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In this issue:
A selection of BDI-funded or part-funded research projects.

The effect of dietary pollen on the health and behaviour of honey bees

Ben Jones, National Bee Unit, Fera and University of Exeter.

The development and maintenance of honey bee colonies depend on adequate nutrition. To maintain healthy honey bee colonies it is essential that they receive sufficient nutrition of the correct type (nutritional value) so as to promote their continuous survival. Previous trials suggest that the addition of pollen to the diet of adult honey bees could enhance immunity, suggesting a link between nutrition and immune function. However, the impact of the components of dietary pollen on the honey bee immune system remain poorly understood. My PhD aims to investigate this knowledge gap. It is jointly supervised by Dr James Cresswell at the University of Exeter and Dr Giles Budge at Fera.

In 2012, the first year of my PhD, my work focussed on the development and optimization of the research tools and methods I would need for my studies. The bulk of experimental work was focused on investigating the dynamics of the immune system of honey bees in order to establish a framework to work within. I was able to establish the temporal dynamics of three immune responses in honey bees: antimicrobial peptide activity (AMPs), activity of glucose oxidase (GOX) and phenoloxidase activity (PO).

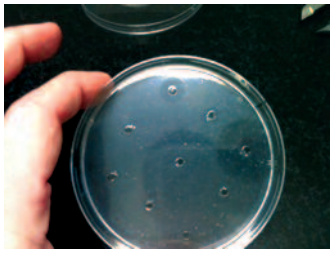

Fig 1. Antimicrobial activity of haemolymph in Apis mellifera. *Injection with lipopolysaccharides (LPS) significantly upregulated AMPs over time compared to placebo controls (injection with a solution devoid of LPS or un-injected controls), $p<0.05$. The 'clear' zones represent bacterial clearance activity of antimicrobial peptides in the bees' haemolymph.*

In 2013 I ran laboratory-based trails to investigate how pollen availability impacts the immune system in honey bees challenged with an immune elicitor (lipopolysaccharides or LPS). In order to gain a comprehensive understanding of the immune response, multiple parameters of immunity at both the personal (PO and AMP activity) and social level (GOX activity) were measured over two time points in cohorts of caged honey bees that were provided with pollen or pollen starved.

My trials in 2014 were aimed at investigating the role of essential amino acids in immune function. I spent the entire 2014 season undertaking numerous laboratory trials to investigate how honey bees regulate their uptake of essential amino acids and any health benefits incurred from uptake of dietary essential amino acids.

Fig 2. Caged honey bees are provided with ad lib access to both mixed pollen and syrup. Cages are kept in constant darkness at 36°C and 60% humidity to mimic colony conditions.

Throughout 2015 I ran field trials to show how the behaviour observed in my feeding trials translated into realistic colony conditions. Using radio-frequency tagged bees I obtained data on over 10000 foraging flights. The data will now be analysed to better understand the links between immunity, feeding behaviour and foraging activity. I intend to submit my results for publication in scientific and beekeeping journals and continue to enjoy researching this fascinating field. My research will help inform beekeepers how the diet of honey bees affects the health of their colonies. ■

Acknowledgements

I would like to thank BDI, SWCJCC and the beekeeping associations as my research has only been made possible by the generous funding received from you.

Background reading

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The unknown role of a symbiont in honey bee health

By Georgia Drew, University of Liverpool and the National Bee Unit.

Pathogen or symbiont?

The interactions between honey bees and bacteria are diverse and complicated, involving a plethora of pathogens and symbionts. While all of these bacteria influence the health of honey bees, it can be difficult to differentiate ally from enemy. A symbiotic bacterium, of the genus *Arsenophonus*, has previously been identified in honey bee colonies of smaller size and generally poorer health (Giles Budge, pers.comm.), yet its true role remains unknown. *Arsenophonus* comprises an intriguing group of bacteria, capable of infecting a diverse range of insects. Species of *Arsenophonus* can act as beneficial vitamin providers right through to parasitic male killers to their obliging insect hosts. These bacteria have been identified in honey bees across the globe, and in a US study *Arsenophonus* was found to increase in hives affected by colony collapse disorder.[1] Despite this, the role of *Arsenophonus* in honey bee colonies remains uncharacterised.

