PROJECT SUMMARY

RURAL INDUSTRIES
Research & Development Corporation

A Genetic Test for Africanised Honey Bees

Introduction

This project has developed a protocol for testing bees and bee semen for Africanisation, or the subspecies *A. m. scutellata* and *A. m. capensis*, to enable importation of semen and bees from countries where Africanised bees have been reported. Australian beekeepers are currently unable to import bees from some nations due to concerns over invasive honey bee strains. Africanised bees are hybrids of European bees and *A. m. scutellata* from Africa that are highly aggressive, unsuitable for beekeeping and extremely invasive. They are extant throughout much of the Americas. Workers of the Cape honey bee *A. m. capensis*, also from Africa, are capable of cloning themselves, and have led to the death of many thousands of colonies each year in South Africa through their unbridled production of workers that spend their time reproducing rather than working.

An A. m. scutellata (yellow) and A. m. capensis (black) worker.

Honey bee breeders have an interest in importing strains of bee from the United States that have been bred for resistance to the Varroa mite and other genetically improved stock, but are prevented from doing so because Africanised bees are present in the some parts of the US.

The results of this project will be of interest to the Australian Queen Bee Breeders Association, Australian Honey Bee Industry Council (AHBIC), Australian Bureau of Agriculture and Resource Economics and Sciences (ABARES), the Australian Government Department of Agriculture and Water Resources and beekeepers.

The honey bee industry is active in all states of Australia and each state has its own association. Nationally AHBIC, the Australian Queen Bee Breeders Association, the National Council of Pollination Associations, the Honey Packers and Marketers Association and Plant Health Australia represent the industry.

There were over 12,150 registered beekeepers with over 521,000 hives in 2013-2014, with approximately 40% of hives and 30% of beekeepers residing in NSW (Plant Health Australia 2016).

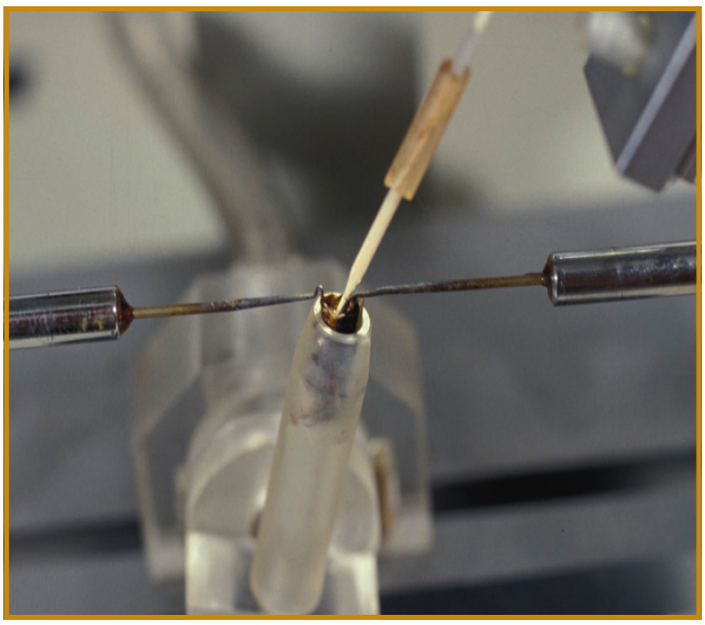
Honey and wax production contribute $92m annually to the Australian economy (ABARES 2013). 35 industries are dependent on honey bee pollination for most of their production and pollination services were valued at $1.7 billion annually in 1999-2000 (Keogh *et al.* 2010) and as much as $6 billion more recently (Plant Health Australia 2016).

Bee breeders, honey, pollen and wax producers and farmers of pollinated crops will all benefit from this research.

Background

Beekeepers wish to import bees/bee semen that have been bred for resistance to the Varroa mite (*Varroa destructor*). Australia currently does not have the Varroa mite, but it is likely that it will get it in the future. The Varroa mite weakens and kills bee colonies, and can decimate populations. Australian bees have little resistance to the Varroa mite, therefore the beekeeping industry and agricultural industries that rely on them for pollination would be severely impacted.

Importing Varroa-resistant stock will likely help to mitigate these effects. However this is currently not possible because Africanised bees are present in the South and South-western states of the US. Africanised bees out-compete strains of bees that have been bred for beekeeping and are themselves not amenable to beekeeping. This project created a genetic test to differentiate Africanised bees from non-Africanised bees to enable importation from countries that have desirable stock, but also have Africanised bees.



Artificial insemination of a queen honey bee.

Aims/Objectives

This project aimed to develop a set of 100 single nucleotide polymorphisms (SNPs) that are diagnostic of African and Africanised bees and establish a testing procedure for importation of queen bees and bee semen that is acceptable to Biosecurity Australia and the beekeeping industry in terms of both cost and security.

