*Article*

# More than just buzz: New Zealand bee research and its impact

**Petra M. Dearden and Peter K. Dearden***
Genetics Otago and Biochemistry Department, University of Otago,
PO Box 56, Dunedin 9054

*The honeybee, Apis mellifera, is a remarkable animal. Honeybees are not only unusual and astounding in their biology, but also vitally important to human agricultural productivity. Despite their nasty sting, they are the poster-child for beneficial insects, and one of the few insects that are well loved. They are regarded with warmth and support. They are part of the soundtrack of a long hot summer, the go-to name when we are working hard, and they provide our flavour of choice at breakfast. They have even been incorporated into Kiwiana; every little Kiwi has had a Buzzy Bee. Honeybees play an important role in New Zealand's economy, production and identity.*

*In this review we aim to present information as to the state and future of New Zealand bees and the beekeeping industry. We would like to review recent advances in bee biology pioneered by New Zealand researchers and show how bee research in New Zealand probes some of the fundamental aspects of bee biology, while also being an exemplar of the way fundamental research often has unforeseen but important applied outcomes.*

## The importance of bees: Biology of bees

Honeybee biology is fascinating to many, paralleling as it does our own society. We see something of ourselves in a society that thrives on the division of labour. While this is an attractive vision, it is illusory. Honeybees are eusocial and humans generally are not. Eusociality is defined by cooperative brood care, often brood care of offspring from other individuals, and division of labour into reproductive and non-reproductive groups. Division of labour produces specialised castes, and one key aspect of eusociality is that individuals of one caste lose the ability to perform another caste's job. In honeybees there is division of labour in the hive, most obviously reproductive division of labour. In the hive there is one queen, who lays all the eggs, and the workers who carry out the rest of the work; cleaning, caring for offspring, guarding the hive, and foraging. Remarkably even these tasks are allocated to different aged worker bees, with young bees starting as nurses, and the oldest only foraging. Worker bees raise the offspring of the queen, which are effectively their sisters. Charles Darwin referred to this arrangement as a the 'one special difficulty' of his theory of evolution by natural selection, as he could not see clearly how it might have evolved (Darwin 1859). Since Darwin, a number of theories (for examples see (Hamilton 1964a,b; Hughes *et al.* 2008; Nowak *et al.* 2010)) have been put forward, but there is still much debate as to which explains the evolution of this remarkable trait.

The complexities and foibles of bee behaviour and biology have recently been well reviewed in an excellent book (Tautz 2008).

Bees are a member of the order Hymenoptera, which includes bees, wasps, ants and sawflies, and they diverged from the lineage leading to the geneticists' workhorse, *Drosophila* (the vinegar fly), about 366 million years ago (Hedges *et al.* 2006). All hymenoptera have a haplodiploid mode of sex determination (Crozier 1977). This means that males (drones), produced from unfertilised eggs, are haploid, while females (workers and queens), produced from fertilised eggs, are diploid. The combination of haplodiploidy and one multiply mated reproductive individual per hive, makes honeybee transmission genetics complex (Figure 1).

Honeybees most notably make honey – a $170 million a year export industry in New Zealand (Statistics New Zealand 2012). Indeed, manuka honey alone is worth $40 million to our economy (Ministry of Primary Industries 2013). Honeybees are also involved in pollination. Recent data shows that around a third of all crop plants require insect pollination (Klein *et al.* 2007), and honeybees play a vital role in supporting pollination

---

* Correspondence peter.dearden@otago.ac.nz

**Peter Dearden** has been at the University of Otago since 2001 and is Associate Professor, Laboratory for Evolution and Development, in the Biochemistry Department, and Director of Genetics Otago. Peter trained initially at Victoria University of Wellington, taking a BSc (Hons) in genetics and molecular biology, and later a PhD in neuro-genetics at Imperial College, University of London. He worked in the Wellcome (now Gurdon) Institute and the Museum of Zoology, University of Cambridge, and then the Zoology Department, University of Western Ontario. He returned to New Zealand and the University of Otago in 2002. His research on the honeybee aims to understand the complexity of their biology, with an eye on providing scientific support for New Zealand's beekeeping industry.

**Petra Dearden** has been a researcher in the Laboratory for Evolution and Development at the University of Otago since 2012.

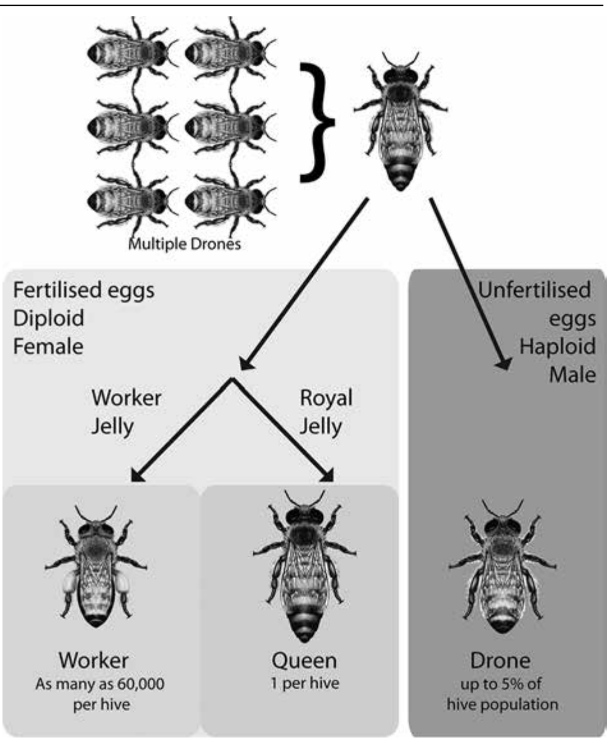

**Figure 1. Honeybee castes and relationships.**

**Honeybees are haplodiploid, meaning that females are diploid and develop from fertilised eggs and males are haploid, developing from unfertilised eggs. Honeybee queens mate with multiple males at the start of their life and can lay either fertilised or unfertilised eggs. Fertilised eggs can produce either workers or queen bees depending on nutrition when they are larvae.**

in horticulture, arable farming and, through clover pollination, pasture health. The National Beekeepers Association estimates that honeybees add $5.1 billion a year to the New Zealand economy through honey, bee products and pollination (National Beekeepers Association 2014). Beekeepers hire out 90,000 hives a year (mainly for kiwifruit and apple producers) and provide pollination services at a cost of $9 million a year (Te Ara: The Encyclopedia of New Zealand). Honeybees are a vital part of our agricultural production.

