# Approaching a novel species in a commercial environment: Lessons from developing a honeybee breeding scheme

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## Summary

With the growing importance of honeybees for the New Zealand primary industries sector, commercial beekeeping has become the next industry that can and should be the target of livestock breeding efforts. While elite queen breeders have been operating both in New Zealand and overseas for a number of years, no efforts have been made to introduce a structured breeding program on the level of commercial beekeeping operations. Commercial beekeepers rely on the breeding efforts undertaken by queen breeders to improve their populations. Currently, neither producers nor queen breeders have a way to verify progress made on a stud level in a commercial operation. In collaboration with a large-scale beekeeping company, we have developed strategies for data collection and genotyping in a commercial population of *Apis mellifera Ligustica* that is continuously supplied with elite queens. We aim to establish a commercial honeybee breeding program and hope to enable honey producer and queen breeder to gain a better understanding of the impact of stud level genetic improvement on a commercial level.

*Keywords: honeybees, breeding program, honey bees, apis mellifera*

## Introduction

While rising demands for managed insects as pollinators are driving the emergence of beekeeping industries worldwide, the New Zealand beekeeping industry presents a special case. Here, high honey prices underpinned by Mānuka honey, a variety that bees produce based on the nectar of a native *Leptospermum* species and that is marketed for its beneficial properties, have enabled the development of a strong and independent honeybee-related industry. In this environment, commercial beekeeping companies can invest in research and development, which in turn other beekeeping sectors around the world can benefit from in the long term.

Approaching genetic improvement in a new species can be a challenging undertaking. In working with honeybees, some biological realities need to be taken into account: the haplo-diploid system of sex determination, eusocial organisation in colonies, and the small individual size of the queens, which hampers the sampling for genotyping. As a result, specific strategies for the genotyping, performance testing and management of genetic resources need to be developed. Here we describe the findings from laying the foundations for a structured honeybee breeding program.

## Material and methods

**Establishment of intensely monitored hive sites**

Compared to most other livestock industries, commercial beekeeping is a low contact operation. Honeybees are handled as colonies, not as individuals, with the only exception being the queen, and they are usually worked on a schedule, leaving days or even weeks between visits. In order to allow focused genotyping and data collection, the concept of "research hives" was created at the collaborating beekeeping company in 2015. Under this concept, 5 hive sites with 40 colonies each were designated for more intensive data collection. Drone samples were collected from each colony in order to generate a genotype for the residing queen.

Due to the strong influence of the environment on honeybee production traits and behaviour (Costa *et al.* 2012), hive sites were selected to represent a diverse range of environments. Over the season 2015/16, it became apparent that while this was necessary, it led to more variation in both the handling of sites as well as the recording of particular data points, because each site had been assigned to a different beekeeping team. As a result, more stringent procedures were introduced for the season 2016/17.

**Development of standard operating procedures**

In order to minimise the impact of personnel on the variation observed between hive sites, all research sites were put under management of a single beekeeping team in 2016. Standard operating procedures were developed for the setup of research hive sites, for the recording of economically important traits (honey production, gentleness, hive strength, brood viability, post-harvest *Varroa* mite count) and to maintain queen traceability. Both standard operating procedures and standard equipment for the collection of drone larva samples were introduced in 2016 as well. This led to clean samples with very little contamination and low levels of specimen degradation.

**Development of DNA extraction and genotyping protocols**

The availability of queen genotypes plays an enormous role in the success of a commercial honeybee breeding program, largely due to two specific aspects of bee biology.

Honeybee queens are polyandrous, they mate in a single event with multiple partners, and store sperm in a designated organ, the spermatheca, for their entire reproductive lifespan (around 2.5 years). These multiple matings, usually involving 6 to 25 drones (Page and Metcalf 1982), have been found to create more vigorous and productive colonies than matings with a low number of effective males (Mattila et al. 2007). Due to this, restrictive matings, for example in the form of single drone artificial insemination, can lead to diminished colony survival and viability. As such, even after the employment of a targeted mating technique such as artificial insemination, paternity of a specific queen usually cannot be determined in the absence of genotyping.

