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Maximising apicultural investments to secure the future of honey bee research

By Giles Budge, National Bee Unit, Fera and Newcastle University.

The funding landscape for honey bee research used to be a bleak place containing very few opportunities to secure money to enable science to help apiculture. The knock-on effects were that there were very few organisations with the necessary understanding of apiculture or with the will to direct their intellectual capital towards honey bee research, leading to precious little relevant discovery science capable of solving the problems we face in apiculture.

More recent times have seen a growth in the number of opportunities, in part because beekeepers and beekeeping-related businesses have invested monies to fund honey bee research. Additional funding from other sources presented a purple patch from 2009–2014, with honey bee research doing well out of schemes like the Insect Pollinators Initiative in which four of nine projects were largely honey bee focussed. But times are becoming leaner as the focus of the funders shifts from bee health to tree health and on to innovation in agriculture. Yet beekeepers and related businesses continue to invest, making their contribution incredibly valuable as other funding pots become harder to access.

We all know how hard it is to earn or accrue money and how many ways there are to spend it! I am a firm believer that we should maximise the current investment in apiculture by finding schemes that allow us to use this precious resource to leverage monies from other pots. I am also convinced that encouraging more researchers in universities and private companies to have a background in apiculture or pollinator science is important for the long-term sustainability of apiculture. Here I present a scheme that I think works well to accomplish both these aims, and provide two completed case studies that show how apiculture has benefitted.

BBSRC iCASE PhD Studentships

Put simply a PhD is a three- or four-year post-graduate training programme that takes an individual graduate and immerses them in a single subject area, providing them with the necessary training, support and nurturing to produce a thesis encapsulating the fruits of their scientific labours. At the end of this process a good student will be able to conduct their own independent research, initially perhaps as a postdoc or researcher in a company, eventually perhaps running their own laboratory in a university. Training students costs money: PhD students have a salary or stipend; university supervision and registration costs need to be paid; and most experiments benefit from having money to spend on consumable items. The total cost varies somewhere between £70k and £100k depending on the scheme and duration of the studentship.

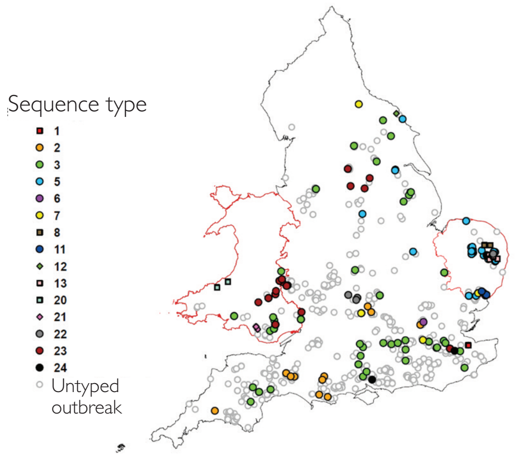
A BBSRC iCase studentship is different from a standard studentship (note: iCASE studentships are also available from other research councils!). The "i" stands for industrial, because this is a project conducted with an industrial partner. The industry partner funds the project at £4,000 per year for four years, £16,000 in total, hosts the student for a few months to help them understand the business area and agrees the subject area of the studentship. In return the student gets a higher monthly stipend, a larger consumables budget and a four-year rather than a three-year PhD. In my experience the top quality students go for these awards and the close working partnership with industry means any outputs are more likely to have positive impacts. I had the privilege of supervising two such studentships after generous industrial sponsorship from Bee Diseases Insurance Ltd (BDI). The £16,000 investment from BDI led to securing the cost of the studentship from BBSRC which is £96,000 and Defra kindly agreed to cover my time supervising, equating to seven times the return on the initial investment from BDI.

The Epidemiology of European Foulbrood

This studentship was won by Edward Haynes, a graduate from Oxford University, and was supervised by Dr Thorunn Helgason from the University of York and I. Ed shared my frustration at not being able to fully understand the epidemiology of European Foulbrood (EFB) caused by the bacterium *Melissococcus plutonius*.

