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Equilibrated clumped isotope signatures of land-snail shells observed from laboratory culturing experiments and its environmental implications

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ABSTRACT

This study reports on the clumped isotope values (Δ_{47}) of land-snail shells precipitated at known temperatures ranging from 20 °C to 30 °C. Our observed shell Δ_{47} values fall into the uncertainties of published Δ_{47}–T calibration line (e.g. Kelson et al., 2017), indicating the shell aragonite of land snails is precipitated at clumped isotopic equilibrium without an obvious Δ_{47} vital effect. The Δ_{47} data are neither related to the various bulk isotopic input sources (both for $\delta^{13}C$ and $\delta^{18}O$) which result from differences in the supplied food and water; nor are they related to the ingested carbonate composition or snail growth rate. Furthermore, we preliminarily applied the Δ_{47}–T calibration (Kelson et al., 2017) to calculate the mean growth temperatures of the parent snails. Results suggest that the clumped isotope values for land-snail shells can be regarded as a useful tool to reconstruct the mean seasonal temperature of snail activity when the environmental parameters are optimum. Future studies should focus on the applicability of this relationship to snails in natural environments where the self-preservation or acclimatization mechanisms are more complex.

1. Introduction

Traditional oxygen isotope thermometers are constrained by both the temperature and isotopic composition of water from which these carbonates precipitated (e.g. Urey, 1947; Epstein et al., 1953; Grossman and Ku, 1986). However, in paleo-studies, the oxygen isotopic composition of water where organisms once lived is always difficult to be elucidated accurately. Furthermore, the vital effect, which might be attributed to the kinetic effect during carbonate precipitation (e.g. Auclair et al., 2003; Yamamoto et al., 2010), increases the difficulty for the application of oxygen isotope thermometry.

Carbonate clumped isotope (Δ_{47}) thermometers are regarded as a more powerful and effective tool to reconstruct paleo-temperatures based on the temperature dependence of the following homogeneous equilibrium (Ghosh et al., 2006).

$$Ca^{13}C^{16}O_3 + Ca^{12}C^{18}O^{16}O_2 \leftrightarrow Ca^{13}C^{18}O^{16}O_2 + Ca^{12}C^{16}O_3$$

In other words, the formation of $^{13}C–^{18}O$ bonds in carbonate minerals is constrained only by the temperature at which these minerals precipitated. During past decade, clumped isotope analytical techniques has been well developed and applied in a variety of carbonate minerals, including both inorganic carbonate (e.g. Ghosh et al., 2006; Dennis and Schrag, 2010; Zaarur et al., 2013; Wacker et al., 2014; Tang et al., 2014; Kluge et al., 2015; Tripati et al., 2015; Kelson et al., 2017) and biogenic carbonate (e.g. Tripati et al., 2010; Thiagarajan et al., 2011; Grauel et al., 2013; Henkes et al., 2013; Spooner et al., 2016; Katz et al., 2017).

Among its applications in biogenic carbonate materials, an interesting topic is the study of land-snail shells, since they are well preserved in Quaternary fossils and provide valuable archival material for investigating the paleo-terrestrial environment (Goodfriend, 1992). However, to date, only a few studies have been devoted to applying clumped isotope thermometry to land-snail shells.

Zaarur et al. (2011) reported that the temperatures derived from the observed clumped isotope composition of land-snail shells are always higher than both the environmental mean annual temperatures and snail activity season temperatures, and they failed to find any relationship between clumped isotope data and relative environmental temperatures. Nevertheless, Eagle et al. (2013) argued that a correlation should exist for terrestrial gastropods living in warm and optimum growth conditions (e.g. summer with a wet environment). Moreover, a recent study observed two land snail species in northern China and found that the shell clumped isotope values correlated well with environmental temperatures (Wang et al., 2016). However, they did observe and offset (3–5 °C) between calculated and observed mean

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temperature values, which may indicate either a species-dependent ecophysiological adaptations or different active seasons/periods.

