Chapter

Diagnostic Radioentomology

Mark Greco

Abstract

Apart from the Neotropical flesh eating Trigona species, all existing bees are pollen feeding. Approximately 5% of these form colonies. In honeybees, colony health is evaluated by measuring seasonal hive weight increases and by visual inspections. However, rather than indicating good colony health, hive weight increases can be attributed to increases in stores from foragers feeding precociously during times of colony stress. Additionally, the subjective nature of these methods, leads to large errors. Visual inspections with stingless bee colonies are particularly invasive. Many bees die during inspections because they drown in spilt honey. Re-sealing the hive also kills bees, and the queen risks being squashed. Nevertheless, studies on bees continue as new, improved methods emerge to replace the old. Diagnostic Radioentomology is an innovative, non-invasive, imaging method for studying insects. Since development, it has been adopted by universities, synchrotron facilities and CT scanners to study morphology, physiology and behaviour of insects and has been hailed as the 'Gold Standard' for honeybee monitoring. In 2008, it was described as an emerging non-invasive technique for behavioural, evolutionary and classical biologists who choose to study animals without harming them. This chapter describes methods and includes examples of research conducted using Diagnostic Radioentomology.

Keywords: Diagnostic Radioentomology, bees, non-invasive imaging, X-rays, anatomy, physiology, behaviour, nest architecture, CT scanning, tomography

1. Introduction

Nearly all existing species of bee are pollen feeding, aculeate (with a stinger) Hymenopterans (membranous wings). There are only three species that do not collect or eat pollen and these are the necrophagic (eat dead or decaying flesh) Neotropical, Trigona species [1–4]. Approximately 5% of all bees are highly social. The highly social bees include species of bumble bees, honey bees and stingless bees. The other species are either semi-social, which occur in aggregations or are solitary. For a full description of these terms, see [5, 6]. With honeybees, colony health has been traditionally evaluated by simple visual inspections and/or by measuring changes in hive weights over time. However, these methods are estimates and subjective. Typically, beekeepers and scientists look for behavioural signs which indicate healthy individuals or colonies, where foragers are regularly bringing in resources. In contrast, they look for weak colonies, where there are usually fewer foragers. These foragers typically exhibit a more lethargic and less purposeful behaviour. However, there are also situations where increases in hive weights can be attributed to increases in pollen and nectar stores due to hyper-collection by foragers that exhibit precocious feeding during times of colony stress [7].

These colonies give the false impression that all is well. In these situations, an increase in hive weight can be misinterpreted as a sign of good colony health yet the colony could be under considerable stress from infection or disease rather than being in good health.

Visual inspections on colonies of stingless bees is particularly invasive because of the central location of the brood and many species are less than 3 mm in size. In stingless bees, colony health can be assessed by manually splitting the hive box apart to view internal structures and any evidence of queen activity [8]. Opening the hive for such inspections invariably damages honey storage pots. This causes honey to spill and many hundreds of bees die because many species are diminutive and will drown in their own spilt honey. Closing the hive after visual inspection also kills bees, and places the queen at risk of being harmed because they can be squashed in the process.

Therefore, the subjective nature of visual inspections and hive weight estimations often leads to errors when assessing colony health. Issues such as these were not so important in previous decades however, with the continued pressure from large scale agriculture, loss of bee habitat and the global increase in bee pathogens and pests [9] it has become paramount that new and more accurate methods are developed.

It is vital that behavioural, morphological and physiological studies on bees continue. However, because they have propensities to live in cavities and traditional methods are often invasive and prone to large errors, new methods for studying them are emerging. These new methods will add accuracy to current estimates on individual bees and colony health parameters which will, in turn, enable better solutions for scientists and beekeepers to improve bee health globally.

This chapter describes one new method termed 'Diagnostic Radioentomology' and includes examples of research conducted using this method.

1.1 Non-invasive imaging

On the 8th of November 1895, Wilhelm Conrad Röntgen (accidentally) discovered an image cast from one of his cathode ray generators. He later repeated the experiment by taking an X-ray photograph of his wife Anna Bertha Ludwig's hand **Figure 1**, which revealed the bones in her hand and her wedding ring on one of her fingers.

The photograph initiated great scientific interest in the new found radiation and because Röntgen did not know what type of radiation it was, he called it 'X-radiation', hence the modern term, X-rays. In general, non-invasive imaging is associated with X-rays or medical imaging, which is a non-invasive method for evaluating anatomy and physiology. Although it is now known that X-rays can be invasive (and can damage biological tissues) at the higher energies, the term non-invasive is based on the fact that, at the lower energies that are used in modern imaging methods, X-rays do not create any damaging biological effects.

