

American Foul Brood PhD Update



by **Barbara Morrissey**



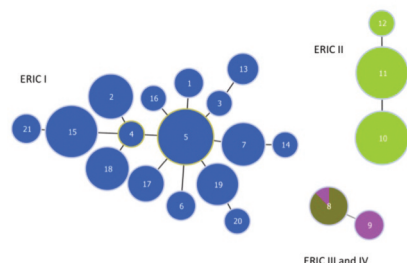
American foul brood (AFB), caused by the spore-forming bacterium, *Paenibacillus larvae*, is a serious and notifiable infection of honey bees. The infection is transmitted to young larvae in food contaminated with spores, which germinate and multiply in the larval gut, and eventually causing the larvae to die of starvation in their sealed cells. Infection spreads easily throughout a colony and all infected colonies will die out. Signs of infection include an uneven or 'Pepper-pot' brood pattern; sunken, greasy or perforated, darkened cell cappings; sticky larval remains that can be drawn out from the cell using a matchstick in rope-like forms; and the appearance of dark scales, which are difficult to remove from cells. You may also notice an unpleasant smell associated with the decaying larvae. More information is available from BeeBase.

If a beekeeper suspects his/her colony has AFB it is his/her legal responsibility to report this to the National Bee Unit (NBU). A NBU inspector will test the suspected larvae and, if AFB is confirmed, issue a Standstill Notice prohibiting removal of bees or equipment from the apiary, to limit disease spread. Because *P. larvae* spores are highly resistant to disinfectants and to extremes of heat and cold, it is a highly persistent infective agent that is managed (but not necessarily eradicated) by destruction of all infected colonies. A number of unique strains of *P. larvae* have been detected across the world, having significant economic, environmental and agricultural impact. Clearly, it is essential to know as much about *P. larvae* strains as possible to better understand how to reduce/prevent spread, and this is the area of research that Barbara Morrissey has been actively investigating, as her report below describes.

I have just started the fourth year of my PhD on the epidemiology of *Paenibacillus larvae*, the causative agent of American foul brood (AFB), split between the University of York and the National Bee Unit at Fera. My work is funded by a BBSRC CASE partnership with Bee Diseases Insurance. I have finished my lab work for the project and am currently spending my time working on analyses and writing my thesis.

One part of my PhD, the new MultiLocus Sequence Typing Scheme (MLST) for *P. larvae*, has been written up and accepted for publication in *Environmental Microbiology*. The paper, titled *Biogeography of Paenibacillus larvae*, the causative agent of American foulbrood, using a new MLST scheme, will be free to access online at: <http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.12625/abstract>.

The paper describes the methods used to create the MLST scheme and how we applied it to test global samples of *P. larvae*. *P. larvae* has already been split into four ERIC (Enterobacterial Repetitive Intergenic Consensus) types using another system but this does not give enough resolution to study disease outbreaks in detail. Briefly, the MLST scheme is used to identify strain types of the bacterium that cause AFB. This is useful for understanding how the disease is spread at a local, as well as a more global level. The scheme is made up of seven genes, which have small DNA sequence differences between strains. We sequence each sample of *P. larvae* seven times, once for each of the genes, and we then look at the combination of DNA sequence



The diagram above shows the 21 different strain types we identified split into groups by ERIC type. The photograph is taken from: *Biogeography of Paenibacillus larvae*, the causative agent of American foulbrood using a new MLST scheme. *Environmental Microbiology*, In Press, Copyright [2014], Wiley Blackwell, Blackwell Publishing.

differences. This gives us the strain type of the bacterium causing the disease.

We have used the MLST to identify over twenty strain types of *P. larvae* using more than 300 samples from 31 different countries. Many of the samples were tested by researchers at the bee research institute in Germany, and others came from the collection at the National Bee Unit at Fera (the Food and Environment Research Agency).

This research has concluded that there were differing global distributions of groups of strain types of AFB. The data showed a correlation between genetic distance and geographic distance of the bacteria in the native range of honey bees. This means that bacteria from countries that are closer together are more genetically similar than bacteria from countries that are further apart. When we looked at samples from outside the native range of honey bees this

pattern disappeared. This may be due to the movement of bees carrying the disease from the native range to new countries that are far away. We also found the same strain types of bacteria caused disease both in the native range of honey bees and outside of the native range. For example, New Zealand had many of the same strain types as the UK. This again suggests movement of the bacteria between these areas.

The MLST scheme is available for other researchers to use at a website developed by Keith Jolley at the University of Oxford (pubmlst.org/plarvae). This website gives all the information a researcher needs to be able to carry out the scheme themselves and we have uploaded the data published in our paper so that it is available to others. We hope that our MLST scheme will be used by others, who will then upload their results so that we can get a clearer picture of this damaging disease and the strains types that cause it.

This summer I attended the Royal Entomological Society conference in York and saw a lot of interesting international speakers. I also presented a poster on my PhD work. In December I will be going to the joint annual meeting of the British Ecological Society and Société Française d'Ecologie in Lille where I will be presenting a talk about my work.

My work is supervised by Dr Thorunn Helgason at the University of York, Dr Giles Budge at Fera (the Food and Environment Research Agency) and Mr Bernard Diaper at BDI (Bee Diseases Insurance). ❀

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