



HYGIENIC BEHAVIOUR IN THE AQBBP

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Hygienic behaviour in 50 AI breeder colonies of the Australian Queen Bee Breeding Program, Queensland Australia. 2014.

BENDIGO VICTORIA

Background

Hygienic behaviour is a heritable genetic trait and a specific type of nest hygiene triggered by smell where 15-18 day old workers can detect, uncap, and remove dead or diseased brood from the nest before the disease enters into a transmittable stage (Arathi et al. 2000, Masterman et al. 2001). Some bees are able to detect diseased brood shortly after it is infected and remove it before the pathogen becomes contagious. Colonies that either begin to or completely remove 95% or more of pupae within 24 hours of death are considered to be “rapid hygienic”.

Hygienic behaviour has been shown to be a natural defense against American Foul Brood and Chalkbrood infections (Sturtevant 1953 as reviewed in Spivak and Gilliam 1998, I, Spivak and Gilliam 1998, II). In preparation to live with the devastating honey bee parasite, *Varroa destructor*, it has been recommended by the world’s leading bee authorities that the Australian Honey Bee Industry begin by selecting and breeding for hygienic behaviour to help combat endemic brood diseases.

Hygienic Behaviour in Australia

Breeding for hygienic behaviour is easily performed a freeze-kill brood test to identify hygienic colonies to be used as breeders. It is being implemented worldwide in countries with substantive apicultural industries.

In populations of honey bees that have not been selected specifically for rapid hygienic behaviour, approximately 10% of the colonies will carry this trait (Spivak personal comm). From preliminary testing in Australia on production colonies, just over 15% meet the requirement for rapid hygienic behaviour. These data demonstrate that Australian Honey bee stocks do exhibit rapid hygienic behaviour, yet a number of questions remain:

- What is the origin of the elevated hygienic propensity? i.e. is it coming from breeding stock, or the feral gene pool or a combination?
- If using field assays (freeze killed brood or pin killed brood) to select for rapid hygienic behaviour is not a common practice in Australia, what techniques or influences are helping this trait be expressed?
- Does hygienic behaviour have an influence on the incidence and management of disease such as AFB and chalkbrood in Australia and/or help a colony defend against small hive beetle?

Australian Queen Bee Breeding Program

The Australian Queen Bee Breeding Program (AQBBP) is an initiative of the Australian Honey Bee Industry Council (AHBIC) and is maintained by commercial queen breeders and producers Laurie and Paula Dewer of Dewer Apiaries in Kalbar Queensland. Currently 14 lines are maintained through Artificial Insemination. 5 breeder queens for each line are produced each time the lines are rejuvenated. The best performing of these queens is selected as the next generation queen mother and subsequent potential breeders are inseminated with pooled drone semen from the best performing colonies in the program. Daughters of the breeder queens from each line are open

mated in the breeding yard and distributed around Australia to commercial beekeepers for evaluation in real-world situations. The evaluations and best performing queens are sent back to Laurie and Paula to include in the following line renewal. AI queens are also available to beekeepers for purchase as breeding stock. These lines were most recently renewed in December 2013 and are due to be renewed again soon.

Queensland "Muck"

Queensland muck is a brood disorder experienced by beekeepers in the forested bottomlands in Queensland. This can occur when soils erode and metals such as aluminum-(bauxite) mix with ground water creating an acid soil situation. The alum gets taken up by plants, which in turn enters the pollen and nectar of the plant. When fed to larvae the alum constipates the developing larvae and kills them giving the appearance of EFB. This disorder usually only happens a few times of the year but can substantially impact the brood development and overall colony productivity.

Methods

On October 30th and 31th, 2014 50 breeder colonies headed by artificially inseminated queens from the 14 lines of Australian Queen Bee Breeding Program were assessed for hygienic behaviour using the freeze-kill brood assay with liquid nitrogen. All colonies were checked ahead of time to confirm the presence of the breeding line mother.

The colonies were on a light honey flow, the skies were mostly sunny with temperatures between upper 20's to low 30's with a slight breeze.

On October 30th, 2014 1 brood frame containing sealed brood aged between pre-pupae and purple eye were removed from each colony and labeled with the line and colony numbers. Empty cells were counted and sections of sealed brood containing on average 173 brood cells were frozen with 10 fluid ounces of liquid nitrogen and placed back in the original colony. Twenty-four hours later, on October 31st, the numbers of totally cleaned and partially cleaned cells were counted and a percent hygienic value was calculated. Pictures were taken through the day documenting the activities.

