

# Asian Honey Bee Transition to Management

## 2011 to 2012

Progress report prepared by Russell Gilmour  
Asian Honey Bee Transition to Management Program  
Biosecurity Queensland  
27 June 2012

© State of Queensland, Department of Agriculture, Fisheries and Forestry, 2012.

The Queensland Government supports and encourages the dissemination and exchange of its information. The copyright in this publication is licensed under a Creative Commons Attribution 3.0 Australia (CC BY) licence.



Under this licence you are free, without having to seek permission from DEEDI, to use this publication in accordance with the licence terms.

You must keep intact the copyright notice and attribute the State of Queensland, Department of Employment, Economic Development and Innovation as the source of the publication.

For more information on this licence visit <http://creativecommons.org/licenses/by/3.0/au/deed.en>

## **Contents**

Contents .....	- 3 -
<b>List of Attachments</b> .....	- 4 -
2011 – 2012 Highlights .....	- 6 -
ATTACHMENT A. Biosecurity Queensland Work Plan to deliver T2M Projects, Progress Report @ 8June 2012 (Back to Top).....	- 9 -
Attachment B. AHB T2M Documents produced 2011/2012 (Back to Top) .....	29
Attachment C. AHB T2M Community Engagement Activities 2011/2012 (Back to Top).....	30
Attachment D. Bee Trap Efficacy (Back to Top) .....	33
Results .....	34
Attachment E. Remote Treatment Trial Update as at 26 June 2012 (Back to Top).....	- 37 -
Attachment F. Cairns Port surveillance strategy and trial - AHB suppression zone around seaport. (Back to Top) .....	- 41 -
Attachment G. Optimising early detection of new incursions of AHB - Collaboration between Australian Government DAFF-Biosecurity and Biosecurity Queensland. (Back to Top) .....	- 43 -
Attachment H. Map of Known Infested Area showing recent detections. (Back to Top) .....	- 45 -
Attachment I. Detection of <i>Apis cerana</i> DNA from bee eater pellets and trap liquor (Back to Top).....	- 46 -
Attachment J May 2012: Short study of microsatellite alleles in Asian honey bees Sourced from PNG/Solomons and north Queensland: summary of data interpretation (Back to Top).....	49
Attachment K. Asian honey bee Known Infested Area 6 Month Intervals 2011-2012 .....	- 62 -

This document is prepared for the Transition to Management Group meeting on 27 June 2012. A number of attachments are included to provide greater detail.

### **List of Attachments**

Attachment	Title	Page
<a href="#">Attachment A</a>	Biosecurity Queensland Work Plan to deliver T2M Projects, Progress Report @ 8 June 2012	7
<a href="#">Attachment B</a>	AHB T2M Documents produced 2011/2012	29
<a href="#">Attachment C</a>	AHB T2M Community engagement activities 2011/2012	30
<a href="#">Attachment D</a>	Bee Trap Efficacy	33
<a href="#">Attachment E</a>	Summary of Remote treatment Trial as at 8 June 2012	37
<a href="#">Attachment F</a>	Cairns Port surveillance strategy and trial - AHB suppression zone around seaport.	41
<a href="#">Attachment G</a>	Optimising early detection of new incursions of AHB - Collaboration between DAFF Biosecurity and Biosecurity Queensland	43
<a href="#">Attachment H</a>	Map of Known Infested Area showing recent detections.	45
<a href="#">Attachment I</a>	Detection of <i>Apis cerana</i> DNA from bee eater pellets and trap liquor	46
<a href="#">Attachment J</a>	May 2012: Short study of microsatellite alleles in Asian honey bees sourced from PNG/Solomons and north Queensland: summary of data interpretation	49
Attachment K	Asian honey bee Known Infested Area 6 Month Intervals 2011-2012	62

Eradication operations ceased on the 30 June 2011 when the Program became an Asian honey bee Transition to Management Program.

<b>Situation at June - November 2011</b>	<b>Situation to be achieved by 30 June 2012</b>	<b>Situation to be achieved by 31 December 2012</b>	<b>Situation to be achieved by 30 June 2013</b>	<b>Situation after 1 July 2013</b>
<ul style="list-style-type: none"> <li>Respond to public reports of nests and swarms</li> <li>Don't provide on-ground respond to foraging bee reports within the known infested area.</li> <li>Destroy all AHB Swarms and Nests</li> <li>Confirm extent of infestation through               <ul style="list-style-type: none"> <li>Surveillance (bee lining, floral sweeping and traps) focussed on edge of known infested areas</li> <li>Dog surveillance supporting bee lining</li> </ul> </li> <li>Monitor traps around movement</li> </ul>	<ul style="list-style-type: none"> <li>Continued engagement of transport industries, utilities and other high risk business</li> <li>Build industry and community capacity.</li> <li>Develop agreed policy/processes to transition to industry and community management</li> <li>Cease destruction of nests and swarms, except those that pose a risk to public safety, social amenity, industry or containment.</li> <li>Cease dog surveillance</li> </ul>	<ul style="list-style-type: none"> <li>Training of transport industries, utilities, and other high risk business. This includes developing appropriate industry codes of practise.</li> <li>Training of pest controllers to destroy nests/swarms that affect public safety and social amenity.</li> <li>Trade implications regarding no Restricted Area resolved.</li> <li>Agreement reached with stakeholders regarding any</li> </ul>	<ul style="list-style-type: none"> <li>Industry management of nests/swarms that impact on industry, including responding to reports of swarm and nests (industry impacts)</li> <li>Pest controllers destroy nests/swarms that affect public safety and social amenity.</li> <li>No Government Permits</li> <li>Agreed industry codes of</li> </ul>	<ul style="list-style-type: none"> <li>AHB T2M Program ceases</li> <li>No Government management of AHB.</li> <li>Landowners and industry responsible for managing relevant AHB impacts.</li> <li>Government responsible for:               <ul style="list-style-type: none"> <li>maintaining pest distribution data on web</li> <li>monitoring AHB distribution</li> <li>exotic mite testing</li> <li>Early detection of mites and new incursions of AHB infestation</li> <li>monitoring ports etc</li> </ul> </li> </ul>

<ul style="list-style-type: none"> <li>hubs</li> <li>• Monitor bee eater roosts around edge of infested area</li> <li>• Maintain Restricted Area</li> <li>• Issue permits</li> <li>• Information updates on status of program</li> </ul>	<ul style="list-style-type: none"> <li>• Up to date information available on web</li> <li>• Develop framework for notification of all nests and swarms (so Government and industry can track spread, distribution and manage impacts)</li> <li>• Maintain a single database of destruction events.</li> <li>• Develop strategies for early detection outside known areas</li> </ul>	<p>future legislative controls.</p> <ul style="list-style-type: none"> <li>• Communication strategy for post June 2013 agreed.</li> <li>• Integrated control strategies developed for consideration</li> <li>• Landowner management of nests/swarms in built environment</li> <li>• Modelling of AHB population dynamics and drivers of spread developed for consideration.</li> <li>• Tools and strategies for suppression of AHB in the natural environment developed for consideration</li> <li>• Identification and detection methodologies that can assist with rapid detection of new incursions of AHB developed for consideration.</li> <li>• Report on pathway analysis for AHB including identification of pathways for long distance spread.</li> <li>• Report on links with activities occurring with the implementation of Varroa Continuity strategy.</li> <li>• No Government destruction of swarms and nests in the Known Infested Area.</li> </ul>	<p>practice developed.</p> <ul style="list-style-type: none"> <li>• Maintain accurate information on web</li> <li>• Cease trap monitoring? Or use improved trap monitoring?</li> </ul>	
--	---	---	--	--

## **2011 – 2012 Highlights**

Shift from Eradication to Management has been achieved through the following

- **Reducing operational surveillance and destruction activity to increase community engagement, industry liaison and scientific research.**
  - New skills were recruited into the T2M Program to achieve the goals of the Asian honey bee Transition to Management program - a Senior Scientist, Scientist, Senior Community Engagement Officer and mapping officer were recruited.
  - 28 community events attended throughout 2011/2012 which was more than double previous year. The purpose of attending these events was to educate the industry/community on the shift in focus as the program transitions to industry/community management and demonstrate how to control Asian honey bees in their area. These were provided to a wide range of audiences, such as Beekeepers, transport industries, environmental and indigenous groups, the community at regional shows, pest controllers and pest forums and local government agencies.
  - A highlighted outcome of these engagement activities has been a greater acceptance and cooperation by the local beekeepers club, who are now working with the program to assist with scientific research trails and are helping to develop educational tools to improve public awareness of Asian honey bees. In addition Biosecurity Queensland is providing the Beekeepers with workshop sessions to better equip them with the tools to help support the community to live with Asian honey bees. Liaison committees at local and state level have been developed and are proving to be beneficial to both parties.
  - The local beekeepers association has stated that their working relationship with the program is the best it has ever been and the new staff and shift in management over the last 6 months has significantly encouraged local beekeepers to become actively involved in the program.
- **Maintaining operational activity until management frameworks, information packages, and transitional policies are developed and agreed upon.**
  - Operational focus continues to be suppressing and containing infestations in and at the edge of known areas of infestation around Cairns, the northern beaches of Cairns, west to the Atherton Tablelands and around Innisfail/South Johnstone. Some operational work has been directed towards scientific research so that more can be learnt about AHB and how to manage its impacts.
  - **Detections –**

Staff attrition, especially in field staff, and changed focus from operations to science and community engagement during 2011/2012 has not hampered the operational effectiveness of the AHB field team. While the number of destroyed nests and swarms in 2011/12 was lower than the number of destructions in 2010/2011 (203 Nests, 92 Swarms), it was achieved with 5 times less operational field staff than there was in 2010/11. However, there has been a consequential decrease in proactive surveillance.

    - As at 22 June 2012, 140 nests and 86 swarms have been detected. Since May 2007, a total of 649 AHB nests and swarms have been detected and destroyed. Significant detections this year have been
      - Wangetti Beach (a swarm 2km outside restricted area)
      - South Johnstone (nests approximately 17 kms south of the restricted area)
      - Biboohra (a swarm 12kms from the nearest detection)
      - Kairi ('in fill' infestation within restricted area)

These detections near and outside the restricted area are not surprising given natural, expected increase and resultant spread of the AHB population in the greater Cairns area. It is important to note that there have been no long distance detections.

- **Public reports –**

The public were still actively reporting bees during the year with a similar number reports received to the previous year.

- 159 suspect bees and 719 suspect swarms have been reported in 2011/12, compared to 103 suspect bees and 829 suspect swarms in 2010/11.

A media strategy has been devised to reinvigorate the level of public reporting. A media release will be issued in late June/early July.

- **Website activity –**

- 1 July 2011 - 31 May 2012)
  - The landing page - 1,262 views
  - AHB and varroa mites - 1,301 views
  - Movement restrictions - 1,197 views
- 17 Jan 2012 - 31 May 2012
  - Report AHB sighting - 77 views
- 21 April 2012 - 31 May 2012
  - Identification and surveillance page - 82 views
  - Transition to management page - 32 views

Feedback from various people through stakeholder and community engagement indicates the website required improvements with emphasis on how to identify and manage AHB. The web pages have been reviewed and new pages created to address these areas of concern and will go live in July 2012.

➤ **Developing agreed transitional and management policies and strategies for industry and community to implement.**

- Tools for the community to manage bee situations and help reduce the spread of Asian honey bees have been documented and training is ongoing with key stakeholders such as, beekeepers, pest controllers, transport industries, rangers, local government and the community.
- Collaboration with Australian Government DAFF-Biosecurity regarding a surveillance strategy trial to detect new incursions of AHB in the Cairns area is in progress.
- Asian honey bee spread analysis mapped out to show expansion of Asian honey bees throughout the Cairns region over time. (See Attachment K)
- AHB data has been reviewed and Geodata sets produced. This will be used to replicate a study regarding the possible spread of AHB.

➤ **Documenting and validating eradication techniques so they can be implemented by community and industry**

- A literature review has been conducted to review the critical points of current knowledge about the Asian honey bee (AHB). It highlighted gaps in available information and will be used to develop management and control strategies for AHB in Australia.
- A report on Asian honey bee behavioural observations was completed and made available on the website.
- A trap efficacy report was completed showing very low trap success rates (0.14% for the period January 2011 - May 2012). The continued use of the traps is currently under review and their use may be modified to reflect the results of the PCR project for genetic detection of AHB DNA in trap liquor. Initial results from the PCR project suggests the trap liquor may be used to detect presence/absence of AHB in an area.

- **Remote nest treatment –**

The Scientific Advisory Groups main point of interest was to determine how many bees are needed to take fipronil back to the nest to suppress a nest of a certain size. They were also interested in off target impact especially in the area of green ants. The results to date indicate:

- The number of bees taking fipronil cannot be used to judge suppression of the nest as SAG expected - results were inconsistent; some nests with large numbers (>1000) of bees taking fipronil did not die, and some with very small numbers did die.
- Instead we used the percentage of bees relative to nest entrance activity and relative to feeding station activity, as an estimate of nest size. SAG wanted the actual nest size, but only three nests were extractable for size estimation (and two of them are small), so nest size could not be used.
- The percentage of bees (relative to feeding station activity) taking back fipronil cannot be used to judge suppression of the nest - results were inconsistent (same as using the number of bees).
- The percentage of bees (relative to nest entrance activity) taking back fipronil can be used to judge suppression of the nest - effectiveness of fipronil increases with increasing percentage of bees taking it back to the nest.
- A target of at least 20% of bees relative to nest entrance activity is advisable.
- Residue testing has commenced to determine off target impact has occurred with the following results
  - Apis cerana (AHB) 0.038µg/bee
  - Apis mellifera (EHB) not detected at or above 0.006µg/bee (too few bees
  - were submitted to get a reading of fipronil)
  - Bee hive comb 0.96 µg/g
- We have been advised that Fipronil has toxicity for bees at an LD50 of 0.004µg/bee. That means treated bees are subjected to 10 times the lethal dose.



## ASIAN HONEYBEE TRANSITION TO MANAGEMENT PROGRAM

## Biosecurity Queensland Work Plan to deliver T2M Projects

Progress Report @ 8June 2012

Current Staff information

<b>* Director BQCC</b>	Neil O'Brien	<b>Program Manager</b>	Russell Gilmour	<b>Program Support officer</b> (part time)	Lorraine Nearey
<b>* Manager Community Engagement BQCC*</b> (as required)	Anthony Wright	<b>Technical Advisor</b>	Following engagement of 2 Scientists, this position will not be filled	<b>Information and Mapping Officer</b>	Mel Smith – commenced April 2012
<b>* Principal Scientist BQ Science*</b> (as required)	Dr Jane Oakey	<b>Community Engagement Officer x 2</b>	Brenda Foley – commenced April 2012 Brett Ross Reid	<b>Scientist x 2</b>	Anna Koetz – commenced April 2012 Natalie Wittmeir
<b>#Manager BQ Intelligence Unit</b> (as required)	Dr Joe Scanlan	<b>Compliance Officer</b>	Vacant – this function is being performed by the Coordinator.	<b>Field assistants x 3</b>	Laurel Cinelli (temp) Dianne Morris, Lisa Leeson, Natalie Wittmeir, Arthur Giblin (casuals)
<b>#Director Science</b> (as required)	Dr David Waltisbuhl	<b>Coordinator</b>	Glen Docherty		
<b>#Animal Biosecurity &amp; Welfare policy</b> (as required)	Dr Rick Symons, Dr Allison Crook, Dr Mark Cozens	<b>Team leader</b>	Lewis De Francisco		

\* these positions are national cost shared fire ant and electric ant positions – any AHB related work performed by these positions are charged to the AHB T2M Program. This is consistent with national cost shared funding principles.

# these positions are funded by core Biosecurity Queensland as part of normal commitments.

Current Financial information

AUSTRALIAN GOVERNMENT FUNDING				
	T2M Project	2011/12 funding	Revenue to Date	Comments
<b>AG1</b>	Limiting impact on urban communities	150,000	<b>525,000</b> (excluding GST)	Australian Government funding includes GST. The revenue available for expenditure on the program excludes the GST component. Invoices will be issued to PHA in mid June 2012.
<b>AG2</b>	Developing and making available a suite of control measures for AHB	350,000		
<b>AG3</b>	Limiting impact on honey production	205,000		
<b>AG4</b>	Limiting impact on natural environment	50,000		
<b>AG5</b>	Optimising early detection of new incursions of AHB	200,000		
<b>AG6</b>	Critical intervention to limit long distance spread	200,000		
<b>Total AG funding</b>		<b>1,155,000</b> (including GST)		
QUEENSLAND GOVERNMENT FUNDING				
<b>QG1</b>	Protecting Queensland social amenity and public assets	250,000	<b>175,000</b>	
<b>QG2</b>	Improving operational efficiency and effectiveness	50,000		
<b>Total QG funding</b>		<b>300,000</b>		
<b>TOTAL FUNDING</b>		<b>1,455,000</b>	<b>700,000</b>	<p>The 2011-12 allocation from government to the two year transition program is \$1,350,000. As at 31 May 2012, total expenditure is \$1,128,000, which sees the program budget on a pro-rata basis with a surplus of \$109,000.</p> <p>The pro-rata surplus is due predominantly to the delayed recruitment of staff into the various projects, this will even out over the course of the program as all facets become fully operational. In addition staff attrition has been high and lag time in recruiting replacement staff has resulted in some savings.</p>

## AG1 – Limiting impact on Urban Communities

Objective – informed community and apiary and pest controller industries able to identify AHB and take actions to reduce the impact of AHB on human health, amenity and honey production

Funding and reporting milestones	Status/comments
<b>Milestone 1 – due 1/8/11 (\$75,000)</b> <ul style="list-style-type: none"> <li>Agreement signed</li> <li>CE Coordinator appointed</li> <li>Facebook and Website established</li> </ul>	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Agreement signed. Invoice issued - payment received</li> <li>CE Officer appointed. A senior CE Officer was appointed in April 2012.</li> <li>Facebook and Website established - website regularly updated</li> </ul>
<b>Milestone 2 – due 1/2/12 (no payment)</b> <ul style="list-style-type: none"> <li>Progress report on activities <ul style="list-style-type: none"> <li>Communications and engagement strategy developed</li> <li>Communication material prepared for landowners, householders and pest eradication industries</li> <li>Evaluation of use of website and facebook by landowners, householders and pest eradication industries</li> </ul> </li> </ul>	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Details of progress are listed below</li> <li>Communications and engagement strategy has been developed, and is in use</li> <li>Learning's from ABARES February 2011 report: <i>an evaluation of the AHB community engagement project</i> were identified and incorporated into the operational plan for community engagement activities.</li> <li>A complete revamp of community engagement documents and website has occurred to communicate available tools for the community (Attachment B).</li> <li>A range of community engagement activities have taken place (Attachment C.).</li> </ul>
<b>Milestone 3 – due 15/6/12 (\$75,000)</b> <ul style="list-style-type: none"> <li>Progress report on activities <ul style="list-style-type: none"> <li>Engagement activities undertaken with community, landowners, householders and pest eradication industries</li> <li>Evaluation of use of website and facebook by landowners, householders and pest eradication industries</li> </ul> </li> </ul>	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Community engagement activities attached (Attachment C).</li> <li>New tools developed are awaiting approval before going live on the web.</li> </ul>
<b>Milestone 4 – due 31/12/12 (\$75,000)</b> <ul style="list-style-type: none"> <li>Communication strategy developed to manage communication and engagement following cessation of this project</li> </ul>	
<b>Milestone 5 – due 15/6/13 (\$75,000)</b> <ul style="list-style-type: none"> <li>Final report on activities undertaken in this project</li> </ul>	

