

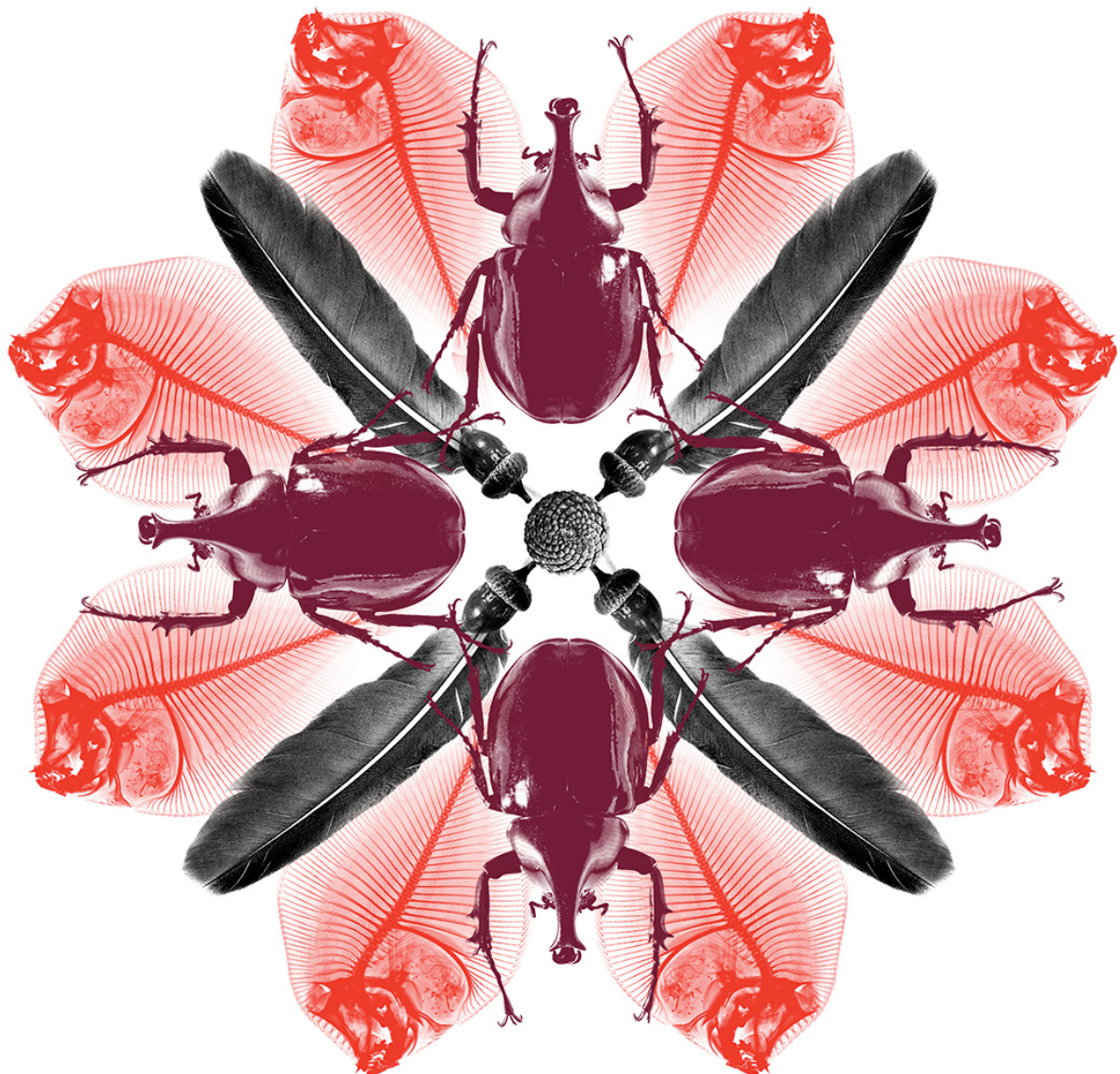


Australian Government
Department of Agriculture
and Water Resources

Importation of honey bee semen

Final review of import conditions

June 2016



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Glossary

Africanised honey bees	Honey bees of the subspecies <i>Apis mellifera scutellata</i> or hybrids between this subspecies and others. Usually denotes this subspecies when found in the Americas
Africanisation	The process of hybridisation of the subspecies <i>Apis mellifera scutellata</i> with other subspecies of <i>A. mellifera</i>
AHA	Animal Health Australia
AHBIC	Australian Honey Bee Industry Council
ALOP	The Appropriate Level Of Protection deemed appropriate by a member of the WTO establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory
apiary	colonies, hives, and other equipment assembled in one location for a beekeeping operation.
Apidae	a family of social bees including the common honey bee and stingless bee.
<i>Apis cerana</i>	the scientific name of the Asian or Eastern honey bee, which is now established in a limited area in Australia
<i>Apis mellifera</i>	scientific name of the European or western honey bee that is naturalised throughout Australia
AUSVETPLAN	Australian Veterinary Emergency Plan
brood	immature honey bees not emerged from their cells: eggs, larvae, and pupae
Cape honey bees	honey bees of the subspecies <i>Apis mellifera capensis</i>
Code	the OIE Terrestrial Animal Health Code (2014)
the department	the Australian Government Department of Agriculture and Water Resources
DNA	nucleic acid (also called deoxyribonucleic acid) that contains the genetic instructions used in the development and functioning of living organisms
drone	male honey bee
EADRA	Emergency Animal Disease Response Agreement
EPPRD	Emergency Plant Pest Response Deed
endemic	belonging to, native to, or prevalent in a particular geography, area or environment
genotype	the genetic makeup of a cell, an organism, or an individual
hive	a box or receptacle with movable frames, used for housing a colony of honey bees
honey flow	a time when nectar is plentiful and honey bees produce and store surplus honey

Import risk analysis	an administrative process through which biosecurity policy is developed or reviewed, incorporating risk assessment, risk management and risk communication
larva	immature honey bee life-stage prior to pupation: white, legless, soft and grub-like. The plural is larvae
microsatellite	repeating sequences of base pairs of DNA used as molecular markers in genetics, for kinship, population and other studies
migratory beekeeping	the moving of colonies of honey bees from one locality to another during a single season to take advantage of two or more honey flows
mitochondria	an organelle found in large numbers in most cells, in which the biochemical processes of respiration and energy production occur
morphometrics	a technique using measurements to determine variation and change in the form (size and shape) of organisms
OIE	World Organisation for Animal Health
OIE Manual	OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2015)
pathway	any means that allows the entry, establishment or spread of a pest
PCR	polymerase chain reaction
pest	any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or animals
pollination	the transfer of pollen from the anthers to the stigma of flowers
pupa	bee life stage that follows the larval stage and takes place within the sealed brood cell. During this stage the adult structures of the honey bee are formed. The plural is pupae
quarantine	official confinement and isolation of regulated articles or animals arriving from abroad for observation or for further inspection, testing or treatment
QAP	quarantine approved premises (also known as approved arrangements)
queen honey bee	a fertile female honey bee, larger and longer than a worker honey bee; able to lay fertilised eggs
restricted risk	risk estimate with mitigation measure(s) applied
RNA	nucleic acid (also called ribonucleic acid) that is involved in protein synthesis and the transmission of genetic information
SNP	single nucleotide polymorphism—a variation at a single position in a DNA sequence among individuals
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures
unrestricted risk	risk estimate in the absence of risk mitigation measures
worker honey bee	a sterile female honey bee that builds, provisions and cleans the hive and feeds the larva
WTO	World Trade Organization

Summary

In response to continued interest from the honey bee industry to import new and diverse genetic material into Australia the Australian Government Department of Agriculture and Water Resources (the department) reviewed the existing conditions for the importation of queen honey bees with reference to the importation of honey bee semen. The *Importation of queen honey bees: Final policy review* (2012) assessed the biosecurity risks to Australia of the importation of queen honey bees (*Apis mellifera*, the European or western honey bee). All disease agents, pests and species of concern were assessed and risk management measures identified to reduce risks to a level consistent with Australia's appropriate level of protection (ALOP).

A draft review was released for 60 days public comment to allow stakeholders time to assess and comment on the proposed conditions under which honey bee semen may be imported. Stakeholder submissions were considered when finalising this review.

The review concludes that honey bee semen imports be restricted in the first instance to those countries approved for the importation of queen honey bees—Canada, the European Union, Japan, New Zealand and the United States of America.

The review concluded that the importation of honey bee semen from approved countries does not achieve Australia's ALOP with respect to Africanised honey bees (*A. m. scutellata* and its hybrids). For Africanised honey bees, risk management measures are required and measures are described depending on the status of the exporting country.

As a result of stakeholders' comments on the draft review the department has reviewed the fungal agent, *Nosema ceranae*. Although present throughout the country a recently completed survey of honey bee pathogens has confirmed that this agent is not present in the major beekeeping area of Western Australia. The review finds that the risk of *N. ceranae* to this honey bee population is such that risk management requires that honey bee semen should not be permitted to enter this state directly unless it is sourced from a country or zone accepted by the department to be free from *N. ceranae*.

Risk management measures for both agents include restrictions on sourcing and/or testing of the honey bee semen as described in Section 1.7, *Biosecurity measures*.

In addition, the Cape honey bee (*A. m. capensis*) is recognised as a hazard and, consistent with the *Importation of queen honey bees: Final policy review* (2012), importation is not permitted from countries where this sub-species is present.

Full details of the risk assessment and the conclusions reached for Africanised honey bees and *N. ceranae* are provided in this review.

1 Introduction

1.1 Australia's biosecurity policy

Australia's biosecurity policies aim to protect Australia against risks that may arise from exotic diseases and pests entering, establishing and spreading, thereby threatening Australia's unique flora and fauna, as well as our agricultural industries that are relatively free from serious diseases and pests.

The risk analysis process is an important part of Australia's biosecurity policy development. It enables the Australian Government to formally consider the risks that could be associated with proposals to import new products into Australia. If the risks are found to exceed Australia's ALOP, risk management measures are proposed to reduce the risks to an acceptable level. However, if it is not possible to reduce the risks to an acceptable level, then trade will not be allowed.

Successive Australian Governments have maintained a conservative, but not zero risk, approach to the management of biosecurity risks. This approach is expressed in terms of Australia's ALOP, which reflects community expectations through government policy and is currently described as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Australia's biosecurity risk analyses are undertaken by the Department of Agriculture and Water Resources (the department), involving technical and scientific experts in relevant fields and consultation with stakeholders at various stages during the process.

The department's assessment may take the form of an import risk analysis, a review of existing policy, or technical advice. Further information about Australia's biosecurity framework is provided the *Biosecurity Import Risk Analysis Guidelines 2016* located on the department's website.

The department recognises that there might be new scientific information and technologies or other combinations of measures that may provide an equivalent level of biosecurity protection for the disease agents identified as requiring risk management. Submissions proposing equivalent measures will be considered on a case-by-case basis.

1.2 This review

Background

This review of the biosecurity risks associated with the importation into Australia of honey bee semen has been undertaken in response to requests by stakeholders to expand the options for importing honey bee genetics beyond the current conditions allowing the importation of queen honey bees. The importation of queen honey bees has been permitted since 2012 under conditions set in *Importation of queen honey bees: Final policy review (2012)* (queen honey bee review). The Australian honey bee industry wants to import new and diverse honey bee genetics to improve production traits and to assist in developing disease resistance against various agents.

Scope

This review assesses the biosecurity risks associated with the importation into Australia of honey bee semen of the species *Apis mellifera* (the Western or European honey bee). This document will be a review of the existing conditions for the importation of queen honey bees, and will incorporate the risk assessments and recommendations made in those conditions and apply them to honey bee semen.

Detailed information was gathered in support of the queen honey bee import conditions through review of the scientific literature and the previous policy. The information has been updated in this review to account for any developments since the release of the queen honey bee review in late 2012.

This review documents the risk assessments of the hazards identified and, where necessary, proposes risk management measures for the importation of honey bee semen. This review applies to semen extracted from the drones in the country of export and transported to Australia, rather than the importation of live drones followed by semen extraction in Australia.

As in the queen honey bee review, 'hazard', 'pathogenic agent' or 'disease agent' can refer to infectious organisms, internal or external parasites, syndromes of unknown aetiology or detrimental species, subspecies or hybrids.