Given the complex and variable terrestrial environmental parameters, it has been challenging to derive a straightforward relationship between observed temperatures and the clumped isotope composition of natural land snails, limiting its application in paleo-climate studies. In this study, we have cultured land snails under controlled temperature conditions, providing the Δ_{47} values of land-snail shell aragonite precipitated at known temperatures. This allows us to discuss the potential application of clumped isotope thermometry to paleo-environmental reconstruction using land snail fossils.

2. Materials and methods

2.1. Culturing of land snails

Acusta despecta is a widely distributed land snail species around Japan and Korea (Azuma, 1995; Lee and Kwon, 1996). It is regarded as a useful reference species for reconstructing the paleo-environment because its fossils can be found in Okinawa and many other islands in southern Japan (Takamiya and Meighan, 1992; Fujie, 2000a, 2000b). *Acusta despecta* has a lifespan around 1 year (Sumikawa, 1962; Okuma, 1982; Takahashi et al., 1992). After emerging from hibernation, the adult snails mate and lay eggs in April and May for several times and then most of them die in June (Sumikawa, 1962). The eggs hatch after 20–25 days and the new generation undergoes active growth until September, whereupon the shell diameter of individual snails can reach 10 mm and the spermatogenesis begins. The snails will go into hibernation in November, and at this time most individuals have reached the adult stage with shell diameter as large as 15 mm. The most frequent copulation period of *Acusta despecta* is May and June (Okuma, 1982), although some of them can mate in November. According to the culture experiment by Kohno (1976), individuals of *Acusta despecta* prefer living at temperatures of 15–30 °C with the optimum temperatures of 25–30 °C.

As described by Zhang et al. (2014), eight adult snails of *Acusta despecta sieboldiana* (a subspecies of *Acusta despecta*) were collected in Suzukakedai, Yokohama, Japan. They were then cultured at room temperature (ca. 25 °C) from January 2012. Similarly, their eggs were hatched in an incubator at 25 °C and the larvae were distributed randomly into small groups at three temperatures of 20 °C, 25 °C, and 30 °C from May 2012 to the beginning of January 2013. Some snails living at 20 °C were cultured until early June of 2013. Furthermore, these snails were living under various controlled conditions, which included: two kinds of food (green cabbage, C_3 plant, $\delta^{13}C = -28.4 \pm 1.2‰$, $n = 12$; corn seed, C_4 plant, $\delta^{13}C = -12.0 \pm 0.7‰$, $n = 4$; VPDB); three kinds of spray water (desalinated ocean water, $\delta^{18}O = -0.1 \pm 0.2‰$; tap water, $\delta^{18}O = -8.2 \pm 0.1‰$; bottled Canadian ice water, $\delta^{18}O = -12.9 \pm 0.0‰$; VSMOW); and two kinds of calcium sources (calcium carbonate powder; calcium phosphate powder).

After a culturing period of 6 months, most of the snails have reached 10 mm in diameter, with spiral numbers of approx. 4.5–5.5. Before collection, most of the land snails are considered to be in the adult stage, which is confirmed by the presence of sexually mature copulatory systems (Zhang et al., 2014).

2.2. Sample preparation and isotopic analysis

Land-snail shells were washed successively with Milli-Q water (pH = 6.998; rinsed three times), acetone (1 h in ultrasonic bath), and 10% H_2O_2 (4 h at room temperature) to remove organic matter. Since the first two internal spirals were inherited from their parents, they were removed from the bulk shell. All the shells were crushed into the homogeneous powder using an agate mortar, and 0.02–0.50 g shell power was recovered for each individual. These samples were then identified as aragonite using X-ray diffractometry (XRD, MXP3TA; Mac Science Ltd.).