Project Number	T2M Deliverable	Actions/strategies	Responsibility	Due Date	Comments/status
A.(i)	Website and facebook site established (adapted from existing sites)	Existing DAFF QLD website maintained	<b>BQCC Cairns</b> CE Officer	Ongoing	<b>COMPLETED AND ONGOING</b> <ul style="list-style-type: none"> <li>Website was updated in April 2012 and again revamped in June 2012. Enhanced identification capacity now available. Website will be maintained monthly and any updates will be documented.</li> <li>Online reporting tool has been added to the website. This allows the public to submit photographs and reports of AHB through the Biosecurity Queensland website. A small number of reports have been received.</li> </ul>
(ii)		New DAFF QLD web and social media features approved	<b>BQCC Cairns</b> CE Officer Coordinator	29.2.12	<b>COMPLETED AND ONGOING</b> <ul style="list-style-type: none"> <li>Biosecurity Queensland facebook page is active <a href="http://www.facebook.com/biosecurityqld">www.facebook.com/biosecurityqld</a></li> <li>A regular social media reporting framework including event calendar has been developed with DAFF QLD's social media unit - regular reports are provided on social media activity including the number of posts from Biosecurity Queensland and the public.</li> </ul>
(iii)		DAFF QLD website is refreshed	<b>BQCC Cairns</b> CE Officer	Initial : 31.3.12 Revised: 31.7.12	<ul style="list-style-type: none"> <li>The website was refreshed in April and June 2012. Final approval of a new website has been delayed because of Machinery of Government changes following a change of government. The revamped website should go live in July.</li> <li>Planning discussions have occurred to develop a Queensland Government Business Industry Franchise to streamline the way clients access information about AHB. Planning is underway to transfer AHB information to this whole of government website.</li> </ul>
B.(i)	Information reviewed and updated	DAFF QLD website is regularly updated as T2M program is	<b>BQCC Cairns</b>	Ongoing	<b>COMPLETED AND ONGOING</b>

	as program outcomes are delivered and information is tested	delivered	CE Officer		<ul style="list-style-type: none"> <li>Website was in March/April 2012. Website will be maintained monthly and any updates will be documented.</li> <li>Online reporting tool added to website in December 2011. This allows the public to submit photographs and reports of AHB through the Biosecurity Queensland website.</li> </ul>
(ii)		PHA website <a href="http://www.asianhoneybee.net.au">www.asianhoneybee.net.au</a> is used as national portal for agreed AHB information, and information is regularly provided to PHA for updating to the website	<b>BQCC Cairns</b> CE Officer Coordinator Program Mgr <b>PHA</b>	First information provided to PHA by mid Feb 2012  ongoing	<b>COMPLETED AND ONGOING</b> <ul style="list-style-type: none"> <li>2 scientific documents placed on PHA website</li> <li>All Management Group minutes are placed on the PHA website</li> <li>All Scientific Advisory Group minutes are placed on PHA website.</li> </ul>
C.(i)	Customer journeys developed for DAFF QLD website	Develop customer journeys for agreed AHB processes	<b>BQCC Cairns</b> CE Officer	30.6.12	<b>COMPLETED FOLLOWING REVIEW</b> <ul style="list-style-type: none"> <li>Initial discussions have been held with DAFF QLD's web designers to identify how to develop 'Customer Journeys'.</li> <li>Next steps will be engaging with key stakeholders to identify and document customer entry points. Customer Journeys will be used throughout the T2M program to identify gaps and opportunities in order to enhance Customer Journey.</li> <li>Following recent Machinery of Government changes following a change of government, the use of customer journeys are being reconsidered.</li> </ul>
D.(i)	Develop comprehensive strategy to manage cessation of government funding of transition by 30 June 2013, underpinned by significant engagement & communications plan	Develop and implement a Community Engagement and Communications Plan.	<b>BQ ABW policy</b> <b>BQCC Cairns</b> CE Officer Coordinator	Developed by 31.12.11  Implementation – ongoing	<b>COMPLETED AND ONGOING</b> <ul style="list-style-type: none"> <li>AHB T2M CE and Communications Plan developed and approved 17 November 2011.</li> <li>CE and communications activities for the AHB T2M (Attachment C.).</li> </ul>
(ii)		Develop staged transition process to implement T2M plan, with key milestones of 30.6.12 and 30.6.13	<b>BQCC Cairns</b> Program Mgr Coordinator BQCC Director	Developed by 29.2.12  Implementation – ongoing	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Staged transition process developed and implementation is ongoing.</li> </ul>
(iii)		Develop T2M Work Plan that identifies timeframes for delivery of the T2M Plan projects and activities.	<b>BQCC Cairns</b> BQCC Director Program Mgr Coordinator <b>ABW policy</b>	Developed and agreed by 29.2.12  Implementation – ongoing	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Work Plan developed – provides a clear line of sight between the Australian Government and Queensland Government projects under the T2M Plan and the work to be delivered by Biosecurity Queensland. Industry funded projects are being managed through a different process.</li> <li>The Work Plan was provided to PHA on 13 January 2012 to coordinate Scientific Advisory Group feedback on the proposed Work Plan. Awaiting further advice from SAG.</li> </ul>
(iv)		Develop an operational plan that integrates all T2M projects and activities under program delivery areas of: program management, community engagement/industry liaison/compliance, operations and science.	<b>BQCC Cairns</b> BQCC Director Program Mgr Coordinator <b>ABW policy</b>	Developed and agreed by 29.2.12  Implementation – ongoing	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>The Operational Plan is an internal Biosecurity Queensland planning document to guide operational delivery of the separate elements of the T2M program.</li> <li>Many T2M Plan activities under the 6 Australian Government projects and 2 Queensland Government projects are inter-related and utilise the same human resources.</li> </ul>
(v)		Decide on geographical areas BQ will respond to a public call out and which areas to refer to pest controller	<b>BQCC Cairns</b> Coordinator Program Mgr BQCC Director <b>ABW policy</b>	Developed by 30.6.12  Implemented by 1.7.12 & ongoing	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Policy has been developed.</li> <li>Currently within the core infested area, the T2M Program is responding to swarms and nests from public reports of AHB. On the edge of the infested area, the T2M Program is proactively conducting surveillance to identify and destroy nests and swarms. Outside of the core infested area, the T2M Program responds to all suspicious AHB reports.</li> <li>Until the tools for others to manage AHB inside the KIA have been fully provided to all stakeholders the T2M Program will continue to respond to all reports, and will continue to destroy known AHB nests and swarms.</li> </ul>

					<ul style="list-style-type: none"> <li>Tools and training to be provided to stakeholders during July 2012</li> </ul>
(vi)		Develop first draft of information and training packages for pest controllers, apiarists and other key stakeholder groups	<u><b>BQCC Cairns</b></u> CE Officer Technical Advisor Coordinator	31.3.12	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Information and training package developed (Attachment C.)</li> </ul>
(vii)		Discuss training package content with industry representatives, incorporate changes and be ready for full launch	<u><b>BQCC Cairns</b></u> Program Mgr CE Officer Coordinator <u><b>BQ ABW policy</b></u>	Finalised by 30.6.12 Implemented – ongoing	<b>COMPLETED AND ONGOING</b> <ul style="list-style-type: none"> <li>Discussions conducted (Attachment C.).</li> </ul>
(viii)		Complete withdrawal of government management and funding, with community/industry having the knowledge to manage AHB	<u><b>BQCC Cairns</b></u>	30.6.13	<ul style="list-style-type: none"> <li>This is the end point of the T2M Program</li> <li>The program remains on track to achieve this by end June 2013.</li> <li>The strategy under AG1.D(i) to manage cessation of government management and funding of AHB will ensure all areas of government, community and industry is empowered to manage AHB impacts.</li> </ul>

## AG2 – Developing and making available a suite of control measures for AHB

Objective – safe and effective control measures are available and approved for use to reduce impacts of AHB on community, honey production and the natural environment

Funding and reporting milestones	Status/comments
<b>Milestone 1 – due 1/8/11 (\$175,000)</b> <ul style="list-style-type: none"> <li>Agreement signed</li> <li>Desk top review of techniques used to control AHB</li> </ul>	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Agreement signed. Invoice issued - payment received</li> <li>AHB Behavioural document completed and available on PHA website</li> <li>Control procedures documented (See Attachment B)</li> <li>AHB Literature review of management strategies around the world undertaken.</li> </ul>
<b>Milestone 2 – due 1/2/12 (no payment)</b> <ul style="list-style-type: none"> <li>Validate efficacy of detection and destruction techniques available for use by commercial pest controllers and for the purposes of integrated control strategies for industry</li> <li>Refine protocols to reduce the risk of non-target poisoning</li> </ul>	<b>IN PROGRESS</b> <p>This milestone has been impacted by the recruitment process of the scientists who did not commence until beginning of April 2012. The priority when they did commence has been the AHB management literature review and the Remote Treatment trials.</p> <ul style="list-style-type: none"> <li>Review of detection procedure using a dog is complete and recommendations implemented.</li> <li>Detection and destruction procedures documented (Attachment B)</li> <li>The process to validate efficacy of detection and destruction techniques has commenced.</li> <li>Trap efficacy report completed</li> <li>Research and trials to validate techniques and reduce the risks to non-target species are in progress.</li> </ul>
<b>Milestone 3 – due 15/6/12 (\$175,000)</b> <ul style="list-style-type: none"> <li>Investigate and report on alternative control measures or attractants for AHB including, but not limited to, the options provided in the AHB T2M Plan</li> <li>Investigate and report optimal design of bait stations for attracting AHB</li> </ul>	<b>IN PROGRESS</b> <p>This milestone has been impacted by the recruitment process of the scientists who did not commence until beginning of April 2012. The priority when they did commence has been the AHB management literature review and the Remote Treatment trials.</p> <ul style="list-style-type: none"> <li>The Literature review of AHB is contributing to this and is expected to be documented by end July.</li> <li>RIRDC and the SAG have identified trials to be conducted by external researchers which will contribute to this.</li> </ul>
<b>Milestone 4 – due 31/12/12 (\$175,000)</b> <ul style="list-style-type: none"> <li>Report on integrated control strategies for industry</li> <li>Report on optimal control strategies for AHB</li> </ul>	
<b>Milestone 5 – due 15/6/13 (\$175,000)</b> <ul style="list-style-type: none"> <li>Final report on activities undertaken in this project</li> </ul>	

Project Number	T2M Deliverable	Actions/strategies	Responsibility	Due Date	Comments/status
A.	Desktop review and analysis to better understand how others manage AHB	Literature review and analysis conducted	<b>BQCC Cairns</b> Technical Adv. <b>BQ Intell. Unit</b> <b>BQ ABW policy</b>	Initial: 31.3.12 Revised due date: 31.7.12	This has been impacted by the recruitment process of the scientists who did not commence until beginning of April 2012. The priority when they did commence has been the AHB literature review and the Remote Treatment trials. In progress <ul style="list-style-type: none"> <li>Library of relevant literature has been collated and a full report will be completed by end July 2012.</li> </ul>
B.	Develop integrated control strategies for different industries to minimise impacts of AHB, including identifying any off target impacts (especially balance between AHB and commercial European honey bee in the same environment to minimise impact on honey production)	SAG to consider and advise on priorities, and provide scientific oversight	<b>SAG</b>	31.1.12	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Was discussed at initial SAG meeting in November 2011 and 18-19 January 2012.</li> <li>At the January meeting, SAG was given an overview of AHB program operations.</li> <li>SAG identified Remote Treatment trials as highest priority.</li> </ul>
		Stakeholder and industry engagement to identify priorities and needs	<b>BQCC Cairns</b> CE Officer Compliance Off. Coordinator Program Mgr <b>BQ ABW policy</b>	Commenced by 31.1.12  Ongoing	<b>COMPLETED AND ONGOING</b> <ul style="list-style-type: none"> <li>Commenced and ongoing (Attachment C.).</li> </ul>
(i)	Validate efficacy of detection and destruction methods and strategies as essential elements of deploying different control methods	Detection and destruction methods and strategies fully documented	<b>BQCC Cairns</b> Coordinator Technical Adv. Team Leader	31.12.12	In progress <ul style="list-style-type: none"> <li>Standard Operating Procedures (SOPs) for each surveillance method and destruction method have been developed (Attachment B.) Formal Approval of these is still required.</li> </ul>



			<b><u>BQ Science</u></b> <b><u>BQ Intell. Unit</u></b>		<ul style="list-style-type: none"> <li>A review of all SOPs has commenced so they can be made available to other Government agencies and industry. This includes adding detail to SOPs such as high quality images, videos and tips.</li> <li>SAG has recommended that video footage of all detection and destruction techniques be uploaded to the web – as important training tools. A video of Remote Treatment has been made for use at Industry meetings. It is to be placed on the web.</li> <li>Video footage has been taken of nests being destroyed and removed from a tree – this footage will be used to develop a training video.</li> </ul>
		Rates of effort and validation of all methods	<b><u>BQCC Cairns</u></b> Technical Adv. Coordinator Spatial/info mgmt officer Scientists <b><u>BQ Intell. Unit</u></b> <b><u>BQ Science</u></b>	31.3.13	<p>In progress</p> <ul style="list-style-type: none"> <li>Based on existing data and field observations, a preliminary assessment of rates of effort has been undertaken.</li> <li>2 scientists have been recruited to conduct this work.</li> <li>Experimental design for validating rates of effort is being developed based on recommendations from SAG's January 2012 meeting.</li> <li>Trials will be conducted to test surveillance techniques around known locations of AHB nests - the design of an exploratory trial is being developed.</li> </ul>
(ii)	Determine the timing of implementing these methods and strategies to maximise effectiveness of control methods	<ul style="list-style-type: none"> <li>Seek advice from stakeholder and industry</li> <li>Develop toolkit</li> </ul>	<b><u>BQCC Cairns</u></b> Coordinator Program Mgr	30.6.12  Reviewed by 30.6.13	<p>In progress</p> <ul style="list-style-type: none"> <li>Relationship established with local beekeepers. (Attachment C.)</li> <li>A training and induction package has been developed for the industry volunteers and has been delivered to over 60 beekeepers. Their evaluation of this package has been used to guide the development of other training packages.</li> <li>New training package developed with training scheduled during July 2012.</li> <li>After the training, stakeholder feedback will be collated.</li> </ul>
(iii)	Determine effectiveness of bait stations, their design and attractant effectiveness	Document bait station use and design	<b><u>BQCC Cairns</u></b> Coordinator Technical Adv. Team Leader Spatial/info mgmt officer Scientists	31.12.12	<p>In progress</p> <ul style="list-style-type: none"> <li>Bait station SOP has been developed (Attachment B.)</li> <li>The process of adding detail to SOPs such as high quality images, videos and tips has commenced.</li> </ul>
		Validate effectiveness	<b><u>BQCC Cairns</u></b> Technical Adv. Spatial/info mgmt officer scientists <b><u>BQ Intell. Unit</u></b> <b><u>BQ Science</u></b>	31.12.12	<p>In progress</p> <ul style="list-style-type: none"> <li>Experimental design for validating rates of effort is being developed based on recommendations from SAG's January 2012 meeting.</li> <li>The senior scientist has conducted an analysis (Attachment D.).</li> <li>Trials will be conducted to test this surveillance technique around known locations of AHB nests - the design of an exploratory trial is being developed.</li> </ul>
(iv)	Understand AHB behaviour to better inform development of targeted control measures	Desktop review and analysis of AHB behaviour in FNQ	<b><u>BQCC Cairns</u></b> Technical Adv. <b><u>BQ Intell. Unit</u></b>	31.1.12	<p><b>COMPLETED</b></p> <ul style="list-style-type: none"> <li>An analysis of AHB behaviour was finalised in January 2012 and is available on the PHA website.</li> </ul>
C.	Investigate alternative control techniques and attractants	SAG to consider and advise on priorities, and provide scientific oversight	<b><u>SAG</u></b>	31.1.12	<p><b>COMPLETED</b></p> <ul style="list-style-type: none"> <li>SAG has made some recommendations to PHA &amp; RIRDC.</li> </ul>
(i)	Finalise development of remote poisoning <ul style="list-style-type: none"> <li>Validate techniques</li> <li>Refine protocols to reduce risk of non-target poisoning and minimise adverse effects on environment and native fauna</li> </ul>	Experimental design for research developed and endorsed	<b><u>BQCC Cairns</u></b> Technical Adv. <b><u>BQ Intell. Unit</u></b> <b><u>BQ ABW policy</u></b> <b><u>BQ Science</u></b>	31.1.12	<p><b>COMPLETED</b></p> <ul style="list-style-type: none"> <li>Research proposal has been developed and was provided to PHA on 27 January 2012 to coordinate SAG's feedback.</li> <li>Research Trials commenced</li> </ul>
		Revised permit is approved by APVMA	<b><u>BQCC Science</u></b>	31.12.11	<p><b>COMPLETED</b></p> <ul style="list-style-type: none"> <li>Approved by APVMA in December 2011</li> <li>The permit requires that only DAFF QLD use the chemical and is</li> </ul>

					<p>valid until 1 July 2015. Discussions with the APVMA regarding the Fipronil permit and how Industry may be able to use the permit or Fipronil in the future indicated</p> <ul style="list-style-type: none"> <li>○ Biosecurity Queensland should write to APVMA advising them we no longer require the permit when this situation arises.</li> <li>○ Biosecurity Queensland may consider applying for industry to be added to the permit.</li> <li>○ Industry could apply for a permit but there would need to be stringent oversight, quality assurance system, to ensure the permit was used appropriately. Use data and operational procedures would be required.</li> <li>○ An alternative option may be government officers using it on behalf of Industry and at their direction.</li> <li>○ APVMA is interested to obtain a copy of any report written regarding the trials.</li> </ul>
		Research trials conducted, techniques validated and final report prepared	<p><b><u>BQCC Cairns</u></b>  Technical Adv. Scientist  Field teams  <b><u>BQ Science</u></b>  <b><u>BQ Intell Unit</u></b></p>	<p>Commenced in January 2012</p> <p>Complete 30.12.12</p>	<ul style="list-style-type: none"> <li>• Research trial has commenced</li> <li>• An early summary of progress has been placed on the PHA website.</li> <li>• Presentations regarding progress have been given to the NSW and QLD bee industries.</li> <li>• An up to date progress summary is attached (Attachment E.).</li> </ul>
(ii)	Tomato dust – a potential control to be researched and validated	SAG to advise on appropriateness	<b><u>SAG</u></b>	31.1.12 – on advice of SAG, will not be conducted	<p><b>TMG APPROVED THAT THIS RESEARCH WILL NOT BE CONDUCTED</b></p> <ul style="list-style-type: none"> <li>• This research was suggested by industry volunteers. SAG discussed the research at the initial SAG meeting in November 2011 and 18-19 January 2012.</li> <li>• SAG recommended that this research is not conducted.</li> <li>• The Transition Management Group approved this recommendation on 24 January 2012.</li> <li>• .</li> </ul>
(iii)	Nectar analysis – analysis of nectar that AHB is foraging on to identify potential attractants for use in traps	SAG to advise on appropriateness	<b><u>SAG</u></b>	31.1.12	<p><b>TMG APPROVED THAT THIS RESEARCH WILL NOT BE CONDUCTED</b></p> <ul style="list-style-type: none"> <li>• Was discussed at initial SAG meeting in November 2011 and 18-19 January 2012.</li> <li>• Initial advice from SAG is that a pollen analysis would provide a better indicator of foraging activity rather than nectar analysis.</li> <li>• SAG has recommended conducting a pollen analysis of honeycomb stored since beginning of the program.</li> <li>• The Transition Management Group approved this recommendation.</li> </ul>
(iv)	Pollen analysis – analysis of pollen in AHB honeycomb determine plants that AHB is foraging on	This research is being conducted by CSIRO/JCU. BQCC Cairns is providing assistance as required.	<p><b><u>Caroline Gross – CSIRO/JCU research project</u></b></p> <p><b><u>BQCC Cairns assisting where necessary</u></b></p>		<ul style="list-style-type: none"> <li>• At its meeting on 18-19 January 2012, SAG recommended a pollen analysis of honeycomb stored since 2007.</li> <li>• In 2007, M K Macphail of the Australian National University conducted a pollen analysis on one AHB nest. The report has been provided to PHA and SAG.</li> <li>• Caroline Gross in conducting CSIRO/UNE research project on the effects of AHB on other pollinators. As part of this research, pollen is being extracted from all nests detected since December 2011.</li> <li>• Biosecurity Queensland has provided field support to the research and will be providing AHB honeycomb to the research team.</li> <li>• SAG identified this as a priority and RIRDC will be supporting this work.</li> <li>• This links with AG4.</li> </ul>

(v)	Research into pheromone use to attract and/or detect AHB in order to increase trap sensitivity	This research is to be conducted by other parties through RIRDC funding that was supported by the SAG.	<b>SAG</b> Funding from Industry projects? TBD TBD	31.1.12	<ul style="list-style-type: none"><li>• Was discussed at initial SAG meeting in November 2011 and 18-19 January 2012.</li><li>• SAG supported a proposal for RIRDC funding of a project that includes Cairns plants that were considered attractive to AHB, as well specific overseas orchids.</li></ul>
-----	--	--	--	---------	--

In addition to the above deliverables identified in the T2M Plan, Biosecurity Queensland has also delivered the following activities:

- Planning and organisation for the Scientific Advisory Group meeting in Cairns on 18 & 19 January 2012. A field trip on 18 January 2012 show cased operational techniques used on the program.
- Worked with local beekeepers to develop an after hours register of beekeepers who can respond to reports of swarms. This provides increased capacity to effectively respond to out of hours call outs and ensures AHB swarms are quickly destroyed if warranted. The state swarm list has also been updated to include additional local beekeepers – this is in direct response to our industry engagement.