Table 1. Breeder sister queens tested for hygienic behaviour from the Australian Queen Bee Breeding Program, Queensland Australia

Line	Number of colonies tested	Colony Labels
1	3	4, 7, 16
2	3	9, 27, 33
3	4	3, 8, 37, 50
4	4	11, 28, 41, 45
5	4	20, 31, 36, MH
7	5	6, 13, 21, 39, 40
8	4	17, 18, 29, 47
9	2	2, 44
10	3	10, 15, 25
11	4	43, 46, 51
12	3	5, 30, 49
13	4	22, 26, 34, 52
14	2	14, MH
15	5	1, 12, 35, 38, 48



Pulling a frame of brood with young pupae from a breeder colony to be tested for hygienic behaviour



Counting missing cells within the test area prior to pouring liquid nitrogen in the test area



Pouring liquid nitrogen into the test area to kill brood. This is done in a screened tent to prevent robbing



Counting the sealed and partially sealed to calculate % of dead brood removed in 24 hour period

Figure 1. The process of testing for hygienic behaviour on the Australian Queen Bee Breeder's Program breeding Stock, Queensland Australia

Hygienic behaviour is reported as a percent having two values (Figure 2):

- *Clean and Partials (CP)*: the number of cells that were completely cleaned out along with the number of cells in the process of being cleaned out as indicated by any disturbance in the cell capping. Research has shown that cells in the process of being cleaned out will be totally cleaned within the next 24 hours and an appropriate measure of hygienic behaviour and is called the Liberal Test
- *Completely Cleaned*: the number of cells totally cleaned out in 24 hours. Using colonies testing above 95% as queen and drone mothers will seed the hygienic trait into the population most rapidly. This is called the Conservative or Strict Test



Many dead brood cells remain untouched after 24 hours. An example on a non-hygienic colony (not from the AQBBP)

Almost all cells are in the process of being cleaned out as indicated by disturbed cell cappings. Passing the Liberal Test for hygienic behaviour. Test area will be completely clean in another 24 hours

All cells are completely cleaned out in 24 hours. A highly hygienic colony passing the Strict Test for hygienic behaviour.

Figure 2. Comparison between non-hygienic colony, a colony passing the liberal test for hygienic behaviour and a colony passing the strict test for hygienic behaviour.

The Liberal Test (CP) can be used to demonstrate the overall hygienic propensity of the line or for the benefit of who raise their own queens as a way of broadening selection criteria for queen and

drone mothers. The Strict Test can be used by in intensive breeding programs such as the AQBBP for queen and drone mother selection to optimize breeding efficiency.

Results

Liberal Test

Of the 50 colonies evaluated, 36 colonies (72% of total) tested 100-95% hygienic averaging 99% hygienic, 8 colonies (16% of total) tested between 94-80% averaging 91% hygienic and 6 colonies (12% of total) tested were below 79% hygienic averaging 71% hygienic (Table 2 and Figure 3).

Table 2. The distribution and average % hygienic of colonies tested for hygienic behaviour using the Liberal Test (clean and partials) in relation to the 50 total colonies tested from the Australian Queen Bee Breeding Program Queensland, Australia

Hygienic Rating	Highest Hygienic 100-95%	Mid Range Hygienic 94-80%	Lower Hygienic 79-0%
number of colonies	36	8	6
average hygienic %	99%	91%	71%
per cent of total	72%	16%	12%

