Recapping and resistance in *Apis mellifera* against the parasite *Varroa destructor*: the queen swap experiment

Georgiana Morgan Webb

MPhil Thesis 2022

School of Science, Engineering and Environment

The University of Salford

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ACKNOWLEDGEMENTS

I would like to thank Professor Stephen Martin for his expert guidance and encouragement. Thank you for organising and designing the present research project and other fundamental research trips. I am grateful that you have helped me to expand my knowledge and interest in this excellent subject.

I would like to thank Isobel Grindrod for helping me to acquire new practical techniques, assisting with the collection and analysis of brood samples and for helping me to understand complex ideas and theories. Thank you for your constant and valued support during this research.

I would like to thank Rhona Toft for all the help that she provided when designing and implementing the queen swap experiment. Thank you for helping us to collect brood samples and for providing detailed information about how the colonies were managing.

I am grateful to all of the beekeepers who have kindly provided brood samples for this study. Thank you for your time and interest in this research.

I would like to profoundly thank the British Beekeepers Association (BBKA) for funding research trips to Pershore, Hawaii and Cuba. We now have an abundance of vital data that would not be possible without their help.

DECLARATIONS

Chapter 1: General introduction

Written and researched by Georgiana Morgan Webb (G. M. W.) with revisions from Stephen John Martin (S. J. M.).

Chapter 2: The queen swap experiment; swapping the queens of mite non-resistant colonies with the queens of resistant colonies

The queen swap experiment was conceived and devised by S. J. M. with support from Rhona Toft (R. T.); R. T. assisted by recording the ongoing experiment; G. M. W., Isobel Grindrod (I. G.) and S. J. M. assisted with field work, collected data and analysed brood samples in the laboratory.

Chapter 3: Understanding the potential relationship between the recapping and infestation levels in 14 apiaries

R. T. assisted with field work and helped to provide further samples from various other apiaries; G. M. W., I. G. and S. J. M. aided with field work, collected data and analysed brood samples in the laboratory.

Chapter 4: Recapping and the targeting of brood cells: why are cells targeted for recapping behaviour?

Brood samples were collected by G. M. W., I. G., S. J. M. and R. T.; analyses of the brood samples were undertaken by G. M. W. and I. G.

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Written and researched by G. M. W. with revisions from S. J. M.

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Appendix 2: BBKA News Article 2021

Written by G. M. W., I. G. and S. J. M.

KEY TERMS

Term	Definition
Recapping	A behaviour which consists of the targeting, uncapping (opening) and recapping of brood cells.
Hygienic behaviour	The targeted uncapping of wax capped cells and removal of dead or diseased brood.
Suppressed mite reproduction (SMR)	Mite non-reproduction caused by the targeted removal of successfully reproducing mites.
Varroa sensitive hygiene (VSH)	A form of hygienic behaviour that specifically targets and removes brood infested by *Varroa* mites.

GENERAL ABSTRACT

Honey bee colonies worldwide are currently facing an unrelenting threat to their survival from the parasitic mite *Varroa destructor*. Although, resistance behaviours; such as recapping; appear to be effective by reducing mite infestations. In our first study, named the queen swap experiment, the queens of mite resistant colonies were swapped with the queens of mite non-resistant colonies. We found that resistant queen colonies displayed higher average recapping levels in comparison to non-resistant queen colonies. Though, the ratio of recapped cells to non-infested normal cells (non-recapped cells) were not significantly different between the resistant and non-resistant queen colonies. The total infestation levels were higher in non-resistant queen colonies in contrast to resistant queen colonies, but with no significant difference. A sugar shake test revealed that non-resistant queen colonies had higher infestation levels in comparison to resistant queen colonies, yet the results indicated no significant difference. Likewise, the mite fall test revealed that there was higher infestation levels in non-resistant queen colonies as opposed to resistant queen colonies, again no significant difference was found. In the second study, we established that of the 14 UK apiaries, nine apiaries displayed a higher average recapping levels in comparison to the average infestation levels. Whereas, five apiaries revealed that these colonies had higher average infestation levels respectively. However, no significant difference was found between the average recapping levels and infestation levels of all 14 apiaries. In our third study, we found that the recapped diameter of infested cells was significantly larger in comparison to that of non-infested cells. Finally, we revealed a significant difference between the lower levels of recapping in mite non-reproductive cells in comparison to the higher levels of recapping observed in mite reproductive cells. Due to these results, it must be considered whether there is either an indirect or direct relationship between recapping and infestation levels. Accordingly, recapping could be deemed to be a detection strategy with the purpose of detecting infested cells. If recapping is found to be an important and heritable trait in terms of resistance to *Varroa* then the measurement of the recapping trait in colonies may predict whether a colony could survive high mite infestations. Subsequently, it is important that the role of recapping should be further explored in this research field.

CHAPTER 1

General Introduction

The parasitic mite *Varroa destructor* (referred to as *Varroa*) is undoubtedly the greatest cause of mortality in honey bee (*Apis mellifera*) populations worldwide (Boecking & Genersch, 2008; Martin et al., 2020). *Apis mellifera* provide vital pollination services for a wide range of food crops, owing to their wide distribution and aptitude for foraging (Calderone, 2012; Hung et al., 2018). Existing beekeeping practices; such as the movement of queen bees for breeding (Seeley & Smith, 2015) and the maintenance of densely packed hives (Lodesani & Costa, 2003); have facilitated the spread of the parasitic mite (Hawkins & Martin, 2021). At present, only regions of Northern Europe, including small isolated islands, remain mite free (Guichard et al., 2020a). Opinions may be divided on the best way to overcome high mite infestations but, without any appropriate action, there is evidence to suggest that a *Varroa* infested colony is predicted to be lost in under two years (Traynor et al., 2020; Moro et al., 2021).

Varroa adapted from its original host, the Asian honey bee (*Apis cerana*), in the 1950's to exploit another host, *A. mellifera* (Oldroyd, 1999; Grindrod & Martin 2021a). Poignantly, *A. mellifera* has struggled to survive high mite infestations as opposed to other honey bee species that appear to be successful in reducing mite infestations (Whitfield et al., 2006; Martin et al., 2020; Traynor et al., 2020). For instance, *A. cerana* boasts multiple defence mechanisms that provide mite resistance (Lin et al., 2016; Lin et al., 2018; Traynor et al., 2020). Likewise, the Africanized honey bee, a hybrid between *A. m. scutellata* from South Africa and East Africa, and European races such as *A. m. ligustica* or *A. m. iberiensis* (Martin et al., 2020) have developed a natural resistance to the mite (Rosenkranz, 1999). However, *A. mellifera* have largely failed to develop the same natural resistance (Fries, Camazine & Sneyd, 1994). Therefore, it is imperative for the future survival of *A. mellifera* that research be conducted to ascertain how this species can reduce deadly mite infestations.

To clarify, the mite does not directly cause the mortality of bees. *Varroa* primarily feed on bee haemolymph and, as more recently discovered, the fat of honey bees which may be essential for mite reproduction (Ramsey et al., 2019; Traynor et al., 2020). By feeding from the pupae, *Varroa* mites increase the susceptibility of honey bees to Deformed Wing Virus

(DWV), which has become an emerging threat with high infestation levels linked to an increased prevalence and ferocity of the virus (Sumpter & Martin, 2004; Brettell et al., 2017; Wagoner et al., 2019; Le Conte et al., 2020). Deformed wings, paralysis and the death of emerging bees characterises this harmful virus (Iqbal & Mueller, 2007; Roth et al., 2020).

Reassuringly, behavioural traits expressed by honey bees can provide resistance against the parasitic mite (Mondet et al., 2020a). The main recognised resistance mechanisms are as follows: recapping, hygienic behaviour, *Varroa* sensitive hygiene (VSH) and suppressed mite reproduction (SMR) (Büchler et al., 2020a). Notably, certain resistance behaviours appear to be more effective than others. Recapping behaviour is purportedly an important aspect of colony survival in *A. mellifera* populations (Oddie et al., 2018a). Recapping involves the worker honey bees partially removing the silk/wax cap of the cell containing a developing pupa, then resealing the cell without removing the pupa (Martin et al., 2020; Mondet et al., 2020a). The act of creating a small hole in the cell cap may allow a detailed investigation of the cell contents (Martin et al., 2020). Worker bees may be able to distinguish a particular stimulus emanating from the mite infested cell and bees that are less sensitive to this stimuli may recap the cell before the mite is detected, hence the infested brood remains in the cell (Martin et al., 2020).

Interestingly, recapping rates have been found to be elevated in *Varroa* infested colonies in previous studies (Martin et al., 2020; Grindrod & Martin, 2021b). What is more, recapping has been observed to decrease mite population growth (Buchegger et al., 2018) and has been linked to a reduction in mite reproductive success (Oddie et al., 2018a). Therefore, recapping behaviour is thought to be expressed in response to the level of mite infestations (Martin et al., 2020; Hawkins & Martin, 2021). Recapping has been observed to be targeted towards mite infested cells (Oddie et al., 2018a; Martin et al., 2020). The diameter of the recapped hole could provide insight into how the underlying mechanisms of recapping behaviour function. Since the recapped diameter of infested cells is found to be larger than that of non-infested cells, as observed in a study by Grindrod and Martin (2021b), this finding provides insight into how bees can identify infested cells.

Alternative research disputes that recapping behaviour alone directly decreases mite infestations. A hypothesis by Martin et al. (2020) determined that recapping may indicate colonies that are capable of becoming resistant rather than the principal cause of resistance

itself. There is little evidence to suggest that recapping behaviour alone is adequate enough to prevent high infestations of *Varroa* mites. Not to mention, there are differing opinions regarding the importance of recapping and whether it should be considered to be an independent resistance behaviour (Oddie et al., 2018a). Despite uncertainty surrounding the exact role of recapping, it may still be a vital behaviour leading to mite resistance (Martin et al., 2020).

Hygienic behaviour is a resistance mechanism performed by worker honey bees to eradicate pests and diseases from the colony (Rothenbuhler, 1964). The term 'hygienic behaviour' is used to define the act of worker bees detecting and later removing dead, diseased and parasitised pupae from wax capped cells (Spivak et al., 2003; Harbo & Harris, 2009). This trait is thought to be inherited rather than learned (Arathi, Burns & Spivak, 2000). Hygienic worker bees detect olfactory cues that permeate through the cell cap from dead or diseased brood (McAfee et al., 2017; Traynor et al., 2020). Crucially, hygienic brood removal disturbs the reproductive cycle of the foundress mite which in turn increases the amount of non-laying female foundresses in the population (Martin et al., 2020; Hawkins & Martin, 2021). However, there is a lack of information about the cues that trigger behavioural defences such as hygienic behaviour (Mondet et al., 2021). Although, research by Mondet et al. (2021) identifies six naturally occurring chemical cues that are found to play a role in the triggering of hygienic behaviour. Worker bees appear not to be sensitive to abnormal olfactory cues, rather they are sensitive to 'unhealthy brood odours' such as dead pupae (Wagoner et al., 2021). Hygienic bees are either highly sensitive to these odorants or they investigate a higher number of suspicious cells (Martin et al., 2020). In addition, *Varroa*-sensitive hygiene (VSH) is another resistance behaviour, similar to hygienic behaviour, although VSH bees specifically target and remove brood infested by *Varroa* mites (Traynor et al., 2020). All worker bees appear to detect different compounds, although research suggests only VSH bees can discriminate between odorants that emanate from infested cells and the cells that are absent of the mite (Mondet et al., 2021).

The resistance behaviour suppressed mite reproduction (SMR) was first described by Harbo and Harris (1999) as a characteristic which causes the non-reproduction of mites, wherein honey bees target the cells of successfully reproducing mites (Harbo & Harris, 2005). To clarify, mites that either 1) produce no offspring, 2) produce only males, 3) produce

offspring too late or 4) die before they can reproduce (Harbo & Harris, 1999) are all examples of SMR. SMR was later described to be a resistance trait expressed by honey bees that can be selected for in breeding programmes (Mondet et al., 2020b). Presently, the core mechanisms behind the SMR trait are not yet clear or well researched, but this trait is implied to reduce mite population growth (Mondet et al., 2020b).