1.2 Techniques available for non-invasive imaging

As a field of scientific investigation, non-invasive imaging constitutes a subdiscipline of biomedical engineering, medical physics or medicine depending on the context. Methods such as nuclear medicine use radioactive materials to diagnose or treat various pathologies and are generally considered to be invasive. Many of the techniques developed for non-invasive imaging such as X-rays, nuclear magnetic resonance imaging (MRI) and ultrasonography (U/S) also have industrial applications, although the energies used in industrial applications are extremely high

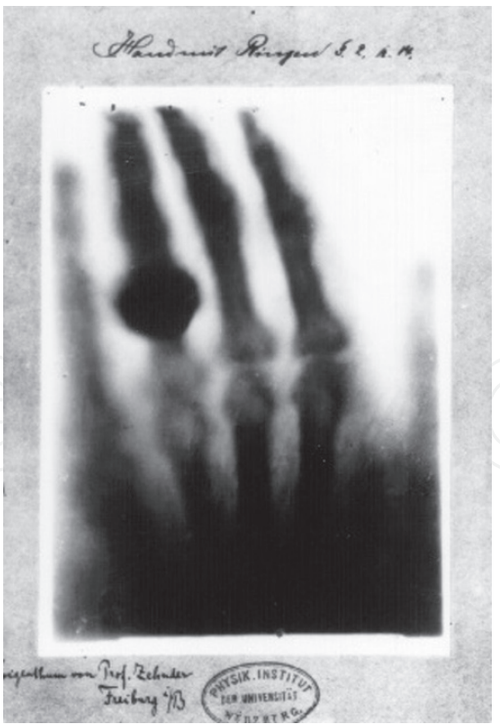

Figure 1.
First medical X-ray by Wilhelm Röntgen of his wife Anna Bertha Ludwig's hand. Wilhelm Röntgen [public domain].

and would be considered to be highly invasive for biological samples. In the case of U/S, the probe emits the beam which consists of ultrasonic pressure waves that return echoes from the various tissue interfaces. The echoes show details of the internal structures. U/S waves do not travel through large interfaces or air and thus limits its use in biological tissue. In the cases of X-rays and MRI, either X-radiation or a magnetic field respectively pass through the tissues to identify, separate and quantify different tissue types such as bone, cuticle, muscle or fat. In general, MRI is the best modality for discerning muscle or fat, is non-invasive and has very long image capture times whereas X-rays are better for discerning smaller structures and have much faster image capture times.

It is the ability to see smaller structures and the fast capture times that led to the development of Diagnostic Radioentomology (DR) to study insects non-invasively. DR is performed on insects using X-ray Computer Tomography Scanners (CT Scanners).

1.3 Diagnostic Radioentomology

The term 'Diagnostic Radioentomology' first came to be used in 2003 during a pollination experiment on the behaviour of the Australian stingless bees *Tetragonula carbonaria* and *Austroplebeia australis* [10]. The term was used because the new method is diagnostic, it uses X-radiation (radio) and is used for studying insects (entomology). **Video 1** gives a brief overview of the methods.

Therefore, DR became the term for an innovative method for studying insect morphology, physiology and behaviour using non-invasive imaging. Since its development, DR has been adopted by the Museum of Natural History in London, Universities, synchrotron facilities and research associations globally.

In 2008, DR was described by The International Bee Research Association (IBRA) as an emerging non-invasive technique for behavioural, evolutionary and classical biologists who need to study insects without harming them.

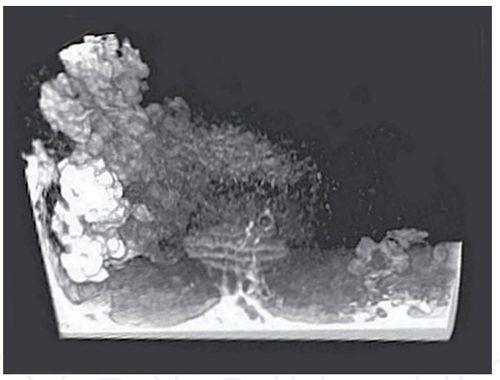

Figure 2.
*The first DR image of a bee colony (*Tetragonula carbonaria*) in a wooden box [11].*

Nowadays, synchrotron beamlines can completely scan and reconstruct 3D images in a matter of seconds and CT scanners can complete a 1-cm scan in as little as one-third of a second, and recent techniques have been developed to enable scanning software to produce 3D images such as in **Figure 2** and 4D movies and physical 3D models which can be downloaded at this address: http://www.radioentomology.com/.

It is generally accepted that for DR studies, the term MacroCT applies to the CT scanning of large items using human body CT scanners and that MicroCT applies to laboratory or Synchrotron CT scanners to study small items at the microscopic level. In recent years, DR has been adopted to visualise macroscopic characteristics of insects and their behaviour [11–15]. Also, with the improvements in spatial resolution and tissue differentiation that are occurring with MacroCT, conventional micro-focus and synchrotron based MicroCT, new methods for the non-invasive imaging of insects are emerging. For an overview of these methods see [16–22].

