Final Report

Opportunities for insecticide-resistant Honey bees for pollination security

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Contents

Public summary

The European Honey bees (*Apis mellifera*) provide significant pollination services for many economically important crops in Australia. The economic value of crops solely reliant on Honey bee pollination is estimated at $5 billion. Maintaining healthy bee populations is therefore important to the economic health of many Australian communities and to ensure food security. Threats including climate change, Varroa mites (*Varroa destructor*), and pesticide exposure, pose major challenges to Honey bee populations and new effective solutions are necessary to overcome them. The main objective of this project was to investigate the potential of using emerging genetic engineering technologies to introduce beneficial traits such as insecticide resistance into Honey bees. The project evaluated the background knowledge, technical feasibility, industry, stakeholder and public perceptions, environmental impacts, and regulatory framework for genetically engineering Honey bees for insecticide resistance.

Pesticides are one of the key factors that contribute to global Honey bee losses. Honey bee pesticide exposure patterns have significantly changed in Australia since last year with the start of using miticides to control Varroa mites. Many pesticides cause lethal or sublethal impacts on Honey bees leading to poor colony health and performance. They could also interact with each other causing synergetic negative impacts on Honey bee adults and the brood. Our study identified advanced genetic engineering technologies such as CRISPR/Cas9 could precisely engineer Honey bees for pesticide resistance with available knowledge, resources and aligning with Australia's gene technology regulations. These advanced gene-editing technologies are highly accurate with minimum off-target effects. Potential pesticide-resistant Honey bees would allow farmers to manage pests and beekeepers to control Varroa mites effectively without negatively impacting Honey bees. Initial communications with industry stakeholders found no direct opposition to gene editing technologies for Honey bees, but the overall value of targeting pesticide resistance was questioned. They prioritised other beneficial traits as potentially valuable (e.g. pest resistance, increased Honey production, improved temperament). The public is willing to consider the development of gene editing technology for use in Honey bees, although risk identification and management were critical considerations. Fifty-four per cent of those surveyed expressed strong support for the technology's development and/or use in Honey bees for resistance to a range of threats (e.g. pests/disease, extreme temperatures, chemical sprays) and increased Honey production. Supportive participants were mainly focused on the potential benefits, such as helping Honey bees to survive and ensuring food security for society. Less supportive participants focused on the consequences of the technology being unknown, uncertain, and potentially negative, such that it could be risky to introduce. The review on regulations around the fair and ethical use of genetic resources for Honey bee research (i.e. 'access and benefit sharing'/ ABS) highlighted the importance of determining the provenance of genetic materials that researchers use and seeking the most current information across relevant jurisdictions that the genetic material is sourced from. Overall, the findings of the study revealed a promising future for Honey bee genetic engineering research to rapidly generate industry-relevant genetic improvement in Australia, thereby securing Honey bees for pollinating horticultural industries.

Keywords

Apis mellifera, CRISPR, transgenesis, genetically modified organisms

Introduction

Various bee species provide essential pollination services for many economically significant Australian crops. The European Honey bee *Apis mellifera* is the most effective pollinator for major crops in Australia including almonds, macadamia, melons, apples, pears, cherries, and avocados and provides over $6 billion/year in economic benefits. Honey bees in Australia face numerous threats including pests, diseases, extreme weather events, pesticide exposure, and starvation and beekeepers estimated around 20% of hive losses in the 2018-19 period (Honey bee health survey 2019). The most recent devastating experience in the Australian Honey bee industry is the varroa mite (*Varroa destructor*) infestation in mid-2022. Initial Varroa elimination strategies euthanised many managed and feral Honey bee hives in Australia. With the movement to Varroa managementl, Australian beekeepers are now permitted to use in-hive miticides to treat Varroa mites. Therefore, Honey bees could potentially be exposed to pesticides within hives other than the agricultural pesticides that they are exposed to. Pesticide residue could accumulate in Honey bee hives causing synergetic interactions that could impact negatively on all stages/castes of the Honey bees including the brood. The essential requirement to manage Varroa mites effectively and timely while minimizing the negative impacts of Honey bees laid the foundation of this project. Also the project was aimed to understand the potential of engineering Honey bees to achieve insecticide resistance for major horticultural insecticides that they exposed to and thereby to minimize the negative impacts on them. Insecticide-resistant bees could enable farmers to manage pest populations promptly while maintaining pollination and crop productivity and pesticide drift from adjacent farms would be less harmful. Hives infected with pesticide-sensitive varroa mites could be treated without negatively affecting the bees. On a broader scale, we were interested in understanding the potential of engineering Honey bees to improve their health to overcome the challenges they face within their natural habitats/to incorporate beneficial traits thereby securing their services.

Since there are genetic components to a bee's resilience to these challenges, advanced genetic engineering tools provide promising opportunities to precisely edit their genome to achieve beneficial traits. Although traditional breeding programs can be useful for selecting for and combining desirable traits, these programs are dependent on existing genetic variation and introgressing alleles can take many generations. Recent advanced biotechnological tools (e.g., CRISPR-based tools) have enabled an unprecedented ability for precise genome editing in many organisms, including bees and can be utilized to rapidly engineer strains to contain elite genetic variants, study existing variations to inform breeding programs, and rationally develop novel traits. The first successful Honey bee genetic engineering was recorded in 2014 (Schulte et al., 2014) using piggyBac transposase. Even though Honey bee genetic engineering is regarded as a complex process due to the complexity of the colonies, difficulties in laboratory rearing, and technological and regulatory challenges, several successful attempts have utilized advanced genetic engineering tools such as CRISPR/Cas9 in engineering Honey bees (Cheng et al., 2023, Chen et al., 2021, Wagner et al., 2022, Hu et al., 2019, Roth et al., 2019, Liu et al., 2019, Nie et al., 2021, Değirmenci et al., 2020). This highlights the potential for using advanced genetic engineering tools to achieve beneficial traits in Honey bees and thereby secure their services for as pollination.

Here, we investigated the potential for engineering Honey bees for insecticide resistance using novel biotechnological tools. Pesticide (i.e. mainly insecticides and miticides) resistance is a commercially important trait of the Honey bee industry that could be engineered for Honey bees relatively easily. The genetic basis of pesticide resistance has been well-studied in numerous insect pest species (Tian et al., 2019, Edwards et al., 2018, Chen et al., 2020, Devonshire, 1998) and to some extent in Honey bees (Kim et al., 2022, Haas et al., 2021, Haas et al., 2022, Haas and Nauen, 2021, Li et al., 2023, Wang et al., 2022). This information could be used to rationally engineer Honey bee genes which should confer pesticide resistance. However, the genetic engineering of bees is technically complex, subject to a variety of regulations that depend on the methods used and is socially controversial. Understanding technical requirements, regulatory principles, and stakeholder perspectives in this techno-social landscape is essential to determine which approaches may be acceptable and have a path to market. Therefore, the present study evaluated background knowledge, technical feasibility, potential environmental impacts, regulatory framework, and social and industry perspectives for genetically engineering Honey bees with a primary focus on pesticide resistance traits. This project is directly aligned with the purpose of the Hort frontiers pollination fund which is to enhance horticulture crop production and resilience through improved pollination.

Methodology

1) *A global review of insecticide sensitivity, resistance, and resistance mechanisms in bees and potential genetic engineering technologies that could be utilized to engineer Honey bees.*

A global review was conducted on bee pesticide sensitivity, resistance and resistant mechanisms (e.g. detoxification pathways and/or target site mutations) and the genetic engineering technologies that have been utilized to engineer insects including bees. We used PubMed, Web of Science, and Google Scholar databases for the literature survey and were mainly interested in the literature produced over the last five years. As there is much literature on pesticide-resistant mechanisms in pest insects, we have also reviewed that literature to infer likely mechanisms of bee pesticide resistance and to identify gaps in bee research. However, due to extensive literature and the limited time availability of the project, priority was given to the review articles as well as recent (within the last two years) research articles. The review was aimed to focus on the European Honey bee *Apis mellifera,* as well as Australian stingless bee species *Tetragonula carbonaria, Tetragonula hockingsi,* and *Austraplebia australis*. As the literature for native stingless bees was very limited, literature on non-Australian bee species was also considered. We comprehensively reviewed the gene-editing technologies used in insects including bees. This includes a comparison of the various genetic engineering tools including transgenesis based on transposases and site-directed nucleases. As CRISPR/Cas9 technology has many advantages over other site-directed nucleases and transposes-based tools and enables precise genome edits in organisms, major attention was given to CRISPR-based systems. The review includes a survey of what tools have been successfully used for editing insects and their methods of delivery (e.g. microinjection of DNA, mRNA, or ribonucleoprotein and embryo vs ovary microinjection approaches). The genetic change required to achieve pesticide resistance was evaluated to determine which gene-editing technology is most suited to introduce the required change. There are only a few laboratories around the world (and no laboratory in Australia to our knowledge) that have edited the Honey bee genome successfully. Therefore, we visited Professors Takeo Kubo at the University of Tokyo, and Tetsuhiko Sasaki at Tamagawa University, Japan who have established Honey bee genetic engineering programs to observe Honey bee transgenesis and determine how Australian PC2 insectary facilities can be used to establish domestic bee genetic engineering program.

2) *Pesticide recommendations*

This activity was aimed to prioritise pesticides/pesticide groups on which any potential future genetic engineering program of Honey bees for pesticide resistance should focus. We reviewed the literature on pesticide and Honey bee interactions, pesticide usage in Australia, potential genetic engineering technologies that could be used to engineer Honey bees, and off-target ecological impacts of genetically engineered organisms to understand the background. Stakeholder (beekeeper and grower) interviews were conducted to learn the pesticide usage in Honey bees and related crop systems, the effects of pesticides on Honey bees, strategies used to mitigate any negative impacts of pesticides and current pain points within the Australian Honey bee industry. We were also interested in seeking information on the stakeholder perception of engineering Honey bees for insecticide resistance and its potential effect on their operations. We developed a project flyer and distributed it among major Honey bee associations and grower groups to attract potential participants for the interviews. We have conducted semi-structured interviews (N = 15) with beekeepers (commercial beekeepers and hobby beekeepers), and growers that rely on Honey bees for pollination services (e.g. almonds and apples) through field visits, and Zoom/phone calls. Additionally, we had discussions (informal) with beekeepers during beekeeper conferences (NSW Apiarists' Association Conference 2024, Wagga Wagga, NSW and 17th Asian Apiculturists' Conference, Perth, WA). In this scoping project, we were interested in conducting comprehensive discussions with a smaller sample size to gather initial insights on pesticide usage and its effect on pollination in our system to make the foundation for any broader future studies. The ethical aspects of this study have been approved by the Macquarie University Human Research Ethics Committee (Approval Number 16055). Further, we communicated with regulatory agencies (OGTR, APVMA, FSANZ, and EPA) to understand the regulatory framework for achieving insecticide resistance in Honey bees in Australia. We also evaluated the potential off-target ecological impacts of gene-edited Honey bees and how these potential changes may affect integrated pest management (IPM) approaches. In the pesticide recommendation report, we have documented the risk management strategies for any potential off-target ecological impacts and provided recommendations on ways to integrate genetically modified Honey bees into existing IPM strategies.

3) *Assessment of industry and public perception of gene editing technologies and potential impediments to research uptake.*

Macquarie University has subcontracted CSIRO for the following activities.

Brief literature review: A brief literature review was conducted on stakeholder perceptions of risks affecting Honey bees, current behaviours used to protect Honey bees, and propensity or readiness to adopt new Honey bee farming practices and innovations. The literature review addressed the status of the Honey bee industry in Australia, partnerships between horticulture and apiculture industries, biosecurity policies and practices, pollination security threats, and genetic innovations in Honey bees.

Preliminary interviews with industry stakeholders: This activity was conducted to gather initial insights from key informant interviews to inform Hort Innovation's future engagement planning on barriers and opportunities for novel research uptake. Following initial scoping conversations with the project reference group (PRG), and the project team, a targeted cohort of individual semi-structured interviews was planned to be held with Australian-based managed pollination service users (growers) and providers (beekeepers). The horticultural sectors selected for the interview included those who relied heavily on pollination services (e.g., almonds) as well as those who engaged in pollination services to improve their yield quality and quantity (e.g., berry growers) and were member industries of Hort Innovation. A total of 14 semi-structured in-depth Interviews were completed between December 2023 and April 2024. The interview sample comprised of 7 growers (users of managed pollination services) and 7 beekeepers (providers of managed pollination services). The CSIRO Social and Interdisciplinary Sciences Human Research Ethics Committee reviewed and approved the research design (CSSHREC Ethics Clearance 184/23). Interviews were mostly between 30-40mins in duration, although a few lasted around one hour. Participants were invited to join the interview using an online video conferencing platform (Microsoft Teams) or via mobile telephone call (initiated by the investigator, using Microsoft Teams). All interviews were automatically transcribed using voice-generated software in Teams and subsequently cleaned for accuracy. All participants consented to be interviewed and for the interviews to be recorded and transcribed.

Preliminary public survey: An online national survey including several metrics was developed to assess a broad range of psychological constructs including but not limited to knowledge, attitudes, emotions, beliefs, and behavioural responses towards a proposed gene engineering solution for increasing resilience in Honey bees. Questions concerning research, development, and implementation also were explored, such as trust in scientists and approval processes, acceptability of different gene editing methods, and confidence in risk management. A set of basic demographic questions were asked after the survey. The average survey completion time was 12 minutes. Participants were provided information about genetic engineering technology in the form of a '*technology storyboard*' which was a PowerPoint-style presentation that provided both visual and textual information. Data collection for the survey was conducted between 8th – 28th March 2024. Participants were recruited via The Online Research Unit (ORU), which is an ISO-accredited company that provides access to the largest research-only consumer panel in Australia. A standard introductory email from The ORU was used to invite participants to the online survey. Upon clicking a link in the email invitation, the preliminary survey entry process commenced, and participants were presented with standard *Participant Information* about the project and its funders, as well as *Participant Consent* material. After reading through and consenting to participation, the online survey was launched. This study received Human Research Ethics approval (*Ethics Clearance 184/23*) from CSIRO's Social and Interdisciplinary Science Human Research Ethics Committee prior to the commencement of data collection.