Currently, the response of researchers and beekeepers alike is directed towards chemical treatments to reduce mite populations (Mondet et al., 2020a). Therefore, only the most intensively treated colonies thrive (Büchler et al., 2020b). Importantly, non-chemical treatment methods are not currently popular amongst beekeepers because they are time consuming and are considered less effective (Büchler et al., 2020b; Moro et al., 2021). Although, the acaricides commonly used often vary in efficacy, potentially contaminate hive products and *Varroa* has been recorded to have developed resistance to certain substances (Milani & Vedova, 2002; Dietemann et al., 2012; Moro et al., 2021). In addition, mite control treatments may restrict the natural evolutionary relationship between host and parasite and prevent coevolution towards a stable relationship (Neumann and Blacquière, 2016; Mondet el al., 2020a; Traynor et al., 2020).

Selective breeding programmes have the advantage to enhance new resistance traits. The queens and colonies that exhibit desirable resistance traits are chosen for breeding programmes (Uzunov, Brascamp & Büchler, 2017). The selection for the hygienic behaviour trait in selective breeding programmes is a common method utilised to reduce other means of mite treatment (Martin et al., 2020). For instance, colonies that have been selected for high hygienic behaviour are more likely to survive mite infestations in comparison to low hygienic colonies (Scannapieco et al., 2016; McAfee et al., 2018). Arguably, selective breeding programmes can be considered an effective long term solution if they are able to enhance resistance traits (Guarna et al., 2017). However, the demands of breeding programmes to prevent disease in honey bee populations may conflict with commercial business demand (Le Conte et al., 2020). Additionally, it is unclear whether recapping is a heritable trait (Guichard et al., 2021b). If this is accurate, recapping may not be a viable trait for selective breeding programmes to focus on.

In the past, natural selection has favoured traits that inhibit the reproductive success of mites including hygienic behaviour, which has been linked with recapping in multiple studies

(Oddie et al., 2018a; Guichard et al., 2021b; Oddie et al., 2021). The stable host-parasite relationship that exists between *A. cerana* and the *Varroa* mite is clearly not yet present in the majority of *A. mellifera* populations (Oldroyd, 1999; Mondet et al., 2020a). *A. cerana* has co-evolved with *Varroa* over millions of years, although the presence of more recent naturally surviving *A. mellifera* populations indicate that an equilibrium can be achieved in a period of less than 100 years (Martin et al., 2020; Le Conte et al., 2020). In Africa, Europe and North America, there are *A. mellifera* populations that are reported to be naturally resistant to the mite (Le Conte et al., 2020; Martin et al., 2020), but most importantly, recapping has been found to exist in naturally surviving *A. mellifera* populations (Oddie & Dahle, 2021). Naturally *Varroa* resistant bees are essential for the genetic diversity of this species (Mondet et al., 2020a). However, attempts to increase populations of naturally resistant honey bees have failed on a wider scale (Mondet et al., 2020b). The ideal solution in regards to controlling mite populations is often debated, whether selective breeding is the answer or indeed permitting colonies to develop natural resistance to the mites is more effective.

Due to controversy surrounding the importance of recapping, the main aim of this thesis is to make further inferences about the role of recapping in honey bee colonies. Of note, this thesis comprises of three separate research studies that all pertain to the resistance behaviour, recapping. In Chapter 2, a queen swap experiment was designed to observe the recapping and mite infestation levels when the queens of *Varroa* naïve colonies (that do not express recapping behaviour) are swapped with the queens of *Varroa* resistant colonies (that are known to express recapping behaviour). Chapter 3 investigates the recapping and infestation levels of 17 UK apiaries to develop an understanding about the dynamics of recapping behaviour. Chapter 4 consists of two independent studies that focus on the process of recapping and how brood cells could be targeted for recapping. The first study involved comparing the diameter of non-infested recapped cells to the diameter of infested recapped cells. The second study was conducted to determine whether mite non-reproductive cells or mite reproductive cells are targeted by recapping. In Chapter 5, the findings of the three studies are summarised and mite resistant behaviours and mechanisms are discussed along with a brief discussion involving the measurement of resistance traits.

CHAPTER 2

The queen swap experiment; swapping the queens of mite non-resistant colonies with the queens of resistant colonies

Abstract

Varroa destructor, the parasitic mite, is currently causing the decline of honey bee populations across the Northern Hemisphere and remains the largest threat to apiculture. Recapping is a behaviour performed by honey bees, involved in resistance against *Varroa*. The main aim of this study was to observe how recapping levels respond after the queens of mite resistant and non-resistant colonies were swapped. This would involve a queen swap between mite naïve colonies from the Island of Colonsay and the high recapping mite resistant colonies from Wadborough, Worcestershire. More specifically, nine non-resistant Colonsay queens were swapped with eight resistant Wadborough queens throughout the course of the experiment. The results of the queen swap experiment present that Wadborough queen colonies displayed a higher average recapping level in comparison to Colonsay queen colonies. The ratio of recapped cells to non-infested normal cells was not significantly different between the Wadborough and Colonsay queen colonies. Notably, the offspring of naïve mite non-resistant queens were capable of recapping when exposed to *Varroa*. The average infestation levels of Wadborough queen colonies were found to be lower than the infestation levels of Colonsay queen colonies, yet the results revealed no significant difference. A sugar shake test revealed that Colonsay queen colonies had higher infestation levels in comparison to Wadborough queen colonies, although no significant difference was found. The mite fall test revealed a similar result with Colonsay queen colonies displaying a higher infestation levels, however no significant difference was found. It is vital, for future research to progress, that there is a greater understanding surrounding the origin of recapping, whether it be a heritable, innate behaviour, and how recapping may affect the infestation levels of colonies, either directly or indirectly.

Introduction

Over the past seven decades the parasitic mite *Varroa destructor* (known simply by *Varroa*) has caused the death and devastation of honey bee (*Apis mellifera*) colonies as it spread worldwide (Rosenkranz, Aumeier & Ziegelmann, 2010; Hawkins & Martin, 2021). Current research puts forward the view that honey bees disrupt the reproductive cycle of *Varroa* and cause lower fecundity of the foundress mite through the resistance behaviour known as recapping (Oddie, Neumann & Dahle, 2019; Mondet et al., 2020a). Recapping involves the worker honey bees open a brood cell containing the developing pupa and subsequently reseal the cell with wax, without removing the pupa (Hawkins & Martin, 2021). Both recapping and the reduced reproductive success of *Varroa* mites seemingly contribute to the long-term resistance and survival of *A. mellifera* colonies in the UK (Hawkins & Martin, 2021). Furthermore, recapping has been linked to lower mite reproductive success in naturally surviving populations (Oddie & Dahle, 2021). Concurrently, the near absence of the recapping trait in *Varroa* naïve colonies is perhaps evidence enough that recapping is specifically associated with mite infestations (Hawkins & Martin, 2021). Previous studies have also commented that recapping behaviour should be regarded as a detection strategy, indirectly involved in the reduction of mite reproductive success (Martin et al., 2020; Grindrod & Martin, 2021a; Hawkins & Martin, 2021).

The queen swap experiment was designed to observe the recapping and infestation levels in colonies when the queens of *Varroa* naïve colonies were swapped with the queens of *Varroa* resistant colonies. The levels of non-infested normal cells (non-recapped cells) would also be analysed to act as a control. It is unknown whether recapping is a heritable trait; an innate behaviour passed from queen to her offspring. For example, hygienic behaviour is an independently heritable trait, targeted in selective breeding programmes (Maucourt et al, 2020). To investigate the levels of recapping and mite infestations in colonies, we swapped the non-resistant queens from an apiary on the island of Colonsay, in the Inner Hebrides of Scotland with resistant queens in Wadborough, Worcestershire, England. The apiary in Wadborough was the location were the queen swap experiment occurred. To elucidate, we introduced mite naïve Colonsay queens into naturally mite resistant Wadborough queen-less colonies (hereafter referred to as Colonsay queen colonies). Simultaneously, we introduced Wadborough naturally resistant queens to queen-less mite naïve Colonsay

colonies (referred to as Wadborough queen colonies). We predicted that the recapping levels of Wadborough queen colonies would increase over the course of the experiment. This would indicate that recapping could be an innate behaviour because the young brood produced by the resistant queen could inherit the recapping trait (Blacquière & Panziera, 2018). Likewise, we predicted that the Colonsay queen colonies would suffer from higher infestations because they had not been exposed to the parasitic mite before. The observation of recapping levels and *Varroa* mite infestation levels in the queen swap experiment may inspire further questions about whether recapping is a viable trait to help protect colonies against *Varroa* infestations and help to deduce more about the role of this resistance trait in colonies.

Methods

A preliminary assessment of recapping levels concluded that the Colonsay colonies were naïve to *Varroa* and did not express the resistance behaviour: recapping. In contrast, Wadborough colonies expressed high levels of recapping and appeared to have resistance to the mite.

Collection of brood samples

On 02/09/20, the queen swap experiment began at the Wadborough apiary in Worcestershire. The queens were provided with a colony number and either a Y-Yellow (2017), R-Red (2018), G-Green (2019), B-Blue (2020) or W-White (2021) identification, which represented the birth year of the queen bee from each colony. To begin, six Colonsay queens were introduced to six queen-less Wadborough colonies. Concurrently, four Wadborough queens were introduced to four queen-less Colonsay colonies. The six new Colonsay queens (Colonies: Colonsay 1B, 2B, 3B, 4B, 5B & 6B) were reported to be accepted by their respective colonies on 27/09/20 (now referred to as Colonsay queen colonies). The same could be said for the four Wadborough queens (Colonies: 8B, 30B, 52B & 74W) that were accepted by their respective Colonsay queen-less colonies (referred to as Wadborough queen colonies). Colonies that were split into two nucs (30B and 74G) or re-queened (52B) after swarming were allowed to mate naturally in the Wadborough apiary to produce daughter queens. Of note, Wadborough colony 8B swarmed and under floor hived, this colony later produced a granddaughter (16W).

The colonies that accepted the queen were monitored and, if obtainable, sealed brood samples (sections of the brood comb) were collected. Brood cells that were thought to contain stretched larvae were excluded from the sample collection. This is because the silk cocoon has not been spun at this pupal stage of development, therefore recapping is not detected (Spivak & Danka, 2020). Likewise, if wax moth damage was visible then brood samples were not collected because the bees may recap these cells, leading to an overestimation of results (Villegas & Villa, 2006; Martin et al., 2020). However, pupae at the white eyed stage of development (Dietemann et al., 2013) and above were included. All collected brood samples were freeze-killed and stored in a minus 80°C freezer before examination in the laboratory at a later date.