Besides the land-snail shells, two synthetic calcium carbonate samples were prepared by mixing solutions of 0.02 mol/L $NaHCO_3$ and 0.01 mol/L $CaCl_2$ in a 2-L flask on a stirrer set in a thermally controlled incubator at the temperatures of 33.0 °C and 61.0 °C. Temperature was monitored and kept stable with ± 0.5 °C. The $NaHCO_3$ and $CaCl_2$ solutions were dripped at the constant rate (typically 60 mL/day) into the flask initially with 1 L of pure water, which was loosely sealed with paraffin film to allow gas exchange but prevent evaporation. Surplus volume of the solution was automatically drained out of the incubator through a tube, and used for pH measurement. The pH was relatively constant around 7.5.

In 2013 and 2014, shell powder samples (7–10 mg) were reacted with 103% H_3PO_4 overnight at 25 °C in McCrea-type reaction vessels. To remove any water completely, the resultant CO_2 was purified by passing through a vacuum line, with the exchange of ethanol/dry ice and liquid nitrogen traps for three times. Then the gaseous CO_2 samples were carried through a Supelco Q-Plot column (30 m long, 0.53 mm ID) in gas chromatography held at −20 °C using He gas (3 mL/min) as the carrier and collected with a duration of 40 min. The column was baked at 200 °C between samples.

In 2017, part of shell samples (7–10 mg) was repeated at the acid digestion temperature of 90 °C, together with the synthetic calcite samples. The samples were allowed to react with 103% H_3PO_4 phosphoric acid for 20 min and the liberated CO_2 was immediately collected in a liquid nitrogen trap to avoid any potential isotope exchange. Then the CO_2 samples were purified with the same method as described for the samples digested at 25 °C.

Clean CO_2 samples were analyzed using a dual-inlet gas source isotope ratio mass spectrometer configured with six Faraday cups (MAT 253; Thermo Fisher Scientific Inc., Bremen, Germany) as described by Huntington et al. (2009) at Tokyo Institute of Technology. During the measurements in 2013 and 2014, the pressures of both bellows were adjusted to ca. 60–70 mbar to achieve 16 V intensity for m/z 44, and the samples were measured for ten acquisitions with ten sample-reference cycles. For each cycle, the integration time was 16 s and the total time was 1600 s. In 2017, the signal of m/z 44 was set to 14 V. Each measurement consisted of eight acquisitions with ten cycles for one acquisition. The total integration time was 1280 s for each CO_2 sample. To avoid any influence caused by the shift of pressure baseline (PBL), all measurements followed the PBL correction protocol suggested by He et al. (2012). A tank of pure CO_2 gas (Oztech, Safford, AZ, USA; $\delta^{13}C_{VPDB} = -10.65‰$; $\delta^{18}O_{VPDB} = 0.21‰$, verified by NBS19) was used as the reference gas. The specific numbers (n) of replicate measurements for each sample are listed in Tables 1–3.

2.3. Standardization

The $\delta^{13}C$ and $\delta^{18}O$ values were calibrated by reference to the NBS-19 and a synthetic calcium carbonate standard, WAKO ($\delta^{13}C_{VPDB} = -9.13‰$; $\delta^{18}O_{VPDB} = -17.02‰$; Wako Pure Chemical Industries Ltd., Osaka, Japan). Acid fractionation factors for oxygen isotope calibration of various minerals and digestion temperatures were cited from Kim et al. (2007). For aragonite digested at 25 °C and 90 °C, this value is 1.01063 and 1.00854, respectively; for calcite, it is 1.01030 and 1.00813, respectively.

Clumped isotope data (Δ_{47}) analyzed in 2013 were firstly projected into Ghosh scale following Huntington et al. (2009) and then calibrated to the absolute reference frame (carbon dioxide equilibrated scale, CDES) using a secondary transfer function suggested by Dennis et al. (2011). The transfer function was established using the equilibrated CO_2 gases and an in-house carbonate standard (WAKO). The "accepted" value for WAKO (0.651 ± 0.020‰; 1σ sd, $n = 18$) was obtained from the measurements from 2014 to 2017, when the Δ_{47} values were directly projected into the absolute reference frame using the equilibrated