Historically, traditional methods for colony health, bee behaviour and the morphological classification of bees have been conducted on apiary hives and with the aid of observation hives and dissecting light microscopes. These techniques are, understandably, limited. The inspection of apiary hives disrupts normal bee behavior, observation hives offer only a view of one side of one frame within an entire hive, dissection obviously kills the bee and the use of light microscopy when used for amber inclusions [23–32], particularly with specimens preserved in opaque amber pieces [33, 34] are grossly limited by the specimens opaqueness. In [35] the authors attempted to address methods of examining insect inclusions within pieces of opaque amber and to supplement traditional light microscopy studies of transparent amber. Those researchers and [36–38] produced traditional radiographs which provided the first, albeit limited, steps toward enhanced visualisation of cryptic bee behaviour and fossil material. More recently, detailed information for the study of bees has been obtained with the use of scanning electron microscopy (SEM) as in [39, 40] and transmission electron microscopy (TEM) as in [41]. While SEM and TEM studies currently provide the highest level of detail, sample preparations are laborious and are often invasive to completely destructive [41]. SEM and TEM can be used for the investigation of amber inclusions as in [39–42] however, these methods are generally not suitable because they require destruction of the material. The development of non-invasive imaging methods such as DR, therefore, offers promise to scientists and beekeepers who need to preserve their specimens or observe behaviour non-invasively.

In 2013, DR was hailed as the 'Gold Standard' for honeybee monitoring [43] and the non-invasive path detailed in the following sections will demonstrate that DR is an ideal method which can be used to study bees and other insects in the most natural of settings. It is also important to mention that the results from the following

experiments can be directly applied to beekeeping husbandry to enhance modern beekeeping methods and enable beekeepers to play a more active role in improving global bee health.

2. Describing an ancient social bee in amber using DR

To help demonstrate the non-invasive, diagnostic advantages of DR it is worthwhile detailing the following experiment on one of the oldest bees known, *Proplebeia adbita*. In the following experiment, we examined the external and internal morphology of an Early Miocene (Burdigalian) stingless bee (Apinae: Meliponini) from the Dominican Republic using non-destructive X-ray microtomography analysis (MicroCT). The study shows the accurate reconstruction of features otherwise obscured or impossible to visualise without destroying/damaging the sample and enables diagnosis of the specimen as a new species of bee [44].

2.1 Materials and methods

Bees have several characteristic morphological attributes such as branched or plumose body setae and broadened metabasitarsi [5, 45]. The highly eusocial stingless bees, the Meliponini are within the corbiculate Apinae for example in [46–49]. In addition to extensive morphological and molecular data such as in [48, 50], the corbiculate apines belonging to a single group has been supported by studies investigating their internal anatomy. For example [39], noted that the proventricular morphology of Euglossini and Bombini consists of long columnar plates, triangular apices in Apini, while the Meliponini have slender and elongated plates. Accordingly, the proventriculus can be used as an important diagnostic structure for bee taxonomy [21], among a suite of other internal anatomical features [45]. The examination of such characters often requires considerable manipulation, dissection, sectioning or even complete destruction of the specimen. Thus, the practical application of such data is at times hampered by the methods employed. In the following experiment, the internal and external morphology of an ancient social bee trapped in amber using non-invasive and non-destructive DR techniques is described in detail.

2.1.1 About the bee

The bee selected for this experiment was collected from the La Bucara mine in the Dominican Republic. This stingless bee was trapped at the widest end of a semi-clear, brown piece of amber that contained many other inclusions **Figure 3**.

The posterior of the bee is at the extreme periphery of the piece's thick end, and the apices of both forewings have broken away from the sample over time.

Age estimates of Dominican amber vary considerably in literature nonetheless, most data indicate that the age of most Dominican amber, including the material in this study, is 16–19 Ma [45–47]. The sample had been polished prior to this study and therefore required no extra preparation.

2.1.2 Non-invasive imaging of the bee

Traditionally, the morphological classification of bees has been conducted with the aid of dissecting microscopes which use light. The technique is understandably limited when used for amber inclusions, particularly with specimens preserved in opaque pieces. Light microscopy was used in this experiment in an attempt to describe its limitations with opaque specimens.

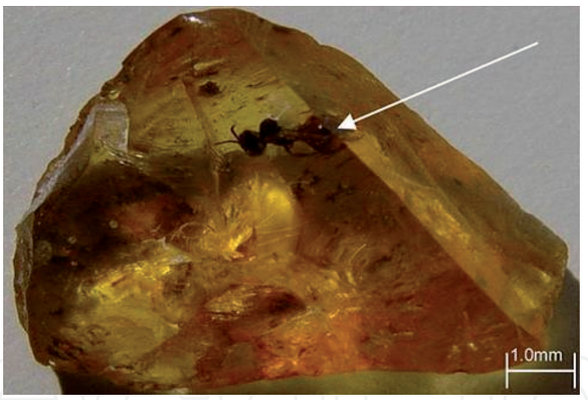

Figure 3.
A piece of semi-clear, brown amber from the Dominican Republic (Early Miocene: Burdigalian), with many inclusions. The stingless bee is at the widest end (arrow) [44].

