Progress Report on the Industry-funded, RIRDC-managed, Asian Honey Bee – Transition to Management projects as at 20 May 2013

Please Note:
Total funding for the below projects equals $377,000 of the $400,000 honey bee industry (AHBIC and FCAAA) contribution to the AHB T2M. AHBIC is currently seeking a RTO to prepare a quote for a training course for beekeepers in the Cairns region, to spend the remaining $23,000 in accordance with objectives of the AHB T2M.

Organisation – The University of Newcastle
Principal Researcher – David Guez
Project Title – Develop an attractant specific to *A. cerana* Java strain
Timeframe – (31/07/2012 – 1/12/2014)
Industry Funding – $131, 000
Project Summary – This project will determine whether or not it is possible to improve the spontaneous visit rate of feeding stations by giving them flower like characteristics and to use this new feeding system to attempt exclusion of *Apis mellifera* and native bees. The researchers will determine if the use of odorants from Lychee, Mad hatter and Coral Vine can improve spontaneous visitation and recruitment to the bait station. The researchers will also determine the attractiveness of Cymbidium species and to see whether the chemical composition of the semio-chemical produced exclusively attracts *Apis cerana*.

Research in Progress report (at 1 May 2013)
As indicated in my previous report, two master students (Nicholas Wall and Dylan Stolzenhein) have been recruited to perform experiment pertaining to the project. Despite administrative delays the students have been trained in Newcastle prior to going to Cairn permanently to perform the experiments.
Student training:
Since I will only be able to make relatively brief trip to Cairn the rational behind training the student in Newcastle is two- fold:

- Insure that the student can confidently and safely manipulate bees.
- Insure that the students have acquired basic skill necessary to the conduct of the experiments (e.g. experimental rigor, and consistency).

Skill training:
The students have been trained to perform both free flying experiment and Proboscis extension reflex. Although, it is anticipated that most if not all experiment performed in Cairn will be free flying experiment, training in PER experiment (Sugar threshold determination and Olfactory conditioning experiment) were also perform because the allowed me to evaluate their capacity to manipulate in such manner that their results were reproducible (independently to weather consideration).
I needed also the student to learn the importance of good experimental record, something that I believe can only be achieved by facing your own error and avoiding them in the future. I believe that was best achieved on experimental work that had no bearing on the studies that need to be performed for this grant. It also allowed me to provide for a progressive learning curve in the difficulty of the experiment to be performed.

Current short term Experimental plan:
The first experiment to be conducted in the next few weeks is to address the issue of sucrose concentration preference. In other words which sucrose concentration induce the best (in term of magnitude and rate) recruitment possible.
The second experiment will address colour and shape preference to enhance spontaneous feeder visitation. For this experiment bees will be trained to a scented feeder (sucrose concentration to be determined from previous experiment). Once bee number on the feeder have stabilized, the feeder

will be removed and replaced by a choice array of artificial flowers differing only by their respective colour (all containing the same sucrose solution and carrying the same scent than the original feeder). Spontaneous landing on the various colour will be recorded. Shape preference will be evaluated in the same manner.

The result of these two experiments will then be used to test the relative attractiveness of different chemical and flower scent extract by measuring the rate recruitment on scented feeders. In parallel trapping experiment will evaluate the spontaneous attractiveness of each scent or chemical.

Brief protocol for experiment 1

Experiment 1: Sucrose content preference (The methods is the same for evaluating fructose or Glucose content)

1. Prepare identification tag for each feeder indicating scent, sugar content, date, location and experimental repetition (to be placed on the top of the feeder for easy photo identification).
2. Test solution: 20%, 30%, 40%, 50% and 60% (weight/weight) sucrose solution.
3. Scent: Lemon, Almond, Rose, peppermint, vanilla (Test solution and scent association will be counterbalanced. See table 1)

Dilution (w/w)	Repetition 1	Repetition 2	Repetition 3	Repetition 4	Repetition 5
20%	Lemon	Vanilla	Peppermint	Rose	Almond
30%	Almond	Lemon	Vanilla	Peppermint	Rose
40%	Rose	Almond	Lemon	Vanilla	Peppermint
50%	Peppermint	Rose	Almond	Lemon	Vanilla
60%	Vanilla	Peppermint	Rose	Almond	Lemon

Table 1: scent counterbalancing

Note: Each repetition will be performed at different site or if not practical with at least a week interval between each repetition if a site need to be reused.