Existing policy

Import policy

Genetic material has been imported into Australia under conditions set in the queen honey bee review.

The conditions currently restrict imports to those countries that can provide a satisfactory level of assurance for certifying to Australia's biosecurity requirements: Canada, the European Union, Japan, New Zealand and the United States.

The queen honey bee conditions require risk management measures for the following hazards that may be present in an approved country and were assessed to be above Australia's ALOP:

- Africanised honey bees (*A. m. scutellata* and its hybrids)
- varroosis
- acarapisosis (tracheal mite)
- *Tropilaelaps* spp.

Risk management measures recommended include that all imported honey bees undergo post-arrival quarantine at a government approved quarantine facility (currently the facility near Mickleham, Victoria). Colonies derived from the imported queen honey bees are propagated and only larvae grafted from those colonies are released from quarantine.

The full import requirements for queen honey bees can be found on the department's website.

Domestic arrangements

The Australian Government is responsible for regulating the movement of animals and their products into and out of Australia. The state and territory governments have primary responsibility for animal health and environmental controls within their jurisdictions. Legislation may be used by state and territory governments to control interstate movement of

animals and their products. Once animals and animal products have been cleared by Australian biosecurity officers, they may be subject to interstate movement controls.

This is the case for honey bees and their products. In particular, Western Australia is free of European foulbrood and prohibits the importation of honey and other honey bee products unless pasteurised or otherwise treated. It is the importer's responsibility to identify and ensure compliance with all requirements.

2 Method

The method used in the assessment of the risks involved in the importation of honey bee semen from countries approved for the importation of queen honey bees is the same as described in Chapter 2 of the queen honey bee review. This follows the principles described by the World Organisation for Animal Health (OIE) in its Terrestrial Animal Health Code (the Code) (OIE 2014).

The components of risk analysis as described in Chapter 2.1 of the Code are:

- hazard identification
- risk assessment (entry assessment, exposure assessment, consequence assessment and risk estimation)
- risk management
- risk communication.

Hazard identification, risk assessment and risk management are sequential steps within a risk analysis. Risk communication is conducted as an ongoing process and includes both formal and informal consultation with stakeholders.

As for the queen honey bee review, this assessment was conducted using a qualitative approach. The likelihood (or probability) that an event will occur was evaluated and reported, using the same qualitative likelihood descriptors for the entry and exposure assessment and the outbreak scenario as used in the queen honey bee review (see Table 1 of the queen honey bee review).

2.1 Risk review

Although not defined or described in the Code, risk review is recognised by risk analysts as an essential component of the risk analysis process (Barry 2007).

Australia applies a process of risk review to the biosecurity risks associated with the importation of an animal or animal product for which relevant current biosecurity measures exist.

Risk review differs from the monitoring and review component of risk management, as described in the Code, in that each component of the risk analysis process (hazard identification, risk assessment and risk management) is reviewed. If a change (either an increase or decrease) in the biosecurity risk associated with an animal or animal product that is currently imported into Australia is identified, risk management measures can be revised accordingly.

2.2 Hazard identification

Hazard identification is described in the Code (Article 2.1.2) as a classification step that is undertaken to identify potential hazards that may be associated with the importation of a commodity.

In accordance with the Code, a disease agent was considered to be a potential hazard relevant to the importation of honey bee semen if it was assessed to be:

- appropriate to honey bees

- OIE listed, emerging and/or capable of producing adverse consequences in Australia
- present in the exporting country and not present in Australia. If present in Australia, the hazard should be a notifiable disease or be subject to an official control program.

Hazards identified as requiring assessment in the queen honey bee review were assessed for significant changes in disease agent attributes and/or geographic distribution that may have occurred since the release of the queen honey bee review. The potential for differing epidemiology between queen honey bees and honey bee semen in the entry and exposure of hazards was also investigated.

The scientific literature was further reviewed for new and emerging diseases of honey bees.

2.3 Risk assessment framework

Risk assessment is defined in the OIE Code as ‘the evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a hazard within the territory of an importing country’. Details of the risk assessment process are provided in Chapter 2.1 of the Code.

The evaluation of disease risks in this review involved estimating the likelihood of susceptible honey bees in Australia becoming exposed to an agent (hazard) and the likely consequences of such exposure. In evaluating the likelihood of susceptible honey bees in Australia becoming exposed to a hazard, the following factors were considered:

- the likelihood of the hazard being released into Australia via imported bee semen (entry assessment) and
- the likelihood of susceptible honey bees in Australia becoming exposed to the hazard via imported bee semen (exposure assessment).

The determination of likely consequences required:

- the identification of the most likely outbreak scenario that could follow exposure to a hazard. Possible outbreak scenarios range from no establishment of the hazard to the hazard establishing and spreading throughout local managed and wild honey bee colonies with further spread to other susceptible populations. Only the most likely outbreak scenario relating to the establishment and/or spread for each hazard was assessed
- estimation of the likelihood of establishment and/or spread for that outbreak scenario
- the effects (health, environmental and socioeconomic) associated with that outbreak scenario.

The risk assessment concludes with an unrestricted risk for each hazard. If the unrestricted risk did not meet Australia’s ALOP, then risk management measures were recommended to reduce the risk in order to meet the ALOP.

2.3.1 Entry and exposure assessment

Entry assessment

The entry assessment considered a single entry scenario defined as the period up to arrival in Australia. A number of factors were taken into account in determining the likelihood of a disease agent entering Australia in bee semen such as:

- if a drone or honey bee colony that is providing semen for export is exposed to the agent

- prevalence of the agent in the exporting country or zone
- tissue distribution of the disease agent
- the effect of storage and transport.

The final outcome of the entry assessment is the likelihood of entry of a potential hazard into Australia.

Exposure assessment

The exposure assessment is the likelihood that a susceptible honey bee in Australia will be exposed to imported honey bee semen infected with a disease agent or containing undesirable genetics. It takes into account the groups of bees most likely to be affected as well as the possible pathways by which exposure of these groups could occur.

The exposure groups recognised in the queen honey bee review were both managed and wild *A. mellifera* colonies. As these groups are closely related, with continuous interactions between the two groups, they were combined to form one exposure group.

Estimation of the likelihood of entry and exposure

The likelihood of entry and exposure was estimated by combining the likelihood of entry and the corresponding likelihood of exposure using the matrix shown in Table 2 of the queen honey bee review.

2.3.2 Consequence assessment

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of a susceptible population has occurred, a number of possible outbreak scenarios could follow, representing a continuum ranging from no spread to widespread establishment.

In this review, the most likely outbreak scenario for each hazard, resulting from the exposure of susceptible honey bees, was considered in a single pathway resulting in infection or infestation and establishment (detailed in the relevant disease chapter).

The likelihood of the outbreak scenario occurring was then estimated to obtain a likelihood of establishment and/or spread.

Determination of the effects resulting from the establishment and/or spread

Potential effects of establishment and/or spread associated with the outbreak scenario may be direct—the life and health of susceptible animals—or indirect—costs of control and the effects on trade, the environment and local communities amongst other factors. These are described in detail in Chapter 2 of the queen honey bee review.

The overall effect of establishment and/or spread associated with the outbreak scenario took into account the geographic level of these effects:

- local—restricted to a single locality or town
- regional—a recognised geographic area
- state or territory
- national

and the magnitude of these effects:

- indiscernible—not usually distinguishable from normal day-to-day variation
- minor significance—recognisable, but minor and reversible
- significant—serious and substantive, but reversible and unlikely to have permanent economic effects
- highly significant—extremely serious and irreversible and likely to have permanent economic effects.

Based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread was determined using the rules described in Table 3 of the queen honey bee review.

Estimation of likely consequences

The likely consequences were estimated by combining the likelihood of establishment and/or spread (associated with the outbreak scenario) with the overall effect of establishment and/or spread using the matrix shown in Table 4 of the queen honey bee review.

2.3.3 Risk estimation

Risk estimation is the integration of likelihood of entry and exposure, with the likely consequences of establishment and/or spread. This derives the unrestricted risk associated with entry, exposure, establishment and/or spread of a hazard introduced by the importation of bee semen.

Combination of the likelihood of entry and exposure with likely consequences of establishment and/or spread was done using the rules shown in the risk estimation matrix in Table 5 of the queen honey bee review.

2.3.4 Evaluation of unrestricted risk

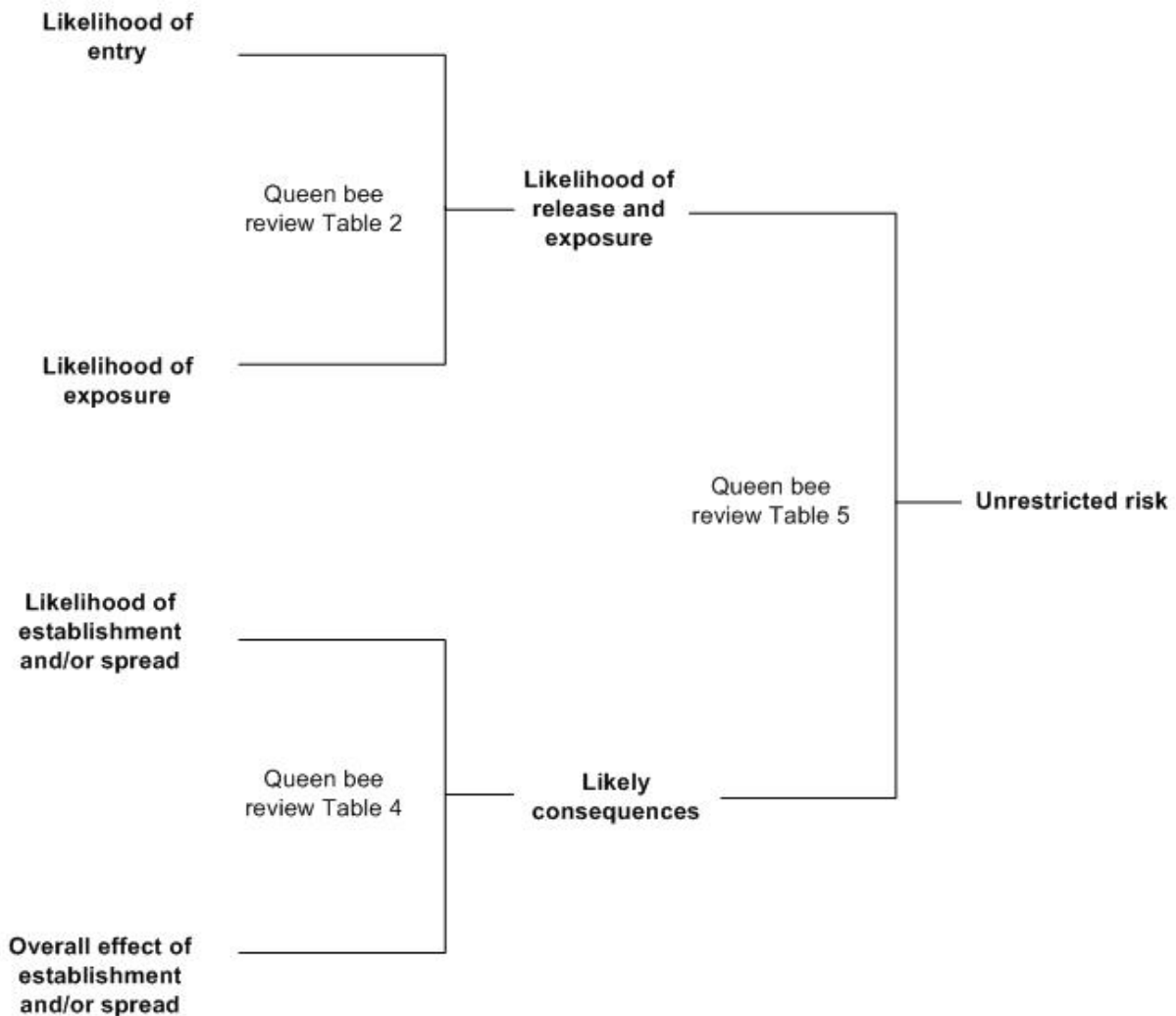
Risk evaluation is described in the OIE Code as the process of comparing the estimated risk with a country's ALOP.