Scoping access and benefit-sharing considerations: An initial desktop study was conducted to understand the access and benefit-sharing considerations applicable to bee genetic research in Australia. Literature relevant to access and benefit-sharing regulation in Australia and overseas was consulted, notably the recent CSIRO guide: *Access and Benefit-Sharing for Australian Synthetic Biologists: A Tool for Risk Management* (2023). The desktop study used a scenario approach, developing three likely scenarios Australian Honey bee researchers could face when undertaking genetic research on bees in Australia: 1) Sourcing genetic material deriving from European Honey bee populations located in Australia, 2) Sourcing genetic material and/or Traditional Knowledge deriving from or related to native bees in Australia, and 3) Sourcing bee genetic material from overseas. Worked examples or use-cases were developed for the latter two scenarios based on the template provided in the CSIRO risk-based decision-making tool.

4) Develop a communication plan on how to appropriately take the industry and public on the journey and mitigate risks for negative perceptions.

A comprehensive Communications and Stakeholder engagement plan was developed at the commencement of the project and was submitted with milestone 102. As part of this process, a stakeholder mapping exercise was undertaken to ensure that the breadth and diversity of stakeholders, inclusive of Industry Representative Bodies, First Nation Representatives, beekeepers, pollination service users, and government agencies, were identified and included, and appropriate strategies developed to ensure that communication outputs from the project are relevant, effective and aligned to partner expectations. The communication plan focused on addressing societal concerns around the advanced CRISPR-based tools by working together to create and share strategies for productive dialogue and opportunities for collaborative engagement with other organisations. It also identified pathways to amplify communication outputs and deliver tangible outcomes to the stakeholders.

Results and discussion

1) A global review on insecticide sensitivity, resistance, and resistance mechanisms in bees and gene-editing technologies that could be used for Honey bee genome engineering.

Bees are exposed to pesticides in many ways such as direct exposure while feeding on flowers that are subject to pesticide treatments, contaminated pollen (Burgarelli et al., 2023), contaminated nectar and water, spraying of non-target flowering plants that bees feed on, and pesticide drifts on bees, flowering plants, and bee hives (Zhang et al., 2023). Pesticide toxicity is considered one of the main contributing factors for massive Honey bee colony collapse (colony collapse disorder) recorded in some parts of the world (vanEngelsdorp et al., 2009). Fortunately, there are no records of Honey bee colony collapse in Australia (APVMA, 2015). However, apart from direct observable lethal effects, pesticides could cause many adverse sublethal impacts in bees including behavioural, cognitive, and physiological changes. The most impacted features are the motor functions, learning and memory, and biochemical aspects (Tosi et al., 2022). Neonicotinoids are a major group of horticultural insecticides famous for their negative impacts on bees. Some neonicotinoids such as imidacloprid, thiamethoxam, and clothianidin are known as highly toxic pesticides to bees causing both lethal and sub-lethal impacts (Di Noi et al., 2021, Tison et al., 2019, Tison et al., 2020, Aguiar et al., 2023, Miotelo et al., 2022). Pyrethroids and organophosphates are heavily used in agricultural pest management and also as in-hive miticides to control Varroa mites. Some pyrethroids and organophosphates are highly toxic to Honey bees and result in lethal and sublethal impacts (Motta et al., 2023, Tosi et al., 2022, Dirilgen et al., 2023, Sabová et al., 2022). Even though some of these chemicals are considered safe (especially Varroa miticides), there is evidence that these can cause long-term sublethal impacts on Honey bees (Reeves et al., 2018, Haarmann et al., 2002, Leska et al., 2021). Further, pesticides may accumulate in bee hives making mixtures of pesticides that could cause synergetic impacts on Honey bees threatening their health, performance, and survival (Johnson et al., 2009).

European Honey bees (*Apis mellifera*) are the main managed bees in Australia that are used for Honey and pollination services. Major crops in Australia such as almonds, apples, pears, and berries depend on Honey bees for pollination. Australia is also rich in native bee diversity with nearly 1650 described species. Most of them are solitary or semi-social bees and 11 described species of eusocial stingless bees belong to genera *Tetragonula* or *Austroplebeia*. They are important pollinators of macadamias, mangoes, blueberries, and lychees and contribute to the pollination of many other crops including strawberries, avocados, and tomatoes (First Australian Native Bee Conference 2018, Hogendoorn et al 2006). The Australian horticulture system utilizes some pesticides that fall under the greatest concern for their effects on bees, those are imidacloprid, thiamethoxam, abamectin, deltamethrin, fipronil, and lambda-cyhalothrin. Even though pesticides cause significant negative impacts on bees, the sublethal effects of many pesticides are still unknown, and current pesticide risk assessment only accounts for the survival of adult Honey bees after exposure, but not the sublethal effects and the impact on other life cycle stages. The other knowledge gap in pesticide-bee interaction is a poor understanding of pesticide impacts on non-*Apis* bees as many studies were based on *Apis* species (mainly on the Honey bee *Apis mellifera* and key non-*Apis* species bumble bees) (Tosi et al., 2022). Recent studies on pesticide vulnerability in bees reported that non-*Apis* bees are more vulnerable to pesticide exposure compared to Honey bees (Schmolke et al., 2021, Tadei et al., 2023, Sampson et al., 2023). This highlights the importance of future research on bee–pesticide interactions to devise effective species-specific conservation tools.

Insects achieve pesticide resistance via two major mechanisms: target site mutations and metabolic resistance/detoxifications. In metabolic resistance, detoxification enzymes break down chemicals into less harmful products and excrete them from the body before reaching their target sites. Cytochrome P450s (CYP) are a large group of detoxification enzymes that mediate resistance to all classes of insecticides (Scott et al., 1998) and overexpression of CYP genes belonging to families CYP4, CYP6, CYP9, and CYP12 is known to be associated with insecticide resistance in insects including bees (Tchouakui et al., 2020, Zhang et al., 2022, Wei et al., 2023, Mao et al., 2011, Djuicy et al., 2020, Wang et al., 2018). However, the Honey bee *(Apis mellifera)* genome contains significantly fewer detoxification genes compared to other insect genomes so far sequenced. Even though target site pesticide resistance is not recorded in Honey bees, it is a widely distributed mechanism by which pest insects achieve pesticide resistance (Kim et al., 2015, Wang et al., 2020, Crossthwaite et al., 2014, Zhao et al., 2023, Zeidabadinezhad et al., 2019, Rameshgar et al., 2019, Perry et al., 2008). Many insects achieve target site resistance via single base pair changes in the DNA sequence encoding the target site which are known as point mutations. When such point mutations happen, it results in a change in the amino acid sequence of the protein they encode that slightly alters its shape and reduces the ability of the pesticide to bind with it and disrupt its normal biological function. Extensive literature on insect pesticide-resistant mechanisms provides opportunities to devise mechanisms to achieve pesticide resistance in Honey bees using genetic engineering technologies.

Advanced genetic engineering techniques such as CRISPR/Cas9 provide tools for precise genome editing in many organisms including Honey bees and could be used to achieve insecticide resistance in them (Wu et al., 2018, Nie et al., 2021, Li et al., 2021, Wu et al., 2020). These techniques are highly accurate with minimal off-target effects and are relatively easy to use (Uddin et al., 2020). Novel biotechnological tools can be effectively harnessed to study the existing genetic variation of Honey bees to inform breeding programs (e.g. through marker-assisted breeding), rapidly introgress beneficial traits that are already present within Honey bees as opposed to traditional breeding programs that take several generations, and to introduce novel genetic variation. Genome editing in Honey bees is a complex and difficult approach due to their unique social structure, difficulty in rearing at laboratory conditions, technical challenges, legal barriers and socio-ethical concerns. Honey bee genetic engineering was first recorded in 2014 (Schulte et al., 2014) followed by later work by Otte et al.(Otte et al., 2018). Schulte et al.(Schulte et al., 2014) used *piggyBac-derived* cassettes to manipulate gene functions in Honey bees with high success (20%-27%) transgenic marker expression rates. To date, few studies have been successful in utilising CRISPR/Cas9 tool to engineer Honey bees (Kohno et al., 2016, Değirmenci et al., 2020, Hu et al., 2019, Cheng et al., 2023, Roth et al., 2019, Nie et al., 2021). For example, the CRISPR/Cas9 tool was successfully utilized to generate knockout mutations in Honey bee major royal jelly protein 1 (Kohno et al., 2016, Hu et al., 2019). These research highlights the potential of using novel advanced biotechnological tools to achieve pesticide resistance in Honey bees.

Please refer to the review report on Honey bee insecticide sensitivity, resistance, and resistant mechanisms and potential genetic engineering technologies that could be utilized in Honey bee genome editing for detailed information (Appendix 1). Further, please refer to the report from the international Honey bee transgenesis facility visit on the process and infrastructure requirements for European Honey bee genetic modification for more information on technical requirements for Honey bee transgenesis (Appendix 2).

2) Pesticide recommendations

The pesticide recommendation activity of the project identified priority pesticides on which any future Honey bee genetic engineering program for insecticide resistance should focus, potential technological tools, and the regulatory environment for Honey bee genome editing in Australia. Honey bees in Australia could be exposed to various pesticides when they are foraging or during foraging trips and within hives making possibilities for pesticide poisoning events. Recently, there was a significant change in pesticide exposure of Australian Honey bees due to the permission of in-hive miticide usage to control Varroa mites. This could lead to changes in pesticide exposure patterns and poisoning of Honey bees, however, the impact will be obvious only after a few years of using miticides. Therefore, it is too early to predict the impacts of Varroa miticides on Honey bee performances in Australia. However, we can get insights from other countries where Varroa has been impacting the Honey bee industry for a long time to make predictions on possibilities. Miticides are the main control strategy against Varroa mites and resistant development of Varroa mites against miticides is one of the key issues facing the Honey bee industry around the world (McGruddy et al., 2023, Bahreini et al., 2020). For example, recent pesticide bioassays determined a 12-fold higher concentration of flumethrin is required to obtain 50% of mite mortality (LC_{50}) compared to that used in 2003 in New Zealand (McGruddy et al., 2023). Even though pyrethroid miticides are known to be less toxic to Honey bees, increased concentrations could impose both lethal and sublethal impacts on Honey bees causing reduced colony performances and eventual death (Li et al., 2022, Johnson et al., 2009). Also, acaricide residue is known to accumulate in bee pollen and wax causing adverse impacts on Honey bee larvae (Flumethrin - Liu et al., 2022, tau-fluvalinate and coumaphos - Zhu et al., 2014). However, if *Varroa* is untreated the Honey bee colonies are expected to collapse (Paynter, 2022).

The Australian horticultural industry that relies on Honey bees for pollination uses pesticides that are known to have negative impacts on Honey bees. We have reviewed pesticide usage in six main crops (almonds, apples, avocados, cherries, melons, macadamias) that rely on Honey bee *Apis mellifera* for pollination, to understand the potential exposure of Honey bees to these pesticides. We found that the Australian horticulture system utilizes some pesticides that fall under the greatest concern for their effects on Honey bees: imidacloprid, thiamethoxam, abamectin, deltamethrin, fipronil, and lambda-cyhalothrin (Tosi et al., 2022). Discussions with beekeepers provided important information on Honey bee-pesticide interactions in Australia where some of them have observed deaths of workers and changes in Honey bee behaviour after providing pollination services in some crops such as almonds. Fortunately, there were no claims of any large-scale losses of Honey bees in Australia due to pesticide poisoning. However, despite the healthy practices conducted by many beekeepers and growers, availability of information, codes of practice, and guidelines, pesticides that are toxic to Honey bees are still used in the Australian agricultural industries in a manner that threatens the Honey bee health and survival due to some of the issues raised by the beekeepers and growers such as limited research on Honey bee -pesticide interactions in Australia, thereby the lack of information on the toxicity of some pesticides, poor communication between pollination service providers and users, extra labour and cost associated with healthy Honey bee-friendly farming practices, lack of transparency on the different pesticides used in the pollination systems, and lack of knowledge on the combined toxicity of pesticides to Honey bees. Further, pollinator conservation sometimes conflicts with effective pest management. For example, there are issues in the apple industry in the management of thrips that attack the blossom when the Honey bees are active and management of apple dimpling bugs, and early fruit caterpillars such as Helicoverpa and loopers. Pesticide usage patterns and exposure of Honey bees within the agricultural system emphasize the potential for Honey bee pesticide poisonings in Australia and the need for effective and efficient conservation strategies.

Achieving insecticide resistance in Honey bees is a complex process not only due to its complex colony structure and caste system but also due to technical requirements and related regulations. However, novel biotechnological tools such as CRISPR have been used within Honey bee systems to precisely edit genes (Chen et al., 2021, Wagner et al., 2022, Hu et al., 2019, Nie et al., 2021, Değirmenci et al., 2020) highlighting the potential for future insecticide-resistant Honey bee programs. CRISPR/Cas9 site-directed nucleases have several advantages over other genetic engineering tools that have been discovered and therefore, will be suitable for any potential Honey bee genetic engineering program. Insecticide resistance in Honey bees could be achieved through various mechanisms that change from simple tweaks in the DNA sequence of the Honey bee genome to transgenes. Target site resistance/mutations is a key mechanism by which insects develop resistance against pesticides they encounter and are well described for pest insects. Many of these mutations are a single nucleotide/base pair change within the DNA sequence which are known as point mutations (Amelia-Yap et al., 2019, Jouraku et al., 2019, Rameshgar et al., 2019, Paula et al., 2021). The introduction of target site point mutations will be one of the ideal approaches to utilize in any future insecticide-resistant Honey bee program as the mechanism is very well understood and it can be achieved through simple changes in the Honey bee genome without introducing foreign DNA using CRISPR base editors. Other possible ways to achieve insecticide resistance in Honey bees is via the introduction/upregulation of detoxification genes to enhance metabolic resistance. Even though CRISPR base editing does not involve the introduction of foreign DNA, the organisms modified through this technology are currently treated as GMOs by OGTR. However, in some countries like the USA, CRISPR base editing is not regulated as GMO and the future of how they may be regulated in Australia is uncertain.

Considering multiple factors including the toxicity of the pesticide, pesticide usage in Honey bee systems and/or potential Honey bee pesticide exposure, genetic engineering technology, and regulatory background, the project made recommendations on pesticides on which any future Honey bee genome editing program for insecticide resistance should focus. When considering all relevant factors, pyrethroids would be an ideal candidate to achieve insecticide resistance in

Honey bees. It is widely used in Honey bee pollinating crop systems in Australia to control insect pests including high-priority pests and used as in-hive miticides (flumethrin, tau-fluvalinate) to control Varroa mites. Some pyrethroids are highly toxic to Honey bees (e.g. deltamethrin, cypermethrin, bifenthrin). Also, low-toxic pyrethroids could combine with other pesticides in real-world applications causing synergetic interactions and making adverse impacts on Honey bees (e.g. tau-fluvalinate). Further, target-site point mutations associated with pyrethroid resistance are well reported and understood in related insect species which makes it a potential group to test simple technologies such as CRISPR base editors to achieve insecticide resistance. Therefore, we ranked pyrethroids as the main class of insecticides followed by organophosphates that any future Honey bee insecticide resistance should achieve. It will allow beekeepers to treat Varroa and farmers to treat insect pests timely and effectively without negatively impacting Honey bees.

