

This is a repository copy of *Earthworm-produced calcite granules : a new terrestrial palaeothermometer?*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/76848/>

Version: Published Version

Article:

Versteegh, Emma, Black, Stuart, Canti, Matt G. et al. (1 more author) (2013) Earthworm-produced calcite granules : a new terrestrial palaeothermometer? *Geochimica et Cosmochimica Acta*. pp. 351-357. ISSN 0016-7037

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

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

Earthworm-produced calcite granules: A new terrestrial palaeothermometer? [☆]

Emma A.A. Versteegh ^{a,*}, Stuart Black ^b, Matthew G. Canti ^c, Mark E. Hodson ^d

^a Department of Geography and Environmental Science, University of Reading, Whiteknights, P.O. Box 233, Reading RG6 6DW, UK

^b Department of Archaeology, University of Reading, Whiteknights, P.O. Box 227, Reading RG6 6AB, UK

^c English Heritage, Centre for Archaeology, Fort Cumberland, Fort Cumberland Road, Portsmouth PO4 9LD, UK

^d Environment Department, University of York, Heslington, York YO10 5DD, UK

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 $\delta^{18}\text{O}$ 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. $\delta^{18}\text{O}$ values were determined on individual calcite granules ($\delta^{18}\text{O}_c$) and the soil solution ($\delta^{18}\text{O}_w$). The $\delta^{18}\text{O}_c$ values reflect soil solution $\delta^{18}\text{O}_w$ values and temperature, but are consistently enriched by $1.51 (\pm 0.12)\text{‰}$ in comparison to equilibrium in synthetic carbonates. The data fit the equation $1000\ln\alpha = [20.21 \pm 0.92] (10^3 \text{ T}^{-1}) - [38.58 \pm 3.18]$ ($R^2 = 0.95$; $n = 96$; $p < 0.0005$). 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.

© 2013 The Authors. Published by Elsevier Ltd. All rights reserved.

1. INTRODUCTION

Many organisms form reliable archives of palaeotemperature or water composition, by precipitating calcium carbonate (CaCO_3) in oxygen isotopic equilibrium with their environment (Wanamaker et al., 2007; Ullmann et al., 2010; Versteegh et al., 2010). The $\delta^{18}\text{O}$ values of the CaCO_3 are controlled by temperature and water isotope composi-

tion (Urey, 1947; Epstein et al., 1953), with equilibrium being defined via a mineral-specific fractionation factor (Kim and O'Neil, 1997). The $\delta^{18}\text{O}$ composition of CaCO_3 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 CaCO_3 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

[☆] This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

* Corresponding author. Tel.: +44 0118 378 7896.

E-mail addresses: e.a.versteegh@reading.ac.uk (E.A.A. Versteegh), s.black@reading.ac.uk (S. Black), matthew.canti@english-heritage.org.uk (M.G. Canti), mark.hodson@york.ac.uk (M.E. Hodson).

