Improving Our Understanding of European Foulbrood (EFB) Transmission

Victoria Tomkies, manager of the National Bee Unit diagnostic laboratory, discusses ongoing research into this notifiable disease

Most honey bee colonies with European foulbrood (EFB) in England and Wales are diagnosed in the field using a lateral flow device, which is a small diagnostic kit that uses technology akin to a pregnancy testing kit. All kits from positive colonies are sent to the National Bee Unit (NBU) laboratory, in York, to determine the genetic makeup of the bacteria causing disease. Understanding the genetic makeup allows us to determine the relatedness between the bacteria causing each outbreak.

The method we use for understanding the genetic relatedness of *Melissococcus plutonius*, the bacterium that causes EFB, is known as multilocus sequence typing (MLST). Similar methods are used to help understand the disease transmission of many human diseases. For *M. plutonius*, the order of individual nucleotides that make up DNA of four genes is determined using a method known as sequencing. The sequence of each gene is compared to a reference database containing the sequences of that gene from all previously sampled *M. plutonius* isolates. Each unique sequence is allocated a number, and the numbers for the four genes represent the MLST type. To date, 35 different MLSTs have been identified worldwide for *M. plutonius*, with 19 of these found in the United Kingdom (UK).

An example of how MLSTs are generated is shown in Table 1. The first DNA sequence obtained from Gene 1 is scored as a 1, the second DNA sequence from Gene 1 is scored as a 2, and so on until the DNA sequence of all four genes is assessed. The pattern shown in Table 1 highlights differences between MLST 1 and MLST 2 generated from differences in Gene 4. Genetic differences can be linked to different clinical presentation in the colony.

MLSTs can be grouped together to form family lineages, known as clonal complexes (CC) (Figure 1). It has been shown that *M. plutonius* from different clonal complexes can share biological properties. For example, some clonal complexes have been shown to cause more severe disease in the field than others, and therefore may be easier to control because clinical symptoms are easier for beekeepers and NBU bee inspectors to see.

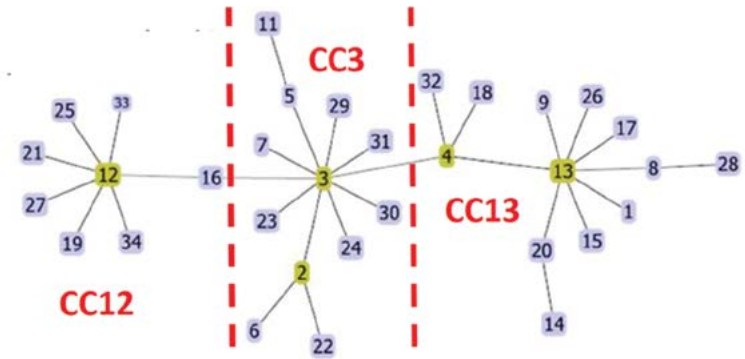
MLST	Gene 1	Gene 2	Gene 3	Gene 4
1	1	1	1	1
2	1	1	1	2
3	1	1	2	2

Table 1. Example of a Multi Locus Sequence Typing (MLST) scheme for an example bacterium. The difference between MLST 1 and MLST 2 is highlighted in red. A further difference between MLST 1 and 2, and MLST 3 is highlighted in green

So why is this information important? The first thing a bee inspector will need to do once discovering EFB is to consider the origin of the disease. Imagine how much easier it would be for a bee inspector if he or she could narrow down the number of potential places from which the disease had been transmitted? Perhaps this would allow him or her to focus inspection efforts in an appropriate area? Or maybe provide evidence as to whether actions are getting on top of a particular outbreak? The NBU operates a risk-based inspection scheme where Beebase data is used to generate inspection lists for inspectors using risk and local outbreak data. However, giving them clues about where to look would both speed things up and potentially get on top of disease more quickly, reducing the ability of EFB to spread. Similarities in the MLST pattern indicate relatedness between the bacteria causing diseases at the sampled sites. In turn, relatedness of bacteria indicate a shared history at some time in the past. NBU bee inspectors can use MLST information to provide important clues about the origin of disease.

Figure 2 shows the EFB positive cases from across England and Wales in 2018. There were 248 cases affecting 148 different apiaries. In the left image (Figure 2a), each positive apiary is represented by an identical red dot, masking any potential differences in bacterial relatedness. Figure 2b shows the same outbreaks on the map, showing the assigned MLSTs. MLST specific information provides more insight into the transmission and pathology

Figure 1. goeBURST diagram using MLST data to show genetic relatedness across three clonal complexes (CC3, CC12 and CC13) occurring in England and Wales. Each circle represents a different MLST. Those MLSTs in yellow indicate 'parent' genotypes from which others have arisen



Budge, et al (2014). *The ISME Journal*; www.pubmlst.org; Nakamura, et al (2016). *Scientific Reports*