## AG3 – Limiting impact on honey production

Objective – beekeepers in areas where AHB is established maintain honey yields and quality

Funding and reporting milestones	Status/comments
<b>Milestone 1 – due 1/8/11 (\$102,500)</b> <ul style="list-style-type: none"> <li>Agreement signed</li> </ul>	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Agreement signed. Invoice issued - payment received.</li> </ul>
<b>Milestone 2 – due 1/2/12 (no payment)</b> <ul style="list-style-type: none"> <li>Literature review and report from apiarists on the impact of AHB on commercial honey production within AHB infested areas</li> <li>Report on behaviour of AHB compared to European honey bee to identify opportunities for control</li> </ul>	<b>IN PROGRESS</b> <ul style="list-style-type: none"> <li>Library of relevant literature has been collated and a full report is in development. Full report on literature review is expected to be completed by end July.</li> <li>A report from local apiarists on the impact of AHB on commercial honey production within AHB infested areas is yet to be developed. Regular meetings have been held with local bee keeping association.</li> <li>An overseas student (Karmi Oxman) volunteered to work on the AHB program in early 2011 to conduct an undergraduate research project on the effects of the AHB eradication program on local bee keepers and farmers. The initial observations from this research have been collated and will be use as a reference when developing a report with local bee keepers about the actual and perceived AHB impacts on local commercial honey production.</li> <li>A full literature review, including impacts observed by local apiarists and an assessment of initial observations from the volunteer researcher, is expected to be completed by end July 2012.</li> <li>An analysis of AHB behaviour was finalised in January 2012 and has been placed on the PHA website. SAG considered and provided feedback on the report on 19 January 2012.</li> </ul>
<b>Milestone 3 – due 15/6/12 (\$102,500)</b> <ul style="list-style-type: none"> <li>Report on management strategies of AHB within the infested area to reduce the impact on commercial honey production and pollination services</li> <li>Evaluation of outputs of project 2 for field application for industry</li> </ul>	<b>IN PROGRESS</b> <ul style="list-style-type: none"> <li>The literature review that will enable this report to be completed is close to completion. The Science team did not commence employment on the program until early April and the Remote Treatment trial has consumed much of their time.</li> <li>The program meets with industry at a local and state level monthly and will progress this on completion of the literature review.</li> </ul>
<b>Milestone 4 – due 31/12/12 (\$87,500)</b> <ul style="list-style-type: none"> <li>Report on modelling of AHB population dynamics and drivers of spread</li> <li>Report on PCR tools for detection of AHB</li> <li>Report on the outcomes of Project 2 for field application for industry</li> </ul>	
<b>Milestone 5 – due 15/6/13 (\$87,500)</b> <ul style="list-style-type: none"> <li>Final report on activities undertaken in this project</li> </ul>	

Project Number	T2M Deliverable	Actions/strategies	Responsibility	Due Date	Comments/status
A.(i)	AHB behaviour research is critical to identify elements of differential control of AHB and European honey bees in the context of honey production. Developing an understanding of AHB behaviour will guide development of targeted control measures through: <ul style="list-style-type: none"> <li>Literature review and engagement with apiarists in the Cairns area who have had experience with both honey bees</li> </ul>	Conduct literature review, conduct analysis, compare behaviour and identify opportunities that support differential control.	<b><u>BQCC Cairns</u></b> Technical Adv. <b><u>BQ Science</u></b> <b><u>BQ Intell. Unit</u></b> <b><u>BQ ABW policy</u></b>	Initial: 31.3.12 Revised: 31.7.12	<ul style="list-style-type: none"> <li>A document regarding observational behaviour of AHB in the Cairns area has been completed and is available on the PHA website.</li> <li>Library of relevant literature has been collated.</li> <li>A full literature review is in progress and hoped to be completed by end July 2012.</li> <li>Impacts observed by local apiarists have been limited until recently when a beekeeper attempting to keep AHB in hives for research purposes observed 3 nests in close proximity to Apis mellifera hives either die out or abscond while a AHB hive not in close proximity to European honey bee hives appears to be healthy.</li> </ul>
(ii)	<ul style="list-style-type: none"> <li>Analysis to understand what is known and not known about AHB in relation to mating, behaviour, foraging habits, weather impacts, etc.</li> </ul>	Desktop review and analysis of AHB behaviour in FNQ	<b><u>BQCC Cairns</u></b>	Initial: 31.1.12 Revised: 31.7.12	<ul style="list-style-type: none"> <li>Refer to AG2.B.(iv)</li> <li>A document regarding observational behaviour of AHB in the Cairns area has been completed and is available on the PHA website.</li> <li>SAG considered the report on 19 January 2012.</li> <li>A full literature review is in progress</li> </ul>
(iii)		Engagement with local apiarists	<b><u>BQCC Cairns</u></b>	Commenced by	<b>COMPLETED AND ONGOING</b>

	<ul style="list-style-type: none"> <li>Compare the behaviour between AHB and European honey bees to identify opportunities that support differential controls</li> </ul>		Technical Adv. Coordinator Program Mgr <b>BQ ABW policy</b>	31.12.11  Completed by 30.6.12	<ul style="list-style-type: none"> <li>Engagement with local apiarists has been an ongoing feature of the program since 2007 – this is continuing (Attachment C.)</li> <li>Since 1 July 2011, the program has attended all local bee keeping association meetings and provided updates on program activities.</li> <li>A report from local apiarists on the impact of AHB on commercial honey production within AHB infested areas is yet to be developed although recent observations were provided.</li> <li>An overseas student (Karmi Oxman) volunteered to work on the AHB program in early 2011 to conduct an undergraduate research project on the effects of the AHB eradication program on local bee keepers and farmers. The initial observations from this research have been collated and will be used as a reference when developing a report with local bee keepers about the actual and perceived AHB impacts on local commercial honey production.</li> </ul>
B.	Development of management strategies <ul style="list-style-type: none"> <li>Based on outcomes of Project 2, develop and test management strategies targeted at limiting impact of AHB on honey production in areas where AHB is established</li> </ul>	Using outcomes of literature review and behaviour analysis, work with industry to develop management strategies for use by industry	<b>BQCC Cairns</b> Technical Adv. Coordinator Program Mgr <b>BQ ABW policy</b>	Strategies developed by 15.6.12  Strategies tested by 31.12.12	<ul style="list-style-type: none"> <li>Also links with AG4</li> <li>The finalised literature review and behavioural analysis will be used as key reference documents to identify additional opportunities for control of AHB. This is on track to be completed by end July 2012.</li> <li>Engagement with local industry has commenced regarding the need of management strategies in the future pending outcomes of the literature review.</li> </ul>
C.	Model the population dynamics and drivers of spread as they impact on the management of EHB hives	SAG to consider and advise	<b>SAG</b> Funding from industry projects? <b>BQ Intell. Unit</b>	31.1.12	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Was discussed at initial SAG meeting in November 2011 and 18-19 January 2012.</li> </ul>
		Report on modelling of AHB population dynamics and drivers of spread	<b>BQCC Cairns</b> Technical Advisor Scientists Spatial/info mgmt officer <b>BQ Intell. Unit</b>	31.12.12	<ul style="list-style-type: none"> <li>In progress</li> <li>2 scientists and a spatial and information management officer were recruited to assist in conducting this work. AHB data has been reviewed and Geodata sets produced. Earlier work conducted by a person external to the program and whilst eradication was being conducted will be replicated using the updated data.</li> </ul>
D.	Develop technology to assist industry to mitigate AHB impacts	SAG to consider and advise on priorities for technology development	<b>SAG</b>	31.1.12	<b>COMPLETED– SEE RIDC APPROVED RESEARCH PROJECTS</b> <ul style="list-style-type: none"> <li>Was discussed at initial SAG meeting in November 2011 and 18-19 January 2012.</li> <li>SAG and RIRDC have addressed this.</li> </ul>
		Stakeholder and industry engagement to identify needs and priorities for technology development	<b>BQCC Cairns</b> Technical Adv. Coordinator Program Mgr <b>BQ ABW policy</b>	Commenced by 31.3.12  ongoing	<b>COMPLETED AND ONGOING</b> <ul style="list-style-type: none"> <li>Consultation process with Apiarist, Transporters, pest controllers, environmental groups etc. (Attachment C.) completed. No new needs identified.</li> <li>Training being provided in June and July 2012.</li> </ul>
		Additional technologies to be developed, as required (to be determined)	TBD Funding from industry projects?		<b>COMPLETED – SEE RIDC APPROVED RESEARCH PROJECTS</b> <ul style="list-style-type: none"> <li>SAG and RIRDC have addressed this. (List SAG /RIRDC research)</li> </ul>
(i)	Develop PCR testing to more quickly detect the presence of AHB in trap syrup etc as indicators of the need for management of European honey bee hives	Report on PCR tools for detection of AHB	<b>BQ Science</b> Jane Oakey	31.12.12	<ul style="list-style-type: none"> <li>At its meeting on 18-19 January 2012, SAG recommended Biosecurity Queensland continue to finalise the PCR research.</li> <li>Significant progress has been made.</li> <li>A test for the specific detection of <i>Apis cerana</i> DNA has been developed. The test is sensitive to approximately 0.5-5 ng of <i>Apis cerana</i> DNA and has shown no cross-reaction to <i>Apis mellifera</i> or any native insects that were trapped when lure syrup was placed in AHB free areas of far north Queensland. DNA extraction methods for whole bee, sugar syrup and wings removed from bee-eater pellets have been investigated and optimised for each sample type. This work provides proof of concept for a molecular</li> </ul>

					surveillance program for AHB in far north Queensland. See Attachment J.
	Undertake preliminary microsatellite work to determine if difference can be observed between Australian, Solomon Islands and PNG populations of AHB Java strain.		<u>BQ Science</u> Jane Oakey	31.12.12	<ul style="list-style-type: none"> <li>On SAG advice, TMG 3 approved - undertake preliminary microsatellite work to determine if difference can be observed between Australian, Solomon Islands and PNG populations of AHB Java strain.</li> <li>Preliminary microsatellite studies showed a distinct difference between AHB in Queensland from those in the sampled populations of PNG. One of the two PNG populations identified included samples from the Solomon Islands. The work identified that there may have been two incursions into QLD, but the number of samples tested was insufficient to be certain. Hence, the pilot study demonstrated the utility of the increased panel of loci and it is recommended that a larger-scale project be undertaken to include all QLD samples (and determine if there has been more than one incursion), and more samples from potential source populations (of the Java strain and others) to identify the most likely source of the QLD incursion(s)." See Attachment K.</li> </ul>
F.	Develop approaches with the honey industry for adoption and implementation of management strategies		<u>BQCC Cairns</u> Technical Adv. Coordinator Program Mgr	Commenced by 31.3.12  ongoing	In progress. Also links with AG1 and Industry project 2 See Attachment C.

In addition to the above deliverables identified in the T2M Plan, Biosecurity Queensland has also delivered the following activities:

- Provided field support to Dr Denis Anderson, CSIRO, who is conducting bee mite and disease research – assistance included locating and removing suitable AHB nests and swarms for the research.
- Assisted Caroline Gross from University of New England/CSIRO, who is conducting a study on the effects of AHB on other pollinators – assistance included locating foraging and nesting Asian honey bees.

## AG4 – Limiting impact on natural environment

Objective – tools and strategies are available for implementation to mitigate the impact of AHB in ecologically significant areas

2011/12 – \$50,000

2012/13 - \$20,000

Funding and reporting milestones	Status/comments
<u>Milestone 1 – due 1/8/11</u> (\$25,000) • Agreement signed	<b>COMPLETED</b> • Agreement signed. Invoice issued – payment received.
<u>Milestone 2 – due 1/2/12</u> (no payment) • Identification of mechanisms of engagement with indigenous communities (incorporating outputs of Project 2)	<b>COMPLETED</b> • Engagement with indigenous communities has commenced and is ongoing. (Attachment A.)
<u>Milestone 3 – due 15/6/12</u> (\$25,000) • Report on efficacy of traps and lures in natural environment	<b>COMPLETED</b> ▪ A report regarding trap efficacy was completed in June 2012. (Attachment D.)
<u>Milestone 4 – due 31/12/12</u> (no payment) • Report on tools and strategies for suppression of AHB in the natural environment (incorporating outputs of Project 2 and linking with Project 3)	
<u>Milestone 5 – due 15/6/13</u> (\$20,000) • Final report on activities undertaken in this project	

Project Number	T2M Deliverable	Actions/strategies	Responsibility	Due Date	Comments/status
A.	Engagement with indigenous communities and environment sectors (linked with AG1)	Meet with indigenous groups and rangers, and environmental groups	<b><u>BQCC Cairns</u></b> CE Officer Compliance Off. Coordinator	First meeting by 31.12.11 Ongoing	Commenced and ongoing • Meetings held with a range of indigenous groups. (Attachment C.) • Discussions held with Queensland Environmental Protection Agency and other ranger groups. (Attachment C.) • Permission was granted by Queensland Parks and Wildlife Service for AHB information posters to be placed in campgrounds in National Parks bordering known infested areas.
		Deliver education/training packages that will foster indigenous and environmental contribution to AHB program	<b><u>BQCC Cairns</u></b> CE Officer Compliance Off.	First training session by 31.3.12 Ongoing	Commenced and ongoing • Comprehensive training package completed. • ‘Taster’ training has been delivered and full training will be provided during July 2012. Attachment C.
B.	Utilise tools and strategies developed for control measures (Project AG2) and limit impact on honey production (AG3)	Indigenous communities and environmental sector utilise tools and strategies to control AHB	<b><u>BQCC Cairns</u></b> Coordinator Program Mgr CE Officer	Ongoing - Tools and strategies utilised as they are developed	Commenced and ongoing. • Tools developed and training to be conducted during July 2012. (Attachment C.)
C. Relates to AG2.C(iv)	Pollen Analysis – analysis of pollen in AHB honeycomb determine plants that AHB is foraging on	Collect pollen from Apis cerana collected in the Known Infested area to determine if competing with Apis mellifera and Native Bees. Also to determine flower preference.	Caroline Gross CSIRO/UNE	TBA	• TMG approved on 24.1.12 to remove nectar analysis research from the T2M Plan and to replace it with pollen analysis under AG4. • This work is being conducted by CSIRO/UNE and the program has provided more than 8 samples to date.

## AG5 – Optimising early detection of new incursion of AHB

Objective – any new incursions of AHB are detected rapidly and tested to determine the presence or absence of emergency bee pests in accordance with provisions of the EPPRD

2011/12 – \$200,000

2012/13 - \$100,000

Funding and reporting milestones	Status/comments
<b>Milestone 1 – due 1/8/11 (\$100,000)</b> <ul style="list-style-type: none"> <li>Agreement signed</li> </ul>	<ul style="list-style-type: none"> <li>Agreement signed. Invoice issued – payment received.</li> </ul>
<b>Milestone 2 – due 1/2/12 (no payment)</b> <ul style="list-style-type: none"> <li>Investigate the establishment of bee free zones around high risk areas</li> <li>Report on assessment of optimal surveillance strategies for detection of AHB</li> <li>Provision of quarterly summary reports throughout the program on surveys of AHB populations for presence of bee pests</li> </ul>	IN PROGRESS <ul style="list-style-type: none"> <li>The focus of meetings with Australian Government DAFF-Biosecurity aim to develop an appropriate way to undertake bee surveillance in port areas with an established population of AHB and ways to conduct differential sensitivity testing for different detection methods. Until this is developed all nests and swarms in the Cairns Port Area that are reported by the public are being destroyed and analysed for the presence of exotic bee pests. These destruction activities are continuing to suppress AHB population around the port.</li> <li>Areas of responsibility for response to AHB reports in the Cairns area has been defined. See Attachment G.</li> <li>A summary report of AHB surveillance and destruction activities and supporting maps, have been produced.</li> </ul>
<b>Milestone 3 – due 15/6/12 (\$100,000)</b> <ul style="list-style-type: none"> <li>Report on assessment of optimal surveillance strategies and risk based surveillance for detection of AHB, including efficacy of detection methods</li> </ul>	IN PROGRESS <ul style="list-style-type: none"> <li>A surveillance trial has been developed and is in progress to assess an optimal surveillance strategy. See Attachment F. Australian Government DAFF-Biosecurity is conducting the floral sweeping surveillance component of the trial while Biosecurity Queensland is conducting the bee lining and destruction components.</li> <li>It is expected the trial will continue until December 2012 so as to provide a good data set for decision makers.</li> </ul>
<b>Milestone 4 – due 31/12/12 (\$50,000)</b> <ul style="list-style-type: none"> <li>Report on identification and detection methodologies that can assist with rapid detection of new incursions of AHB</li> </ul>	
<b>Milestone 5 – due 15/6/13 (\$50,000)</b> <ul style="list-style-type: none"> <li>Final report on activities undertaken in this project</li> </ul>	

Project Number	T2M Deliverable	Actions/strategies	Responsibility	Due Date	Comments/status
A.	Conduct differential sensitivity testing to determine the comparative effectiveness of all available detection methods eg. sentinel hive strategy vs strategic sampling of surveillance traps		<b>BQCC Cairns</b> Technical Advisor Coordinator Field teams <b>NAQS</b> <b>BQ Intell. Unit</b> <b>BQ Science</b>	31.12.12	In progress <ul style="list-style-type: none"> <li>Discussions have been held with Australian Government DAFF-Biosecurity on developing an appropriate way to undertake bee surveillance in port areas with an established population of AHB and ways to conduct differential sensitivity testing for different detection methods. 4 meetings to date.</li> <li>Floral sweeping by Australian Government DAFF-Biosecurity commenced in April 2012 and a Bee trap efficiency paper (Attachment D) has been produced.</li> <li>SAG advice is also to be obtained.</li> <li>In the Cairns Port area all nests and swarms that are reported by the public are being destroyed. These destruction activities are continuing to suppress AHB population around the port.</li> </ul>
B.(i)	Determine efficacy of surveillance strategies and techniques to determine likely detection rate in bee free zones and around ports in the context of established AHB populations	Surveillance strategies and techniques fully documented	<b>BQCC Cairns</b> Coordinator Technical Advisor Field teams <b>BQ Intell. Unit</b> <b>BQ Science</b>	31.3.12	Links with T2M AG2.B.(ii) <ul style="list-style-type: none"> <li>Standard Operating Procedures (SOPs) for each surveillance and destruction method have been developed. (Attachment B.)</li> <li>A review of all SOPs has commenced so they can be made available to other Government agencies and industry. This includes adding detail to SOPs such as high quality images, videos and tips.</li> <li>SAG has recommended that video footage of all detection and destruction techniques be uploaded to the web – as important training tools.</li> <li>Video footage has been taken of nests being destroyed and</li> </ul>

					<p>removed from a tree – this footage will be used to develop a training video.</p> <ul style="list-style-type: none"> <li>A surveillance trial has been developed and is in progress to assess an optimal surveillance strategy. See Attachment G. Australian Government DAFF-Biosecurity is conducting the floral sweeping surveillance component of the trial while Biosecurity Queensland is conducting the bee lining and destruction components.</li> </ul>
(ii)		Rates of effort, efficacy and validation of surveillance methods	<u><b>BQCC Cairns</b></u> Coordinator Technical Adv. Field teams Scientists Spatial/info mgmt officer <u><b>BQ Intell. Unit</b></u> <u><b>BQ Science</b></u>	31.12.12	<p>In progress</p> <ul style="list-style-type: none"> <li>Discussions held with SAG and Biosecurity Queensland Intelligence Unit to determine methodology for collating evidence to determine rates of effort and validation of techniques</li> <li>In April 2 scientists and a Spatial and Information Management Officer to collate information and analyse the surveillance methods were employed.</li> </ul>
	<b>Develop an appropriate scientific methodology for floral sweep netting in the outer areas of the containment area to gain greater confidence of absence or presence of AHB</b>	Floral sweep netting at edge of known infested area data analysed and documented.	<u><b>BQCC Cairns</b></u> <u><b>Coordinator</b></u> <u><b>Technical Adv</b></u> <u><b>Scientists</b></u> <u><b>BQ Intell Unit</b></u> <u><b>BQ Science</b></u>		<ul style="list-style-type: none"> <li>TMG 3 approved - develop an appropriate scientific methodology for floral sweep netting in the outer areas of the containment area to gain greater confidence of absence or presence of AHB</li> <li>The program has a targeted floral sweep netting procedure it is using and reviewing with a view to improve it. The program has shared this with the Commonwealth DAFF Biosecurity who are working with us to develop a general sweep netting technique.</li> </ul>
C.	Establish a strategy for laboratory analysis of AHB detections within bee free zones and around ports as an early detection strategy for any new incursions that could carry mites or viruses		<u><b>BQCC Cairns</b></u> Technical Adv. Scientist Coordinator <u><b>NAQS</b></u> <u><b>BQ Science</b></u> <u><b>BQ ABW policy</b></u>	Strategy developed by 31.3.12	<p><b>Completed</b></p> <ul style="list-style-type: none"> <li>Strategy used during the eradication phase of the program is continuing to be utilised. Areas of responsibility for the Cairns port area have been agreed. A joint surveillance trial is in progress in the high risk sea port area.</li> <li>All AHB detections are routinely tested for presence/absence of mites and diseases. No mites/diseases have been detected.</li> </ul>
D.	Partner with the Northern Australian Quarantine Strategy (NAQS) program of DAFF to develop integrated operations focussed on early detection of new AHB incursions and any quarantine pests that they may carry	Develop and implement integrated operational plan and surveillance strategy with NAQS	<u><b>BQCC Cairns</b></u> Coordinator Program Mgr <u><b>NAQS</b></u> <u><b>BQ ABW Policy</b></u>	<p>Initial discussions held by 31.12.11</p> <p>Strategy and plan developed by 30.4.12</p> <p>Integrated operations commenced by April 2012 and ongoing</p>	<p>Commenced and ongoing</p> <ul style="list-style-type: none"> <li>Regular discussions with Australian Government DAFF-Biosecurity have been held to coordinate surveillance activities. 4 meetings to date.</li> <li>The focus of recent meetings with Australian Government DAFF-Biosecurity has been to identify current operational procedures and resources focussed on early detection of new AHB incursions and current laboratory capacity, including identifying areas of responsibility in the Cairns Port area (Attachment H.)</li> </ul>



## AG6 – Critical intervention to limit long distance spread

Objective – critical interventions and their points are identified and analysed to inform the Varroa action plan; awareness and control information is in place to support spread threat reduction strategies before significant long distance spread occurs; AHB impact is limited as much as possible in strategic areas as elements of long distance spread intervention strategies

2011/12 – \$200,000

2012/13 - \$50,000

Funding and reporting milestones	Status/comments
<b>Milestone 1 – due 1/8/11 (\$100,000)</b> <ul style="list-style-type: none"> <li>Agreement signed</li> </ul>	<b>Completed</b> Agreement signed. Invoice issued – payment received.
<b>Milestone 2 – due 1/2/12 (no payment)</b> <ul style="list-style-type: none"> <li>Commence development of an industry managed certification system for movement of European honey bees and risk material such as containers, machinery, vehicles and freight</li> </ul>	<ul style="list-style-type: none"> <li>A review of strategies to limit long distance spread has assisted in the development of tools and training for transport industry. This will enable transport companies to integrate AHB awareness and procedures into their systems</li> <li>Discussions have been held with the bee industry about an industry managed certification system for moving European honey bees within the restricted area.</li> </ul>
<b>Milestone 3 – due 15/6/12 (\$100,000)</b> <ul style="list-style-type: none"> <li>Report on cost, compliance and efficacy of movement controls to limit spread of AHB that could occur with European honey bee hives and risk material such as containers, machinery, vehicles and freight</li> </ul>	<ul style="list-style-type: none"> <li>The cost, compliance and efficacy of movement controls have been considered and a consultation document, “Biosecurity Precautions for Beekeepers Moving European Bee Hives Within and Out of the Asian Honey Bee Restricted Area” produced for discussion with industry. Following discussions with industry and Australian Government DAFF-Biosecurity, it was agreed to retain the current Restricted Area and movement controls. It is planned to have further discussions with industry and Canadian Government representatives.</li> </ul>
<b>Milestone 4 – due 31/12/12 (no payment)</b> <ul style="list-style-type: none"> <li>Report on pathway analysis for AHB including identification of pathways for long distance spread</li> <li>Develop and report on evaluation of training for transporter businesses</li> <li>Report on links with activities occurring within implementation of Varroa Continuity Strategy</li> <li>Implementation of an industry managed certification system for movement of European honey bees and risk material such as containers, machinery, vehicles and freight</li> </ul>	<ul style="list-style-type: none"> <li>A strategy to minimise the spread of Asian honey bee through pathways including the maintenance of bee suppression zones has been developed.</li> </ul>
<b>Milestone 5 – due 15/6/13 (\$50,000)</b> <ul style="list-style-type: none"> <li>Final report on activities undertaken in this project</li> </ul>	

