BY CHRISSIE GILES

PROTECTING THE POLLINATORS

Insect pollinators, including honeybees, bumblebees and hoverflies, are in decline. The £10 million Insect Pollinators Initiative – part-funded by the Wellcome Trust – has been launched to find out why. We met researchers from three of the nine projects funded to hear about their plans.

False-coloured scanning electron micrograph of a honeybee (winner of a 2011 Wellcome Image Award – see page 22). *David McCarthy and Annie Cavanagh/Wellcome Images*

We all know that bees make honey, but they do much more for the food we eat. Bees and other insects, including butterflies and hoverflies, pollinate plants. By transferring pollen from the male parts of flowers to the female parts, they are a vital part of the process that eventually leads to fruit, nut and seed production.

For some crops, such as melons, no pollinators means no fruit. For others, it means a lesser harvest. This widespread role of insects in food production is reflected in insect pollinators' economic value – estimated at €153 billion (£130bn) globally in 2005.[1]

Pollinators are under threat, though. Research published in 2006 indicates that the diversity of wild bees – a key pollinator group – has declined severely since the 1980s.[2] Another major pollinator, honeybees, are prone to a number of diseases. The mite *Varroa destructor*, for example, carries viruses that can quickly destroy entire colonies, and has spread almost completely around the world in the last 30 years.

While disease is a serious risk, it is not the only one pollinators face. "There's likely a smorgasbord of problems," says Professor Jane Memmott from the University of Bristol, a lead investigator on one of the projects funded by the Initiative. "And they probably interact in different ways too – if bees are not properly fed, then they're more likely to catch diseases, and so on."

The nine projects funded through the Insect Pollinators Initiative are setting out to understand these threats better. We have looked at three below, and details of all nine can be found at **www.wellcome.ac.uk/pollinators**.

How do diseases affect the honeybee, and could they spread to other bee species?

"We've picked what we think are the most important disease organisms for the honeybee," says Dr Robert Paxton, from Queen's University Belfast and the University of Halle, Germany. His team is studying deformed wing virus, carried by the *Varroa destructor* mite, and a fungus-like microorganism called *Nosema ceranae*.

"Before the *Varroa* mite came to the UK, deformed wing virus was found in maybe 1 in 10 000 colonies," says Robert. This changed after its discovery in the UK in 1992, when the amount of virus carried by bees increased dramatically.

Varroa is a rusty-coloured mite, which feeds on the haemolymph (circulatory fluid) of adult and pupal bees. This increases the amount of deformed wing virus carried by bees and can lead to symptoms including – as the name suggests – misshapen wings that prevent bees from flying. The grounded bees are taken by predators and the colonies suffer as their numbers drop.

Robert says at least half of the colonies in the UK contain clinical symptoms of deformed wing virus – severe infection with which can lead to the collapse of colonies.

Nosema, meanwhile, has spread from East Asia in the last 10–15 years to the western honeybee. Robert suspects that the interaction between this and deformed wing virus may act as a "double whammy", greatly increasing the ill-effects on honeybees. Not only honeybees are at risk: these diseases also affect bumblebees, and there are fears that they will be transmitted to other pollinators too.

Dr Mark Brown and his team at Royal Holloway, University of London, will be investigating how bumblebees come to be infected with deformed wing virus and *N. ceranae*, and the impact that these emergent diseases have on individuals and colonies of the important native bumblebee species.

In their project, researchers are studying how the diseases affect the bees physically, and whether they have any impact on insects' flight behaviour, orientation and learning – so-called sub-lethal effects, which don't kill the bees but affect how they function.

"We're working with Juliet Osborne's team at Rothamsted Research that has very refined methods for tracking how individual bees fly," says Robert. "It will be really nice to understand the impact of these disease organisms on individuals."

Professor Vincent Jansen, also at Royal Holloway, will use the data collected to model the spread of the

Bumblebee (*Bombus pascuorum*) foraging on red clover. © *Claire Carvell, Centre for Ecology and Hydrology*

"The mite Varroa destructor *carries viruses that can quickly destroy entire colonies"*

disease organisms in the pollinator community, to try to understand the threat to both honeybees and bumblebees.

On top of all these efforts, the team will also attempt to find ways to treat these infections. Robert and his colleagues will be testing the use of RNAi (RNA interference) methods in controlling deformed wing virus, an RNA virus. The technique involves blocking the multiplication of the virus and has been effective in other RNA viruses in honeybees, though it is not completely understood how.

They are also looking in some detail at the bacterial species associated with the honeybee gut. Researchers have only recently discovered that insect guts hold a huge variety of lactic acid bacteria and related species, the kind you find in probiotic yoghurts. They will investigate whether the two diseases have an impact on these bacteria, and whether these bacteria can help overcome the disease symptoms, particularly those caused by *Nosema*, which bees contract by swallowing spores.

How do pesticides and other chemicals affect bees' behaviour?