## Where do bees in New Zealand come from?

Though New Zealand does have native bees (Te Ara: The Encyclopedia of New Zealand), they are solitary, non-eusocial and do not produce honey. Honeybees, as we know them, were first brought to New Zealand from Sydney to Hokianga by a Mrs Bumby in 1839 (hives in Australia having been established in 1822 via hives from England) (Ministry for Culture and Heritage 2014). Later shipments came into New Zealand directly from Europe in the early years of settlement. Bees were apparently transported in the hold of ships while sailing, and were moved up on deck and let out of the hive during periods of calm.

New Zealand bee imports have likely come from multiple sources including Carniolan, Italian and British bees. Now New Zealand exports 20 tonnes of worker and queen bees a year worldwide (National Beekeepers Association 2014).

## The beekeeping industry

Currently the New Zealand beekeeping industry is experiencing a boom, with numbers of hives up approx. 150,000 since 2009, and export earnings more than doubling over the same period (Figure 2). This boom has been triggered by the high export prices for manuka honey, which has transformed the industry. Prices for bees and queens are increasing, and more people are joining the beekeeping industry (Statistics New Zealand). Pollination requirements for horticulture and seed production are also increasing. A recent parliamentary report painted a rosy view of the New Zealand industry (Primary Production Committee 2014).

Beekeeping is a complex and difficult art, as is bee breeding. For effective bee breeding and artificial selection for improved stocks, bees need to have artificial insemination (AI). AI provides a significant barrier (both in equipment and technical skills) to selective breeding programmes, so stock improvement in bees is lagging behind breeding of other production animals. In New Zealand we are lucky to have two breeding programmes that use AI to control genetics and select for improved stock (so-called closed populations). Daykel Apiaries concentrate on breeding Carniolan bees and Betta Bees Research Ltd produce selected lines of Italian bees. While these two programmes undertake careful scientifically based selective breeding for a range of production traits, little stock improvement occurs elsewhere. Breeders produce small numbers of AI breeder queens, which are placed in hives and large numbers of new queens are then raised from their offspring. These queens, named production queens, produce the backbone of the bee industry.

## Challenges to beekeeping in New Zealand

Despite the rosy situation of the New Zealand honey industry(Primary Production Committee 2014), bees worldwide are facing significant challenges. Overseas two very widely publicised threats, Colony Collapse Disorder (CCD) (Vanengelsdorp *et al.* 2009; Williams *et al.* 2010; Dainat *et al.* 2012) and Pollinator Decline (Packer & Owen 2001; Zayed *et al.* 2004; Gallai *et al.* 2009), appear to be doing significant damage to bee populations.

In North America and parts of Europe, CCD, a disease that causes bees to abandon their hives, has caused drastic declines since 2006. The exact cause of colony collapse is disputed, but blame has been laid at the door of changing beekeeping practices, pesticides, infections with Varroa and other mites, malnutrition, immunodeficiency, loss of habitat, or a combination of factors (Reviewed in (Williams *et al.* 2010)).

Pollinator decline is a broader issue in which pollinators in general appear to be declining. Again many factors have been proposed as causing this effect, but insecticides, especially neonicitinoids, have been proposed as key factors.

### *Varroa mite*

While New Zealand does not currently appear to have CCD or a general decline in pollinators (Primary Production Committee 2014), it is struggling with the recent accidental introduction of the Varroa mite (*Varroa destructor*). This bee parasite has affected bee populations worldwide and it arrived in New Zealand in about 2000. Sadly it has now spread the length of the country (Figure 3). Varroa mite is a particularly pernicious parasite, causing colonies to die without significant intervention. Varroa mite suck the hemolymph of adult bees, leaving open wounds prone to infection, and are spread between them. The mite females lay their eggs on developing bee brood and the eggs are sealed in the brood cell and emerge from the cell at the same time as the hatching bee brood. Varroa appears to have recently skipped host from the Eastern honeybee (*Apis cerana*) (Rosenkranz *et al.* 2010) where it has little effect on populations.

The effect of Varroa is two-fold. Clearly mites damage the population through damaging bees, but they also are very effective vectors of viruses (Bowen-Walker *et al.* 1999; Kleespies *et al.* 2000; Neumann *et al.* 2012; Francis *et al.* 2013; Ryabov *et al.* 2014), causing common viruses to be more infectious and damaging to the hive than pre-Varroa. It is also apparent that the interaction between bees, Varroa and viruses has led to novel viruses (Ryabov *et al.* 2014), produced through viral recombination, that are virulent and deadly to bees. Varroa is a very significant, and currently intractable, problem.

Varroa are controlled either with mitecides applied to the hive (Rosenkranz *et al.* 2010), or organically with a range of compounds. Both methods significantly increase the cost of honey production per hive. With honey prices increasing, beekeepers have been able to absorb this extra cost, but Varroa makes marginal operations uneconomic. During the first years of Varroa in New Zealand many hobbyist and small business beekeepers exited beekeeping (Statistics New Zealand and Figure 2). It is also clear that resistance is developing to the compounds used to control Varroa, with reports of resistance in New Zealand (Primary Production Committee 2014), and more worrying reports coming from overseas, where mites have been found with resistance to many of the compounds for control (Miozes-Koch *et al.* 2000; Martin *et al.* 2002; Maggi *et al.* 2009; Gonzalez-Cabrera *et al.* 2013). Such treatments also have been shown to have significant effects on the bees themselves (Mondet *et al.* 2011) The development of new control methods is vital in the face of this increasing resistance.

Without control, Varroa kills entire beehives. This has caused a worldwide loss of feral (non-managed) bees. Without mitecide treatments, all the ‘wild’ colonies, mainly escaped from beekeeping operations, have died. This extinction event has left New Zealand without feral bees, except perhaps on outlying islands such as the Chatham islands that are currently Varroa-free. While little research has gone into the impact of this loss, it presumably has led to the loss of a great deal of ‘free’ pollination capacity and perhaps a reduction in the gene pool.

Alongside the effect of Varroa on management practice, the New Zealand industry has responded to Varroa in a number of ways. Varroa triggered the formation of Betta Bees Research