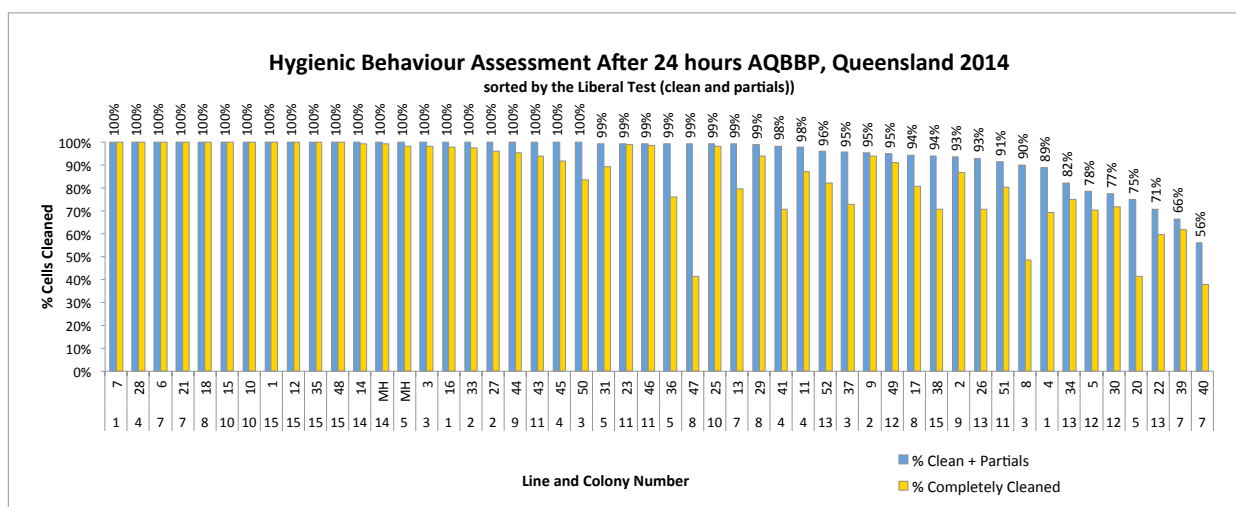


Figure 3. Results from hygienic testing with the freeze kill brood assay. The blue bars show the Liberal Test (number of cells cleaned and partially cleaned out by worker bees in 24 hours). The yellow bars show the Strict test (number of cells totally cleaned out in 24 hours). Data labels at the top of the blue bars reflect scores of the Liberal Test.

Strict Test

Of the 50 colonies evaluated, 22 colonies (44% of total) tested 100-95% hygienic averaging 99% hygienic, 12 colonies (24% of total) tested between 94-80% averaging 88% hygienic and 16 colonies (32% of total) tested were below 79% hygienic averaging 64% hygienic (Table 3 and Figure 4).

Table 3. The distribution and average % hygienic of colonies tested for hygienic behaviour using the Strict Test (completely

clean) in relation to the 50 total colonies tested from the Australian Queen Bee Breeding Program Queensland, Australia

Hygienic Rating	Highest Hygienic 100-95%	Mid Range Hygienic 94-80%	Lower Hygienic 79-0%
number of colonies	22	12	16
average hygienic %	99%	88%	64%
per cent of total	44%	24%	32%

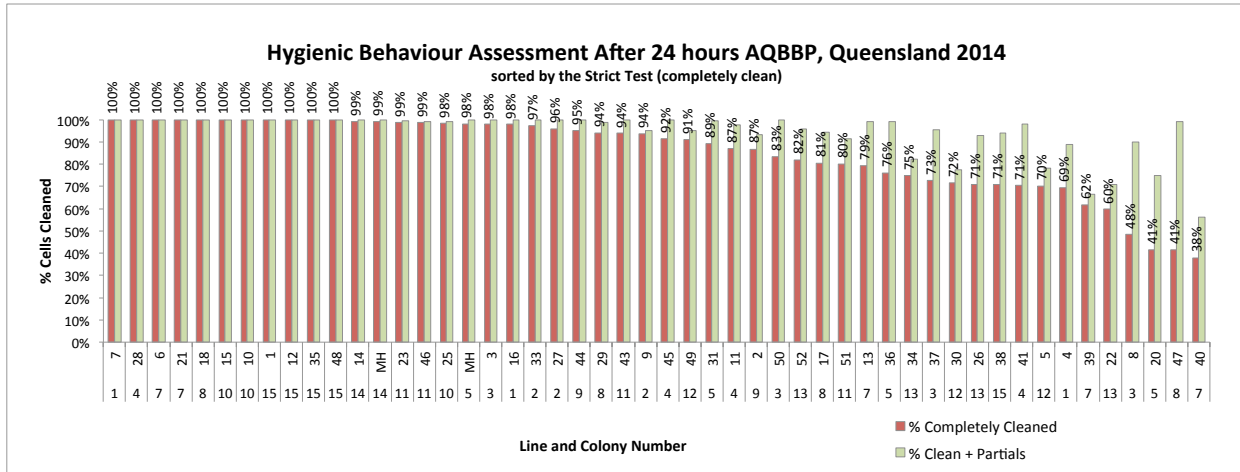


Figure 4. Results from hygienic testing with the freeze kill brood assay. The red bars show the Strict Test (number of cells totally cleaned out in 24 hours). The green bars show the Liberal test (number of cells cleaned and partially cleaned out by worker bees in 24 hours). Data labels at the top of the red bars reflect scores of the Strict Test.