An estimated risk that was either 'very low' or 'negligible' was considered sufficient to achieve Australia's ALOP. This provided a benchmark for evaluating risk and determining whether risk management was required.

If the unrestricted risk was 'negligible' or 'very low', then it achieved Australia's ALOP and risk management measures were not required. If the unrestricted risk was above 'very low', risk management measures were required. This was considered the final output of the risk assessment.

Figure 1 shows a summary of the risk assessment pathways.

Figure 1 Summary of risk assessment pathways



2.4 Risk management

Once the unrestricted risk for a particular hazard has been evaluated and assessed as exceeding Australia’s ALOP, measures to manage and reduce that risk are considered and proposed.

The imposition of a particular risk management measure or a combination of measures produces the restricted risk. The aim of risk management measures is to meet Australia’s ALOP by reducing the restricted risk to ‘very low’ or ‘negligible’.

Risk management options proposed in this report aim to reduce the likelihood that the imported honey bee semen would lead to the entry, exposure, establishment and spread of disease agents or honey bee genetics of biosecurity concern in Australia. These may be imposed pre-border, to reduce the likelihood of hazards entering Australia in imported bee semen, or post-arrival, aiming to prevent the exposure and/or establishment and spread of the hazard in susceptible local populations.

The specific measures recommended for the hazard with an unrestricted risk that did not achieve Australia's ALOP are described in detail in section 1.7 of this review.

2.5 Risk communication

Risk communication is defined in the Code as 'the interactive transmission and exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions among risk assessors, risk managers, risk communicators, the general public and other interested parties.

In conducting import risk analyses and reviews the department consults, where relevant, with the Australian Government Department of Health to ensure that public health considerations are included in the development of Australia's animal biosecurity policies.

A formal process of consultation with external stakeholders is a standard procedure for all import risk analyses and policy reviews to enable stakeholder assessment and feedback on draft conclusions and recommendations about Australia's animal biosecurity policies.

3 Hazard identification

Hazard identification for the importation of queen honey bees is described in Chapter 3 of the queen honey bee review. A preliminary list of honey bee pests and diseases that were identified as potential hazards was generated from the scientific literature, previous work produced by the department and the OIE disease list for Apidae.

The OIE lists six diseases or pests of Apidae of importance for international trade—tracheal mite (*Acarapis woodi*), American foulbrood, European foulbrood, small hive beetle, *Tropilaelaps* spp. and *Varroa* spp. (OIE 2014).

The nature of the commodity means that the risk associated with the importation of the three pathogenic mites—*A. woodi*, *tropilaelaps* and *varroa*—is greatly decreased compared to live honey bees. The risk of contamination of semen with these agents by an infested drone is negligible.

Viruses of honey bees can be transmitted by semen (Da Cruz-Landim, Roat & Fernandez 2012; Yue et al. 2006) and a number of viruses associated with colony loss overseas do not appear to be present in this country. However the queen honey bee review found that without the presence of *varroa* acting as a vector the risk that these viruses pose is less than Australia's ALOP.

The hazard list was refined by applying the criteria described in Chapter 2 of the queen honey bee review.

For this review the hazards retained for risk assessment in the queen honey bee review were considered along with potential hazards that have either emerged since the publication of the queen honey bee review in 2012 or where there are new findings relevant to agents that were considered in the queen honey bee review (see Tables 1 and 2).

Table 1 Hazards retained for risk assessment in the queen honey bee review

Hazards retained for risk assessment in queen honey bee review	OIE ¹ listed	Results of risk assessment in queen honey bee review	Present in an approved country	Present in Australia	Retained for risk assessment in review of importation of honey bee semen—yes/no
American foulbrood (<i>Paenibacillus larvae</i>)	Yes	Found not to require risk management	Yes	Yes—under official control	No. The queen honey bee review found that American foulbrood is present in Australia and concluded that a risk assessment was not required.
European foulbrood (<i>Melissococcus plutonius</i>)	Yes	Found not to require risk management	Yes	Yes—under official control	No. The queen honey bee review found that European foulbrood is present throughout large areas of Australia and concluded that a risk assessment was not required.
<i>Nosema ceranae</i>	No	Found not to require risk management	Yes	Regional freedom	Yes—a recent survey has confirmed that the major beekeeping area of Western Australia is free of this agent ²
Braula fly	No	Found not to require risk management	Yes	Tasmania only	No. The queen honey bee review found that the likely consequences of Braula fly introduction to be 'negligible'.
Phorid fly (<i>Apocephalus borealis</i>)	No	Found not to require risk management	Yes	No	No. The queen honey bee review found that the likely consequences of Phorid fly introduction to be 'negligible'.
Small hive beetle	Yes	Found not to require risk management	Yes	Yes—Eastern seaboard only	No. Endemic in eastern Australian mainland and negligible likelihood of being present in semen.
Acarapisosis	Yes	Risk management measures are required for the importation of queen honey bees	Yes	No	No. Parasite of the respiratory system and only survives a few hours outside of respiratory system. Extremely low likelihood of being present in semen.
Tropilaelaps	Yes	Risk management measures are required for the importation of queen honey bees	Yes	No	No. External parasite. Negligible likelihood of being present in semen.

¹ World Organisation of Animal Health

² Roberts, J, Anderson, D & Durr, P 2015, *Upgrading knowledge on pathogens (particularly viruses) of Australian honey bees*, publication no. 15/095, Rural Industries Research and Development Corporation, Canberra, available at <https://rirdc.infoservices.com.au/items/15-095>.

Hazards retained for risk assessment in queen honey bee review	OIE ¹ listed	Results of risk assessment in queen honey bee review	Present in an approved country	Present in Australia	Retained for risk assessment in review of importation of honey bee semen—yes/no
Varroosis	Yes	Risk management measures are required for the importation of queen honey bees	Yes	No	No. External parasite. Negligible likelihood of being present in semen.
Acute paralysis virus	No	Found not to require risk management	Yes	No	No. There is evidence that honey bee viruses may be transmitted by via semen ³ . However the queen honey bee review found that in the absence of the varroa mite as a vector, the unrestricted risk of this virus was 'negligible'.
Deformed wing virus	No	Found not to require risk management	Yes	No	No. There is evidence that honey bee viruses may be transmitted by via semen. However the queen honey bee review found that in the absence of the varroa mite as a vector, the unrestricted risk of this virus was 'negligible'.
Slow paralysis virus	No	Found not to require risk management	Yes	No	No. There is evidence that honey bee viruses may be transmitted by via semen. However the queen honey bee review found that in the absence of the varroa mite as a vector, the unrestricted risk of this virus was 'negligible'.
Africanised honey bee (<i>Apis mellifera scutellata</i> and its hybrids)	No	Risk management measures are required for the importation of queen honey bees	Yes	No	Yes. The queen honey bee review concluded that the likely consequences of entry were 'high' and the unrestricted risk 'moderate'.
Cape honey bee (<i>Apis mellifera capensis</i>)	No	Risk management measures are required for the importation of queen honey bees	No	No	No. The queen honey bee import conditions require country freedom and no currently approved country is affected. No risk assessment required but Cape honey bee to be retained as a hazard and country freedom is required for risk management.
Colony collapse disorder	No	Found not to require risk management	No	No	No. The queen honey bee review concluded that this did not constitute a separate honey bee hazard.

³ Da Cruz-Landim, C, Roat, TC & Fernandez, FC 2012, 'Virus present in the reproductive tract of asymptomatic drones of honey bee (*Apis mellifera* L.), and possible infection of queen during mating', *Microscopy Research and Technique*, vol. 75, no. 7, pp. 986-90.; Yue, C, Schröder, M, Bienefeld, K & Genersch, E 2006, 'Detection of viral sequences in semen of honeybees (*Apis mellifera*): evidence for vertical transmission of viruses through drones', *Journal of Invertebrate Pathology*, vol. 92, no. 2, pp. 105-8.

Table 2 New or emerging diseases not considered in the queen honey bee review

New or emerging diseases not considered in queen honey bee review	OIE listed	Present in an approved country	Present in Australia	Retained for risk assessment—yes/no
<i>Crithidia mellificae</i>	No	Yes	Yes	No—present in Australia ⁴
Lake Sinai virus 1 & 2	No	Yes	Yes	No—present in Australia ⁵
<i>Spiroplasma melliferum</i>	No	Yes	Yes	No—present in Australia ⁶
<i>Apicystis bombi</i>	No	Yes	unknown	No—primarily an agent of bumble bees so unlikely to be present in mainland Australia. Unlikely to cause significant adverse effects.
Varroa destructor Macula-like virus	No	Yes	unknown	No—unlikely to cause significant adverse effects
Big Sioux River virus	No	Yes	unknown	No—unlikely to cause significant adverse effects
Aphid lethal paralysis virus	No	Yes	unknown	No—unlikely to cause significant adverse effects
Israeli acute paralysis virus	No	Yes	Yes	No—strains are present in Australia

⁴ Langridge, DF & McGhee, RB 1967, '*Crithidia mellificae* n. sp. an acidophilic Trypanosomatid of the honey bee *Apis mellifera*', *The Journal of Protozoology*, vol. 14, no. 3, pp. 485-7.

⁵ Roberts, J, Anderson, D & Durr, P 2015, *Upgrading knowledge on pathogens (particularly viruses) of Australian honey bees*, publication no. 15/095, Rural Industries Research and Development Corporation, Canberra, available at <https://rirdc.infoservices.com.au/items/15-095>.

⁶ Djordjevic, SP, Noone, K, Smith, L & Hornitzky, MZ 1998, 'Development of a hemi-nested PCR assay for the specific detection of *Melissococcus pluton*', *Journal of Apicultural Research*, vol. 37, no. 3, pp. 165-74.

Conclusion

On the basis of the hazard refinement process, which includes the hazard refinement in the queen honey bee review, the following pathogenic agents were identified as hazards:

- Africanised honey bee (*A. m. scutellata* and its hybrids)
- Cape honey bee (*A. m. capensis*).
- *Nosema ceranae*

The Africanised honey bee and *N. ceranae* were retained for further assessment.

The Cape honey bee was not retained for further risk assessment as the queen honey bee review import conditions require country freedom.

4 Risk review—Africanised honey bee

Background

The Western honey bee, *Apis mellifera*, originated in Africa and expanded into Eurasia at least twice (Whitfield et al. 2006). These expansions resulted in the four major lineages of honey bee diversity: the Western European lineage (M), the Eastern European lineage (C), the Middle Eastern lineage (O) and the African lineage (A) (Franck et al. 2001; Franck et al. 1998). Modern beekeeping is mainly based on bees of the C lineage, particularly *A. m. ligustica* and *A. m. carnica*.

Within the A lineage there are more than seven named subspecies (Schneider, DeGrandi-Hoffman & Smith 2004) including *Apis mellifera scutellata*. The natural range of *A. m. scutellata* (hereafter called ‘Scutellata’) is the savannah region of sub-Saharan Africa. Scutellata is characterised by behavioural traits that appear to be adaptations to life in the savannah: high reproductive rates, extreme defensiveness and regular migration (Rinderer 1988).

From 1954 to 1956 there were various imports of Scutellata queen honey bees into Brazil. The goal of these importations was to develop a strain of honey bee that was more suitable for tropical beekeeping than the existing M and C lineage honey bees that were available in Brazil at the time (Schneider, DeGrandi-Hoffman & Smith 2004). Descendants of these imports formed the basis of a massive wild population of A lineage Scutellata honey bees that has replaced honey bees of European lineage (hereafter ‘European’) throughout the neo-tropics and the southern United States (Clarke et al. 2002; Pinto et al. 2005; Schneider, DeGrandi-Hoffman & Smith 2004; Whitfield et al. 2006; Winston 1992).

Following the initial importation, Scutellata expanded its range by 200–500 km per year (Taylor 1977; Winston 1992). By 1986 it had reached Mexico and it first invaded the United States in 1992 (Guzmán-Novoa & Page 1994). This is one of the most successful biological invasions of all time (Winston 1992).