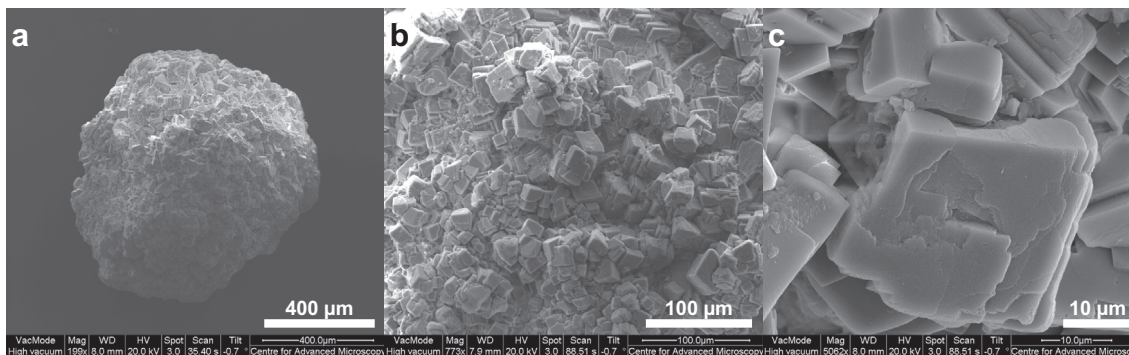


Fig. 1. SEM pictures of a *L. terrestris*-produced calcite granule. (a) Entire granule, (b) surface calcite crystal structure, (c) individual calcite crystal. Images are back-scattered electron images taken using a FEI Quanta FEG 600 Environmental Scanning Electron Microscope. (a) and (b) are taken from Lambkin et al. (2011).

worm *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 calciferous 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; Pearce, 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 $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_w$) value from the replicates of the same treatment. Calcite granules were individually analysed for $\delta^{18}\text{O}_c$ values, using a Thermo Delta V Advantage IRMS with a GasBench II. The raw $\delta^{18}\text{O}_c$ values were converted into the VPDB scale after normalising against NBS18 and NBS19 carbonate standards. The long-term standard deviation of a routinely analysed in-house CaCO_3 standard 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. Ca^{2+} concentrations in soil solutions were determined using a Perkin Elmer Optima 3000 ICP-OES. Operational blanks had Ca^{2+} concentrations below detection (16 $\mu\text{g/l}$). 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 4 week exposure, soil solution $\delta^{18}\text{O}_w$ values ranged from -10.2 to -5.3 ‰ VSMOW. Some influence of evaporation was observed, causing higher $\delta^{18}\text{O}_w$ values for higher temperatures. This is most pronounced for the Hamble soil, probably because of its lower water holding capacity (Table 1, Fig. 2). $\delta^{18}\text{O}_c$ values of individual granules ($n = 931$) vary between -10.10 and -3.21 ‰ VPDB. Within-treatment $\delta^{18}\text{O}_c$ values of granules are normally distributed. The variability in $\delta^{18}\text{O}_c$ values between granules produced by an individual earthworm within a single month is on average 0.61‰ (range 1.44‰). Higher soil solution $\delta^{18}\text{O}_w$ values yield higher $\delta^{18}\text{O}_c$ values, and higher temperatures result in lower $\delta^{18}\text{O}_c$ values (Fig. 2).

4. DISCUSSION

For each replicate the expected $\delta^{18}\text{O}_c$ value for equilibrium was calculated from the $\delta^{18}\text{O}_w$ value and temperature according to the equation of Kim and O'Neil (1997):

$$1000 \ln \alpha = 18.03(10^3 T^{-1}) - 32.42 \quad (1)$$

The resulting predicted $\delta^{18}\text{O}_c$ ($\delta^{18}\text{O}_{\text{pred}}$) values are plotted with average measured $\delta^{18}\text{O}_c$ per replicate in Fig. 3. A linear regression shows that earthworm granules are systematically enriched in ^{18}O by $1.51 (\pm 0.12 \text{ s.d.})$ ‰ in comparison to equilibrium:

$$\delta^{18}\text{O}_c = 1.02\delta^{18}\text{O}_{\text{pred}} + 1.51 \quad (2)$$

with $R^2 = 0.98$; $n = 96$; $p < 0.001$. Subsequently, the fractionation factor α was derived:

$$\alpha_{\text{calcite-water}} = \frac{[1000 + \delta^{18}\text{O}_c \text{VSMOW}]/[1000 + \delta^{18}\text{O}_w \text{VSMOW}]}{\quad} \quad (3)$$

and plotted against $10^3 T^{-1}$ (K). Regression analysis yields the palaeotemperature relationship:

$$1000 \ln \alpha = [20.21 \pm 0.92](10^3 T^{-1}) - [38.58 \pm 3.18] \quad (4)$$

with $R^2 = 0.95$; $n = 96$; $p < 0.001$. Quoted errors on the slope and intercept are reported at the 95% confidence interval. Our results are compared to equations by Kim and O'Neil (1997; synthetic carbonates) and Wanamaker et al. (2006; 2007; Blue mussel, *Mytilus edulis*) in Fig. 4. *M. edulis* data are slightly offset from equilibrium (<0.2‰), but this difference is not statistically significant (Wanamaker et al., 2007). $\delta^{18}\text{O}_c$ values of calcite granules produced by *L. terrestris* reflect soil solution $\delta^{18}\text{O}_w$ values and temperature, but are enriched in ^{18}O in comparison to equilibrium in synthetic carbonates (Kim and O'Neil, 1997) and the bivalve *M. edulis* (Wanamaker et al., 2007).