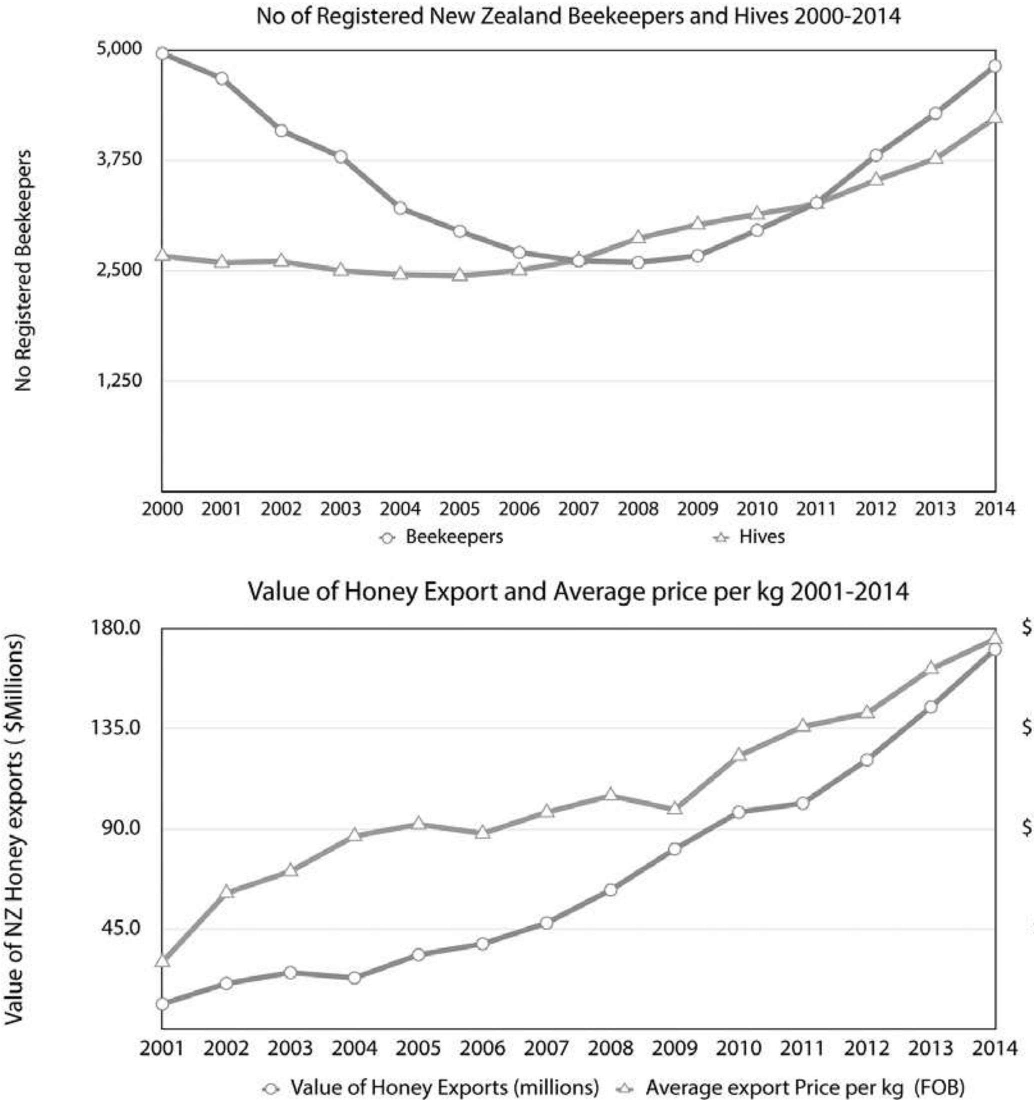

**Figure 2: Statistics New Zealand data for New Zealand apiculture.**

**Statistics for number of registered hives, numbers of registered beekeepers, export earning of the New Zealand honey crop and average price of honey per kg in the last decade.**

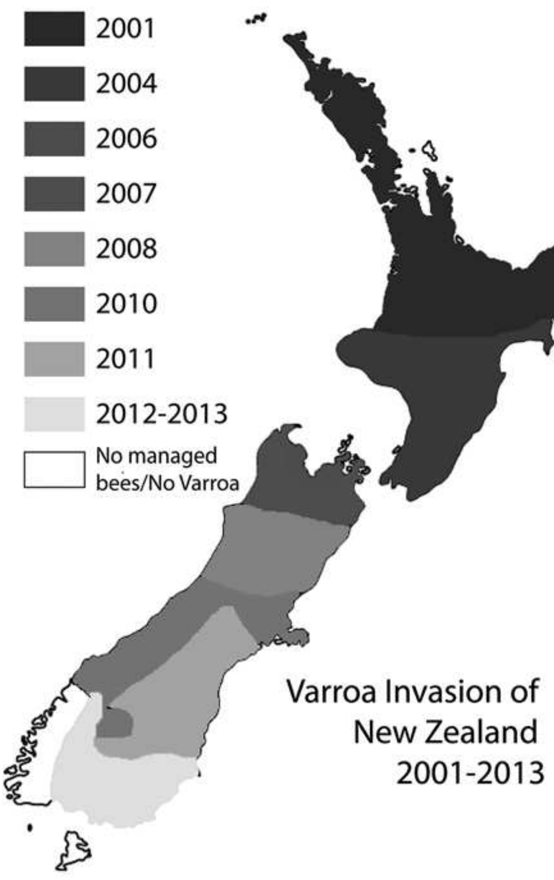

**Figure 3: Spread of Varroa mite in New Zealand.**

**Map of New Zealand showing the reported spread of Varroa mite. Redrawn from Mondet *et al.* (2014).**

Ltd (BBR), as Varroa-free South Island beekeepers found they couldn't import queens from the infected North island, which provided the impetus to carry out the breeding themselves. The industry, together with the Ministry of Primary Industries (MPI), funded Plant and Food Research Ltd (PFR) to selectively breed Varroa-tolerant bees, using protocols shown to be effective overseas (Ward *et al.* 2008; Danka *et al.* 2011). This programme produced a stock of bees (now named Mercury Island Bees) with increased tolerance to Varroa. A new enterprise, Rainbow Honey, is now attempting to maintain this stock and provide it to the industry. BBR is also evaluating these bees for Varroa tolerance with an aim to incorporating it into its own selective breeding population. It remains to be seen if this stock of bees can be effectively used in the New Zealand industry to improve colony loss from, and costs of, Varroa mite.

### American Foulbrood

Before the arrival of Varroa, the biggest disease issue of bees in New Zealand was American Foulbrood (AFB), a highly contagious, spore-forming, bacterial infection of bees (Genersch 2010). AFB, caused by infection with *Paenibacillus larvae* ssp. *larvae*, leads to colony death and is spread easily between hives. The spores of this infection remain for long periods (up to 40 years) in soil, honey and beekeeping equipment, so it recurs easily without management. Control of AFB in New Zealand is regulated by the AFB National Pest Management Strategy (National Pest Management Strategy, undated). This agency ensures beekeepers are registered, well trained, and can identify AFB in hives. AFB-infected hives and contaminated equipment are burned to control the spread of this disease. Disease levels currently fluctuate between 0.31% and 0.26% of hives.

PFR, led by Dr Mark Goodwin, has been instrumental in developing methods to control AFB, training beekeepers in its detection and techniques to control outbreaks (Goodwin 2006). This research has been transformative for the control of AFB in New Zealand.