Scutellata is now present throughout sub-Saharan Africa and Central America. In South America, Scutellata has extended as far south as Argentina, however it is only present in the north of the country. South of Buenos Aires province the country is considered to be free of Scutellata. The region between these two areas is a transition zone where Scutellata forms a stable hybrid with honey bees of the C and M lineages (Kerr, de Leon del Rio & Barrionuevo 1982; Sheppard et al. 1991; Whitfield et al. 2006).

In the United States, Scutellata had been identified in a number of states at the time of the release of the queen honey bee review—Alabama, Arizona, Arkansas, California, Florida, Georgia, Louisiana, Nevada, New Mexico, New York, Oklahoma, South Carolina, Texas, Utah and Virginia (Georgia Department of Agriculture 2010; Louisiana Department of Agriculture and Forestry 2009; Utah Department of Agriculture and Food 2010). Since then there have been identifications in at least two other states—Tennessee and Colorado (Erdahl 2014; Platt 2012).

Scutellata was expected to increase its range into states to the north, especially along the eastern seaboard of the United States (Taylor 1977; Winston 1992). However as time goes on this expansion seems less likely.

Africanised honey bees or ‘Africanised bees’ are notifiable as a disease in New South Wales, South Australia, Tasmania, Victoria, Western Australia and the Australian Capital Territory. Scutellata is not present in Australia. There were some imports of *A. m. iberiensis* into Western Australia early last century, but these appear to have been of M lineage, not A lineage (Chapman,

Lim & Oldroyd 2008). Scutellata is not an OIE listed disease and there are no recommendations in the OIE Code on measures for safe trade.

Technical information

Epidemiology

Scutellata bees spread and replace European honey bees primarily by producing larger numbers of reproductive swarms and drones. Otis (1991) observed that a Scutellata colony can produce 6–12 reproductive colonies per year and, via the offspring of the offspring colonies, up to 60 descendent colonies per year. Other processes that contribute to the ‘Africanisation’ of colonies are described below.

Scutellata drones enter European colonies, and by their presence, suppress drone rearing by the host colony (Rinderer et al. 1985). This process increases the proportion of Scutellata drones present in the hive and therefore the likelihood that queen honey bees will mate with Scutellata drones rather than European drones. Other factors that contribute to a Scutellata mating advantage include seasonal patterns of drone production that favour queens mating primarily with Scutellata drones at certain times of the year and the possible use of Scutellata spermatozoa at higher rates by queens mated to both Scutellata and European bees (Schneider, DeGrandi-Hoffman & Smith 2004).

Scutellata drones often enter European colonies where they suppress drone production merely by their presence. In contrast, Scutellata colonies almost ever accept foreign drones (Rinderer et al. 1985). By processes not yet understood, the Scutellata queen honey bee replaces the European queen honey bee, converting the previously European colony into a Scutellata one (Danka et al. 1992; Vergara et al. 1989).

The development time of Scutellata queen honey bees is shorter than European queen honey bees, therefore a queen honey bee that is mated to both Scutellata and European drones is more likely to produce offspring queen honey bees that are of Scutellata paternity. This is because the first offspring queen honey bee to hatch seeks out and kills her sisters that are still in their pupal queen cells. The first queen honey bee to hatch is most likely to have Scutellata paternity and this is the bee most likely to inherit the colony (Schneider & DeGrandi-Hoffman 2003).

Scutellata is resistant to some of the major pests of European honey bees, particularly small hive beetle (SHB) and varroa. European honey bees suffer more damage from SHB infestations, often resulting in colony collapse (Caron 2001; Cuthbertson et al. 2013; Hood 2004). In a study comparing Africanised and European honey bees, European brood and adult bees were twice as likely to be infected with varroa (Guzman-Novoa et al. 1996).

Scutellata’s adaptations to the African savannah make it undesirable for modern beekeeping as practised in Australia because:

- Scutellata colonies have high absconding rates—forming a migratory swarm that seeks better environmental conditions when the colony faces starvation (Otis 1991). Scutellata will continue to breed even when there is a lack of food available in the field, utilising resources for reproduction rather than honey storage.
- Scutellata colonies are difficult to work with, requiring full honey bee suits and heavy gloves at all times. When beekeepers work in Scutellata apiaries the colonies become extremely defensive and attack any mammal or bird within a few hundred metres. Scutellata apiaries are recommended to be situated a minimum of 200–500 metres away from places occupied by humans, livestock or companion animals (Caron 2001).
- If introduced into Australia, Scutellata may increase the size of the wild honey bee population. An increased density of wild honey bees may have negative effects on the native fauna that compete with wild honey bees for nesting sites and floral resources. They pose a significant risk to the public via incidental stinging attacks. The attacks involve hundreds of

stinging honey bees, and death by stinging or the consequences of panic is possible. In Mexico between 1988 and 1998 there were 360 honey bee related accidents, involving over 5000 people. From 1988 to 2009 there were 480 deaths recorded as being due to multiple honey bee stings (Becerril-Ángeles et al. 2013).

- The individual stings are no more venomous than honey bees of other subspecies (Schumacher & Egen 1995). However, both private and public landowners are likely to be more reticent to allow beekeeping activities on their properties because of the possibility of stinging incidents, stock losses, and fear of litigation. This would have a significant negative impact on beekeepers and landowners who hire honey bees for pollination.
- Scutellata are unsuitable for paid pollination services. It is dangerous to have large numbers of 'killer bees' in orchards where untrained and unprotected people are likely to come in contact with the colonies. However Ratnieks and Visscher (1996) reported that in Mexico regular re-queening of colonies with European queen honey bees and changes in management practices has allowed managed pollination to be maintained.

Identification

Extreme defensive behaviour and stinging incidents are often the first indication of the presence of Scutellata colonies. Assessment of defensive behaviour, the amount and pattern of brood (more with Africanised colonies) and the difficulty in locating the queen honey bee have been used in the field as indications of the presence of Scutellata (Spivak 1991).

Scutellata are small yellow bees and are not sufficiently different from honey bees of other subspecies to be reliably distinguishable by eye. Large colonies are extremely aggressive but this too is not a reliable diagnosis—so are some European colonies. Small colonies of Scutellata are often not aggressive and this can lead to misdiagnosis. Scutellata that have developed in isolation on the island of Puerto Rico show markedly less aggression than is generally reported (Rivera-Marchand, Oskay & Giray 2012).

Testing

Comb measurement

If comb has been constructed naturally (i.e. has not been built with the aid of foundation comb), a rough diagnosis can be obtained by measuring the width of 10 consecutive worker cells. For this procedure, each cell is defined as having one cell wall. The outside of the wall of the first cell is included in the measure. The last cell is measured up to the edge of the eleventh wall. Only fully formed worker cells in the central portion of a comb should be measured (Rinderer et al. 1986).

Scutellata cells average 4.8–4.9 cm (range 4.5–5.0 cm) whereas European cells average 5.2–5.3 cm (range 5.0–5.5 cm). Colonies producing worker comb having an average of three measurements of 4.9 cm or below are almost certain to be Africanised. Colonies producing worker comb having an average of three measurements of 5.2 cm or above are almost certain to be European. Colonies with intermediate values should be considered unidentifiable by this procedure, but suspected to be Africanised (Rinderer et al. 1986).

Morphometrics

Morphometrics, the study of size and shape of organisms, can provide a diagnosis of Africanisation (Daly & Balling 1978). Although morphometry can detect hybrids as well as purebred individuals, the sensitivity and accuracy of this method declines as the level of Africanisation decreases (Guzmán-Novoa, Page & Fondrk 1994).

Morphometrics is a laboratory procedure involving precise measurement of forewing length using a dissecting microscope fitted with a calibrated ocular micrometer. Ten forewing lengths of adult worker honey bees are measured (see Figure 14 in the queen honey bee review) and the

average calculated. The average forewing length of Scutellata honey bees is 8.87 mm, and the average forewing length of European honey bees is 9.20 mm. The probability that the sample is Scutellata and the probability that the sample is European can then be estimated (Rinderer et al. 1986).

Evidence of Africanisation in the mitochondrial genome

Testing of honey bee maternally inherited mitochondrial genome is available. Deoxyribonucleic acid (DNA) is extracted by a standard protocol (Holmes et al. 2010).

A diagnostic restriction site for Scutellata has been identified using a PCR amplified DNA assay and the procedure validated confirming it is a simple, rapid test suitable for screening large numbers of samples (Crozier, Koulianos & Crozier 1991; Pinto et al. 2003). The restriction site was scored as present (not Scutellata) or absent and the test was performed on a sample of 451 colonies from the southern United States with most being able to be classed as honey bees of A lineage or bees of C and M lineage. The only exception was that some honey bees of the *A. m. iberiensis* type from southern Spain and France were diagnosed as being of A lineage and therefore, Scutellata (Franck et al. 1998; Pinto et al. 2003).

A diagnosis of Africanisation cannot be based solely on the mitochondrial genome as it only provides information about maternal ancestry. For example, if a queen honey bee of C lineage mates with Scutellata males, the offspring will show C lineage mitochondria (inherited maternally), but will be 50 per cent Scutellata in the nuclear genome. If there are repeated backcrosses to Scutellata males, it is possible to have a colony that is Scutellata in phenotype and nuclear genotype, but still showing C lineage in the mitochondria. Therefore it is important to test both the nuclear and mitochondrial genomes for evidence of Scutellata genetic background. Evidence of A lineage in either the nuclear or mitochondrial genome is sufficient for a positive diagnosis.

Evidence of Africanisation in the nuclear genome

Whitfield et al identified seven single nucleotide polymorphisms (SNP) that are at high frequency in Scutellata and low frequency in honey bees of the C lineage. However, all these SNPs occur with high frequency in honey bees of the M lineage and would give a high false positive rate in wild honey bees tested from both Australia and the US. Therefore the use of SNPs was considered unsuitable for the diagnosis of Africanisation in the queen honey bee review (Whitfield et al. 2006).

Significant advances have been made in SNP genotyping technology and a project at the University of Sydney has developed a panel of 96 SNP loci that can differentiate between the three honey bee lineages (A, C and M). This panel detects all subspecies of the A lineage including *A. m. intermissa*, as well as Scutellata and the Cape honey bee. The efficacy of the panel has been tested by genotyping individuals from a number of distinct populations—Australian wild and domestic populations, Africanised bees from the United States and from Brazil, Scutellata from South Africa, Cape honey bees from South Africa and a panel of bees from Europe of both C and M lineages (Chapman et al. 2015). This methodology will be assessed in further detail as it has the potential to be used as a diagnostic test for the importation of both honey bee semen and queen honey bees from areas where Africanised bees are present.

Control

The history of Scutellata management in the Americas show that establishment and spread is rapid and there is no possibility of eradication (Schneider, DeGrandi-Hoffman & Smith 2004) (Rinderer 1988). Restriction of movement (other than either into or from Western Australia or Tasmania) is likely to be futile, and would cause significant disruption to the beekeeping industry.

Possible management measures and restrictions include:

- requeening with non-Scutellata stock
- methods of transport including mandatory netting of loads
- restriction of beekeeping activities in urban areas
- location of apiaries, including increasing distances from roads, dwellings, and walking tracks
- appropriate warning signage around apiaries
- development of reliable sources of non-Scutellata stock. This could include establishment of queen honey bee breeding businesses in colder regions as African bees have a reduced capacity for winter survival.

Current biosecurity measures

There are current biosecurity measures in place in the conditions for the importation of queen honey bees. These are either country freedom from Africanised bees or in the case of Canada, which shares a border with the US where Africanised bees are present, morphometrics and mitochondrial testing is required.

Conclusion

- *A. m. scutellata* and its Africanised hybrids are not present in Australia and are likely to be present in some exporting countries.
- Africanised bees are not OIE listed and there are no recommendations in the OIE Code on measures for safe trade.

Therefore, the department concluded that a risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see section 1.2. A summary of the risk assessment is shown in Figure 2.

Entry assessment

The following factors were considered relevant to the estimate of the likelihood of Scutellata genes being present in imported honey bee semen:

- Scutellata is now present throughout sub-Saharan Africa and Central America. In South America, Scutellata extends as far south as 34° South, where it forms a stable hybrid zone with honey bees of the C and M lineages (Sheppard et al. 1991).
- In the United States, Scutellata has been identified in the states of Alabama, Arizona, Arkansas, California, Colorado, Florida, Georgia, Louisiana, Nevada, New Mexico, New York, Oklahoma, South Carolina, Tennessee, Texas, Utah and Virginia.
- Scutellata are not sufficiently different from honey bees of a number of other *Apis mellifera* subspecies to be reliably distinguishable by eye. Large colonies can be extremely aggressive but this too is not a reliable indication of Africanisation.