Project Number	T2M Deliverable	Actions/strategies	Responsibility	Due Date	Comments/status
A.	Maintain existing movement controls for an interim period and collect information on their efficacy, cost, compliance etc.	Maintain Restricted Area and existing movement controls, until movement control policy has been reviewed	<b>BQCC Cairns</b> Compliance Off. Coordinator Program Mgr <b>BQ ABW policy</b>	Ongoing, until determined	Ongoing. <ul style="list-style-type: none"> <li>A Restricted Area remains in force and movement licenses are being predominately issued to move bees or beekeeping equipment into or within the restricted area.</li> <li>77 applications for movement licences were assessed and approved. No movement licences were declined.</li> <li>10 AHB staff are appointed as Inspectors under the <i>Exotic Diseases in Animals Act 1981</i></li> <li>Discussions with Biosecurity Queensland Apiary Officers from South East Queensland and local bee keepers regarding various bee pests including AHB are ongoing.</li> <li>Investigated two alleged breaches of Queensland legislation and movement controls               <ul style="list-style-type: none"> <li>One alleged breach of moving AHB honeycomb outside the restricted areas was not substantiated.</li> <li>The other alleged breach of moving dead AHB material outside the restricted area was substantiated. No further action was taken as the movement posed minimal biosecurity risk of spreading AHB or exotic bee mites as bees and honeycomb were dead and had been sprayed with chemical.</li> </ul> </li> </ul>
		Develop strategy to cease/amend Restricted Area and movement	<b>BQ ABW policy</b>	Strategy	In progress

		controls to transition to management <ul style="list-style-type: none"> <li>Amend Restricted Area, as required</li> </ul>		developed by 30.6.12 RA rescinded by 30.6.13	<ul style="list-style-type: none"> <li>Restricted area will be maintained until this strategy is developed</li> <li>A Biosecurity QLD internal paper has considered the Legislative Options re the Restricted Area.</li> <li>Following discussions with industry and Australian Government DAFF-Biosecurity it has been decided to leave the restricted area as it currently stands.</li> <li>Discussions are planned with trading representatives from Canada.</li> </ul>
		Collect information and conduct analysis of movement control efficiency, cost and compliance	<b><u>BQCC Cairns</u></b> Compliance Off. Coordinator Scientists Spatial/info mgmt officer <b><u>BQ Intell. Unit</u></b> <b><u>BQ ABW policy</u></b>	Commenced by 31.1.12  Completed by 31.12.12	In progress <ul style="list-style-type: none"> <li>Analysis of current data and strategies to limit long distance spread has commenced. This will guide the ongoing discussions with industry about the development of any certification system.</li> <li>The cost, compliance and efficacy of movement controls have been considered and a consultation document, "Biosecurity Precautions for Beekeepers Moving European Bee Hives Within and Out of the Asian Honey Bee Restricted Area" produced for discussion with industry. Following discussions with industry and Australian Government DAFF-Biosecurity, it was agreed to retain the current Restricted Area and movement controls. It is planned to have further discussions with industry and Canadian Government representatives.</li> </ul>
		Finalise policy on movement of European honey bee hives from within the Restricted Area to outside the Restricted Area	<b><u>BQCC Cairns</u></b> <b><u>BQ ABW policy</u></b>	31.3.12	<b>Completed</b> <ul style="list-style-type: none"> <li>A draft Protocol to Allow Movement of Hives out of the Asian honey bee Restricted Area was developed in consultation with local beekeepers. The draft protocol has been placed on hold after receiving advice from Australian Government DAFF-Biosecurity about the possible trade implications if bees moved outside the restricted area.</li> <li>Discussions have been held with the bee industry about an industry managed certification system for moving European honey bees within the restricted area</li> </ul>
B.	Conduct pathway analysis to better understand likely pathways and potential for spread through pathways implicated in the long distance spread of AHB with a primary focus on effective strategies to minimise long distance spread through transport vectors	<ul style="list-style-type: none"> <li>Advice from SAG</li> <li>Collate data</li> <li>Analyse data</li> <li>Develop strategies</li> </ul>	<b><u>SAG</u></b> <b><u>BQCC Cairns</u></b> Compliance Off. Coordinator Program Mgr <b><u>BQ Intell. Unit</u></b>	Commenced by 31.3.12  Completed by 31.3.13	<ul style="list-style-type: none"> <li>A strategy to address concerns of countries that import Australian honeybees has been supported by SAG &amp; RIRDC, and a specific research project is underway.</li> <li>Biosecurity Queensland's 2 scientists and a spatial and information management officer are reviewing were recruited in April 2012 to assist in conducting this work</li> <li>All AHB data to date has been collated into Biosirt and Geodat sets established.</li> <li></li> </ul>
C.	Implement operations to minimise spread through those pathways, including developing bee free zones around transport hubs, ports, etc and review and revise their efficacy after one year	Develop and implement strategy to minimise spread through pathways, including strategy to create and maintain bee free zones	<b><u>BQCC Cairns</u></b> Compliance Off. Coordinator Program Mgr Field staff <b><u>BQ ABW policy</u></b>	Strategy developed by 31.3.12  Implementation - ongoing	<ul style="list-style-type: none"> <li>A strategy to minimise the spread of Asian honey bee through pathways including the maintenance of bee suppression zones has been developed. (Attachment H.)</li> <li>All nests and swarms that are reported by the public are being destroyed, and the T2M Program's compliance officer is continuing to actively engage with local transport industries. These destruction activities are continuing to suppress AHB population around transport hubs and the port.</li> <li>A review of the dispersal pathways from Cairns suggests current strategies are effective as there has been no known long distance dispersal from Cairns. Ongoing engagement and training with transport industries is effective. Further training of industry groups is underway.</li> <li>A formal bee free zone is yet to be established – A joint trial with Australian Government DAFF-Biosecurity and Biosecurity Queensland has commenced to assess the feasibility of a bee suppression zone.</li> <li>The remote poisoning trials will be trialled around the transport</li> </ul>



		Review strategy and revise efficacy within 12 months of implementation	<b>BQCC Cairns</b> Compliance Off. Coordinator Program Mgr <b>BQ ABW policy</b>	31.3.13	hubs and port. To be commenced <ul style="list-style-type: none"> <li>This is linked back AG2 (B) and is in progress.</li> </ul>
D.	Work with transporter businesses in the development of monitoring systems, providing training in the recognition of AHB and what to do when AHB is suspected	Review existing transport industry systems and develop any monitoring systems (that may minimise spread through pathways) for implementation by the transport industry	<b>BQCC Cairns</b> Compliance Off. Coordinator Program Mgr	30.6.12 ongoing	<ul style="list-style-type: none"> <li>Discussions with industry are ongoing. (Attachment C)</li> <li>A guide and training for transporters has been developed and will be provided in July 2012. (Attachment B.)</li> </ul>
		Deliver information and training packages for transport industries	<b>BQCC Cairns</b> Compliance Off. Coordinator CE Officer	1 <sup>st</sup> session delivered by 31.3.12, ongoing	In progress <ul style="list-style-type: none"> <li>Information and training package developed, based on the engagement with transport industry undertaken since 2010.</li> <li>Discussions held with 50 high risk businesses with relevant AHB literature placed in their offices. This will encourage staff of businesses that are at a high risk of inadvertently moving bees to look out for and report AHB. These companies are at a high risk of inadvertently moving bees and are an effective passive surveillance tool to report suspect AHB.</li> <li>The engagement process with transport industries has shifted since 1 July 2011 – engagement now focuses on the important role transport companies play in preventing the spread of AHB from Far North Queensland.</li> <li>Extension materials, such as ID card, brochures and posters, are supplied to transport companies.</li> <li>Training is planned for July 2012.</li> </ul>
E.	Review the varroa mite management plan and revise, as appropriate  <i>(There is a reference to DAFF's Varroa Continuity Strategy which mentions the need for movement controls. PHA chairs the Management Committee for implementation of the Continuity Strategy - so this T2M deliverable will link in with PHA and the Varroa Continuity Strategy)</i>		<b>BQ ABW policy</b>	Revise by 30.6.12 Revise by 30.6.13	<ul style="list-style-type: none"> <li>Discussions have taken place between the Animal Biosecurity &amp; Welfare program and Plant Health Australia.</li> <li>Further discussions are planned for June/July 2012.</li> </ul>

## QG1 – Protecting Queensland’s social amenity and public assets

Objective – Queensland's social amenity and assets are protected

This project also contributes to Australian Government funded Projects AG1-6.

2011/12 - \$250,000

2012/13 - \$250,000

Project Number	T2M Deliverable	Actions/strategies	Responsibility	Due Date	Comments/status
A.(i)	Contain AHB to the vicinity of known infested areas  Suppress AHB infestations in strategic areas  Destroy AHB infestations around edges of infested area and in areas with high social amenity value	Conduct operations to contain, suppress and destroy AHB <ul style="list-style-type: none"> <li>Develop revised operational strategy and plan to deliver T2M operations</li> <li>Develop revised destruction policy &amp; surveillance strategy to reflect T2M focus</li> </ul>	<b>BQCC Cairns</b> Program Mgr Coordinator Field teams	Ongoing	Ongoing <ul style="list-style-type: none"> <li>A T2M Work Plan has been completed and implemented. – See AG1D.(iii)</li> <li>A revised operational plan is completed, implemented and updated as required. See AG1D.(iv)</li> <li>A summary report of AHB Surveillance and Destruction activities since 1 July 2011, and maps, has been completed quarterly.</li> <li>3 staff trained to be licensed Pest Controllers. 2 staff have since resigned.</li> <li>All AHB nests and swarms detected from public callouts or detected along the edge of the known infested area are destroyed.</li> <li>Since 1 July 2011, 136 nests and 86 swarms have been detected, as at 15 June 2012.</li> <li>Since May 2007, a total of 645 AHB nests and swarms have been detected and destroyed, as at 15 June 2012.</li> <li>Surveillance activities have primarily consisted of trap maintenance and targeted floral sweeping activities along the edge of the known infested area so as to detect any spread of AHB.</li> <li>Targeted floral sweeping surveillance has focussed along the edge of the known infested areas - the tablelands, Innisfail/Sth Johnstone and northern beaches.</li> <li>Surveillance suggests the AHB infestation is becoming more established in known infested areas and new detections have occurred increasing the size of the known infested area. (Attachment I.)</li> <li>87 traps are maintained and checked fortnightly.</li> <li>Active bee eater roosts are monitored and pellets are collected for analysis of AHB presence/absence.</li> </ul>
(ii)		Develop strategy to cease government intervention, respond to fewer public call outs, undertake less surveillance	<b>BQCC Cairns</b> Program Mgr Coordinator Director BQCC <b>BQ ABW policy</b>	Strategy developed by 31.3.12  Complete cessation of operational activity by 30.6.13	<ul style="list-style-type: none"> <li>A strategy has been documented and is in the final stages of approval.</li> </ul>
B.	Conduct surveillance to determine extent of known infested area	Develop and implement staged surveillance strategy to deliver on T2M projects QG1, AG1-6	<b>BQCC Cairns</b> Program Mgr Coordinator Field teams <b>BQ ABW policy</b>	Strategy developed by 31.3.12  Complete cessation of operational activity by 30.6.13	<p>During May, there were several extensions of the known infested area to the north (at Wangetti Beach), to the south (at South Johnstone) and to the north west (at Bibhoora), as well as 'in-fill' infestation at Koah. These extensions of the infested area are to be expected and will become more frequent as the AHB population within the known infested area builds and spreads in all directions.</p> <p>Since January 2012, the shift in operational focus of the Transition to Management Program (from proactive detection and destruction of AHB to validating remote treatment research, developing operational</p>

					tools for use by industry and community to minimise the impacts of AHB, and engaging industry and community to understand the role they play in managing AHB) means there is less resources available for operational detection and destruction activity - the net effect of less detection and destruction is an increase in AHB population that will spread laterally in all directions. At this stage, our continued compliance and industry engagement activities appears to be effective as there is no known long distance spread of AHB through transport pathways.
--	--	--	--	--	--

## QG2 – Improving operational efficiency and effectiveness

Objective – determine efficacy of current operational techniques and operational delivery

This project also contributes to Australian Government funded Projects AG2-6.

2011/12 – \$50,000

2012/13 - \$50,000

Project Number	T2M Deliverable	Actions/strategies	Responsibility	Due Date	Comments/status
A.	Review of efficacy of odour detection dog surveillance. With eradication no longer being the focus of the program, determine whether odour detection dog is a cost effective operational tool	<ul style="list-style-type: none"> <li>Review conducted by Coordinator and BQCC Senior Dog Handler, with input from AHB Program Dog Handler and BQ Senior Dog Handler</li> <li>Recommendation to be made to GM ABW for decision</li> </ul>	<u>BQCC Cairns</u> Coordinator Program Mgr <u>BQ ABW policy</u>	Finalise review by 31.12.11  Final decision by 31.1.12	<b>COMPLETED</b> <ul style="list-style-type: none"> <li>Review found that it was not cost effective to use the odour detection dog for T2M activities. The dog has ceased operational duties on the AHB program and has been re-trained for use in other Biosecurity Queensland programs.</li> </ul>
B.	Quantify efficacy of current operational techniques	Refer to AG2 B.(ii)-(iv)			<ul style="list-style-type: none"> <li>Refer to AG2 B.(ii)-(iv)</li> <li>This is ongoing as research and analysis is conducted as the team transition through the different phases of the program.</li> </ul>
C.	Undertake spatial analysis of current AHB infestation to guide to future surveillance activities	<ul style="list-style-type: none"> <li>Identify, collate and review all information</li> <li>discuss information required to conduct spatial analysis needs</li> <li>conduct spatial analysis</li> </ul>	<u>BQCC Cairns</u> Info/mapping Off. Coordinator <u>BQ Intell. Unit</u>	Commence analysis by 1.3.12 Finalise by 30.8.12	<ul style="list-style-type: none"> <li>2 scientists and a spatial and information management officer were recruited in April 2012 to assist in conducting this work.</li> <li>AHB data has been reviewed and Geodata sets produced but this work has been hampered by the information management officer also having to assist with Remote Treatment trials.</li> <li>The study previously undertaken by a person external to the program is going to be replicated with the updated data in BioSIRT.</li> </ul>
D.	Undertake spread analysis of current AHB infestation to guide future management strategies	<ul style="list-style-type: none"> <li>Identify, collate and review all information</li> <li>discuss information required to conduct spread analysis needs</li> <li>conduct spread analysis</li> </ul>	<u>BQCC Cairns</u> Info/mapping Off. Coordinator <u>BQ Intell. Unit</u> <u>BQ ABW policy</u>	Commence analysis by 1.3.12 Finalise by 30.8.12	<ul style="list-style-type: none"> <li>2 scientists and a spatial and information management officer were recruited in April 2012 to assist in conducting this work.</li> <li>AHB data has been reviewed and Geodata sets produced but this work has been hampered by the information management officer also having to assist with Remote Treatment trials.</li> <li>The study previously undertaken by a person external to the program is going to be replicated with the updated data in BioSIRT.</li> </ul>
E.	Undertake technical analysis of all nests and honeycomb to guide the spread and spatial analysis		<u>BQCC Cairns</u> Technical Adv. <u>BQ Science</u>	ongoing	Ongoing <ul style="list-style-type: none"> <li>Of the 140 detections of nests a significant number could not be extracted (more than 100) and therefore could not be analysed. Nest have been analysed for <ul style="list-style-type: none"> <li>age, queen cells, estimation of drones and workers, assessment of pollen and nectar.</li> </ul> </li> <li>Nests and swarms are analysed for exotic mites. Some results are pending but all results received to date have been negative.</li> <li>Swarms were analysed to estimate size.</li> <li>Honeycomb detected since December 2011 are being analysed as part of a UNE/CSIRO research project – refer to AG2C.(iv).</li> </ul>

## **Attachment B. AHB T2M Documents produced 2011/2012 ([Back to Top](#))**

### **1. Standard Operational Procedures**

- After Hours and Weekend On Call Response Procedures
- Bee Traps Construction and Sample Procedure
- Bee Lining Procedure
- Floral Surveillance Procedure
- Nest Destruction Procedure
- Nest and Swarm management Procedure
- Swarm Destruction Procedure

### **2. Community Engagement**

- Asian honey bee Fact Sheet (**Apr 2012**)
- Asian honey bee Community Guide Fact Sheet (**Jun 2012**)
  - Website updated to show a clear shift in focus with tools for community
- Guidelines to Destroy a Swarm and Nest of Asian honey bees (**Jun 2012**)
  - Workshop presentation and training package developed
- Guidelines to Destroy a Swarm and Nest of Asian honey bees (**Jun 2012**)
  - Workshop presentation and training package developed
- Guide to managing Asian honey bees for Pest controllers and beekeepers (**Jun 2012**)
  - Pest controller workshop presentation and training package developed
  - Beekeepers workshop presentation and training package developed
- Asian honey bee destruction guidelines for government agencies and council staff (**Jun 2012**)
  - QPWS Ranger workshop presentation and training package developed
  - Indigenous Ranger & Traditional owners presentation and training package developed
- Guide to managing Asian honey bees for the transport industry (**Jun 2012**)
  - Transport workshop presentation and training package developed
- Asian Honey bee launch plan developed and approved
- Tools for Responding to an Asian honey bee incursion – Standard operating procedures booklet for national biosecurity centres (draft form)

### **3. Science**

- **Asian Honey Bee (*Apis cerana javana*) in Cairns, Far North Queensland**, Foraging, nesting and swarming behaviour, Report of field observations April 2007-September 2011 Report by Shirin Hyatt, BQCC, Technical Advisor. Available on Plant Health Australia website.
- **Asian Honey Bee Transition to Management Program, Remote Poisoning Trials**, EXPERIMENTAL DESIGN AND PROCEDURES
- **Asian honey bee Remote Nest Treatment**, Results from five nests (and two attempted trials) current at 2 April 2012, Progress report prepared by Shirin Hyatt, BQCC, Technical Advisor
- **Asian honey bee (*Apis cerana javana*) literature review**. Written by Dr. Anna Koetz, BQCC, Senior Scientist. (First Draft completed)
- **Trap Efficacy Report**. Written by Dr. Anna Koetz, BQCC, Senior Scientist.

## **Attachment C. AHB T2M Community Engagement Activities 2011/2012 ([Back to Top](#))**

### **1. Website**

- Online reporting tool established for the public.
- Known Infested Area Map updated and put online – continual updates to occur to reflect known spread.
- Revamped website that includes more conclusive tools for the public's use. Tools such as Identification of the pest, swarm, & nest.

### **2. Facebook & Twitter - [www.facebook.com/biosecurityqld](http://www.facebook.com/biosecurityqld)**

- March/April information regarding an interactive BQ stand at "Toad Day Out".
- June - information regarding an interactive BQ stand at "Festival of the Knob".
- June - information regarding an interactive BQ stand at "World Environment Day – Kuranda".
- Full outline and content for Facebook and Twitter "bee alert" series developed to be run out over a 5 day period from the 2/7 – 6/7/2012

### **3. Bee keeping industry**

- Development of a training and induction package for the industry volunteers has been delivered to over 60 beekeepers across Australia. Their evaluation of this package has been used to guide the development of other training packages. (2011/2012)
- An established engagement process has been developed with local beekeepers. The T2M Program attends monthly local beekeeper meetings.
- Attended Townsville Beekeepers association with update on AHB T2M program (Feb 2012)
- An established engagement process has been developed with beekeepers at a state level. (2011/2012)
- Demonstration delivered to Cairns Beekeeping Association – Demonstration of swarm removal and destruction (March 2012)
- June – Developing live AHB display for use at this year's FNQ show Circuit and future events (June 2012)
- First full training workshops for local beekeepers being held on the 26.6.2012.
- Local Beekeeping industry will be assisting BQ with the FNQ Show circuit 2012 post June.

### **4. Indigenous rangers and communities**

- Taster training session provided to Yarrabah Community - (22.3.2012)
- Taster training session provided to Djunbunji Rangers (15.5.2012)
- Meeting with the regional indigenous liaison officer –to develop future training to empower indigenous Rangers and Traditional owners in Cairns and the Traditional Country areas around the region (Meeting – 20.4.2012).
  - First full training workshops for Indigenous rangers and Traditional Owners being held on the 27.6.2012.

### **5. Environmental groups including Government agencies e.g. Rangers Delivered AHB T2M objectives and program updates**

- Meeting With QPWS at the Mossman & Daintree Office (March 2012)
- Board members and QPWS staff (Mar 2012)
- Andrew Millard (QPWS) and Wet Tropics Management Authority – meetings held to discuss T2M objective. Workshops to be delivered (Feb 2012)
- Terrain (Barron river catchment) – discussions held regarding workshop delivery (Apr 2012)

### **6. Local & State Government Meetings – to provide program updates and change in direction information sessions designed to foster support for T2M (April 2012)**

- Russel Wild (CRC)
- Trevor Sides (ROC)
- Andrew Maclean – (WETMA)
- Andrew Millerd – (QPWS Rangers)
  - First workshops for council being held on the 4.7.2012.
  - First workshops for QPWS rangers being held on the 28.6.2012.