### Pesticides

Here, and overseas, there is significant concern about insecticides used in primary production and their off-target effects on bees. Recent studies have shown significant effects on honeybees and bumblebees of sub-lethal doses of neonicotinoid insecticides (Thompson & Maus 2007; Gobin *et al.* 2008; Yang *et al.* 2008; Belien *et al.* 2009; Smodis Skerl *et al.* 2010; Berry *et al.* 2013; de Almeida Rossi *et al.* 2013). This, combined with evidence that these insecticides are more environmentally stable than first thought (Bonmatin *et al.* 2014), has led to them being seen as the major culprit in pollinator decline (Chagnon *et al.* 2014; van der Sluijs *et al.* 2014). Some neonicotinoids are now temporarily banned in Europe as a result of assessments carried out by the European Food Safety Authority (European Food Safety Authority 2013).

Insecticide use is a particularly difficult problem because of their impact on pollinators. As stated earlier, one-third of the food we eat depends on pollination (Gallai *et al.* 2009), and thus is adversely affected by the use of insecticides. Unfortunately insects are responsible for damaging crops and stored food, meaning insecticides are a key tool in primary production. Balancing insecticide use and protecting beneficial insects is a huge challenge for primary production.

In New Zealand we do not have strong evidence for insecticide use being a problem for bees (Primary Production Committee 2014). Clearly some bee deaths are due to insecticide use in surrounding areas, but this doesn't seem to be a widespread problem, nor do we seem to be suffering pollinator decline. The experience overseas, combined with remarkable modelling experiments carried out in New Zealand (Davie-Martin *et al.* 2013), make it vital that we begin to research the impact of insecticides on pollinators used in New Zealand primary production. Only then can we support either strict bans or continued general use.

### Manuka honey identification

The increasing value of manuka honey has helped the New Zealand honey industry weather the storm of Varroa and its resulting economic downturn. The manuka honey industry is based on long-term research carried out at the Honey Research Lab, led by Prof Peter Molan, Waikato University, to identify and quantify the unique factors in manuka that give it its favourable antibacterial properties (Willix *et al.* 1992; al Somal *et al.* 1994; Wood *et al.* 1997; Cooper *et al.* 2001; English *et al.* 2004). This work has identified the unique factor methylglyoxal (Adams *et al.* 2009), and Waikato University continue to work on understanding the metabolism of manuka that allows it to produce this chemical in honey. The high value of the honey, as well as its use in high-value products, depends entirely on this research. The story of this scientific success and its impact on industry has been described in a recently published book (Van Eaton 2014).

By understanding the product, the price of manuka honey exports has increased. In recent years, however, it has become clear that the markets are beginning to question the quality and provenance of manuka honey (Wynn *et al.* 2013). Manuka (*Leptospermum scoparium*) is very similar to kanuka (*Kunzea ericoides*), which does not appear to produce methylglyoxal, but gives honey with similar qualities. MPI is currently investigating scientific solutions to distinguish manuka honey from kanuka honey, and to produce tests that can assure customers that the high-value honey they are purchasing is what it says it is on the jar.

Manuka honey, however, has unusual characteristics that make it fail tests for C4 sugars. International markets require testing of honey to ensure that it is not adulterated with sucrose, but high-quality manuka honeys often fail those tests. Dr Karen Rogers and team at GNS Science have developed and validated new tests that will improve market access for our honeys (Rogers *et al.* 2010).

The New Zealand honey industry is in good health, but this is entirely due to the high prices for its products. Vigilance and good science are needed to maintain and improve bee health, and to provide surety of the quality and traceability of our products to ensure market access.

## New Zealand honeybee science

Honeybee biology has fascinated humans since our first interactions with them in around 2400 BC; Aristotle wrote in 350 BC on the social behaviour of honeybees. Bee research has undergone a renaissance in the last few years, mainly due to the sequencing of the honeybee genome in 2006 (The Honey Bee Genome Sequencing Consortium (includes P.K. Dearden) 2006). This was done by an international consortium (including two New Zealand groups) and has provided an excellent sequence, as well as an understanding of the complement of genes in honeybees (Dearden *et al.* 2006). It has expanded the possible ways we can research bee biology and has provided a number of breakthroughs.

### *Honeybee behaviour*

Honeybee behaviour, and their remarkable navigation and learning abilities, have been a subject for research for many years. In New Zealand, two groups have been active in producing world-class honeybee behavioural research.

Dr Guy Warman at the University of Auckland has been using bees to investigate time sense and its response to anaesthetics. Bees have an innate time sense, based on their internal circadian clock, which they consult to modify their navigation as the position of the sun changes over the day. Dr Warman's team showed that anaesthesia disrupted that time sense, causing the bees to wrongly predict the position of the sun. Anaesthesia thus effectively caused the bees to be 'jetlagged', and the team showed that this disruption occurred in the molecular circadian clock (Cheeseman *et al.* 2012). Given that anaesthetics have broadly similar effects in different animals, and the conservatism of the circadian clock machinery, it seems likely that humans undergoing surgery also suffer this jetlag phenomenon, perhaps affecting their recovery. Dr Warman's work in bees implies that anaesthetic treatments might be better administered during the night, a time at which the bees showed no loss of time sense.

Further experiments examining the behaviour of bees after anaesthesia also provided insights into their cognitive processes during navigation (Cheeseman *et al.* 2014). Mammals have been shown to have a metric cognitive map that helps in continuously updating their position in space. By examining the way bees navigate after wrongly predicting the position of the sun, Dr Warman and his team showed that bees, too, appear to have a cognitive map (Cheeseman *et al.* 2014).

Prof Alison Mercer, at the University of Otago, has been investigating a number of aspects of bee behaviour, concentrating on how a queen bee signals to worker bees to control reproduction. This signal is carried by a pheromone, Queen Mandibular Pheromone (QMP), which acts to modify worker bee behaviour and reproductive activity. Prof Mercer has investigated how this pheromone impacts on the brains of worker bees to modify their behaviour, learning and physiology (Beggs *et al.* 2007; Vergoz *et al.* 2007, 2009; Beggs & Mercer 2009). Prof Mercer's team has shown that QMP appears to act through dopamine receptors, one of the components of QMP, homovanillyl alcohol (HVA) (Beggs *et al.* 2007) having structural similarity to dopamine. HVA causes signalling through one of the dopamine receptors in the honeybee antenna, and this signalling modulates the behaviour of workers (Beggs & Mercer 2009). One remarkable finding is that QMP blocks aversive learning in young worker honeybees (Vergoz *et al.* 2007), making them unable to learn to associate a stimulus with an electric shock. This block in aversive learning did not extend to appetitive learning (Vergoz *et al.* 2007), whereby bees learn to associate an odour with a food reward. It appears as if the queen bee not only represses the reproduction of worker bees, but also brainwashes them to like her.

