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Environmental implication from isotopic signatures of land snail shell carbonate based on laboratory culturing experiment

Land snails are strictly local indicators of terrestrial environment and are considered as archive materials in studying the paleo environment, especially in the Quaternary period. Up to now, isotopic tools such as $\delta^{13}C$, $\delta^{18}O$ and clumped isotope thermometry (Δ_{47}) are widely applied in reconstructing the environment conditions such as paleo-climate changes, shift of vegetation distribution, rainfall amount, and so on. However, the knowledge of the relationships between these isotopic signatures and relative environment conditions are still very poor, which could limit their application in better understanding the paleo environment. Therefore, laboratory cultivation of land snails under various controlled environment is necessary.

In this study, eight adult land snails of *Acusta despecta sieboldiana*, which is widely distributed around Japan and Korea, were collected from Yokohama, Japan. Their offspring were cultured from eggs to adults around 6–8 months under various controlled environments. Briefly, the snails grew up at three different temperatures (20 ℃, 25 ℃, and 30 ℃), two kinds of diet (Cabbage, $\delta^{13}C$ = -28.4‰; Corn, $\delta^{13}C$ = -12.0‰), three kinds of water (Tap water, $\delta^{18}O$ = -8.2‰; Canadian ice water, $\delta^{18}O$ = -12.9‰ and Oceanic deionized water, $\delta^{18}O$ = -0.1‰), and two kinds of calcium sources (calcium carbonate and calcium phosphate). Finally, shell $\delta^{13}C$, $\delta^{18}O$ and clumped isotope values were measured by isotope ratio mass spectrometry (IRMS, MAT 253 or Delta XL); organic $\delta^{13}C$ and water $\delta^{18}O$ values were measured by two different cavity ring-down spectroscopy (CRDS, Picarro), or by IRMS (Delta V).

For carbon isotopic composition ($\delta^{13}C$) of land snail shell carbonate, we confirmed it derives from three sources: diet, atmospheric CO_2, and ingested carbonate (limestone). Herein, we consider the influences of metabolic rates and temperature on the carbon isotopic composition of the shell carbonate. Based on previous works and on results obtained in this study, a simple but credible framework is presented for discussion of how each source and environmental parameter can affect shell carbonate $\delta^{13}C$ values. According to this framework and some reasonable assumptions, we have estimated the contributions of different carbon sources for each snail individual: for cabbage (C_3 plant) fed groups, the contributions of diet, atmospheric CO_2 and ingested limestone respectively vary as 66–80%, 16–24%, and 0–13%. For corn (C_4 plant) fed groups, because of the possible food stress (lower consumption ability of C_4 plant), the values vary respectively as 56–64%, 18–20%, and 16–26%. Moreover, we present new evidence that snails have discrimination to choose different plants as food. Therefore, we suggest that food preferences must be considered adequately when applying $\delta^{13}C$ in paleo-environment studies.

For oxygen isotopic composition ($\delta^{18}O$), we discussed the effectiveness of flux balance model raised by Balakrishnan and Yapp (2004). However, the complex and variable environmental parameters, such as relative humidity and ingested water isotope composition, can restrict its application in the modern and paleo studies. Secondly, we observed a fairly good and common relationship (slop = 0.69; R^2 = 0.98) between snail shell $\delta^{18}O$ values and body water $\delta^{18}O$ values at various temperatures, suggesting the shell is directly precipitated from the body fluid. Nevertheless, the deviation of this slope from unity (slope = 1; isotopic-equilibrated

precipitation), indicates that potential non-equilibrated precipitation or vital effect exists. In addition, these relations seem to be only controlled by the environmental parameter such as temperature and evaporation, rather than the ingested limestone, input water (e.g. different diet) and growth rate. Thirdly, snails living in some non-optimal conditions (e.g. low temperature) can adapt their biological behaviours to be accustomed to the environment, which will affect the oxygen isotope compositions in body fluid and shells. Additionally, we observed that evaporation effect can largely enrich ^{18}O in snail body water, and in further produce a more enriched $\delta^{18}O$ values than expected values of isotopic equilibrated precipitation.

For the clumped isotope (Δ_{47}), we present the first empirical calibration of the clumped isotope thermometer based on land snails in-laboratory cultured in a temperature range of 20 ℃ to 30 ℃. The data were normalized into the absolute reference frame by the empirical transfer function (ETF) suggested by Dennis et al. (2011). The slope is consistent with the published ones digested at 25 ℃; and different to those digested at 90 ℃. Besides, our data show a discrepancy (~0.02‰) to those re-calibrated data using two-point secondary transfer functions (e. g. Ghosh et al., 2006; Dennis and Schrag, 2010), however, the values are consistent with those directly referenced to the ETFs (e.g., Henkes et al., 2013; Wacker et al., 2014), suggesting the absolute reference frame based on isotopic equilibrated CO_2 gas at various temperatures is effective. Therefore, we encourage more studies about empirical calibration curves should be carried out based on directly referencing to the absolute frame. Moreover, we confirmed that land snail shells are precipitated in isotopic equilibrium condition (for clumped isotopes), and the clumped isotope data are not related to the various bulk isotopic sources such as different food, or water sources, and also has no relation to the ingested carbonate or snail growth rate. In addition, we confirmed the size effect reported by Wacker et al. (2013), that is, to those digested in phosphoric acid at 25 ℃, the sample size less than 6.5 mg will present more positive data, which sometimes can be as large as 0.07‰.

In summary, this study permits us to comprehensively understand the relation between isotopic signatures of land snail shells and their living environment. The next step is to test these relations from studying the living snails in natural and then try to apply these implications in the Quaternary land snail fossils. All in all, our researches prompt the field of paleo environment reconstruction from studying the isotopic composition of land snail fossils.