The beekeeping industry and agricultural industries that rely on bees for pollination would be severely impacted if either Africanised bees or the Varroa mite were introduced to Australia. The test would keep Africanised bees out of Australia while enabling genetically improved stock that has been bred for resistance to the Varroa mite to be introduced.

Methodology

SNPs (single nucleotide polymorphism) genetic markers (n = 144) were selected via whole genome sequencing to differentiate between 3 ancestral lineages of honey bee: African (A), Eastern European (C) and Western European (M). SNPs were tested on a panel of reference bees from Europe and *A. m. scutellata* from Africa.

Samples from the Australian feral and commercial populations, US commercial and Varroa-resistant populations, the commercial Canadian population, Africanised bees from Brazil and the US were typed with 144 SNPs to assign the proportion of ancestry from the ancestral lineages using the programme STRUCTURE. Ultimately 95 SNPs produced useable data.

False negative and false positive frequencies were calculated for various thresholds at which a sample would be declared Africanised.

Results/Findings

A genetic test was created based on 95 SNPs for differentiating between African/Africanised bees and European-derived bees. When samples with greater than 15% African ancestry are declared Africanised the false negative rate was 0% and the false positive rate was 4.7%, resulting in a small proportion of non-Africanised bees being declared Africanised that would be denied entry.

It was found that enough DNA can be extracted from queen wingtips to perform the test. While the test can be performed directly on semen, the test cannot pick up a small contribution of Africanised semen in mixed semen samples.

A cheaper 40 SNP test was also created, that appears to perform comparably to the 95 SNP test, based on preliminary analysis.

In addition, protocols for testing samples at the Australian Genomic Research Facility, and provide price estimates has been developed.

The creation of a test for Africanised bees provides the tools needed to differentiate desirable strains of bees from undesirable (Africanised, *A. m. scutellata* and *A. m. capensis*) strains of bees. Honey bee import conditions are under review and the present draft enables importation of semen from countries that have Africanised bees if they pass a suitable test for Africanisation.

Implications

The creation of the test for Africanised bees opens the opportunity for industry to import honey bee queens and semen from countries that have Africanised bees. Honey bees are not native to Australia, but much of our agriculture is reliant upon them. Australia is currently free of the Varroa mite and a number of other pests and diseases. Opening new avenues for importation opens the possibility of bringing new pests and diseases into the country, however the importation of lines of bees that have been bred for resistance to the Varroa mite may improve the resilience of our honey bees should Varroa be introduced. Queen bees can already be imported from a number of countries where Africanised bees are not present, but the Varroa mite and other pests and diseases are present. If Africanised bees can be excluded, the import of sperm is safer than the import of queens, because no mites are present in sperm. It is also cheaper. The ability to import stock that is popular overseas may also open new markets for the sale of queen bees, as beekeepers overseas may wish to only buy bees adapted to their conditions, or Varroa-resistant bees.

The implication for Australia is that aggressive Africanised bees will be kept out of the country, while enabling the industry to continue to improve their stocks. Honey bees are extremely important to agriculture and declining bee populations are a great cause for concern as our human population continues to expand.

The main implication for policy makers is that a test that satisfies the recommendations of the draft policy review for the importation of honey bee semen has been created.

Recommendations

1. That the Australian Government Department of Agriculture and Water Resources consider permitting imports of semen and queen bees from the United States and other nations with Africanised bees, provided that the imported material is tested with the SNP panel and found to have less than 15% African ancestry.
2. Should the beekeeping industry wish to pursue importing lines of bees that have been bred for Varroa resistance then queen breeders and the broader beekeeping industry should consider some means of communal funding for the genetic testing and distribution of Varroa resistant bees. While there could be considerable financial benefit to any one queen breeder having a monopoly on imported stock, this would be at considerable expense and financial risk and would perhaps not result in the wide distribution of Varroa-resistant stock. A communal approach would spread both the risk and the costs.
3. Australia is the last bastion of a Varroa-free honey bee population. While Australia's biosecurity programs have thus far been successful at excluding Varroa, it should be remembered that other countries also have strong biosecurity programs, but nonetheless the Varroa mite did become established. A breeding program is currently underway to improve 'hygenic behaviour' in Australian honey bees, which has been linked to improved resistance to Varroa, but without exposure to Varroa it is impossible to determine the effectiveness of this program in terms of Varroa resistance. Strains with proven tolerance to Varroa exist overseas.
4. Import conditions must minimise time in transit and be easily adhered to by the exporter.

More Information

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The following scientific journals have been published:

Hybrid origins of Australian honeybees (Apis mellifera)
http://link.springer.com/article/10.1007/s13592-015-0371-0

A SNP test to identify Africanized honeybees via proportion of 'African' ancestry
http://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12411/abstract

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