#### **7. Pest Control industry – Presentations and mock kill demonstrations delivered to local Government pest controllers, Land protection officers, QPWS Rangers, & Property owners**

- Pest Annual Forum (Tablelands) Presentation and Mock demonstration on how do Bag/collect/remove swarm of AHB & kill a nest of AHB using BQ techniques. (Nov 2011 public event)
- Pest Annual Forum (Cassowary Coast, Mission Beach) Message delivered to audience through a presentation. “Living with Asian honey bees”. Provided Known infested area map, Identification knowledge of the pest, and differences between a swarm and nest. (Feb 2012 public event)
- Cape York Peninsular Pest Management advisory group (April 2012) Presented project and community engagement update)
- Pest Annual Forum (Cairns) Presentation delivered to local Government pest controllers, Land protection officers, QPWS Rangers, & Property owners with regards to be on the look out for the pest. (August 2011 public event)
- Presentation and information provided to 70 Pest Controllers at a Garrards Pest Controllers Road show regarding their industry involvement in managing the pest, (May.2012 Industry event)
- Pest Annual Forum (Ingham) Presentation delivered to local Government, pest controllers, Land protection officers, QPWS Rangers, & Property owners with regards to be on the look out for the pest and how to control. (May 2012 public event)
- List of local private pest controllers has been compiled for engagement purposes to organise and provide workshops to the industry – empowering industry with skills and knowledge to manage the pest in the future
- Engaged local pest controllers already working with the AHB program to identify their information and training needs. (Apr 2012)
  - First workshop for pest management industry being held on the (5.7.2012)

#### **8. Transport industries engaged regarding limiting long distance spread and responsibilities**

- Transport and Workers Union - Presentation delivered to union workers regarding how to integrate bee watch into track safe procedures (Mar 2012)
- Trades Labour Council - presentation regarding AHB T2M progress and working with the transport industry (Mar 2012)
- Meetings with Main transport companies were held to provide information on up coming training and how the program can work with the industry to limit the long distance spread (NQX freight, Perkins, Sea swift, HMAS Cairns, Toll priority, Northline, Woolworths and Coles, QR National (Apr 2012)
  - First workshop for Transport Industry being held on the (2.7.2012)

#### **9. Public Events/reports Shows**

- Malanda Show – 8,9,10<sup>th</sup> of July 2011 (Static display with BQ officers on hand for information and questions at all regional shows)
- Atherton Show – 11,12<sup>th</sup> of July 2011
- Innisfail Show – 14<sup>th</sup> of July 2011
- Cairns Show – 20,21,22 of July 2011
- Mossman Show – 24,25<sup>th</sup> of July 2011

- Austropex (Cairns convention centre) Presentation delivered to Cairns businesses and Agriculture industry. (12.8.11)
- Carnival on Collins (Cairns Festival) 4<sup>th</sup> of September 2011
- Tablelands Garden Expo 24,25<sup>th</sup> of September 2011
- Toad Day Out 1<sup>st</sup> of April 2012 – Public event for all things pest related. Delivered message to public through one to one conversations. Message – “Living with Asian honey bees” 42 People engaged

#### **10. Media Releases**

- Toad day out – come and see BQ officers at the Toad day out in relation to Asian honey bees (Cairns Post 3.4.12)
- Call to the public and bird watchers too be on the lookout for Rainbow Bee eater roosts outside of the Known infested area (April 2012)
- Expansion of AHB infestation- increase public reports-Call for nests in the area for research purposes to assist science operations (June 2012)

#### **11. Interviews**

- Radio interview – ABC Cairns, with regards to calling on the public for Rainbow Bee eater roosts (27<sup>th</sup> of March)
- Media interview – “Reportage” website regarding Remote Treatment Trials. (May 2012)

#### **12. Drop-In Events - informal activities that had a festive/fun element to help generate enthusiasm for the project and obtain feedback**

- Gordonvale shopping centre (Jul, Aug & Sept 2011)
- Smithfield shopping plaza (Aug 2011)
- Innisfail shopping centre (Nov 2011)
- Mareeba central plaza (Nov 2011)
- Malanda shopping centre (Nov 2011)
- Port Douglas shopping centre (Nov & Dec 2011)



## **Attachment D. Bee Trap Efficacy** ([Back to Top](#))

### **Introduction and methods**

Since 2010, bee traps have been used for surveillance of Asian honey bees. Traps contain sugar syrup and lavender to create an attractive food source to lure bees. The addition of gelatine to the mixture provides a 'gluggy' consistency to the syrup so that foraging bees may become trapped whilst they are feeding.

Traps were placed around the edges of the then known infested area and within 'high risk' areas (namely the port area of Cairns and around major transport nodes). The current trap placement of 101 traps has been in place since September 2011 (Table 1; APPENDIX A). Approximately one-third of traps were placed each in urban environment (including industrial areas), agricultural environment and relatively natural environment (defined as very low or no housing surrounded by large tracts of uninterrupted forest; Table. 2). Field personnel perform surveillance and inspect the traps and remaining sugar syrup levels fortnightly.

The aim of this report was to review the efficacy of bee traps to detect Asian honey bees.

Table 1. General locations of 101 Asian honey bee traps in the greater Cairns area

Area	No of traps
Cairns industrial area	9
Portsmith, Cairns	8
Northern beaches, Cairns	13
Innisfail	25
Malanda	13
Mareeba	33
<b>Total</b>	<b>101</b>

Table 2. Environment types that traps were placed in

Habitat type	No of traps	% of traps
Urban (incl. industrial)	33	32.7
Agricultural	40	39.6
Natural environment	28	27.7
<b>Total</b>	<b>101</b>	

### **Methods**

The efficacy of bee traps to detect Asian honey bees was calculated in several ways:

1. For the period between January 2011 and August 2011 (previous 70 trap locations) the following was calculated:
  - a. The percentage of positive Asian honey bee detections based on the total number of traps checked;
2. For the period between January 2011 and May 2012 (current 101 trap locations) the following was calculated:
  - a. The percentage of positive Asian honey bee detections based on the total number of traps checked;
  - b. The trap run effort (person hours and days);
  - c. The effort needed to detect one Asian honey bee;
  - d. The percentage of total operations time taken up by trap runs.
3. The time periods in 1. and 2. were also combined to determine a combined trap success rate between January 2011 and May 2012.
4. Trap efficacy for traps in different environments (using the current 101 traps) was also analysed.

## Results

Trap success was found to be exceedingly low (Table 1). Only four Asian honey bee detections were made in traps between January 2011 and May 2012, leading to a success rate of 0.14% (Table 1).

The operations team currently has 48 person-days available per week (4 OOs – 10 days/fortnight, 1 OO – 8 days/fortnight). It takes an estimated six person-days/fortnight (or 43.5 hours) to do a trap run. Therefore, the field team spends 12.5% of their time (each fortnight) checking traps.

Three of the four Asian honey bees detected in traps were found in industrial areas, whereas one was found in natural environment (Eucalypt woodland; Table 2). Therefore, trap success was lowest for agricultural environments (0%). Traps in natural and urban environments were 0.2% and 0.6%, respectively (Table 3).

**Table 1.** Trap success rates for two different time periods as well as the combined period.

Time period	No of traps checked	No of AHB found	Trap success
January - August 2011	1120	4	0.36%
September 2011 - May 2012	1818	0	0%
January 2011 - May 2012 (combined)	2938	4	0.14%

**Table 2.** Locations of the four Asian honey bees found in traps

Date	Trap number & location	Vegetation
17/08/2011	AHB13388, Kuranda	Eucalypt woodland
5/08/2011	AHB13403, Portsmith, Cairns	Industrial
11/07/2011	AHB13403, Portsmith, Cairns	Industrial
11/07/2011	Hargreaves Rd, Edmonton	Industrial/agricultural

**Table 3.** Trap success for different environment types between September 2011 and May 2012.

Environment type	No. of traps	No of traps checked	No of AHB found	Trap success
Urban (incl. industrial)	33	528	3	0.6%
Agricultural	40	640	0	0%
Natural environment	28	448	1	0.2%

## Discussion

The bee traps as they are currently used are not an effective way of detecting Asian honey bees. 0.36% for an eight-month period, and 0.14% for the entire 17-month period between January 2011 and May 2012 is a very low success rate, at a rather high effort required by the operations team.

As Asian honey bees have spread, some traps are no longer at the edge of the known infested area but within it. Interestingly, despite Asian honey bees likely being present in areas where traps are located, no Asian honey bees have been trapped recently.

The low success rate can most likely be linked to several factors based on field experience by the operations team:

- Sugar syrup stations (traps) are not an attractive food source for honey bees, and bees are unlikely to feed on them unless they are trained to do so. This involves spraying the bees with sugar syrup, which may then result in the bees going onto the station. This initial training is crucial in getting bees to feed on a feeding station, but it means that detection of wild Asian honey bees is unlikely.
- Traps are often placed randomly in the landscape. However, even when they are placed directly underneath an Asian honey bee nest or next to floral sources with Asian honey bee foragers, they will prefer flowers to the sugar syrup.

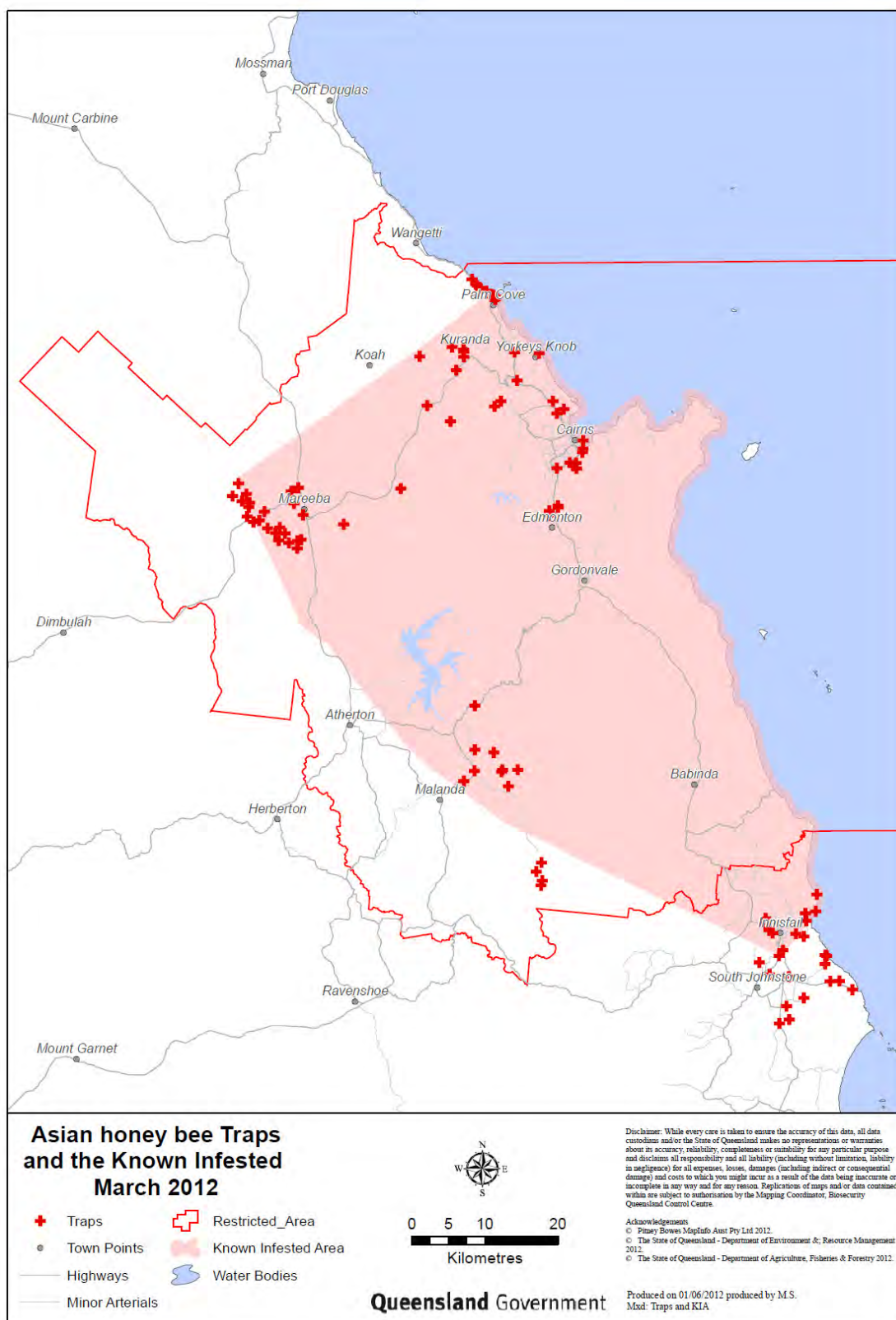
- Despite being covered, traps may dry out in dry weather or flood and dilute in wet weather (especially the wet season) within the two weeks between trap runs. Dry traps do not attract bees, and diluted trap syrup does not trap bees.

Traps syrup may be used to detect Asian honey bees that have fed on it using genetic techniques. It has been confirmed that this can be done when in excess of 50 bees have fed on the syrup, and tests are currently underway to determine whether fewer bees (e.g. one or two) can also be detected. If so, feeding stations (without being sticky or gluggy) could be left in the field, and syrup liquor could be collected periodically to be genetically checked for Asian honey bee DNA. Rainbow bee-eater roosts are used in a similar way to detect presence of Asian honey bee. However, unlike feeding stations that need to be topped up regularly, rainbow bee-eater roosts require no maintenance.

Based on this analysis, it would be advisable to cease the trap runs in the current locations and using the current method, until better attractants are found. Targeted floral sweeping appears to be a quicker and more time (and hence cost) effective way to survey large areas for Asian honey bees.

The floral sweeping technique involves collecting seasonal blossom data from within the region of surveillance for all flowering trees, plants, shrubs and weeds that honey bees and Asian honeybees in particular have previously been found to prefer foraging on. These floral sources are then mapped and surveyed frequently by motor vehicle at appropriate times of the year (and day) for the presence of Asian honeybees. This process also allows a potentially huge spatial area to be surveyed effectively in a relatively short period of time.

## APPENDIX A: Map of current trap locations



# **Attachment E. Remote Treatment Trial Update as at 26 June 2012 ([Back to Top](#))**

Trial #	Nest locality	Date of treatment/s	Nest Activity prior to trial and/or nest size S,M,L	Target	Achieved No. of bees (% relative to nest activity) Foraging distance Exposure time	Result <b>Red – nest dead</b> Grey – aborted <b>Green – still active</b>	Hours prepare/ conduct trial (for 2 x FO)	Noteworthy Observations
Trial 1 IP556	House cavity	28.2.12	3692 bees entered nest in 60 min/Large	No. bees - 1000 Foraging distance – 80m Exposure time – 60mins	No. bees – 973 (26.4%) Foraging distance – 82m Exposure time – 60mins	<b>Nest DEAD at 24 hrs</b>	26.25hrs	Strong foraging before trial on regular station, reduced foraging on bait station at 30min, no foraging at 45 min
Trial 2 IP 563	House cavity	5.3.12				<b>Trial abandoned</b>	14.5hrs	Prior to start of trial, nest was being attacked by Green Ants – nest destroyed by predators
Trial 3 IP 569	Tree cavity	6.3.12	Nest activity not able to be counted due to height of nest/Nest busy - Large?	No. bees – 500 Foraging distance – 80m Exposure time – 60mins	No. bees – 201 Foraging distance – 76m Exposure time – 60mins	<b>Trial aborted</b>	81.75 hrs	Strong foraging on regular station before trial, reduced foraging on bait station at 30min, 3 foragers at 45min. Monitoring at 24 hrs found that foragers remaining on feeding station are from a different nest (confirmed by beelining) – <b>when nest was checked at 49 days mellifera were found in the nest. There is a possibility that mellifera may have taken over the weakened cerana nest.</b>
Trial 4 IP 557	House cavity	5.3.12  13.3.12	1600 bees entered nest in 60 min/Medium?  1300 bees entered nest in 60 min/Medium?	No. bees – 150 Foraging distance – 80m Exposure time – 60mins  No. bees – 150 Foraging distance – 80m Exposure time – 60mins	No. bees – 150 (9.4%) Foraging distance – 74m Exposure time – 20mins  No. bees – 128 (12.4%) Foraging distance – 74m Exposure time – 30 mins	<b>Nest DEAD at 31 days after initial treatment (21 days after second treatment)</b>	54.75 hrs	Strong foraging before trial and for duration of time bait station in field. Significantly lowered nest activity at 24 hrs, nest under attack by Green Ants at 48 hrs. Activity appeared to increase after 48 hrs (green ants numbers less). Second treatment conducted at 240 hrs with nest activity since remaining low (green ants observed again at 336 hours following 2 <sup>nd</sup> treatment).
Trial 5 IP 567	House cavity	8.3.12	2198 bees entered nest in 60 min/Large?	No. bees – 500 Foraging distance – 80m Exposure time – 60mins	No. bees – 322 (14.6%) Foraging distance – 80m Exposure time – 60mins	<b>Nest manually destroyed at 64 days</b>	36.25 hrs	Strong foraging on station before trial, reduced foraging at 30min, 2 foragers at 40min, 0 foragers at 60min  Very low nest activity between 24 hrs and 33 days. Significantly increased activity from 34 days. 2 <sup>nd</sup>

								treatment was planned but bees could not be trained back onto station. Nest was destroyed at 64 days.
Trial 6 IP 562	House cavity	13.3.12	5524 bees entered the nest in 60 min/Large?	No. bees – 500 Foraging distance – 80m Exposure time – 60mins	No. bees – 400 (7.4%) Foraging distance – 80m Exposure time – 30mins	<b>Nest DEAD at 37 days (888hrs)</b>	52.25 hrs	Strong foraging activity on regular station before trial, reduced foraging on bait station by 30 minutes. Significantly lowered nest activity at 24hrs and activity has remained low and steady since; declined to zero by 888hrs.
Trial 7 IP566	Bird box	21.3.12	606 bees entered nest in 60 min/very small (approx. 500-1000 bees in colony)	No. bees – 50 Foraging distance – 80m Exposure time – 60mins	No. bees – 42 (6.9%) Foraging distance – 25m Exposure time – 12mins	<b>Nest DEAD at 6 days</b>	28.75 hrs	Couldn't move station to 80m, bees would not cooperate. Trial conducted at 25m instead. Low foraging activity on station before trial, low foraging activity at 12 minutes. Inspection at 24 hrs – very low activity (lots of drones flying around entrance) Inspection at 48 hrs and 120 hrs – low activity 20-30 bees remaining on comb. Nest dead at 6 days (144hrs). Ants consumed most dead bees at bottom of box, only exoskeleton parts remaining.
Trial 8 IP 578	In tree cavity	4.4.12  24.05.12	Busy. Count could not be done as 12m high in tree	No. bees – 600 Foraging distance – 80m Exposure time – 60mins  No. bees – 1000 Foraging distance – 80m Exposure time – 60mins	No. bees – 580 (% na) Foraging distance – 80m Exposure time – 40mins  No. bees – 921 (23% na) Foraging distance – 80m Exposure time – 40mins	<b>Nest DEAD at 57 days after <u>initial</u> treatment (7 days after second treatment)</b>	279.5 hrs	Strong foraging on station before 1 <sup>st</sup> trial, reduced foraging at 20min, 5 foragers at 40min. Nest activity decreased dramatically, stayed low until day 16. Starting to increase in numbers. Strong foraging on station before 2 <sup>nd</sup> trial, reduced foraging at 20min. Nest activity decreased dramatically and did not recuperate. Nest was extracted by contractor.
Trial 9 IP589	In tree cavity	10.4.12	Busy. Count could not be done as 12m high in tree	No. bees – 1200 Foraging distance – 80m Exposure time – 60mins	No. bees – 1110 (% na) Foraging distance – 84m Exposure time – 40mins	<b>Nest DESTROYED by contractor after 55 days.</b>	203 hrs	Strong foraging on station before trial, reduced foraging at 30min, 25 foragers at 30min, 0 bees at 40 minutes. Nest activity decreased dramatically, increasing from day

								16. Targeted for 2 <sup>nd</sup> treatment but staff shortages and re-allocation of priorities led to the decision to destroy this nest and forgo the 2 <sup>nd</sup> treatment.
Trial 10 IP593	In house cavity	17.4.12	3390 bees entered nest in 60 minutes	No. bees – 800 Foraging distance – 80m Exposure time – 60mins	No. bees – 800 (23.6%) Foraging distance – 80m Exposure time – 34mins	<b>Nest DEAD at 24 hrs</b>	20.75 hrs	Busy nest and strong foraging on station before trial. Reduced foraging by 20 mins. No activity was seen from 24 hrs. Checked with endoscope at 9 days, confirmed dead.
Trial 11 IP591	In compost bin	18.4.12	3096 bees entered nest in 60 minutes	No. bees – 700 Foraging distance – 80m Exposure time – 60mins	No. bees – 484 (15.6%) Foraging distance – 80m Exposure time – 60mins	<b>Nest DEAD at 24 hrs</b>	18.75 hrs	Bees started showing signs of poisoning at 20 minutes. 2-5 bees on station by 40 minutes, 0 by 50 minutes. No activity seen at 24hrs. Nest extracted from bin at 6 days.
Trial 12 IP558	In incinerator	19.4.12	5916 bees entered nest in 60 minutes	No. bees – 1200 Foraging distance – 80m Exposure time – 60mins	No. bees – 1022 (17.3%) Foraging distance – 80m Exposure time – 60mins	<b>Nest manually destroyed at 48hrs</b>	10.75 hrs	Busy nest and strong foraging on station before trial. Reduced foraging by 30 mins. Nest activity severely reduced at 24hrs (2 bees entering nest). Mellifera found to enter nest at 48hrs. Decision made to abort to avoid off-target impact from mellifera robbing honey.
Trial 13 IP606	In tree cavity	16.05.12	1060 bees entered nest in 60 minutes	No. bees – 200 Foraging distance – 80m Exposure time – 60mins	No. bees – 60 (6%) Foraging distance – 80m Exposure time – 60mins	<b>Nest still ACTIVE after 41 days</b>	96 hrs	Strong foraging activity before trial, medium nest activity. Few bees on station once trial commenced (5-16 at any one time). Reduced foraging activity from 10 minutes. Nest activity was slightly higher at 24 hrs after treatment and has been going strong since. 2 <sup>nd</sup> treatment with high target percentage planned for 26.06.12.
Trial 14 IP615	On support post for a fish tank	28.05.12	3024 bees entered nest in 60 minutes	No. bees – at least 600 Foraging distance – 80m Exposure time – 60mins	No. bees – 742 (25%) Foraging distance – 80m Exposure time – 60mins	<b>Nest DEAD after 8 days</b>	73 hrs	Strong foraging and nest activity before trial. 40-60 bees on bait station during first 30 minutes of trial. Bees showed effects of fipronil from 20 minutes, reduced foraging 40 minutes. Nest activity dramatically reduced within 24 hrs, no activity from day 3.

Trial 15 IP609	In tree cavity	30.05.12	1294 bees entered nest in 60 minutes	No. bees – as many as possible in 60 minutes Foraging distance – 80m Exposure time – 60 mins	No. bees – 222 (17%) Foraging distance – 80m Exposure time – 60 mins	<b>Nest DEAD at 21 days after <u>initial</u> treatment (2 days after second treatment)</b>	193.5 hrs	Medium foraging and nest activity before trial. 30 to 50 bees on the original bait station immediately prior to trial. Bees seemed uninterested in the remote treatment station, most were hesitant to drink from the station. 222 bees left station during the remote treatment trial but it seemed that very few took Fipronil syrup back with them. Nest activity reduced at 48 hrs but increased greatly from day 5. Strong foraging and nest activity before 2 <sup>nd</sup> trial. Nest entrance activity dramatically reduced 24 hrs after 2 <sup>nd</sup> treatment. No activity from day 2.
		18.06.12	3576 bees entered nest in 60 minutes	No. bees – 1250 Foraging distance – 80m Exposure time – 60 mins	No. bees – 1250 (35%) Foraging distance – 80m Exposure time – 39 mins			
Trial 16 IP608	In house cavity	05.06.12	1132 bees entered nest in 60 minutes	No. bees – 600 Foraging distance – 80m Exposure time – 60mins	No. bees – 573 (51%) Foraging distance – 15m Exposure time – 60mins	<b>Nest DEAD 7 days after treatment</b>	62 hrs	Medium foraging and nest activity before trial. 20-30 bees on station at any one time. Bees start being affected by fipronil after 30 minutes, reduced foraging from 40 minutes. No nest entrance activity from 48 hrs.



