

A typing scheme for *Melissococcus plutonius*, the causative agent of European foulbrood of honey bees (*Apis mellifera*)

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Introduction

European foulbrood (EFB) is a larval disease of the honey bee, *Apis mellifera*. It is the most prevalent bacterial disease of honey bees in the UK, and is found on every continent where honey bees are kept. EFB is caused by the gram-positive bacterium *Melissococcus plutonius*, which typically infects 4-5 day old larvae in early summer. Sick larvae first become displaced in their cells, a process usually followed swiftly by larval death, discolouration and decomposition (Figure 1). Aside from such basic symptomatological observations, surprisingly little is known about how *M. plutonius* spreads between apiaries at a landscape level and details of disease pathology are poorly documented. Major advances in our understanding of these processes and how to impede them, will come from creating typing schemes to identify strains of *M. plutonius*. Identifying these genetic differences will allow pathogen spread to be tracked.

Methods

We developed a Multi Locus Sequence Typing (MLST) scheme to describe DNA sequence variation of internal fragments of multiple genes responsible for essential metabolic functions in the bacterium. Each gene represents a different locus and each different sequence at a particular locus represents a unique allele. The allelic profile across all loci then gives an isolate its individual sequence type (ST), which is assigned a unique numerical identifier.

Results and Conclusions

- An MLST scheme was successfully developed that was able to distinguish 24 strains from UK and international isolates (Figure 2).
- The type strain (ST1), first isolated decades ago in the UK, is still present in the wild in several countries (Figure 2).
- The UK appears to contain many genetically different types of *M. plutonius*.
- The "Atypical" *M. plutonius* type, recently identified in Japan, was separated into five STs (10, 13, 14, 21 and 24) and found on three additional continents (Figure 2).
- We have shown that genetic markers can be used to identify different strains of *M. plutonius*. Strains will likely demonstrate differences in pathology and, in turn, may differ in their response to the available treatments.
- Phylogenetic and population genetic inferences can potentially track infections in epidemiological studies.



Figure 1. Top: healthy brood, attended by adult nurse bees. Note larvae's pearly white colouration and plump appearance. Bottom: EFB-infected brood. Larvae appear sunken and displaced in cells. They are discoloured, with a 'melted' texture

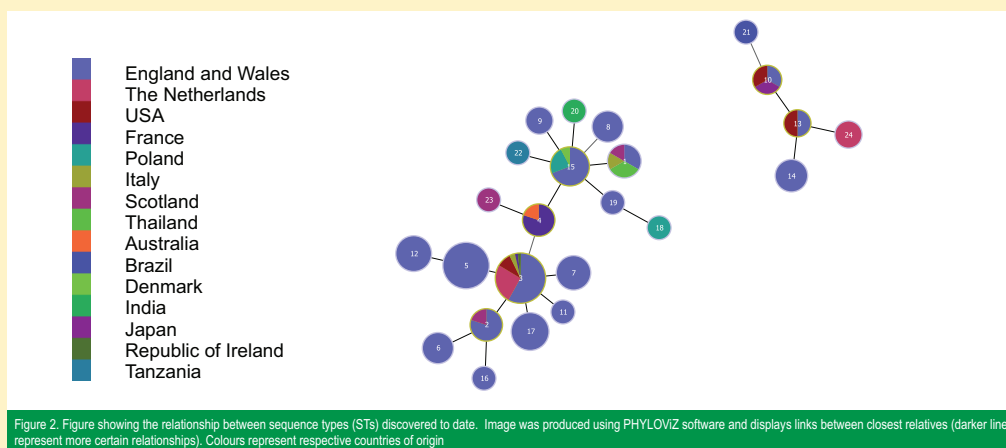


Figure 2. Figure showing the relationship between sequence types (STs) discovered to date. Image was produced using PHYLOVIZ software and displays links between closest relatives (darker lines represent more certain relationships). Colours represent respective countries of origin

Acknowledgements