Conclusion: based on this information, the likelihood of entry of Scutellata associated with the importation of honey bee semen was estimated to be **moderate**.

Exposure assessment

The exposure group assessed is managed and wild honey bee colonies and the most likely exposure pathway is the introduction of imported semen with Africanised genetics to a managed honey bee colony.

The nature of the commodity means that Africanised genes imported in semen are most likely to be introduced directly into the managed honey bee population through artificial insemination of local queen honey bees.

Conclusion: based on this information, the likelihood of susceptible honey bees being exposed to Africanised honey bee genetics via imported honey bee semen was estimated to be **high**.

Estimation of the likelihood of entry and exposure

The likelihood of entry and exposure is estimated by combining the likelihood of entry and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (see Table 2 in the queen honey bee review). With the likelihood of entry estimated to be 'moderate' combined with the likelihood of exposure estimated to be 'high', the likelihood of entry and exposure was estimated to be **moderate**.

Consequence assessment

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by describing the likely extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to Africanised honey bee genetics is considered to be establishment and/or spread to populations of honey bees within multiple states and territories.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of honey bees to Africanised honey bee genetics:

- Following importation into Brazil, descendants of *Scutellata* imports have formed the basis of a very large wild population of *Scutellata* that has replaced European honey bees throughout the tropical areas of Central and South America and in southern United States (Clarke et al. 2002; Pinto et al. 2005; Schneider, DeGrandi-Hoffman & Smith 2004; Whitfield et al. 2006; Winston 1992). From the initial importation in 1954, *Scutellata* has expanded its range by 200–500 km per year (Taylor 1977; Winston 1992).
- *Scutellata* is characterised by behavioural traits that are adaptations to life in the African savannah: high reproductive rates and extreme defensiveness to counter frequent predation, and regular migration to exploit highly ephemeral nectar sources (Rinderer 1988).
- *Scutellata* are small, yellow honey bees and are not sufficiently different from honey bees of a number of other *A. mellifera* subspecies to be reliably distinguishable by eye. Large colonies are extremely aggressive but this is not a reliable indication of Africanisation. A colony with Africanised genetics present, particularly a small nucleus colony, may not appear unusually aggressive.
- The pattern of distribution of *Scutellata* in South Africa, southern United States and Argentina strongly suggests that *Scutellata* could establish large wild populations in all dry savannah regions of Australia north of 34° South. Thus the majority of Australia, including

most of the preferred beekeeping country in Australia, is vulnerable to the establishment of a wild *Scutellata* population.

- The experiences of *Scutellata* management in the Americas show that, once established, there is very little possibility of eradication.

Conclusion: based on these considerations, it was determined that the likelihood of establishment and/or spread of Africanised honey bee genetics for the exposure group was **high**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect effects of Africanised honey bee genetics were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria. The following factors were considered relevant when determining the effects of the establishment and/or spread of Africanised honey bee genetics.

Direct effects

The effect on the life or health (including production effects) of susceptible animals including public health consequences:

- *Scutellata* spreads and replaces European honey bees primarily by producing larger numbers of reproductive swarms and drones. A *Scutellata* colony can produce 6–12 reproductive colonies per year and via the offspring of the offspring colonies, up to 60 descendent colonies per year (Otis 1991). There are also other more subtle processes that contribute to Africanisation.
- *Scutellata* is resistant to some of the major pests of C lineage honey bees, particularly the small hive beetle and varroa.
- Replacement of European (C and M) lineage wild honey bees with *Scutellata* throughout all of Australia north of 34° South is likely to occur.
- *Scutellata* pose a significant risk to the public via incidental stinging attacks. When beekeepers work in *Scutellata* apiaries the colonies become extremely defensive and can attack any mammal or bird within a few hundred metres. The attacks involve hundreds of stinging honey bees, and death by stinging or the consequences of panic is possible. From 1988 to 2009 there were 480 deaths recorded in Mexico as due to multiple honey bee stings (Becerril-Ángeles et al. 2013). A recent analysis of the public health costs if *Apis cerana* became established in Australia suggested that the annual direct public health costs would be A\$7.4 million (Ryan 2010). The costs associated with an endemic *Scutellata* population would likely be higher.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment:

- If introduced into Australia, *Scutellata* may increase the size of the wild honey bee population. An increased density of wild honey bees may have negative effects on the native fauna that compete with wild honey bees for nest sites, pollen and nectar.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs:

- Scutellata is not nationally notifiable in Australia. However Scutellata is notifiable in several states and in the Australian Capital Territory and is included in the AUSVETPLAN *Disease Strategy: Bee Pests and Diseases* (AHA 2010).
- Under the current emergency response arrangements:
 - controls over honey bee movements would be imposed on managed apiaries within designated areas until further decisions were made (AHA 2010)
 - movement controls and/or inspections may be imposed on vehicles (e.g. trucks, trains) leaving the Scutellata control zone
 - if the decision was made not to attempt eradication but to recommend that control practices be initiated, state or territory and/or industry-based control measures may be initiated. This may include encouraging industry to develop its own long-term policies and procedures such as:
 - a recommended frequency of mandatory re-queening with non-Scutellata stock
 - transport methods such as mandatory netting of loads
 - restrictions on beekeeping activities in urban areas
 - location of apiaries, including increasing the distance from roads, dwellings, and walking tracks
 - appropriate warning signage around apiaries (AHA 2010).

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries:

- Under the current emergency response arrangements, the detection of Scutellata would result in movement restrictions that would be imposed until tracing and surveillance were completed and results analysed (AHA 2010).
- If eradication was not attempted, control measures may include interstate movement restrictions (AHA 2010).
- Scutellata apiaries are recommended to be situated a minimum of 200-500 metres away from places occupied by humans, livestock or companion animals (Caron 2001).
- Scutellata's behavioural and productive characteristics make it undesirable for modern beekeeping as practised in Australia.
- The costs of production of honey products and other honey bee products may increase.
- Both private and public landowners are likely to become more reticent to allow beekeeping activities on their properties because of the possibility of stinging incidents, stock losses and fear of litigation. This would have a significant negative impact on beekeepers and landowners who need to hire bees for pollination.
- Scutellata are likely to be unsuitable for paid pollination services.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand:

- The presence of Scutellata in Australia may reduce international consumer demand for Australian queen and package honey bees and if Scutellata were to become established, renegotiation of existing trade conditions would be necessary.
- It is unlikely to have an effect on the export of Australian honey and other bee products

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems:

- Wild *Scutellata* colonies would compete with native fauna for nesting sites and floral resources. Although similar to existing wild colonies of other *A. mellifera* subspecies, the density of wild *Scutellata* colonies is likely to be higher, exacerbating any adverse environmental effects.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures:

- The financial cost of beekeeping may increase due to expenses associated with the management of *Scutellata*.
- The introduction of *Scutellata* into Australia would likely see many beekeepers, both hobbyist and commercial, exiting the industry, as has occurred in South and Central America (Winston 1992). Working with *Scutellata* honey bees can be extremely unpleasant, potentially dangerous, and requires trained personnel.

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated, after referral to Table 3 in the queen honey bee review, to be **high**. The effect is likely to be significant at the national level, highly significant within affected zones, and be of national concern.

Estimation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario and, using the matrix in Table 4 of the queen honey bee review, the likely consequences were estimated.

The likelihood of establishment and/or spread (**high**) is combined with the estimate of the overall effect of establishment and/or spread (**high**) which results in **high** likely consequences.

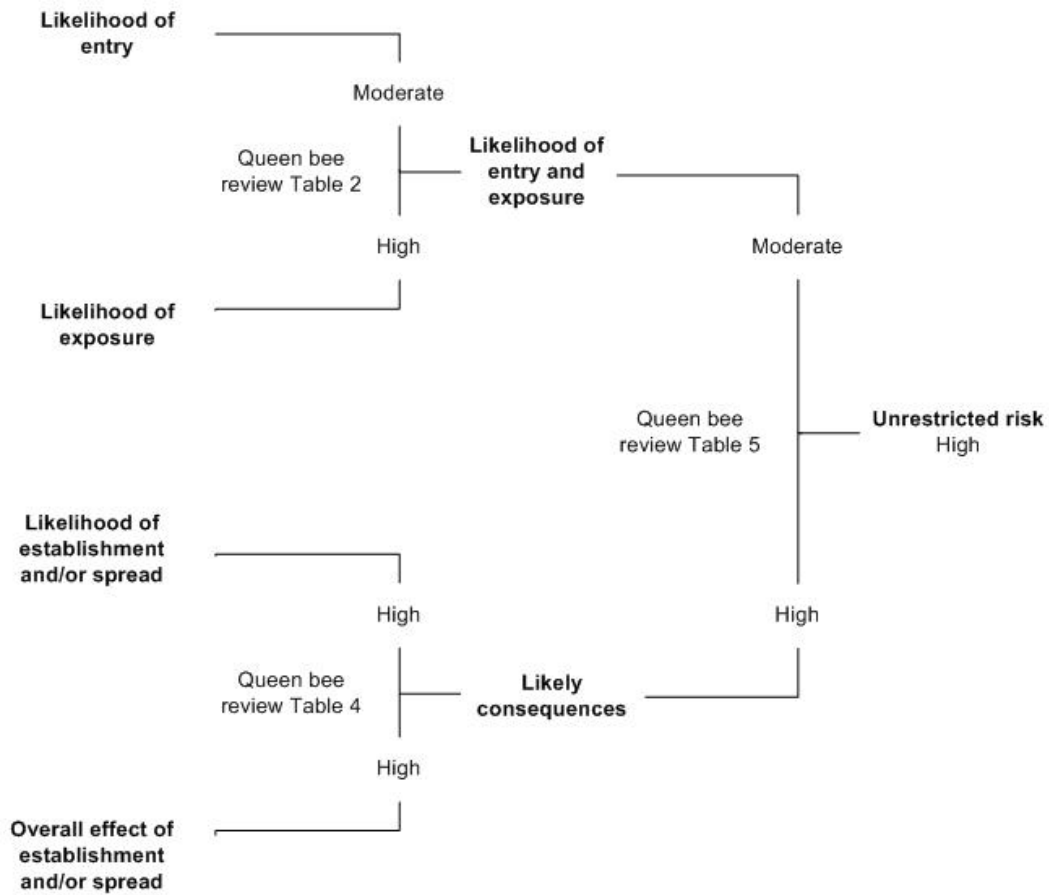
Evaluation of unrestricted risk

Risk estimation is the integration of likelihood of entry and exposure with likely consequences of establishment and/or spread to derive the risk associated with entry, exposure, establishment and/or spread of *Scutellata* introduced into Australia by imported honey bee semen.

Using the matrix in Table 5 of the queen honey bee review, the likelihood of entry and exposure (**moderate**) is combined with the likely consequences of establishment and/or spread (**high**), resulting in a risk estimation of **high**. For a summary see Figure 2.

Therefore, as the unrestricted risk associated with Africanised honey bees exceeds Australia's ALOP of 'very low', risk management was considered necessary.

Figure 2 Risk assessment pathways for Africanised honey bees



5 Risk review—*Nosema ceranae*

Background

N. ceranae is a microsporidian parasite of several *Apis* species including *Apis mellifera*. Both *N. ceranae* and the closely related *N. apis* infect all classes of honey bees. *N. apis* was first described in 1909 and has a worldwide distribution. *N. ceranae* was first described in the Asian honey bee (*Apis cerana*) in 1996 (Fries 2014) and there are indications that *N. ceranae* is replacing *N. apis* in some honey bee populations (Huang et al. 2015). Studies subsequent to its initial identification have shown that *N. ceranae* had infested European honey bees for some decades before it was first identified as such and that some earlier reports of *N. apis* infections, identified by light microscopy only, may have been unrecognised *N. ceranae* (Paxton et al. 2007; Teixeira et al. 2013).

