brown et al., 2005; Dietl and Flessa, 2011).

A challenge in this process is the accurate determination of sample ages, especially in very young specimens (less than a few hundred years) for which the rapid influx of ^{12}C into the atmosphere with the combustion of fossil fuels since industrial revolution and the near doubling of ^{14}C in the atmosphere with the detonation of nuclear warheads (especially in the 1960s) have complicated the use of radiocarbon dating (Bronk Ramsey, 2008; and references therein). Amino acid racemization (AAR) provides a reliable, affordable, and rapid method for dating many specimens. The extent of AAR in a fossil (or sub-fossil: the remains of a recent organism which may or may not be incorporated into the fossil record) is determined by the ratio of D- to L-enantiomers (or the interconversion of diastereoisomers in the case of isoleucine) and is used to infer the age of a specimen at a given temperature. The rate of racemization not only varies with temperature, but can also vary taxonomically (*e.g.*, within genus or family), position within the specimen, and can be influenced by a variety of diagenetic

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processes (*e.g.*, bacterial activity). Nevertheless, AAR is a useful geochronological tool once the rate of racemization for a given study area and taxon has been calibrated with ^{14}C (for specimens greater than several hundred years old) or other independent dating methods (though it is also useful as a stratigraphic tool without age calibration given similar thermal histories of samples). AAR has previously been applied to marine deposits that contain shelly mollusks, where the rate of AAR has been calibrated by radiocarbon (Kosnik et al., 2008 provides a recent tabulation of such studies). Calibration of the rate of AAR by use of radiocarbon in freshwater mollusks is less common, despite their obvious geochronological potential.

This study focuses on the genus *Lampsilis,* a freshwater mussel that is endemic to North America. Its species are known from 16 of 17 freshwater mussel faunal provinces, all except the Pacific province (Haag, 2010; Haag, Personal communication 2010). The genus has been identified in archaeological and paleontological samples as well. Five *Lampsilis* species are endangered, three are threatened, and four are species of concern (http://www.fws.gov/endangered); accessed 10 February 2011.

The goal of this study is to calibrate the rate of AAR using historically collected specimens of the genus *Lampsilis*. Laboratory heating experiments, combined with the known ages of historically collected specimens, are used to derive Arrhenius parameters, which provide the basis for age equations that can be adapted to a range of thermal histories and, thus, collections of *Lampsilis* throughout North America.

2. Materials and methods

2.1. Materials

Thirty individuals of the freshwater mussel genus *Lampsilis* were selected from the collections of the US National Museum of Natural History, the Carnegie Museum, and from our own field-collected specimens. The specimens were collected from the Potomac and Pamunkey Rivers in Virginia and Washington DC, the Clinch River in Tennessee, and the New River in Virginia between 1885 and 2006 (Table 1; Fig. 1). The specimens were collected as dead shells from modern muskrat middens along the river banks. We use the collection year to approximate the time of death as only very well preserved valves (*i.e.*, paired valves with very little shell degradation and intact periostracum) were used.

2.2. Sample preparation

Because the extent of AAR can vary within a (sub-) fossil shell, all samples were taken from the postero-dorsal margin of the valve (avoiding muscle attachment scars). The periostracum and thin primary prismatic aragonite layer were removed with a Dremel rotary tool fit with a silicon carbide bit to expose the remaining secondary nacreous layer. Approximately 150–200 mg of nacre was extracted as a small fragment for the analysis of amino acids.

All shells were subjected to two preparation techniques to determine which procedure provided the best results for age

Table 1
Taxonomy, collection locality, and D/L values for specimens used in this study.