These experiments investigate the fundamental nature of the eusocial structure of honeybee society, showing that the reproductive division of labour is a highly regulated and complex construct of pheromones and neurochemistry. This research also is likely to provide novel management solutions for beekeeping, as pheromone control of behaviour may be an effective way to trigger desirable behaviour in managed colonies.

### *Honeybee embryonic and larval development and plasticity*

The sequencing of the honeybee genome and the development of techniques to manipulate the expression of honeybee genes (Dearden *et al.* 2009), have allowed use of the honeybee as a model to study the evolution of developmental pathways. Our lab has used bees (and other insects) to study the evolution of axis formation and segmentation mechanisms within insects (Wilson & Dearden 2009, 2011, 2012; Wilson *et al.* 2010). These pathways are well known in the fruit fly *Drosophila*, and we, and others, have studied how the pathways we know about in *Drosophila* have evolved.

Axis formation involves the determination of the anterior, posterior, dorsal and ventral parts of the embryo and, in most animals, occurs very early in development (Lynch 2014). In insects these pathways provide information that is used by segmentation genes to divide the embryo into the metameric segments diagnostic for the insect body plan. Beginning with understanding which genes are encoded in the genome, we discovered that genes that act early in axis formation are less conserved in sequence (Dearden *et al.* 2006), expression and function (Wilson & Dearden 2009, 2011, 2012; Wilson *et al.* 2010) than genes that act later – a counter-intuitive finding. This pattern has now been found in other systems, such as sex determination (Gempe & Beye 2011; Bopp *et al.* 2014), and we are working to discover how variation in early-acting pathways might still integrate with more conserved, later-acting, processes.

One consequence of this research has been the discovery of a range of genes that act in axis formation in insects, and a list of genes and pathways that are missing from, or are substantially different, in honeybees. We are now using this information of genes and pathways that are present in most insects but absent in honeybees as possible insecticide targets. We hope with this knowledge we will be able to reduce the impact of 'insecticide collateral damage' in primary production pollinators such as honeybees.

Honeybees are holometabolous insects, which means they have two phases of development. The first is embryonic development, the second larval or imaginal development. This second phase takes place in specialised tissues in the larvae, which come to replace larval tissue in the pupa to form the final adult structures. Bees are remarkable in that this second phase of development is strongly influenced by the diet of the larvae.

Larvae destined to be queen bees are fed royal jelly, which triggers a remarkable series of changes leading to an adult with different morphology, behaviour and physiology from worker bees. We examined how gene expression differs between larvae fed royal jelly and those that are not, to try and identify genes and pathways involved in making a queen (Cameron *et al.* 2013). Our data shows that different gene expression in worker and queen larvae occurs after only 6 hours of being fed royal jelly. We also identified a range of processes that appear different between worker and queen larvae, and, using RNA interference, demonstrated that some of them are critical to produce queens (Cameron *et al.* 2013). We also showed apparent differences in DNA methylation, confirming a role for epigenetic changes in this example of developmental plasticity.

Understanding developmental plasticity in a model such as a bee is vitally important, as it is suggested as having major roles in evolutionary change (West-Eberhard 2003) and in human health (Gluckman & Hansen 2004).

## New Zealand as a laboratory in which to study the spread of honeybee disease

The relatively recent incursion of Varroa mite into New Zealand has provided an opportunity to study the dynamics of virus infections in bees as the Varroa invasion front travelled (Mondet *et al.* 2014). This work, carried out by Dr Fanny Mondet in a collaboration between Prof Mercer's group at Otago and Avignon University in France, showed the clear association between virus titres in bees and the invasion front of Varroa (Mondet *et al.* 2014). By sampling bees before and after the advent of Varroa, and looking at virus titres in bees and mites, the team was able to determine how Varroa affects the dynamics of viral infections in bees. Varroa certainly increases the infection rates of some viruses, but particularly seems to cause infections with multiple viruses. Infection with some of these viruses seem to be crucial for the pathogenicity of Varroa, showing a deadly interplay between Varroa, the bee viruses and its vectors (Mondet *et al.* 2014).

## Genetics and selective breeding

Honeybees determine sex by haplodiploidy (Figure 4). In bees, females are diploid while males are haploid. This method of sex determination relies on the ability of bees to detect their own ploidy, a process that depends on genetic diversity (Beye 2004). At one locus in the genome, named *complementary sex determiner* (*csd*), bees count the number of alleles they have. If they find two different *csd* alleles, the bee must be diploid and develops as a female. If only one allele is present, the bee is either haploid or homozygous diploid (i.e. both alleles the same, so it can only detect one) and develops as a male. In recent years the *csd* locus has been identified and the gene responsible examined (Beye *et al.* 2003). This gene has a hypervariable region in its coding sequence that evolves rapidly, ensuring many alleles of this locus occur, thus reducing the risk of diploid male bees (Hasselmann *et al.* 2008). As inbreeding increases, or genetic diversity decreases, more and more diploid bees have the same *csd* alleles and inappropriately turn male. These bees are killed by workers in the hive, reducing brood viability. Genetic variation at the *csd* locus is thus vital for the long-term health of honeybee populations.

Given that all bees in New Zealand have been imported, likely producing a genetic bottleneck, and with the loss of our feral bees, we wondered if the number of *csd* alleles in the New Zealand bee population was adequate for long-term health. Springing from our more fundamental work on honeybee genetics we developed genetic tests for *csd* (Hyink *et*

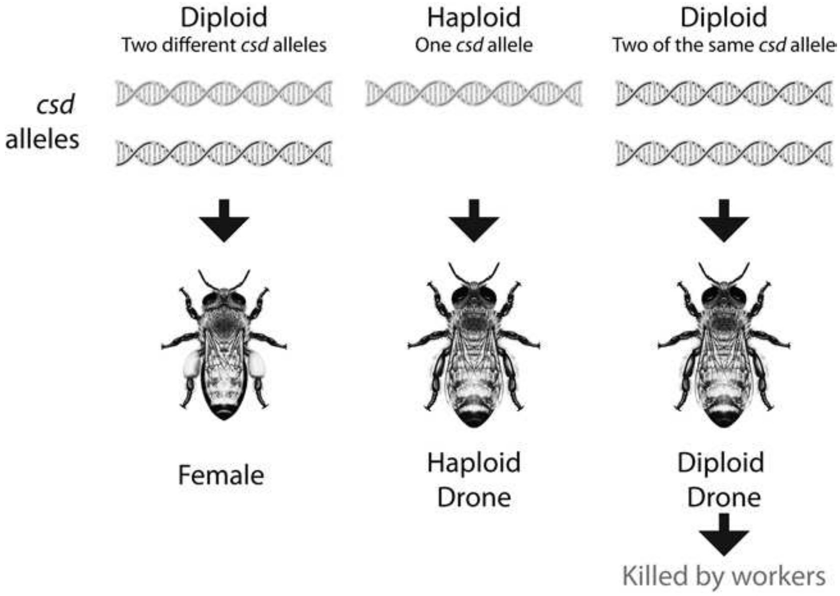

**Figure 4: Sex determination in bees.**

**Sex in bees is determined by the number of alleles at the complementary sex determiner (*csd*) locus. If a bee has two alleles that differ in DNA sequence, then the bee develops as a female. If one allele is present then the bee develops as a drone. A single allele could be caused by the bee being haploid (the normal situation) or being inbred and having two alleles the same at *csd*. Diploid bees with two alleles the same also develop as drones, but are detected and killed by worker bees.**

*al.* 2013) and then deployed them, funded by the Sustainable Farming Fund (SFF) for the Ministry for Primary Industries, to survey commercial beekeepers for *csd* alleles. We found that New Zealand has more than enough alleles for the population, but that closed breeding programmes need to take care to not narrow their gene pools.