**Attachment F. Cairns Port surveillance strategy and trial - AHB suppression zone around seaport. ([Back to Top](#))**

**Cairns Port surveillance strategy and trial - AHB suppression zone around seaport.**

The broad strategy involves creating a “bee suppression zone” around the International Seaport in Cairns, which has been identified as the most likely area where a new incursion of Asian honey bee would occur.

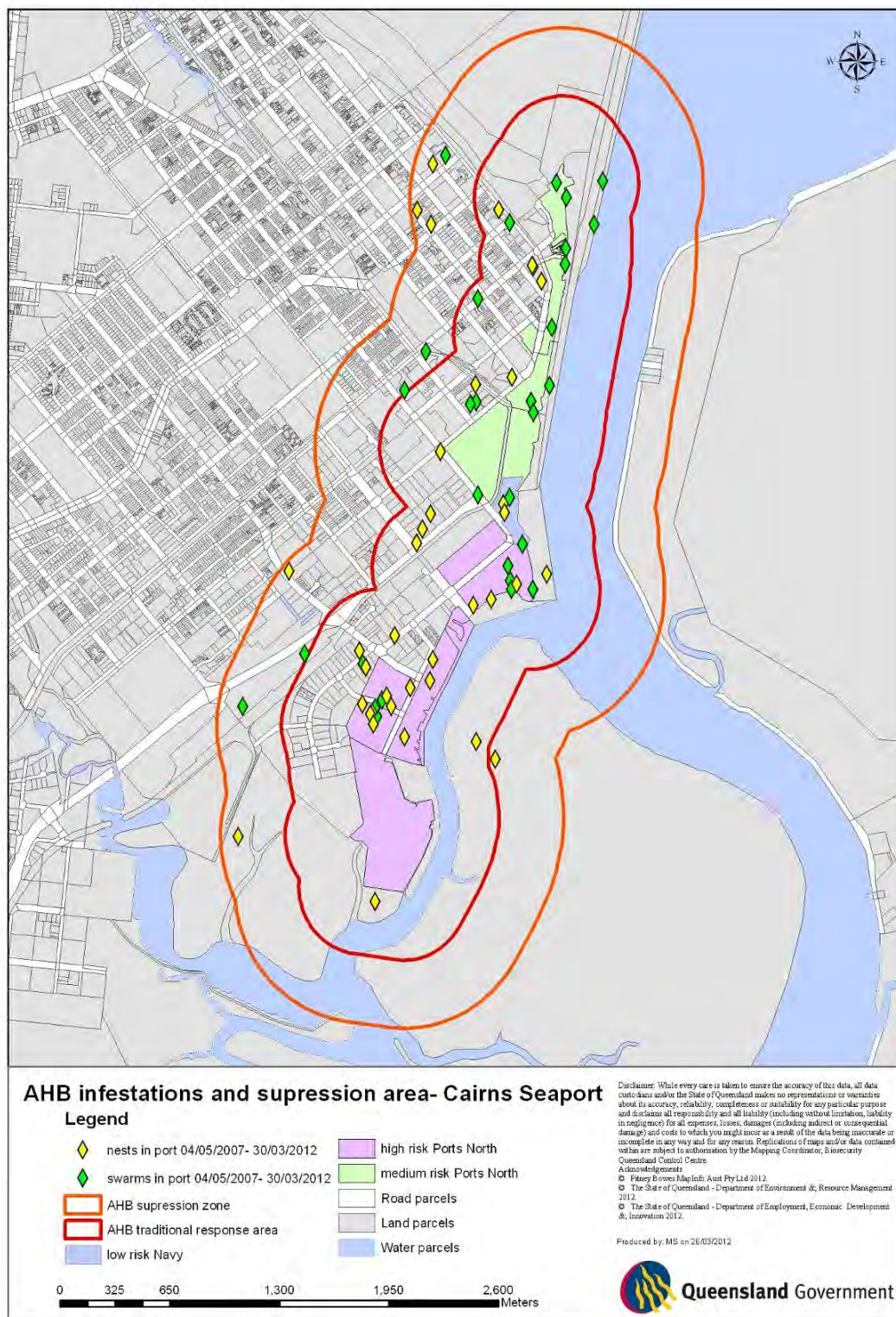
Australian Government DAFF Biosecurity will conduct active bee surveillance and Biosecurity QLD will conduct subsequent response activity until 31 December 2012. The aims of this concept are to detect the presence of exotic bee parasites that may establish in the *Apis cerana* population through subsequent incursions and potentially to demonstrate the benefit of fipronil baiting to suppress a bee population in a port area. The risks for new *Apis cerana* introductions into different areas of Cairns Port were discussed.

- It was agreed that the suppression zone would be based around the port area associated only with international sea cargo and Torres Strait sea cargo.
- DAFF QLD data relating to distance between *Apis cerana* nests and feeding stations will be analysed to determine the appropriate radius around the port area for the suppression zone. DONE
- Within the suppression zone, DAFF will initiate appropriate responses to referrals of bees that occur within the immediate port area and DAFF QLD will initiate appropriate responses in the remainder of the zone. IN PROGRESS

A joint surveillance strategy for the detection of bee mites is being tested in an area around Cairns Port where *Apis cerana* has established. NAQS staff have implemented regular floral surveillance designed to detect foraging *Apis cerana*. DAFF QLD staff then use these detections to identify nest locations, capture bees and collect nest material. NAQS staff examine collections for the presence of bee pest mites and DAFF QLD staff may conduct PCR analysis of samples for new *Apis cerana* genetic material where considered necessary.

The project will examine the feasibility of this strategy as an alternative surveillance option to that of sentinel *Apis mellifera* hives. It may also provide a quantitative measure of the resource required to achieve freedom and or management of *Apis* species around a port area.

This activity contributes to the delivery of projects B(i) and D under the AG5 component of the Transition to Management Program. Also, inherent within this activity is development of a protocol re use of sweep netting to provide confidence of absence/presence of AHB and a protocol regarding the laboratory analysis of AHB detections that meets the delivery requirements of project C.



**Attachment G. Optimising early detection of new incursions of AHB - Collaboration between Australian Government DAFF-Biosecurity and Biosecurity Queensland.**  
**(Back to Top)**

**Optimising early detection of new incursions of AHB - Collaboration between Australian Government DAFF-Biosecurity and Biosecurity Queensland**

Australian Government DAFF-Biosecurity data (from its Operational Science Program) indicates that international air cargo does not represent a significant risk pathway for *Apis cerana*. NAQS data indicates the same in regard to domestic Torres Strait aircraft pathways. As a result the following has been agreed

- Cairns Airport should not be considered as relevant to AG5 for the “Bee Free Zone”.
  - International terminal – Australian Government DAFF-Biosecurity has an active strategy around the international terminal – and would respond to any bee reports in this area.
  - Domestic terminal – due to proximity to international terminal, there would be an expectation that Australian Government DAFF-Biosecurity would still respond – this would be reviewed once tools are developed and education with CAA and relevant aviation businesses has occurred
  - General aviation - agreed that Biosecurity Queensland would respond to general aviation – until the tools are developed and education with general aviation business has occurred
- Cairns Seaport - was the highest risk area.

**AHB suppression around seaport**

- The northern seaport is recreational vessels and local transportation, whereas the southern seaport is the international cargo area – each area has differing levels of risk
- International pathway is the risk that needs to be managed
- Biosecurity Queensland has identified the risks of the different areas of the seaport
- Biosecurity Queensland has used existing data to map suitable floral resources around the port – this will focus the targeted surveillance.
- As a general rule:
  - DAFF Biosecurity will respond to reports within the seaport
  - Biosecurity Queensland will respond to reports outside the seaport
  - Both Biosecurity Queensland & DAFF Biosecurity will assist each other where appropriate and when requested
- To gain confidence about absence or presence of mites on AHB
  - Swarms – analysis of swarm bees will give high confidence of absence/presence of mites
  - Foraging bees – only a small percentage of foraging bees carry mites, so analysing single bee will provide low confidence of absence/presence of mites
  - Nests – strategically sampling X number of *A. mellifera* & AHB nests within proximity of port will provide high confidence of absence/presence of mites
- If we want to test/trial anything in a population carrying mites, then it should be conducted in the Torres Strait Islands





## Asian Honey Bee Response Authorities-Airport



Queensland Government

### Legend

- airport land
- BQCC response area
- AQIS response area
- Motorways
- Highways
- Minor Arterials



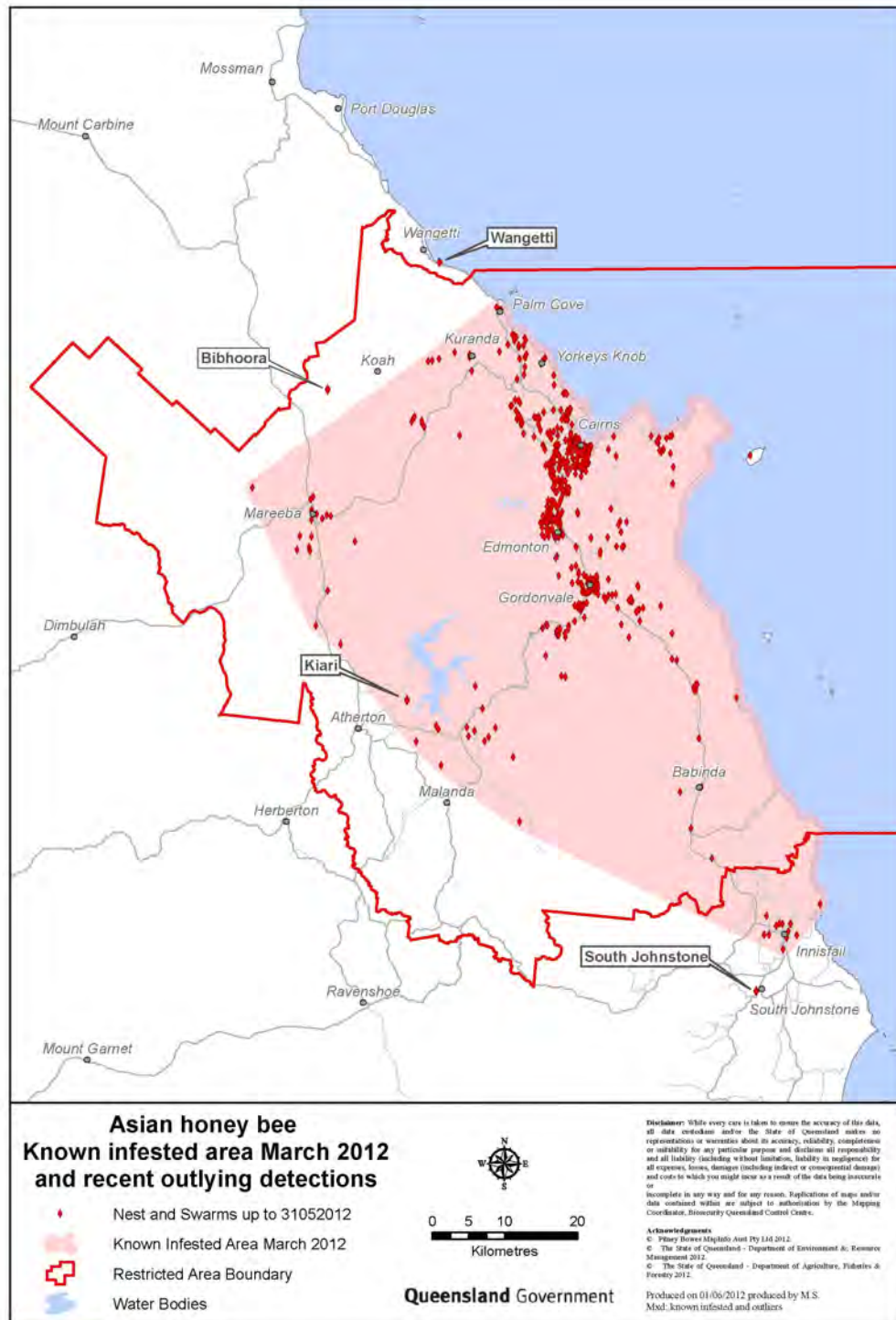
0 250 500 1,000 1,500 2,000 Meters

Disclaimer: While every care is taken to ensure the accuracy of this data, all data custodians and/or the State of Queensland makes no representations or warranties about its accuracy, reliability, completeness or suitability for any particular purpose and disclaims all responsibility and all liability (including without limitation, liability in negligence) for all expenses, losses, damages (including indirect or consequential damage) and costs to which you might incur as a result of the data being inaccurate or incomplete in any way and for any reason. Replications of maps and/or data contained within are subject to authorisation by the Mapping Coordinator, Biossecurity Queensland Control Centre.

Acknowledgements  
© Piney Bowes MapInfo Aust Pty Ltd 2012  
© The State of Queensland - Department of Environment & Resource Management 2012  
© The State of Queensland - Department of Employment, Economic Development & Innovation 2012

**Map Produced By M.S. 27/03/2012**

**Attachment H. Map of Known Infested Area showing recent detections. ([Back to Top](#))**





## **Attachment I. Detection of *Apis cerana* DNA from bee eater pellets and trap liquor**

### **(Back to Top)**

#### **Detection of *Apis cerana* DNA from bee eater pellets and trap liquor**

B.Pease and J.Oakey

#### **Tasks**

Biosecurity Queensland were tasked to develop a test for the detection of *Apis cerana* (asian honey bee or AHB) DNA. The test is required to assist in the surveillance program by providing either a more efficient or more sensitive detection tool than current methods. Advice from the surveillance team was to concentrate on two sample types:

1. Bee eater pellets, which could contain skeletal remains of consumed insects. Current work relies upon the presence of intact wings that can be morphologically identified. Wings can be quickly identified by an entomologist, the limiting factor being the necessity to find an intact wing. It would be advantageous if a DNA test could provide diagnosis in the absence of whole wings.
2. Material from the surveillance feeding traps. The traps of sugar syrup and sand rely upon a bee being caught in the syrup and morphologically identified. Ineffective capture rates left many traps empty despite bees feeding in them. It would be advantageous if a DNA test could detect when AHB have fed though not been caught.

#### **History**

In 2007 BQ were tasked with development of a simple PCR that would react with DNA from *A. cerana* but not DNA from any other species. The test material was intended to be wings and wing fragments within honey-eater pellets. The resulting test was shown to detect approx. 5-50 pg *A. cerana* DNA and did not react with *Apis mellifera*. The test necessitates the separation of wings and other insect parts from the pellets prior to analysis and it was suspected the pellet material contained some PCR inhibitors. Wings are separated from the pellets with a simple floating technique. This test is ready for operational use, pending confirmation that reaction components that have superseded those from 2007 perform sufficiently.

#### **Samples**

1. Bee eater pellets were obtained from bee eater roosting sites in areas of known AHB infestation in far north Queensland. Some pellets were identified as positive by BQ entomologists while others were tested without prior processing.
  - Pellet 1 – Machan's beach. Location (-16.85221,145.74518)
  - Pellet 2 – McLeod St. Location (-16.92386, 145.77218)
  - Pellet 3 – Kuranda. Sample processed at TAAHL and positive for AHB wings.
2. Trap samples were obtained from BQ staff in Cairns, traps were exposed to feeding bees for an hour then the top 5 mm of sand and all of the liquor were removed and stored at 4 degrees until processed. Negative samples were exposed to uninfested sites overnight prior to sampling.
3. *Apis cerana* samples were obtained from infested sources in far north Queensland. Two samples were taken from the following locations:
  - IP8 – Nest located at Greenhill, Qld 4865
  - IP9 – swarm located Greenhill, Qld 4865
  - IP10 – Nest located at Aloomba, Qld 4871
  - IP15 – Nest located at Portsmouth, Qld 4870
4. *Apis mellifera* samples were obtained from the Varroa mite detection team at the Biosecurity Sciences Laboratory.

#### **Confirmation and validation of test designed in previous study by BQ**

The test designed in a previous study by Biosecurity Queensland was used as a starting point. The study had designed primers to the cytochrome B gene located on the mitochondrial DNA of *Apis cerana*. Comparison of this work and later published<sup>1</sup> sequence of the entire mitochondrial DNA from *Apis cerana* (Chinese origin) indicated a possible redesign of one of the two primers and a change of reagents may yield a more sensitive test.

---

<sup>1</sup> Tan et al. PLoS ONE 6 (8), E23008 (2011) The Complete Mitochondrial Genome of the Asiatic Cavity-Nesting Honeybee *Apis cerana*

The two sets of primers and two reagent chemistries were tested with respect to annealing temperature and serial dilution of template to determine the optimal combination for detection. Detection of AHB DNA was found to be most sensitive and robust using MyTaq DNA polymerase (Bioline, Alexandria, NSW) and the redesigned primers at 63 °C annealing. No cross reaction with *Apis mellifera* was observed and sensitivity was increased by 1 order of magnitude to detect approximately 0.5-5pg of DNA.

#### **Bee eater pellets**

A bee wing identified as *A. cerana* from a pellet was used as template to determine the viability of DNA having passed through the gut of the bee eater. Two single wings were extracted using the standard insect DNA extraction procedure developed for the Electric ant eradication program (Biosecurity Queensland) requiring a modification of the manufacturers recommended procedure applied to DNEasy Blood & Tissue Kit (Qiagen, Doncaster, VIC). Viable DNA was extracted and tested positive to *Apis cerana* by PCR and identification was confirmed through DNA sequencing of the amplicon. Wings found in bee eater pellets collected in AHB-free areas produced a negative result.

The insect extraction method was compared with the DNeasy Stool DNA extraction kit, commonly used where PCR inhibitors are likely to be present. One pellet sample was spiked with an *Apis cerana* wing. Minor changes were made to the manufacturers recommended procedure to allow for a large volume of sample, and 1 gram of pellet was used. The increased starting volume was required to accommodate homogenisation of the sample to ensure that subsampling did not exclude the small amount of *Apis cerana* skeletal tissue expected to be in the sample. It was not possible using these methods to sufficiently reduce inhibitors to allow downstream PCR processing, therefore the test can currently be used only on separated wing samples. Information has been provided by Qiagen as to a possible alternative procedure however due to a short timeframe and limited number of samples available this investigative direction has not been fully exhausted.

The results have proven the ability of the test to definitively distinguish *Apis cerana* wings from *Apis mellifera*. The test is more expensive and time consuming than morphological identification therefore has little application in its present form, though will provide a secondary analysis confirmation when morphological characteristics such as a brown streak, vein length and spurs are obscured or conflicting. Further investigation into inhibitor removal would enable the test to be undertaken without the necessity of wing removal. Should this prove successful the test could be used to screen large numbers of samples efficiently. As the DNA extraction preserves the morphology of the wings this would enable both forms of identification to be used, and a record of the find to be retained.

### Trap samples

The traps essentially consist of a “takeaway food container” that is half filled with sand to provide a landing area for the bees and an amount of sugar syrup with floral attractant for the bees to feed on at the other end.

Initially, it was reported that bees were unidentifiable due to degradation as they lay trapped in sugar syrup in the field for up to a week before collection. Attempts to recreate this scenario in the lab proved unsuccessful. A fresh bee was placed in the sugar syrup and incubated at 37 °C for a month. While organism growth was observed through the increase in biomass and the production of gases, the bee itself did not require DNA analysis as it remained morphologically identifiable after this time and may have been preserved by the sugar concentration in the syrup. Field trials were setup by Cairns field staff although were impeded by wet weather so no results are available.

It was decided to apply the DNA test to the scenario in which AHB have landed on the trap, fed on the syrup and flown away. Samples of sugar syrup and sand that had been placed in a AHB-free area were used to prove the concept. Syrup sample #1 was spiked with 2 µL (1 µg) of AHB DNA; syrup #2, with 10 µL (5 µg) of AHB DNA; syrup #3, with a single leg of an AHB; and syrup #4 remained AHB free, though would have contained native insect DNA collected in the field. Sand sample #1 was spiked with 2 µL (1 µg) AHB DNA; Sand #2 was unexposed sand; and sand #3 was sand that had absorbed syrup but not exposed to AHB. Samples were extracted with DNeasy blood and tissue kit. It was found that all spiked syrup and sand samples provided positive reactions.

To challenge the DNA test with field samples BQ staff prepared a trap and allowed AHB from a research colony to feed freely for a period of an hour, it was estimated that over fifty bees fed on the trap. Three replicates of 3 mL of AHB exposed syrup and a 3mL sample of unexposed AHB syrup were extracted with the DNeasy blood and tissue kit. The top 5mm of sand that had AHB land on it was washed in buffer EB (DNeasy kit component) and this was extracted using the blood and tissue kit. All replicates of the syrup tested positive for AHB DNA, however both sand samples tested negative, controls indicated reliable results.

These results demonstrate the DNA test can be used to determine if AHB have fed on a syrup sample. Further field trials to test the limitations of the test in terms of number of bees required to feed and duration of feeding have been hindered by recent weather conditions and results are not available at present. When this has been completed, the results to date will be used to develop a standardised testing regime based on DNA detection from syrup samples that can be applied in the surveillance program.

### Conclusions

A test for the specific detection of *Apis cerana* DNA has been developed. The test is sensitive to approximately 0.5-5 ng of *Apis cerana* DNA and has shown no cross-reaction to *Apis mellifera* or any native insects that were trapped when lure syrup was placed in AHB free areas of far north Queensland. DNA extraction methods for whole bee, sugar syrup and wings removed from bee-eater pellets have been investigated and optimised for each sample type. This work provides proof of concept for a molecular surveillance program for AHB in far north Queensland. The test has been optimised on the following sample types:

1. Whole bee (Diagnostic speciation) - \$9 per sample (includes bidirectional sequence)
2. Bee wing extracted from bee-eater pellet (Diagnostic speciation) - \$9 per sample
3. Sugar syrup (Detection of presence/ absence) \$22 per sample + \$6 sequence for positives



**Attachment J May 2012: Short study of microsatellite alleles in Asian honey bees Sourced from PNG/Solomons and north Queensland: summary of data interpretation (Back to Top)**

**May 2012: Short study of microsatellite alleles in Asian honey bees sourced from PNG/Solomons and north Queensland: summary of data interpretation**

**Specimens:**

5 X DNA from *A. cerana* sourced in Thailand provided by Dr. B. Oldroyd (U Sydney), outgroup  
5 X bees from single site/sample sourced from Solomons, provided by AQIS  
2-5 X bees from each of 11 samples sourced from PNG, provided by AQIS  
3-5 X bees from each of 3 samples sourced from Torres Strait, provided by AQIS  
100 X bees representing 1 – 4 individuals from 29 early infested sites in Cairns

**Microsatellite loci studied:**

A preliminary study in 2009 using eight loci showed this number of loci to be insufficient for population assignment. In 2011, a second study using 20 loci showed that 17 were consistently amplified from the Queensland samples. These 17 loci were used in this study. Two loci were monomorphic for all samples, but were retained to serve as positive controls for allele size.