Pesticides and other agricultural chemicals used to maximise crop yields may also be affecting the health of bees. The treatment of honeybees with pesticides – miticides – to try to prevent infestation with the *Varroa* mite could be detrimental to their wellbeing.

Dr Chris Connolly is a neurobiologist at the University of Dundee. Though usually found investigating the human brain, he is now applying his expertise to bees.

"I was thinking about pesticides and realised that, although people have looked at the concentrations that kill pest and non-pest species, so-called sub-lethal doses may affect how bees behave. Moreover, there have been no studies to investigate whether the sub-lethal effects of multiple pesticides might create the 'perfect storm' through synergistic interactions," Chris says.

"I decided that this is more or less the kind of thing we've been doing on mammalian brain cells, so if we could apply this to the bee brain, we could find out if these chemicals have sub-lethal effects at the level of individual cells, neural networks, whole animals or entire colonies." There are fears that these exposures may affect the bees' abilities to move, communicate and find food.

The *Varroa*-killing miticides are a reformulation of the pesticides used in the field and one of the prime targets for synergistic toxicity. "If the bees encounter this and then another pesticide, then the double hit might be the problem," Chris says.

His team is designing assays to study the effects of different combinations of pesticide on brain cells. "Our next step will be to look at neural networks and to study the effects on the insects' abilities to learn at the neuronal level," he says.

To assess the impact of pesticides, research in the lab of Dr Geraldine Wright at Newcastle University (see below) will investigate how such chemicals affect learning and memory in both honeybees and bumblebees. "After the bees have been exposed to chemicals, we can ask: are they slower at learning? Do they forget what they've learned?" says Chris.

This part of the project will involve the radiotagging of 6000 bees, overseen by Dr Nigel Raine at Royal Holloway. A scanner will monitor bees as they enter and leave the hive. And the bees will be weighed, allowing researchers to work out not only each individual bee's contribution to the hive throughout its life but also the performance of the whole colony throughout a season.

Are British bees getting the right diet?

Like doing the grocery shopping for your family, foraging worker bees have to pick food that's right not only for them but also for those back home. The bee's shopping list is simpler though: pollen is the main source of protein and nectar the main source of carbohydrate.

"How worker bees choose food is not well understood," says Dr Geraldine Wright from Newcastle University. She is investigating bee nutrition, and how a bee's nutritional state affects how it forages.

Geraldine is working with Dr Annie Borland, a plant physiologist at Newcastle, and Dr Phil Stevenson, from the Royal Botanic Gardens at Kew, to measure the nutritional content of nectar and pollen from different agricultural, horticultural and native UK species.

"We're looking to find out if bees need to forage from one or several different species to achieve their optimal carbohydrate-to-protein ratio," says Geraldine. "One thing that's emerging from the work of our collaborator, Professor Sue Nicolson at the University of Pretoria in South Africa, is that workers don't survive well on a high-protein diet, but this is in fact what the brood [the immature bees] need. It will be really interesting to find out how much the honeybee has to disregard its own nutritional state while it's foraging."

While bee nutrition is the focus of the project, research by Geraldine's laboratory is mainly around understanding the mechanisms of learning and memory. She and her colleagues have been using the honeybee as a model for some time, and are bringing that knowledge to their new project.

"We don't know a lot about nutrition and how it influences learning and memory. This project will allow us to understand exactly what honeybees and bumblebees need, in terms of pollen and nectar, but also how their foraging can feed back on to what they do when they're learning." For example, she says, if a bee is low on protein in its diet, is it more likely to learn to associate a floral scent with an amino acid (which proteins are made of) than with a sugar?

With Professor Sharoni Shafir at the Hebrew University, Israel, Geraldine will be investigating how bees weigh rewards – or, as she describes it: "We're going to look at how nutrition affects the cognitive behavioural decisions that forager bees have to make when they're out doing the shopping."

- Dr Robert Paxton is working with Dr Mark Brown (Royal Holloway, University of London) and Dr Juliet Osborne (Rothamsted Research).
- Dr Chris Connolly is working with Dr Jenni Harvey (University of Dundee), Dr Nigel Raine (Royal Holloway), Dr Geraldine Wright (Newcastle University) and Professor Neil Millar (UCL).
- Dr Geraldine Wright is working with Dr Phil Stevenson (Royal Botanic Gardens, Kew), Dr Annie Borland (Newcastle University), Prof. Sue Nicolson (University of Pretoria), Prof. Sharoni Shafir (Hebrew University of Jerusalem) and Prof. Steve Simpson (University of Sydney).

1. Gallai N et al. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics 2009;68(3):810–21.
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The Insect Pollinators Iniatitive is supported by the Biotechnology and Biological Sciences Research Council, the Department for Environment, Food and Rural Affairs, the Natural Environment Research Council, the Scottish Government and the Wellcome Trust, under the auspices of the Living With Environmental Change partnership.