Please refer to the pesticide recommendation report for more information on Honey bee pesticide interactions, priority pesticides, potential off-target ecological impacts of gene-edited Honey bees and how they could be integrated with existing IPM strategies (Appendix 3).

3) ***Assessment of industry and public perception of gene editing technologies and potential impediments for research uptake.***

Literature review on stakeholder perceptions of risks affecting Honey bees, current behaviours used to protect Honey bees, and propensity or readiness to adopt new Honey bee farming practices and innovations.

The Honey bee industry is estimated to contribute $14.2 billion annually to the Australian economy (Honey bee Industry Council, 2022), contributing across multiple agricultural industries requiring crop and feedstock pollination. Honey and wax production contribute an additional $147 million at the farm gate, with the industry producing approximately 37,000 tonnes of Honey each year (Clarke, 2023; Clarke & Le Feuvre, 2021; Department of Agriculture, 2022; Karasiński, 2012). In terms of food production, approximately 35 industries depend on pollination services for most of their production (bees as well as other insects) such as almonds, apples, avocadoes and blueberries (Keogh et al., 2010). Pollinator-dependent and pollinator-responsive crops currently make up between 25-75% of Australians' diets and include some of Australia's most popular horticultural products including almonds, avocados, melons and apples (Baer & Anderson, 2016; Rader et al., 2016). Achieving pollination security is influenced by several factors which extend beyond the management of commonly identified biosecurity threats to Honey bees (Evans et al., 2023; Winfree, 2008). While the focus for exploration of industry perspectives in this project remains on the relationships between managed pollination service providers (beekeepers) and pollination service users (horticultural commodity growers), there are broader challenges faced by both industries linked to global and external influences which shape industry dynamics (Cavigliasso et al., 2021). These include extreme weather patterns affecting both wild pollinator populations and demand for managed pollination services; changing land use and horticultural practices; and a plummeting Honey prices leading to shifts in Australia's apiculture industry (Bloom et al., 2021; Clarke & Le Feuvre, 2021; Eeraerts et al., 2020; Vercelli et al., 2021; Willcox et al., 2023). The discovery of varroa mites on Australian shores in 2022 is also likely to intensify pressure on the livelihoods of managed pollination providers and other beekeepers to maintain healthy hives. The threat of wild pollinators succumbing to varroa infestation will become a significant future concern for both horticultural and pollination industries. Understanding the pollination security challenges unique to Australia requires applying a broader systems lens to incorporate consideration of the human, climate, and governance drivers of effective pollination services. Of the commonly identified pests and diseases currently threatening Australian apiculture, varroa mite, small hive beetle, and American foulbrood (AFB) remain of significant concern (Department of Agriculture, 2024).

There is currently heavy reliance on some Australian commodity sectors (e.g. almonds and avocados) on the availability of quality professional pollination services (Clarke & Le Feuvre, 2022; Keogh et al., 2010). Without access to organised pollination services, these sectors would be at high risk of production failure. For other horticultural sectors (e.g. berries and lychees), securing reliable pollination services can influence production outcomes including yield quantity and quality at harvest, yet partnerships between beekeepers and growers are less organised and can occur on an *ad hoc* basis (Keogh et al., 2010). Globally, farmers' awareness of the importance of bees to pollination security is high, yet adoption of pollinator-supporting practices is mixed, especially in relation to promoting wild pollinators during horticulture production (Bloom et al., 2021; Eeraerts et al., 2020; Osterman et al., 2021). The emergence of formalised service arrangements in the form of contractual agreements between growers and beekeepers along with the growth of beekeeping brokering services has assisted the professionalisation of the Australian pollination industry. There is only one legislative requirement in Australia for apiarists to inspect and record the status of their hives, and that falls under the Australian Biosecurity Code of Practice. The mandatory Code, developed for bee biosecurity and nationally endorsed by the bee industry, outlines a clear framework for Australian beekeepers to engage in best-practice biosecurity, to ensure future sustainability and viability of the Honey bee industry in this country (Hauxwell, 2024; Plant Health Australia, 2016b). More generally, beekeepers and growers are provided with adequate, easy-to-find resources for understanding relevant pests and diseases for Honey bees (e.g. the BeeAware website's biosecurity page; the Australian Government's Department of Agriculture, Fisheries and Forestry page for Honey bee pests and diseases), as well as pathways for reporting suspicious hives. There are also various websites providing access to The Biosecurity Manual for Beekeepers (Plant Health Australia, 2016a) and biosecurity toolkits for actioning recommended actions such as implementing biosecurity farm signage and apiary production record templates (e.g. Honey bees - Farm Biosecurity). The Australian Department of Agriculture, Fisheries and Forestry (DAFF) resource centre for bee biosecurity also includes a section on agricultural chemical regulation, which highlights the Australian Pesticides and Veterinary Medicines Authority (APVMA) as being aware of concerns in Europe and the USA related to agricultural chemicals for use in Australia that may negatively impact the health of Honey bees.

Research into the Honey bee genome has proliferated over the last decade, as researchers try to understand more about the role of genetics in developing Honey bee resilience. In many cases, researchers have been interested in advanced gene-based selective breeding techniques for protecting Honey bees from threats and ensuring the survival of these key agricultural pollinators. Several studies have been conducted investigating the genetic mechanisms for improving Honey bee health and disease resistance (Faber et al., 2021, Gabel et al., 2023; Hoppe et al., 2020, Yokoi et al., 2018, Türkiye et al., 2023). The 2021 Plan Bee Survey conducted by AgriFutures (Chapman & Frost, 2022) asked a sample of 109 national beekeepers for their attitudes and opinions related to a national genetic improvement program for Honey bees in Australia. Overwhelmingly, these industry stakeholders agreed that a genetic improvement program was important, with 73% of beekeepers agreeing that modern genetic techniques would increase the chances of a successful breeding program and 82% agreeing that there was value in this type of bee improvement program (Chapman & Frost, 2022). Honey production was cited as the most important genetic trait amongst beekeepers, and resistance to small hive beetle and bee temperament was identified as the second and third most important genetic trait identified. Thus, it was concluded that the industry saw value in a national genetic breeding program for Industry growth and the use of modern breeding innovations would increase the chances of success. However, among the traits explored in the Bee Plan Survey, insecticide resistance was not included. Therefore, it is not clear as to whether beekeepers view a gene-based innovation for insecticide resistance in Honey bees as acceptable or necessary; the present study explores these attitudinal perspectives more amongst Australian beekeepers.

In considering genetic innovations for protecting Honey bees, it is important to first understand public perceptions of Honey bees and the public's perceived need to conserve and protect the species, regardless of what those efforts may entail. Prior research suggests that people may be less willing to protect species that they perceive are not likeable, unpopular, raise negative emotions (e.g., fear and disgust) and attitudes, or are associated with myths and superstitious beliefs (for a review, see Schonfelder & Bogner, 2017). A few studies have explored public perceptions of bees (e.g., of bees in general or unspecified, Honey bees, solitary bees, and bumblebees) among school and university students, adults, online participants, and beekeepers (e.g. Cass et al., 2022; Nicholls et al., 2020; Ojija & Leweri, 2022; van Vierssen Trip et al., 2020). Synthesising findings across these studies, it is observed that knowledge, understanding and/or awareness of bees tend to be low in the general (non-beekeeper) population, yet people still hold positive views towards bees and are motivated to help them (Nicholls et al., 2020; van Vierssen Trip et al., 2020; Wilson et al., 2017). Even in the presence of some negative attitudes and feelings (e.g., people view bees as a conditional danger, and some may hold intense fear due to the experience of bee stings), people still hold remarkably positive perceptions towards the species (Hall & Martins, 2020; Schonfelder & Bogner, 2017; Stanisavljević & Stanisavljević, 2017) and are aware that bees are under threat (e.g., from pesticides), express concern about declining bee populations, support bee conservation, and are willing to protect bees (Hall & Martins, 2020; Nicholls et al., 2020; Schonfelder & Bogner, 2017; Stanisavljević & Stanisavljević, 2017). According to the CSIRO 2018 national survey with over 8,000 members of the Australian public showed that support for genetically engineering endangered insect and invertebrate species was heterogeneous – 38% expressed higher levels of support, 22% reported little or no support, while 39% held moderate support (CSIRO Synthetic Biology Future Science Platform, 2021). Despite the slight trend towards supporting the use of synthetic biology technology in insects and other invertebrates, many participants still expressed caution and concern regarding the use of the technology to help endangered species in general.

People expressed concern about the long-term effects on humans, animals, and the natural environment; and the possibility of the technology being inadvertently misused, or purposefully used for bad purposes. Building on this research, the current study examined public perceptions of genetically engineering Honey bees for use in agriculture.

Please refer to the industry and stakeholder perception report (Part I) for more information (Appendix 4).

Preliminary interviews with industry stakeholders:

A range of economic and political factors shape industry perspectives on gene editing technology for Honey bees. These include current enterprise challenges, sector-specific drivers of specific technology use as well as domestic and global transitions affecting both horticulture and apiculture industries more broadly. These contextual considerations influence stakeholders' interest and capacity to imagine the technologies' utility. Relationships among beekeepers and growers across the managed pollination services sector vary significantly. Trust, loyalty and respect between growers and beekeepers are key to strong and productive relationships, which in some cases are valued above formalised contracts in relation to keeping bees safe from pesticide exposure. Among beekeepers interviewed, there was no direct opposition to gene editing technologies for Honey bees but the overall value of developing specific pesticide resistance was questioned. Other desirable traits for gene editing were preferred including pest resistance, increased Honey production and improved temperament. Among growers interviewed, technology rendering pesticide-resistance among Honey bees had merit although the technology was not considered pivotal to securing pollination security at this time. In fact, pesticide exposure was not viewed as a high-priority problem, with growers already engaging in several farming practices to effectively control pesticide usage. Both beekeepers and growers viewed gene editing technologies for pest resistance as having considerable future value as pests such as varroa mites impact on the wider pollination security ecosystem. While pesticide resistance was not an identified priority for either growers or beekeepers, other beneficial traits (e.g., pest resistance, increased Honey production, improved temperament) were suggested as potentially valuable, opening the door to further innovation pathways.

Please refer to the industry and stakeholder perception report (Part II) for more information (Appendix 4).

Preliminary public survey:

Through the online public survey, it was observed that ~55% were more supportive, ~30% were moderately supportive, and the remainder (~15%) were not at all, or only slightly supportive of engineering Honey bees for beneficial traits. Respondents strongly supported using genetic engineering to increase Honey bees' resistance to a range of threats (pests and disease; extremes of temperature; agricultural chemical sprays) and for increased Honey production but were slightly less supportive of changing their temperament to make Honey bees calmer. Supportive participants were mainly focused on the potential benefits – helping bees to survive and ensuring food security for society. In contrast, less supportive participants focused on the consequences of the technology being unknown, uncertain, and potentially negative, such that it could be dangerous and risky to introduce. Overall, the survey findings revealed that the general public is willing to consider the development of gene editing technology for use in Honey bees. However, risk identification and management are critical considerations. All participants regardless of whether they supported or did not support the development and/or use of genetic technology raised concerns regarding the potential for unintended negative impacts.

Please refer to the industry and stakeholder perception report (Part III) for more information (Appendix 4).

Scoping access and benefit-sharing considerations:

A brief overview of access and benefit-sharing considerations for the genetic engineering of Honey bees was also conducted as part of this scoping study. We note that research utilising genetic material obtained from native bees would require meaningful engagement with Indigenous groups to comply with Australia's access and benefit sharing (ABS) obligations.
Currently in Australia, ABS laws (of all jurisdictions) only apply to genetic material derived from native species. Consequently, they would only apply to genetic material derived from native bees and not that derived from European

Honey bees, whether domesticated or wild. Thus, when considering how ABS might apply to bioengineering research on bees in Australia, there appear to be three main types of situations relevant to the accessing of genetic material: 1. Sourcing genetic material deriving from European Honey bee populations located in Australia 2. Sourcing genetic material and/or Traditional Knowledge deriving from or related to native bees in Australia 3. Sourcing bee genetic material from *overseas.* We have discussed these three situations in the report.

Please refer to the industry and stakeholder perception report (Part IV) for more information on success and benefit-sharing considerations under each of the three identified situations (Appendix 4).

4) ***Develop a communication plan on how to appropriately take the industry and public on the journey and mitigate risks for negative perceptions.***

The communication plan identified effective communication strategies with the wider stakeholders of the Honey bee industry in Australia. It identified the Australian horticulture industries, Honey bee industry (commercial beekeepers), regulatory agencies (APVMA, OGTR, EPA, FSANZ), other research institutes/groups working on Honey bees, the Australian government, and the public including First Nations as stakeholders/audience the project. The communication plan provides information on how the project objectives, findings, outputs, and outcomes were communicated to its stakeholders throughout the project lifecycle.

Please refer to the communication plan for more information (Appendix 5).

Outputs

Table 1. Output summary

Output	Description	Detail
Report - review report on pesticide sensitivity, resistance, and resistance mechanisms of bees and biotechnological tools that could be utilized to engineer Honey bees.	We reviewed the literature on pesticide sensitivity, resistance, and resistance mechanisms in bees and the biotechnological tools that could be used to engineer Honey bees to achieve beneficial traits such as insecticide resistance.	We used PubMed, Web of Science and Google Scholar databases for the literature survey and were mainly interested in the literature produced through the last five years. As there is much literature on pesticide-resistant mechanisms in pest insects, we have also reviewed that literature to infer likely mechanisms of bee pesticide resistance and gaps in bee research. Please see ***Appendix 1*** for the complete review report.
Report - a report on technology and technical requirements for Honey bee transgenesis based on the visit to international Honey bee transgenesis facilities in Japan.	We visited Professors Takeo Kubo at the University of Tokyo, and Tetsuhiko Sasaki at Tamagawa University who have established Honey bee genetic engineering programs. We consulted with them and visited their facilities to observe Honey bee transgenesis and determine how Australian PC2 insectary facilities can be used to establish a domestic bee genetic engineering program.	Please refer to the report on the process and technical requirements for Honey bee genetic engineering program compiled through the international Honey bee transgenesis facility visits – ***Appendix 2***
Report - pesticide recommendations	The pesticide recommendation report includes detailed information on Honey bee pesticide interactions in Australia, priority pesticides on which any future Honey bee gene editing program on insecticide resistance should focus, and Australian regulatory requirements for Honey bee genetic engineering compiled through literature reviews, stakeholder interviews/discussions, and regulatory meetings.	We conducted a literature review on pesticide usage patterns in Honey bee pollinating crops in Australia, Honey bee pesticide exposure, current mitigate strategies and pain points in pest management and pollinator conservation and qualitative interviews with stakeholders (beekeepers and growers) to understand the pesticide -Honey bee interactions in Australia. We also participated in industry conferences to understand the requirements of the Australian Honey bee industry and the potential for novel research uptake by the industry. We communicated with the regulatory agencies to understand the regulatory requirements for Honey bee genetic engineering in Australia. Further, we explored the potential off-target ecological impacts of genetically engineered Honey bees and how they could be incorporated into the existing IPM programs. Information collected through literature review, stakeholder and regulatory meetings were used to prioritise the pesticides on which any future Honey bee genetic engineering program for insecticide resistance should focus. Please see ***Appendix 3*** on the pesticide recommendation report for more information.