**Data collection methodology in brief**

DNA was extracted from partial bees, using either legs, head or abdominal slices, depending upon the state of the sample. DNA was extracted using a commercial kit (Qiagen DNeasy blood and tissue). Amplification was conducted using a commercial master mix (Qiagen Multiplex PCR) with all loci multiplexed into two reactions following application of the software Multiplex Manager. Alleles were resolved using Applied Biosystems Genetic Analyser 3500xL, with inclusion of Genescan 600 as a size standard. Data were visualised and proofread using GeneMarker software (Soft Genetics). Each set of data contained a negative control with no addition of DNA, and a positive control containing DNA from a bee previously tested to demonstrate consistency of allele presence and size.

**Summary of data analyses**

**1. Cluster analysis**

The software STRUCTURE<sup>2</sup> was applied to the data firstly to determine optimal number of genetic clusters (determination of  $k$ ).  $K$  was determined using the method described by Pritchard *et al*<sup>1</sup>, and by the method described by Evanno *et al*<sup>3</sup>. Both methods inferred a total of six clusters from the data. STRUCTURE was repeated for 100 independent runs using K6 and the runs were combined into a single assignment table using the software CLUMPP<sup>4</sup>. Assignments were independently evaluated using the method of Paetkau<sup>5</sup> in GenAlEx<sup>6</sup> and the reassignment resulted in the same clustering. Assignments are depicted in Table 1. In summary, Cluster 1 contains samples from PNG and Torres Strait, and cluster 2 contains samples from PNG, Torres Strait and Solomon Is. One sample site, sai594 contained individuals from both clusters and one specimen sai594-1 had equal likelihood of assignment to either cluster. Clusters 3, 4 and 6 contain the samples from the Cairns area. The sample from IP1 (Q1-1, Q1-3 and Q1-4) contained individuals from clusters 1 and 6, while the other 28 IPs could be assigned to a single cluster. The sample included as a presumed outgroup formed a distinct cluster 5.

**2. Differentiation of clusters**

a.  $F_{ST}$  was calculated using GenAlEx<sup>5</sup> and is depicted in Figure 1. For ease of interpretation, those with lower  $F_{ST}$  (arbitrarily selected as  $<0.1$ ) are shaded pink, and those with higher  $F_{ST}$  (arbitrarily selected as  $>0.2$ ) are shaded blue and unshaded boxes represent interim  $F_{ST}$ . As expected, cluster 5 (the Thai outgroup) had the highest pairwise  $F_{ST}$ s with all the other clusters. The lowest  $F_{ST}$ s were between clusters 1 and 2 (PNG, Torres Strait and Solomon Is), and clusters 3 and 4 (Cairns). The  $F_{ST}$  between the three Cairns clusters was  $<0.1$ , but the  $F_{ST}$  between the Cairns clusters and the PNG/Solomons clusters was in the intermediate-high range, with cluster 2 and cluster 4  $F_{ST}$  being comparable to the difference between the outgroup and any other sample.

b. The likelihood of assignment tests (Paetkau) are depicted graphically to provide a graphical representation of the differentiation of clusters (Figure 2). As with the  $F_{ST}$ , the clusters

<sup>2</sup> Pritchard *et al.* 2000, *Genetics* **155**: 945-959.

<sup>3</sup> Evanno *et al.* 2005, *Molecular Ecology* **14**: 2611-2620

<sup>4</sup> Jakobsson & Rosenberg 2007, *Bioinformatics* **23**: 1801-1806

<sup>5</sup> Paetkau *et al.* 2004, *Molecular Ecology* **13**: 55-65

<sup>6</sup> Peakall & Smouse 2006, *Molecular Ecology Notes* **6**: 288-295

1 and 2 appear more close to each other than to other clusters; clusters 3 and 6 appear the closest pair within the Cairns clusters, followed by 3 and 4 (subjective observation), while 4 and 6 appear more discrete. The Cairns clusters and the PNG/Solomons clusters appear to be discrete also. As expected, the most discrete cluster is cluster 5 (Thai outgroup).

c. Cavalli-Sforza & Edwards Chord distance<sup>7</sup> and Nei's standard genetic distance<sup>8</sup> were calculated with 1000 replicates using the software MSA<sup>9</sup>. A consensus matrix was created using consense option in the software PHYLIP<sup>10</sup>. A neighbour-joining unrooted tree was created in PHYLIP. Both genetic distance metrics resulted in a tree of identical topology. The Chord distance tree is depicted in Figure 3. The differentiation/relationships between clusters largely resembles that inferred by the likelihood of assignment tests and  $F_{ST}$  in that the two PNG clusters group together, the three Queensland clusters group together, with clusters 3 and 6 being more similar to each other than to cluster 4. The outgroup shows no inferred association with any cluster.

d. The data was examined for presence of private alleles using GenAlEx<sup>5</sup>. In two loci, clusters 3 and 6 (QLD1 and QLD3) had alleles observed in both these clusters but no other cluster. In another two loci, cluster 4 (QLD2) showed three alleles that were not observed in the other QLD clusters but were observed in the PNG and Thai clusters.

### 3. Interpretation, conclusions and recommendations

a. Based upon the small number of samples tested here, the data do not support a conclusion that the Cairns outbreak is related to either of the two PNG/Solomon Is/Torres Strait clusters. As these two clusters are genetically similar but geographically distinct, it is likely that other *A. cerana* populations in the PNG region may be similar also. Hence, it must be considered that PNG/Solomon Is/Torres Strait may not be the most likely source of the Cairns infestation.

b. There are three genetically similar clusters of *A. cerana* sampled from Queensland (using the small number of samples in this study). In contrast to the previous study with eight loci, only one sample contained individuals from more than one cluster. Of interest, this sample was obtained from a mast of a boat at Portsmith, Cairns. This boat had been in Darwin some time previously and is not thought to have left Australian waters. The data suggest that two clusters (3 and 6) are more similar to each other than to cluster 4, but of these 2, cluster 3 is more similar to cluster 4 than is cluster 6.

There is insufficient data (samples) to make any conclusion as to whether these represent more than one incursion from a single or related source(s). However, spatially, there appears a trend for the three clusters, with cluster 3 tending towards the region south of Cairns and east of Gordonvale; cluster 4 tending towards the city with some representation down to Gordonvale; and cluster 6 tending towards the west of the city and down to the western side towards Gordonvale (Figure 4). If the spatial clustering is correct, then it seems probable that all clusters comprise a single incursion that has spread in different directions. Given the  $F_{ST}$ , likelihoods of assignment and the phylogenetic tree, it might be proposed that the initial incursion would have been in cluster 3 (QLD1). However, given the low sample size, it must be considered that cluster 4 (QLD2) may yet prove to be a concurrent yet separate incursion.

c. **It is recommended that further samples are tested to determine source, and that these samples represent the Java strain from other regions within and outside PNG (eg Indonesia).**

<sup>7</sup> Cavalli-Sforza & Edwards. 1967. *Evolution* **32**: 550-570

<sup>8</sup> Nei 1983. *Journal of Molecular Evolution* **19**: 153-170

<sup>9</sup> Dieringer *et al* 2003. *Molecular Ecology Notes* **3**: 167-169

<sup>10</sup> Felsenstein: <http://evolution.genetics.washington.edu/phylip.html>

**d. It is recommended that the study is extended to include higher numbers of Cairns samples to determine/confirm the number of incursions and will likely establish patterns of spread through the monitoring of cluster assignments.**

#### **Acknowledgments**

Technical assistance was provided by Mr Evan Harris.

This work described in this document has been reviewed by Dr. Ben Oldroyd.

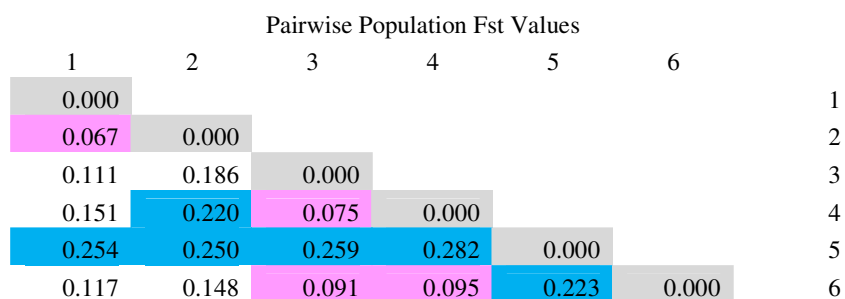
**Table 1:** assignment of specimens into clusters defined by STRUCTURE. Where two population identities are stated, this individual showed approx. equal likelihood of assignment into either cluster

by sample ID			by cluster			by location		
ID	pop	CoO	ID	pop	CoO	ID	pop	CoO
1222-1	1	PNG 1992	1222-1	1	PNG 1992	21-1_C	3	cairns
1222-2	1	PNG 1992	1222-2	1	PNG 1992	Q1_1	3	cairns
1222-3	1	PNG 1992	1222-3	1	PNG 1992	Q10-1	3	cairns
1222-4	1	PNG 1992	1222-4	1	PNG 1992	Q10-3	3	cairns
1320-1	1/2	PNG 1992	1320-1	1/2	PNG 1992	Q10-4	3	cairns
1320-2	2	PNG 1992	1598-1	1	PNG 1993	Q10-5	3	cairns
1320-3	2	PNG 1992	1598-2	1	PNG 1993	Q11-4	4	cairns
1320-4	2	PNG 1992	1598-3	1	PNG 1993	Q11-5	4	cairns
1369-1	2	PNG 1992	1598-4	1	PNG 1993	Q12-1	4	cairns
1369-2	2	PNG 1992	1598-5	1	PNG 1993	Q12-3	4	cairns
1369-3	2	PNG 1992	1637-1	1	PNG 1993	Q12-4	4	cairns
1436-1	2	PNG 1992	1637-2	1	PNG 1993	Q12-5	4	cairns
1436-2	2	PNG 1992	1637-3	1	PNG 1993	Q1-3	6	cairns
1436-3	2	PNG 1992	1637-4	1/2	PNG 1993	Q13-3	3	cairns
1436-4	2	PNG 1992	1825-1	1	PNG 1993	Q13-4	3	cairns
1598-1	1	PNG 1993	1825-2	1	PNG 1993	Q13-6	3	cairns
1598-2	1	PNG 1993	1825-3	1	PNG 1993	Q1-4	3	cairns
1598-3	1	PNG 1993	1825-4	1	PNG 1993	Q14-1	4	cairns
1598-4	1	PNG 1993	1825-5	1	PNG 1993	Q14-2	4	cairns
1598-5	1	PNG 1993	1905-1	1	PNG 1993	Q14-3	4	cairns
1637-1	1	PNG 1993	1905-2	1	PNG 1993	Q14-4	4	cairns
1637-2	1	PNG 1993	1905-3	1	PNG 1993	Q15-1	4	cairns
1637-3	1	PNG 1993	2556-1	1	PNG 1995	Q15-2	4	cairns
1637-4	1/2	PNG 1993	2556-2	1	PNG 1995	Q15-3	4	cairns
1825-1	1	PNG 1993	2556-5	1	PNG 1995	Q16-1	4	cairns
1825-2	1	PNG 1993	3996-2	1	Torres St 1996	Q16-2	4	cairns
1825-3	1	PNG 1993	3996-3	1	Torres St 1996	Q16-3	4	cairns
1825-4	1	PNG 1993	3996-4	1	Torres St 1996	Q16-4	4	cairns
1825-5	1	PNG 1993	502-1	1	Torres St 2002	Q16-5	4	cairns
1905-1	1	PNG 1993	502-2	1	Torres St 2002	Q17-2	4	cairns
1905-2	1	PNG 1993	502-3	1	Torres St 2002	Q17-3	4	cairns
1905-3	1	PNG 1993	502-4	1	Torres St 2002	Q17-4	4	cairns
1949-1	2	PNG 1993	502-5	1	Torres St 2002	Q18-2	6	cairns
1949-2	2	PNG 1993	6366-1	1	Torres St 2002	Q18-4	6	cairns
1968-1	2	PNG 1993	6366-2	1	Torres St 2002	Q18-5	6	cairns
1968-2	2	PNG 1993	6366-3	1	Torres St 2002	Q20-2	3	cairns
1968-3	2	PNG 1993	6366-4	1	Torres St 2002	Q20-5	3	cairns
1968-4	2	PNG 1993	sai594-3	1	new guinea 1994	Q2-1	4	cairns
1968-5	2	PNG 1993	sai594-4	1	new guinea 1994	Q21-2	3	cairns
21-1_C	3	cairns	sai594-5	1	new guinea 1994	Q21-4	3	cairns
2556-1	1	PNG 1995	1320-2	2	PNG 1992	Q21-5	3	cairns
2556-2	1	PNG 1995	1320-3	2	PNG 1992	Q2-2	4	cairns
2556-3	2	PNG 1995	1320-4	2	PNG 1992	Q22-1	3	cairns
2556-4	2	PNG 1995	1369-1	2	PNG 1992	Q22-10	3	cairns
2556-5	1	PNG 1995	1369-2	2	PNG 1992	Q22-2	3	cairns
3996-1	2	Torres St 2002	1369-3	2	PNG 1992	Q22-3	3	cairns
3996-2	1	Torres St 1996	1436-1	2	PNG 1992	Q23-1	3	cairns
3996-3	1	Torres St 1996	1436-2	2	PNG 1992	Q23-3	3	cairns
3996-4	1	Torres St 1996	1436-3	2	PNG 1992	Q23-4	3	cairns
3996-5	2	Torres St 2002	1436-4	2	PNG 1992	Q23-5	3	cairns
502-1	1	Torres St 2002	1949-1	2	PNG 1993	Q24-1	3	cairns
502-2	1	Torres St 2002	1949-2	2	PNG 1993	Q24-3	3	cairns
502-3	1	Torres St 2002	1968-1	2	PNG 1993	Q24-4	3	cairns
502-4	1	Torres St 2002	1968-2	2	PNG 1993	Q24-5	3	cairns
502-5	1	Torres St 2002	1968-3	2	PNG 1993	Q25-1	6	cairns
6366-1	1	Torres St 2002	1968-4	2	PNG 1993	Q25-2	6	cairns

6366-2	1	Torres St 2002	1968-5	2	PNG 1993	Q25-3	6	cairns
6366-3	1	Torres St 2002	2556-3	2	PNG 1995	Q25-4	6	cairns
6366-4	1	Torres St 2002	2556-4	2	PNG 1995	Q26-2	6	cairns
Q1_1	3	cairns	3996-1	2	Torres St 2002	Q26-3	6	cairns
Q10-1	3	cairns	3996-5	2	Torres St 2002	Q26-4	6	cairns
Q10-3	3	cairns	sai594-1	1/2	new guinea 1994	Q27-1	3/4	cairns
Q10-4	3	cairns	sai594-2	2	new guinea 1994	Q27-2	3	cairns
Q10-5	3	cairns	sol404-1	2	solomon is 2004	Q27-3	3	cairns
Q11-4	4	cairns	sol404-2	2	solomon is 2004	Q27-5	3	cairns
Q11-5	4	cairns	sol404-3	2	solomon is 2004	Q28-1	6	cairns
Q12-1	4	cairns	sol404-4	2	solomon is 2004	Q29-1	4	cairns
Q12-3	4	cairns	sol404-5	2	solomon is 2004	Q29-2	4	cairns
Q12-4	4	cairns	21-1_C	3	cairns	Q29-3	4	cairns
Q12-5	4	cairns	Q1_1	3	cairns	Q29-4	4	cairns
Q1-3	6	cairns	Q10-1	3	cairns	Q29-5	4	cairns
Q13-3	3	cairns	Q10-3	3	cairns	Q30-2	3	cairns
Q13-4	3	cairns	Q10-4	3	cairns	Q30-3	3	cairns
Q13-6	3	cairns	Q10-5	3	cairns	Q31-4	3	cairns
Q1-4	3	cairns	Q13-3	3	cairns	Q32-2	3	cairns
Q14-1	4	cairns	Q13-4	3	cairns	Q32-3	3	cairns
Q14-2	4	cairns	Q13-6	3	cairns	Q32-4	3	cairns
Q14-3	4	cairns	Q1-4	3	cairns	Q32-5	3	cairns
Q14-4	4	cairns	Q20-2	3	cairns	Q33-1	6	cairns
Q15-1	4	cairns	Q20-5	3	cairns	Q33-2	6	cairns
Q15-2	4	cairns	Q21-2	3	cairns	Q33-3	4/6	cairns
Q15-3	4	cairns	Q21-4	3	cairns	Q33-4	6	cairns
Q16-1	4	cairns	Q21-5	3	cairns	Q6-1	4	cairns
Q16-2	4	cairns	Q22-1	3	cairns	Q6-2	4	cairns
Q16-3	4	cairns	Q22-10	3	cairns	Q6-3	4	cairns
Q16-4	4	cairns	Q22-2	3	cairns	Q6-4	4	cairns
Q16-5	4	cairns	Q22-3	3	cairns	Q7-1	4	cairns
Q17-2	4	cairns	Q23-1	3	cairns	Q7-4	4	cairns
Q17-3	4	cairns	Q23-3	3	cairns	Q8-1	4	cairns
Q17-4	4	cairns	Q23-4	3	cairns	Q8-2	4	cairns
Q18-2	6	cairns	Q23-5	3	cairns	Q8-3	4	cairns
Q18-4	6	cairns	Q24-1	3	cairns	Q8-4	4	cairns
Q18-5	6	cairns	Q24-3	3	cairns	Q8-5	4	cairns
Q20-2	3	cairns	Q24-4	3	cairns	Q9-2	4	cairns
Q20-5	3	cairns	Q24-5	3	cairns	Q9-3	4	cairns
Q2-1	4	cairns	Q27-1	3/4	cairns	Q9-4	4	cairns
Q21-2	3	cairns	Q27-2	3	cairns	Q9-5	4	cairns
Q21-4	3	cairns	Q27-3	3	cairns	sai594-1	1/2	new guinea 1994
Q21-5	3	cairns	Q27-5	3	cairns	sai594-2	2	new guinea 1994
Q2-2	4	cairns	Q30-2	3	cairns	sai594-3	1	new guinea 1994
Q22-1	3	cairns	Q30-3	3	cairns	sai594-4	1	new guinea 1994
Q22-10	3	cairns	Q31-4	3	cairns	sai594-5	1	new guinea 1994
Q22-2	3	cairns	Q32-2	3	cairns	1222-1	1	PNG 1992
Q22-3	3	cairns	Q32-3	3	cairns	1222-2	1	PNG 1992
Q23-1	3	cairns	Q32-4	3	cairns	1222-3	1	PNG 1992
Q23-3	3	cairns	Q32-5	3	cairns	1222-4	1	PNG 1992
Q23-4	3	cairns	Q11-4	4	cairns	1320-1	1/2	PNG 1992
Q23-5	3	cairns	Q11-5	4	cairns	1320-2	2	PNG 1992
Q24-1	3	cairns	Q12-1	4	cairns	1320-3	2	PNG 1992
Q24-3	3	cairns	Q12-3	4	cairns	1320-4	2	PNG 1992
Q24-4	3	cairns	Q12-4	4	cairns	1369-1	2	PNG 1992
Q24-5	3	cairns	Q12-5	4	cairns	1369-2	2	PNG 1992

