EFB Research

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EFB melted down larvae. Photo courtesy of The Food and Environment Research Agency (Fera), Crown Copyright; images supplied by the National Bee Unit at Fera.

Four years after starting my PhD at the University of York and the Food and Environment Research Agency (Fera), I have now finished my research and completed my thesis. My project was to investigate the epidemiology of European Foul Brood (EFB) using molecular techniques, and I am pleased to take this opportunity to give an overview of some of my findings.

EFB is an important brood disease of honey bees, and is caused by the bacterial pathogen *Melissococcus plutonius*. The most significant advance I have made over the last four years is the creation of a typing scheme, or a way of distinguishing between genetically different subtypes of *M. plutonius*. This may not sound immediately arresting, but was actually a technically difficult, yet important improvement to our ability to understand the behaviour of this bacterium. *M. plutonius* has traditionally been described as genetically homogenous, with many different authors suggesting that one bacterium was pretty much identical to another. If this is correct, this lack of genetic variation causes a problem for epidemiologists who need diversity to track transmission. Having the genetic fingerprint of a criminal is no good if all criminals share the same fingerprint!

After some initial discussions with my supervisors and some preliminary investigations, the method I settled on for a typing scheme was something called Multi Locus Sequence Typing, or MLST. In many studies this has proved to be a powerful way of distinguishing between variants of lots of different species of bacteria and fungi. This technique looks at the DNA sequences (i.e. the order of As, Ts, Gs and Cs in the genetic code) in several of the pathogen's genes. The genes normally looked at in MLST are those, showing differences, that are involved in essential metabolic processes. However, when I looked at the sequences of these genes in *M. plutonius*, they turned out to be quite uniform, and so they were useless for distinguishing between lineages. To get around this problem, I used cutting-edge sequencing techniques to obtain all the genetic code from several isolates of *M. plutonius*, i.e. the entire genome. These were then compared, using sophisticated computer programs, to look for the very few genes that showed higher levels of variability.

I finally identified four genes that were much more variable than the others, and by sequencing these in a wide variety of samples from around the world we have started to get a clearer picture of the diversity of this pathogen. I have now identified many different types of *M. plutonius*, some of which are distributed widely around the world while others are confined to certain countries. In terms of disease spread, we identified several instances where bees were sold around the UK, after which bees belonging to both the seller and the buyer came down with EFB. Using this MLST scheme we were able to confirm that, in these case studies, both the sellers and buyers had the same, often rare, version of the bacterium, implicating trade in bees in the spread of disease. Over greater distances we have seen evidence of the import of a novel type of *M. plutonius* into the UK from continental Europe, again in a consignment of live bees. Thinking more globally, we recently identified a subtype of *M. plutonius* from Japan both in the UK and the USA, again implying quite extensive movement of some types around the world.

These advances have the potential to alter the way we respond to EFB outbreaks in this country. Not only can we use this as a confirmatory tool when looking at suspected transmission events, but we can use our understanding of which types are found in what parts of the country (and which are not found here at all) to make predictions about where an introduction of disease has come from, and perhaps stop it occurring again. More speculatively, it is likely that different variants of *M. plutonius* have different characteristics, or phenotypes; some may cause more severe disease symptoms, others might be better at persisting after a treatment event. These differences may, one day, lead to different treatment options being enacted for different types.

Closer to home, I visited apiaries around England and collected samples from lots of different places where we thought *Melissococcus* could be lurking, ready to re-infect honey bees. The samples taken included apparently healthy larvae, soil and water samples as well as swabs of various pieces of beekeeping equipment. Additional samples were sent in to me by the Fera Bee Inspectors. All the samples were then taken back to the laboratory and subjected to newly validated techniques for extracting *M. plutonius* DNA. The DNA extracts were then subjected to a very sensitive assay for the presence of a *M. plutonius*-specific DNA sequence. This 'qPCR' test is able to amplify up tiny fragments of target DNA to detectable levels, and then tell us how much pathogen is present. The results showed that *M. plutonius* is frequently found in asymptomatic larvae. This has previously been demonstrated in other studies, but for the first time we were able to follow these symptomless colonies through to confirm the development of disease in the hive. We also found bacteria on a few pieces of equipment, such as smokers or vehicles, which suggests that increased apiary hygiene may be important in some cases. None of our soil or water samples contained detectable *M. plutonius*, which might mean that they are not environmental reservoirs for the pathogen.

These are just a flavour of my results, and I hope to publish more in the future as time allows, so keep an eye on the NBU website. Further developments of the work are likely to include rolling the technique out to a larger dataset, to get more information about the spatial distribution of *M. plutonius* variants, and characterising their different phenotypes. On a personal note, I will be staying at Fera, but moving teams. I have recently been awarded a Research Fellowship in Molecular Epidemiology, to apply some of the same techniques I have learned on *Melissococcus* to foodborne pathogens of humans, such as *Campylobacter* or *Listeria*.

I want to take this final opportunity to thank everyone who has helped me throughout my PhD, including all the beekeepers and Bee Inspectors who have generously given their time and efforts. Special thanks must also go to my supervisors, Dr Thorunn Helgason and Prof J Peter W Young at the University of York and Dr Giles Budge and Dr Richard Thwaites at Fera, as well as Bernard Diaper at BDI, whose help and support have been invaluable.