Although the relationship is strong, the range of $1000 \ln \alpha$ for a given temperature is about twice that observed for *M. edulis* (Wanamaker et al., 2007) translating into an average offset in reconstructed temperature of 0.73 °C. A possible explanation for this increased variation is that some evaporation occurred during the experiment, progressively increasing soil solution $\delta^{18}\text{O}_w$ values throughout the 4-week experiment. In addition, some of the variation could have been caused by the variability of the controlled temperature chambers in which the experiments were performed at ± 0.5 °C from the set temperature.

The enrichment in ^{18}O by 1.51‰ compared to equilibrium is statistically indistinguishable from the equation for synthetic calcite precipitated from a solution with initial $[\text{Ca}^{2+}]$ and $[\text{HCO}_3^-]$ of 15 mM (Fig. 4; Kim and O'Neil, 1997). The soil solutions collected after the experiment had $[\text{Ca}^{2+}]$ values varying between 8.30 (± 1.02 s.d.) mM and 16.79 (± 1.42 s.d.) mM with higher values for higher temperatures, for mineral waters with higher initial $[\text{Ca}^{2+}]$, and for the Red Hill soil. Average pH values were 6.5 ± 0.8 s.d. (Fiji), 6.8 ± 0.5 s.d. (England) and 7.0 ± 0.3 s.d. (Norway; Table 1). pH did not significantly differ between temperatures or soils. At the measured pH values, most DIC will be present in the form of HCO_3^- (Zeebe and Wolf-Gladrow, 2001). HCO_3^- was not determined directly, but concentrations of dissolved inorganic carbon (DIC) are likely to have been high (up to 32 mM based on equivalence of charge of DIC with Ca^{2+}), due to earthworm and microorganism respiration (601–1329 $\mu\text{mol/mol}$ CO_2 in soil air, own data). As such, it appears that elevated initial $[\text{Ca}^{2+}]$ and $[\text{HCO}_3^-]$ caused the ^{18}O enrichment in earthworm granules, through the same unknown mechanism as in synthetic calcite. Earthworms do not produce calcite granules at low $[\text{Ca}^{2+}]$ (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.

Table 1
Experimental conditions.