Colony 7 from Line 1 was so hygienic that they tore down all of the comb and left a hole in the wax foundation (Figure 5).



Figure 5. Colony 7 from line 1 removed the dead pupae along with all of the wax on either side of the frame, Queensland

Line Specific Information

LONGEVITY

Longevity is an indication of the number of original queens surviving since the last line renewal. Lines 7 and 15 each contained five sister queens while lines 3, 4, 5, 8, 11 and 13 contained four. The averages for the Liberal and Strict Tests of line 7 were 84% and 76% respectively. The averages of the Liberal and Strict Tests for line 15 were 99% and 94% respectively (Table 4). Although both lines contained the highest number of queens, line 15 was far more hygienic than line 7.

BROOD PATTERN

The quality of brood pattern is reported in per cent of the 173 test cells that were sealed with pupating brood. 100% would indicate that every cell in the test contained sealed brood. The line with the most sealed brood was line 4 (initially from the Better Bees Breeding Program in Western Australia) averaging 84% while line 7 was the lowest with 65% sealed brood. The overall average was 76% sealed brood in test areas.

HYGIENIC PROPENSITY

Of the fourteen lines tested all but two lines contained one or more sister queens that tested 95% or greater on for the Strict Hygienic Test. The two lines not passing the Strict Test, still passed the Liberal Test (Figure 6 and Table 4).

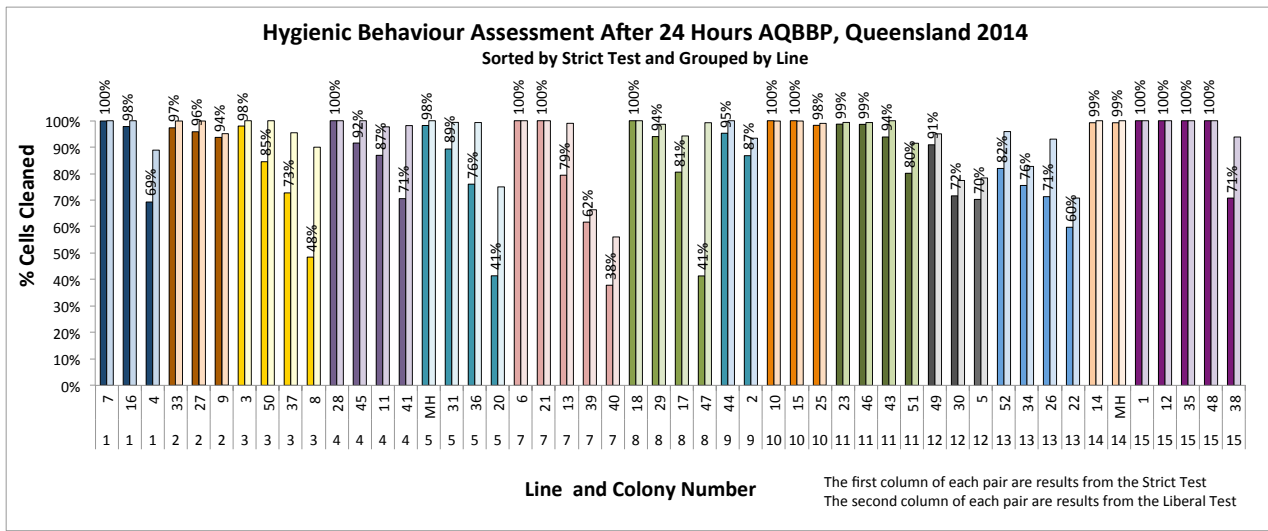


Figure 6. Results from hygienic testing with the freeze kill brood assay. The first column of each pair (dark colour) shows results from the Strict Test with the bars labelled accordingly. The second column of each pair shows the results of the Liberal Test. The data are grouped by line and colour coded accordingly.