Lab ID (UAL)	Sample	Museum ID[a]	*Lampsilis* species	Collection year	D/L Asp[b]	D/L Glu[b]	River	State	Rejection criterion[c]
5509	P1885	USNM47760	*L. radiata*	1885	0.157	0.048	Potomac	Washington, DC	–
5492	P1889-1	CM61.11035-1	*L. radiata*	1889	0.141	0.044	Potomac	Washington, DC	–
5504	P1889-2	CM61.11035-2	*L. radiata*	1889	0.200	0.055	Potomac	Washington, DC	–
5491	P1889-3	CM61.11035-3	*L. radiata*	1889	0.193	0.051	Potomac	Washington, DC	–
5493	P1889-4	CM61.11035-4	*L. radiata*	1889	0.179	0.050	Potomac	Washington, DC	–
5508	P1889-5	CM61.11095-1	*L. cariosa*	1889	0.252	0.062	Potomac	Washington, DC	–
5494	P1889-6	CM61.11095-2	*L. cariosa*	1889	0.214	0.060	Potomac	Washington, DC	–
5497	P1889-7	CM61.11095-3	*L. cariosa*	1889	0.223	0.056	Potomac	Washington, DC	–
5498	P1890-1	USNM227692-1	*L. cariosa*	1890	0.192	0.051	Potomac	Washington, DC	Sub-samples > 15%
5502	P1890-2	USNM227692-2	*L. cariosa*	1890	0.213	0.058	Potomac	Washington, DC	AA conc. > 3SD
5496	P1890-3	USNM227698	*L. radiata*	1890	0.188	0.051	Potomac	Washington, DC	–
5500	P1895-1	CM61.6979-1	*L. radiata*	1895	0.229	0.061	Potomac	Washington, DC	–
5501	P1895-2	CM61.6979-2	*L. radiata*	1895	0.199	0.054	Potomac	Washington, DC	–
5505	P1895-3	CM61.6979-3	*L. radiata*	1895	0.207	0.057	Potomac	Washington, DC	Sub-samples > 15%
5507	P1896-1	CM61.11037-1	*L. radiata*	1896	0.167	0.045	Potomac	Virginia	Sub-samples > 15%
5506	P1896-2	CM61.11037-2	*L. radiata*	1896	0.177	0.046	Potomac	Virginia	Sub-samples > 15%
5499	P1896-3	CM61.11037-3	*L. radiata*	1896	0.174	0.048	Potomac	Virginia	Sub-samples > 15%
5510	P1896-4	CM61.11036-1	*L. radiata*	1896	0.130	0.043	Potomac	Virginia	–
5495	P1896-5	CM61.11036-2	*L. radiata*	1896	0.135	0.039	Potomac	Virginia	–
5503	P1896-6	CM61.11036-3	*L. radiata*	1896	0.196	0.056	Potomac	Virginia	–
5483	T1969-1	USNM853618-1	*L. ovata*	1969	0.092	0.028	New	Virginia	–
5485	T1969-2	USNM853618-2	*L. ovata*	1969	0.132	0.029	New	Virginia	–
5482	T1970	CM69094	*L. ovata*	1970	0.161	0.033	Clinch	Tennessee	Sub-samples > 15% D/L > 2SD
5484	P1972-1	USNM711155-1	*L. cariosa*	1972	0.119	0.026	Pamunkey	Virginia	Sub-samples > 15%
5481	P1972-2	USNM711155-2	*L. cariosa*	1972	0.103	0.022	Pamunkey	Virginia	Sub-samples > 15%
5486	T1974	USNM853656	*L. sp.*	1974	0.097	0.020	Clinch	Tennessee	Sub-samples > 15% AA conc. > 2SD
5487	T2006-1	Neves	*L. ovata*	2006	0.056	0.018	Clinch	Tennessee	–
5488	T2006-2	Neves	*L. ovata*	2006	0.051	0.020	Clinch	Tennessee	–
5489	T2006-3	Neves	*L. ovata*	2006	0.083	0.022	Clinch	Tennessee	–
5490	T2006-4	Neves	*L. ovata*	2006	0.058	0.018	Clinch	Tennessee	Sub-samples > 15%

[a] USNM: National Museum of Natural History of the Smithsonian Institution; CM: Carnegie Museum of Natural History; Neves: from the collection of Richard J. Neves. The number following the dash indicates different individuals from the same lot.

[b] D/L Asp and D/L Glu are the mean values of the sub-samples from the bleached treatment. See Appendix A for complete set of D/L data.

[c] Eleven valves were rejected based on one or more of the following: (1) the two sub-sample D/L Asp or D/L Glu values differed by >15% of the mean value (sub-samples > 15%) for either the bleached or non-bleached shells; (2) the RMA residual value from the regression of mean amino acid concentrations (Asp v. Glu; bleached preparation) was greater than two standard deviations from the mean RMA residual value (AAconc. > 2SD); or (3) the residual value from the regression of mean D/L values (Asp v. Glu; bleached preparation) was greater than two standard deviations from the mean residual value (D/L > 2SD).