Report - the Industry and Stakeholder Perception Report on Honey bee genetic engineering in Australia for beneficial traits.	The industry and stakeholder perception report includes a brief review of prior research exploring public perceptions of bees and conservation efforts by focusing explicitly on genetically engineering Honey bees for specific traits that will enable them to better withstand threats they encounter. It also includes industry and public perception of Honey bee genetic engineering in Australia and a brief overview of access and benefit-sharing considerations for the genetic engineering of Honey bees.	A brief literature review on the industry and stakeholder perception of bees, threats to Honey bees, conservation strategies, and readiness for novel technologies such as genetic engineering was conducted. Qualitative interviews with beekeepers and growers that rely on Honey bee pollination (N = 14) and an online public survey (1,220 participants) were conducted to understand the industry and public perception of Honey bee genetic engineering in Australia. Further, access and benefit-sharing considerations for the genetic engineering of Honey bees under different scenarios were also studied. Please refer to ***Appendix 4*** for the detailed report on industry and stakeholder perception.
Stakeholder communication plan	A stakeholder communication plan was developed to ensure the breadth and diversity of stakeholders, inclusive of Industry representative Bodies, First Nation representatives, beekeepers, pollination service users, and government agencies, were identified and included. The engagement plan was focused on addressing societal concerns around the advanced CRISPR-based tools by working together to create and share strategies for productive dialogue and help identify opportunities for collaborative engagement with other organisations.	Please refer to ***Appendix 5***, which details communication strategies used for effective project delivery and collaborations with stakeholders regarding project objectives, findings, outputs, and outcomes.
Conference abstract - Asian Apiculturists' Association Conference 2024 abstract	The project postdoctoral researcher presented the research at the 17th Asian Apiculturists' Conference which was held in Perth, WA from 12th to 14th June 2024.	An oral presentation was given at the 17th Aian Apiculturists' Association conference on the project overview - **Appendix 6.**
Industry news article – AFG Winter 2023	Project leader Dr. Maciej Maselko has shared the research with AFG (Australian Fruit Grower) Winter 2023 which is the leading publication for apple and pear growers.	The article discussed the objectives of the project and its potential benefits to the Honey bee industry – **Appendix 7**.

Outcomes

Table 2. Outcome Summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
A thorough understanding of the opportunity landscape for genetically modified bees, stakeholder perspectives, regulatory frameworks, environmental considerations, and technical requirements which all provide critical knowledge for any future investments.	***Outcome 1: Improved management of European Honey bee for pollination*** *Strategic Investment Priorities - SIP(ies)* *- Future-proof against endemic and exotic pests and diseases.* *- Increase the effectiveness of hive management and services.* *- Develop and test superior bee genetics.* *KPIs - Recommendations based on R&D available for best practice management of European Honey bees.* *- Adoption rates of research and development outputs, by both growers and apiarists.* ***Outcome 2: Crop pollination requirements are understood and integrated into best practice*** *SIP(ies)* *- Develop adaptive and tailored strategies to meet pollination requirements.* *- Integrate effective pollination into horticulture production systems.* *KPIs* *- Strategies for effective pollination developed and their uptake by industry* *- Practical information available to horticulture industries on*	The project laid foundation for any future Honey bee genetic engineering program, through better understanding of the knowledge, technology, regulatory requirements, and stakeholder perceptions for engineering Honey bees for beneficial traits. The project revealed the possibility of initializing Honey bee gene editing program within the Australian context with available resources, technology, experts, and skills with high level of social acceptance.	Review report that includes detailed information on background knowledge on pesticide sensitivity, resistance, and resistant mechanisms of bees and biotechnological tools that could be utilized to engineer Honey bees - **Appendix 1**. A report on process and technical requirements for Honey bee transgenesis based on the visit to international Honey bee transgenesis facilities in Japan - **Appendix 2**. Pesticide recommendation report has identified the priority pesticides for any future genetically engineering program for pesticide resistance, and Australian regulatory requirements for Honey bee genetic engineering - **Appendix 3**. The industry and stakeholder perception report includes detailed information on the Australian industry, stakeholder and public perception on Honey bee genetic engineering to achieve beneficial traits and readiness for novel research uptake - **Appendix 4**.
Sets the foundation for future research in the area to develop agricultural innovations that are informed by the needs of end users and the Australian public.		The project was successfully capable of identifying the beneficial traits that the Australian Honey bee industry expects in any future Honey bee gene-editing	We have conducted stakeholder interviews with beekeepers and growers to understand their perception on Honey bee genetic engineering and the traits that the industry is looking for in a

	considerations for effective pollination within crop management systems. *- Adoption rates of research and development outputs, by both growers and apiarists.* ***Outcome 3: Alternate pollination options developed for increased productivity*** *SIP(ies)* *- Increase capability and industry capacity to utilise alternative pollinators.* *- Develop and enable novel technologies to support pollination.* *- Pollination options developed to meet the diverse needs of horticulture crop production.* *KPIs* *- Alternate pollinator options for horticulture crops identified* *- Partnerships established to develop novel pollination technologies*	program through effective stakeholder communication. Further, the project identified the stakeholders and public perception of Honey bee genetic engineering. This information will aid in designing any future Honey bee genetic engineering program in a manner to addresses key issues in the Australian Honey bee industry with high stakeholder and public acceptance.	potential Honey bee genetic engineering program. We also participated in industry conferences and actively engaged with stakeholders to share the research. Further, the project evaluated the perception of the Australian public on Honey bee genetic engineering – **Appendices 3, 4, 5, 6, 10, 11, 12, and 13**. Honey bee Workshop was conducted to bring the diverse project team and the project reference group members including representatives from main stakeholder groups to discuss the findings of the current project and to plan any future potential studies to address the needs of the industry- **Appendix 13**.
Ensure the design and delivery of biotechnical solutions have a greater chance of public/stakeholder acceptance and uptake		Stakeholder engagements in the current project enhance the awareness of different genetic engineering technologies that could be utilized in any future Honey bee genetic engineering program. It enhanced the knowledge of stakeholders on a wide variety of genetic engineering technologies including simple tweaks in the genome to transgenesis. The project also makes recommendations to utilize some of the simple gene-editing technologies such as CRISPR base editors that do not involve in introduction of foreign DNA which may have higher stakeholder and public acceptance.	The research was effectively communicated to the industry and other stakeholders **(Appendix 5**) through participating in industry conferences (**Appendix 6**), producing industry newsletters (**Appendix 7**), communicating with industry partners and conducting stakeholder interviews (**Appendices 3, 4, 10, 11, 12**), a public survey **(Appendix 4),** project reference group meetings **(Appendices 8, 9),** and a Honey bee workshop **(Appendix 13)** which aid in increasing the chances of stakeholder acceptance for any future Honey bee genetic engineering research.

Enhanced industry security and enhanced competitiveness of Australia's agricultural industries		The project has successfully identified the potential challenges of pesticide usage that could be imposed on the Honey bee industry, especially with the recent movement of using in-hive miticides to control Varroa mites. It also identified current pain points in managing agricultural pest insects and safeguarding Honey bees. The current project identifies enhanced biotechnological approaches such as CRISPR/Cas as a potential mechanism to improve Honey bee health and enhance the production of Australian agriculture.	The literature review and pesticide recommendation report provided information on opportunities in using novel advanced biotechnological tools to improve Honey bee resilience to the challenges they impose within the Australian ecosystem - **Appendices 1 & 3**
Positive environmental outcomes with the efficient use of insecticides		Potential gene-edited Honey bees for insecticide resistance will allow effective and timely usage of pesticides to control Varroa mites and economically important agricultural pest insects that will result in reduced spread of pest insects and potential outbreaks which in turn minimizes the pesticide usage.	The pesticide recommendation report identifies potential environmental outcomes of any future gene-edited Honey bees. It provides details on how potential gene-edited Honey bees could lead to a reduction of overall chemical pesticide usage if growers follow healthy farming practices with effective communication - **Appendix 3**.

Monitoring and evaluation

Table 3. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
To what extent did the project identify the potential, stakeholder perception, regulatory frameworks, environmental impacts, and technical requirements for creating genetically engineered Honey bees?	The project team was capable of successfully achieving the project's key outcomes by identifying the potential pesticide-resistant mechanisms and regulatory genes, potential biotechnological tools and approaches that might be able to utilize to engineer Honey bees using available technology resources in Australia, regulatory and license requirements for Honey bee genome editing, potential environmental impacts of genetically engineered Honey bees, and more importantly, preliminary insights into stakeholder and public perception on Honey bee gene editing. Overall, the project outcomes reveal promising technical, socio-ethical, and regulatory opportunities for Honey bee genetic engineering in Australia for beneficial traits.	Enhanced stakeholder engagements through information sessions/workshops for the industry stakeholders and participating in industry meetings. Preliminary laboratory trials on Honey bee genetic engineering to test the protocols within the Australian context. More contextualised analysis of social acceptability, risk and benefit perceptions influencing and/or driving uptake of future Honey bee engineering, amongst representative samples of stakeholders and the general public is needed.
To what extent the project identified the level of current and future issues/threats of pesticides on Honey bees and pollination and to what extent genetically engineered Honey bees could impact the operations of end-users?	The global literature review has identified the impact of pesticide poisoning on Honey bee and pollination in Australia. It also predicts how the recent shift to Varroa miticide usage in Australia could impact the Honey bees negatively through potential synergetic interactions. Through stakeholder communication, we gathered real information on Honey bee pesticide interactions in Australia. Beekeepers who engage in pollination services acknowledge that pesticide risk is one of the issues they are facing in the industry. There are no large-scale Honey bee hive losses due to pesticide poisoning in Australia, however, pesticides have caused negative impacts on Honey bee behaviours/services and worker mortality. Even though pesticide resistance is not regarded as the current primary concern in the Honey bee industry, some beekeepers expressed their willingness to adopt genetically engineered Honey bees for pesticide resistance in their operations and they predicted it would have a positive outcome in the industry. Further, they were keen on traits such as Varroa resistance, hygiene behaviour, and disease resistance in	Regular and extensive communication with beekeepers is essential to understanding the changes in pesticide - Honey bee interactions within Australia, especially with the recent introduction of Varroa miticides. Future research on how to utilize genetic engineering approaches to introduce disease and/or Varroa resistance, and hygiene behaviour in Honey bees. Future research on bees (Honey bee and native bee) – pesticide interactions and synergetic impacts of pesticides on bees needs to be conducted within the Australian context.

	any future Honey bee genetic engineering program.	
To what extent did the project directly communicate with its stakeholders including industry, beekeepers, growers, and regulatory bodies?	The project team has successfully engaged with the stakeholders using different approaches such as project reference group meetings, qualitative interviews, participation in industry conferences, and meetings with regulatory agency representatives. We conducted two meetings with the project reference group and used email communications with them to receive feedback on the project activities throughout its life cycle. We have completed 15 stakeholder interviews for the pesticide recommendation activity mainly including commercial beekeepers and some hobby beekeepers and growers. We have also participated in industry conferences (NSW Apiarists' Association conference and 17th Asian Apiculturists'' conference) which gave us opportunities to share our research with the industry stakeholders and to receive their perceptions. We also conducted meetings with regulatory agencies to understand the regulatory environment for Honey bee gene editing in Australia. Further, the CSIRO project team has conducted 14 qualitative interviews with the beekeepers and growers that rely on Honey bees for pollination services to understand challenges in the industry and their perception of Honey bee genetic engineering.	Organize information sessions/workshops, participate in country industry meetings and produce industry newsletters to better engage the beneficiaries/stakeholders. In-situ investigation of issues affecting different relevant stakeholders, to create a responsible and end-user-focused approach to technology development (best practice), aiding fit-for-purpose technology development and deployment
To what extent was the project able to get the acceptance and willingness to adopt the proposed biotechnological approaches to create insecticide-resistant Honey bees?	Through the preliminary stakeholder interviews, it was revealed that both beekeepers and growers are more open to the use of advanced biotechnological tools to improve Honey bee health. More than 50% of interview participants (beekeepers and growers) of the pesticide recommendation activity (N = 15), stated that they highly prefer/prefer to use future gene-edited Honey bees for pesticide resistance in their operations. Through the qualitative stakeholder interviews conducted by the CSIRO project team (N = 14), it was evident that among growers interviewed, technology rendering pesticide resistance among Honey bees had merit and they expressed their willingness to adopt the proposed insecticide resistance Honey bees in their systems. However, pesticide	Future studies could investigate the potential for engineering Honey bees for high-priority beneficial traits that the Australian Honey bee industry is looking for such as Varroa resistance, disease resistance or hygiene behaviour. This would involve well-planned and resourced engagement with relevant stakeholders using qualitative methodology for responsible technology development.

	resistance was not considered the immediate requirement of the Honey bee industry to secure pollination security. Pesticide exposure was not viewed as a high-priority problem, with growers already engaging in several farming practices to effectively control pesticide usage. While pesticide resistance was not an identified priority for either growers or beekeepers, other beneficial traits (e.g., pest resistance, increased Honey production, improved temperament) were suggested as potentially valuable, opening the door to further innovation pathways.	
How well is the project output communicated to its stakeholders?	The project team made their best effort to engage with the stakeholders and to communicate project outputs. The project was composed of a seven-member reference group that includes industry representatives. There were experts in the field and representatives from the NSW Department of Primary Industries, the Australian Honey Bee Industry Council, the NSW Apiarists' Association, Amateur Beekeepers Australia, and the almond industry. We shared project documents (interview schedules, and storyboard that were incorporated with the public survey to share project information) via email with the project reference group and received their feedback. After the main project activities, we conducted a project workshop (hybrid module) to share the findings with the project reference group and receive their feedback. We contacted the main industry bodies/associations such as Apiarists' associations in different states, amateur beekeeper associations, grower associations of crops that rely on Honey bees for pollination such as almonds, apples, melons and berries, and project reference group members to spread the information of the project within their membership and to attract potential interview participants. Participating in industry conferences also gave us opportunities to share our research with the industry stakeholders. We also submit timely milestone reports to Hort Innovation with project outputs.	Prepare industry newsletters, organize webinars, and participate in industry meetings to share the project outputs with its stakeholders.
How well does the project use the skills and knowledge of project team members and the stakeholder	The project integrated the knowledge, skills and experiences of its interdisciplinary team members in achieving the project outcomes.	Organize group discussion forums/workshops to share knowledge within the

feedback to achieve its outcomes?	The project team was composed of experts in biotechnology, entomology, ecotoxicology, integrated pest management, environmental law and social scientists. The project members communicated through emails, project meetings, sub-activity meetings, and the project workshop to collaborate effectively and efficiently to deliver project outcomes. We also frequently communicated with industry representatives and the project reference group to get their feedback on project activities.	interdisciplinary project team and the stakeholder industry representatives.