Table 4. Results for the % capped brood and Strict and Liberal Tests for hygienic behaviour from 50 colonies headed by AI breeder queens representing 14 lines from the Australian Queen Bee Breeding Program. Within line duplicates are sister queens. Queensland Australia, 2014. N=No Muck, NR=No Report, Y=Muck Present

Line	Colony	Colonies in Line	% Capped Brood	Capped Brood Avg	Liberal Test	Liberal Average	Strict Test	Strict Average	Muck
1	7	3	66%	79%	100%	96%	100%	89%	N
1	16		83%		100%		98%		NR
1	4		88%		89%		69%		Y
2	33	2	65%	77%	100%	98%	97%	96%	N
2	27		83%		100%		96%		N
2	9		83%		95%		94%		NR
3	3	4	86%	74%	100%	96%	98%	76%	N
3	50		82%		100%		85%		NR
3	37		51%		95%		73%		N
3	8		75%		90%		48%		NR
4	28	4	79%	84%	100%	99%	100%	87%	N
4	45		89%		100%		92%		NR
4	11		75%		98%		87%		N
4	41		94%		98%		71%		NR
5	MH	4	64%	79%	100%	93%	98%	76%	N
5	31		92%		99%		89%		NR
5	36		84%		99%		76%		N
5	20		74%		75%		41%		N
7	6	5	69%	65%	100%	84%	100%	76%	Y
7	21		49%		100%		100%		Y
7	13		59%		99%		79%		NR
7	39		62%		66%		62%		NR
7	40		86%		56%		38%		N
8	18	4	68%	78%	100%	98%	100%	79%	NR
8	29		87%		99%		94%		N
8	17		80%		94%		81%		N
8	47		77%		99%		41%		NR
9	44	2	73%	67%	100%	97%	95%	91%	NR
9	2		61%		93%		87%		N
10	10	3	91%	66%	100%	100%	100%	99%	NR
10	15		42%		100%		100%		N
10	25		66%		99%		98%		NR
11	23	4	90%	73%	99%	98%	99%	93%	NR
11	46		84%		99%		99%		NR
11	43		38%		100%		94%		NR
11	51		82%		91%		80%		NR
12	49	3	70%	82%	95%	84%	91%	78%	NR
12	30		90%		77%		72%		Y
12	5		86%		78%		70%		Y
13	52	4	71%	77%	96%	86%	82%	72%	NR
13	34		80%		83%		76%		NR
13	26		66%		93%		71%		NR
13	22		89%		71%		60%		N
14	14	2	76%	76%	100%	100%	99%	99%	NR
14	MH		76%		100%		99%		NR
15	1	5	83%	79%	100%	99%	100%	94%	N
15	12		87%		100%		100%		N
15	35		83%		100%		100%		NR
15	48		66%		100%		100%		NR

15	38	75%	94%	71%	N
Averages		76%	94%	85%	

QUEENSLAND MUCK

The first day of testing we did not notice any Muck in the colonies, the second day of testing we began seeing Muck in colonies and noticed that some colonies were more severely infested than others (Figure 7). Unfortunately, we did only collect data on about half of the colonies with respect to the presence/ absence of Muck. However, from the casual observation, it appears as though colonies that were less hygienic had higher Muck infestations. This avenue clearly needs more careful study.



Figure 7. Queensland Muck caused by larval death due to excess alum uptake by forage plants

Discussion and Recommendations

World Class Stock

This hygienic testing was an exploratory exercise to understand the hygienic propensity within the breeding stock of the Australian Queen Bee Breeding Program with a view of helping to inform a future direction for the program and breeding focus. However, surprisingly these results are unprecedented against a background of unselected stock. The results are more reflective of breeding stock from a program with a long focus of selecting and breeding for hygienic behaviour than that of preliminary testing for gathering baseline data (Table 5).

Table 5. The AQBBP 2014 hygienic tests compared to the established (2004-2009) AI Minnesota Hygienic breeder queens and three commercial operations producing open mated Minnesota Hygienic production queens.

	Colonies Tested	Liberal	Strict
MN Hygienic Breeders 2009	171	75%	36%
Beekeeper 1 2009	118	79%	29%
Beekeeper 2 2009	123	63%	24%
Beekeeper 3 2009	87	62%	24%
AQBBP Breeders 2014	50	72%	44%

These initial tests confirm the high potential of the stock from the Australian Queen Bee Breeding Program with respect to the source of hygienic behaviour. It also raises some important questions about the potential benefits for breeding Australian hygienic stock with respect to best practice pest and disease management and Varroa preparedness. These results also raise caution directed towards the common perception for the need of foreign stock importation to seed hygienic traits into existing Australian honey bee populations.

It has been recommended from the world's renown honey bee experts that Australia prepare for living with Varroa by initiating and sustaining an active hygienic selection program that actively promotes hygienic behaviour in production colonies. These results indicate that unbeknownst to the industry, that process is well underway.

The bees from Australian Queen Bee Breeding Program are carefully scrutinized and evaluated under commercial production conditions and are selected for traditional desirable traits (honey production, temperament, low swarming tendencies, disease resistance, etc.). By incorporating hygienic selection as an additional breeding criterion, it is possible to further enhance the program providing bees that are proven and adapted to Australian conditions.