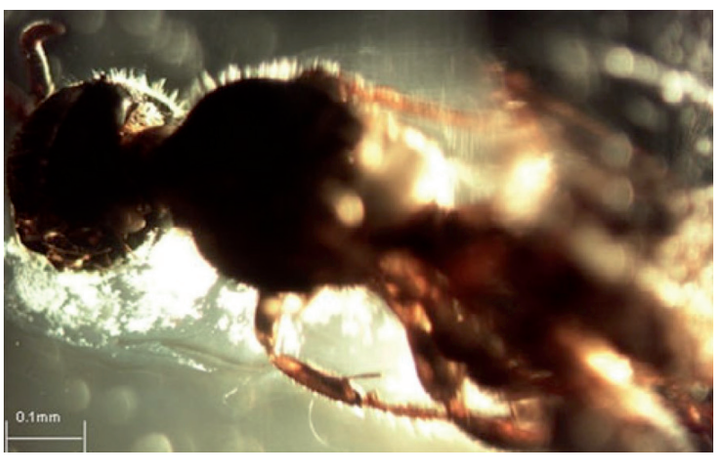

Figure 4.
Air bubbles, fractures and general thickness of the amber prevent adequate visualisation of the metasoma, posterior mesosoma and wings. Image taken under optimal optical conditions (increasing or decreasing light intensity further degraded image quality) [44].

2.1.3 Light microscopy

For light microscopy, the bee was viewed using a Leica MZ12 stereomicroscope, Leica Microsystems GmbH Ernst-Leitz-Strasse 17–37 35578 Wetzlar. The Leica MZ12 has distortion-free 109 eyepieces with a resolution of 375 line-pairs per mm.

Ideally, because of the thickness, air bubble inclusions and **Figure 4**, it would have been better to slice the fractures present in the amber piece prior to light microscopy examination. However, the sample was intentionally preserved to enable visualisation of the other biological inclusions using DR in future studies.

The colour of the bee was brown to dark-brown; however, it is possible that the bee was black when alive and that the cuticular melanin was altered over time. Moreover, newly moulted adult stingless bees are often lighter in coloration and so the more brownish colour of the specimen cannot be considered diagnostic. Gross external morphological features of the bee such as the chaetae, coxae, trochanter and tibiae were visible to about the level of the mesothorax. The air bubbles, fractures and general thickness of the amber piece prevented adequate visualisation of the more posterior including a lack of detail of the wings **Figure 4**. Increasing light intensity created image degradation due to light diffracting from cracks, air bubbles and generalised opacity of the amber. Decreasing light intensity made it difficult to optically visualise the bee's morphological features.

2.1.4 DR scanning

We need to keep in mind that this experiment was conducted during early testing phases for the potential applications of X-rays to insect morphology. Therefore, to assess their potential, three different apparatuses were used. X-ray MicroCT scans were performed a commercial benchtop system, a custom designed X-ray scanner and the facility for MicroCT available at the SYRMEP beamline of the Elettra Light Source Synchrotron in Trieste (Italy).

For a full description of these methods see [45]. Prior to scanning, the sample was placed in a 20.5 mm cylindrical sample holder between the X-ray source and the image detector **Figure 5**. This simple positioning procedure for the scanning phase of a DR examination can be adapted for all X-ray apparatuses. Scanning produces 2D images which are then converted to 3D images with specialised software.

As with light microscopy, gross external morphological features of the bee such as the Chaetae, the articulations of the coxae, trochanters, tibiae, and tarsi, including the corbiculae of the metatibiae and the broadened metabasitarsi, were well visualised in the 3D reconstructions **Figure 6**. In addition, gross internal structures, such as the brain (including details of its anatomical regions), direct and indirect flight muscles and a loaded rectum were accurately represented, **Figure 7**. **Video 2** (Ancient bee Proplebeia abdita trapped in amber approximately 20 million years ago) will highlight these features and also provide an understanding of what can be achieved during DR, 3D processing. Considering the specimen's age (16–19 Ma), the brain of this bee was

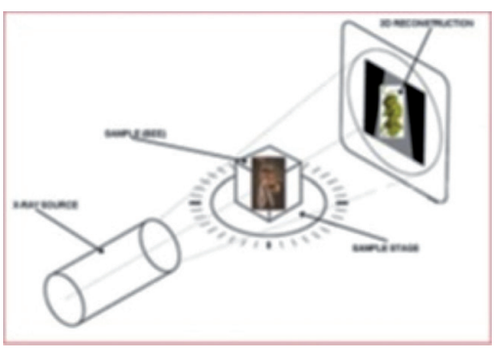

Figure 5.
A schematic diagram of sample positioning for DR. Essentially, the only preparation required is that the sample (bee) is positioned securely on the sample stage so that it remains motionless during the scan [44].