He was interested in developing a scheme that might highlight the relationships between EFB outbreaks to help Bee Inspectors from the National Bee Unit (NBU) and beekeepers appreciate how this damaging disease was moving around our country. Ed turned to a technology known as multi locus sequence typing (MLST) which had been used widely in the area of human medicine to track bacterial infections. This method identifies regions of the heritable genetic code of *M. plutonius* that vary between isolates and uses these data to highlight relationships between outbreaks. The journey was long and arduous, other professors internationally recognised for their work on honey bee pathology, had tried and failed to develop such a scheme. After much hard work, Ed was successful and his methods published.[2] We then made the method available to all international researchers on the pubmlst website (pubmlst.org) hosted by Dr Keith Jolley at Oxford University.[4] Key impacts from this studentship were:

Ed Haynes.



Map highlighting the diversity of M. plutonius across England and Wales from 2009-2011 (from Budge et al 2014).[1]

- ♦ The scheme was used to demonstrate that there are at least fifteen different sequence types (STs) of *M. plutonius* circulating in England and Wales and many are highly local.[1]
- ♦ For the last three years, the NBU has obtained the ST from nearly every outbreak of EFB in England and Wales, helping to track and trace infections. Some infections have come from national trade in honey bees, others from international honey bee movements.
- ♦ These data suggest local outbreaks can be eradicated and disease persistence over several seasons can be a result of a new type entering an area.
- ♦ Internationally, researchers have used the scheme to type *M. plutonius* isolates from across the world, including Asia, Europe, North and South America.
- ♦ Dr Ed Haynes now works as a Food Safety Authority Fellow at Fera tracking human infections using whole genome sequencing methods.

The Epidemiology of American Foulbrood

This studentship was won by Barbara Morrissey after impressing us with a great Master's thesis and was again supervised by Dr Thorunn Helgason from the University of York and I. Barbara followed in Ed's footsteps using similar methods to develop an MLST scheme for the causative agent of American foulbrood (AFB), the bacterium *Paenibacillus larvae*. Her methods were published[3] and provided new insights into the epidemiology of this damaging honey bee disease, including:

Barbara Morrissey.

- ♦ AFB outbreaks in the UK, including Northern Ireland and Scotland, are caused by between four and six different types of *P. larvae* with local clusters being highly related.
- ♦ Despite millennia of honey bee movements, the MLST data indicate that structure exists in populations of *P. larvae* only within the native range. This suggests that strains may be adapted to infect local honey bees.
- ♦ The large AFB outbreak in Jersey was caused by multiple STs, suggesting the pathogen had arrived on the island several times, not just once as previously thought.
- ♦ Some locations with persistent AFB contain different types of *P. larvae* over time suggesting a high risk of reinvasion.
- ♦ Dr Barbara Morrissey now works as a Molecular Biologist for The Rivers and Lochs Institute in Inverness.

A word from Mike Brown – Head of the National Bee Unit

The BDI-sponsored studentships have made an unbelievable difference to our understanding of both foulbrood diseases. Before embarking on the studentships little did we imagine the level of diversity and complexity in these bacterial infections, nor the potential scope and significance of the findings. A really brilliant example of the practical appliance of science.

I hope you share my enthusiasm for these studentships and thank the BBSRC and Bee Diseases Insurance Ltd, in particular Bernard Diaper, for all their support over the years. I look forward to seeing the new crop of PhD students develop and discover new knowledge, maximising the investment from apiculture to benefit honey bee health. ■

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Factors affecting European Foulbrood virulence

Nicola Burns, University of York and National Bee Unit, Fera.

My current PhD title is 'The genetic and environmental factors affecting virulence of European Foulbrood (EFB), a bacterial pathogen of honey bees', a four-year project funded by the BBSRC and by Bee Diseases Insurance (BDI) through an industrial CASE partnership with Fera. I am co-supervised by Dr Thorunn Helgason and Dr Ville Friman at University of York and by Dr Giles Budge at Fera.