Future Breeding Efforts

To incorporate hygienic behaviour into the breeding program, it is important to only use the most hygienic queens as queen mothers and highly hygienic queens as drone mothers. This means colonies scoring 95% or better for the Strict Test after two hygienic tests separated by at least a month for queen mothers and scoring in the high 90s on the liberal test for drone mothers. It is extremely important that the hygienic results be consistent regardless of brood clutch (cycle), environmental conditions, or other potentially contributing factors.

Continuing to select for other desirable traits under production conditions is also critical. Keep in mind that it is okay and even recommended to keep colonies in the breeding program that do not meet the high standards for hygienic behaviour if they meet other breeding criteria. Since only 50% of the drones that a virgin mates with need to be from hygienic mothers in order for the trait to be expressed, it is advantageous to keep those other superior colonies in the program regardless of their hygienic propensity.

Line Rejuvenation

With respect to the AI breeder queen mothers, it will be advantageous to rear daughters from the most hygienic of the line queens collecting and pooling drone semen from all of the most hygienic queens. **As many of these AI queens are old and showing signs of failing it is critical that these lines be renewed as soon as possible.** This initial line rejuvenation should proceed directly without testing the breeder colonies twice with the view to establish a regular protocol next renewal.

Production Queens/ Casual Breeders

It is possible to advance the hygienic propensity of open mated queens with only queen mother selection, However to speed up the seeding of this desired trait, adding hygienic drone mother colonies to the vicinity of a the program mating yard will be advantageous. Because of the

devastation of feral colonies by Small Hive Beetle in Southern Queensland, it will be quite possible for open mated production queens to achieve high hygienic standards rather quickly.

Field Testing and Evaluations

In order to understand the effectiveness of the hygienic breeding program on seeding rapid hygienic behaviour into production colonies and subsequent impact on honey bee pest and disease mitigation, a testing and monitoring program would need to be established. Evaluating beekeepers can be trained to test for hygienic behaviour and to monitor pest and disease loads within the colonies. This information can be reported back to the breeding program and the effectiveness can be assessed.

Stock Advertisement, Dissemination and Availability

The queens produced by the Australian Queen Bee Breeding Program are a known asset to the Australian Honey Bee Industry with prospects of laying the foundation to prepare for living with Varroa. Intensive breeding programs such as the AQBBP are expensive to maintain, requiring intensive input for the desired stock. The financial support for maintaining the program either needs to be reflected in the price of the queens sold or be subsidized by industry or other supporting organizations. Charging the true cost of the queens may make them prohibitively expensive inaccessible to the very beekeepers the stock is being bred for.

It is important for the Australian Honey Bee Industry Council to decide the level of long term support and commitment they are prepared to dedicate towards building and developing an industry beekeeper driven accessible stock improvement plan to ensure the perpetuation of this promising stock.

Concluding Remarks

It is arguable that the Australian honey bee industry is at the crossroad to move forward with supporting industries' capability to address both endemic pests and diseases and prepare for living with Varroa through a sustained breeding focus. Quoting Sir Isaac Newton, we have the opportunity to "stand on the shoulders of giants" learning from the experiences and research conducted globally and apply it to our unique Australian environment.

Selecting and breeding for hygienic behaviour is easily done and the stock contained in the AQBBP is the perfect place to exercise our focused breeding efforts aimed at endemic and exotic pest and disease resilience. As technology advances it may become possible to select for traits for that will contribute further to pest and disease resistance in our honey bee stocks, but without the

"Your percentages in Australia are very remarkable and impressive. I do encourage you to test each colony twice, but it seems like Australia has a very good genetic base from which to select for Varroa -resistant bee stocks.

Congratulations on the great work!"

-Dr. Marla Spivak University of Minnesota

network of selecting, breeding, evaluating, and ultimately getting those traits into commercial production operations, no amount of technology will make a difference for industry.

The AQBBP has some exceptional stock that should be given a chance to be bred in a focused way for hygienic behaviour and assessed for the effectiveness of the stock against endemic pests and diseases and also against Varroa. This is a very exciting time and there is much work to be done.

Prepared for Laurie and Paula Dewar of Dewar Apiaries, stock managers for and on the behalf of the Australian Honey Bee Industry Council, for the Australian Queen Bee Breeders Group and the Australian Honey Bee Industry Council, 2014.

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