Both species are present in Australia. A survey of pathogens present in Australian honey bees carried out between 2013 and 2015 indicates that *N. apis* and *N. ceranae* are present in all states and territories except the Northern Territory with a general tendency for *N. ceranae* to be more prevalent in the north of the country and *N. apis* in the south (Roberts, Anderson & Durr 2015). This reflects a general European and North American pattern where *N. ceranae* is the more prevalent species in the hotter south of the continent and *N. apis* in the cooler north (Fries 2014). The isolated and relatively pest-free honey bee population of south-west Western Australia (WA) is free of *N. ceranae* (Roberts, Anderson & Durr 2015).

Technical information

Nosema are obligate intracellular parasites. Transmission is oral-faecal with the spores germinating in the cells of the mid-gut of the infected honey bee.

Severe *N. apis* infections are associated with diarrhoea manifested as faecal stains on the hive; *N. ceranae* does not appear to produce these clinical signs to the same extent (Higes et al. 2008). Colonies can be heavily infected with either species without showing clinical signs.

A number of studies have provided evidence that *N. ceranae* is more virulent at a colony level than *N. apis* and that severe infections can lead to colony losses (Higes et al. 2008). There is also evidence that *N. ceranae* may be more virulent on an individual basis (Paxton et al. 2007) either by itself or in conjunction with other pathogens (Zheng et al. 2015). There is recent experimental evidence that *N. ceranae* can infect the larvae of honey bees and that this can affect the longevity of the infected individuals (Eiri et al. 2015).

However the evidence is contradictory and in some instances *N. ceranae* has either been shown to be long-present in honey bee populations without noticeable effect (Teixeira et al. 2013) or shown to be less infective and less virulent than *N. apis* (Huang et al. 2015).

N. apis can be present in semen (Peng et al. 2015) and it can be assumed that this would also be the case with *N. ceranae*. There is also evidence that anti-microbial factors present in honey bee semen will inactivate *N. apis*, should it be present (Roberts et al. 2015) but the authors caution about extending the conclusions beyond the lineage of bees and the *Nosema* species studied without further work.

Diagnosis

Light microscopy can be used to diagnose infections with *Nosema* spp. and an estimate of the infection level can be made by counting spores in a sample of bees (Fries 2014). PCR is used to distinguish between the two species (Roberts, Anderson & Durr 2015).

Conclusion

Both *Nosema* spp. are present and widespread in Australia but the recently completed survey of honey bee pathogens (Roberts, Anderson & Durr 2015) has confirmed previous findings that *N. ceranae* is not present in the major beekeeping region in the south-west of WA. WA has legal instruments in place to control movement of honey bees and bee products into this defined region (Biosecurity and Agricultural Management Regulations 2013). This region is also free from European foulbrood and small hive beetle (*Aethina tumida*).

Given that the south-west of WA has been confirmed to be free of *N. ceranae* and that the literature does provide evidence as to the potential pathogenicity of this agent, a risk assessment of the entry of *N. ceranae* into WA is required.

Risk assessment

For details of the method used in this risk assessment see section 1.2.

Entry assessment

The following factors were considered relevant to the estimate of the likelihood of *N. ceranae* being present in imported honey bee semen:

- *N. ceranae* has a worldwide distribution
- *Nosema* spp. can be present in bee semen

Conclusion: Based on this information the likelihood of the entry of *N. ceranae* associated with the importation of honey bee semen was estimated to be **moderate**.

Exposure assessment

- Infected semen would be inseminated directly into susceptible queen bees

Conclusion: Based on this, the likelihood of susceptible queen honey bees being exposed to infected semen was estimated to be **high**.

Estimation of the likelihood of release and exposure

The likelihood of entry and exposure is estimated by combining the likelihood of entry and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (see Table 2 in the queen honey bee review). With the likelihood of entry estimated to be moderate combined with the likelihood of exposure estimated to be high, the likelihood of entry and exposure was estimated to be **moderate**.

Consequence assessment

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario following introduction of *N. ceranae* into the south-west of WA is spread through that region. Spread beyond that region is not relevant.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of honey bees to *N. ceranae*:

- *N. ceranae* has spread worldwide
- It is not easily distinguishable from *N. apis* which is endemic in south-west WA
- Infections, even relatively heavy, may not cause overt clinical signs

Conclusion: based on these considerations, it was estimated that the likelihood of establishment and spread of *N. ceranae* through the region was **high**.

Determination of the effects resulting from establishment and spread

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect effects of *N. ceranae* were estimated at the national, state or territory, district/region and local levels.

The following factors were considered relevant when determining the effects of the establishment and/or spread of *N. ceranae*:

Direct effects

The effect on the life or health (including production effects) of susceptible animals including public health consequences and the effect on the living environment, including life and health of wildlife, and any effects on the non-living environment:

- *N. ceranae* has been associated with lethal and sub-lethal effects at an individual and colony level.
- There are no public health consequences
- *N. ceranae* is restricted to Apis species—there are unlikely to be effects on wildlife or the non-living environment.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs; the effect on domestic trade or industry, including changes in consumer demand; the effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand and the effect on communities, including reduced rural and regional economic viability and loss of social amenity.

- *Nosema* infections are not nationally notifiable. *N. ceranae* is listed on the Western Australia Organism List as a declared pest and is prohibited. Detection may lead to control measures being taken in that state.
- Controls imposed in a control program in this region may interfere with normal movement and commerce.
- Given that *N. ceranae* is endemic in the rest of the country, there will be no effect on interstate trade should this agent become established in south-west WA.
- There will be no effect on international trade in honey bees or honey bee products. Although Japan in particular has restrictive requirements for *Nosema*, this applies to both species and *N. ceranae*-freedom does not confer a more favourable status.

Conclusion for overall direct and indirect effects: there will be only a limited geographical impact of the introduction of *N. ceranae* into this region. Within this region the effect is likely to be recognised and may be significant to the affected parties. Based on the limited geographical impact of the introduction of *N. ceranae* into this region and that the effects of this agent are equivocal, the overall effect across Australia is estimated to be **very low**. However the effects on the naïve population in WA will be higher. These are estimated as **low**.

Estimation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario and, using the matrix in Table 4 of the queen honey bee review, the likely consequences were estimated.

The likelihood of establishment and/or spread (**high**) is combined with the estimate of the overall effect of establishment and/or spread. On a national basis this is **very low** which results in likely consequences being estimated as **very low**. On a local basis the effects are estimated as low which results in **low** likely consequences

Evaluation of unrestricted risk

Risk estimation is the integration of likelihood of entry and exposure with likely consequences of establishment and spread to derive the risk associated with entry, exposure, establishment and spread of *N. ceranae* introduced into south-west WA by imported honey bee semen.

Using the matrix in Table 5 of the queen honey bee review, the likelihood of entry and exposure (**moderate**) is combined with the likely consequences of establishment and/or spread. Again, on a national basis the consequences are **very low**, resulting in a final risk estimation of **very low**, below the level where risk management is indicated. However, the local consequences are **low** and the estimation of risk in this case is **low**, indicating that management of the risks of this agent to this local population is indicated.

6 Risk management

Risk management measures considered in this review aim to reduce the likelihood that imported honey bee semen would lead to the entry, exposure, establishment and/or spread of hazards of biosecurity concern. The method used here for risk management is consistent with that used in the queen honey bee review and the OIE Code(OIE 2014).

To achieve Australia's ALOP, the biosecurity risk associated with imported honey bee semen must be no higher than **very low**. If the unrestricted risk estimate did not achieve Australia's ALOP, risk management options to reduce the risk to an appropriate level, i.e. **very low** or **negligible**, were considered. In this case, the Africanised honey bee (*A. m. scutellata* and its hybrids) did not achieve Australia's ALOP and risk management measures are considered necessary to reduce the risk to an appropriate level. After assessment of *N. ceranae*, risk management measures are considered necessary for Western Australia.

The effect of risk management measures is assessed as the change in the risk estimate and expressed as 'restricted risk'. When the combined effect of the applied risk management measures reduces the risk estimate to **very low** or **negligible**, the measure, or combination of measures, are appropriate.

When deciding on risk management measures, equivalent measures that would achieve Australia's ALOP were considered and those that would be least trade restrictive were identified.

6.1 Risk management options

The efficacy and feasibility of risk management measures that could be applied in this review take the following into account:

- honey bee semen is viable, which limits treatments associated with non-viable product, e.g. heat treatment
- some honey bees infected with disease agents may not show clinical signs of infection (i.e. they have a subclinical infection or a latent carrier state) or carry undesirable genetics that may not be immediately evident and therefore detection of disease or hybridisation through visual observation and examination is unreliable
- the sensitivity of some diagnostic tests is not always optimal (i.e. infection or infestation may not be detected)
- treatments are not always reliable in preventing disease or stopping shedding of disease agents
- honey bees are essentially an uncontrolled commodity. The status of honey bee pests and diseases within a country may change and be undetected for an extended period of time.

Pre-entry risk management options

- Approved countries
- Country or zone freedom
- Premises status
- Diagnostic testing
- Visual inspection.

Post-arrival risk management options

- Diagnostic testing
- Restricted post-quarantine release.

Contingency measures

In the event that imported honey bee semen does not meet Australia's import requirements then the affected consignment and any honey bees deemed to have been in contact with it may be destroyed.

6.2 Risk management for Africanised honey bees

The unrestricted risk for Africanisation associated with the importation of honey bee semen was estimated to be **high**. Risk management options that could be applied to achieve Australia's ALOP were considered.

The likelihood of entry of semen containing Africanised genes was estimated to be **moderate** and the likelihood of exposure of Australian honey bees to Africanised genes in imported semen was estimated to be **high**. The following options were considered as risk management measures to reduce the likelihood of entry of, and/or exposure to, Africanised genes.

Pre-entry measures

Approved countries

The queen honey bee review approved a number of countries for export to Australia. This was after consideration of a number of criteria including the animal health status of the country, animal health legislation, the effectiveness of systems in place for certification of animals and products, the effectiveness of veterinary and laboratory services, and the standard of reporting of disease outbreaks to the OIE.

Conclusion

The countries approved to export queen honey bees to Australia—Canada, the European Union, Japan, New Zealand and the United States—are also considered suitable to export honey bee semen.

If other countries with a history of trade with Australia wish to be added to the list of approved countries for either queen honey bees, honey bee semen, or both, an assessment of the above criteria would be required to determine if Australia's import requirements could be met.

Country or zone freedom

- Scutellata are now present throughout sub-Saharan Africa and Central America. In South America, Scutellata extends as far south as 34° South in Argentina, where it forms a stable hybrid zone with bees of the C and M lineages (Sheppard et al. 1991). In the United States, Scutellata have been identified in the states of Alabama, Arizona, Arkansas, California, Colorado, Florida, Georgia, Louisiana, Nevada, New Mexico, New York, Oklahoma, South Carolina, Tennessee, Texas, Utah and Virginia (Cooperative Agricultural Pest Survey (CAPS) 2011; Erdahl 2014; Platt 2012).
- Scutellata are not sufficiently different from honey bees of the other subspecies present in Australia to be reliably distinguishable by eye. Large colonies are extremely aggressive but this too is not a reliable indication of Africanisation.

Conclusion

Based on this information, the department considered that country or zone freedom is a suitable risk management option for Africanised honey bees.

Consideration of country or zone freedom from *Scutellata* may be agreed if declaring freedom in accordance with Article 1.4.6 of the OIE Code. Supporting documentation, including evidence of adequate surveillance to demonstrate freedom, may be required. Factors to be considered may include, but are not limited to, the presence of an early detection system for *Scutellata* and measures to prevent introduction.

The Africanised honey bee status within a country can vary depending on factors such as prevailing weather and internal migratory beekeeping practices. The status can change and go without detection for an extended period of time. Due to the difficulty in detecting the presence of *Scutellata*, country or area freedom alone was not considered sufficient to reduce the likelihood of entry from some approved countries.

Premises status

- *Scutellata* are not sufficiently different from honey bees of other subspecies to be reliably distinguishable by eye.
- Large *Scutellata* colonies can be extremely aggressive but this is not a reliable indication of Africanisation.