4. Collect forager bee from flower in tubes (5 bees per tubes).
5. Place bee on feeders and release the bees while they are feeding.
6. Record bee population on each feeder every (5 min) by taking a photo (manual count to be done from photos)

Note: It may be interesting to run the same experiment using fructose solution since fructose solution do not seems to induce recruitment in Apis mellifera (to be tested concurrently).

Organisation – The University of Sydney

Principal Researcher – Ben Oldroyd

Project Title – Inter-specific matings between *A. cerana* and *A. mellifera*?

Timeframe – 2 (29/05/2012-15/05/2014)

Industry Funding – $56, 230

Project Summary: This project will quantify the threats to the Australian honey bee industry associated with interspecific matings by the following experiments: 1) In Cairns, the researchers will perform reciprocal artificial inseminations of *A. cerana* and *A. mellifera*. The researchers will study the eggs of the queens to determine if there is embryogenesis. The researchers will allow some brood to emerge in an incubator to quantify the proportion of offspring that are haploid males, inviable hybrids and thelytokous females. The researchers will use microsatellites to confirm the maternity and (lack of) paternity in the offspring. 2) In the Solomon Islands where there are extremely dense populations of the Java strain of *A. cerana*, the researchers will determine the drone flight time of the males of both species to see if there is overlap. If logistically feasible, the researchers will determine the location of DCAs of *A. mellifera* and *A cerana*. Finally, the researchers will examine the offspring of *A. mellifera* queens that we allow to naturally mate with *A. cerana* males.

Research in Progress report (at 1 May 2013)
20% of a sample of Mellifera queens from China had Cerana semen in their spermatheca. We are waiting on a shipment of A. mellifera queens mated in Cairns, which should arrive next week.
We artificially inseminated 2 Mellifera queens with Cerana sperm in Cairns. We found no evidence of thelytokous reproduction. We DID induce thelytoky in a control queen inseminated with saline. Thus it may be manipulation of the genital tract, rather than heterospecific matings that induces thelytoky. The finding that thelytoky can be induced by inseminating with saline is exciting, with potential applications in breeding and importation.
20 Cerana queens from DAFF collections did not show any heterospecific semen. Thus we suspect that Cerana males can mate with Mellifera queens but not vice versa.
We determined that there is overlap between the drone flight times of Cerana and Mellifera in Cairns. Drone trapping attempts suggested that the congregation areas overlap (i.e. we saw but did not trap).
Bruce White has been unable to contact any beekeeper in the Solomons. I feel that it would be better to devote the funds to working on the Cairns population rather than an attempt to set up work in the Solomons from scratch. [RIRDC has approved this approach.]

Organisation – AgEconPlus Pty Ltd
Principal Researcher – Michael Clarke
Project Title – A strategy to address concerns of countries that import Australian honeybees
Timeframe – (1/6/2012 – 28/09/2012)
Industry Funding – $30, 000

Completed
Final report provided to the AHB T2M Management Group on 4 October 2012.

Organisation – CSIRO
Principal Researcher – John Roberts
Project Title – Establishing the disease status of *A. cerana* Java strain in the Cairns region
Timeframe – (1/06/2012-4/06/2013)
Industry Funding – $109, 212
Project Summary – The objectives for this study are to establish the disease status of the Asian honeybee and the European honeybee in the Cairns region. With this information the researchers will aim to identify the possible transferability of pathogens from the Asian honeybee to the European honeybee in the Cairns region. Identification of honeybee pathogens will involve a two-pronged approach. One approach (1) will engage metagenomic sequencing while the other approach (2) will use standard laboratory procedures as described by Anderson (1990, J. Apic. Res. Vol 29: 53-59) and Chen (2004, J. Inv. Path. Vol 87: 84-93). Metagenomic sequencing of DNA and RNA from pooled samples of *A. cerana and A. mellifera* will be performed at the Biomedical Research Facility based at the Australian National University. Genomic sequence data will be analysed and compared with public sequence databases to assemble partial genomes and identify known and unknown pathogens. The second approach will use bioassays involving the injection of honeybee extracts into pupae and adults of both *A. cerana* and *A. mellifera* to propagate viruses. PCR and serology techniques will be used to identify known viruses. Injected bees that show signs of disease, but are negative in PCR and serology tests, will be further tested to isolate novel pathogens.