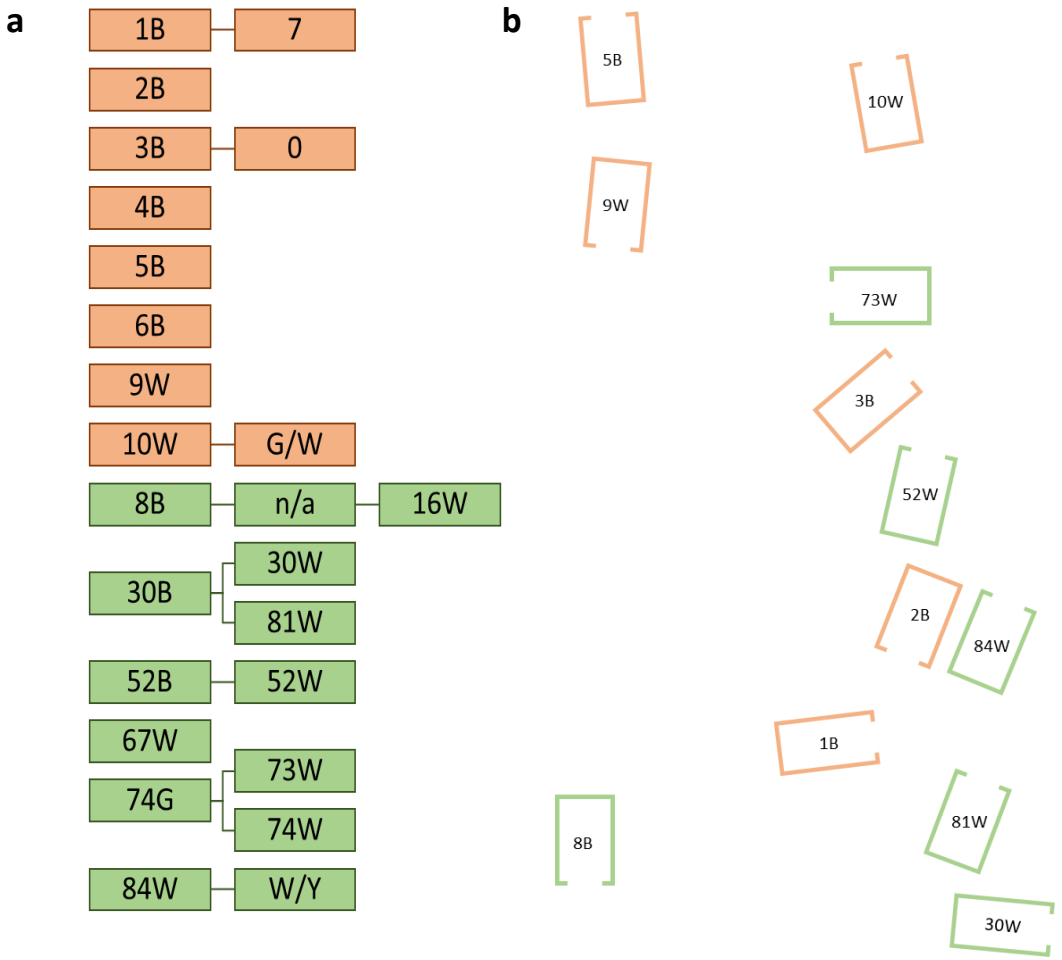

Figure 1. a) A family genealogy illustrating the relationships of Colonsay queen colonies (orange) and Wadborough queen colonies (green). b) A plan of the queen swap experiment hives at the Wadborough Apiary, Worcestershire 2021. To include

Wadborough queen colonies (green) and Colonsay queen colonies (orange). The hive entrance is represented by the gap in the hive diagram.

The queen swap experiment continued into 2021 and samples were collected up until the October of that year. Colony 6B had suffered from a failing Wadborough queen and after an attempted supersedure (the replacement of an old queen by a young superior queen), the colony was lost and removed from the experiment. On 13/07/21, three queen-less and three queen right nucs (5 frame hives) arrived from Colonsay to the Wadborough Apiary. Subsequently, the three Colonsay queens were introduced to three queen-less Wadborough colonies (0, 9W & 10W) and four Wadborough queens were introduced to four queen-less Colonsay colonies (52W, 67W, 74W & 84W). However, two of the introductions (0 and 9W) were not successful and Colonsay 0 was removed from the trial. Colonsay 9 was allowed to mate in Wadborough naturally with local drones and then continued in the trial. The relationships of colonies in the queen swap experiment and a plan of the queen swap experiment in 2021 are displayed in Figure 1 (a, b). Brood samples of colonies in the trial were collected again and subsequently frozen at minus 80°C for future analysis. It is important to note that the results may be slightly affected by Colonsay 9W because the brood samples were taken soon after the queen was introduced to the colony.

Examination of brood samples

In the laboratory, all previously frozen samples collected for the queen swap experiment were investigated. In total, 4451 cells (16 samples) from Wadborough queen colonies and 4613 cells (19 samples) from Colonsay queen colonies were analysed for this experiment (Table S1). To identify whether a cell had been recapped a binocular microscope (x 16) was

used and a bright light source which facilitated the detailed observation of each wax cell cap. Fine forceps were used to cautiously peel back the wax cap of each cell.

Figure 2. Image of a recapped cell. The red circle highlights a recapped brood cell visible by the matte, grainy area on the underside of the wax cap. The wax cap has been fully recapped. Author's own image.

A matte, grainy area on the underside of the cap is a clear indicator that the cap has previously been repaired, which is referred to as recapping (Hawkins & Martin, 2021). This is where the silk lining of the pupa's cocoon has been disturbed and then repaired (Spivak & Danka, 2020), visible in Figure 2. The number of recapped and non-recapped cells was subsequently recorded. At the same time, the pupae were removed from each cell using fine forceps and their age was recorded based on the eye and body colour, further detailed by Dietemann et al. (2013). Possible signs of *Varroa* mites were identified; such as the white excrement on the walls of the cell and the pupae were examined for mites which are commonly found between the abdominal sternites (Mondet et al., 2018). The number of cells that were infested by *Varroa* mites and the number of cells that did not contain the mites (non-infested) for each brood sample was recorded. By the end of the experiment, 9064 total cells had been analysed from 35 brood samples. Of note, some of the samples were taken from the same colony but were collected on separate dates (see Table S1).

The average recapping level of Wadborough queen colonies was calculated by dividing the total recapped cells by the number of total cells analysed (Table S1). The equivalent calculation was made for Colonsay queen colonies. The same method was used to calculate the average non-infested normal (non-recapped) level for both Wadborough and Colonsay queen colonies. Likewise, the average infestation level of Wadborough queen colonies was calculated by dividing the total infested cells by the number of total cells analysed. Again, the equivalent calculation was made for the infestation level of Colonsay queen colonies.

Sugar shake test

There are several ways to assess the infestation level of a colony. For this experiment a sugar shake test (Gregorc, Alburaki & Sampson, 2018) was conducted, on 14/10/21, at the Wadborough apiary for each of the colonies that still remained in the queen swap experiment (Table S2). In total, seven Wadborough queen colonies and six Colonsay queen colonies were sampled. The aim of this method was to substantiate the results from the infestation levels of Colonsay and Wadborough queen brood samples. The plastic sugar shake container (plastic container with a mesh top and lid) was weighed on a small electronic scale. Approximately 700 bees were collected from the hive and put into the sugar shake container which was then sealed and weighed again. One tablespoon of icing sugar was placed into the container through the mesh lid. The container was sealed with the lid and then was shaken and rotated for approximately two minutes. Again, one tablespoon of icing sugar was added at this point. Following this, the container was shaken for another one minute period. The lid was removed and the container was shaken over a large white wash bowl to allow the mites to fall through the mesh lid along with the icing sugar. A small amount of water was added to the bowl to dissolve the sugar icing. The remaining mites were then counted. This method was repeated for each of the queen swap experiment colonies. The weight of the container was subtracted from the weight of the bees in the container to indicate a more precise measurement to estimate the amount of bees in each sample. The average number of bees was calculated to be 6.5 per one gram. This was used to calculate the number of bees in each sample. Next, the number of mites was divided by the number of bees and multiplied by 100 (Table S2) to determine the number of mites per 100 bees.

Mite fall test

Again, to corroborate the results for the infestation levels of colonies, mite fall was assessed on 18/10/21 for all existing colonies at the Wadborough apiary in the queen swap experiment (Table S3). This comprised of five Wadborough queen colonies and six Colonsay queen colonies. Colonies were treated with Api-Bioxal (oxalic acid) and monitoring trays were put in the full sized colonies and removed 33 hours later. The mite fall was subsequently counted for each colony. The number of seams of bees treated (the line of bees between two hive frames) varied from colony to colony (visible in Table S3). To standardise the results, the mite fall was divided by the seams of bees treated. Notably, both the Wadborough and Colonsay colonies had not previously been chemically treated for *Varroa*, until this application of treatment. The chemical was applied after all of the brood samples had been collected in 2021.

Statistical analyses

All statistics were calculated using the website Social Science Statistics (Social Science Statistics, 2022). A Chi-square Test compared the association of two categorical variables which in this case were the recapped cells and non-infested normal cells (non-recapped) of Wadborough queen colonies and Colonsay queen colonies. A comparison was made between the average infestation levels of Wadborough queen and Colonsay queen colonies. The data was not normally distributed, thus a Man-Whitney U Test was performed. The number of mites per 100 bees in Wadborough queen colonies were compared to the number of mites per 100 bees in Colonsay queen colonies. A Kolmogorov-Smirnov Test of Normality revealed that all data were normally distributed, therefore a T-test was performed. Finally, the mites per seam of bees treated with Api-bioxal in Wadborough queen colonies was compared to the result for Colonsay queen colonies. Since the data was found to be normally distributed, a T-test was performed.

Results

Recapping and infestation levels

In the queen swap experiment, 714 cells were recapped of the 4451 total cells analysed in Wadborough queen colonies. In Colonsay queen colonies, 430 out of the 4613 total cells were recapped.

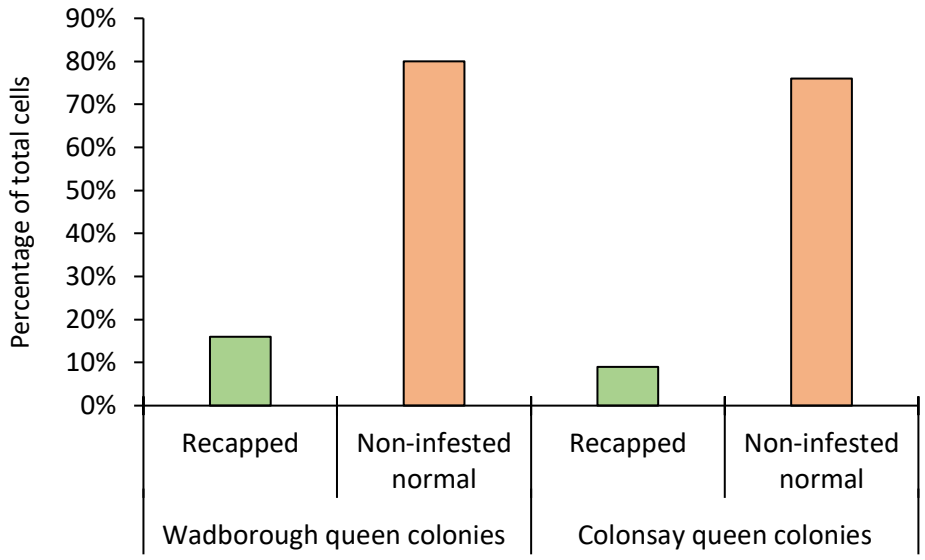

Figure 3. The average recapped cells (%) and non-infested normal cells (%) of Wadborough queen colonies and Colonsay queen colonies. The ratio of recapped cells to non-infested normal cells was not significantly different between the Wadborough and Colonsay queen colonies.

Interestingly, in Figure 3, the average recapped cells of Wadborough queen colonies (16%) is higher than that of Colonsay queen colonies (9%). The average non-infested normal levels of Wadborough queen colonies (80%) was found to be slightly higher than that of Colonsay queen colonies (76%). When compared in a Chi-square Test, the ratio of recapped cells to non-infested normal cells displayed no significant difference between the Colonsay and Wadborough queen colonies ($X^2 = 1.3992$, $p = .236854$). In summary, there appears to be higher levels of recapping in Wadborough queen colonies in contrast to Colonsay queen colonies.