Conclusion

The department considered that premises status was a risk management option for Africanised honey bees. Although *Scutellata* is not sufficiently different from other subspecies of *A. mellifera* to be reliably distinguishable by eye, a requirement for the honey bee semen to have been sourced from an apiary free from visible evidence of *Scutellata* infestation, including behavioural, at the time of sourcing was considered appropriate.

However, due to the difficulty in detecting the presence of *Scutellata*, premises status alone was not considered sufficient to reduce the likelihood of entry.

Diagnostic testing

- The testing of mitochondria will give information on the maternal lineage of the tested honey bee. As it cannot give information on the paternal line, this method, used alone, cannot give a definite diagnosis of freedom from Africanisation. However evidence of *A* lineage in the mitochondrial genome is sufficient for a positive diagnosis.
- There are no known DNA microsatellites that show fixed difference between honey bees of African origin and honey bees of C and M lineage (Clarke et al. 2002). Therefore DNA microsatellites cannot be used for definitive diagnosis.
- Morphometrics (Daly & Balling 1978) can provide a diagnosis of Africanisation; however the sensitivity and accuracy of the method declines as the level of hybridisation decreases (Guzmán-Novoa, Page & Fondrk 1994).
- The use of single nucleotide polymorphisms (SNP) was not recommended for the identification of Africanised honey bees in the queen honey bee review. However knowledge and research in this area has increased since the release of that review (Chapman et al. 2015). This new methodology can test the level of Africanisation in samples from drones providing semen for export and has the potential to allow the import of semen from countries such as the United States where Africanisation is endemic.

Conclusion

Based on this information, the department considered that diagnostic testing is a risk management option for Africanised honey bees.

A test of the mitochondrial lineage could be performed on a random sample of worker or drone honey bees from the same colony from which the semen for export will be sourced. However, testing of mitochondrial DNA alone was not considered sufficient to reduce the likelihood of entry.

SNP testing could provide risk management sufficient to reduce the likelihood of entry even from countries or regions where Africanisation is endemic. The department intends to address this methodology in a separate discussion paper so the benefits and risks and the quarantine arrangements that may be required can be fully discussed and assessed before it is recommended as a risk management measure.

Apiary and colony inspection

Provided all other import requirements are met, honey bee semen will only be eligible for export if it is derived from colonies that have been inspected and certified by an officer from the relevant authority within 30 days of export and assessed as clinically healthy and free from the hazards of concern.

The drones themselves should themselves be free of physical or behavioural abnormalities and show no visible evidence of the hazards of concern.

Post-arrival measures

No post-arrival measures are recommended—it is expected that after border clearance imported honey bee semen will go directly to the place of insemination, which in most cases will be honey bee breeding operations.

Diagnostic testing

A validated test of the nuclear genome could be performed in Australia. Testing would be of genetic material associated with the semen, for example drone cadavers, rather than the semen itself. Imported honey bee semen could be held in an appropriate QAP until the consignment is approved by the department following the results of testing, but policies and procedures would need to be developed to accommodate this. Alternatively, semen could be imported after testing in Australia confirms the closed exporting colonies to be free of Africanisation.

Restricted post-quarantine release

The progeny of honey bee semen may be restricted to certain regions in Australia to comply with internal movement restrictions for particular hazards.

Overall conclusion

The unrestricted risk associated with Africanised honey bees was estimated to be **high**.

In order to achieve Australia's ALOP of **very low**, the likelihood of entry and exposure needs to be reduced to **extremely low**. For this to occur, the likelihood of entry for Scutellata needs to be reduced from **moderate** to **extremely low**.

The reduction of the unrestricted risk to meet Australia's ALOP can be achieved by:

- importing from countries that are free from Africanised honey bees and where there is negligible chance of contamination from neighbouring areas infested with Africanised honey bees

OR

- importing from countries or zones that are either within or adjacent to Africanised honey bee infested areas but can demonstrate absence of Africanised honey bees and diagnostic mitochondrial DNA testing has shown no evidence of Africanisation

OR

- importing from any approved country regardless of its status as long as the semen has been shown not to be Africanised by a validated and approved test of the nuclear genome.

6.3 Risk management for *Nosema ceranae*

The unrestricted risk for *N. ceranae* associated with the importation of honey bee semen into Australia was assessed as **very low**. However the unrestricted risk for WA was estimated to be **low**. Risk management options that could be applied importation into WA to achieve Australia's ALOP were considered.

The likelihood of entry of semen containing *N. ceranae* was estimated to be **moderate** and the likelihood of exposure of honey bees in WA to *N. ceranae* genes in imported semen was estimated to be **high**. The following options were considered as risk management measures to reduce the likelihood of entry of, and/or exposure to, *N. ceranae*.

Pre-entry measures

Country or zone freedom

- *N. ceranae* has a worldwide distribution.
- There are regional variations in its distribution within countries.

Conclusion

Based on this information, the department considered that country or zone freedom is a suitable risk management option for *N. ceranae*.

Consideration of country or zone freedom from *N. ceranae* may be agreed if declaring freedom in accordance with Article 1.4.6 of the OIE Code (OIE 2014). Supporting documentation, including evidence of adequate surveillance to demonstrate freedom, will be required to be forwarded to and assessed by the department before importation under this condition could proceed. Factors to be considered may include, but are not limited to, the presence of an early detection system and measures to prevent introduction.

The *N. ceranae* status within a country or region can vary depending on factors such as migratory beekeeping practices or importation. The status can change and go without detection for an extended period of time. Due to these issues, country or area freedom alone was not considered sufficient to reduce the likelihood of entry from some approved countries.

Premises status

- Importation from colonies within a country or zone known to be infected was considered. Given the free-flying nature of honey bees and their constant interactions with individuals from other colonies, both managed and wild, the health status of colonies established by testing in this situation can only be transitory.

Conclusion

The department considered that premises status was not a risk management option for *N. ceranae*.

Diagnostic testing

- Light microscopy can be used to diagnose infections with *Nosema* spp. and an estimate of the infection level can be made by counting spores in a sample of bees (Fries 2014).
- PCR can be used to detect *Nosema* and to distinguish between the two species (Roberts, Anderson & Durr 2015).
- Semen collected for artificial insemination will be pooled from a number of drones and testing of a sample of semen from a collection vessel will not verify the disease status of all the drones contributing to the collection.

Conclusion

N. ceranae can be relatively easily identified. However, given the way semen is collected, transported and administered, the department considered that diagnostic testing is not a wholly effective risk management option for *N. ceranae* in honey bee semen.

Post-arrival measures

For consignments of semen originating in countries or recognised zones accepted by the department to be free from *N. ceranae* no post-arrival measures are recommended.

Restricted post-quarantine release

The progeny of honey bee semen may be restricted to certain regions in Australia to comply with internal movement restrictions for particular hazards.

Overall conclusion

The unrestricted risk associated with *N. ceranae* in WA was estimated to be **low**.

The reduction of the unrestricted risk to meet Australia's ALOP can be achieved by:

- importing from countries or zones that are free from *N. ceranae* and where there is negligible chance of contamination from neighbouring areas infected with *N. ceranae*

7 Biosecurity measures

7.1 Requirements for the importation of honey bee semen

Eligibility

Importation under these conditions is restricted to honey bee semen from approved countries only.

Documentation

- a) A written application to import honey bee semen must be lodged with the department prior to any import proceeding.
- b) Each consignment must be accompanied by:
 - i. a valid import permit
 - ii. an original international veterinary health certificate consistent with the OIE Code, signed by a Government Apiary Officer or an Official Veterinarian of the country of export (requirements of this certification are specified in Attachment 1).
- c) Any inadequacies in certification may result in the consignment being returned to the country of origin at the importer's expense or the destruction of the semen without recompense.

Hazards

For the purposes of these requirements, the word 'hazard' refers to the pests and pathogens listed below:

- Africanised honey bees (*Apis mellifera scutellata* and its hybrids)
- Cape honey bees (*A. m. capensis*)
- *Nosema ceranae*.

Review

The requirements of importation may be reviewed if there are any changes in the animal health status and/or the import policy of the exporting country, or at any time at the discretion of the Director of Biosecurity

7.2 Proposed Health Certificate requirements for honey bee semen exported to Australia

The certificate must accompany the consignment of honey bee semen.

The Government Apiary Officer or Official Veterinarian must sign, date and stamp with the official government stamp each page of the certificate.

The certificate must include a statement showing the exporting country and the name of the Government Apiary Officer or Official Veterinarian and contain a declaration as to the following with respect to the source of the honey bee semen for export to Australia:

1. The colonies from which the honey bee semen for export was sourced have been examined by a Government Apiary Officer or Official Veterinarian within 30 days prior to export and found to be clinically healthy and free from visible evidence of the hazards listed at clause 1.7.1 of the Requirements above.
2. The country of export is free from Cape honey bees (*A. mellifera capensis*).
3. The country of export meets one of the following three conditions:
 - i) The country of export is free from Africanised honey bees (*A. mellifera scutellata* and its hybrids) and the country does not share a land border with a country where Africanised honey bees are known to be present.

OR

- ii) The country of export is free from Africanised honey bees but the country shares a land border with a country where Africanised honey bees are known to be present OR the semen for export has been sourced from colonies in a zone considered free from Africanised honey bees within a country where Africanised honey bees are known to be present.

AND

Testing of mitochondrial DNA within three months prior to export of a random selection of at least 10 worker or drone honey bees from the colonies from which the semen for export was sourced; or testing of nuclear DNA by a method approved by the Australian Department of Agriculture and Water Resources has shown the colonies not to be Africanised.

AND

The exporter has confirmed that the drones producing the semen in the consignment have been confined to the colonies of origin by a queen excluder and have not been permitted free flight prior to semen collection.

OR

- iii) The honey bee semen for export has been sourced from colonies in a country or zone not considered free from Africanised honey bees and testing of nuclear DNA by a method approved by the Australian Department of Agriculture and Water Resources has shown the colonies not to be Africanised.

AND

The exporter has confirmed that the drones producing the semen in the consignment have been confined to the colonies of origin by a queen excluder and have not been permitted free flight prior to semen collection.

4. For importation into Western Australia, the semen has originated from a country or zone recognised by the Australian Department of Agriculture and Water Resources to be free from *Nosema ceranae*.

