TRACKING INFECTIONS AND DIFFERENCES BETWEEN COUNTRIES

Distinguishing Between EFB Outbreaks

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I am now in the final year of my PhD studying the causative organism of European foul brood (EFB), a bacterium called *Melissococcus plutonius*. Having finished the vast majority of my lab studies I am now concentrating on data analysis and writing up, so this seemed like a good opportunity to write about one of the most interesting aspects of my findings.

The objective of this part of my project was to find a way to distinguish between different types of the EFB pathogen. This is important, as if one is able to say that the type of *M. plutonius* found in one infected apiary is the same as that found in a nearby apiary, it's possible that the disease has spread between the two. Conversely, if the bacteria found in one outbreak are different from those found in another outbreak, a connection between them can be ruled out. In this manner routes of transmission can be confirmed, or hypotheses developed.

Genetically Homogenous Organism

The task of differentiating between different types of *M. plutonius* is one I've been working on since the start of my PhD. It has proved quite challenging, as *M. plutonius* has been described in the literature as an extremely genetically homogenous organism (Djordjevic et al, 1999) – I'll try to explain what this means. The genetic material of an organism like *M. plutonius* contains all the necessary information to make all the protein building blocks required to construct a bacterial cell. Like us, the genetic information of *M. plutonius* is encoded in material called DNA, which can be seen as a string of letters that contain instructions to make the proteins. Researchers in the past have found that this genetic information is virtually identical in *M. plutonius*, no matter if you are looking at a sample from York or New York! If there are no genetic differences between different lineages of the pathogen, then it is clearly impossible to distinguish between them and we can't hope to use genetics to say whether outbreaks are connected.

DNA Sequencing

It is possible to use molecular biology techniques to look at the sequence of letters that code for an individual protein (this sequence is known as a gene). By comparing these gene sequences between different samples of the same bacterial species, researchers have traditionally found small differences in the DNA that allow different variants to be distinguished. In *M. plutonius* however, when I looked at the genes that are traditionally used to distinguish between types of common bacterial pathogens, I found very little difference between our *M. plutonius* samples. I therefore decided to use a different approach where I used advanced, high-throughput DNA sequencing techniques to obtain the sequences of all two thousand genes present in each of several samples of bacteria taken from around the country. I was then able to use specialist software to compare the sequences that came from the different samples, and look for the very rare genes that were the most variable between isolates.

Using these new, hypervariable DNA sequences, I was able to develop a scheme

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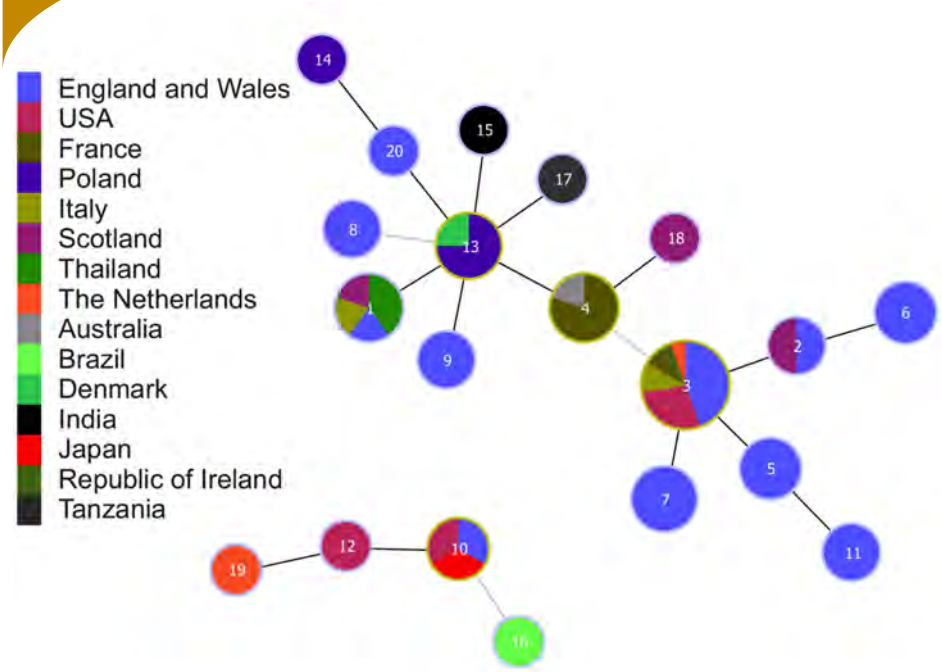