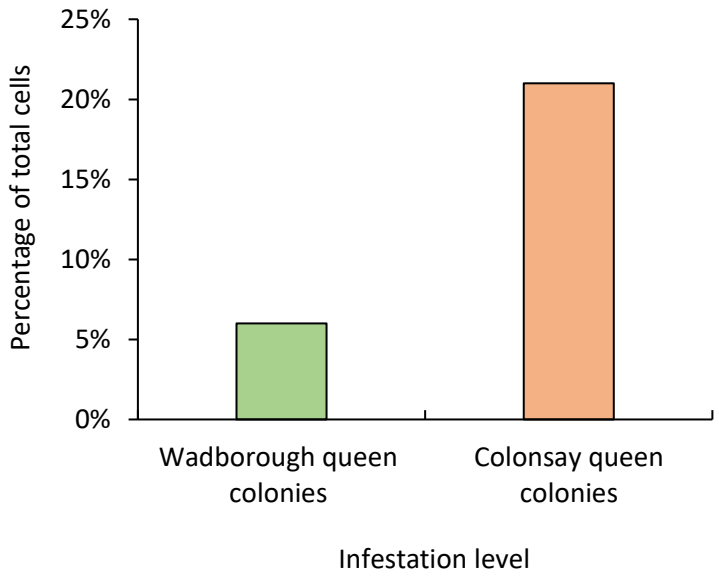

Figure 4. The average infestation level (%) of Wadborough queen and Colonsay queen colonies. A comparison of the average infestation levels of Wadborough queen and Colonsay queen colonies revealed no significant difference.

Of the 4451 cells that were analysed of Wadborough queen colonies, 261 cells were infested. For Colonsay colonies, 957 cells were infested out of the 4613 total cells analysed. The average infestation level of Wadborough queen colonies (6%) was found to be lower than that of Colonsay queen colonies (21%) in Figure 4. A Man-Whitney U Test determined that the results were not statistically different ($U = 134$, $z = 0.57948$, $p = .56192$).

Sugar shake and mite fall test

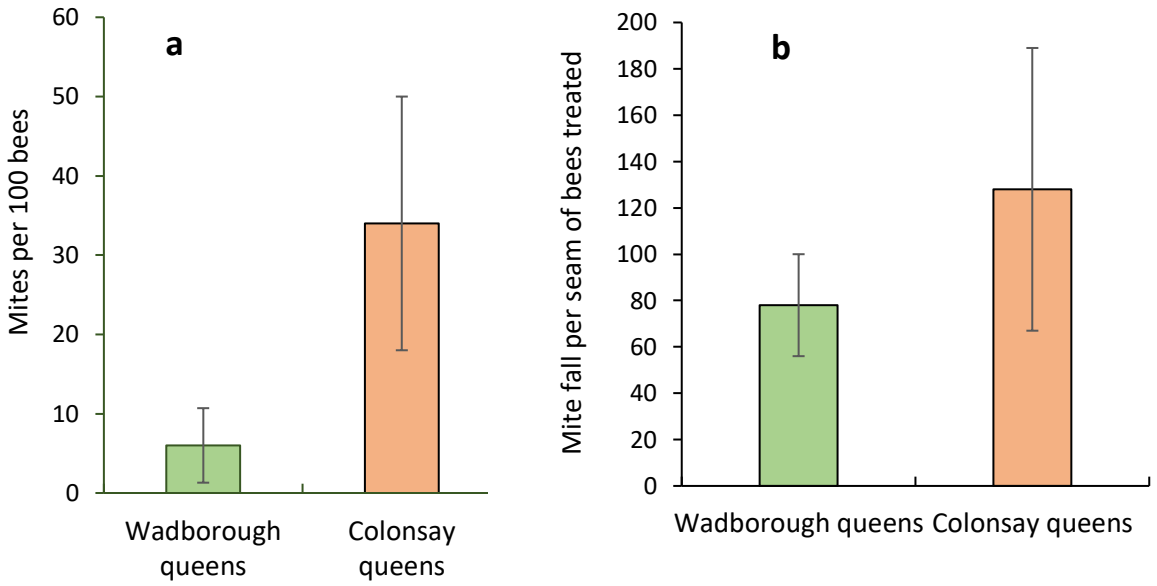

Figure 5. a) The sugar shake results for the average mites per 100 bees in Wadborough queen colonies (n = 7) and Colonsay queen colonies (n = 6). Colonsay queen colonies had higher average mites per 100 bees (34) in comparison to Wadborough queen colonies (6), although a T-test revealed no significant difference. The error bars represent the standard deviation. **b) Average mite fall per seam of bees treated for Wadborough queen (n = 5) and Colonsay queen colonies (n = 6) after 33hrs of trickling Api-Bioxal.** There is a higher average mite fall for Colonsay queen colonies (128) than Wadborough queen colonies (78) per seam of bees treated, however no significant difference was found. The error bars represent the standard deviation.

Since analysing the data available in Table S2, it is apparent from Figure 5 (a) that Colonsay queen colonies had the highest average mites per 100 bees (34) in the sugar shake test. Whilst, the average mites per 100 bees for Wadborough queen colonies remained lower (6). However, the result was not significantly different (t = 0.2582, p = .400148). In Figure 5 (b), the Colonsay queen colonies have a higher average mite fall (128) per seam of bees in comparison to the result for Wadborough queen colonies (78). Accordingly, this result conveys a higher infestation level of Colonsay queen colonies, however no significant difference was found (t = 1.5731, p = .075073). Data from this table is comparable to Figure

3 to corroborate the finding that Colonsay queen colonies had a higher infestation level of mites than Wadborough queen colonies.

Discussion

The focus of this research was to investigate further the role of recapping by perceiving the recapping and infestation levels in colonies when the queens of naturally mite resistant colonies were swapped with the queens of mite naïve non-resistant colonies. Expectantly, it would then be possible to infer whether recapping could hypothetically be an innate behaviour. Interestingly, the results of Figure 3 present that the average recapping level of Wadborough queen colonies (16%) was higher than the result for Colonsay queen colonies (9%). The average level of non-infested normal cells was 80% for Wadborough queen colonies and 76% for Colonsay queen colonies respectively. Although, the ratio of recapped cells to non-infested normal cells was not significantly different between the Wadborough and Colonsay queen colonies. The average infestation level of Wadborough queen colonies (6%) was revealed to be lower than that of Colonsay queen colonies (21%) in Figure 4. This result showed no significant difference. Similarly, the findings in Figure 5 provide further evidence that Colonsay queen colonies had higher infestation levels as opposed to Wadborough queen colonies. In the sugar shake test, the Colonsay queen colonies exhibited the highest average mites per 100 bees (34) in comparison to Wadborough queen colonies that revealed a lower result (6) yet there was no significant difference found. Similarly, in the mite fall test, Colonsay queen colonies demonstrated a higher average mite fall (128) to that of Wadborough queen colonies (78) after chemical treatment with Api-Bioxal. Again, no significant difference was found. Both the sugar shake test and mite fall test corroborate the findings of the brood sample analysis that the infestation levels were higher in non-resistant colonies.

As to an explanation for these results, the higher infestation levels of Colonsay queen colonies suggests that they are not as successful in reducing mite infestation levels in comparison to Wadborough queen colonies. This was correctly predicted as it was expected that Colonsay queen colonies would require more time to adjust to a parasite they had only recently been exposed to. Evidently, Wadborough queen colonies had lower levels of mite infestations. On these grounds it can be argued that Wadborough colonies may be

expressing more resistance behaviours or a higher efficiency of resistance behaviours than Colonsay queen colonies, thereby reducing the population of *Varroa*. Incidentally, the results also reveal that the offspring produced by the newly introduced *Varroa* naïve Colonsay queens were in some way capable of recapping even though they had not previously been exposed to the mite before the experiment.

Taken together, the higher average infestation levels of Colonsay queen colonies suggests that although the colonies are capable of recapping, they may struggle to cope with high mite infestations. This could be due to low efficiency of the process of recapping or that they do not display other resistance behaviours such as brood removal to reduce mite levels (Grindrod & Martin 2021a). Once more, this could be expected of a host that has not been exposed to a parasite before. An alternative explanation could be that if infestation levels are already low in Wadborough colonies then it is not necessary to perform recapping or other resistance behaviours as it would be an unnecessary waste of energy.

It is challenging to conclude whether recapping is a heritable trait considering there is a lack of research in this area (Maucourt et al. 2020). Equally, it is difficult to determine whether recapping is an innate behaviour due to the short time in which the experiment was conducted. To elucidate, the queen does not directly contribute to behavioural performance traits but influences the colony through genetics (Oxley & Oldroyd 2010; Maucourt et al., 2020). Since Wadborough colonies displayed higher recapping levels, this finding could suggest an innate behaviour because the offspring of the resistant queens could have inherited the recapping trait (Blacquière & Panziera, 2018). Thus recapping could be considered an instinctive behaviour. On the contrary, there is a possibility that recapping could be a learned behaviour. Perhaps because of the close proximity of the Colonsay queen colonies and the Wadborough queen colonies (Figure 1b), the Colonsay queen bees could be learning recapping through what they have observed in the Wadborough queen hives nearby. Moreover, the results could also be interpreted by the suggestion of a combination of innate and learned behaviour. After all, the interaction and expression of both learned and innate behaviours play a role in insect navigation (Buehlmann, Mangan, & Graham, 2020; Goulard et al., 2021). However, it is important to express that the adaptations promoting survival in one colony may differ in other colonies (Oddie et al., 2018b). If

recapping is an innate behaviour in one colony it could potentially be a learned behaviour in another.

Recapping could not be linked directly to *Varroa* resistance in a study by Guichard et al (2021b). The authors also found low heritability of the recapping trait. However, the results of this study are not robust due to small sample sizes and the infrequent evaluation of recapping over the study period. If recapping is not the direct cause for the reduction of mite infestation levels, it could still have an important role to play indirectly. Guichard et al. (2021b) still found an association between recapping and hygienic behaviour. What is more, recapping has been observed to be positively correlated with brood removal in multiple previous studies (Oddie et al., 2018a: Martin et al., 2020; Grindrod & Martin 2021a). Based on the present study and previous studies (Martin et al., 2020; Grindrod & Martin, 2021b; Hawkins & Martin, 2021) these findings could provide further support to the idea that recapping is consistently found alongside *Varroa* mites and other resistance behaviours in colonies. Thereby stressing the importance of this behaviour.

Before the mite fall test, both Wadborough and Colonsay colonies were not chemically treated. Permitting *A. mellifera* to develop novel adaptations to the parasitic mite without need for chemical treatments would theoretically be the ideal long term solution for natural mite resistance, but only if this solution is economically viable for independent and commercial beekeeping. The Bond method (Fries, Imdorf & Rosenkranz, 2006) and Darwinian beekeeping (Blacquière et al., 2019) involve the management technique of leaving colonies untreated, then the surviving colonies are chosen for future breeding (Mondet et al., 2020b). The limited use of pesticides could allow bees to develop current resistance mechanisms. A survey paper by Moro et al. (2021) brought to attention the emergence of 44 reports (18%) of managed untreated colonies that presently have stable equilibrium with the mite. However, naturally surviving untreated populations often present undesirable characteristics to beekeepers such as frequent swarming, low productivity which prevents their large-scale use (Mondet et al., 2020a).

Surprisingly, it is possible that honey bees could develop a new resistance trait through natural selection or a sustained equilibrium with the mite in less than 100 years (Martin et al., 2020; Le Conte et al., 2020). For instance, the natural selection of VSH behaviour may evolve in a short time period (Panziera et al., 2017). If there is a relatively short period of

time in which honey bees can develop resistance then permitting colonies to breed naturally without human intervention could potentially lead to some *A. mellifera* populations becoming independently resistant to *Varroa*. Van Alphen and Fernhout (2020) consider a colony to be resistant when it is able to reduce the population size of *Varroa* to a level that does not cause mortality and ensures survival of the colony (Oddie et al., 2018a). Tolerance is the act of limiting the damage caused by the parasite burden (Blacquière & Panziera, 2018). Tolerance to the mite could prove to be a more realistic objective to achieve instead of complete resistance. Perhaps *Varroa* could still be present in colonies but at a level that allows both host and parasite to thrive in an equilibrium (Oddie & Dahle, 2021).

There is often high variability found when measuring the recapping levels of colonies (Martin et al., 2020; Hawkins & Martin, 2021). Replacing the queens in colonies every two years is a common practise in most countries, this reduces the vertical transmission of the parasite from mother to daughter bees (Blacquière & Panziera, 2018). However this practice can impede the process of natural selection for resistance against the mite (Van Alphen & Fernhout, 2020). Therefore, the mite is confronted with a new queen genotype and possibly new drone genotypes, if there is natural mating at the apiary (Neumann & Blacquière, 2016). Consequently, the host-parasite interactions reset when a foreign queen is introduced (Blacquière & Panziera, 2018). This may explain why recapping levels in the queen swap experiment are variable (visible in Table S1).