T (°C)	Soil	Mineral water added	Water holding capacity (%)	Soil solution pH	Soil solution $[Ca^{2+}]$ (mM)	CaCO ₃ production per earthworm (mg/day)	$\delta^{18}O_w$ (‰ VSMOW)	N^*
Pure mineral waters		Fiji		7.8	0.46		-6.3 ± 0.2	1
		England		7.4	1.20		-7.3 ± 0.2	1
		Norway		7.7	0.18		-10.0 ± 0.2	1
10	Hamble	Fiji	33	7.2 ± 0.4	10.93 ± 0.89	1.45 ± 0.28	-5.93 ± 0.05	5
10	Hamble	England	33	6.7 ± 0.9	10.44 ± 0.62	1.66 ± 0.43	-7.11 ± 0.04	6
10	Hamble	Norway	33	5.5 ± 1.0	8.30 ± 1.02	1.24 ± 0.47	-9.60 ± 0.05	6
10	Red Hill	Fiji	56	6.9 ± 0.1	15.86 ± 2.09	1.82 ± 0.21	-6.04 ± 0.11	6
10	Red Hill	England	56	6.7 ± 0.1	13.30 ± 0.82	1.77 ± 0.26	-7.24 ± 0.03	6
10	Red Hill	Norway	56	6.9 ± 0.1	15.21 ± 0.92	1.76 ± 0.46	-9.85 ± 0.06	6
16	Hamble	Fiji	33	7.3 ± 0.2	12.13 ± 1.52	2.30 ± 0.24	-5.72 ± 0.14	5
16	Hamble	England	33	7.1 ± 0.2	11.22 ± 1.31	2.11 ± 0.25	-7.10 ± 0.08	6
16	Hamble	Norway	33	6.7 ± 0.8	9.09 ± 0.87	1.79 ± 0.37	-9.44 ± 0.10	6
16	Red Hill	Fiji	56	6.9 ± 0.1	15.82 ± 0.84	2.56 ± 0.27	-6.20 ± 0.12	5
16	Red Hill	England	56	6.7 ± 0.2	13.44 ± 1.45	2.75 ± 0.27	-7.24 ± 0.09	6
16	Red Hill	Norway	56	6.7 ± 0.2	15.25 ± 0.49	2.19 ± 0.49	-9.94 ± 0.10	6
20	Hamble	Fiji	33	7.0 ± 0.4	12.34 ± 1.35	2.39 ± 0.47	-5.43 ± 0.10	6
20	Hamble	England	33	6.9 ± 0.7	11.39 ± 0.58	2.06 ± 0.60	-6.74 ± 0.04	6
20	Hamble	Norway	33	5.7 ± 0.5	11.38 ± 0.43	1.92 ± 0.47	-9.02 ± 0.11	5
20	Red Hill	Fiji	56	6.8 ± 0.2	16.79 ± 1.42	2.46 ± 0.27	-5.85 ± 0.05	6
20	Red Hill	England	56	6.8 ± 0.3	13.81 ± 0.53	2.89 ± 0.31	-7.02 ± 0.05	5
20	Red Hill	Norway	56	6.9 ± 0.2	15.10 ± 0.89	2.70 ± 0.53	-9.60 ± 0.10	4

* N is number of replicates per treatment. Average soil solution pH, $[Ca^{2+}]$, CaCO₃ production and $\delta^{18}O_w$ values are all means \pm s.d.

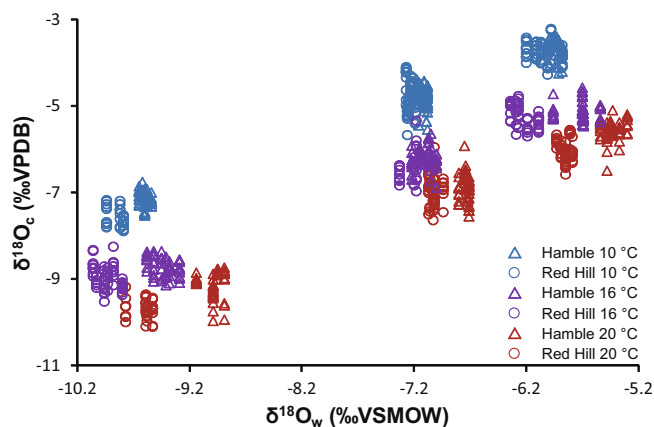


Fig. 2. $\delta^{18}O_c$ values of individual granules and soil solution $\delta^{18}O_w$ values. Granules produced at 10 °C (blue), 16 °C (purple) and 20 °C (red) in Hamble soil (triangles) and Red Hill soil (circles). Higher $\delta^{18}O_w$ values result in higher $\delta^{18}O_c$ values and higher temperatures give lower $\delta^{18}O_c$ values.