My scientific background

I completed my undergraduate degree in Biology (BSc) at the University of York in 2014. My interest in microbiology started during a summer studentship before my final year, working with *Pseudomonas aeruginosa*. I learnt all my basic microbiology knowledge while undertaking this project, and gained confidence in a laboratory environment. This project resulted in a publication 'Polysogeny magnifies competitiveness of a bacterial pathogen *in vivo*'.[1]

My third year project 'Sequencing and comparison of *Paenibacillus larvae*, American Foulbrood' set off my interest in

Nicola Burns.

bee disease and health, and gave me skills in genetic techniques and bioinformatics. I was also able to visit the Food and Environment Research Agency (Fera) during this project which I found exciting as I had never experienced work in a Government laboratory.

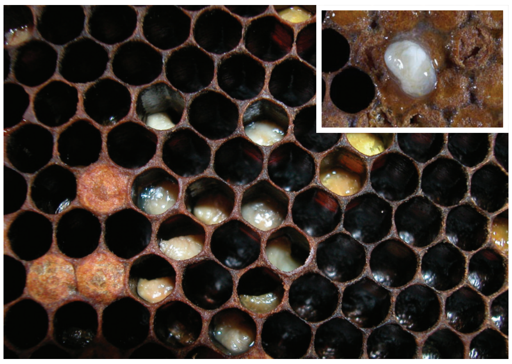
After my degree, I worked at The University of Sheffield as research technician for a year-and-a-half on a project entitled 'Population dynamics of *Staphylococcus aureus* infections'. During my employment, I was able to learn novel techniques, such as making microinjections into zebrafish. It was during this time that I knew I wanted to start a PhD and continue to work on honey bee disease. I successfully applied for my current project, which began in October 2016.

My PhD project

As Dr Giles Budge has described in the predeeding article, European Foulbrood (EFB) is a disease of honey bee, *Apis mellifera*, larvae. It is caused by the bacterium, *Melisococcus plutonius*, infecting the larval gut and killing the larva at four to five days old. EFB disease is distributed worldwide, and it impacts on agricultural services and honey production. Infections can spread through contaminated equipment used by beekeepers or through natural movement of bees between hives, such as during swarming or honey robbing.

There are three possible treatments for EFB. The first is shook swarm, transferring adult bees to a new hive containing new foundation and destroying the old combs. The second is treatment with an antibiotic, oxytetracycline, something rarely used these days. The third is colony destruction by burning.

Previous research on EFB suggested that there is very little genetic difference between strains of *M. plutonius* but more recent research has suggested that this may not be true with typical and 'atypical' types of bacteria being discovered. Multi-locus sequence typing (MLST) characterises bacteria isolated from individual outbreaks into sequence types (STs). The MLST scheme for EFB[2] was developed by Dr Edward Haynes during his PhD, which was also funded with help from BDI. It is useful to characterise bacterial isolates into STs because genetic relationships can result from common movements telling us something about how EFB may have spread between apiaries. However, MLST schemes



Main photo: comb containing EFB-infected larvae. Inset: a higher power image of a larva infected with EFB. Note the whitish yellow colour of the infected larval gut. Photos courtesy of Nicola Burns.

are based on core 'housekeeping' genes that will not inform us of the physical characteristics of isolates. To see if there are genetic differences that explain this variation I will need to carry out comparative genomics techniques, comparing features and changes across all 2,000 bacterial genes. I am looking forward to meeting many of you and discussing my results, when I have some!

