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Article:
Versteegh, Emma, Black, Stuart, Canti, Matt G. et al. (1 more author) (2013)

https://doi.org/10.1016/j.gca.2013.06.020

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Earthworm-produced calcite granules: A new terrestrial palaeothermometer?

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Received 19 December 2012; accepted in revised form 12 June 2013; available online 27 June 2013

Abstract

In this paper we show for the first time that calcite granules, produced by the earthworm *Lumbricus terrestris*, and commonly recorded at sites of archaeological interest, accurately reflect temperature and soil water δ18O values. Earthworms were cultivated in an orthogonal combination of two different (granule-free) soils moistened by three types of mineral water and kept at three temperatures (10, 16 and 20 °C) for an acclimatisation period of three weeks followed by transfer to identical treatments and cultivation for a further four weeks. Earthworm-secreted calcite granules were collected from the second set of soils. δ18O values were determined on individual calcite granules (δ18Oc) and the soil solution (δ18Ow). The δ18Oc values reflect soil solution δ18Ow values and temperature, but are consistently enriched by 1.51 (± 0.12)% in comparison to equilibrium in synthetic carbonates. The data fit the equation 1000ln a = [20.21 ± 0.92] (T/°C)0.5 [38.58 ± 3.18] (R² = 0.95; n = 96; p < 0.0005). As the granules are abundant in modern soils, buried soils and archaeological contexts, and can be dated using U–Th disequilibria, the developed palaeotemperature relationship has enormous potential for application to Holocene and Pleistocene time intervals.

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1. INTRODUCTION

Many organisms form reliable archives of palaeotemperature or water composition, by precipitating calcium carbonate (CaCO3) in oxygen isotopic equilibrium with their environment (Wanamaker et al., 2007; Ullmann et al., 2010; Versteegh et al., 2010). The δ18O values of the CaCO3 are controlled by temperature and water isotope composition (Urey, 1947; Epstein et al., 1953), with equilibrium being defined via a mineral-specific fractionation factor (Kim and O’Neil, 1997). The δ18O composition of CaCO3 produced by some biomineralising taxonomic groups shows a systematic offset from equilibrium, and as such is still useful as a proxy (Ziveri et al., 2003; Rosenheim et al., 2009; Ford et al., 2010). The CaCO3 minerals produced by other taxonomic groups are not in equilibrium with the environment, but exhibit deviations, called vital effects (Owen et al., 2002; Juillet-Leclerc et al., 2009; Correa et al., 2010). Therefore, when a biomineral is investigated as a potential palaeoenvironmental proxy, it is important to develop species-specific and well-constrained palaeotemperature relationships.

Although not widely appreciated, many earthworm species are true biomineralisers (Briones et al., 2008b) and produce calcite granules in specialised glands (Canti, 1998; Lee et al., 2008b) first noted by Darwin (1881; Fig. 1). The lob...
Earthworm *Lumbricus terrestris* is common in Europe and increasingly as an invasive species in the USA and Canada. It is one of the major producers of earthworm-secreted calcite granules in temperate soils. As such a large majority of these distinctly shaped granules, that are commonly found in soils, will have been produced by this species (Canti, 2007). The granules are formed in the calciiferous glands of the earthworm, occurring in segments 10–12 as three pairs of swellings off the oesophagus (Canti, 1998). The function that granule secretion serves is unknown, with suggestions ranging from excretion of excess calcium as a reaction against calcium toxicity of soils, to neutralisation of gut pH and regulation of CO₂ (Darwin, 1881; Robertson, 1936; Crang et al., 1968; Piearce, 1972; Bal, 1977; Becze-Deák et al., 1997). Data show that granules incorporate both dietary and atmospheric C (Briones et al., 2008b; Canti, 2009). They have been reported from the Pleistocene (Meijer, 1985; Green et al., 2006), but can likely be preserved for longer (Lambkin et al., 2011). Preliminary data suggest they can be dated using U–Th disequilibria: granules recovered from Silbury Hill, a Neolithic monument in Wiltshire, UK (Atkinson, 1967), which has an earliest date of 4400 BP produce a U–Th disequilibrium age of 4670 ± 440 years (own data). Further U–Th analyses are ongoing and applications and limitations will be discussed in a future publication. ¹⁴C dating of earthworm granules is possible if enough material is available from the same stratigraphic unit and yields ages that agree with other carbonates (Pustovoytov and Terhorst, 2004). Due to their abundance and good preservation, calcite δ¹⁸O (δ¹⁸O_C) values from these granules form a potentially powerful palaeotemperature proxy. A limited number of oxygen isotope analyses have recently been published on earthworm calcite, showing similar values as directly precipitated secondary carbonates, but no systematic investigation into temperature relationships has yet been performed (Pustovoytov and Terhorst, 2004; Koeniger et al., 2012). Therefore, the aim of the experimental work presented here was to investigate this potential by testing the hypothesis that the δ¹⁸O values recorded in the earthworm secreted granules vary systematically with soil solution δ¹⁸O values and temperature.