## The future

Honeybees are a key part of our primary production-based economy, and one where good science can make a huge difference, both in supporting the honey industry, but also supporting and improving pollination by bees and others. There are many remaining questions for scientists working with honeybees, many of which will have impacts on the industry. we believe that it is time we used the genomic tools developed for selective breeding of mammalian production animals to improve stocks in bees. Honeybees' short generation time and the lack of intensive selective breeding, mean that significant genetic gains must be possible by deploying appropriate and well researched whole-genome selection techniques.

Alongside this we need to understand that New Zealand's production ecosystems are unique and simplified. We simply do not know enough about the interactions between bees, pesticides and pathogens in the New Zealand environment, and it is not enough to rely on overseas research to provide solutions. Knowledge of the impacts on, and the value of, bees in New Zealand is vital if we are to continue to have healthy bee populations.

Honeybee-related science, especially in New Zealand, is a remarkable case study of the value of basic science to produce applied outcomes. More than that, it shows how questions posed by industry problems can trigger some of the most interesting fundamental research. In the case of manuka honey, this research has transformed the industry. Honeybees provide insights into a wide range of biological questions as well as providing a high-value industry. Innovative science is required to unlock that biological knowledge for the benefit of the environment and human health as well as supporting and improving this remarkable and valuable little insect.

## References

Adams, C.J.; Manley-Harris, M.; Molan, P.C. 2009. The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydrate Research 344(8)*: 1050–1053.

al Somal, N.; Coley, K.E.; Molan, P.C.; Hancock , B.M. 1994. Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. *Journal of the Royal Society of Medicine 87(1)*: 9–12.

Beggs, K.T.; Glendining, K.A.; Marechal, N.M.; Vergoz,V.; Nakamura, I.; Slessor, K.N.; Mercer, A.R. 2007. Queen pheromone modulates brain dopamine function in worker honey bees. *Proceedings of the National Academy of Sciences USA 104(7)*: 2460–2464.

Beggs, K.T.; Mercer, A.R. 2009. Dopamine receptor activation by honey bee queen pheromone. *Current Biology 19(14)*: 1206–1209.

Belien, T.; Kellers, J.; Heylen, K.; Keulemans, W.; Billen, J.; Arckens, L.; Huybrechts, R.; Gobin, B. 2009. Effects of sublethal doses of crop protection agents on honey bee (*Apis mellifera*) global colony vitality and its potential link with aberrant foraging activity. *Communications in Agricultural and Applied Biological Sciences 74(1)*: 245–253.

Berry, J.A.; Hood, W.M.; Pietravalle, S.; Delaplane, K.S. 2013. Field-level sublethal effects of approved bee hive chemicals on honey bees (*Apis mellifera* L.). *PLoS One 8(10)*: e76536.

Beye, M. 2004. The dice of fate: the *csd* gene and how its allelic composition regulates sexual development in the honey bee, *Apis mellifera*. *Bioessays 26(10)*: 1131–1139.

Beye, M.; Hasselmann, M.; Fondrk, M.K.; Page, R.E.; Omholt, S.W. 2003. The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. *Cell 114(4)*: 419–429.

Bonmatin, J.M.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D.P.; Krupke, C.; Liess, M.; Long, E.; Marzaro, M.; Mitchell, E.A.; Noome, D.A.; Simon-Delso, N.; Tapparo, A. 2014. Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Science and Pollution Research International* Epub, 7 Aug 2014.

Bopp, D.; Saccone, G.; Beye, M. 2014. Sex determination in insects: variations on a common theme. *Sex Dev. 8(1–3)*: 20–28.

Bowen-Walker, P.L.; Martin, S.J.; Gunn, A. 1999. The transmission of deformed wing virus between honeybees (*Apis mellifera L.*) by the ectoparasitic mite *Varroa jacobsoni* Oud. *Journal of Invertebrate Pathology 73(1)*: 101–106.

Cameron, R.C.; Duncan, E.J.; Dearden, P.K. 2013. Biased gene expression in early honeybee larval development. *BMC Genomics 14*: 903.

Chagnon, M.; Kreutzweiser, D.; Mitchell, E.A.; Morrissey, C.A.; Noome, D.A.; Van der Sluijs, J.P. 2014. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environmental Science and Pollution Research International* Epub, 19 Jul 2014.

Cheeseman, J.F.; Millar, C.D.; Greggers, U.; Lehmann, K.; Pawley, M.D.; Gallistel, C.R.; Warman, G.R.; Menzel, R. 2014. Way-finding in displaced clock-shifted bees proves bees use a cognitive map. *Proceedings of the National Academy of Sciences USA 111(24)*: 8949–8954.

Cheeseman, J.F.; Winnebeck, E.C.; Millar, C.D.; Kirkland, L.S.; Sleigh, J.; Goodwin, M.; Pawley, M.D.; Bloch, G.; Lehmann, K.; Menzel, R.; Warman, G.R. 2012. General anesthesia alters time perception by phase shifting the circadian clock. *Proceedings of the National Academy of Sciences USA 109(18)*: 7061–7066.

Cooper, R.A.; Molan, P.C.; Krishnamoorthy, L.; Harding, K.G. 2001. Manuka honey used to heal a recalcitrant surgical wound. *European Journal of Clinical Microbiology & Infectious Diseases 20(10)*: 758–759.

Crozier, R.H. 1977. Evolutionary genetics of the Hymenoptera. *Annual Review of Entomology 22*: 263–288.

Dainat, B.; Vanengelsdorp, D.; Neumann, P. 2012. Colony collapse disorder in Europe. *Environmental Microbiology Reports 4(1)*: 123–125.

Danka, R.G.; Harris, J.W.; Villa, J.D. 2011. Expression of Varroa sensitive hygiene (VSH) in commercial VSH honey bees (Hymenoptera: Apidae. *Journal of Economic Entomology 104(3)*: 745–749.

Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection.* London, UK, J. Murray.