Funding and Support

This research is supported by the BBSRC and Bee Diseases Insurance Ltd, and it is conducted at the University of York and the National Bee Unit at Fera. ■

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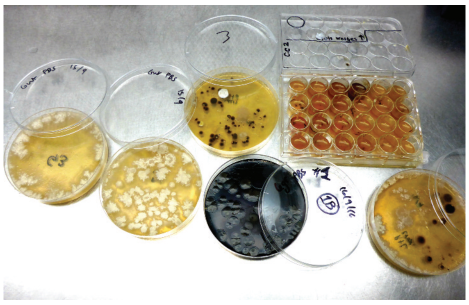
Friend, foe or free rider: An uncertain honey bee symbiont

Georgia Drew, University of Liverpool and National Bee Unit, Fera.

Worker bees ready for an experiment. All photos by Georgia Drew.



Growing bacteria from bees: attempts to grow Arsenophonus bacteria under different conditions.

A genus of bacteria, known as *Arsenophonus*, has recently been identified in honey bee colonies of poor health,[1] including those affected by the unresolved colony collapse disorder.[2] *Arsenophonus* bacteria infect many different insect species, and are capable of forming diverse symbioses with their insect partners. These bacteria can range from parasites that disrupt the reproduction of an insect, right through to symbionts that manufacture vital nutrients for their insect host.[3] Yet the nature of the *Arsenophonus* and honey bee interaction remains relatively unknown, despite the association of infection with poor colony health. To begin characterising this interaction, we have been screening managed honey bee colonies across the UK for the past three years. Here, we report some of our findings to date.

Arsenophonus bacteria are widespread in honey bees

Arsenophonus bacteria appear to be common in UK honey bees, being present in 58% of the 130 colonies tested. This presence does not appear to be limited by geography, with infected bees found in ten different counties. *Arsenophonus* bacteria probably boast a global distribution in bees, with their presence also being reported by researchers in America, Israel and Switzerland.

Arsenophonus in honey bees is distinct from previously studied strains

The genome of the *Arsenophonus* strain recovered from honey bees shows notable similarity to another strain of *Arsenophonus*, known as *A. nasoniae*. This strain infects parasitoid jewel wasps and can be vertically transmitted from mother to offspring. It has a powerful effect, manipulating the wasp's reproduction to kill 80% of male offspring. By extraction and amplification of DNA from the eggs, larvae and pupae of *Arsenophonus*-infected honey bee colonies, we have established that *Arsenophonus* is not vertically transmitted in honey bees and does not appear to induce the killing of male offspring. These notable differences between the two *Arsenophonus* strains are interesting in the light of their highly similar genomes.

Body tissues affected by *Arsenophonus*

To begin uncovering potential pathological effects of *Arsenophonus*, we are dissecting and testing different honey bee tissues. We are working to elucidate if the bacteria target specific organs or tissues, or if infection disseminates throughout the bee. Using fluorescence *in situ* hybridisation (FISH) analysis we can section 3μm slices from bee specimens and incubate these with fluorescent probes specific to *Arsenophonus* bacteria. This is followed by microscopy to visualise the probe locations in the honey bee. We are hoping to localise the bacteria in honey bee tissues at a high resolution, which will provide us with a detailed characterisation of the possible sites of action of the bacteria.

Arsenophonus in British solitary bees

Among other bee genera *Arsenophonus* have also been identified in solitary Colletes bees in Germany.[4] The life of a solitary Colletes bee contrasts with that of the eusocial honey bee and its complex colony structure and cooperative care. These differences in lifestyle will undoubtedly present *Arsenophonus* bacteria with different challenges on invasion of its host and will affect the transmission opportunities available to the bacteria.

On a new project at the University of Liverpool we are also working to survey solitary bee species for *Arsenophonus*. We hope this will improve our understanding of potential transmission of bacteria between wild bees and managed honey bees.

Funding and Support

We would like to thank the many beekeepers that have generously donated their time and samples, to this project.

This research is supported by the BBSRC and Bee Diseases Insurance Ltd, and it is conducted at the University of Liverpool and the National Bee Unit at Fera. It is supervised by Prof Greg Hurst and Dr Giles Budge. ■

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