

S cience careers are funny things. Fifteen years ago I completed my geology PhD on why some igneous rocks on the southern tip of Greenland have stripes. At the time, earthworm poo had never entered my mind.

If someone had told me then that a decade or so later Denise Lambkin, my postdoc, and I would spend significant periods of time sifting through earthworm poo I would have thought they were having a laugh.

But my story starts much earlier than this. It really began in 1881. After more than 40 years of observation Darwin completed and published his final great work *The Formation of Vegetable Mould, Through the Action of Worms, with Observations on Their Habits.*' As an indication of Victorian reading tastes, the book sold faster than *On the Origin of Species*, at least to begin with.

My collision with Darwin's great text really goes back to 2002. I had been working on how metal pollution in contaminated soils affects earthworms for a mere two years when I found myself at an International Earthworm Symposium in Cardiff.

Back then I was unaware of the book and unaware that earthworms excrete calcium carbonate. But all that was about to change. An archaeologist called Matt Canti illustrated a talk with images that spoke straight to anyone with a love of visually stunning rocks and minerals – this is what led me to study those wonderful Greenlandic rocks back as a PhD student.

He showed some fantastic images of balls of calcite. These are clusters of rhombahedral crystals, all stuck together into granules up to two millimetres in

diameter. Incredibly these granules had been excreted by earthworms

The combination of a link to Darwin, the beauty of the granules and my

favourite soil-dwelling organism was just too tempting. I wanted to find out more about these granules. And so I began collecting earthworm poo and picking bits out of it.

In 1881 Darwin suggested that the earthworms might use the granules to neutralise acid in their stomachs, a bit like a self-produced Rennie, or to excrete excess calcium. There are unresolved issues

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with both these theories, and the function of the granules remains a mystery, but environmental scientists are showing a growing interest in earthworm granules.

For Matt Canti, as an archaeologist, the appeal of earthworm granules is that since they accumulate near the soil surface, large numbers of granules at depth might indicate a buried soil surface. So careful analysis may show, for example, the original soil surface before a burial mound or defensive wall was built.



If the calcite granules survive for significant periods of time, and their presence in buried soils suggest that they can, their composition opens up the possibility of using them as a dating tool. Possible approaches are carbon dating, amino-acid dating, or uranium-thorium dating. Uranium is an impurity in calcium carbonates. It decays to the element thorium over time, so the more thorium in a sample the older it is. Or it might be possible to work out the environmental conditions when the granules formed by looking at their oxygen isotope compositions.

Before we can start really addressing any of the above fascinating possibilities we need to know in detail what the granules are made of, how rapidly they are produced and how long they are likely to last in the soil. We are investigating these issues in my laboratory.

Like slicing Maltesers

Our first experiment simply consisted of collecting some granules from the soil, cutting them into slices and looking at the slices with an electron microscope, rather like slicing through a Malteser to look at the centre of the sweet. This approach immediately paid dividends with some rather spectacular results.

We showed for the first time that the granules appear to have a non-calcium-carbonate core. We already knew the granules started off with the formation of minuscule 'spherites' in a gland known as the calciferous gland, but how these spherites coalesced to form the granules was unknown. It now looks like the granules form when the spherites stick onto bits of mineral present in the soil ingested by the earthworms. The ingested mineral grains form the cores of the granules.

The revelations didn't stop there, however. Our images also show that the calcite granules often have a radial structure, centring on their core. In other cases the calcite granules have a concentric structure with layers of quartz and feldspar crystals trapped within the calcite.

The granules kept their best surprise until last. Martin Lee at the University of Glasgow used a technique called Electron Back Scattered Diffraction to check the composition of the calcite in the granules. To our surprise some of the calcite disappeared when we tried to analyse it this way. The 'missing' bits of granule were definitely calcium carbonate but they weren't calcite – the most stable form of calcium carbonate. Instead, we had discovered something extremely unusual –

amorphous calcium carbonate. This stuff contains calcium and carbonate ions just like calcite but the ions aren't organised in a regular repeating pattern.

Impossible poo?

The intriguing thing about amorphous calcium carbonate is that strictly it shouldn't exist, or at least it shouldn't exist for long enough for us to have spotted it in the earthworm-produced granules.

Amorphous calcium carbonate is so unstable that it is almost impossible to make in the laboratory and once made converts to calcite within minutes. The mystery of how this amorphous calcium carbonate is

An electron microscope image of a slipe through a grantile of earntworm pro-showing the quarty grain (grey) at its centre. The coloried abouting to their orientation and aspear to radiate from the quartz core.

surviving in the granules is something we are actively investigating. It is a question of interest to material and biomedical scientists who would like to make micron-scale devices out of amorphous materials due to their structural properties.

So now we know a lot more about the structure and composition of the granules that earthworms secrete. The second part of our study concerns how rapidly the granules are produced and how long they last in soils.

To date we have monitored the mass of calcite produced by individual *Lumbricus* terrestris earthworms in a series of soils with different properties, for example, some of the soils contain more organic matter, some are more acidic and they all have differing compositions. We removed any existing granules from the soils and then added some earthworms and waited. Several weeks later we extracted and weighed the granules

freshly secreted into the soil.

One thing that is very clear is that, just like people, earthworms are all individuals. There is a huge amount of variation in the mass of calcite produced by *Lumbricus terrestris* in any given soil. In one soil our earthworms produced between 0.2 and 4.3 milligrams of calcite per earthworm per day.

If those numbers sound small, once you take into account average earthworm densities in soil you find that earthworm-produced calcite could lock up 564kg of carbon per hectare of soil per year. Put into context, typical soil-based carbon sequestration techniques such as using cover crops, organic composts and manures, and converting degraded soils into forests could sequester 300 to 800kg of carbon per hectare per year.

Of course not all earthworms are such superb producers of calcite as our 4.3 milligrams per day individual, and production rates vary with soil chemistry. As you might expect we find more calcium carbonate granules in alkali soils and fewer in acid soils though this may be due to the granules dissolving relatively rapidly in the acid soil rather than differences in production rate. But none the less this is a significant amount of carbon. The question then becomes, of course, how long do these granules last? That tantalising question is the subject of ongoing work the results of which could give us a whole new respect for the role of the earthworm in the terrestrial carbon cycle.

For me its been a long journey from studying igneous rock layering in Greenland to collecting calcium carbonate from earthworm poo, but this project has it all – sexy minerals, potential dating and environmental-indicator tools and a new take on the terrestrial carbon cycle. I'm sure that Darwin would have approved.

*No, not that one. The Formation of Vegetable Mould, Through the Action of Worms, with Observations on Their Habits sold 6000 copies in its first year, selling faster than On the Origin of Species by Means of Natural Selection when it was first published.

MORE INFORMATION

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