Assessing the prevalence and transmission of *Arsenophonus* in UK honey bees

We have been assessing the current infection status of UK honey bees by setting up a DNA typing scheme and screening colonies from across the UK. This typing scheme focuses on the DNA sequences of six core bacterial genes in order to accurately identify the microbe and determine if there are multiple strains of the bacteria circulating in honey bees. Of the 96 colonies screened, 52 were identified as *Arsenophonus* positive and exist as one major strain type (Table 1). This gives *Arsenophonus* spp. a prevalence of 54% among managed UK honey bee colonies (95% confidence intervals 44–64%) .

Location	Total colonies screened	*Arsenophonus*+ colonies
N Yorkshire (FERA colonies)	47	30
Exeter	25	11
Liverpool	6	0
Gr Manchester	10	4
Lancashire	1	1
Cheshire	2	1
Derbyshire	1	1
W Yorkshire	1	1
Other	3	3
Total	96	52

Table 1. Arsenophonus *spp. positive colonies found within each sampling area, based on PCR detection.*

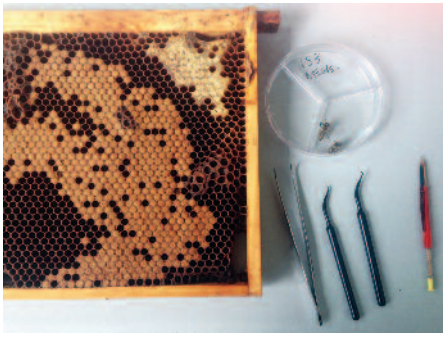
Despite this high prevalence of *Arsenophonus* in UK honey bees, the bacteria are not commonplace in other bee species. Extensive screening programmes have revealed only solitary Colletes bees and the Alfalfa leafcutter bee to be infected with these bacteria,[2,3] posing some interesting questions regarding host specificity and transmission.

Sample	n	*Arsenophonus* prevalence (%)
Queen	2	0
Workers	35	86
Drone	17	47
Newly emerged workers	34	6
Pupae	23	0
Larvae	35	0
Eggs	9	0
Pollen	3	0
Honey	3	0

Table 2. Arsenophonus *prevalence in colony components. Samples of caste members and different life stages, and hive components were tested. All samples were taken from colonies that had previously tested positive for* Arsenophonus, *based on sampling of worker bees within the same month.*

In other insect hosts closely related symbionts are often vertically transmitted via eggs, but horizontal transmission of *Arsenophonus* from the environment or other insects is also possible. Based on our preliminary tests *Arsenophonus*

Newly emerged worker bees in the lab. All photos courtesy of Georgina Drew.



Collecting samples for Arsenophonus testing.

appears far more abundant in adult members of the colony, commonly being found in both workers and drones (Table 2). This strongly suggests against maternal transmission from the queen, and allows us to investigate alternative routes. Determining the transmission route is pivotal as it will help us to adjust bee husbandry accordingly in order to prevent or encourage the bacteria.

Future plans

Over the coming months we will continue to study colonies infected by *Arsenophonus*, monitoring the presence of the bacteria and looking for effects on■ colony health. Alongside this we hope to determine the honey bee organs and tissue infected by *Arsenophonus* and investigate the transmission of these intriguing bacteria between colony members.

Acknowledgements

This work is split between the University of Liverpool,and the National Bee Unit, Fera. It is supervised by Greg Hurst (UoL), Giles Budge (Fera), Alistair Darby (UoL), and supported by the BBSRC and Bee Diseases Insurance Ltd. Thank you to all beekeepers who donated samples. Please contact g.drew@liverpool.ac.uk if you are interested in contributing samples to this research.

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RiViVe: Rolling out the evolution of resistance to varroa and DWV

By Jessica Kevill, a new postgraduate researcher at the University of Salford. Jessica is working on the RiViVe (Rolling out the evolution of resistance to varroa and DWV) project, funded by the BDI and the CB Dennis trust. The research aims to look at interactions between *Varroa destructor*, Deformed wing virus (DWV) and European honey bees. This UK-wide research initiative will provide data on DWV viral emergence, seasonal changes in virus populations and will give an indication of causes of overwinter mortalities in the UK.

