



## Factors controlling carbon isotopic composition of land snail shells estimated from lab culturing experiment

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Carbon isotopic composition ( $\delta^{13}\text{C}$ ) of land snail shell carbonate is widely applied in reconstructing the C3/C4 vegetation distribution of paleo-environment, which is considered to reflect variations of some environmental parameters [1][2][3]. Land snail shell carbon has three potential sources: diet, atmospheric  $\text{CO}_2$  and ingested carbonate (limestone) [4]. However, their relative contributions to shell carbonate have not been understood well yet [4][5][6][7][8]. More researches are necessary before we could apply this tool in paleo-environment reconstruction, especially inter-lab culturing experiment.

A kind of land snail species, *Acusta despecta sieboldiana*, was collected at Yokohama, Japan and cultured under suitable environment to lay eggs. The second generations were growing up from eggs to adults around 6-12 months at the temperature of  $20^\circ$ ,  $25^\circ$  and  $30^\circ$ , respectively. All of the snails at  $25^\circ$  and  $30^\circ$  and most of those at  $20^\circ$  were fed by cabbage (C3 plant) during their life span while others were fed by corn (C4 plant). To investigate the effect of ingested carbonate, some of them were fed by  $\text{Ca}_3(\text{PO}_4)_2$  powder while others were fed by  $\text{CaCO}_3$  powder.  $\delta^{13}\text{C}$  of shells were analyzed by an Isotope Ratio Mass Spectrometry (Thermo Finnigan MAT 253);  $\delta^{13}\text{C}$  of food and snail tissue were measured by a Cavity Ring-Down Spectroscopy (Picarro G1121-i). At the same time,  $\delta^{13}\text{C}$  of eggshell and new born snails were analyzed by a Continuous Flow Isotope Ratio Mass Spectrometry (GasBench II).

We confirmed that diet, atmospheric  $\text{CO}_2$  and ingested limestone could be important sources controlling shell  $\delta^{13}\text{C}$  values. And the temperature could affect shell carbonate  $\delta^{13}\text{C}$  values, too. A simple but credible frame was raised to discuss the mechanism of how each possible source and environmental parameter could affect shell carbonate  $\delta^{13}\text{C}$  values based on previous works [4][6][8] and this study. According to this frame and some reasonable assumptions, we have estimated the contribution of different carbon sources for each snail individual: to cabbage (C3 plant) fed groups, the contributions of diet, atmospheric  $\text{CO}_2$  and ingested limestone vary in a range of 66~80%, 16~24% and 0~13%, respectively. And to corn (C4 plant) fed groups, because of the possible food stress (lower consumption ability of C4 plant), they vary in 56~64%, 18~20% and 16~26%, respectively. We will discuss how these results could be consistent to the observations, which suggests our calculations are suitable and believable. In addition, we will discuss the carbon isotope fractionation during egg laying and hatching of land snails, too.

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