A Letter from America…

Listening to Organ Music in Ladies Wear
(or Making Transgenic Worms in Philadelphia)

It's normal to have worms in your guts – only WEIRD people (Western, Educated, Industrialised, Rich, Democratic) don't. Nematodes have repeatedly evolved to be parasites and for one, *Strongyloides rattii,* we had just discovered the genes that it uses to be a parasite. But, we had no idea what most of these genes did.

As we were doing this work in Bristol, my US colleague James 'Sparky' Lok had just worked out how to make transgenic *Strongyloides*. You might think that this isn't a big deal – we've been able to make transgenic *C. elegans* for 30 years – but that's the point: until now, transgenesis of any other nematode, especially a parasite, hasn't been possible. The Leverhulme Trust funded me to go to Sparky's lab to learn to make transgenic *Strongyloides,* and to start studying its 'parasitism genes'.

Sparky is in the vet school of the University of Pennsylvania. 'Penn', as it's usually known, is in Philadelphia, the city of brotherly love. It's a private, Ivy League institution, and one of the US's oldest universities, founded in 1740 by Benjamin Franklin, a polymath and signatory of the US Declaration of Independence (and look at all the trouble that caused).

Penn's rich – its endowment is worth a tad under $10 billion, and so it gives effectively free education to very many of its 25,000 students. Despite this wealth its bean counters are avaricious at extracting money from research labs – I hesitate to tell tales, to save giving cunning ideas to Bristol's accountants…

The vet school at Penn has a rich parasitological heritage. A long-time departmental chair was E.L. Soulsby who wrote several classic parasitology text books. He's now in the House of Lords. Gerry Schad was also at Penn and did classic work on human hookworm infection in the southern US and India with the Rockefeller Foundation. My favourite paper of his measured gut transit time of mice by pulse-chase feeding them iron fillings, then assaying what came out the other end with a magnet. It was Schad who started Penn's work with *Strongyloides.*

Penn is named after its home state, one with strong links to Bristol. Pennsylvania was founded eponymously by William Penn, on land given by Charles II repaying a loan to Penn's unimaginative father, William Penn Sr. Penn Jr married in Bristol, Penn Sr is buried in St Mary Redcliffe.

S. stercoralis infects at least 100 million people, but it can also infect dogs. In the 1980s Schad came across a local canine outbreak and started working on it; ever since it's been maintained in dogs at Penn. The dogs are given a low level, non-pathological infection and also lightly immunosuppressed to prolong the infection. This immunosuppression can lead to a type of Cushing's syndrome (no, not a yearning to act in horror films) and when this happens they're taken off the immunosuppression regime, are dewormed and then retired as pets.

The vet school feels very unlike SoBS. In the US veterinary science is a graduate programme and the students are noticeably older, which gives a very different (and not unpleasant) feel to the place. Many staff and students bring their dogs to work, and there's a small animal clinic too. It really feels like a vet school.

There was a nice balance of common sense and care when it comes to health and safety. In Sparky's lab there was plenty of potential harm, with large quantities of dog faeces

containing thousands of human-infective *Strongyloides* larvae. With these everyone took care to wear gloves, though lab coats were optional. We carefully used a separate lab sink to wash our coffee mugs.

Last century when I was a post doc I had learnt how to make transgenic *C. elegans*, so I was confident that what I wanted to do with *Strongyloides* would be a doddle. Oh dear no. It's bloody fiddly making transgenic *Strongyloides*. The theory's simple – you inject a DNA construct into the ovaries of free-living *Strongyloides* worms and then search for their rare progeny expressing the construct. But here's the rub: the worms are 1 mm long, 50 µm in diameter, containing a pair of ovaries perilously close to the worm's gut, so the microinjections have to be very precise. And nematodes are under positive pressure, so microinjecting them is like trying to inject an inflated balloon. To get round this you mount worms on dried agar pads that partially dries them out, so that they shouldn't explode when injected. Dry them out too much – that's lethal too. Most things I injected exploded or had a terminal gonadal hernias. It was all pretty dispiriting stuff, especially for the worms.

Philadelphia is to New York City, as Bristol is to London – and each pair are about 100 miles apart. Philly was the US's temporary capital while Washington DC was being constructed. Philadelphia grew and grew, trading across the continent and the world. It peaked in the nineteenth century as a major industrial centre, but this was followed by a long decline to a 1980s nadir. It's since turned a corner and is a thriving, forward-looking city.

Now the really interesting thing about Philadelphia is that it's home to two enormous pipe organs – both products of Philadelphia's nineteenth century wealth. Of course organ size isn't everything. Indeed playing a big organ can be distracting because there's so much to fiddle with, that one stops concentrating on making beautiful music. One of Philly's large organs, the Wannamaker organ, is the centre piece of a grand department store. The console's 6 keyboards, pedal board and 729 stops control 28,500 pipes, that are arranged over six floors of the store's atrium. There are twice daily concerts and the best viewing's to be had in ladies wear.

The Wannamaker's 'string' division is of particular note, producing a vast but gentle, ethereal sound that fills the store, but from an unknowable source. Pipe organ 'strings' are actually two slightly out of tune pipes that 'beat' giving the aural impression of a string. (String stops are standard in English church organs – for example, the *Vox Celesta* – typically used during meditative parts of the liturgy.) The Wannamaker has string stops in spades, with the de-tuned pipe pairs in both sharp and flat versions, adding great texture and depth to the timbre.

On my daily walk to the local coffee shop I looked down the gridded street to a cluster of the city's skyscrapers a couple of miles away. They would gleam in the sun, or were covered in mist, or reflected the snow. These were built by the American 'can do' spirit. It was an inspiring sight: in America you can do anything – even microinjecting *Strongyloides*.

I slowly got better at injecting the worms, and the females began to produce some fluorescent, glowing eggs, but these eggs weren't viable. The DNA constructs were being expressed in dying embryos. We fiddled around with different genes, different DNA constructs, to try and get some glowing eggs that would hatch. Eventually we did – and it was all down to DNA concentration. It seemed we'd been injecting far too much construct, which was probably sucking-up all the transcription factors in the cells causing the embryos to die.

I'm now about to swap my year of worm injecting for a busy Bristol teaching year. Sitting, injecting worms for a few hours each day is a great time to think and ponder. Indeed, I think I

may have had my most brilliant (or unhinged) research idea ever. I've also daily pondered the many mutated genes that together turned free-living nematodes into parasites, genes that we're now slowly beginning to understand. Of course, organs have 'mutation' stops too – *Tierce*, *Nasard, Quint* – but that's another story…

Mark Viney
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