Calcite precipitation in the calciferous gland likely takes place along a pathway of dissolved CO₂ transforming to HCO₃⁻ and CO₃²⁻, then to amorphous CaCO₃, which finally stabilises into calcite through a dissolution-precipitation mechanism (Briones et al., 2008a; Lee et al., 2008a). If calcite precipitation were very fast, there would be incomplete fractionation between HCO₃⁻ and CaCO₃, resulting in ¹⁸O 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 (\pm 0.47 s.d.) and 2.89 (\pm 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 $\alpha_{\text{calcite-water}}$ 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-

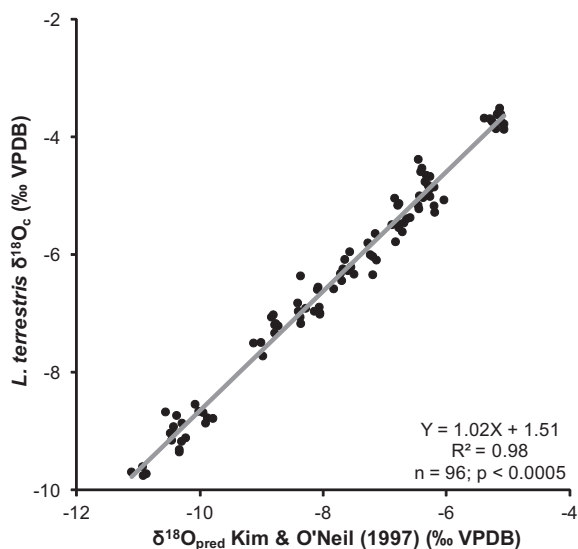


Fig. 3. Comparison of $\delta^{18}\text{O}_{\text{pred}}$ values according to Kim and O'Neil (1997) and measured $\delta^{18}\text{O}_{\text{C}}$ values for earthworm-produced calcite. *L. terrestris* CaCO_3 granules are enriched in ^{18}O by 1.51‰.

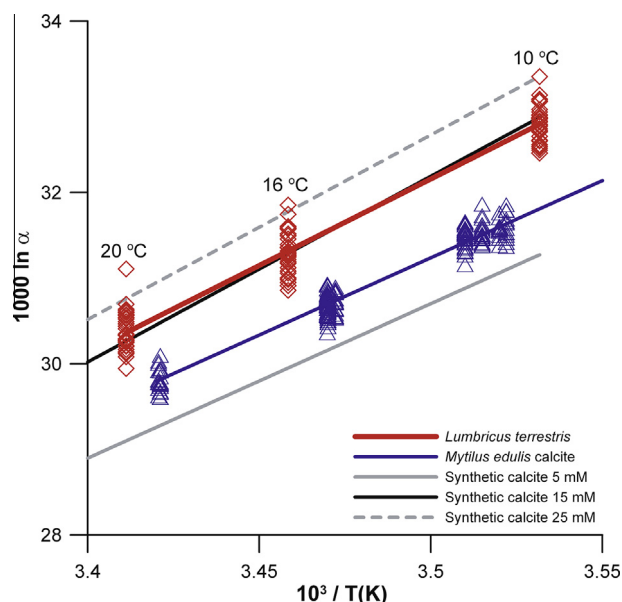


Fig. 4. Relationship between $10^3 \ln \alpha$ and temperature for several types of calcite. *L. terrestris* granules (red diamonds; this study), equilibrium (grey solid line), synthetic at initial $[\text{Ca}^{2+}]$ and $[\text{HCO}_3^-]$ of 15 mM (black solid line), synthetic at initial $[\text{Ca}^{2+}]$ and $[\text{HCO}_3^-]$ of 25 mM (grey dashed line; Kim and O'Neil (1997)), and *M. edulis* shells (blue triangles; Wanamaker et al. (2007)). Earthworm granules are enriched in ^{18}O compared to equilibrium, in a similar way as synthetic carbonates precipitated at elevated initial concentrations of Ca^{2+} and HCO_3^- .