Davie-Martin, C.L.; Hageman, K.J.; Chin, Y.P. 2013. An improved screening tool for predicting volatilization of pesticides applied to soils. *Environmental Science & Technology 47(2)*: 868–876.

de Almeida Rossi, C.; Roat, T.C.; Tavares, D.A.; Cintra-Socolowski, P.; Malaspina, O. 2013. Brain morphophysiology of Africanized bee *Apis mellifera* exposed to sublethal doses of imidacloprid. *Archives of Environmental Contamination & Toxicology 65(2)*: 234–243.

Dearden, P.K.; Duncan, E.J.; Wilson, M.J. 2009. RNA interference (RNAi) in honeybee (*Apis mellifera*) embryos. *Cold Spring Harbour Protocols 2009(6)*: pdb prot5228.

Dearden, P.K.; Wilson, M.J.; Sablan, L.; Osborne, P.W.; Havler, M.; McNaughton, E.; Kimura, K.; Milshina, N.V.; Hasselmann, M.; Gemp, T.; Schioett, M.; Brown, S.J.; Elsik, C.G.; Holland, P.W.; Kadowaki, T.; Beye, M. 2006. Patterns of conservation and change in honey bee developmental genes. *Genome Research 16*: 1376–1384.

English, H.K.; Pack, A.R.; Molan, P.C. 2004. The effects of manuka honey on plaque and gingivitis: a pilot study. *Journal of the International Academy of Periodontology 6(2)*: 63–67.

European Food Safety Authority 2013. Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid. *EFSA Journal 11(1)*: 3068.

Francis, R.M.; Nielsen, S.L.; Kryger, P. 2013. Varroa-virus interaction in collapsing honey bee colonies. *PLoS One 8(3)*: e57540.

Gallai, N.; Salles, J.M.; Settele, J.; Vaissiere, B.E. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics 68(3)*: 810–821.

Gempe, T.; Beye, M. 2011. Function and evolution of sex determination mechanisms, genes and pathways in insects. *Bioessays 33(1)*: 52–60.

Genersch, E. 2010. American Foulbrood in honeybees and its causative agent, *Paenibacillus* larvae. *Journal of Invertebrate Pathology 103 Suppl 1*: S10–19.

Gluckman, P.; Hansen, M. 2004. *The Fetal Matrix: Evolution, Development and Disease*. Cambridge University Press.

Gobin, B.; Heylen, K.; Billen, J.; Arckens, L.; Huybrechts, R. 2008. Sublethal effects of crop protection on honey bee pollination: foraging behaviour and flower visits. *Communications in Agriculture and Applied Biological Sciences 73(3)*: 405–408.

Gonzalez-Cabrera, J.; Davies, T.G.; Field, L.M.; Kennedy, P.J.; Williamson, M.S. 2013. An amino acid substitution (L925V) associated with resistance to pyrethroids in *Varroa destructor*. *PLoS One 8(12)*: e82941.

Goodwin, M. 2006. *Elimination of American Foulbrood Disease Without the Use of Drugs*. National Beekeepers Association of New Zealand. http://www.nba.org.nz/publications/

Hamilton, W.D. 1964. The genetical evolution of social behaviour. I. *Journal of Theoretical Biology 7(1)*: 1–16.

Hamilton, W.D. 1964. The genetical evolution of social behaviour. II. *Journal of Theoretical Biology 7(1)*: 17–52.

Hasselmann, M.; Vekemans, X.; Pflugfelder, J.; Koeniger, N.; Koeniger, G.; Tingek, S.; Beye, M. 2008. Evidence for convergent nucleotide evolution and high allelic turnover rates at the *complementary sex determiner* gene of Western and Asian honeybees. *Molecular Biology and Evolution 25(4)*: 696–708.

Hedges, S.B.; Dudley, J.; Kumar, S. 2006. TimeTree: a public knowledge-base of divergence times among organisms. *Bioinformatics 22(23)*: 2971–2972.

Hughes, W.O.; Oldroyd, B.P.; Beekman, M.; Ratnieks, F.L. 2008. Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science 320(5880)*: 1213–1216.

Hyink, O.; Laas, F.; Dearden, P. 2013. Genetic tests for alleles of *complementary-sex-determiner* to support honeybee breeding programmes. *Apidologie 44(3)*: 306–313.

Kleespies, R.G.; Radtke, J.; Bienefeld, K. 2000. Virus-like particles found in the ectoparasitic bee mite *Varroa jacobsoni* Oudemans. *Journal of Invertebrate Pathology 75(1)*: 87–90.

Klein, A.M.; Vaissiere, B.E.; Cane, J.H.; Steffan-Dewenter, I.; Cunningham, S.A.; Kremen, C.; Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings Royal Society of London B. Biological Sciences 274(1608)*: 303–313.

Lynch, J. 2014. Diversity of molecules and mechanisms in establishing insect anterior–posterior polarity. *Current Opinions in Insect Science 1*: 39–44.

Maggi, M.D.; Ruffinengo, S.R.; Damiani, N.; Sardella, N.H.; Eguaras, M.J. 2009. First detection of *Varroa destructor* resistance to coumaphos in Argentina. *Experimental and Applied Acarology 47(4)*: 317–320.

Martin, S.J.; Elzen, P.J.; Rubink, W.R. 2002. Effect of acaricide resistance on reproductive ability of the honey bee mite *Varroa destructor*. *Experimental and Applied Acarology 27(3)*: 195–207.

Ministry for Culture and Heritage. 2014. Honey bees brought to NZ. http://www.nzhistory.net.nz/mary-bumby-brings-the-first-honey-bees-in-new-zealand

Ministry of Primary Industries 2013. Ministry of Primary Industries information on pollinator security for the Local Government and Environment Committee. www.parliament.nz/resource/mi-nz/50SCLGE_EVI_49DBHOH_PET3075_1_A347361/e387f4d64bb700c8d2dad551cf77fec9f4a1298d

Miozes-Koch, R.; Slabezki, Y.; Efrat, H.; Kalev, H.; Kamer, Y.; Yakobson, D.A. 2000. First detection in Israel of fluvalinate resistance in the varroa mite using bioassay and biochemical methods. *Experimental and Applied Acarology 24(1)*: 35–43.

Mondet, F.; de Miranda, J.R.; Kretzschmar, A.; Le Conte, Y.; Mercer, A.R. 2014. On the front line: quantitative virus dynamics in honeybee (*Apis mellifera* L.) colonies along a new expansion front of the parasite *Varroa destructor*. *PLoS Pathogens 10(8)*: e1004323.

Mondet, F.; Goodwin, M.; Mercer, A. 2011. Age-related changes in the behavioural response of honeybees to Apiguard(R), a thymol-based treatment used to control the mite *Varroa destructor*. *Journal of Comparative Physiology A. Neuroethology, Sensory, Neural and Behavioral Physiology 197(11)*: 1055–1062.