I studied at the University of Salford for five years and am in the first year of my PhD. My previous studies concentrated on wildlife conservation and, as a beekeeper, I knew I wanted to stay on at university and pursue a career in pollinator conservation. I conduct a lot of work within the Salford community, helping people access beekeeping and bee products, who would normally not have the opportunity to be educated in this field. I have been a beekeeper for five years and have an in-depth understanding of the plight facing our bees. The research conducted as part of my PhD will be funded by the ReViVe project and I will be working under Professor Stephen Martin's supervision. I will be studying mechanisms for resistance in honey bees which have been left untreated for varroa, as well as understanding the relationship between DWV, varroa, honey bees and over-wintering losses in the UK. The goal of this research is to help understand the current status of our bees, how we can help contribute to their resistance and what actions we can take in the future to limit on-going chemical treatments within our apiaries. Since starting my PhD in October I have met with many beekeepers from all over the UK and have collected lots of samples for analysis. My first goal is to ascertain which strains of DWV infect which colonies, in which areas. Colony losses will be recorded and these samples analysed in more detail to determine which DWV strains are lethal and if any patterns of emergence occur. From here, I will look at the mechanisms for resistance in the untreated colonies which have survived for more than four years. This research is very exciting and will help provide answers and contribute to scientific knowledge.

The problem

Over the winter of 2014–2015, almost twice (17.5%, over 31 countries) the number of European honey bee colonies perished compared to previous years (COLOSS, 2015). The reasons for these declines is unclear, however there is evidence to support DWV and the effects of long-term varroa infestation as being causative. DWV has three known master variants, group types A, B and C, each of which has varying effects upon colony health. Variant type A is considered to be the most lethal, type B has been linked to colony survivorship via super-infection exclusion and type C is relatively unknown as it has only recently been discovered.

Work completed so far

The work conducted by myself over the last few months, has concentrated on creating a method of detection for types A, B and C using molecular biology techniques. This has been developed using a new primer set of short nucleic acid sequences, which synthesise RNA. The primers are specific to each type of DWV and build upon specific viral RNA fragments found in honey bee samples. The use of a QPCR machine (Rotor gene 6000) allows for the quantification of viral particles and for a distinction to be made between a chronic or acute infection, as well as detecting which types of virus are present in a colony of bees.

The samples

Honey bee samples will be provided from funding associations across the UK at different times of the year. These samples will then be tested for each variant of DWV and quantified. This will allow me to ascertain the seasonal changes between viral loads and types of DWV across the UK. It is predicted that as varroa become phoretic over the winter period, DWV loads will increase, in some cases causing colony collapse from the effects of varroa feeding but also from viral infection. Varroa is the perfect vector for honey bee pathogens, as European honey bees have not developed resistant traits, such as varroa sensitive hygiene and grooming behaviours. Varroa feed on the hemolymph of bees. When they feed on an infected bee, virus particles enter the varroa mite and this may then feed on another bee. In this way the virus is spread throughout the colony. Whether DWV accumulates in the gut or replicates in the body tissue of varroa is disputed. Either way varroa cause an increase in viral particulate in bees. DWV was here in the UK before varroa although virus loads were low; it is varroa and DWV combined which seem to cause overwinter losses in the UK.

The future

This study aims to provide a clearer picture as to the cause of honey bee losses and the effects of DWV in overwintering colonies. The role of super-infection exclusion in the safeguarding of seemingly resistant colonies will also be investigated, as well as the impacts of DWV type C. Furthermore, feral and managed colonies persisting without varroa treatment will be tested for DWV variants and conclusions upon their longevity will be made. This is a very exciting research project to be involved in and I will keep you posted with my results. ■