tion $\delta^{18}\text{O}_{\text{w}}$ values and evapotranspiration, these vary over time and with depth (Hsieh et al., 1998). However, in a high-rainfall temperate climate, like that of the British Isles, the influence of evaporation is likely to be small. This is supported by data showing that the $\delta^{18}\text{O}_{\text{w}}$ composition of

groundwater reflects that of local precipitation within 0.5‰ (Darling et al., 2003) and that $\delta^{18}\text{O}_{\text{C}}$ values of modern soil carbonate are correlated with $\delta^{18}\text{O}_{\text{w}}$ 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, $\delta^{18}\text{O}_{\text{w}}$ values of precipitation are often not well-constrained and the earthworm-calcite palaeothermometer can only be used if an independent reconstruction of soil water $\delta^{18}\text{O}_{\text{w}}$ values is available. Alternatively, the equation can serve as an independent proxy for soil water $\delta^{18}\text{O}_{\text{w}}$ values (and hence $\delta^{18}\text{O}_{\text{w}}$ 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 $\delta^{18}\text{O}_{\text{C}}$ 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

- Atkinson R. J. C. (1967) Silbury hill. *Antiquity* **XLI**, 259–262.
- Avery B. W. (1980) *Soil Classification for England and Wales [higher categories]*. Rothamsted Experimental Station, Harpenden.
- Bal L. (1977) The formation of carbonate nodules and intercalary crystals in the soil by the earthworm *Lumbricus rubellus*. *Pedobiologia* **17**, 229–240.
- Becze-Deák J., Langohr R. and Verrecchia E. P. (1997) Small scale secondary CaCO_3 accumulations in selected sections of the European loess belt. Morphological forms and potential for paleoenvironmental reconstruction. *Geoderma* **76**, 221–252.
- Briones M. J. I., López E., Méndez J., Rodríguez J. B. and Gago-Dupont L. (2008a) Biological control over the formation and storage of amorphous calcium carbonate by earthworms. *Mineral. Mag.* **72**, 227–231.
- Briones M. J. I., Ostle N. J. and Pearce T. G. (2008b) Stable isotopes reveal that the calciferous gland of earthworms is a CO_2 -fixing organ. *Soil Biol. Biochem.* **40**, 554–557.