Annual Progress Report (as at October 2012)
Bee samples were collected from the Cairns region between 16-7-12 and 27-7-12. Several hundred adult bees were collected from 7 Apis cerana and 14 Apis mellifera colonies, and larvae were

collected from 3 and 5 of these colonies, respectively. An additional 10 adult A. cerana samples (10-20 bees each) were provided by DEEDI. Brood comb was inspected from 4 A. cerana and 14 A. mellifera colonies and samples taken of suspected diseased brood. Chalkbrood and possibly American foulbrood and sacbrood disease were detected in A. cerana colonies, while chalkbrood and possibly European foulbrood and sacbrood disease were detected in A. mellifera colonies. Small hive beetle was also found in A. mellifera colonies and from one dead A. cerana colony provided by a local beekeeper. In addition, adult A. cerana have been tested microscopically for Nosema, tracheal mites and malpighamoeba, with low levels of Nosema found in 4 A. cerana colonies.

Bioassays to propagate viruses in pupae were conducted in Cairns using adult and larval extracts from 3 A. cerana and 5 A. mellifera colonies. All injected pupae and extracts have been tested against available antisera, which detected Kashmir Bee Virus in A. cerana and A. mellifera samples and Black Queen Cell Virus in A. mellifera samples. Positive samples have been confirmed by diagnostic PCR.
Extracts of viral RNA from adult A. cerana and A. mellifera are currently being prepared for deep sequencing using Illumina sequencing technology.

Collection of bee samples from the Cairns region is completed, although it was hoped that more A. cerana colonies with brood would be available. Access to live brood was essential for the bioassays to propagate viruses and for brood comb inspections. Despite attempts to have several suitable A. cerana colonies identified by DEEDI prior to arriving in Cairns, only one colony was available. The other 6 colonies collected were found through public reports during our trip and only 3 colonies were suitable for a bioassay. However, the distribution of these samples across the Cairns restricted area will be suitable to meet the project objectives.

Bioassays and testing against antisera has been completed for all A. cerana and A. mellifera samples. Only few positive samples to common viruses (Kashmir Bee Virus and Black Queen Cell Virus) have been detected with available antisera despite signs of viral infection in many injected pupae. More sensitive testing of these samples through virus purification and deep sequencing is expected to reveal the presence of any undetected viruses.

The planned research was presented at the Queensland Beekeeper's Association at Bribie Island, QLD in June 2012.

Organisation: CSIRO
Principal Researcher – Simon Barry
Project Title – Risk assessment of ports for bee pests and pest bees
Timeframe – (15/06/2012-1/10/2013)
Industry Funding – $50, 600
Project Summary – This project will estimate the relative likelihood of establishment of pest bees and/or bee pests at Australian ports based on the best available information. The researchers will do this by combining likelihood of entry with likelihood of establishment. To estimate likelihood of entry, the researchers will analyse shipping records and combine this with available interception data. The researchers will develop a species distribution model for A. *mellifera* and *A. cerana* to underpin estimates of likelihood of establishment across Australia's ports. This project directly links with other research conducted on this topic, including the recently completed ABARES report 'A benefit-cost framework for responding to an incursion of Varroa destructor'.

Research in Progress report (at 1 May 2013)

The project is in full swing now after key staff have delivered on other contractual commitments.

Accessing data on port layout:

The desktop nature of the study precludes site visits to no more than a few Australian ports of first call, we have subscribed to the IHS Maritime Sea-Web Ports Online module to facilitate access to the physical layout (berthing locations etc.) of all the ports or interest.

Building species distribution models:

From each state and territory we have contacted both an industry and government stakeholder to start an exchange of information on the distribution of feral bees in their state as means of calibrating better species distribution models for A. mellifera and A. cerana. We have also organised spatial GIS layers to underpin this modelling.

Assessing risk models:

We have had discussions with AQIS staff to gather information factors relating to commodities, countries and ports that influence the risk of pest bees or bee pests successfully mounting an incursion.

Analysing shipping data:

We have undertaken preliminary analysis of the arrival pressure of shipping containers by port. We note that industry belief regarding is highly context specific, and this may not be reflected in broadscale analysis of ship movements. For example, cargo from NZ is a particular concern for an introduction of Varroa on A. mellifera, yet small scale supply vessels operating out of Northern Australia are considered a major risk for introduction of A. cerana and Varroa.