### 2. METHODS

We investigated the utility of calcite δ¹⁸O_C values of the earthworm-secreted granules as a palaeothermometer by means of a laboratory experiment with an orthogonal combination of two different types of soil, three types of mineral water (initial δ¹⁸O values −10.0, −7.3 and −6.3 (± 0.2)‰ VSMOW) and three temperatures (10, 16 and 20 °C) with 6 replicates (individual earthworms) per treatment.

Soils were collected from agricultural fields in Berkshire, UK: Hamble (SU 61968 70235) a Hamble series Typical Argillic Brown Earth with 1.3 weight% Ca, and Red Hill (SU 56060 80033) a Yattendon series Typical Argillic Brown Earth with 0.6 weight% Ca (Jarvis, 1968; Avery, 1980). Following our established methodology for the collection of earthworm secreted granules produced over the course of an experiment, the soils were air-dried and sieved to 250 µm prior to use (Lambkin et al., 2011). This ensures soils are granule-free initially, and facilitates granule recovery at the end of the experiments. For each replicate, 300 g of soil were mixed with one of 3 different types of mineral water to 65% water holding capacity (BS ISO, 1998). This was put in a zip-lock bag with 5 g air-dried horse manure rehydrated with 10 ml of the same mineral water. One adult *L. terrestris* was added to each bag, and they were placed in one of three constant temperature rooms at 10, 16, or 20 °C in darkness. There were 6 replicates for each treatment. A scoping study indicated that within 3 weeks exposure to new isotopic conditions the oxygen isotopic composition of the granules had reached a steady state. Therefore earthworms were acclimatised for three weeks, and then transferred to an identical treatment bag containing the same type and mass of soil, manure and water at the same temperature. After 28 days earthworms were removed and soil solution was extracted using rhizon samplers (Van Walt Micro Rhizon; Kölling et al., 2005; Seeberg-Elverfeldt et al., 2005; Dickens et al., 2007). The samplers produce water with a water vapour pressure of 2340 Pa at ambient temperature (20 °C) and hence any fractionation of the water isotopes during this process will be minimal. The soil was wet-sieved to 500 µm to retrieve granules, which were air-dried and weighed.
Oxygen isotope analyses on the original mineral waters added and soil solutions extracted at the end of the experiment were performed on a Picarro L2120-i Isotopic Water Analyzer with an A0211 High-precision Vaporizer and ChemCorrect software. Values were calibrated against reference standards IA-R052, IA-R053 and IA-R054 from Iso-Analytical Limited. Long-term reproducibility was <0.2‰. Nine soils did not yield enough water for analysis. In order to incorporate granule data from these in our analysis we used the average water δ¹⁸O (δ¹⁸O_water) value from the replicates of the same treatment. Calcite granules were individually analysed for δ¹⁸O_water values, using a Thermo Delta V Advantage IRMS with a GasBench II. The raw δ¹⁸O_water values were converted into the VPDB scale after normalising against NBS18 and NBS19 carbonate standards. The variability in δ¹⁸O_water values were calculated from the Advantage IRMS with a GasBench II. The raw δ¹⁸O_water values were converted into the VPDB scale after normalising against NBS18 and NBS19 carbonate standards. The long-term standard deviation of a run was <0.1‰. Soil solution pH was measured with a combination micro-electrode connected to a Hanna pH21 pH/mV meter and calibrated using pH 4.0 and 7.0 buffers. Ca²⁺ concentrations in solutions were determined using a Perkin Elmer Optima 3000 ICP-OES. Operational blanks had Ca²⁺ concentrations below detection (16 μg/l). Data were calibrated via analysis of 1, 50 and 100 ppm dilutions of the Merck ICP multi-element standard solution IV and accuracy determined through analysis of an in house standard.