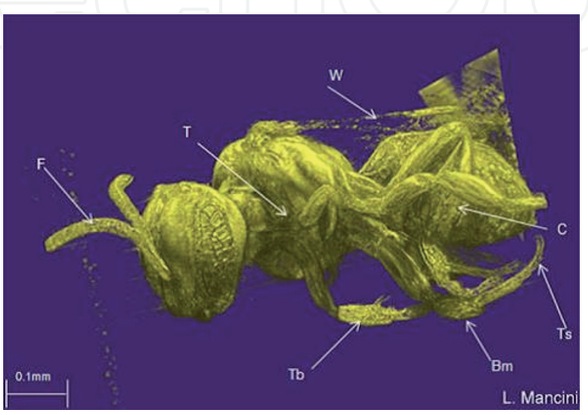

Figure 6.
Volume rendering image of the holotype worker of Proplebeia abdita in Early Miocene (Burdigalian) Dominican amber. Wings (W), flagellomeres (F), base of trochanter (T), tibiae (Tb), tarsi (Ts), the corbicula (C) of the metatibia and the broadened metabasitarsi (Bm) are all well visualised [44].

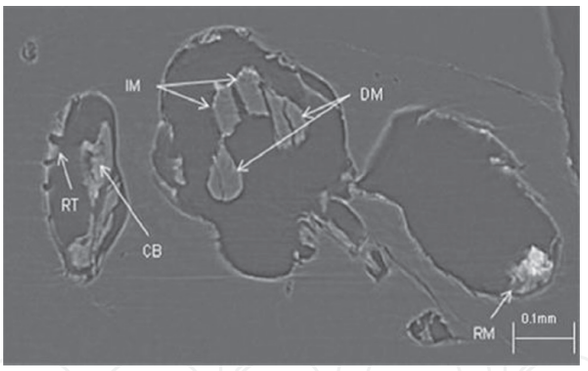

Figure 7.
A 2D view of Proplebeia abdita. Gross internal structures such as the central body of the brain (CB), retinal zone of the compound eyes (RT), direct (DM) and indirect (IM) flight muscles and a loaded rectum (RM) were accurately visualised [44].

particularly well preserved. The optic and antennal lobes were well reconstructed along with the dense central body and the protocerebral lobes. The retinal zone was also well preserved. Adhesion of the retinal zone to the proximal surface of the compound eyes and the corresponding region on the distal surface of the medullae was evidenced by a thin, dense film of tissue.

2.1.5 Discussion and results

Diagnostic Radioentomology permitted the comprehensive examination of this ancient specimen, where other methods were (in the case of light microscopy) and would be (in the case of SEM or TEM) found to be less reliable or unsuitable because of their limitations and/or destructive nature. The bee's anatomical characteristics were accurately assessed and precise morphometric measurements were performed with on-screen linear measuring callipers. As a result, details of a previously undescribed species, *P. abdita* Greco and Engel were described [44]. This experiment demonstrated that all three apparatuses were appropriate for accurately visualising the bee. Thus, entomologists can consider which facility would provide the best option for them. In addition to the application of DR to this particular bee, its more extensive use on historical type material (e.g. the holotype of *P. dominicana*, other amber preserved bees or even unique specimens of rare modern species) will permit a more complete characterisation of these bees and comprehensive comparisons between them and their modern counterparts. Improved anatomical understanding of these bees will greatly enhance phylogenetic reconstructions utilising paleontological data and potentially revise our paleoecological perspectives of early pollinators. It is hoped that by highlighting the utility of DR for characterising an ancient social bee that these techniques might be more broadly applied to social bee biology and anatomy, much in the tradition of [37] earlier applications of novel imaging methods and in the way it has been applied to the study of termites and living stingless bees [10, 13], as well as solitary bee species [11, 21].

3. Discovering new bee behaviour via DR methods

As mentioned above, DR offers new ways of studying known behaviours and features of bees. As it turned out in the following experiment, DR also introduced us to some new behaviours that were totally unexpected. We know that decision making in honeybees is based on information which is acquired and processed in

order to make choices between two or more alternatives [51]. These choices lead to the expression of optimal behaviour strategies such as floral constancy [52]. Optimal foraging strategies such as floral constancy improve a colony's chances of survival, however, there has been no research on decision making based on optimal storage strategies.

The following DR experiment describes how decision making in storer bees is influenced by nectar sugar concentrations and that, within 48 hours of collection, honeybee workers store carbohydrates in groups of cells with similar sugar concentrations in a non-random way. We can surmise that this behaviour, as evidenced by patchy cell distributions, would help to hasten the ripening process by reducing the distance between cells of similar sugar concentrations [52]. Therefore, colonies which exhibit optimal storage strategies such as these would have an evolutionary advantage and improved colony survival expectations over less efficient colonies and it is plausible that beekeepers could select colonies that exhibit these preferred traits.

3.1 Materials and methods

During an unrelated DR experiment, in an attempt to mark and track Varroa destructor within a honeybee colony, an unexpected pattern appeared on the honey comb images. Bees from several different colonies were fed marked and unmarked sucrose solution ad libitum. The bees then stored this sucrose freely without any restrictions.