National Beekeepers Association. 2014. http://www.nba.org.nz.

National Pest Management Strategy [undated] American Foulbrood Pest Management Strategy. http://afb.org.nz

Neumann, P.; Yanez, O.; Fries, I.; de Miranda, J.R. 2012. Varroa invasion and virus adaptation. *Trends in Parasitology 28(9)*: 353–354.

Nowak, M.A.; Tarnita, C.E.; Wilson, E.O. 2010. The evolution of eusociality. *Nature 466(7310)*: 1057–1062.

Packer, L.; Owen, R. 2001. Population genetic aspects of pollinator decline. *Conservation Ecology 5(1)*: 4.

Primary Production Committee 2014. Briefing on the health of bees. http://www.parliament.nz/en-nz/pb/sc/business-summary/00DBSCH_INQ_12262_1/briefing-on-the-health-of-bees

Rogers, K.M.; Somerton, K.; Rogers, P.; Cox, J. 2010. Eliminating false positive C4 sugar tests on New Zealand Manuka honey. *Rapid Communications in Mass Spectrometry 24(16)*: 2370–2374.

Rosenkranz, P.; Aumeier, P.; Ziegelmann, B. 2010. Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology 103 Suppl 1*: S96–119.

Ryabov, E.V.; Wood, G.R.; Fannon, J.M.; Moore, J.D.; Bull, J.C.; Chandler, D.; Mead, A.; Burroughs, N.; Evans, D.J. 2014. A virulent strain of deformed wing virus (DWV) of honeybees (*Apis mellifera*) prevails after *Varroa destructor*-mediated, or in vitro, transmission. *PLoS Pathogens 10(6)*: e1004230.

Smodis Skerl, M.I.; Kmecl, V.; Gregorc, A. 2010. Exposure to pesticides at sublethal level and their distribution within a honey bee (*Apis mellifera*) colony. *Bulletin of Environmental Contamination and Toxicology 85(2)*: 125–128.

Statistics New Zealand 2012. *Agricultural Yearbook 2012*. http://www.stats.govt.nz/~/media/Statistics/yearbook/tables/agri-yrbook-2012.xlsx Last modified Wednesday 3 July 2013.

Tautz, J. 2008. *The Buzz about Bees*. London, Springer.

The Honey Bee Genome Sequencing Consortium 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature 443*: 931–949.

Thompson, H.M.; Maus, C. 2007. The relevance of sublethal effects in honey bee testing for pesticide risk assessment. *Pest Management Science 63(11)*: 1058–1061.

van der Sluijs, J.P.; Amaral-Rogers, V.; Belzunces, L.P.; Bijleveld van Lexmond, M.F.; Bonmatin, J.M.; Chagnon, M.; Downs, C.A.; Furlan, L.; Gibbons, D.W.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D.P.; Krupke, C.; Liess, M.; Long, E.; McField, M.; Mineau, P.; Mitchell, E.A.; Morrissey, C.A.; Noome, D.A.;

Pisa, L.; Settele, J.; Simon-Delso, N.; Stark, J.D.; Tapparo, A.; Van Dyck, H.; van Praagh, J.; Whitehorn, P.R.; Wiemers, M. 2014. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. *Environmental Science and Pollution Research* DOI 10.1007/s11356-014-3229-5.

Van Eaton, C. 2014. *Manuka: The biography of an extraordinary honey*. New Zealand, Exisle Publishing.

Vanengelsdorp, D.; Evans, J.D.; Saegerman, C.; Mullin, C.; Haubruge, E.; Nguyen, B.K.; Frazier, M.; Frazier, J.; Cox-Foster, D.; Chen, Y.; Underwood, R.; Tarpy, D.R.; Pettis, J.S. 2009. Colony collapse disorder: a descriptive study. *PLoS One 4(8)*: e6481.

Vergoz, V.; McQuillan, H.J.; Geddes, L.H.; Pullar, K.; Nicholson, B.J.; Paulin, M.G.; Mercer, A.R. 2009. Peripheral modulation of worker bee responses to queen mandibular pheromone. *Proceedings of the National Academy of Sciences USA 106(49)*: 20930–20935.

Vergoz, V.; Schreurs, H.A.; Mercer, A.R. 2007. Queen pheromone blocks aversive learning in young worker bees. *Science 317(5836)*: 384–386.

Ward, K.; Danka, R.; Ward, R. 2008. Comparative performance of two mite-resistant stocks of honey bees (Hymenoptera: Apidae) in Alabama beekeeping operations. *Journal of Economic Entomology 101(3)*: 654–659.

West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution*. Oxford, Oxford University Press.

Williams, G.R.; Tarpy, D.R.; vanEngelsdorp, D.; Chauzat, M.P.; Cox-Foster, D.L.; Delaplane, K.S.; Neumann, P.; Pettis, J.S.; Rogers, R.E.; Shutler, D. 2010. Colony Collapse Disorder in context. *Bioessays 32(10)*: 845–846.

Willix, D. J.; Molan, P.C.; Harfoot, C.G. 1992. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *Journal of Applied Bacteriology 73(5)*: 388–394.

Wilson, M.J.; P. K. Dearden, P.K. 2009. Tailless patterning functions are conserved in the honeybee even in the absence of Torso signaling. *Developmental Biology 335(1)*: 276–287.

Wilson, M.J.; Dearden, P.K. 2011. Diversity in insect axis formation: two orthodenticle genes and hunchback act in anterior patterning and influence dorsoventral organization in the honeybee (*Apis mellifera*). *Development 138(16)*: 3497–3507.

Wilson, M.J.; Dearden, P.K. 2012. Pair-rule gene orthologues have unexpected maternal roles in the honeybee (*Apis mellifera*). *PLoS One 7(9)*: e46490.

Wilson, M.J.; Havler, M.; Dearden, P.K. 2010. Giant, Kruppel, and caudal act as gap genes with extensive roles in patterning the honeybee embryo. *Developmental Biology 339(1)*: 200–211.

Wood, B.; Rademaker, M.; Molan, P. 1997. Manuka honey, a low cost leg ulcer dressing. *New Zealand Medical Journal 110(1040)*: 107.

Wynn, K.; Milne, J.; Leake, J. 2013. The land of bad milk and fake honey. *New Zealand Herald* 25 Aug 2013.

Yang, E.C.; Chuang, Y.C.; Chen, Y.L.; Chang, L.H. 2008. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *Journal of Economic Entomology 101(6)*: 1743–1748.

Zayed, A.; Roubik, D.W.; Packer, L. 2004. Use of diploid male frequency data as an indicator of pollinator decline. *Proceedings of the Royal Society B. Biological Sciences 271*: S9–S12.