3. RESULTS

Results are shown in Table 1 and Fig. 2. At the end of the week exposure, soil solution δ¹⁸O_water values ranged from −10.2 to −5.3‰ VSMOW. Some influence of evaporation was observed, causing higher δ¹⁸O_water values for higher temperatures. This is most pronounced for the Hamble soil, probably because of its lower water holding capacity (Table 1, Fig. 2). δ¹⁸O_water values of individual granules (n = 931) vary between −10.10 and −3.21‰ VPDB. Within-treatment δ¹⁸O_water values of granules are normally distributed. The variability in δ¹⁸O_water values between granules produced by an individual earthworm within a single month is on average 0.61‰ (range 1.44‰). Higher soil solution δ¹⁸O_water values yield higher δ¹⁸O_water values, and higher temperatures result in lower δ¹⁸O_water values (Fig. 2).

4. DISCUSSION

For each replicate the expected δ¹⁸O_water value for equilibrium was calculated from the δ¹⁸O_water value and temperature according to the equation of Kim and O’Neill (1997):

\[ \Delta_{\text{calcite-water}} = \left( \frac{[1000 + \delta^{18}O_{\text{VSMOW}}]}{[1000 + \delta^{18}O_{\text{VSMOW}}]} \right) \]

and plotted against 10⁵ T⁻¹ (K). Regression analysis yields the palaeotemperature relationship:

\[ 1000 \ln \Delta = 18.03 \times 10^{0.1} - 32.42 \]

The resulting predicted δ¹⁸O_water (δ¹⁸O_water,pred) values are plotted with average measured δ¹⁸O_water per replicate in Fig. 3. A linear regression shows that earthworm granules are systematically enriched in ¹⁸O by 1.51 (± 0.12 s.d.)‰ in comparison to equilibrium:

\[ \Delta_{\text{calcite-water}} = 1.02 \delta^{18}O_{\text{pred}} + 1.51 \]

with \( R^2 = 0.98; n = 96; p < 0.001 \). Subsequently, the fractionation factor \( \Delta \) was derived:  

\[ \Delta_{\text{calcite-water}} = \left( \frac{[1000 + \delta^{18}O_{\text{VSMOW}}]}{[1000 + \delta^{18}O_{\text{VSMOW}}]} \right) \]

for each replicate in ¹⁸O. Nine soils did not yield enough water for analysis. As such, it appears that elevated initial [Ca²⁺] and [HCO₃⁻] caused the ¹⁸O enrichment in earthworm granules, through the same unknown mechanism in as synthetic calcite. Earthworms do not produce calcite granules at low [Ca²⁺] (own data) or low pH (Lambkin et al., 2011), and thus the range of these parameters in soils containing granules will be constrained at the lower end. Therefore, different soils are unlikely to yield radically different palaeotemperature equations.

A possible alternative or additional explanation for the 1.51‰ offset of the granules lies in precipitation kinetics.
Calcite precipitation in the calciferous gland likely takes place along a pathway of dissolved CO$_2$ transforming to HCO$_3^-$ and CO$_3^{2-}$, then to amorphous CaCO$_3$, which finally stabilises into calcite through a dissolution-reprecipitation mechanism (Briones et al., 2008a; Lee et al., 2008a). If calcite precipitation were very fast, there would be incomplete fractionation between HCO$_3^-$ and CaCO$_3$, resulting in $\delta^{18}$O enrichment of earthworm granules in comparison to equilibrium (kinetic effect; Mickler et al., 2004; Lachniet, 2009). The production rate of the calcite granules varied between 1.24 (± 0.47 s.d.) and 2.89 (± 0.31 s.d.) mg/day and was higher for higher temperatures and the Red Hill soil, and lower for Norway water (Table 1). However, no relationship between precipitation rate and $\delta^{18}$O$_c$ values was found.