This diagram shows the relationships between the different types of M. plutonius *I've discovered so far.*
Each circle on the diagram represents a different variant of the bacterium (the numbers in the circle are just the names of these different types). Rather like a family tree, the lines between circles link types to their closest relatives. Black lines indicate close relationships, grey lines more distant ones, and circles that are ringed with yellow are thought to be 'founders' of multiple other types. Finally, the colours within each circle show the countries in which that particular type of M. plutonius *has so far been found. The larger cluster of variants all belong to the 'typical'* M. plutonius *group, and the smaller cluster are all 'atypical'.*

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to reliably distinguish between types of *M. plutonius* (Haynes et al, 2013). This has led to some very interesting insights. Firstly, when I compared bacteria from around the world, I found that the distribution of disease variants is not the same in different countries. As the figure shows, lots of varieties were found in England and Wales that were not found elsewhere, and there were also several types that were found in other countries but not in the UK. This is quite different from the earlier literature on *M. plutonius*. Recent work in Japan (Arai et al, 2012) demonstrated that *M. plutonius* could be split into two types: the normal, or 'typical', *M. plutonius*, and an 'atypical' type that was able to grow more easily in the lab and seemed to cause more severe disease symptoms. My genetic technique is able to distinguish between these typical and atypical types, and to identify finer level differences within each. I have also demonstrated that this atypical type is not confined to Japan, as had appeared to be the case, but has been found in the UK, the USA, the Netherlands and Brazil.

Disease Transmission Events

From a more applied point of view, I have also used this scheme to confirm some disease transmission events, and to hypothesise about others. In one case, bees were identified that had been sold from one beekeeper to another 50 km away and in an area free of EFB for 10 years. Both parties developed disease, and when I looked at the *M. plutonius* present I found it was an extremely rare type, only seen in these two samples. This is compelling evidence that the pathogen was sold in a consignment of bees. In another case the situation is much the same, but with different beekeepers. In this instance the distance from seller to buyer was 80 km, and the bacteria in both apiaries was found to be another rare type. A further example of using the scheme to investigate movement involves the rapid appearance of EFB in an apiary after all bees and equipment were imported into the UK from abroad. When investigated, this type was different to anything found before in the UK. It is therefore quite likely that this type was brought into the UK with the imported bees.

Reducing the Burden of EFB

I hope that this tool that I have developed will be useful in reducing the burden of EFB for British Beekeepers. As shown already, it has the potential to allow us to track infections and treat their ultimate source. In the future, with more work, it is likely that these different pathogen types will prove to have different abilities to spread or cause more or less severe disease. Perhaps some particularly persistent types will need a whole apiary shook swarm, or even destruction? Or maybe types that spread less rapidly won't require such a large area to be inspected after they're found. These are questions that I leave for my supervisor Giles Budge to hopefully answer!

Thanks

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References

Arai R, Tominaga K, Wu M, Okura M, Ito K, et al (2012) Diversity of *Melissococcus plutonius* from Honeybee Larvae in Japan and Experimental Reproduction of European Foulbrood with Cultured Atypical Isolates. PLoS ONE 7(3): e33708. doi:10.1371/journal.pone.0033708

Djordjevic SP, Smith LA, Forbes WA and Hornitzky, MA (1999) Geographically diverse Australian isolates of *Melissococcus plutonius* exhibit minimal genotypic diversity by restriction endonuclease analysis. *FEMS Microbiology Letters*, **173**: 311–318. doi: 10.1111/j.1574-6968.1999.tb13519.x

Haynes E, Helgason T, Young JPW, Thwaites R and Budge GE (2013) A typing scheme for the honeybee pathogen *Melissococcus plutonius* allows detection of disease transmission events and a study of the distribution of variants. *Environmental Microbiology Reports*. doi: 10.1111/1758-2229.12057