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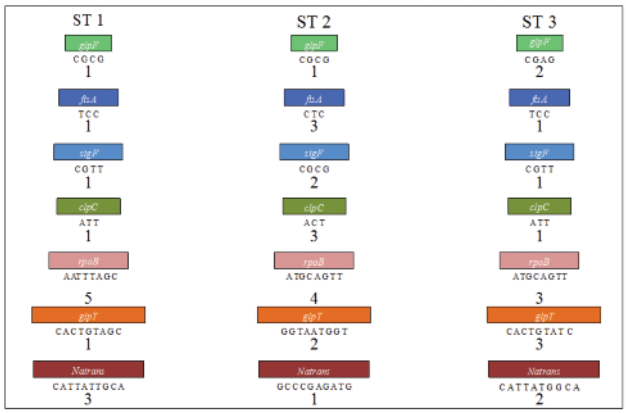
The epidemiology of *Paenibacillus larvae*

By Barbara Morrissey, who has recently completed her PhD on the epidemiology of *Paenibacillus larvae*, the causative agent of American foul brood. Barbara's time has been split between the University of York and the National Bee Unit at Fera. Her work is funded by a BBSRC CASE partnership with Bee Diseases Insurance.

American foul brood (AFB) is an extremely damaging disease of honey bees which, if left untreated will lead to the death of the colony. It is found worldwide everywhere that honey bees are kept. It is under statutory control in the UK and infected colonies must be destroyed. My work focussed on the creation of a typing scheme to differentiate among strain types of the bacteria causing disease and looking at the genetic differences between these strain types.

The first of my chapters was published in *Environmental Microbiology* (Morrissey *et al.*, 2015) and described the development of a MultiLocus Sequence Typing scheme (MLST) to differentiate among strain types of *P. larvae*. MLST is based on small DNA sequence differences in a number of essential genes between strain types. Sequencing these genes shows the base differences (or alleles) and the combination of alleles belonging to the isolate tells you which sequence type (ST) the bacterial isolate belongs to.



This diagram shows the sequence and allele difference between esch ST. Each box represents one gene that was sequenced for each of the three shown isolates. The number under each box is the allele number, allocated based on the DNA sequence differences (letters). The combination of alleles gives the ST.

The scheme was created in collaboration with researchers at the bee research institute in Germany. In the first PhD thesis chapter the scheme was used to look at the global patterns of distribution of *P. larvae* STs. This was the first time that *P. larvae* distribution could be described in such detail. Using the scheme we have identified over twenty STs from over three hundred isolates tested. The STs were found to vary in their distribution, some were found on nearly every continent, others have been found in only a few sites.

We also found that *P. larvae* had a significant population structure within the native range of the honey bee (*Apis mellifera*). This pattern broke down when we looked outside of the native range. This meant that populations of *P. larvae* that were closer together were genetically more similar than those that were further apart.

The MLST scheme formed the basis for the rest of my thesis and I used it for the research described in each of the next chapters.

In the second data chapter I used the MLST scheme to describe the distribution of STs causing disease in the UK. In a recent paper by Mill *et al.* (2013) AFB was found to occur in disease clusters in the UK. These clusters were found to recur for a few years before being eradicated by the inspection regime. However, some clusters were more persistent than others. I used the MLST scheme to identify the STs of *P. larvae* causing these disease outbreaks for the first time and was able to identify whether clusters were composed of a single ST, which would indicate a single infection event and local spread, or if clusters were composed of multiple STs, which would suggest multiple infection events.

The MLST scheme was used to choose diverse *P. larvae* isolates to genome sequence. Previous schemes split *P. larvae* into four groups so the new MLST scheme, with over 20 types, allows us to study the genetic differences within and between strain types in more detail. Genome sequencing captures the complete DNA sequence of an organism. The *P. larvae* genome is between four and five million bases long. By comparing the genome sequences I was able to find some genetic difference between strains and it is possible that these differences may affect the behaviour of these strain types. Further work will have to be done to determine whether this is true.

The *P. larvae* MLST scheme is available online at pubmlst.org/plarvae. Hopefully, other researchers will add their results to the database and we will get a clearer picture of the global distribution and spread of this damaging and costly disease.