Recommendations

- Record keeping and frequent monitoring of Honey bee hives to understand/early detection of the impacts of Varroa miticides is crucial.
- Future research should address the impacts of pesticide combinations (especially Varroa miticides)/synergetic impacts of pesticides on Honey bees.
- Future research to understand the impacts of multiple stressors on Honey bee health, survival, and performance.
- CRISPR/Cas9 technology (especially point mutations through CRISPR base editors) could be used to introduce precise genome editing in Honey bees to achieve beneficial traits through any future Honey bee genetic engineering programs.
- Any future research on genetically engineering Honey bees for insecticide resistance could focus on the development of resistance against pyrethroids as it was identified as the high-priority pesticide group in the current project.
- Targeted participatory workshops and focus group discussions with industry groups (including horticulture) to consider the potential for gene editing technology as a tool for futureproofing pollination and food security. These workshops and focus group discussions could be followed up with a broad-scale survey to ascertain industry views/perceptions across a larger and more representative sample of the population.
- Building on the public perceptions survey, targeted/place-based focus group discussions could be held with residents of horticultural districts to consider the potential introduction of gene-edited Honey bees in their local area. This could be supplemented with a more experimental and targeted public survey approach to carry out a contextualized assessment of acceptability and risk.
- Maintain effective communication with stakeholders in developing and commercializing any potential genetically engineered Honey bees to avoid unnecessary outcomes such as changes in pesticide usage.
- Always follow precautions and healthy farming practices to prevent/minimize Honey bee pesticide interactions (even if the Honey bees are genetically engineered for pesticide resistance in future).
- Any future potential pesticide-resistant Honey bees would be ideal to utilize for pollination purposes. However, if they are used for Honey, measures should be taken to maintain the standards and ensure Honey follows the minimum pesticide residue limits. Targeted engagement with end users including Honey packers should be considered in this scenario.
- Evaluate each intended genetic modification of Honey bees independently within the laboratory and in field settings before release commercially to investigate any potential trade-offs on important traits.
- Distribute genetically engineered mated queens that are homozygous for the edited trait (i.e. insecticide resistance) to minimize the dilution of the edited trait through matings with unedited drones in the field.
- Establish proper and regulated mechanisms for the commercialization of gene-edited Honey bees.

Refereed scientific publications

None to date. The manuscript (a perspective article) is in preparation.

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Intellectual property

No project IP or commercialization to report

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Appendices

Major project reports and outputs

1. Global review report on Honey bee pesticide sensitivity, resistance, resistance mechanisms and genetic engineering technologies used in insects including Honey bees
2. Report on visit to international Honey bee transgenesis facility
3. Pesticide recommendations
4. Stakeholder perception report – delivered by CSIRO
5. Stakeholder communication plan
6. Conference abstract and slides – 17th Asian Apiarists' Association conference
7. Industry news article – AFG Winter 2023 (Page 54)

Other supplementary documents

8. Email invitation to join the PRG
9. Project reference group members
10. Stakeholder interview invitation – pesticide recommendation activity
11. Stakeholder interview schedule – pesticide recommendations activity
12. Project flyer
13. Honey bee workshop report

Appendix 1: Global review report on Honey bee pesticide sensitivity, resistance, resistance mechanisms and genetic engineering technologies used in insects including Honey bees

Confidential, provided separately

Appendix 2: Report on visit to international Honey bee transgenesis facility

Process and Infrastructure Requirements for European Honey Bee Genetic Modification.

Introduction

The majority of industry-relevant genetic engineering of Honey bees will first require establishing modified strains that must be maintained and studied under laboratory conditions and be strictly confined. The complex life history, reproductive biology, eusocial behaviours, and hive structure makes Honey bees far more challenging to engineer and rear in a lab environment than other commonly studies insect species. It was therefore important to understand if these challenges could realistically be overcome in the Australian context in case a Honey bee genetic engineering research program is deemed worth establishing.

Professors Takeo Kubo at the University of Tokyo and Tetsuhiko Sasaki at Tamagawa University both have Honey bee genetic engineering programs and Dr Maselko visited their labs in November, 2023 to learn about genetic engineering protocols and the required infrastructure/equipment. The overall purpose of the research programs in Japan are to study the neurobiology and evolution of eusociality. Although European Honey bee genetic manipulation was limited to CRISPR/Cas9 mutagenesis in these labs, the protocols would differ only slightly for transgenesis and the infrastructure/biocontainment requirements would be identical in the Japanese context.

Engineering European Honey Bees

Heritable germline modifications of any animal requires the introduction of DNA modification reagents (e.g. CRISPR/Cas9, piggyBac plasmids and transposase, etc.) into either gametes or their progenitor cells. This is typically accomplished via microinjection of a solution containing these reagents into early-stage embryos within a few hours of fertilization. These reagents enter the nuclei of various undifferentiated stem cells and the resulting insect is a chimera with varying degrees of modification in its body. If all goes well, some of the pole cells (germ cell progenitors) are edited. Offspring of a chimera that inherit an edit via a modified sperm or egg are heterozygous for the mutation which is present in every cell of their body. The mutation is inherited just like any other sequence of nuclear DNA in subsequent generations. In the case of Honey bees, haploid drones can be produced from virgin queens. Establishing homozygous strains requires crossing between heterozygotes and screening for homozygotes (Fig. 1). Both CRISPR/Cas9 methods to generate gene knockouts and piggyBac transposase transgenesis to introduce exogenous DNA sequences have been demonstrated in European Honey bees[1].

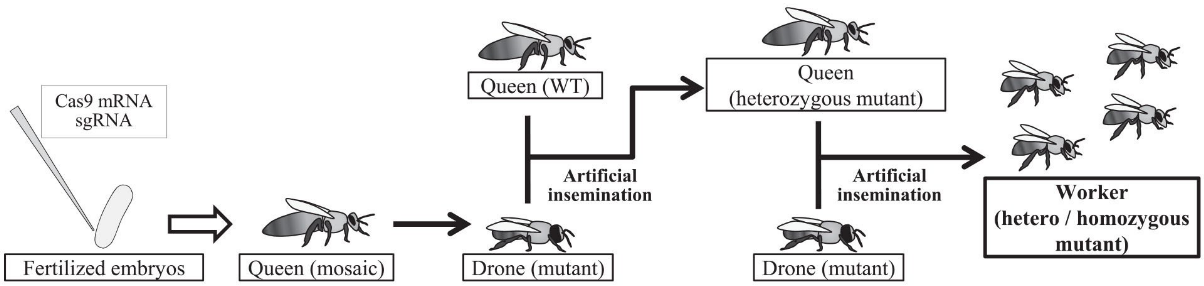

Figure 1: Overview of Honey be genetic modification process from Prof Takeo Kubo's lab[2].

The first stage in the process is collecting embryos. A queen is placed in a small plastic oviposition chamber with wells for oviposition (e.g., Jenter egg collection system). Alternatively, fresh embryos can be found in the wells of the hive. These are checked every few hours. Freshly laid eggs are collected, aligned on a microscope slide, and microinjected with a solution of CRISPR/Cas9 or piggyBac transposon reagents via standard insect embryo microinjection procedures (Fig. 2). The specialized equipment required for microinjection includes a capillary needle puller and a microinjection station comprising a microscope, microinjector, and micromanipulator.

Figure 2: Collecting embryos (Left), microinjection (Middle), and small hive box (Right).

Injected embryos are incubated for 3 days and hatched larvae are transferred to petri dishes containing media with royal jelly for up to 5 more days and then grafted into a queenless colony in a small hive box with plastic queen cells and nurse bees. Capped queen cells are removed and emerged queens are housed with workers.

Laying unfertilized eggs by virgin queens can be stimulated with CO_2 treatment which produce haploid male drones. If the queen had a transgene integrated, then the drones are evaluated for the presence of a selectable marker (e.g., GFP eyes). Otherwise, DNA extracted from a portion of a wing or leg must be performed to identify gene knockouts. Large deletions (50+ nt) are easy to detect via PCR and gel electrophoresis, but Sanger sequencing is necessary to identify smaller mutations and to determine their precise nature.

Once genetically modified drones are identified, wild-type queens are artificially inseminated using a queen bee artificial insemination instrument (Fig. 3). Inseminated queens are placed in a hive to produce heterozygous workers or fertilized eggs can be reared on royal jelly and placed in queenless hives to generate heterozygous queens. Producing a homozygous colony requires another set of artificial inseminations to transgenic drones and isolation of homozygous queens (Fig. 1).

Figure 3: Artificial insemination. (Left) Semen is collected from a drone and (Middle) microinjected into a queen. (Right) Indoor Honey bee colony.

Although this is a labour intensive process, the efficiency of genetic engineering is surprisingly high compared to other species of insects. Transgenesis with hyperactive piggyBac transposase results in 19-44% of chimeric queens producing transgenic offspring[3], while injection of CRISPR/Cas9 results in around 10% mutagenesis[2] although improved methods that result in highly efficient biallelic knockouts in the injected embryos[4] will likely result in increased rates of transgenesis.

Hives of transgenic bees can be maintained indoors (Fig. 3, Right) where they are kept in hives within climate controlled rooms with multiple layers of netting for biocontainment and fed an artificial diet, however, these colonies often struggle to thrive. Worker bee populations drop and must be supplemented with workers from outdoor wild-type colonies. This has not been a major hindrance for researchers thus far due to the nature of the mutations they have been studying.

Any work to introduce mutations that may have industrial benefits will need the development of more sophisticated invertebrate PC2 greenhouse habitats that allow for foraging. On the other hand, knockout strains generated via CRISPR/Cas9 in the absence of a repair template would not be subject to the same biocontainment requirements from the OGTR as transgenic strains. It may be acceptable to use hives where mated queens are contained while workers may forage though the biosafety and social acceptance of such experiments would need to be carefully evaluated before proceeding.

<u>Conclusion</u>

Although more complicated than many other insects, European Honey bee genetic engineering could be achieved using existing Australian insect genetic engineering capabilities. Currently, certified invertebrate PC2 labs could be used for microinjection, screening for mutants, establishing transgenic strains, and evaluating limited sets of phenotypes (possibly including hygiene behaviour). However, specialized contained facilities will be needed to evaluate the performance of engineered Honey bees for many industrially relevant traits such as foraging behaviour and Honey productivity. A subsequent report will include information on indoor habitats suitable for Honey bee rearing and performance evaluation after consulting with additional Honey bee experts.

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(3) Otte, M.; Netschitailo, O.; Kaftanoglu, O.; Wang, Y.; Page, R. E.; Beye, M. Improving Genetic Transformation Rates in Honey bees. *Sci. Reports 2018 81* **2018**, *8* (1), 1–6. https://doi.org/10.1038/s41598-018-34724-w.

(4) Roth, A.; Vleurinck, C.; Netschitailo, O.; Bauer, V.; Otte, M.; Kaftanoglu, O.; Page, R. E.; Beye, M. A Genetic Switch for Worker Nutrition-Mediated Traits in Honey bees. *PLOS Biol.* **2019**, *17* (3), e3000171. https://doi.org/10.1371/JOURNAL.PBIO.3000171.

Appendix 3: Pesticide recommendation report

Confidential, provided separately

Appendix 4: Stakeholder perception report – delivered by CSIRO

Confidential, provided separately

Appendix 5: Stakeholder Communication Plan

High-value communications plan

Project title:

Opportunities for insecticide-resistant Honey bees for pollination security

Project code:

PH22000

Project leader:

Dr. Maciej Maselko

Delivery partner:

Macquarie University

Date:

30 November 2024

Contents

Purpose

This working communications strategy acts as a guiding document for all communications staff to refer to throughout the life of the project. This document:

- Establishes a roadmap for communication to ensure all partners are on the same page
- Streamlines messaging
- Ensures all available partner and industry stakeholder communication tools are being utilised
- Details processes and attribution requirements.

Current situation

Insect pollination services are essential for many crops, including $6 billion/year of Australian horticultural products. European Honey bees provide more than half of that, and various native bee species provide substantial value as well. Maintaining healthy bee populations is therefore important to the economic health of many Australian communities and ensuring food security. Threats including climate change, varroa mites, and pesticide exposure pose major challenges to bee populations and new solutions are necessary to overcome them.

The main objective of this project was to investigate the potential of using emerging genetic engineering technologies to introduce beneficial traits such as insecticide resistance into Honey bees and thereby to provide recommendations to the Australian horticulture industries on which may be technically feasible, desirable to farmers, environmentally sound, allowed by existing regulatory frameworks, and publicly acceptable. The recommendations were developed by an interdisciplinary group of biologists and social scientists who directly engaged with stakeholders and reviewed the latest scientific literature.

The findings of the project make important contributions to the aims of the Hort Innovation Pollination Fund whose purpose "... is to enhance horticulture crop production and resilience through improved pollination". More specifically, this project directly addresses two of the three key investment priorities: Improve the management of European Honey bees for pollination and Optimise crop pollination.