Moreover, fluctuating climatic conditions and food resources may lead to specific local adaptations or genotypes based on unique environments (Büchler et al., 2014; Büchler et al., 2020a). As a result, the transfer of resistant queens from one population to another can fail to maintain such a high level of resistance (Büchler et al., 2014; Büchler et al., 2020a). This could have been a factor in the queen swap experiment. Furthermore, mechanisms underlying mite population may be colony specific and can differ in and among apiaries (Wagoner et al., 2021). Due to the relatively close proximity of apiaries in this apiary (Figure 1b), it could be said that the infestation levels would only fluctuate if influenced by factors such as the local climate or indeed the expression of resistance traits. These factors may also account for the variability in the recapping and infestation levels of colonies in the queen swap experiment (Table S1).

Understanding whether recapping is a learned or innate behaviour is important for selective breeding programmes. If it is a learned behaviour then this takes pressure off breeding programmes and new strategies in the field can potentially encourage this behaviour. If recapping is discovered to be an innate behaviour, breeders can select the queens that have the ability to produce high recapper bees based on the recapping levels of colonies and queen genetics. Additionally, the role of recapping may be considered integral in resistance against *Varroa* if this behaviour is specifically linked to hygienic brood removal (Martin et al., 2020). For the above reasons, it is imperative that the exact role of recapping is understood in order for future research to progress.

CHAPTER 3

Understanding the potential relationship between the recapping and infestation levels in 14 apiaries from the UK

Abstract

The global spread of the ectoparasitic mite *Varroa destructor* has presented a major threat to honey bee populations across the world. It is now acknowledged that some naturally surviving honey bee colonies perform the resistance behaviour known as recapping. Here, we investigated the average recapping levels along with the average infestation levels of 14 apiaries in the UK. The results of this study found that nine apiaries displayed higher average recapping levels in comparison to lower infestation levels. Whereas, five apiaries displayed lower average recapping levels in comparison to infestation levels. There was no significant difference found between the average recapping levels and infestation levels of all 14 apiaries. Understanding the dynamics of recapping in colonies is vital for future research and more specifically, for comprehending how this resistance behaviour could have an effect on infestation levels. Furthermore, it is beneficial for selective breeding programmes to, if possible, identify colonies that have a greater chance of surviving mite infestations by displaying an increase in resistance behaviours.

Introduction

The greatest threat honey bees now encounter is from a parasitic mite, named *Varroa destructor* (referred to as *Varroa*), that is causing the mortality of untreated managed honey bee (*Apis mellifera*) colonies (Rosenkranz et al., 2010; Martin et al., 2020) and contributing to colony losses throughout the Northern hemisphere (Hawkins & Martin, 2021). Honey bees are vital for the pollination of common crops (Boecking & Genersch, 2008), therefore they are an incredibly integral species in our ecosystem. The recapping behaviour of honey bees is a form of resistance against the mite whereby a cell is uncapped then later recapped without the removal of the developing pupa within (Martin et al., 2020). This behaviour is common in honey bee colonies as determined by multiple studies (Villegas & Villa, 2006; Harris, Danka & Villa, 2010; Martin et al., 2020, Hawkins & Martin, 2021). Previous research has demonstrated that high recapping levels can be linked to mite resistant populations (Oddie et al., 2018a; Martin et al., 2020; Grindrod and Martin, 2021a).

In a study by Hawkins and Martin (2021) the frequency of recapping was higher in naturally *Varroa* resistant populations and, notably, mite infested brood cells were targeted. Similarly, Oddie, Dahle & Neumann (2017) observed low rates of mite reproduction in four European mite-resistant populations, which is comparable to the low rates of reproduction found in African and Africanised bee (AHB) colonies (Martin et al., 2020). Oddie et al. (2018a) later linked this low mite reproduction with high recapping (Martin et al., 2020). Assuredly, if recapping reduces mite reproductive success then it could be expected that there would be an increase in the frequency of recapping and the targeting of infested cells in surviving populations, in comparison to populations that are more susceptible to *Varroa* (Oddie et al., 2018a; Grindrod & Martin, 2021a).

Selective breeding programmes contribute to the resistance of honey bees against *Varroa*. Originally, many beekeepers began to breed from the stock that survived the mite infestations (Martin et al., 2020). This approach was not successful for the most part because colonies did not appear to have the necessary defence adaptations or they were not able to cope with the overwhelmingly high numbers of *Varroa* mites (Martin et al., 2020). A lack of sufficient research and knowledge about the mechanisms of honey bee resistance, particularly recapping, may be the reason why there is little progress in the

selection of honey bees that are known to survive *Varroa* infestations (Guichard et al., 2020a).

However, the underlying mechanisms of recapping are debated, likewise so is this trait's importance in resistance against *Varroa* (Oddie et al., 2018a; Grindrod & Martin, 2021a). Hawkins & Martin (2021) found that recapping was not the principal cause of failed mite reproduction, as they did not observe a negative correlation between the proportions of infested cells along with the total mite reproductive success. They proposed that the trait was more likely to be involved in the detection and removal of infested brood as reported in previous studies (Martin et al., 2020). Therefore, the main aim of this study was to infer more about the role of recapping whilst gaining an understanding of how recapping and infestation levels could interact. For this study we collected brood samples from 17 apiaries in the UK. These samples were analysed to determine the recapping and infestation levels of colonies. One hypothesis would be that the infestation levels would decrease if recapping levels increased in a colony if recapping is to be considered a resistance behaviour.

Methods

Collection and examination of brood samples

In total, 88 brood samples (sections of the brood comb) were obtained from 17 apiaries and a total of 22,153 cells were analysed (Table S4) over a period of two years from 2020 to 2021. Brood samples were collected from beekeepers in Worcestershire and from the University of Salford campus apiary. All samples were stored in a minus 80°C freezer for future inspection. Recapping in the brood samples was identified by inverting the wax cap of each cell using fine tweezers under a binocular microscope (x 16) and a bright light source. The number of cells that had been recapped, visible by the matte and grainy area on the underside of the cap, was recorded along with the number of cells that were not recapped (non-recapped). After removing the pupae with fine forceps and inspecting the abdomen and the inside of the cell for signs of the mites (such as white excrement), the number of cells that contained mites (infested) was noted along with the number that contained no mites (non-infested).

Apiaries with less than two samples (Little Aston, Netherton and Weston Subedge) were excluded from the results to standardise the data (Table S4). This would leave 85 samples from 14 apiaries. The number of brood samples for each apiary were: Beckford (n = 6), Besford Bridge (n = 8), Bishampton (n = 4) Bredon's Norton (n = 11), Charlton (n = 2), Church Lench (n = 2), Croome (n = 13), Harvington (n = 2), Harvington Salford Lodge (n = 3), North Piddle (n = 2), Pershore College (n = 11), UOS (n = 14), Wadborough (n = 3) and Wishaw (n = 4). For each sample, the total number of recapped cells was divided by the total number of cells analysed. Likewise the total number of infested cells was divided by the total cells analysed. An average percentage for the levels of recapped cells and infested cells was calculated by combining the results for each sample per apiary. If the samples were collected from the same area then the infestation levels are unlikely to be affected by varying climatic conditions. Therefore, in theory, the monitoring of infestation levels could be more dependent on the expression of resistance behaviours and mechanisms.

Statistical analyses

A One-Way ANOVA test was conducted to compare the average result for recapping and infestation levels in all apiaries to determine if there was a significant difference. Statistics were calculated using the website Social Science Statistics (Social Science Statistics, 2022).

Results

Recapping and infestation levels of UK apiaries

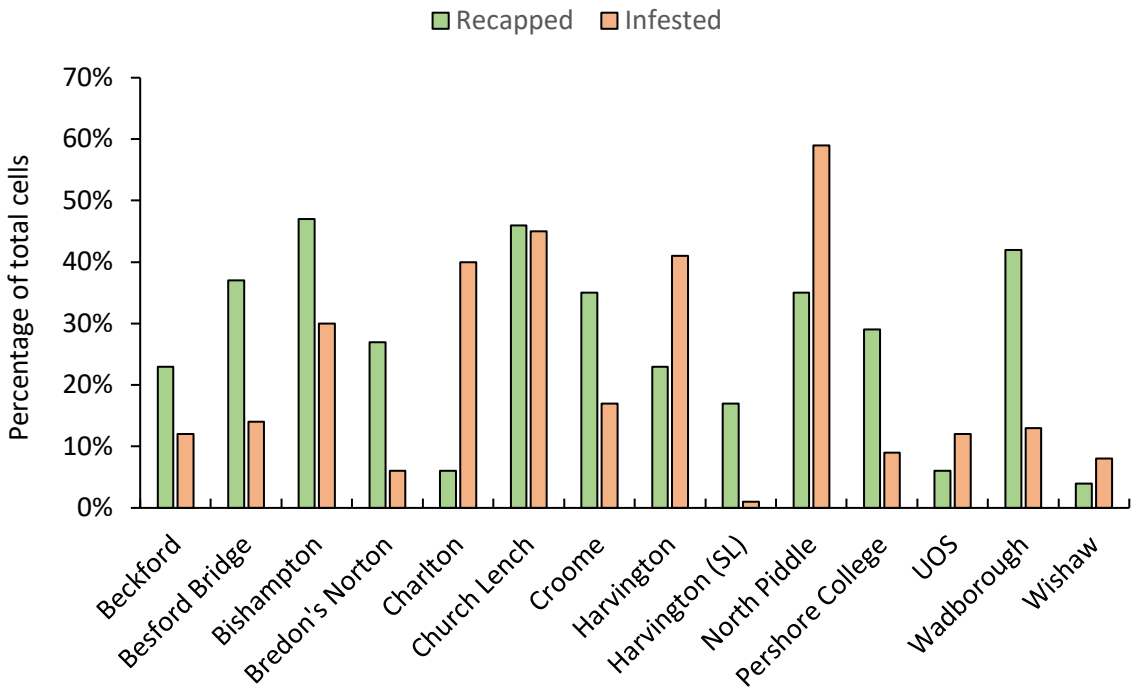

Figure 6. Average total cells recapped (%) and total cells infested (%) in 14 UK apiaries. Nine apiaries displayed higher average recapping levels in comparison to lower infestation levels. Whereas, five apiaries showed higher average infestation levels compared to lower recapping levels. There was no significant difference found between the average recapping levels and infestation levels of all 14 apiaries.

Of the 14 apiaries sampled, nine apiaries displayed higher average recapping levels than infestation levels. This would leave five apiaries with higher average infestation levels in comparison to recapping levels. The samples from Bishampton had the highest average recapping level (47%) coupled with a lower infestation level (30%) in Figure 6. The average infestation level of North Piddle is the highest result (59%) but with a lower recapping level (35%). Interestingly, Charlton displayed a considerably lower average recapping level (6%) in contrast to a high average infestation level (40%). Notably, the results from Church Lench present that the average recapping level (46%) was very similar to the infestation level (45%). The results from the University of Salford (recapping level: 6%, infestation level: 12%) and Wishaw (recapping level: 4%, infestation level: 8%) displayed similar results for the average recapping and infestation levels. Harvington Salford Lodge exhibited the lowest average infestation level (1%) along with a comparatively higher recapping level (17%). In a comparison between the average recapping levels and infestation levels of all 14 apiaries,

no significant difference was found (p = .424056). Overall, the results provide important insight into the dynamics of recapping and infestation levels in colonies.