- BS ISO (1998) *Soil Quality – Determination of the Water-retention Characteristic*. BS ISO, Geneva.
- Canti M. G. (1998) Origin of calcium carbonate granules found in buried soils and quaternary deposits. *Boreas* **27**, 275–288.
- Canti M. G. (2007) Deposition and taphonomy of earthworm granules in relation to their interpretative potential in quaternary stratigraphy. *J. Quatern. Sci.* **22**, 111–118.
- Canti M. G. (2009) Experiments on the origin of ^{13}C in the calcium carbonate granules produced by the earthworm *Lumbricus terrestris*. *Soil Biol. Biochem.* **41**, 2588–2592.
- Cerling T. E. (1984) The stable isotopic composition of modern soil carbonate and its relationship to climate. *Earth Planet. Sci. Lett.* **71**, 229–240.
- Coplen T. B. (2007) Calibration of the calcite–water oxygen-isotope geothermometer at Devils Hole, Nevada, a natural laboratory. *Geochim. Cosmochim. Acta* **71**, 3948–3957.
- Correa M. L., Montagna P., Vendrell-Simón B., McCulloch M. and Taviani M. (2010) Stable isotopes ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$), trace and minor element compositions of recent scleractinians and Last Glacial bivalves at the Santa Maria di Leuca deep-water coral province, Ionian Sea. *DSR* **57**, 471–486.
- Crang R. E., Holsen R. C. and Hitt J. B. (1968) Calcite production in mitochondria of earthworm calciferous glands. *Bioscience* **18**, 299–301.
- Darling W. G., Bath A. H. and Talbot J. C. (2003) The O and H stable isotope composition of freshwaters in the British Isles. 2. Surface waters and groundwater. *HESS* **7**, 183–195.
- Darwin C. (1881) *The Formation of Vegetable Mould, Through the Action of Worms, with Observations on their Habits*. The Echo Library, Teddington.
- Day C. C. and Henderson G. M. (2011) Oxygen isotopes in calcite grown under cave-analogue conditions. *Geochim. Cosmochim. Acta* **75**, 3956–3972.
- Dickens G. R., Koelling M., Smith D. C. and Schnieders L. (2007) Rhizon sampling of pore waters on scientific drilling expeditions: an example from the IODP Expedition 302, Arctic Coring Expedition (ACEX). *Sci. Drill.* **4**, 22–25.
- Dietzel M., Tang J., Leis A. and Köhler S. J. (2009) Oxygen isotopic fractionation during inorganic calcite precipitation – effects of temperature, precipitation rate and pH. *Chem. Geol.* **268**, 107–115.
- Epstein S., Buchsbaum R., Lowenstam H. A. and Urey H. C. (1953) Revised carbonate–water isotopic temperature scale. *Bull. Geol. Soc. Am.* **64**, 1315–1326.
- Feng W., Banner J. L., Guilfoyle A. L., Musgrove M. and James E. W. (2012) Oxygen isotopic fractionation between drip water and speleothem calcite: a 10-year monitoring study, central Texas, USA. *Chem. Geol.* **304–305**, 53–67.
- Ford H. L., Schellenberg S. A., Becker B. J., Deutschman D. L., Dyck K. A. and Koch P. L. (2010) Evaluating the skeletal chemistry of *Mytilus californianus* as a temperature proxy: effects of microenvironment and ontogeny. *Paleoceanography* **25**.
- Green C. P., Branch N. P., Russell Coope G., Field M. H., Keen D. H., Wells J. M., Schwenninger J.-L., Preece R. C., Schreve D. C., Canti M. G. and Gleed-Owen C. P. (2006) Marine isotope stage 9 environments of fluvial deposits at Hackney, north London, UK. *Quatern. Sci. Rev.* **25**, 89–113.
- Hsieh J. C. C., Chadwick O. A., Kelly E. F. and Savin S. M. (1998) Oxygen isotopic composition of soil water: quantifying evaporation and transpiration. *Geoderma* **82**, 269–293.
- Jarvis R. A. (1968) *Soils of the Reading District*. Agricultural Research Council, Harpenden.
- Juillet-Leclerc A., Reynaud S., Rollion-Bard C., Cuif J. P., Dauphin Y., Blamart D., Ferrier-Pagès C. and Allemand D. (2009) Oxygen isotopic signature of the skeletal microstructures in cultured corals: identification of vital effects. *Geochim. Cosmochim. Acta* **73**, 5320–5332.
- Kim S.-T. and O'Neil J. R. (1997) Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochim. Cosmochim. Acta* **61**, 3461–3475.
- Koeniger P., Barta G., Thiel C., Bajnóczi B., Novothny Á., Horváth E., Techmer A. and Frechen M. (2012) Stable isotope composition of bulk and secondary carbonates from the quaternary loess-paleosol sequence in Süttő, Hungary. *Quatern. Int.*
- Kölling M., Seeberg-Elverfeldt J. and Schlüter M. (2005) Rhizon – an excellent pore water sampler for low maintenance collection and filtration of small volume samples. *Geophys. Res. abstr.* **7**.
- Lachniet M. S. (2009) Climatic and environmental controls on speleothem oxygen-isotope values. *Quatern. Sci. Rev.* **28**, 412–432.
- Lambkin D. C., Gwilliam K. H., Layton C., Canti M. G., Pearce T. G. and Hodson M. E. (2011) Production and dissolution rates of earthworm-secreted calcium carbonate. *Pedobiologia* **54**, S119–S129.
- Lee M. R., Hodson M. E. and Langworthy G. N. (2008a) Crystallization of calcite from amorphous calcium carbonate: earthworms show the way. *Mineral. Mag.* **72**, 257–261.
- Lee M. R., Hodson M. E. and Langworthy G. N. (2008b) Earthworms produce granules of intricately zoned calcite. *Geology* **36**, 943–946.
- McDermott F., Atkinson T. C., Fairchild I. J., Baldini L. M. and Matthey D. P. (2011) A first evaluation of the spatial gradients in $\delta^{18}\text{O}$ recorded by European Holocene speleothems. *Global Planet. Change* **79**, 275–287.
- Meijer T. (1985) The pre-Weichselian non-marine molluscan fauna from Maastricht-Belvédère (Southern Limburg, The Netherlands). In *Maastricht-Belvédère: Stratigraphy, Palaeoenvironment and Archaeology of the Middle and Late Pleistocene Deposits*. Geological Survey of The Netherlands, Haarlem, pp. 75–103.
- Mickler P. J., Banner J. L., Stern L., Asmerom Y., Edwards R. L. and Ito E. (2004) Stable isotope variations in modern tropical speleothems: evaluating equilibrium vs. kinetic isotope effects. *Geochim. Cosmochim. Acta* **68**, 4381–4393.
- Owen R., Kennedy H. and Richardson C. (2002) Isotopic partitioning between scallop shell calcite and seawater: effect of shell growth rate. *Geochim. Cosmochim. Acta* **66**, 1727–1737.
- Pearce T. G. (1972) The calcium relations of selected lumbricidae. *J. Anim. Ecol.* **41**, 167–188.
- Pustovoytov K. and Terhorst B. (2004) An isotopic study of a late quaternary loess-paleosol sequence in SW Germany. *Rev. Mexic. Cie. Geol.* **21**, 88–93.
- Robertson J. D. (1936) The function of the calciferous glands of earthworms. *J. Exp. Biol.* **XIII**, 279–297.
- Rosenheim B. E., Swart P. K. and Willenz P. (2009) Calibration of sclerosponge oxygen isotope records to temperature using high-resolution $\delta^{18}\text{O}$ data. *Geochim. Cosmochim. Acta* **73**, 5308–5319.
- Seeberg-Elverfeldt J., Schlüter M., Feseker T. and Kölling M. (2005) Rhizon sampling of pore waters near the sediment/water interface of aquatic systems. *Limnol. Oceanogr. Methods* **3**, 361–371.
- Tremaine D. M., Froelich P. N. and Wang Y. (2011) Speleothem calcite formed in situ: modern calibration of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ paleoclimate proxies in a continuously-monitored natural cave system. *Geochim. Cosmochim. Acta* **75**, 4929–4950.
- Ullmann C. V., Wiechert U. and Korte C. (2010) Oxygen isotope fluctuations in a modern North Sea oyster (*Crassostrea gigas*) compared with annual variations in seawater temperature: implications for palaeoclimate studies. *Chem. Geol.* **277**, 160–166.