References

- AHA 2010, *Disease strategy: bee diseases and pests (version 3.2)*, Australian Veterinary Emergency Plan (AUSVETPLAN), Primary Industries Ministerial Council, Canberra, available at [http://www.animalhealthaustralia.com.au/fms/Animal%20Health%20Australia/AUSVETPLAN/BEES3_2-07PROOF\(16Feb2010\).pdf](http://www.animalhealthaustralia.com.au/fms/Animal%20Health%20Australia/AUSVETPLAN/BEES3_2-07PROOF(16Feb2010).pdf).
- Barry, M 2007, *Effective approaches to risk assessment in social work: an international literature review*, Education Information and Analytical Services, Scottish Executive, Edinburgh.
- Becerril-Ángeles, M, Núñez-Velázquez, M, Arias-Martínez, MI & Grupo del Programa de Control de la Abeja Africanizada, S 2013, 'Mortalidad en México relacionada con picaduras de abeja de 1988 a 2009' (Mortality related to honey-bee stings in Mexico from 1988 to 2009), *Revista Alergia Mexico*, vol. 60, no. 2, pp. 58-62. (Abstract only)
- Caron, D 2001, *Africanized honey bees in the Americas*, The A.I. Root Company, Medina.
- Chapman, NC, Harpur, BA, Lim, J, Rinderer, TE, Allsopp, MH, Zayed, A & Oldroyd, BP 2015, 'A SNP test to identify Africanized honeybees via proportion of 'African' ancestry', *Molecular Ecology Resources*, vol. 15, no. 6, pp. 1346-55.
- Chapman, NC, Lim, J & Oldroyd, BP 2008, 'Population genetics of commercial and feral honey bees in Western Australia', *Journal of Economic Entomology*, vol. 101, no. 2, pp. 272-7.
- Clarke, KE, Rinderer, TE, Franck, P, Quezada-Euán, JG & Oldroyd, BP 2002, 'The Africanization of honeybees (*Apis mellifera* L.) of the Yucatan: a study of a massive hybridization event across time', *Evolution*, vol. 56, no. 7, pp. 1462-74.
- Cooperative Agricultural Pest Survey (CAPS) 2011, 'Record summary: Africanized honey bee - *Apis mellifera scutellata*', *National Agricultural Pest Information System (NAPIS) Pest Tracker*, available at <http://pest.ceris.purdue.edu/pdf/pdfdata.php?pestcode=ISAEAEA>.
- Crozier, YC, Koulianos, S & Crozier, RH 1991, 'An improved test for Africanized honeybee mitochondrial DNA', *Cellular and Molecular Life Sciences*, vol. 47, no. 9, pp. 968-9.
- Cuthbertson, AGS, Wakefield, ME, Powell, ME, Marris, G, Anderson, H, Budge, GE, Mathers, JJ, Blackburn, LF & Brown, MA 2013, 'The small hive beetle *Aethina tumida*: a review of its biology and control measures', *Current Zoology*, vol. 59, no. 5, pp. 644-53.
- Da Cruz-Landim, C, Roat, TC & Fernandez, FC 2012, 'Virus present in the reproductive tract of asymptomatic drones of honey bee (*Apis mellifera* L.), and possible infection of queen during mating', *Microscopy Research and Technique*, vol. 75, no. 7, pp. 986-90.
- Djordjevic, SP, Noone, K, Smith, L & Hornitzky, MZ 1998, 'Development of a hemi-nested PCR assay for the specific detection of *Melissococcus pluton*', *Journal of Apicultural Research*, vol. 37, no. 3, pp. 165-74.
- Eiri, DM, Suwannapong, G, Endler, M & Nieh, JC 2015, 'Nosema ceranae can infect honey bee larvae and reduces subsequent adult longevity' *PLoS ONE*, vol. 10, no. 5, pp. e0126330, available at <http://dx.doi.org/10.1371/journal.pone.0126330>.
- Erdahl, K 2014, 'Killer bees discovered in Colorado for first time', *FOX31 Denver*, FOX31 Denver KDVR-TV, Denver, Colorado, USA, available at <http://kdvr.com/2014/05/14/killer-bees-discovered-in-colorado-for-first-time/>.
- Franck, P, Garnery, L, Loiseau, A, Oldroyd, BP, Hepburn, HR, Solignac, M & Cornuet, J-M 2001, 'Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data', *Heredity*, vol. 86, no. 4, pp. 420-30.

- Franck, P, Garnery, L, Solignac, M & Cornuet, JM 1998, 'The origin of west European subspecies of honeybees (*Apis mellifera*): new insights from microsatellite and mitochondrial data', *Evolution*, vol. 52, no. 4, pp. 1119-34.
- Fries, I 2014, 'Microsporidia', in *Bee health and veterinarians*, Ritter, W (ed), World Organisation for Animal Health, Paris.
- Georgia Department of Agriculture 2010, *Africanized honeybees found in Georgia*, available at <http://medicalboard.georgia.gov/portal/site/AGR/menuitem.f298b492a7ec98007eff626ed03036a0/?vgnnextoid=53eb52d02aecb210VgnVCM100000bf01010aRCRD&vgnnextchannel=5a0121e7ea1e5210VgnVCM100000bf01020aRCRD>.
- Guzmán-Novoa, E & Page, RE, Jr. 1994, 'The impact of Africanized bees on Mexican beekeeping', *American Bee Journal*, vol. 134, no. Suppl. 2, pp. 101-6.
- Guzmán-Novoa, E, Page, RE, Jr. & Fondrk, MK 1994, 'Morphometric techniques do not detect intermediate and low-levels of Africanization in honey-bee (Hymenoptera, Apidae) colonies', *Annals of the Entomological Society of America*, vol. 87, no. 5, pp. 507-15.
- Guzman-Novoa, E, Sanchez, A, Page, RE, Jr. & Garcia, T 1996, 'Susceptibility of European and Africanized honeybees (*Apis mellifera* L) and their hybrids to *Varroa jacobsoni* Oud', *Apidologie*, vol. 27, no. 2, pp. 93-103.
- Higes, M, Martín-Hernández, R, Botías, C, Bailón, EG, González-Porto, AV, Barrios, L, Del Nozal, MJ, Bernal, JL, Jiménez, JJ, Palencia, PG & Meana, A 2008, 'How natural infection by *Nosema ceranae* causes honeybee colony collapse', *Environmental Microbiology*, vol. 10, no. 10, pp. 2659-69.
- Holmes, MJ, Oldroyd, BP, Allsopp, MH, Lim, J, Wossler, TC & Beekman, M 2010, 'Maternity of emergency queens in the Cape honey bee, *Apis mellifera capensis*', *Molecular Ecology*, vol. 19, no. 13, pp. 2792-9.
- Hood, WMM 2004, 'The small hive beetle, *Aethina tumida*: a review', *Bee World*, vol. 85, no. 3, pp. 51-9.
- Huang, W-F, Solter, L, Aronstein, K & Huang, Z 2015, 'Infectivity and virulence of *Nosema ceranae* and *Nosema apis* in commercially available North American honey bees', *Journal of Invertebrate Pathology*, vol. 124, pp. 107-13.
- Kerr, WE, de Leon del Rio, S & Barrionuevo, MD 1982, 'The southern limits of the distribution of the Africanized honey bee in South America', *American Bee Journal*, vol. 122, pp. 196-8.
- Langridge, DF & McGhee, RB 1967, '*Crithidia mellificae* n. sp. an acidophilic Trypanosomatid of the honey bee *Apis mellifera*', *The Journal of Protozoology*, vol. 14, no. 3, pp. 485-7.
- Louisiana Department of Agriculture and Forestry 2009, *Distribution of Africanized honeybees in Louisiana*, LSU AgCenter, available at http://www.lsuagcenter.com/en/environment/insects/bees_wasps/Distribution+Of+Africanized+Honeybees+In+Louisiana.htm.
- OIE 2014, *Terrestrial Animal Health Code 2014*, World Organisation for Animal Health (OIE), available at <http://www.oie.int/international-standard-setting/terrestrial-code/>.
- Otis, GW 1991, 'Population biology of the Africanized honey bee', in *The "African" honey bee*, Spivak, M, Fletcher, DJC & Breed, MD (eds), Westview Press, Oxford.
- Paxton, RJ, Klee, J, Korpela, S & Fries, I 2007, '*Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*', *Apidologie*, vol. 38, no. 6, pp. 58-565.

- Peng, Y, Baer-Imhoof, B, Harvey Millar, A & Baer, B 2015, 'Consequences of *Nosema apis* infection for male honey bees and their fertility' *Scientific Reports*, vol. 5, pp. 10565, available at <http://dx.doi.org/10.1038/srep10565>.
- Pinto, MA, Johnston, JS, Rubink, WL, Coulson, RN, Patton, JC & Sheppard, WS 2003, 'Identification of Africanized honey bee (Hymenoptera: Apidae) mitochondrial DNA: validation of a rapid polymerase chain reaction-based assay', *Annals of the Entomological Society of America*, vol. 96, no. 5, pp. 679-84.
- Pinto, MA, Rubink, WL, Patton, JC, Coulson, RN & Johnston, JS 2005, 'Africanization in the United States: replacement of feral European honeybees (*Apis mellifera* L.) by an African hybrid swarm', *Genetics*, vol. 170, no. 4, pp. 1653-65.
- Platt, J 2012, 'Africanized bees found in Tennessee for first time', *Mother Nature Network*, available at <http://www.mnn.com/earth-matters/animals/stories/africanized-bees-found-in-tennessee-for-first-time>.
- Ratnieks, F & Visscher, PK 1996, 'Living with the Africanized bee: Sinaloa beekeepers adapt pollination to Africanized bees', *California Agriculture*, vol. 5, no. 4, pp. 24-8.
- Rinderer, TE 1988, 'Evolutionary aspects of the Africanization of honey-bee populations in the Americas', in *Africanized honey bees and bee mites*, Needham, GR, Page, RE, Jr., Delfinado-Baker, M & Bowman, CE (eds), Ellis Horwood, Chichester.
- Rinderer, TE, Hellmich, RL, Danka, RG & Collins, AM 1985, 'Male reproductive parasitism: a factor in the Africanization of European honey-bee populations', *Science*, vol. 228, no. 4703, pp. 1119-21.
- Rinderer, TE, Sylvester, HA, Brown, MA, Villa, JD, Pesante, D, Collins, AM, Spencer, R, Kleinpeter, S & Lancaster, V 1986, 'Field and simplified techniques for identifying Africanized and European honey bees', *Apidologie*, vol. 17, no. 1, pp. 33-48.
- Rivera-Marchand, B, Oskay, D & Giray, T 2012, 'Gentle Africanized bees on an oceanic island', *Evolutionary Applications*, vol. 5, no. 7, pp. 746-56.
- Roberts, J, Anderson, D & Durr, P 2015, *Upgrading knowledge on pathogens (particularly viruses) of Australian honey bees*, publication no. 15/095, Rural Industries Research and Development Corporation, Canberra, available at <https://rirdc.infoservices.com.au/items/15-095>.
- Roberts, KE, Evison, SEF, Baer, B & Hughes, WOH 2015, 'The cost of promiscuity: sexual transmission of *Nosema* microsporidian parasites in polyandrous honey bees' *Scientific Reports*, vol. 5, pp. 10982, available at <http://dx.doi.org/10.1038/srep10982>.
- Ryan, T 2010, *Estimating the potential public costs of the Asian honey bee incursion*, 10/026, RIRDC project no PRJ-005542, Rural Industries Research and Development Corporation, Canberra.
- Schneider, SS & DeGrandi-Hoffman, G 2003, 'The influence of paternity on virgin queen success in hybrid colonies of European and African honeybees', *Animal Behaviour*, vol. 65, no. 5, pp. 883-92.
- Schneider, SS, DeGrandi-Hoffman, G & Smith, DR 2004, 'The African honey bee: factors contributing to a successful biological invasion', *Annual Review of Entomology*, vol. 49, no. 1, pp. 351-76.
- Schumacher, MJ & Egen, NB 1995, 'Significance of Africanized bees for public health: a review', *Archives of Internal Medicine*, vol. 155, no. 19, pp. 2038-43.
- Sheppard, WS, Rinderer, TE, Mazzoli, JA, Stelzer, JA & Shimanuki, H 1991, 'Gene flow between African- and European-derived honey bee populations in Argentina', *Nature*, vol. 349, no. 6312, pp. 782-4.

- Spivak, M 1991, 'The Africanization process in Costa Rica', in *The "African" honey bee*, Spivak, M, Fletcher, DJC & Breed, MD (eds), Westview Press, Boulder.
- Taylor, OR, Jr. 1977, 'The past and possible future spread of Africanized honeybees in the Americas', *Bee World*, vol. 58, no. 1, pp. 19-30.
- Teixeira, EW, Santos, LG, Sattler, A, Message, D, Alves, MLTMF, Martins, MF, Grassi-Sella, ML & Franco, TM 2013, '*Nosema ceranae* has been present in Brazil for more than three decades infecting Africanized honey bees', *Journal of Invertebrate Pathology*, vol. 114, no. 3, pp. 250-4.
- Utah Department of Agriculture and Food 2010, *African bee colonies expand in southern Utah*, available at <http://ag.utah.gov/news/africanmainpage.html>.
- Whitfield, CW, Behura, SK, Berlocher, SH, Clark, AG, Johnston, JS, Sheppard, WS, Smith, DR, Suarez, AV, Weaver, D & Tsutsui, ND 2006, 'Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*', *Science*, vol. 314, no. 5799, pp. 642-5.
- Winston, ML 1992, 'The biology and management of Africanized honey bees', *Annual Review of Entomology*, vol. 37, pp. 173-93.
- Yue, C, Schröder, M, Bienefeld, K & Genersch, E 2006, 'Detection of viral sequences in semen of honeybees (*Apis mellifera*): evidence for vertical transmission of viruses through drones', *Journal of Invertebrate Pathology*, vol. 92, no. 2, pp. 105-8.
- Zheng, H-Q, Gong, H-R, Huang, S-K, Sohr, A, Hu, F-L & Chen, YP 2015, 'Evidence of the synergistic interaction of honey bee pathogens *Nosema ceranae* and deformed wing virus', *Veterinary Microbiology*, vol. 177, no. 1-2, pp. 1-6.