In addition, lack of genetic diversity has a very direct impact on a honeybee population because of their haplo-diploid system of sex determination. A gene cascade lead by the gene *csd*, the complimentary sex determiner, decides the sex of a developing embryo based on the number of *csd* alleles present (Kaskinova and Nikolenko 2017). While diploid individuals develop into female workers and queens, haploid embryos turn into male drones. In the case of a matched mating, however, where a queen mates with a drone carrying one of

her *csd* alleles, half of the diploid offspring are homozygous at the *csd* locus and develop into diploid males. These "accidental" males present a drain on the colony, both in terms of resources for larva production and in missing workers, leaving inbred colonies weakened.

To address these issues on the basis of DNA evidence, standardised protocols for the extraction of DNA and for the process of genotyping itself are needed. As a cost-effective genotyping method with very little development costs attached, genotyping-by-sequencing (GBS) was chosen. GBS involves the enzymatic digest of genomic DNA, and calls for high quality of the submitted material. Direct transfer of existing protocols for the extraction of genomic DNA proved to be difficult, despite the common use of insects as model organisms in diverse research areas. A high-yielding method using the "Quick-DNA™ Tissue/Insect 96 Kit" (Zymo Research, Irvine, CA, USA) turned out to be severely limited by the amount of material that could be subjected to the extraction process, but was eventually successfully modified to fit the purpose. Genotyping itself was performed using a double digest in an optimised version of the GBS protocol originally developed by Elshire *et al.* (2011). (Further details can be found in Petersen *et al.* (2017))

Additional genotyping targeting specifically the *csd* locus using an established high-throughput protocol (Hyink and Dearden, unpublished), is underway.

## Discussion and outlook

*Apis mellifera* has been domesticated for thousands of years, and locally adapted subspecies, such as the Italian Bee, *A. m. Ligustica*, show very specific sets of characteristics. However, the targeted breeding efforts that have led to the establishment of separate breeds in other livestock species have largely remained absent. With the emergence of pathogens such as American Foulbrood and the parasitic mite *Varroa destructor*, breeding schemes focusing on disease resistance traits gained momentum, proving that selection for specific traits is possible (Ward *et al.* 2008). Simultaneously, the successful creation of the composite Buckfast Bee illustrates the potential opportunities for driven individual breeders working to improve overall honeybee performance.

The initial assumption was that a direct transfer of knowledge on genetic improvement gained in working with other species would be possible. Strategies for both phenotyping and genotyping would have to be adjusted, but the bulk of research and development had already been done. Unfortunately, this turned out to be only partially true. Despite the fact that New Zealand beekeepers in particular are aware of their shortcomings in the department of genetic improvement and are eager to participate in any developments in this sector, communication with the beekeepers themselves and implementation of standardised, comparable protocols for performance testing required a lot of time spent building trust and mutual respect. This included the acceptance of selection decisions based on personal preference or emotional aspects[1] comparable to type traits in other species alongside with more obviously economically relevant traits.

Similarly, in working directly with a commercial beekeeping company, development of standardised operating procedures turned out to be necessary to create reliable records. During this process it became clear that these need to be revised on a regular basis to fit specific operations or circumstances, but present a good way to establish a basic framework for phenotyping in a naïve industry.

The development of a commercially accessible genotyping protocol took longer than expected, and is still not entirely completed. Due to the small size and relatively short life span of honeybee queens, high costs cannot be justified and a good balance between the

information made available by genotyping and the cost per individual needs to be found. Affordable genotyping methods open the door to managing the genetic diversity in commercial populations, an important aspect due to the pronounced impact of inbreeding on honeybee colony performance. By obtaining information on the relationships between queens based on their DNA, this is possible even in the absence of paper records or a stringent system of queen traceability, or while these are still being established.

One requirement for the implementation of a structured breeding program is the necessity to control matings. This can be established either by using artificial insemination, or by restricting drone access to virgin queens in isolated mating stations or by managing drone and queen flight times (Oxley *et al.* 2010).

Most commercial honey producers in New Zealand purchase elite queens from designated queen breeders and rely on the breeding efforts undertaken by these to improve their populations. Currently, neither producers nor queen breeders have a way to verify progress made on a stud level in a commercial operation, or select individuals suited for a specific environment. With our approach of using a small subset of a large commercial population as a source of information we aim to enable the honey producer to establish a breeding scheme fitting their specific needs, with special emphasis on maintaining or even increasing genetic diversity. At the same time, both the commercial operator and the queen breeder will be supplied with information to help understand the flow of improved genetics into a wider operation and increase the penetration of genetic improvement.

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