Discussion

Pointedly, a study by Grindrod and Martin (2021a) discovered that there were significantly high levels of recapping, brood removal and mite infertility found in colonies resistant to *Varroa*. Their framework for the development of *Varroa* resistance helps to visualise the relationships between resistance behaviours and highlights the differences between mite susceptible populations in comparison to mite resistant populations. For instance, resistant colonies recap infested cells significantly more than susceptible colonies which they propose indicates an increased detection of *Varroa*. If recapping is a successful detection strategy, then an increase in this behaviour could potentially increase hygienic brood removal (Oddie et al., 2018a, Grindrod & Martin, 2021a). Since no significant difference was found between the average recapping levels and infestation levels of all 14 apiaries, this could provide evidence that recapping levels do not have a direct effect on infestation levels, otherwise it would be expected to observe a significantly higher result for recapping levels in comparison to infestation levels. Nonetheless, recapping levels may still have an indirect effect on infestation levels. In Figure 6, Bishampton displayed the highest average recapping level (47%) in comparison to a lower result for the average infestation level (30%). As these colonies were performing high levels of recapping on average, this could suggest that they are expressing an appropriate behavioural resistance response if infested cells are being successfully detected, possibly through recapping, and infested brood is removed thereby decreasing the infestation level (Martin et al., 2020; Grindrod & Martin, 2021). That is not to say that if infestation levels are low that recapping should still be noticeably higher. Rather, if there are low infestation levels in a colony then bees may waste energy by increasing recapping behaviour more than is necessary. An example of this would be the results from UOS (recapping level: 6%, infestation level: 12%) and Wishaw (recapping level: 4%, infestation level: 8%). If the levels of infestations are low alongside low recapping levels then this colony could be considered to be expressing the appropriate response to control mite population levels. In fact, although Church Lench has a considerably high result for the average infestation levels (45%) the recapping levels are very high (46%) which could also suggest that these colonies are capable of controlling mite infestation levels. Conversely, the

average infestation level is considerably higher in the Charlton apiary (40%) compared to the result for the average recapping level (6%) of this apiary. Similarly, the average infestation level for North Piddle (59%) is higher than the average recapping level (35%). These results could suggest that colonies are struggling to cope with mite infestations as the bees may not be efficiently detecting infested cells. Harvington Salford Lodge displayed a very low result for average infestation levels (1%) compared to a relatively higher result for average recapping levels (17%). Seemingly, these colonies appear to be reducing mite populations well, possibly indirectly through recapping. Of most significance, it is apparent that the recapping level is elevated in all colonies infested by *Varroa* in this study (Figure 6). This finding is similar to results presented by Martin et al. (2020). Interestingly, the recapping levels of Besford Bridge (37%) and Croome (35%) are similar to the recapping levels of North Piddle colonies (35%) however the infestation levels are considerably lower (14% and 17% respectively) in comparison to North Piddle (59%). The sample size for North Piddle colonies is low, however this result could suggest that these colonies are in the process of responding to high infestation levels by increasing recapping levels. By monitoring these colonies over a longer period of time it may be possible to observe a decrease in colony infestation levels. Thus further indicating that recapping is likely to be a fluctuating behaviour, similar to the fluctuating hygienic behaviour determined by Al Toufailia et al. (2018).

By contrast to the present study, a paper by Guichard et al (2021b) stated that recapping is not linked to improved resistance against *Varroa* and that recapping levels are independent of infestation levels. The same findings were also expressed in a study by (Buchegger et al., 2018). However, even if recapping does not have a direct effect on mite populations then it can at least be an indicator for the survivability of a colony (Oddie et al., 2021). For instance, recapping may be a useful trait to assess a colony's ability to resist the mite (Oddie & Dahle, 2021). This behaviour could be successfully monitored and can provide an indication as to whether the cells have previously been targeted for brood removal (Hawkins & Martin, 2021).

Selection programmes have proved to be effective in enhancing resistance traits to reduce *Varroa* mite infestations in many previous studies (Palacio et al., 2010; Kirrane et al., 2015; Scannapieco et al., 2016; Gerdts et al., 2018). Oddie et al. (2018a) claim that the recapping

trait may be more effective to selectively breed for in contrast to other resistance traits as it appears to mitigate mite infestations. Although, if the recapping trait shows low heritability (Guichard et al., 2021b), this may pose a problem for future selective breeding programmes. What is more, an effective selection programme is time consuming and requires more effort, expertise and expense and impractical for wide-scale apicultural use (Leclercq et al., 2018; Mondet et al., 2020b; Wagoner et al., 2021). Furthermore, as demonstrated by the present study, resistance traits are difficult to measure due to the changing season, the availability of worker brood and fluctuating infestation levels (Mondet et al., 2020b; Grindrod & Martin, 2021a; Moro et al., 2021). This undoubtedly is challenging for selection programmes (Guichard et al., 2020a). Comparatively, a study by Al Toufailia et al. (2018) comments that an explanation for fluctuating levels of hygienic behaviour may be that it is only necessary to express in high amounts when the levels of mites are high and low levels when disease is rare (Al Toufailia et al., 2018). This hypothesis could also apply to recapping behaviour. Whilst it is still not proven that recapping has a direct effect on infestation levels, the present study does indicate that infestation levels and recapping levels are interacting.

A drawback of targeted selection is that when new pests occur a new breeding programme with specifically selected traits will have to be devised (Blacquière & Panziera, 2018). A further issue is if the desired traits change faster than the several years it may take for selective breeding programmes to become effective (Guichard et al., 2020a). Populations that have naturally adapted to reduce *Varroa* infestations will have the advantage in this case because they can develop new resistance mechanisms whilst still making use of previous resistance traits. For the preceding reasons it may be challenging to select traits for recapping. Conclusively, understanding the relevance of the recapping trait and whether this trait is heritable is critical for honey bees to survive the ongoing fight against *Varroa* mites.

CHAPTER 4

Recapping and the targeting of brood cells: why are cells targeted for recapping behaviour?

Abstract

Given that the mite *Varroa destructor* is perhaps the largest threat to the honey bee, *Apis mellifer*a, there has been an increase in research surrounding the resistance behaviours of honey bees. These resistance behaviours are known to help decrease the infestation levels of *Varroa*. One particular resistance behaviour, recapping, has expressly gained interest but little is understood about how and why brood cells are specifically targeted for recapping. Understanding whether the contents of a brood cell has an effect on the detection of infested cells and recapping levels is an important step in understanding the specific cues that trigger these behaviours. As found in previous studies, we established that the recapped diameter of infested cells was significantly larger in contrast to that of non-infested cells. Of particular interest, there was a significant difference found between the lower levels of recapped mite non-reproductive cells in comparison to the higher levels of recapped reproductive cells. The results of this study highlight that infested cells are targeted for recapping and conclude that the presence of mite offspring may influence whether a cell is recapped.

Introduction

Varroa destructor (commonly referred to as *Varroa*) is an ectoparasitic mite that is currently threatening the survival of the honey bee, *Apis mellifera*, populations in the Northern Hemisphere (Traynor et al., 2020; Hawkins & Martin et al., 2021). Recapping is a behavioural trait that is thought to be involved in providing resistance against the mite (Oddie et al., 2018; Martin et al., 2020; Guichard et al., 2021a). This behaviour involves the detection, uncapping and recapping of cells without removing the developing pupae within (Hawkins & Martin, 2021). Previous research has found that recapping is increased in colonies that survive *Varroa* infestations, and that infested cells are specifically targeted (Oddie et al., 2018a; Hawkins & Martin, 2021; Oddie & Dahle, 2021).

To gain a deeper understanding about the role of recapping, we conducted two studies to deduce how and why cells are targeted by worker bees. Firstly, there is an argument that mite infested brood cells have a larger recapped diameter in contrast to non-infested cells (Grindrod & Martin, 2021b; Hawkins & Martin, 2021). To elucidate, the uncapping of cells by bees may improve the detection of olfactory cues that trigger hygienic behaviour (Grindrod & Martin, 2021b). An explanation for the recapping of non-infested cells is suggested to be that bees are checking areas in the proximity of infested cells or that a signal has diffused from an infested cell into a neighbouring cell (Grindrod & Martin, 2021). Currently, there is little data on this subject therefore we investigated and compared the recapped diameters of non-infested and infested brood cells to observe whether infested cells are recapped more than non-infested cells.

Secondly, the origin of the specific cue that triggers resistance behaviours remains unknown, whether it be emanating from the foundress mother, her offspring or indeed the pupa itself. It is of significant interest to determine where the cue that instigates recapping behaviour originates from. Furthermore, identifying the chemical compounds that instigate hygienic behaviour (the removal of dead or infested brood from cells) in honey bees is equally as important because this discovery may help improve research into the cues instigating recapping behaviour, especially if they are found to be instigated by the same cue (Grindrod & Martin, 2021a). A paper by Sprau et al. (2021) determined that the presence of offspring does not have an influence on recapping levels and therefore the

offspring alone is not significant in the recapping process. To test this hypothesis, we compared the levels of recapped mite non-reproductive cells (cells containing a single foundress mite) to the levels of recapped reproductive cells (cells containing a foundress and offspring) with the intention of inferring why cells may be targeted for recapping behaviour.

Methods

Worker brood samples were collected from 17 apiaries in the UK. Predominantly, samples were collected from apiaries in Worcestershire, in the West Midlands and from the University of Salford (UOS) campus in the North West of England. Other brood samples were donated by beekeepers. Brood samples that contained vast amounts of stretched larvae or signs of wax moth were not included in this study because it is difficult to identify recapping in these cells (Villegas & Villa, 2006; Martin et al., 2020; Spivak & Danka, 2020). The samples were stored in a minus 80°C freezer for future analyses.

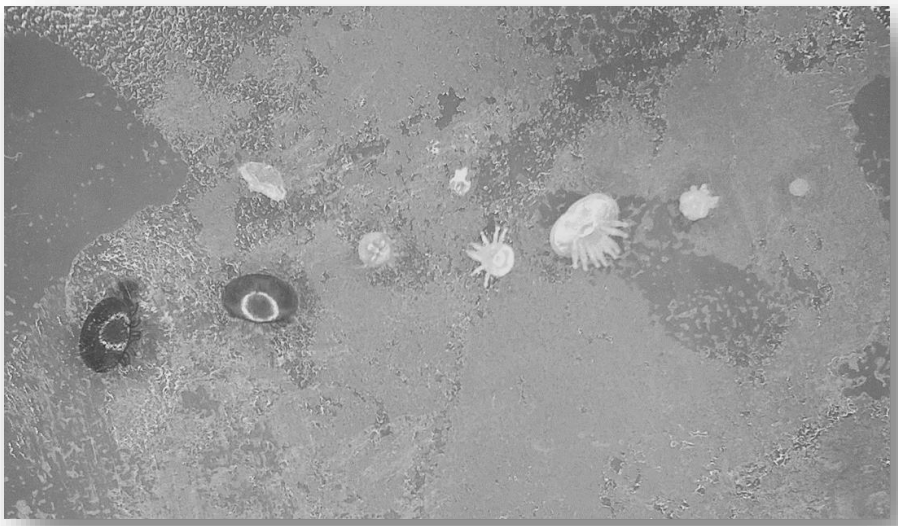

Figure 7. Image of a *Varroa* mite family that have been removed from an infested cell. From left to right: A foundress mother (dark brown) and her offspring: an adult female with a skin (above), an adult male (brown legs), a deutronymph male with a skin (above), a female deutronymph, a protonymph and an egg. Author's own image.

In the laboratory, the status of the underside of the wax cap was identified using fine forceps, a binocular microscope (x 16) and a bright light source. A matte, grainy area

indicated that the silk lining had been disturbed and the cell was therefore 'recapped'. The size of the recapped area was recorded using a scale of 1-5 which approximately equates to a scale of 1 mm to 5 mm in worker brood cells. The average recapped diameter (1-5 mm) was later calculated for each brood sample. This would include 5138 non-infested cells from 98 samples and 1569 infested cells from 77 samples. After pupae were removed using the fine forceps, their development age was noted according to Dietemann et al. (2013).