Audiences

Key audiences:

- Horticulture industry
- Growers who use Honey bees for pollinating their crops
- Commercial beekeepers

Other parties:

- The Australian regulatory agencies (e.g. APVMA, OGTR, FSANZ)
- Potential research partners
- The Australian Government
- General public

Overarching key messages

Table 1. Overarching key messages

Audience	What they need to know	Key communications messages
Horticulture industry	• Project objectives, outputs, and outcomes • Importance of advanced biotechnological tools in achieving beneficial traits such as pesticide resistance in Honey bees. • Priority pesticides on which any future Honey bee genetic engineering program for pesticide resistance should focus. • Stakeholder and public perception of Honey bee genetic engineering in Australia.	• Pesticides are one of the main contributors to the decline of Honey bee health and survival globally. • Advanced biotechnological tools could be used to precisely engineer Honey bees for beneficial traits such as pesticide resistance. • Pyrethroids would be an ideal candidate to achieve insecticide resistance in Honey bees in any future genetic engineering program. • Industry stakeholders had no direct opposition to gene editing technologies for Honey bees, but they prioritised other beneficial traits (e.g. pest resistance, increased Honey production, improved temperament) as potentially valuable. • The general public is willing to consider the development of gene editing technology for use in Honey bees, although risk identification and management were their critical considerations.
Growers who use Honey bees for pollinating their crops	• Project objectives, outputs, and outcomes • Importance of advanced biotechnological tools in achieving beneficial traits such as pesticide resistance in Honey bees. • Priority pesticides on which any future Honey bee genetic engineering program for pesticide resistance should focus. • Stakeholder and public perception of Honey bee genetic engineering in Australia.	• Pesticides are one of the main contributors to the decline of Honey bee health and survival globally. • Advanced biotechnological tools could be used to precisely engineer Honey bees for beneficial traits such as pesticide resistance. • Pyrethroids would be an ideal candidate to achieve insecticide resistance in Honey bees in any future genetic engineering program. • Industry stakeholders had no direct opposition to gene editing technologies for Honey bees, but they prioritised other beneficial traits (e.g. pest resistance, increased Honey production, improved temperament) as potentially valuable. • The general public is willing to consider the development of gene editing technology for use in Honey bees, although risk identification and management were their critical considerations.
Commercial Beekeepers	• Project objectives, outputs, and outcomes	• Pesticides are one of the main contributors to the decline of Honey bee health and survival globally.

	• Importance of advanced biotechnological tools in achieving beneficial traits such as pesticide resistance in Honey bees. • Priority pesticides on which any future Honey bee genetic engineering program for pesticide resistance should focus. • Stakeholder and public perception of Honey bee genetic engineering in Australia.	• Advanced biotechnological tools could be used to precisely engineer Honey bees for beneficial traits such as pesticide resistance. • Pyrethroids would be an ideal candidate to achieve insecticide resistance in Honey bees in any future genetic engineering program. • Industry stakeholders had no direct opposition to gene editing technologies for Honey bees, but they prioritised other beneficial traits (e.g. pest resistance, increased Honey production, improved temperament) as potentially valuable. • The general public is willing to consider the development of gene editing technology for use in Honey bees, although risk identification and management were their critical considerations.
The Australian regulatory agencies (e.g. APVMA, OGTR, FSANZ)	• Objectives of the project • Potential technologies and traits that will be used in any future Honey bee genetic engineering program	• CRISPR/Cas9 technologies (e.g. CRISPR base editors) could be used to achieve beneficial traits such as pesticide resistance in Honey bees.
Potential research partners	• Project objectives, outputs, and outcomes • Importance of advanced biotechnological tools in achieving beneficial traits such as pesticide resistance in Honey bees. • Priority pesticides on which any future Honey bee genetic engineering program for pesticide resistance should focus. • Stakeholder and public perception of Honey bee genetic engineering in Australia.	• Pesticides are one of the main contributors to the decline of Honey bee health and survival globally. • Advanced biotechnological tools could be used to precisely engineer Honey bees for beneficial traits such as pesticide resistance. • Pyrethroids would be an ideal candidate to achieve insecticide resistance in Honey bees in any future genetic engineering program. • Industry stakeholders had no direct opposition to gene editing technologies for Honey bees, but they prioritised other beneficial traits (e.g. pest resistance, increased Honey production, improved temperament) as potentially valuable. • The general public is willing to consider the development of gene editing technology for use in Honey bees, although risk identification and management were their critical considerations.
The Australian Government	• Project objectives, outputs, and outcomes • Importance of advanced biotechnological tools in achieving	• Pesticides are one of the main contributors to the decline of Honey bee health and survival globally.

	beneficial traits such as pesticide resistance in Honey bees. • Priority pesticides on which any future Honey bee genetic engineering program for pesticide resistance should focus. • Stakeholder and public perception of Honey bee genetic engineering in Australia.	• Advanced biotechnological tools could be used to precisely engineer Honey bees for beneficial traits such as pesticide resistance. • Pyrethroids would be an ideal candidate to achieve insecticide resistance in Honey bees in any future genetic engineering program. • Industry stakeholders had no direct opposition to gene editing technologies for Honey bees, but they prioritised other beneficial traits (e.g. pest resistance, increased Honey production, improved temperament) as potentially valuable. • The general public is willing to consider the development of gene editing technology for use in Honey bees, although risk identification and management were their critical considerations.
General public	• Project objectives, outputs, and outcomes • Importance of advanced biotechnological tools in achieving beneficial traits such as pesticide resistance in Honey bees. • Priority pesticides on which any future Honey bee genetic engineering program for pesticide resistance should focus. • Stakeholder and public perception on Honey bee genetic engineering in Australia.	• Pesticides are one of the main contributors to the decline of Honey bee health and survival globally. • Advanced biotechnological tools could be used to precisely engineer Honey bees for beneficial traits such as pesticide resistance. • Pyrethroids would be an ideal candidate to achieve insecticide resistance in Honey bees in any future genetic engineering program. • Industry stakeholders had no direct opposition to gene editing technologies for Honey bees, but they prioritised other beneficial traits (e.g. pest resistance, increased Honey production, improved temperament) as potentially valuable. • The general public is willing to consider the development of gene editing technology for use in Honey bees, although risk identification and management were their critical considerations.

Communication methods, activities and platforms

Table 3. Communication method, activities and platforms

Date	Announcement/ Opportunity	Communication method	Who is responsible?
At the start of the project (June 2023)	Email invitations were sent to relevant stakeholders to join the project reference group.	Email communication with major related industries e.g. Apiarists' associations, relevant grower associations (e.g. almond, apple), NSW Department of Primary Industries and potential partner universities	Project leader/postdoctoral researcher
25th July 2023	The first Project reference group meeting was conducted to receive feedback on the objectives, procedures, and timeline of the project.	Virtual Meeting	Project leader and project team members
1st August 2023	A project monitoring and evaluation plan was submitted with milestone 102 and is available to stakeholders through the Hort Innovation website.	Report	Project leader and project team members
August 2023	A newsletter on the use of advanced biotechnological approaches to engineer Honey bees for beneficial traits (that discussed the background, research needs and objectives of the project) was published in AFG Winter 2023 magazine.	Industry newsletter	Project lead
1st November 2023	A milestone report (Milestone 103) that communicates the project findings was submitted to Hort Innovation. It included a review report on Honey bee-pesticide interactions and genetic engineering technologies that could be utilized to engineer Honey bees.	Report	Project postdoctoral researcher and team members
November 2023 to July 2024	Email invitations were sent to potential industry stakeholders to participate in interviews for pesticide recommendation activity which was aimed at understanding the pesticide-Honey bee interactions and challenges in the Australian context.	Email communications, phone calls	Project postdoctoral researcher
8th to 28th March	A public perception survey was conducted.	Online survey	CSIRO project team
December 2023 to April 2024	Semi-structured interviews were conducted to understand the industry stakeholder perceptions on Honey bee genetic engineering in Australia.	Semi-structured interviews	CSIRO project team
April 2024	Email communications with EPA to understand regulatory requirements for genetically engineering Honey bees for pesticide resistance.	Email communications	Project leader and project postdoctoral researcher

22nd to 24th May 2024	Participated in the NSW Apiarists' Association annual conference and communicated research with commercial beekeepers.	Conference attendance	Project postdoctoral researcher
30th May 2024	A joint meeting was conducted with OGTR and FSANZ to understand the regulations related to Honey bee genetic engineering in Australia.	Virtual Meeting	Project leader and project postdoctoral researcher
12th to 14th June 2024	Presented the research findings at the 17th Asian Apiculturists' Conference. The presentation titled 'Genetically engineering Honey bees for beneficial traits'	Conference presentation	Project postdoctoral researcher
July 2024	Email communications with APVMA to understand regulatory requirements for genetically engineering Honey bees for pesticide resistance.	Email communications	Project leader and project postdoctoral researcher
1st August 2024	A milestone report (Milestone 104) that communicates the findings of the project was submitted to Hort Innovation. It included a pesticide recommendation report that prioritizes pesticides on which any future Honey bee genetic engineering program for pesticide resistance should focus.	Report	Project postdoctoral researcher and team members
19th August 2024	A Honey bee workshop was conducted to discuss project findings with the interdisciplinary project team and the PRG.	Meeting (in-person + Online)	Project leader and project team members
30th November 2024	A report on stakeholder and public perception of Honey bee genetic engineering in Australia was submitted with the final report.	Report	CSIRO team
30th November 2024	Outcomes of the project that communicate all outputs and outcomes of the project (milestone 190).	Final report	Project leader and project team members
To be informed	A journal publication on perspectives on genetically engineering Honey bees for beneficial traits will communicate the major findings of the study to the broader scientific and non-scientific community.	Journal article	Project leader and project team members

Table 4. Partners' available communications platforms

Organisaton	Available platforms and frequency
Hort Innovation	MEDIA: National grower-facing media database (more than 550 journalists) E-NEWSLETTER: Growing Innovation (monthly e-newsletter) SOCIAL: Facebook, Twitter, Instagram and LinkedIn INDUSTRY COMMUNICATIONS: Manage industry communication projects (magazines, newsletters, e-newsletters, social media, etc. for all industries nationwide) WEBSITE: News and media section
Macquarie University	MEDIA: Internal magazine such as The Lighthouse; Research-focused news articles SOCIAL: Facebook, Twitter WEBSITE: News and media section
Project team	PUBLICATIONS IN SCIENTIFIC JOURNALS SOCIAL: Facebook, Twitter, LinkedIn WEBSITE: Lab website SCIENTIFIC- AND INDUSTRY-FOCUSED CONFERENCES: online or face-to-face presentation of progress and results at national and international conferences

Table 5. Relevant industry communications platforms

Industry	Available platforms and frequency
Commercial beekeepers and Growers that rely on Honey bees for pollination (e.g. almonds, apples, berries, melons, avocados)	E-NEWSLETTER: The Australian Honey Bee Industry Council newsletter (monthly e-newsletter) INDUSTRY MAGAZINES: e.g. Australian Fruit Grower Magazine (published quarterly) FACT SHEETS: The Wheen Bee Foundation, The professional Extension Aus beekeepers' website, Almond Board, Apple and Pear Australia Limited SOCIAL MEDIA, Facebook, Twitter, and LinkedIn

Media liaison

In all instances, Hort Innovation provides oversight over the communications on projects on which it is the contract manager. Partner communications staff will liaise to promote project stories and harness media opportunities.

Media protocol

- The communications contacts are the communications/media liaison contacts listed in Appendix 1 for each organisation. Hort Innovation is the nominated 'lead'
- All requests for media interviews or comments must be referred to the Hort Innovation nominated lead immediately. Hort Innovation will liaise with the appropriate partner contact(s) to determine how the enquiry will be handled
- Where possible, Hort Innovation will seek approval from all organisations before issuing any media releases, statements or other media communications. Commentators will be determined by Hort Innovation on a case-by-case basis
- Organisations may nominate a media spokesperson in Appendix 1. They may be required to provide a quote for communications content where applicable for activity relevant to their organisation's research component.

Attribution requirements

The funding statement along with the appropriate branding must be included at some point in all R&D project communication outputs and marketing media releases. The statement recognises industry levy investment, co-investment details and any Australian Government contributions.

Logo: The single, dedicated Frontiers must be included in all relevant R&D project communication outputs. The logo is available via the delivery partner section of Hort Innovation's website.

In addition to the logo and funding statement, it is a requirement to also weave project acknowledgement and naming into your communications narrative. A project must be referred to by its full name and code in the first instance, for example: The project < insert project name > (< insert project code >) is an investment through Hort Innovation Frontiers. Media releases are an exception as they do not require a full project name and code.

The funding statement for the project:

PH22000 *'Opportunities for insecticide resistant Honey bees for pollination security'* is funded through Frontiers developed by Hort Innovation, with co-investment from Macquarie University and contributions from the Australian Government.

Evaluation

At each milestone report, communications achievements should be reviewed, with any communications gaps identified. Opportunities for the next six months should be updated in the Communication Strategy.

Media coverage reports, and other communications updates, should be provided to Hort Innovation as part of milestone reporting.

NOTE: This should be considered as part of project monitoring and evaluation.

Appendix 6: Conference abstract and slides – 17th Asian Apiarists' Association conference

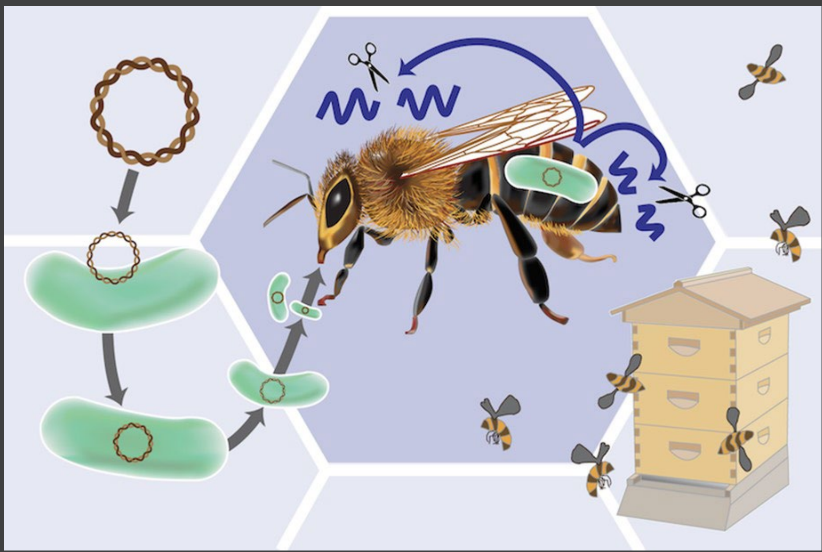
Genetically engineering Honey bees for beneficial traits

Anu Jayaweera and Maciej Maselko
School of Applied BioSciences, Macquarie University, Sydney, Australia

The European Honey bee (*Apis mellifera*) is a critical species in the Australian Honey bee industry – producing some of the world's highest-quality Honey and providing essential pollination services. Nearly 65% of Australian agricultural production depends on pollination by Honey bees. Maintaining healthy bee populations is therefore important to the economic health of many Australian communities and to ensure food security. Climate change, Varroa mites, and pesticide exposure pose major challenges to Honey bee populations and new solutions are necessary to overcome them. This project will study the potential of using emerging technologies of genetic engineering to introduce beneficial traits into bees and provide recommendations to the Australian horticulture industries on which may be technically feasible, desirable to farmers, environmentally sound, allowed by existing regulatory frameworks, and publicly acceptable. Advanced genetic engineering techniques such as CRISPR/Cas9 provide tools for precise genetic editing in many organisms including Honey bees. These tools may be used to rapidly engineer strains to contain elite genetic variants, study existing variations to inform breeding programs, and rationally develop novel traits such as pesticide resistance. We will be presenting these different potential approaches to Honey bee genetic engineering ranging from the introduction of simple point mutations to transgenes in the conference. Future gene-edited Honey bees for beneficial traits may allow beekeepers and growers to maintain healthy Honey bee colonies and to secure their services, especially in pollination.