- Urey H. C. (1947) The thermodynamic properties of isotopic substances. *J. Chem. Soc. (Resumed)*, 562–581.
- Versteegh E. A. A., Vonhof H. B., Troelstra S. R., Kaandorp R. J. G. and Kroon D. (2010) Seasonally resolved growth of freshwater bivalves determined by oxygen and carbon isotope shell chemistry. *GGG* **11**, 16.
- Wanamaker, Jr., A. D., Kreutz K. J., Borns, Jr., H. W., Introne D. S., Feindel S. and Barber B. J. (2006) An aquaculture-based method for calibrated bivalve isotope paleothermometry. *GGG* **7**, 13.
- Wanamaker, Jr., A. D., Kreutz K. J., Borns, Jr., H. W., Introne D. S., Feindel S., Funder S., Rawson P. D. and Barber B. J. (2007) Experimental determination of salinity, temperature, growth, and metabolic effects on shell isotope chemistry of *Mytilus edulis* collected from Maine and Greenland. *Paleoceanography* **22**, 12.
- Zeebe R. E. and Wolf-Gladrow D. (2001) *CO₂ in Seawater: Equilibrium, Kinetics, Isotopes*. Elsevier, Amsterdam.
- Ziveri P., Stoll H., Probert I., Klaas C., Geisen M., Ganssen G. and Young J. (2003) Stable isotope ‘vital effects’ in coccolith calcite. *Earth Planet. Sci. Lett.* **210**, 137–149.

Associate editor: F. McDermott