In the second part of the study, for 50 brood samples (Table S6), it was noted whether a single foundress (non-reproductive cell) or a foundress with offspring was found in the cell (reproductive cell), identifiable in Figure 7. Whether the cells had been recapped was also noted. In total, 12,042 cells were analysed in this study. The number of recapped mite non-reproductive cells were divided by the total number of cells containing non-reproductive mites. The same calculation was made for mite reproductive cells and an average result was determined for each of the results to form a percentage.

Statistical analyses

All statistics were calculated using the website Social Science Statistics (Social Science Statistics, 2022). Since a Kolmogorov-Smirnov Test of Normality revealed that all data were normally distributed, a T-test calculated whether there was a significant difference between the average recapped diameter of non-infested and infested cells (Table S5). For the second study, as data was found to not be normally distributed, a Mann-Whitney U test compared the number of recapped cells of single foundress infested cells to the number of mite non-reproductive cells of mite reproductive cells (Table S6).

Results

Recapping diameters of infested and non-infested brood cells

A T-test revealed that there is a significant difference ($t = -4.72587$, $p = .00001$) between the larger recapped diameter of infested cells, median 2.6 mm, ($n = 98$) in contrast to the smaller recapped diameter of non-infested cells, median 2 mm, ($n = 77$). Worker bees appear to be creating larger holes in the wax cap of cells that are infested with *Varroa*.

Recapping levels of mite non-reproductive and mite reproductive cells

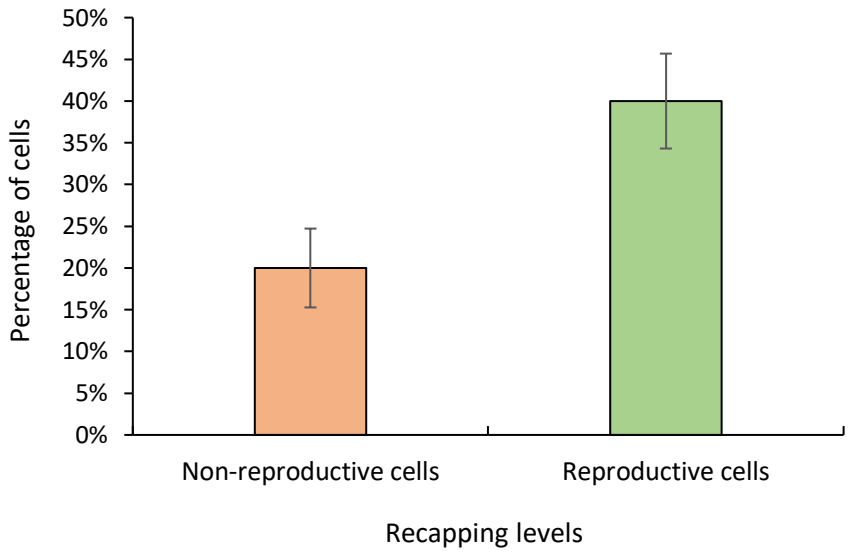

Figure 8. The recapping levels of mite non-reproductive cells (%) and mite reproductive cells (%). A significant difference was revealed when the recapping levels of non-reproductive cells were compared to the recapping levels of reproductive cells. The error bars represent the standard error.

Of the 12,042 cells analysed, 217 cells contained a non-reproductive mite, 43 of those cells were recapped. Whereas, 943 cells contained reproductive mites, 394 of those cells were recapped. With data available in Table S6, it is clear from Figure 8 that the average recapped mite reproductive cells is higher (40%) than that of non-reproductive cells (20%). The infested cells containing an adult female mite and her offspring are recapped more in comparison to cells that only contain a single foundress female in this study. The level of recapped mite reproductive cells was found to be statistically different to that of recapped non-reproductive cells ($U = 835$, $z = -2.85749$, $p = .00424$).

Discussion

According to Martin et al. (2020) if a non-infested cell is opened in error, the recapped diameter remains small (1-2 mm). If the cell is infested then the hole made by uncapper bees would be enlarged (3-4 mm) to gain better access to the brood for a detailed inspection (Hawkins & Martin, 2021). In the present study, a significant difference was found between the larger recapped diameter of infested cells, median 2.6 mm, in comparison to the smaller recapped diameter of non-infested cells, median 2 mm. Grindrod and Martin (2021b) reported similar results in their study. The recapped diameter of

infested cells (median, 3.07 mm) was found to be significantly greater than the recapped diameter of non-infested cells (median, 2.15 mm). Likewise, in a paper by Hawkins and Martin (2021), analysis revealed a significant difference in the frequency distributions of the recapped diameters of infested and non-infested cells in natural *Varroa* resistant colonies. Seemingly, it would be unnecessary and a waste of energy to create a larger hole in cells where the bees do not detect mites.

Grindrod and Martin (2021b: 707-716) asserted that 'the uncapping and recapping of non-infested cells is being driven by the presence of mite infested cells'. In this study, both the recapping of non-infested cells and the recapping of infested cells increased simultaneously. The positive correlation between the recapping of infested cells and non-infested cells suggests that individuals in naturally resistant colonies that are superior in detecting *Varroa* are also more likely to investigate non-infested cells in close proximity to the infested cells. The detection of infested cells could either be through trial and error, or because the chemical cues emanating from infested cells are permeating into non-infested cells nearby (Grindrod & Martin, 2021b). As a result of this, infested cells could be increasingly difficult to target, hence why recapping appears to occur in clusters (Grindrod & Martin, 2021b). If this is correct, it would explain why the recapping levels of non-infested cells in some colonies is relatively high (visible in Martin et al., 2020). However this process operates, it is vital for future research to test this theory to discover the underlying mechanisms of this resistance behaviour, including why the recapping of non-infested cells is necessary.

Varroa resistant colonies potentially have an enhanced sensitivity to cues from infested cells (Grindrod & Martin, 2021b). This could be because they have a higher number of cells that are detected by bees that uncap, or that a higher number of bees uncap the cells for a thorough inspection of its contents (Grindrod & Martin, 2021b). The present paper tested whether mite reproductive cells were targeted as opposed to mite non-reproductive cells with the intention of deducing where the cue that triggers recapping behaviour may originate from. The average recapping level of mite reproductive cells was found to be higher (40%) and statistically different to the recapping levels of non-reproductive cells (20%), as portrayed in Figure 8. Since this result indicates that reproductive cells were being targeted more than non-reproductive cells, it is possible that the offspring of *Varroa* mites may produce more pronounced olfactory cues that bees are able to detect.

Comparable to recapping behaviour, the chemical signals associated with hygienic behaviour appear to have low volatility and can be pinpointed to specific cells (Wagoner et al., 2019). This low volatility and high localisation to a specific cell of the signal may be beneficial, as explained in an evolutionary sense, because there is a high cost to the colony when excessive amounts of brood are removed from cells (Wagoner et al., 2019). Bees may respond to the foreign toxicity of the odorant or the production of a strong signal (McAfee et al., 2018). Regardless, since recapping has been found to be positively correlated with brood removal in previous studies (Oddie et al., 2018a: Martin et al., 2020), perhaps a suggestion of a common trigger would not be so implausible (Grindrod & Martin 2021a).

Hawkins & Martin (2021) discovered that mites in the cell wall trapped by the pupal cocoon did not elicit increased recapping behaviour in comparison to non-infested cells, therefore suggesting that a volatile odour is likely to be the cause. Of note, the majority of cells studied were recapped and contained successfully reproducing mites in this study. This could indicate that the cells containing offspring had higher levels of recapping because of a specific cue that they produce. As the offspring ages, sheds the skin, then develops a hard outer shell (Roth et al., 2020), the strength of this odorant cue could lessen. Therefore the female foundress mother could develop chemical camouflage, as described in a study by Le Conte et al. (2015), allowing her to remain undetected and capable of laying more eggs. On the contrary, Wagoner et al. (2019) argue that it is in fact the cuticular hydrocarbons (heptacosene [$C_{27}H_{24}$] and tritriacontane [$C_{33}H_{66}$]) found on the pupa that are responsible for the uncapping of infested brood (Hawkins & Martin, 2021). This would indicate that the pupae elicit the trigger. If this is the case, when the offspring feed from the pupae, this cue could transfer from the mites to the pupa.

Contrary to the findings of this paper, Sprau et al. (2021) found no difference between the recapping rates of mite non-reproductive cells in comparison to reproductive cells. However this study used artificial mite insertion and produced only a small sample size which the authors claim is because the mites were escaping the cell after insertion, before the cell was recapped. Only protonymphs and eggs were recorded after eight days of monitoring which indicated a delayed start of reproduction. If a cue is produced by the offspring, it could be produced later in their life cycle. Eight days of monitoring may not be sufficient to monitor where the cue that stimulates recapping behaviour emanates from. As a result of this

finding, Sprau et al. (2021) concluded that the monitoring of VSH behaviour should be preferred to the monitoring of recapping behaviour.

Similarly to Sprau et al., (2021) Kim et al. (2018) determined that VSH bees targeted and uncapped cells that contained multiple foundresses and higher numbers of mite offspring. Thus indicating that these cells were distinguishable from both cells that contained less foundresses and offspring and from non-infested cells. Although, the mite offspring levels in targeted and non-targeted cells were similar in this study which could suggest that the presence of offspring alone does not influence whether a cell is targeted with VSH behaviour or not and therefore would perhaps not be targeted for recapping behaviour. Whilst it remains unknown where the stimulus for eliciting recapping behaviour originates from, it is important that further research focuses on this subject. Future research could ascertain more about the underlying mechanisms of this behaviour and why cells are targeted for recapping.

CHAPTER 5

General discussion

The main aim of this thesis was to surmise more about the role of recapping as a resistance behaviour. In the queen swap experiment (Chapter 2, Figure 3), the average recapping level of Wadborough colonies (16%) was higher than the recapping level of Colonsay queen colonies (9%). Though, the ratio of recapped cells to non-infested normal cells was not statistically different between the Wadborough and Colonsay queen colonies. The predicted result of the queen swap experiment was that Wadborough queen colonies would have higher recapping levels than that of Colonsay queen colonies and that Colonsay queen colonies would suffer from higher mite infestations. Both predictions were found to be correct, although further data is required. The lower infestation levels of Wadborough queen colonies observed in the brood samples, the sugar shake test and the mite fall test is important to recognise (Figure 4, 5). Although, both Wadborough queen and Colonsay queen colonies were expressing recapping, Wadborough queen colonies potentially mitigated the mite population with more advanced or efficient resistance behaviours or mechanisms. Perhaps the most significant finding is that the mite naïve Colonsay queens are capable of producing offspring that recap cells. To elucidate, recapping appears to exist in mite naïve bees that have no prior need to express this trait. Although the results of this study do advocate that recapping could be an innate behaviour, it is challenging to prove considering the fluctuating levels of recapping in sampled colonies. Although critical in terms of beginning to understand the role of recapping, on close analysis, the results from the queen swap experiment must be considered preliminary due to the short time period in which the experiment was conducted. The queen swap experiment should be conducted over a longer period of time with multiple years of data to observe whether the results are consistent.

Evidently, although recapping is present in naturally surviving populations which is promising (Oddie et al., 2018a), there still needs to be more thorough investigations to further understand the heritability and relevance of recapping in the resistance of honey bee populations. Both of these findings would be important for a successful selective breeding programme. However, by attempting to control the dynamics of this already

volatile relationship it is easy to overlook the basics of natural selection and host-parasite relationships. If the focus is solely directed towards selective breeding as a solution then it removes the possibility for bees to develop a natural resistance to the parasite or indeed any future devastating parasite. Indeed, 'sustainable solutions for the apicultural sector can only be achieved by taking advantage of natural selection and not by attempting to limit it' (Neumann & Blacquière, 2016, p.288).