Several recent publications on speleothems and inorganically precipitated calcite suggest that $\alpha_{calcite-water}$ is greater than the commonly accepted value by up to 1.5‰ (Coplen, 2007; Dietzel et al., 2009; Day and Henderson, 2011; Tremaine et al., 2011; Feng et al., 2012). Although these systems are very different from the earthworm calciferous gland, it is possible, that they have a common cause for disequilibrium, such as fractionation reactions at the crystal surface.

For application in palaeotemperature reconstructions, a good estimate of soil water $\delta^{18}$O$_w$ values is needed. Due to the combined influence of seasonal variations in precipita-

### Table 1

<table>
<thead>
<tr>
<th>Soil</th>
<th>$T$ (°C)</th>
<th>Water holding capacity (%)</th>
<th>Soil solution pH</th>
<th>Soil solution [Ca$^{2+}$] (mM)</th>
<th>CaCO$_3$ production per earthworm (mg/day)</th>
<th>$\delta^{18}$O$_w$ (% VSMOW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiji</td>
<td>10</td>
<td>7.2 ± 0.4</td>
<td>7.8</td>
<td>0.46</td>
<td>1.45 ± 0.28</td>
<td>$-6.3 ± 0.2$</td>
</tr>
<tr>
<td>England</td>
<td>10</td>
<td>6.7 ± 0.9</td>
<td>7.4</td>
<td>1.20</td>
<td>1.66 ± 0.43</td>
<td>$-7.3 ± 0.2$</td>
</tr>
<tr>
<td>Norway</td>
<td>10</td>
<td>5.5 ± 1.0</td>
<td>7.7</td>
<td>0.18</td>
<td>1.24 ± 0.47</td>
<td>$-10.0 ± 0.2$</td>
</tr>
</tbody>
</table>

$N$ is number of replicates per treatment. Average soil solution pH, [Ca$^{2+}$], CaCO$_3$ production and $\delta^{18}$O$_w$ values are all means ± s.d.
groundwater reflects that of local precipitation within 0.5‰ (Darling et al., 2003) and that δ18Ow values of modern soil carbonate are correlated with δ18Ow values of local meteoric water (Cerling, 1984). For the past 4000 years, modern values for the isotopic composition of precipitation can be used (McDermott et al., 2011). For earlier Holocene and Pleistocene time intervals, δ18Ow values of precipitation are often not well-constrained and the earthworm-calcite palaeothermometer can only be used if an independent reconstruction of soil water δ18Ow values is available. Alternatively, the equation can serve as an independent proxy for soil water δ18Ow values (and hence δ18Ow values of precipitation) in the presence of existing palaeotemperature reconstructions.

5. CONCLUSIONS

In summary, our study shows that the relationship between temperature and earthworm calcite δ18O values is significant and predictable. In combination with the wide distribution, good preservation, and direct U–Th dating of the granules, these will make a novel terrestrial temperature proxy. Important questions to be addressed are if this relationship holds under field conditions when evaporation of soil moisture may occur, and for other earthworm species. Future work will investigate these issues and also include application of the developed palaeothermometer to granules retrieved from various Holocene and Pleistocene locations and archaeological sites in Great Britain, the Netherlands and Germany.

ACKNOWLEDGEMENTS

This research was funded by a NERC Standard Research Grant (M.E.H. and S.B.; NE/H021914/1). We would like to thank Yan Gao for assistance with stable isotope analyses, Anne Dudley and Martin Heaps for help with the Ca analyses, and Alan Wanamaker for making available his M. edulis data. In addition, we thank Frank McDermott, Tim Atkinson and an anonymous reviewer for constructive comments that were of great help in improving the manuscript.

REFERENCES


*Associate editor: F. McDermott*