Acknowledgements

My work was 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 Disease Insurance). The project is funded by a BBSRC CASE partnership with BDI. ■

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Integrated control of honey bee diseases

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Pests and diseases are the greatest current challenge faced by honey bees and beekeeping. Hasan Al Toufailia began his PhD on Integrated Control of Honey Bee Diseases in Apiculture at LASI, the Laboratory of Apiculture and Social Insects at the University of Sussex, under the supervision of Professor Francis Ratnieks in April 2012 and submitted his thesis on 18 April 2016. Hasan is from Syria, where he is a lecturer at the University of Damascus. The University of Damascus funded him to take his Masters and Doctoral degrees at LASI, so that he could become Syria's national honey bee expert. BDI (Bee Diseases Insurance) provided additional support for Hasan during the writing up of his PhD thesis. Hasan's research comprises eleven projects in two main areas, varroa control and hygienic behaviour.

Hasan uncapping cells in frozen comb to count the numbers of varroa mites in worker and drone cells.

Over the past six years LASI has been carrying out a wide-ranging project called the Sussex Plan for Honey Bee Health & Well Being. The Sussex Plan is focused on the two main challenges faced by honey bees and beekeeping, pests and diseases, and food supply (flowers). An important feature of the Sussex Plan is that it is focused on research of practical significance with many research topics determined in consultation with the BBKA and other beekeepers. We decided to focus Hasan's PhD on two important honey bee pests and diseases (varroa and deformed wing virus) and four methods of disease control: hygienic behaviour, oxalic acid, Apistan and drone trapping. Hasan's PhD thesis will contain eleven research chapters, each presenting the results of one experiment, which will be published as scientific papers. Three have already been published. The others have been accepted or are submitted for publication in scientific journals. Below are some of the highlights of his PhD research.

Hasan in Brazil inspecting a colony of African bees for small hive beetle. All photos by F Ratnieks.

Hasan's research on varroa control shows that applying oxalic acid via sublimation is better than spraying or trickling. Sublimation is more effective at killing varroa at lower doses, causes no harm to the colonies and results in stronger colonies in spring. One treatment of 2.25 grams to a broodless hive in December or January kills 97% of the varroa, enough for one year's management. Two treatments, at an interval of 10 days, kills 99.6%. Hasan has also shown that December is the month when most hives are broodless, but that there is no winter month when all are broodless. This means that beekeepers must inspect their hives before applying oxalic acid and remove sealed brood. Even a small patch of 500 sealed cells will greatly reduce the duration of varroa control, as oxalic acid does not kill varroa in sealed brood cells.*

Hasan has also carried out research on

Hasan extracting varroa mites from a sample of worker bees to determine the number of mites/100 bees.

hygienic behaviour, a natural form of disease resistance via 'improved public health' in which hygienic worker bees remove dead and diseased brood from capped cells, thereby reducing disease transmission. His research shows that varroa build-up over one year is reduced by 57%, on average, in hygienic colonies. Hygienic colonies also had deformed wing virus levels that were on average 10,000 times lower than non-hygienic colonies. In addition, hygienic behaviour can 'save' a diseased colony. Colonies producing workers with shrivelled wings, an overt sign of deformed wing virus and which is usually a harbinger of colony collapse, have high survival when requeened with a hygienic queen (11/15 colonies survived one year) versus a non-hygienic queen (2/15 survived). This was without any other form of disease control.

Hasan's research is already known to many UK beekeepers. He has given numerous talks to beekeeper groups, including to the 2015 BBKA Spring Convention. He has also taught in LASI workshops on varroa control and helped write popular articles for beekeeping magazines. His recent research paper on the control of varroa mites using oxalic acid has been downloaded a record number of times from the *Journal of Apicultural Research*. He has also discovered small hive beetles in bee hives in Brazil, the first record of this pest in South America. In short, Hasan's PhD is one of great practical importance to beekeepers, both in the UK and other countries.

*The UK Veterinary Medicines Directorate (VMD) has now approved oxalic acid treatment with Api-Bioxal. This is the only oxalic acid based treatment for varroosis that is now legal for beekeepers to use in the UK.

Hasan teaching at a LASI workshop on integrated control of Varroa mites.

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LASI Information Sheet from Hasan's PhD Research

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