Genetically engineering honey bees for beneficial traits

Dr. Anu Jayaweera & Dr. Maciej Maselko
Applied Biosciences, Macquarie University, Sydney

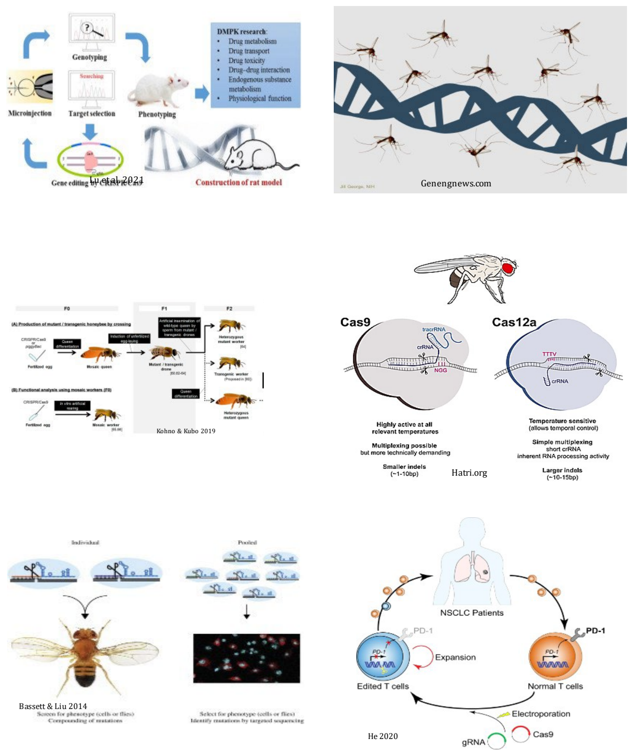


The Australian horticulture industry greatly depends on the European honey bee *Apis mellifera* for pollination services.


ats, including
extreme weather patterns, pathogens, parasites, and pesticides.
Image source: Science.org.au


• The Australian honey bee industry faces one of the most challenging times ever…..
Image source: Australian Honey bee Industry Council

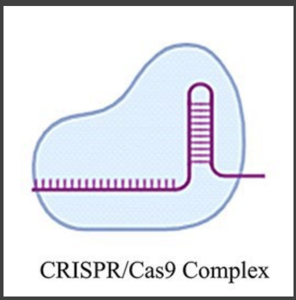
- Recent advances in biotechnology have enabled precise genome editing in many organisms, including bees.

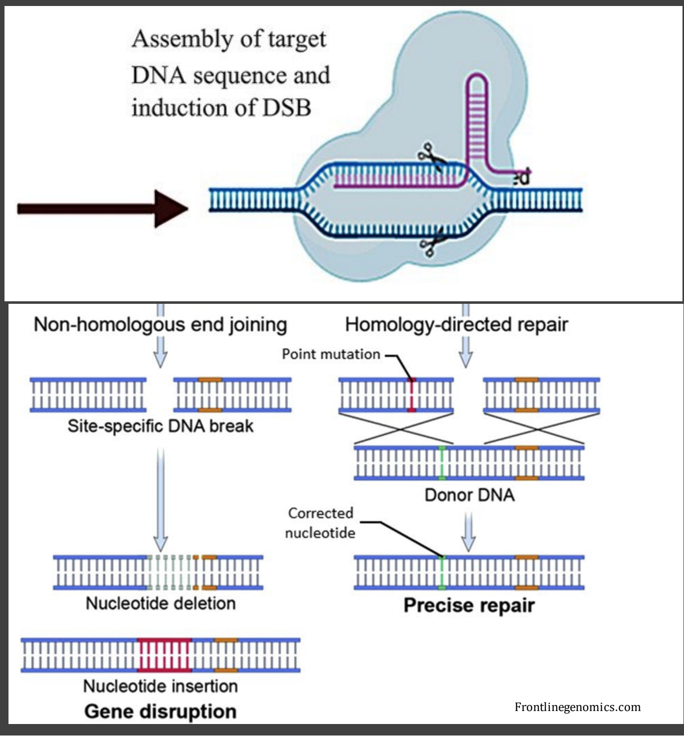


Genetically engineering honey bees for beneficial traits

- The project aims to study the opportunity landscape for honey bee genetic engineering for beneficial traits such as insecticide -resistance.
- **We do not perform any honey bee genetic engineering in the current project.**

CRISPR/Cas9 technology





Potential applications of CRISPR technology in honey bees

- Study existing genetic variation to inform breeding programs

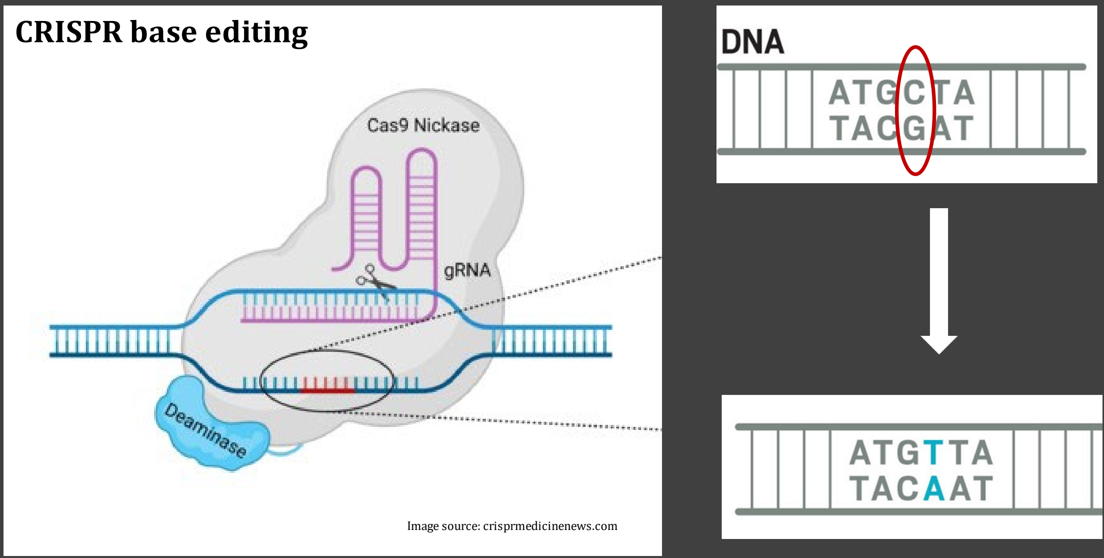


Image source: crisprmedicinenews.com

- Rapidly engineer strains to contain elite genetic variants.

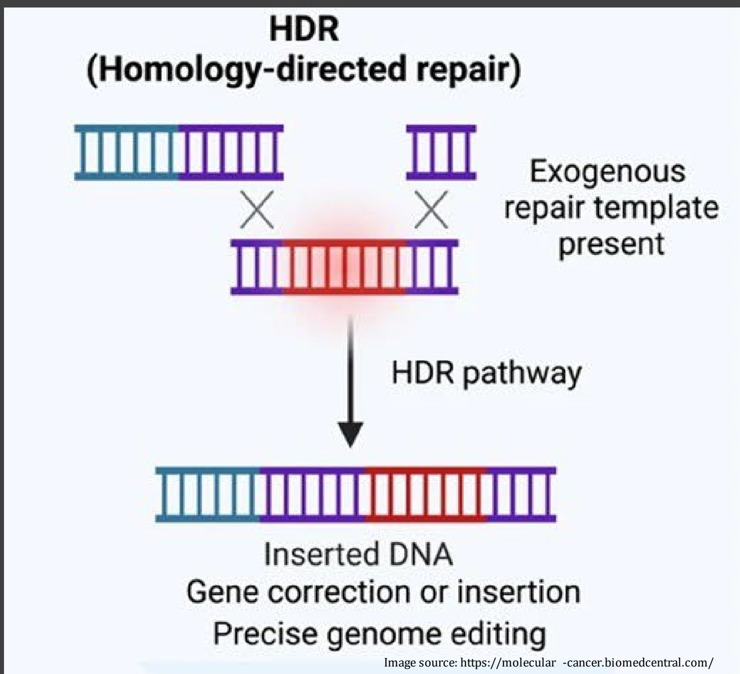
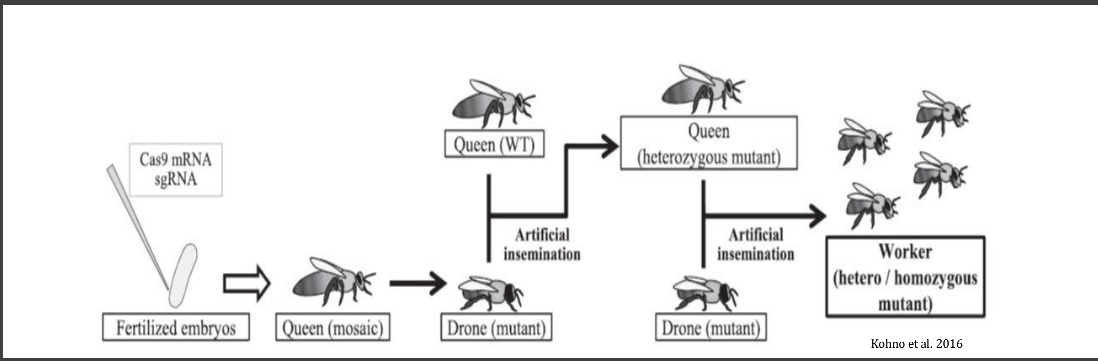


Image source: https://molecular -cancer.biomedcentral.com/

- Rationally develop novel traits



- Several studies succeeded in utilizing CRISPR/Cas9 in producing knockout mutations in honeybees (Kohno et al. 2016, Hu et al. 2019, Roth et al. 2019).

Participate in our stakeholder interviews……..

Dr. Anu Jayaweera
anu.jayaweera@mq.edu.au
0477540775

The ethical aspects of this research have been approved by the Macquarie university Human Research Ethics Committee (HREC).
HREC approval No. 16055

Appendix 7: Industry news article – AFG Winter 2023

How genetics could bolster honey bee defences

APAL

A new study is looking into the possibility of breeding honey bees to be disease resistant and more heat tolerant in the face of climate change.

Key points

- A new project will explore the potential of using emerging technologies to introduce beneficial traits to honey bees.
- It will provide recommendations to the Australian horticulture sector on what options are technically feasible, desirable to growers, environmentally sound, supported by existing regulatory frameworks and publicly acceptable.
- Researchers are interested in the possibility of breeding honey bees to be resistant to insecticides and diseases, and be more heat tolerant in the face of climate change.

The detection of the deadly Varroa mite in honey bees in New South Wales last year has been the catalyst for a new project aimed at bolstering the defences of Australia's precious pollinators.

Apples are dependent on pollinators such as honey bees and native bees for production, while one third of Australian food that ends up on our plate is dependent on honey bee pollination.[1]

Macquarie University synthetic biologist and research group leader, Dr Maciej Maselko, said in the wake of the Varroa mite detection, conversations with colleagues about how to help strengthen pollination security led to a new research project now underway.

The Hort Frontiers Pollination Fund project, *Opportunities for insecticide resistant honey bees for pollination security* (PH22000), will explore the potential of using emerging technologies to introduce beneficial traits to honey bees.

[1] **Source:** https://agrifutures.com.au/rural-industries/honey-bee-pollination/

What does the project involve?

Maciej is leading the project which will provide recommendations to the Australian horticulture sector on what options are technically feasible, desirable to growers, environmentally sound, supported by existing regulation and publicly acceptable.

"This project is a one-year study to understand the opportunity and risk landscape and see whether or not it makes sense to proceed with breeding honey bees using emerging genetic technologies," Maciej said. "We are in the early stages of the project, designing the surveys and identifying stakeholders we will engage with.

"We want to have meaningful discussions with industry stakeholders and the public so that they understand the scope of options and get an understanding of what, if any, breeding of honey bees using emerging genetic technologies would they find to be acceptable.

"We also want to understand how these different types of traits might impact existing agricultural practices, what modifications are likely to have a net environmental benefit, and which ones may cause harm and we should avoid.

"For example, if we breed honey bees to be pesticide resistant to support them through issues like Varroa mite, how is that likely to impact production practices? We want to understand views across our stakeholders including growers and the public."

"But these are hypotheses we want to go out and test by having conversations with farmers who are having to make these sorts of decisions all the time."

Potential benefits

Maciej said while the Varroa mite issue was one of the factors that spurred the project, researchers are also interested in the possibility of breeding honey bees to be resistant to other types of diseases and be more heat tolerant in the face of climate change.

Using tools to accelerate breeding gains is another significant potential benefit.

"For example, if you have a strain of honey bee that has several particular traits that you like, and another strain that has other traits that you would like to introduce, normally, you would have to crossbreed and backcross over multiple generations, which can be very time consuming," Maciej said.

"But if we understand the nature of these traits and their genetic basis, then potentially we can introduce a few genetic changes that you want to a strain in one step to produce strains with a combination of desirable traits in much shorter timeframes.

"We need to know what those actual markers are, and some of that will be improved through a more thorough understanding of the basic biology of these bees."

Global insights

The project will see researchers conduct a global review of current research into insecticide resistant honey bees and stingless bees, focusing on emerging genetic technologies like gene-editing. Maciej said he knows of two research groups in Germany and Japan who have successfully bred honey bees using gene-editing.

With tools already existing for emerging genetic technologies like gene-editing in European honey bees, Maciej said these tools would likely be the main focus of research to understand their feasibility and grower and public perception and views on this approach, while the research team would also survey the potential of other species including native bees.

