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An update on the ReVive project.

ReVive: The First Ten Months

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The first ten months of the ReViVe (Rolling out the Evolution of resistance to Varroa and DWV) project have been the usual research roller-coaster. Often things appear to be going well, then, unexpectedly, it takes weeks or even months to sort out a single problem. All this 'behind the scenes' hard work is rarely seen by the general public; it is only the nice publication or exciting results presented at a talk that people see. Hence, it always appears to take ages from providing samples until the results appear. However, a good researcher battles on, never gives up, learns from their failures and always gets there in the end.

DWV analysis: sample collection and processing

The great news is that we have had an amazing response to the call for bee samples from beekeepers. To date, we have received over 180 samples from right across England and Wales (see Fig. 1). The samples fall roughly into equal numbers of those from beekeepers that are treating for varroa, and those that have not used any mite control measures for many years. Much of the spring and early summer has been spent crushing bees and extracting the RNA. This is an expensive, boring and very time-consuming step, but essential for the detection of DWV. This will allow us to determine if DWV is present in the sample, and if it is, the amount and viral strain types, A, B or C, that are present. We have just received two new fridge/freezers to increase our storage capacity, so please do not forget about re-sampling again at the end of August - beginning of September. The extracted RNA is stored at minus 80°C until it is analysed.

Experimental 'trials and tribulations'

The development of a new molecular method that can detect all three DWV strains in a single go has taken much longer to get working than was first expected. We have run into a variety of problems that had to be solved, including dealing with the unusual problem of being sent faulty molecular kits by the manufacturer. The method is now working but we need to resolve a couple of final minor issues before we can turn to analysing all the samples that beekeepers have sent us. So please be patient for a little while longer. It is important that we get this key, preliminary, analytical stage right. As soon as the new primers are working, (primers are specific molecules that allow us to identify each of the DWV strains) sample screening will begin.

Geographical distribution of bee samples received

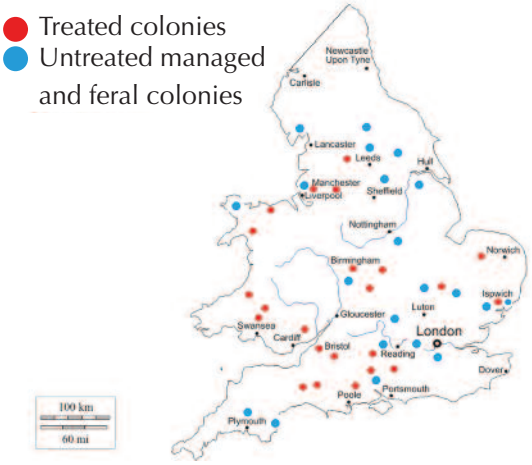

Figure 1. The distribution of adult honey bee samples received from beekeepers, with untreated colonies been shown in blue and treated colonies shown in red. This indicates an excellent spread of samples sent in from all around the country.
All photos courtesy of Jessica Kevill and Stephen Martin.

Varroa resistance studies

We also have collected bees from the long-term varroa tolerant honey bee population in the Arnot forest (USA) studied by Tom Seeley. Preliminary results, using our old molecular methods, looked interesting, but we are waiting for the completion of the new, improved molecular method before re-running the samples. **We hope the new method will give us greater specificity and sensitivity.** In the meantime, we continue to collaborate with David Peck, Tom Seeley's PhD student, who is collecting further samples.

Stephen was fortunate enough to get out to Fernando de Noronha, Brazil again in May to complete his study into the longest known varroa-resistant population of European honey bees. Although this work is funded by the Brazilian Government, it

Jessica: in bold above I have introduced a sentence to explain why you are improving the method - just a guess, so you might want to change it? But, it would be nice to have something explanatory here if possible.

Figure 2. Jessica helping hive a swarm.

dovetails neatly into the central aim of the ReViVe project of looking at the role of DWV in long-term varroa-resistant populations. While Stephen was there, he collected a further set of bee samples for molecular analysis, in addition, to collecting a very detailed set of mite reproductive data. This is the only known remaining population of the Japanese haplotype of *Varroa destructor*, which is believed to have poor reproductive abilities, i.e. it is less virulent, relative to the Korean haplotype, which is now present right across the globe. So, over the summer this work will be written up for open access publication so everyone can see the results.

Funds awarded for a complementary project

The other big news is that Stephen has been awarded funding from the Apis M healthy hive 2020 fund. Although this funding stream is totally unconnected with the ReViVe funding, it will provide a more global view of the strain diversity of DWV and its relation to honey bee health. It is nice to see the British beekeepers leading the way, and others following.

The aim of the Apis M work is to look at the DWV strain within the USA honey bee population, the funding is entirely dedicated to a DWV strain survey that will be conducted by a MSc research student at Salford. So in the next one to two years we will have a very clear idea of the connection between DWV strains and honey bee health in both the UK and USA.

Swarm calls and precarious bee collections

In between sample preparation and conducting molecular work, Jessica has been busy responding to lots of swarming honey bee calls. Roughly about 80% of these calls have been in response to people who have bumblebee nests. So far, seven swarms of honey bees have been collected from various locations including a barn owl box (30ft above ground level) and a huge birch tree. These bees have been placed in the Salford university apiary and also in her back garden (see Fig. 2).

Communicating findings and discussing projects benefits all

Jessica has also given several talks about honey bee declines and how the ReViVe project aims to answer many questions about DWV and honey bee winter losses. One talk was given at a Silverdale pupil referral unit, to some of the most under-privileged teenagers in Salford. Many questions were asked, including how they could become involved in research and pupils really enjoyed seeing the work which is being conducted at the University of Salford. Ormskirk BKA also received a talk about the aims of the project and the implications of the research, which resulted in a fantastic question and answer session.

Several conferences have also been attended. Jessica presented a poster at the BBKA spring convention and also at the Salford post-graduate conference. Later Jessica gave a talk at the post-graduate symposium also at the University of Salford. She has also attended RNA workshops at the Molecular Biology Society annual conference and was able to see Gideon Mordecai present some of his fascinating work, which is also centred on DWV. This was an excellent networking opportunity and very beneficial to her learning. She also attended the Royal Entomological society annual conference at Harper Adams University and learnt a great deal from the other delegates.

Although this all sounds like good fun, lots of training and professional development has been undertaken. All laboratory protocols and procedures have been learnt from scratch. Some of this training has been conducted in-house, but some training was required to be taken in Plymouth at the Marine Biological Association. Researchers here conduct lots of work on viruses and have great expertise in working with single-stranded RNA viruses, such as DWV. Other training, such as the use of statistics packages, has also been undertaken.

Next steps

Moving forward Jessica and collaborators will solve the final issue with the new primer set. Once this has been resolved, she will start to screen the honey bee samples already received. Analysis of these samples is expected to be well underway by winter 2016. Once analysis is completed, bee samples from the Aug-Sept batch will be prepared and screened.

Sampling reminder

On the note above, we would like to remind beekeepers involved in sampling not to forget to send in your honey bee samples for mid August–early September to us please. Please provide 20+ bees per sample, because twenty is the minimum number of bees which can be screened. Samples of less than twenty bees do not provide an accurate colony-level result and will be excluded from analysis.

Associations which are involved in the research should already have their August–September collection kit. If not, then please contact Jessica at J.kevill@edu.salford.ac.uk. ■