3.1.1 The interesting discovery

Soon we discovered patterns that were previously unreported appearing on the honeycomb. Some colonies formed these patterns and some did not. **Video 3** (Flying through an apidea hive) and **Figure 8** show examples of these patterns.

Now, for these marking experiments, there are only two possible pathways that a cell can have only 50% sucrose solution or 70% sucrose solution in it. For a full description of these pathways see [52] and **Figure 9**.

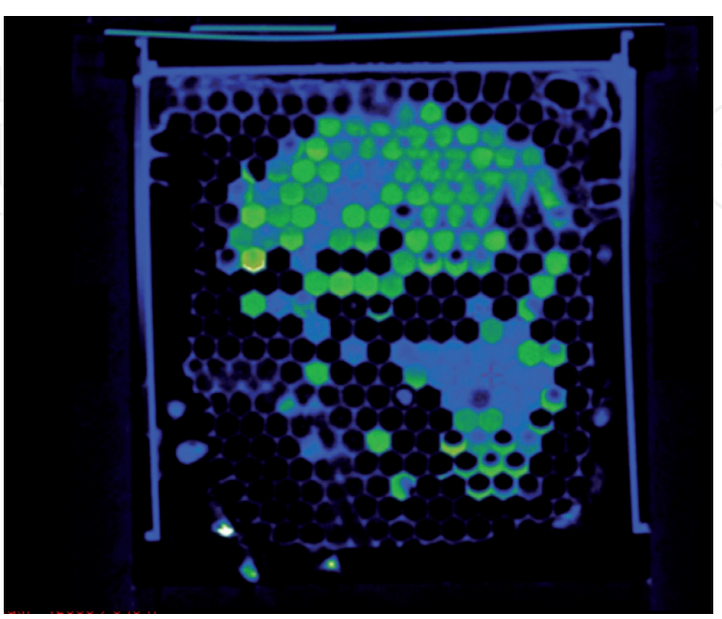

Figure 8.
A DR scan of a honey comb showing patchy distribution of cells containing honey with differing sugar concentrations. The marked 'green' cell patches contained only 70% sucrose syrup and the 'blue' unmarked cell patches contained only 50% sucrose syrup [52].

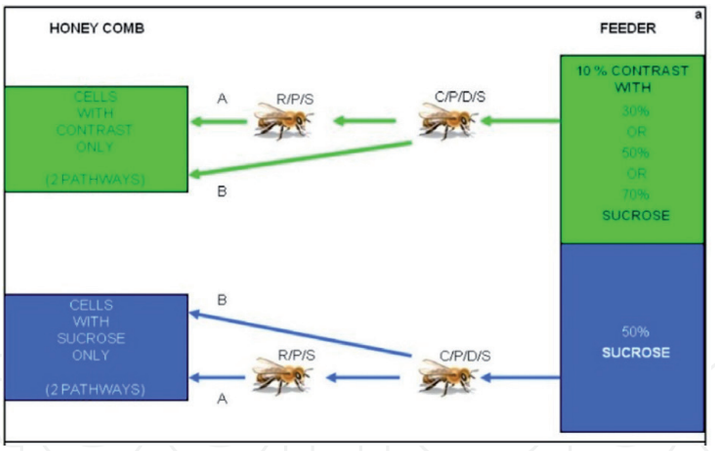

Figure 9.
A schematic diagram of the two possible pathways for either marked (green) or unmarked (blue) sucrose solutions to enter a cell unmixed [52].

3.1.2 Discussion and results

The data from the experiment, showed significant differences in the green/blue/mixed patch ratios. This implies possible behavioural influences on patch ratios. These behavioural influences are likely to be actions by bees making decisions on where to place honey of similar sugar concentrations. As in [53], it is likely that bees from some colonies deposit nectar according to contextual information, such as the location of other cells in the hive containing honey of similar sugar concentrations, and that bees from other colonies do not.

3.1.3 Some honeybee colonies are more efficient than others

These behaviours are influencing the honey storage patterns and are probably based on achieving optimal storage strategies. In this experiment the data indicate, as do those of [54–56], that honeybee colonies show a preference for storing honey according to sugar concentrations in the nectar. Therefore, one optimal storage strategy would be for storer bees to return to cell patches containing cells with similar sugar concentrations until all the cells in those patches were full. This strategy would reduce search time and thus increase storing behaviour efficiency. The DR images in this study clearly show that honeybees are producing these similar sugar concentration cell patches [52].