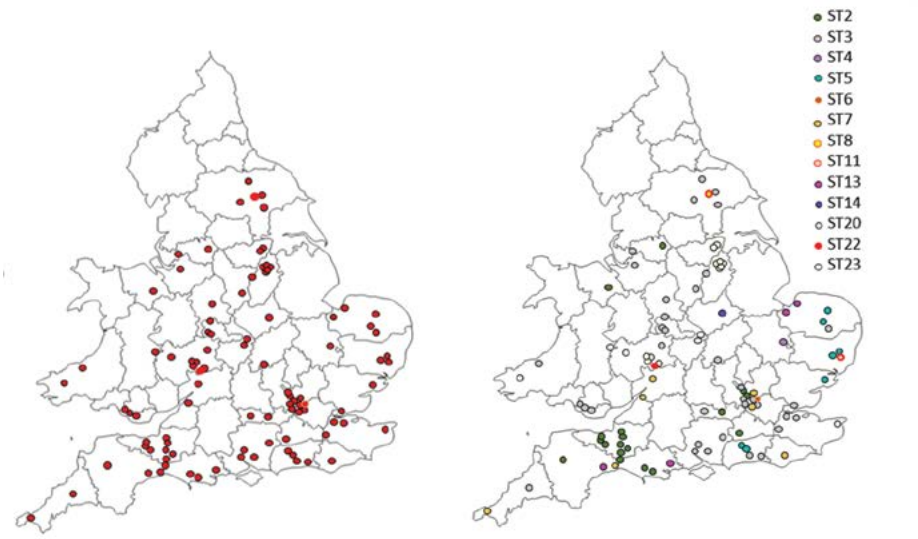


Figure 2. Outbreak locations of EFB in England and Wales in 2018. Figure 2a (left) shows all MLST types, figure 2b (right) shows outbreaks broken down into separate MLSTs

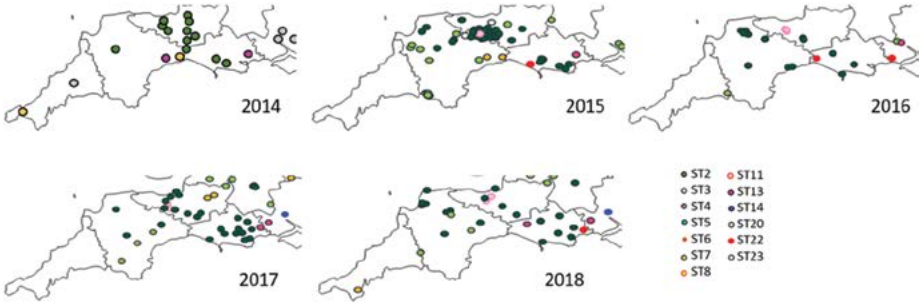


Figure 3. Annual MLST results for EFB outbreaks in south-west England from 2014 to 2018

of the disease, and enables inspectors to answer questions such as whether disease is persistent in an area, or from where disease may have originated.

Figure 3 shows the EFB cases in the south-west of England over five years, 2014 to 2018. There is a local persistent problem with ST2 (green circles) in this region. Is this a particularly hard MLST to get on top of? Or, perhaps there is the local problem from one source? ST2 is only commonly found in this region, with occasional cases in East Anglia. These results enable the bee inspectors to build a regional picture of historic transmission events.

This past season (2019) has been a very busy year for EFB diagnosis in the laboratory. In England and Wales, we have had 479 cases of EFB affecting 359 apiaries. The whole country maps are not available yet, but we have a snapshot of the south-east region of England. This area has seen a lot of EFB over the past few years and has not escaped the high numbers we have seen nationally in 2019.

Figure 4 shows the different STs seen across an area of Greater London in 2019. It shows clustering of specific STs, with ST2 and ST6 clustering in the northern part, and ST7 clustering in the south. ST3 appears in both clusters, suggesting there may be a common link between them. This would be a factor for the local inspector to consider when trying to track the source(s) of infection.

Since 2014, we have been able to build a comprehensive picture of MLST types of EFB in England and Wales. Looking back at Figure 1, MLSTs within CC12 and CC13 are not seen as often in England and Wales, and are generally quite localised, suggesting they have not been present for as long. MLSTs found within CC3 are far more common and have a much wider geographical distribution. Indeed, many of these appear endemic within England and Wales. We are understanding that some MLSTs persist in certain regions (ST2 in the South West, ST13 in East Anglia), some are spread throughout the landscape (ST3 and ST5), and others only appear sporadically from time to time. We have had cases of certain MLSTs being found that had not previously been seen in England and Wales (ST14 in Nottinghamshire and ST20 in Worcestershire) and were able to advise the local bee inspector about possible routes of transmission.

We are beginning to use these data to help us understand how MLST types respond to treatment by monitoring specific treatment outcomes. For example, observations indicated that ST5 seemed resistant to treatment using the (now rarely used) antibiotic oxytetracycline (OTC); laboratory experimental work has confirmed this.

The combination of scientific understanding of the genetic relatedness of *M. plutonius* and the expertise of bee inspectors on the ground gives us multiple weapons in the fight against EFB. These data are helping us to build a picture of EFB disease at the landscape level, more rapidly identify disease sources and remove some of the historic mystery about how EFB spreads. Future links between MLST type and treatment efficacy will help control disease more rapidly, which can only be good news for beekeeping in England and Wales. □

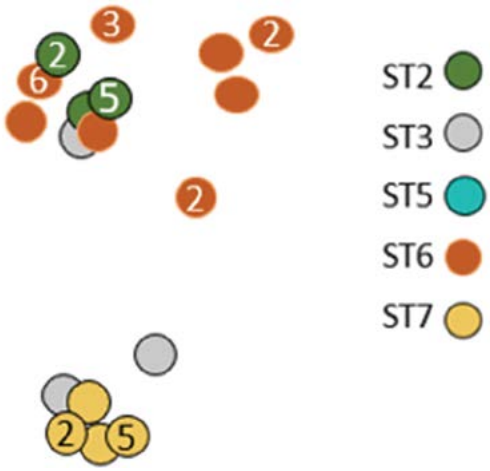


Figure 4. Map showing the distribution of EFB positive cases across Greater London in 2019. Numbers within circles represent the number of positive colonies at that location

References

Budge, et al (2014). Molecular epidemiology and population structure of the honey bee brood pathogen *Melissococcus plutonius*. *The ISME Journal*, **8**(8), 1588–1597. doi: 10.1038/ismej.2014.20

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