The project will also see researchers consult with the horticulture and beekeeping industries to develop a prioritised list of the insecticide groups of concern that Australian work should focus on. AFG

Dr Maciej Maselko, synthetic biologist and research group leader, Macquarie University.

Acknowledgement
The Opportunities for insecticide resistant honey bees for pollination security (PH22000) project is funded by the Hort Frontiers Pollination Fund, part of the Hort Frontiers strategic partnership initiative developed by Hort Innovation, with co-investment from Macquarie University and contributions from the Australian Government.

Updates on this project will be communicated in future editions of *AFG*.

Appendix 8: Email invitation to join the PRG (example evidence)

11/18/24, 10:10 AM Mail - Anu Jayaweera - Outlook

Outlook

Project Reference Group (Project: Opportunities for insecticide resistant honey bees)

From Anu Jayaweera <anu.jayaweera@mq.edu.au>
Date Fri 6/23/2023 12:20 PM
To info@nswaa.com.au <info@nswaa.com.au>
Cc Maciej Maselko <maciej.maselko@mq.edu.au>

Dear [redacted]

I am Anu, a researcher in the Biotechnology group, Applied BioSciences, at Macquarie University. I am working on a industry funded research project to explore the opportunities for genetically engineered honey bees for insecticide resistance. This one-year project does not include any bee engineering, rather, it is a scoping study that investigates the technical, industrial, social, and regulatory dimensions of doing so in order to determine under what circumstances, if any, would it be appropriate to engage in honey bee genetic engineering in Australia.

We are currently establishing a Project Reference Group (PRG) to get their valuable feedback and guidance on the project activities to accomplish desired project outcomes. We would be very grateful if NSWAA representative could be a member of our PRG as we strongly believe your feedback will significantly impact our project. Please let us know if you are happy to become a PRG member of the project.

Please do not hesitate to contact me if you need any further information.

Thank you,
Best Regards,
Anu

Anu Jayaweera, PhD
Postdoctoral Research Fellow,
Applied BioSciences,
Macquarie University,
NSW 2109, Australia.

https://outlook.office.com/mail/sentitems 1/1

Appendix 9: Project reference group members

Project reference group members

Name	Role and Organization
Dr. Maciej Maselko	Project Leader, Macquarie University
Dr. Anu Jayaweera	Project postdoctoral research fellow, Macquarie University
Prof. Phil Taylor	Team member, Macquarie University
Dr. Mary Whitehouse	Team member, Macquarie University
Prof. Anwar Sunna	Team member, Macquarie University
Prof. Grant Hose	Team member, Macquarie University
A/Prof. Peter Davies	Team member, Macquarie University
Prof. Ken Cheng	Team member, Macquarie University
Prof. Lisa Wynn	Team member, Macquarie University
Prof. Andrew McGregor	Team member, Macquarie University
Dr. Jon Symons	Team member, Macquarie University
Dr. Aditi Mankad	Team member, CSIRO
Dr. Lucy Carter	Team member, CSIRO
Dr. Elizabeth V. Hobman	Team member, CSIRO
Dr. Barton Loechel	Team member, CSIRO
Lesly Scanlan	Team member, CSIRO
Dr. Emily Remnant	PRG member, Lecturer and Academic Fellow, School of Life and Environmental Sciences, University of Sydney
Danny Le Feuvre	PRG member, Chief executive officer, Australian Honey Bee Industry Council (AhBIC)
Elizabeth Frost	PRG member, Technical Specialist Bees, Department of Primary Industries (DPI), NSW
Ray Hull	PRG member, Executive Councilor NSW Apiarists' Association Inc. (NSWAA)
Wayne Andreatta	PRG member, Development Manager, Kooba Station, Darlington Point, NSW
Doug Purdie	PRG member, Editor, Amateur Beekeepers Association (ABA)
Ashley Zamek	Hort Innovation R&D Manager

Appendix 10: Stakeholder interview invitation – pesticide recommendation activity

SCHOOL OF APPLIED BIOSCIENCES
Faculty of Science and Engineering

MACQUARIE University SYDNEY·AUSTRALIA

14 November 2023

Invitation to participate in a stakeholder interview.
Project: Opportunities for insecticide resistant honey bees for pollination security

My name is Anu Jayaweera, a researcher in Applied Biosciences, at Macquarie University. I am part of a team working on a Hort Innovation-funded research project to investigate the potential of using modern genetic engineering tools to engineer honey bees for beneficial traits such as pesticide resistance. We are not performing any genetic engineering. We are only evaluating the technical, regulatory, and socio-ethical landscape to determine the prudence of doing so. This will be achieved via various research activities including reviewing the relevant literature, conducting stakeholder and regulatory meetings, and a public survey.

One of our project activities aims to understand the pesticide usage patterns in Australia, their effects on honey bees, and strategies used to mitigate the negative impacts of pesticides on honey bees by conducting stakeholder (beekeepers and growers who use managed honey bees for pollination services) interviews. Further, we are also interested in understanding how beekeepers and growers anticipate any modified honey bees would impact their operations in the future.

If you are a beekeeper or a grower (who uses managed honey bees for pollination) you can be a part of our research. Please let us know if you would like to participate in our stakeholder interviews. Your feedback will be very important for us and in any honey bee research to understand the current situation and thereby safeguard them and their services. Please find attached the project flyer for more information and please do not hesitate to contact me if you need further information.

Thank you,

Best Regards,

Anu

Anu Jayaweera, PhD
Postdoctoral Research Fellow,
Applied Biosciences,
Macquarie University,
NSW 2109, Australia.
Email: anu.jayaweera@mq.edu.au
Phone: +61(02) 9850 6262

Macquarie University, NSW 2109, Australia E: anu.jayaweera@mq.edu.au

Appendix 11: Stakeholder interview schedule – pesticide recommendations activity

End-user (beekeeper and grower) interview schedule

PH22000: Opportunities for insecticide resistant honey bees for pollination security

Objective	Draft questions
How existing pesticide use patterns affect bee pollination services or honey production?	1) What best describes your contribution to the honey bee industry? (Beekeeper/grower)
	2) How long have you been engaged with the honey bee industry?
	3) What services do you get from managed honey bees?
	4) What are the main issues you are facing in the beekeeping industry?
	5) What kind of pollinator-dependent crops have you grown in the last 5 years?
	6) What are the main issues you are facing in your crop system?
	7) Within the last 5 years, have you applied any pesticides in your farming system?
	8) What chemicals do you use in your farming system? Please specify the chemicals and the method and frequency of application.
	9) In your opinion what are the threats imposed on honeybees in your farming practices?
	10)Have you ever noticed a decline in honeybees or a reduction in their services? If Yes; What did you observe? What would be the likely cause for any honey bee losses?
What are the strategies used to mitigate negative impacts of pesticides on honey bees and current pain points?	1) What actions have you taken to minimize pesticide usage or to minimize exposure of honey bees to pesticides?
	2) How often do honey bee pollination services prevent you from effectively managing a pest?
	3) What will be the impact on your farming system if Varroa is established in Australia?
	4) In your opinion what measures are possible to take to save honey bees?
How beekeepers/growers anticipate bees resistant to various pesticides would impact their operations?	1) How will you rank your understanding of genetic engineering technology?
	2) How would you feel about bees that would be resistant to chemical sprays in crops?
	3) What do you think about engineering honey bees for insecticide-resistance?

	4) In the future if honeybees were genetically engineered to carry insecticide resistance, how likely you would use genetically engineered honeybees in your operations?
	5) What are the other beneficial traits you would like to be engineered into honey bees in the future?
	6) Do you have any other concerns on honey bee genetic engineering or their impact/s?

Appendix 12: Project flyer

Honey bees are at greater risk…

Honey bees are exposed to various threats including extreme weather, pathogens, parasites such as Varroa mites, and pesticides. The Australian horticulture industry is greatly dependent on bees for pollination services; therefore, maintaining healthy populations is critically important. Genetic improvement is one of the best ways to deal with these challenges. Current breeding programs are dependent on existing genetic variation which can take many generations to establish.

Therefore, novel solutions are crucial to safeguard honeybees and thereby to secure pollination. Recent advances in biotechnology have enabled precise genome editing in many organisms, including bees. These tools may be used to rapidly engineer strains to contain elite genetic variants, study existing genetic variation to inform breeding programs, and rationally develop novel traits.

Our project is aimed to,

study the opportunity landscape (stakeholder perspective, regulatory barriers, technical requirements, and environmental impacts) for honey bee genetic engineering for insecticide -resistance. It is a commercially valuable trait that could be engineered for honey bees, which enables farmers to manage pests effectively without interrupting pollination services, also if Varroa mites were established in Australia, it could be treated without negatively affecting the bees.

We will conduct various activities including a review of recent literature on honey bee pesticide sensitivity/resistance, industry and stakeholder interviews to understand the current situation of the Australian honey bee industry and the pesticide usage patterns, meetings with regulatory agencies to understand regulatory requirements, and a public survey to get their perception on honey bee gene-editing technologies. This will make a foundation for any future research studies on developing gene-edited honey bees for beneficial traits such as insecticide resistance, temperature tolerance, and improved honey production ….

How can you contribute?

Spare 20-30 minutes to share your experience in the honey bee industry and opinions on how potential honey bee gene-editing would impact your operations.

This will help us to understand the current situation of honey bees and pesticide usage in Australia, the priority pesticides on which insecticide-resistant honey bees should achieved, and the stakeholder perspective for honey-bee genetic engineering.

The ethical aspects of this study have been approved by the Macquarie University Human Research Ethics Committee (HREC). HREC approval No. 16055

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Appendix 13: Honey Bee Workshop Report

Workshop - Genetically engineering Honey bees

PH 22000: Opportunities for insecticide-resistant Honey bees for pollination security

On 19th July 2024 at the seminar room (G28), Applied Biosciences, Macquarie University

Attendees

	Name	Role and Institution	Participation (In-person/Online)
	Project team members		
1	Dr. Maciej Maselko	Project lead, MQ	In-person
2	Dr. Anu Jayaweera	Project postdoctoral fellow, MQ	In-person
3	Prof. Phil Taylor	Project team member, MQ	Excused
4	Dr. Mary Whitehouse	Project team member, MQ	In-person
6	Prof. Ken Cheng	Project team member, MQ	In-person
7	Prof. Grant Hose	Project team member, MQ	In-person
8	Prof. Anwar Sunna	Project team member, MQ	Online
9	A/Prof. Peter Davies	Project team member, MQ	In-person
10	Prof. Lisa Wynn	Project team member, MQ	Excused
11	A/Prof. Andrew McGregor	Project team member, MQ	In-person
12	Dr. Jonathan Symons	Project team member, MQ	In-person
13	Dr. Aditi Mankad	Project team member, CSIRO	In-person
14	Dr. Lucy Carter	Project team member, CSIRO	In-person
15	Dr. Elizabeth Hobman	Project team member, CSIRO	In-person
16	Dr. Loechel Barton	Project team member, CSIRO	In-person
17	Lesley Scanlan	Project team member, CSIRO	In-person
	Project reference group members		
18	Ashley Zamek	R & D Manager, Hort Innovation	Online
19	Dr. Emily Remnant	Lecturer and Academic Fellow, University of Sydney	In-person
20	Elizabeth Frost	Technical Specialist Bees, Department of Primary Industries (DPI), NSW	Online
21	Danny Le Feuvre	Chief executive officer, Australian Honey Bee Industry Council (AhBIC)	Online
22	Ray Hull	Executive Councilor NSW Apiarists' Association Inc. (NSWAA)	Online
23	Doug Purdie	Editor, Amateur Beekeepers Association (ABA)	In-person
24	Wayne Andreatta	Development Manager, Kooba Station, Darlington Point, NSW	In-person

Workshop: Genetically engineering Honey bees for beneficial traits

PH 22000: Opportunities for insecticide-resistant Honey bees for pollination security

On 19th July 2024 at the seminar room (G28), Applied Biosciences, Macquarie University

Morning program (10.00 a.m. to 12. 30 p.m.)

10.00 – 10. 15 – Welcome and introduction

10.15 – 10.50 – Potential technologies & regulations for Honey bee genetic engineering

10.50 – 11.10 – Tea break

11.10 – 11.40 – Honey bee pesticide sensitivity, resistance, resistant mechanisms, and pesticide recommendations

11.40 – 12.30 – Stakeholder & public perception on Honey bee genetic engineering

Lunch break (12.30 p.m. to 1.30 p.m.)

Afternoon Program for project team members (1.30 p.m. to 3.30 p.m.)

1.30 – 2.30 – Potential publications

2.30 – 3.30 – Future directions

Workshop: Genetically engineering Honey bees for beneficial traits

We held a one-day project workshop on Friday, 19th July 2024 to bring together our interdisciplinary research project team and the project reference group (PRG) members (Figure 1) to discuss the project findings and future directions. It was held in the seminar room, Applied Biosciences at Macquarie University with a total of 21 participants (16 in-person and 5 virtual).

Both project team members and PRG members participated in the morning session (10.00 a.m.– 12.30 p.m.) which was aimed to discuss the major findings of the project. After welcoming and self-introductions, the project leader Dr. Maciej Maselko, presented the findings on potential technologies & regulations for Honey bee genetic engineering which was followed by an active discussion with the participants. Next, the project post-doctoral researcher, Dr. Anu Jayaweera, presented the findings of the literature review on pesticide-Honey bee interactions and pesticide recommendation activity. The findings were discussed with the participants after the presentation. Finally, the CSIRO project team presented their findings on the different project activities they conducted; stakeholder perception of Honey bee genetic engineering, public perception of Honey bee genetic engineering and scoping access and benefit sharing (ABS) considerations followed by an active discussion. The session was very successful in communicating the major findings of the study with the team members and the project reference group which was comprised of the major industry stakeholders and subject experts.

After the networking session during lunch, the project team members gathered to discuss the potential publications that could be produced from the study and future directions. Team members discussed the possibility of publishing a perspective paper on Honey bee genetic engineering for beneficial traits to communicate the potential, requirements, technology, regulations, and socio-ethical environment. Also, they discussed how we can shape future research in a manner to address the major challenges of the Honey bee industry in Australia.

Figure 1. In-person participants of the project workshop

Front row (Left to right): *Dr. Elizabeth Hobman, Lesley Scanlan, Dr. Marry Whitehouse, Dr. Aditi Mankad, Dr. Emily Remnant, Doug Purdie, Dr. Anu Jayaweera*

Back row (Left to right): *Prof. Andrew McGregor, A/Prof. Peter Davies, Dr. Loechel Barton, Dr. Lucy Carter, Dr. Jonathan Symons, Prof. Ken Cheng, Wayne Andreatta, Dr. Maciej Maselko, Prof. Grant Hose*