3.1.4 Can this behaviour help save the colony?

Storing honey in cell patches has benefits other than for those of ripening honey. Nectar collected by honeybees from different foraging patches (either natural or agricultural patches) will have differing sugar concentrations simply because the plants in these patches are growing under different local ambient conditions. In light of the current trend in global colony losses, it is crucial to mention here that the nectar from these plants might also contain other differences in constituents such as lethal or sub-lethal levels of toxins from agrichemicals and other sources [57–59]. Honey storage strategies, like those shown in this experiment, would be based on information such as sensing the sugar concentrations in incoming nectar and that of the ripening honey in the cells. Although it is not clear whether honeybees can detect agrichemicals in nectar or honey, as was shown in this experiment, they might store toxin-containing nectars separately from toxin-free nectars indirectly by way of sensing the nectars' different sugar concentrations. This would be an effective way to prevent all the honey from being contaminated and it would

reduce widespread toxin contamination in the hive and thus help prevent bee losses. The data from [60] also supports that honeybees store pollen with high levels of chlorothalonil separately in entombed cells which is a phenomenon similar to the patchy honey storage pattern behaviour shown in this experiment.

We should also consider that there are plants in several genera from at least 11 families [61, 62] that naturally produce nectar which contain constituents that have varying degrees of toxicity to bees and humans. There are also plants that produce toxic pollen [63, 64]. Forager bees bring these naturally occurring nectar and pollen back to the hive. In evolutionary terms, these naturally occurring toxins in pollen and nectar have provided the selective pressure for honeybees to improve their food storage strategies. Thus colonies that exhibit storage strategies which separate toxic from non-toxic food would have an evolutionary advantage over colonies whose bees store food indiscriminately [52].

3.1.5 How is this recent discovery relevant to beekeepers?

These storage behaviour efficiencies will have important implications for the long term survival of honeybee colonies. The data from the above experiment show that bees from some colonies exhibit efficient selective storage strategies and that bees from other colonies do not. These strategies have the potential to directly or indirectly separate toxins and pathogens with the hive. If beekeepers can determine which bees exhibit more effective storage strategies they will be able to select colonies that exhibit such preferred traits.

The DR experiments above and the in the following section were conducted using non-invasive, state of the art 'High Tech' Science. They have shown behaviour that is not apparent to the naked eye because humans cannot visually detect different sugar concentrations in honey comb cells. The next section will describe a simple method for beekeepers to select bees/Queens from one colony in preference over bees from another colony. This simple method will place beekeepers at the forefront of protecting their colonies at the grassroots level by improving honeybee husbandry.

3.1.6 A simple selection method for the modern beekeeper

The DR experiment above, indicated that honey bees show preferences when storing food and importantly, when feeding other bees via trophallaxis. As a secondary effect, some honeybees might also 'preferentially' spread pathogens/medication, which is contained in nectar/syrup, to other bees within their hive. The experiment below demonstrates that bees from certain colonies show 'preferences' while feeding other bees and that bees from other hives do not. The simple method, developed during the experiment for assessing food and pathogen transmission in bees, will help beekeepers to select and breed bees that have a higher propensity for spreading damaging pathogen or if required for spreading invaluable medication within a hive. This will help place beekeepers in a position to select more efficient bees and use their own breeding programs to help mitigate global bee declines, at the grass roots level.

3.1.7 Testing and selecting bees

To test whether bees show preference when feeding other bees, a simple segregation cup was developed, **Figure 10**.

Collection cups have been used previously to study bees [66] however this new system is the first system to segregate bees within the cup. Segregating bees within the cup enables one group of bees to interact bees from another section and prevents them from interacting with bees from a third section. The mode of

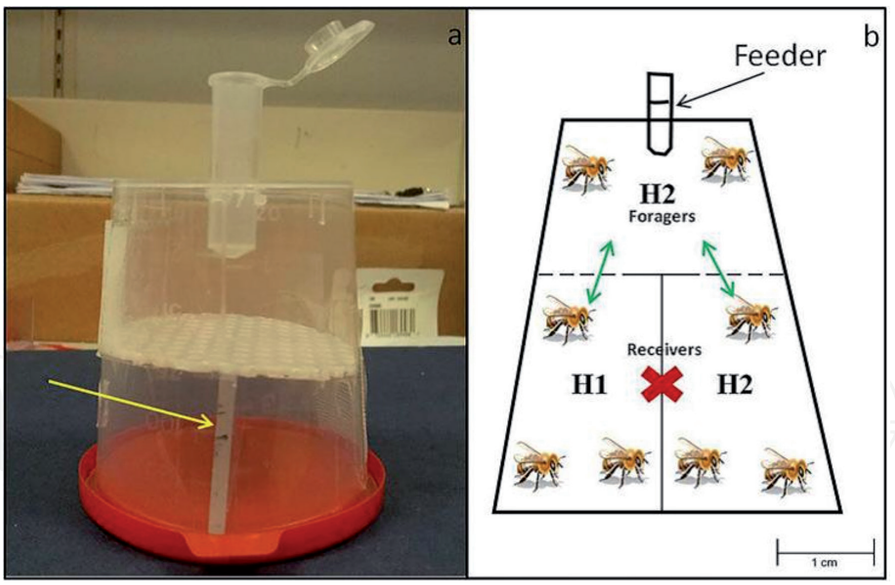

Figure 10.
(a) A segregation cup featuring the unique 'T' piece (yellow arrow) which separates three groups of bees. (b) The 'T' piece allows trophallaxis between bees in upper chamber and lower chambers (green arrows) and prevents all contact between bees in the two lower chambers (red cross) [65].

segregation is provided by the unique 'T' piece shown in **Figure 10**. The 'T' piece is inserted in the cup before the bees are collected. The horizontal portion of the 'T' piece is a 3 mm mesh and the vertical portion is solid Perspex. For a full description of the new segregation cup system see [65].