Q25-1	6	cairns	Q14-1	4	cairns	1369-3	2	PNG 1992
Q25-2	6	cairns	Q14-2	4	cairns	1436-1	2	PNG 1992
Q25-3	6	cairns	Q14-3	4	cairns	1436-2	2	PNG 1992
Q25-4	6	cairns	Q14-4	4	cairns	1436-3	2	PNG 1992
Q26-2	6	cairns	Q15-1	4	cairns	1436-4	2	PNG 1992
Q26-3	6	cairns	Q15-2	4	cairns	1598-1	1	PNG 1993
Q26-4	6	cairns	Q15-3	4	cairns	1598-2	1	PNG 1993
Q27-1	3/4	cairns	Q16-1	4	cairns	1598-3	1	PNG 1993
Q27-2	3	cairns	Q16-2	4	cairns	1598-4	1	PNG 1993
Q27-3	3	cairns	Q16-3	4	cairns	1598-5	1	PNG 1993
Q27-5	3	cairns	Q16-4	4	cairns	1637-1	1	PNG 1993
Q28-1	6	cairns	Q16-5	4	cairns	1637-2	1	PNG 1993
Q29-1	4	cairns	Q17-2	4	cairns	1637-3	1	PNG 1993
Q29-2	4	cairns	Q17-3	4	cairns	1637-4	1/2	PNG 1993
Q29-3	4	cairns	Q17-4	4	cairns	1825-1	1	PNG 1993
Q29-4	4	cairns	Q2-1	4	cairns	1825-2	1	PNG 1993
Q29-5	4	cairns	Q2-2	4	cairns	1825-3	1	PNG 1993
Q30-2	3	cairns	Q29-1	4	cairns	1825-4	1	PNG 1993
Q30-3	3	cairns	Q29-2	4	cairns	1825-5	1	PNG 1993
Q31-4	3	cairns	Q29-3	4	cairns	1905-1	1	PNG 1993
Q32-2	3	cairns	Q29-4	4	cairns	1905-2	1	PNG 1993
Q32-3	3	cairns	Q29-5	4	cairns	1905-3	1	PNG 1993
Q32-4	3	cairns	Q6-1	4	cairns	1949-1	2	PNG 1993
Q32-5	3	cairns	Q6-2	4	cairns	1949-2	2	PNG 1993
Q33-1	6	cairns	Q6-3	4	cairns	1968-1	2	PNG 1993
Q33-2	6	cairns	Q6-4	4	cairns	1968-2	2	PNG 1993
Q33-3	4/6	cairns	Q7-1	4	cairns	1968-3	2	PNG 1993
Q33-4	6	cairns	Q7-4	4	cairns	1968-4	2	PNG 1993
Q6-1	4	cairns	Q8-1	4	cairns	1968-5	2	PNG 1993
Q6-2	4	cairns	Q8-2	4	cairns	2556-1	1	PNG 1995
Q6-3	4	cairns	Q8-3	4	cairns	2556-2	1	PNG 1995
Q6-4	4	cairns	Q8-4	4	cairns	2556-3	2	PNG 1995
Q7-1	4	cairns	Q8-5	4	cairns	2556-4	2	PNG 1995
Q7-4	4	cairns	Q9-2	4	cairns	2556-5	1	PNG 1995
Q8-1	4	cairns	Q9-3	4	cairns	sol404-1	2	solomon is 2004
Q8-2	4	cairns	Q9-4	4	cairns	sol404-2	2	solomon is 2004
Q8-3	4	cairns	Q9-5	4	cairns	sol404-3	2	solomon is 2004
Q8-4	4	cairns	thai1	5	thailand	sol404-4	2	solomon is 2004
Q8-5	4	cairns	thai2	5	thailand	sol404-5	2	solomon is 2004
Q9-2	4	cairns	thai3	5	thailand	thai1	5	thailand
Q9-3	4	cairns	thai4	5	thailand	thai2	5	thailand
Q9-4	4	cairns	thai5	5	thailand	thai3	5	thailand
Q9-5	4	cairns	Q1-3	6	cairns	thai4	5	thailand
sai594-1	1/2	new guinea 1994	Q18-2	6	cairns	thai5	5	thailand
sai594-2	2	new guinea 1994	Q18-4	6	cairns	3996-2	1	Torres St 1996
sai594-3	1	new guinea 1994	Q18-5	6	cairns	3996-3	1	Torres St 1996
sai594-4	1	new guinea 1994	Q25-1	6	cairns	3996-4	1	Torres St 1996
sai594-5	1	new guinea 1994	Q25-2	6	cairns	3996-1	2	Torres St 2002
sol404-1	2	solomon is 2004	Q25-3	6	cairns	3996-5	2	Torres St 2002
sol404-2	2	solomon is 2004	Q25-4	6	cairns	502-1	1	Torres St 2002
sol404-3	2	solomon is 2004	Q26-2	6	cairns	502-2	1	Torres St 2002
sol404-4	2	solomon is 2004	Q26-3	6	cairns	502-3	1	Torres St 2002
sol404-5	2	solomon is 2004	Q26-4	6	cairns	502-4	1	Torres St 2002
thai1	5	thailand	Q28-1	6	cairns	502-5	1	Torres St 2002
thai2	5	thailand	Q33-1	6	cairns	6366-1	1	Torres St 2002
thai3	5	thailand	Q33-2	6	cairns	6366-2	1	Torres St 2002

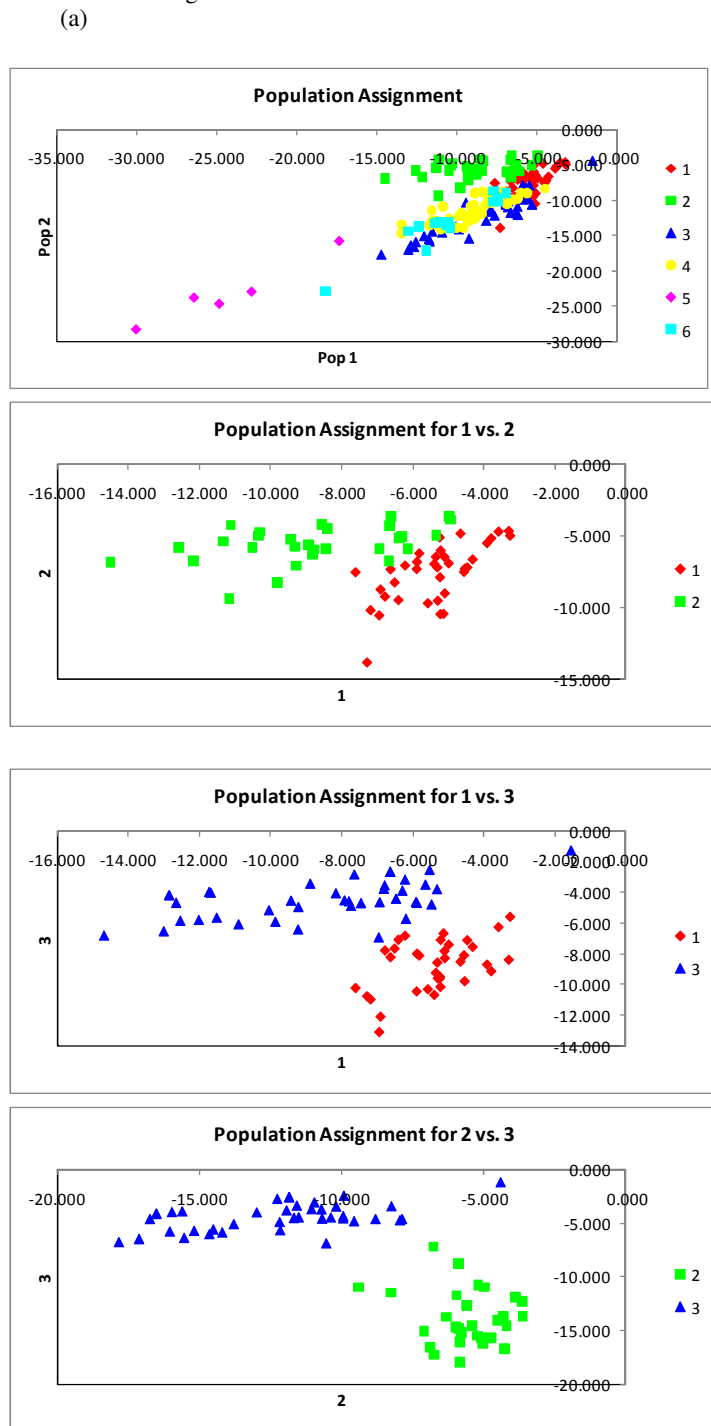
thai4	5	thailand		Q33-3	4/6	cairns		6366-3	1	Torres St 2002
thai5	5	thailand		Q33-4	6	cairns		6366-4	1	Torres St 2002

**Figure 1:** Pairwise  $F_{ST}$  between clusters identified by STRUCTURE

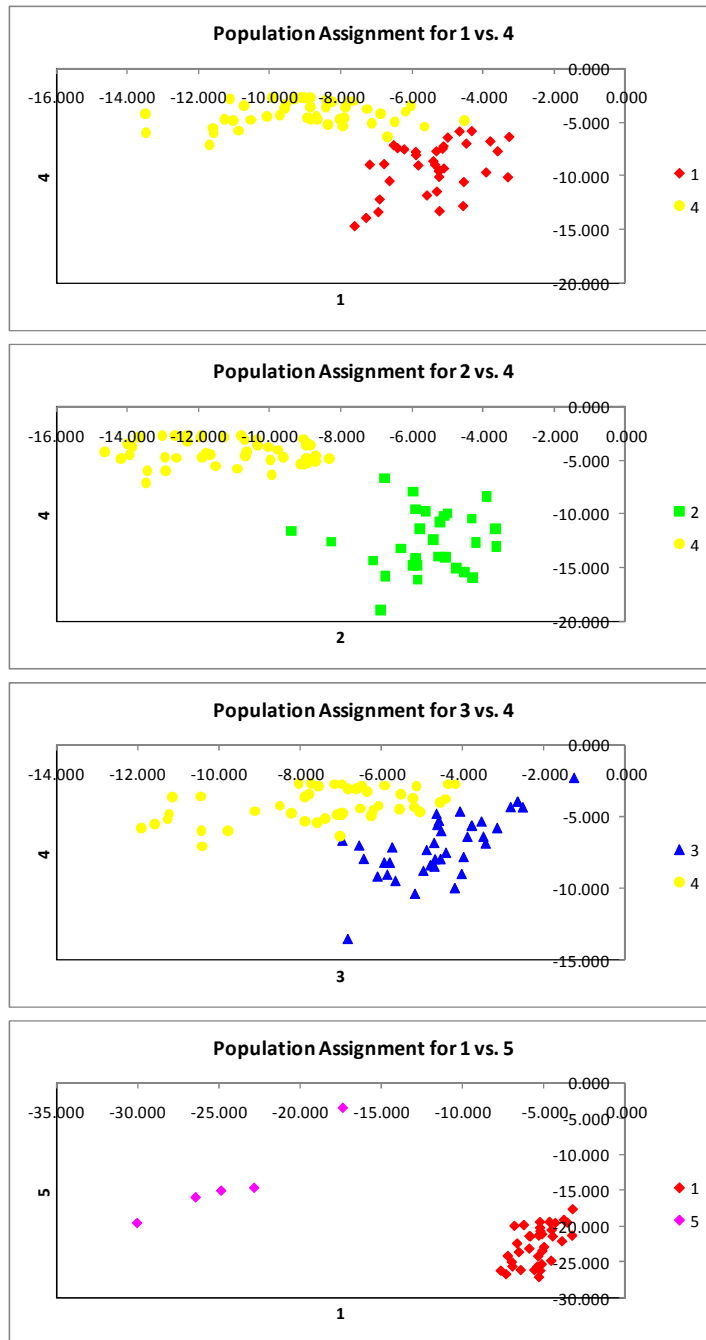
Fst Values below diagonal.

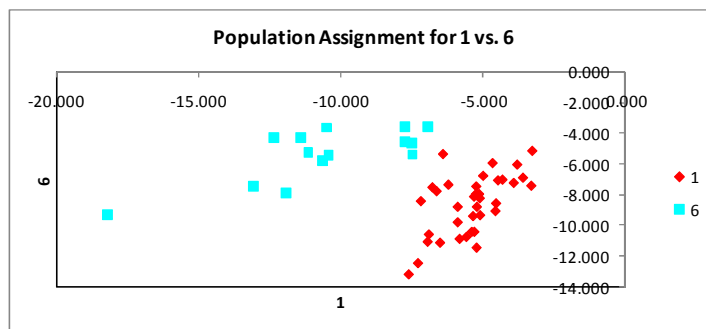
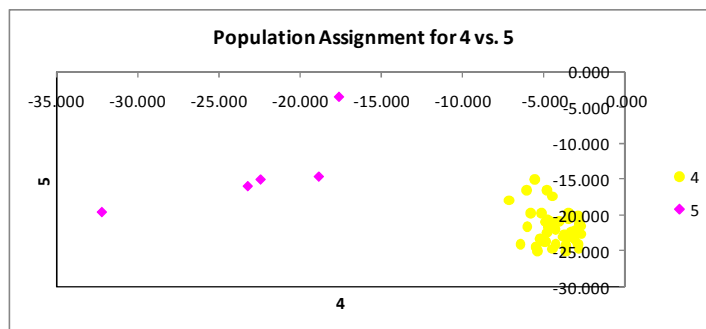
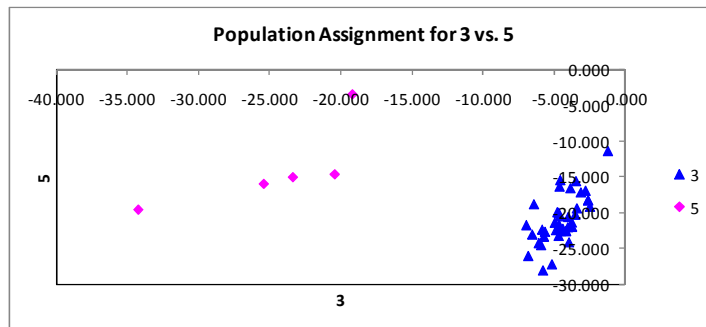
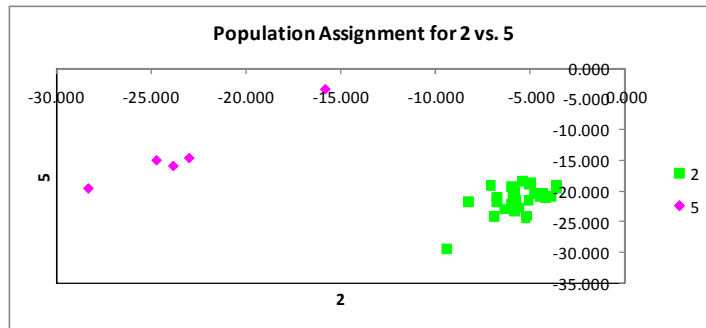
- 1 PNG, Torres Strait
  - 2 PNG, Solomons
  - 3 cairns
  - 4 cairns
  - 5 thailand
  - 6 cairns
- Pink <0.1, blue > 0.2, white 0.1-0.2

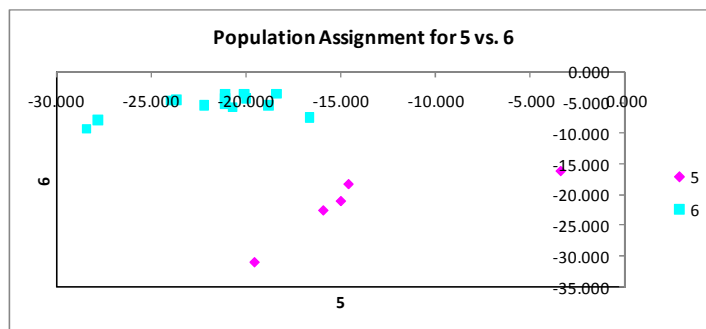
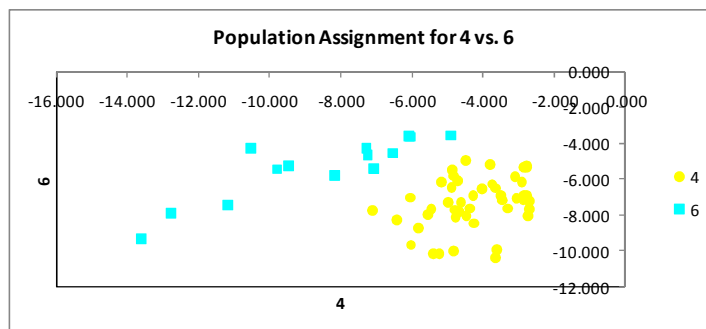
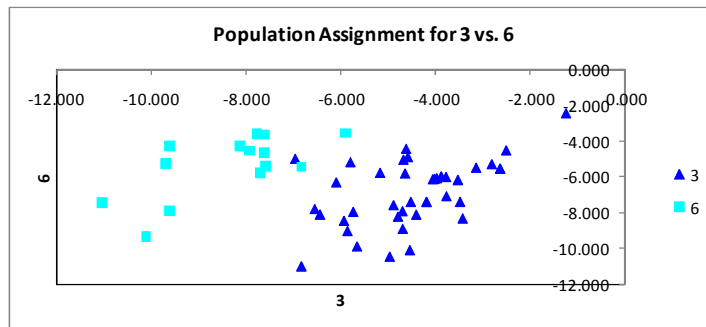
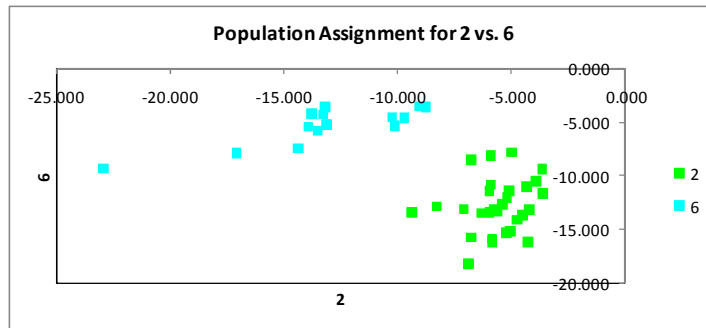
**Figure 3:** graphical representation of cluster assignments using Paetkau method. (a) all clusters, (b) pairwise likelihoods of assignment











**Figure 3:** Cavalli-Sforza & Edwards Chord Distance (X1000), neighbour-joining tree. PNG1 = cluster 1, PNG-Sol= cluster 2, QLD1 = cluster 3, QLD2 = cluster 4, Thai = cluster 5, QLD3 = cluster 6.

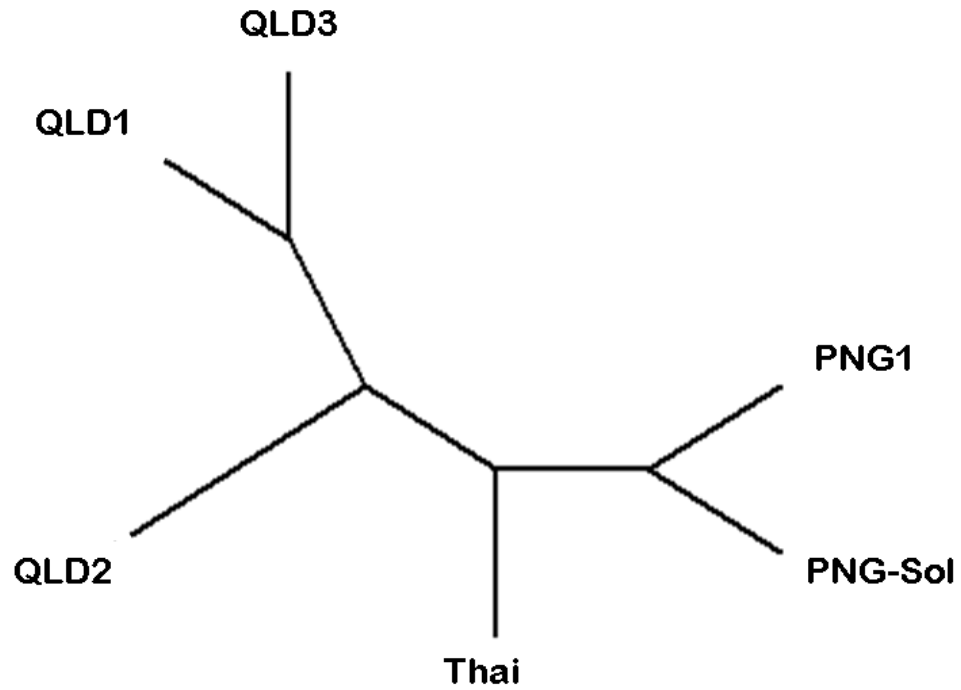
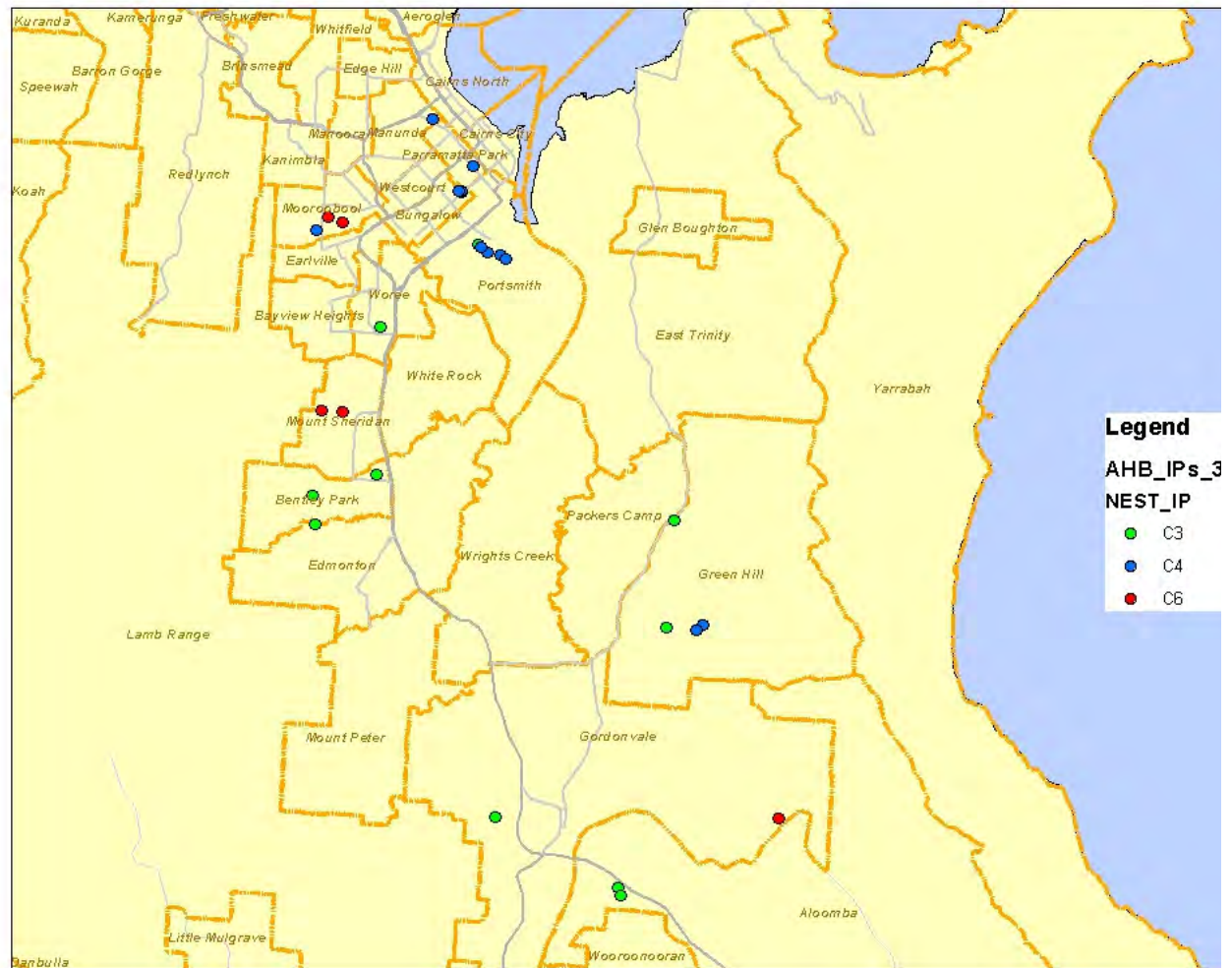


Figure 4: Spatial depiction of the IPs used in this study with colour cided cluster assignment



**Attachment K. Asian honey bee Known Infested Area 6 Month Intervals 2011-2012**