The segregation cup system enables beekeepers to collect one, two or three different groups of bees. **Figure 10** shows a schematic diagram of bees from hive 2 (H2) in the top section and bees from hive 1 (H1) and hive 2 (H2) in the bottom sections. The top section has a syrup feeder which means that the bees in the bottom sections can only receive food from bees in the top section through the mesh via trophallaxis. Bees that do not receive food from the top group commence starving within a few hours.

This simple system quickly demonstrates to the beekeeper which bees the top group prefers to feed via trophallaxis. The group of bees in the section that does not starve are the preferred bees.

3.1.8 Selecting bees for improved treatment

The recent finding that lithium chloride could be used as a medication added to syrup to treat Varroa destructor infestations [67] would be a good example of improved treatment by utilising better distribution of medication within the hive.

The new segregation cup system showed that when bees from H2 were placed in the top section, they had a significant trophallactic preference for H2 bees and tended to ignore H1 bees which subsequently starved. However, when bees from H1 were placed in the top section, they did not show a trophallactic preference. Bees from H1 fed both bottom section groups equally and bees from both H1 and H2 in the bottom sections survived for as long as the bees in the top section.

It has been established that, due to bees drifting in an apiary, there are commonly bees from other hives present in all hives. In fact, there can be as many as 38% at any given time [68].

As gauged by the level of H1 bee mortality [65], H2 foragers preferentially fed H2 bees over H1 bees. H1 type bees showed fewer preferences and will feed more

bees within the hive trophallactically. Therefore, beekeepers can choose to breed from H1 'type' bees that will spread medication in syrup via trophallaxis to more bees within the hive. After breeding these colonies, beekeepers can then assess whether there are also greater survival rates with those colonies.

3.1.9 Selecting bees for improved health

In light of the current trend in global colony losses, it is crucial to mention here that nowadays, nectar brought in by forager bees might also contain constituents such as lethal or sub-lethal levels of toxins from agrichemicals or pathogens [9]. Although it is not clear whether honeybees can detect agrichemicals or pathogens in nectar, H2 type bees would pass on toxins/pathogens that are in nectar preferentially via trophallaxis. This would be an effective way to prevent up to 38% of bees [68] receiving toxins/pathogens and it would reduce widespread contamination in the hive and thus help prevent bee losses. In addition, there are plants in several genera from at least 11 families [61, 62] that naturally produce nectar which contain constituents that have varying degrees of toxicity to bees. Foragers bring these naturally occurring nectars back to the hive. Thus colonies containing H2 type bees that show more preference would have an evolutionary advantage over bees such as those with H1 type bees.

During times when the environment is less conducive to colony health, such as when agrichemicals are used on crops or when EFB, AFB and Nosema are prevalent, H2 type bees would bring these back in the nectar and spread them within the hive via trophallaxis with less efficiency than H1 type bees because H2 type bees show preferences for H2 bees only. H1 type bees show fewer preferences and are likely to feed all bees within the colony via trophallaxis. This behaviour will spread the incoming nectar more rapidly throughout the colony. The new segregation cup system can test for this behaviour and beekeepers can select H2 type bees for better colony health/survival over H1 type bees.

It is important to mention that on some occasions beekeepers might select H1 type bees and on other occasions they might want to select H2 type bees. The new segregation cup system can enable beekeepers to make these choices. Once the choice is made, beekeepers can then develop their own breeding programs by breeding queens from those colonies to propagate the desired behaviours in subsequent colonies.

4. Conclusions

This chapter described new DR methods and how they can help beekeepers make informed choices. The chapter detailed how current knowledge can be studied in novel ways non-invasively and how DR methods brought to light unexpected new knowledge that is beneficial to the preservation of honeybees globally.

Descriptions and examples were given to describe DR at the cutting edge of state of the art technology. DR methods helped to discover a new species, previously undescribed bee behaviour and a new selection system to help beekeepers improve their hive management knowledge.

DR is an applied science that has a direct impact on modern beekeeping. Although it is very high tech, it also provides links to new methods which help beekeepers make their own informed decisions to improve bee husbandry methods and colony health at the grass roots level.

Video materials

The video materials referenced in this chapter are available at: https://bit.ly/2BV3DnO.

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Author details

Mark Greco
Charles Sturt University, Wagga Wagga, Australia

*Address all correspondence to: radioentomology@gmail.com

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