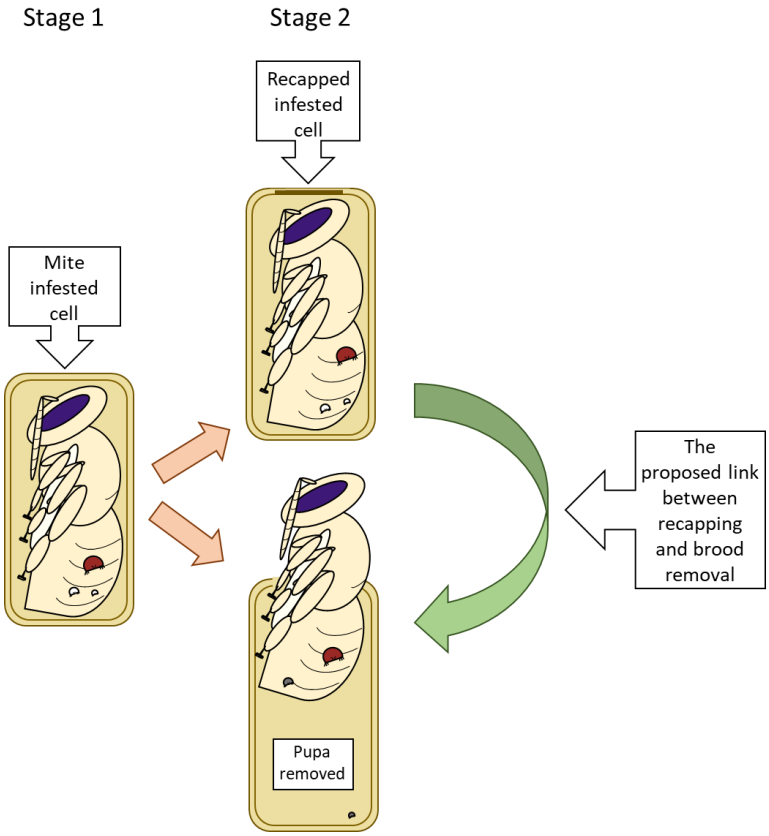

Figure 9. Stage 1. A cell that is infested by a foundress mite and her offspring. Stage 2. The cell is recapped after failed brood removal or the pupa is removed after detection of the mites. The green arrow represents the proposed link between recapping and brood removal, found to be correlated in a study by Martin et al. (2020).

In Chapter 3, the recapping and infestation levels of 14 apiaries from the UK were analysed to make further inferences about the role of recapping in colonies and the traits potential interaction with infestation levels. In Figure 6, the elevated recapping levels alongside the elevated infestation levels determined that this trait is likely to be linked to *Varroa*

infestations. Generally, it could be concluded that if recapping levels are higher than infestation levels, such as Harvington (Salford Lodge) (recapping level: 17%, infestation level 1%), then these colonies could be controlling mite infestation levels well. It could be argued that the colonies that have low levels of recapping and infestation overall such as the UOS (recapping level: 6%, infestation level: 12%) and Wishaw (recapping level: 4%, infestation level: 8%) are not currently at risk of failing. However, apiaries such as Charlton (recapping level: 6%, infestation level: 40%) and North Piddle (recapping level: 59%, infestation level: 35%) that display much higher average infestation levels in comparison to recapping levels could be predicted to fail. Since no significant difference was found between the average recapping levels and infestation levels of all 14 apiaries, this could provide evidence that recapping levels do not have a direct effect on infestation levels, otherwise it would be expected to observe a significantly higher result for recapping levels in comparison to infestation levels. This idea supports findings in a paper by Martin et al. (2020). If recapping is a detection behaviour and can be used to assess whether a colony is predicted to survive mite infestations (Oddie & Dahle, 2021) and can provide an indication as to whether hygienic brood removal is successful (Martin et al., 2020; Grindrod & Martin, 2021b), it would be a beneficial trait to select for in breeding programmes and can provide an indication as to whether a colony is predicted to survive future mite infestations. The relationship between recapping and hygienic behaviour is illustrated in Figure 9.

Chapter 4 focused on how and why specific cells may be targeted for recapping. Comparable to previous studies (Grindrod & Martin, 2021b; Hawkins & Martin, 2021), the results of this paper found that the median recapping diameter of infested cells (2.6 mm) was statistically different to the larger recapped diameter of non-infested cells. As previously mentioned, a thorough inspection of the cell contents would explain the larger recapped diameters of infested cells (Hawkins & Martin, 2021). The theory that smaller holes are created when a weak chemical cue from a nearby infested cell may have permeated into the next cell, may also explain why recapping appears to occur in clusters (Grindrod & Martin, 2021b). However, further research is required to determine how cells are targeted for recapping. Additionally, more data should be gathered to test the clustering hypothesis for non-infested cells.

In Figure 8, the results of the recapping levels of mite non-reproductive and reproductive cells revealed that mite reproductive cells had higher recapping levels (40%) in contrast to non-reproductive cells (20%). The result was significant. For that reason, it can be assumed that the cells that contained offspring were being specifically targeted by recapper bees. Hence, the cue for recapping a cell may originate from the offspring. It is still debated whether the presence of multiple foundress mites (Sprau et al., 2021), the offspring of the mites (as found by the present study) or indeed the pupae (Wagoner et al., 2019) are responsible for producing the cue that triggers recapping. Perhaps a larger study that monitors the recapping rates of mite non-reproductive and reproductive cells during each stage of a *Varroa* mite's life cycle is necessary to discern where the cue that triggers recapping behaviour originates from.

Correspondingly, it is important to consider whether other resistance traits are more successful at reducing mite infestation levels. The *A. m. scutellata* population in Kenya studied by Nganso et al. (2018) presented evidence of resistance towards mite infestations through SMR due to mite infertility, similar results were also found by Strauss et al. (2015). In a paper by Buchegger et al. (2018), SMR and recapping had the strongest influence on the decrease in mite population growth. The authors imply that they are promising resistance characteristics, which can be important for selective breeding against *Varroa*. Unfortunately, in this experiment it was not proven if colonies are able to reduce high mite infestations. The recapping of infested cells was highly correlated to SMR in a study by Büchler et al. (2020a). As of yet, the presence of a direct link between SMR and recapping has not been found, however both traits are often found together in surviving populations (Oddie & Dahle, 2021). Similarly, more research should be conducted to determine whether recapping should be an independent trait or whether it is linked to other resistance traits. Natural VSH behaviour was found to be the focal trait associated with strong mite resistance in a study by Panziera et al. (2017). Mondet et al. (2020a) comment that further research is required to determine if recapping is indeed a separate trait or whether it is a part of the VSH process. In hindsight, the definitions of terms such as hygienic behaviour and VSH behaviour should be concise to standardise results across a range of studies (Guichard et al., 2020a). In fact, referring to VSH as a separate trait to hygienic behaviour may prove to be

confusing as they both involve the same behaviours of detecting, uncapping and brood removal (Spivak & Danka, 2020).

The clear lack of consistency in the measurement of resistance behaviours is somewhat a pitfall for assessing the successfulness of these traits in reducing mite numbers. In addition, small sample sizes are a common feature of this research subject. Undeniably, if there was a direct method of measurement for each resistance behaviour that future studies adhered to then the results would be more comparable and easier to interpret. A challenging problem is that resistant traits are difficult to measure under field conditions (Mondet et al., 2020b; Grindrod & Martin, 2021a). To illustrate, the analysis of SMR requires further training and laboratory access to identify the mite compositions in brood cells (Büchler et al., 2017; Guichard et al., 2021b). Furthermore, if recapping only affects the success of mites mating and not the laying capability of mites then there could be a delay before the true impact of recapping can be monitored (Oddie et al., 2021). Appropriate bioassays should be utilised to assess hygienic behaviour in colonies (Leclercq et al., 2018). Freeze-killed and pin-killed brood assays (described in Leclercq et al., 2018) are widely used across Europe to measure hygienic behaviour and are useful to assess the levels of removed brood (Guichard et al., 2021b). It would be beneficial to measure recapping alongside this assessment to gain a better understanding of a colony's ability to become truly resistant (Hawkins & Martin, 2021). It is important to note that the management of colonies by beekeepers and scientists may differ and that is why it is imperative to monitor traits under field conditions to establish novel, useful selection traits (Guichard et al., 2021b). Finally, the data should be recorded on specific dates annually to identify trait evaluation periods, therefore the measurements can be repeated (Guichard et al., 2021b).

In summation, perhaps the most compelling argument is that recapping does not have a direct effect on mite population levels, but instead is a detection strategy for infested cells and a way for researchers to identify colonies that have a high resistance to the mite (Hawkins & Martin, 2021). The results of the present study would further support that recapping is specifically associated with infested colonies, thereby signifying that recapping is an important aspect of *A. mellifera* resistance against *Varroa*. Yet, the exact role of recapping in colonies is still not entirely clear. Due to the complex relationship between the honey bee and the *Varroa* mite, perhaps a combination of traits are ultimately instrumental

in the establishment of a stable equilibrium between host and parasite (Rosenkranz et al., 2010, Locke et al., 2016; Hawkins & Martin, 2021). In terms of *Varroa* resistance in the future, it is important to discuss the value of traits and their heritability for use in selective breeding programmes. Likewise, if more resistance traits prove to be heritable, permitting honey bee colonies to adapt naturally to *Varroa* could provide a long term and substantial solution to prevent the loss of many colonies. The contributions of the present study have wide applicability and provide further insight into the role of recapping and into this ever-expanding area of research.

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APPENDIX 1: Raw data tables

The letter after the colony number represents the birth year of the queen: Y-Yellow (2017), R-Red (2018), G-Green (2019), B-Blue (2020) and W-White (2021).

Table S1. Raw data for the recapping and infestation levels of colonies in the queen swap experiment.

Colony queen	Apiary location	Colony ID	Collection date	Total cells	Non-infested normal	Non-infested recapped	Infested normal	Infested recapped	Infested recapped (%)	Non-infested recapped (%)	Total recapped (%)	Total infested (%)
Wadborough queen colonies	Croome	8B	Sep-20	360	162	159	1	38	97	50	55	11
	Wadborough	30B	Apr-21	310	308	0	2	0	0	0	0	1
	Wadborough	52B	Apr-21	341	336	5	0	0	0	1	1	0
	Wadborough	74G	Apr-21	276	211	65	0	0	0	24	24	0
	Wadborough	30B	Jul-21	307	210	62	17	18	51	23	26	11
	Wadborough	8B	Jul-21	202	193	0	7	2	22	0	1	4
	Wadborough	30W	Jul-21	189	181	2	6	0	0	1	1	3
	Wadborough	73W	Jul-21	190	139	51	0	0	0	27	27	0
	Wadborough	52W	Jul-21	250	244	0	5	1	17	0	0	2
	Wadborough	74W	Jul-21	163	56	107	0	0	0	66	66	0

	Croome	84W	Jul-21	237	202	35	0	0	0	15	15	0
	Wadborough	81W	Sep-21	458	313	31	88	26	23	9	12	25
	Wadborough	52W	Sep-21	259	242	15	2	0	0	6	6	1
	Wadborough	67W	Sep-21	341	320	18	3	0	0	5	5	1
	Wadborough	84W	Sep-21	328	252	72	1	3	75	22	23	1
	North Piddle	16W	Oct-21	240	196	3	40	1	2	2	2	17
Colonsay queen colonies	Wadborough	2B	Aug-20	129	129	0	0	0	0	0	0	0
	Wadborough	1B	Sep-20	179	179	0	0	0	0	0	0	0
	Wadborough	4B	Sep-20	184	184	0	0	0	0	0	0	0
	Wadborough	1B	May-21	130	123	0	7	0	0	0	0	5
	Wadborough	2B	May-21	151	142	9	0	0	0	6	6	0
	Wadborough	3B	May-21	129	113	14	2	0	0	11	11	2
	Wadborough	3B	May-21	191	159	31	1	0	0	16	16	1
	Wadborough	6B	May-21	202	202	0	0	0	0	0	0	0
	Wadborough	1B	Sep-21	191	109	0	82	0	0	0	0	43
	Wadborough	3B	Sep-21	58	26	3	12	17	59	10	34	50
	Wadborough	3B	Sep-21	432	164	45	143	80	36	22	29	52

	Wadborough	5B	Sep-21	358	166	0	177	15	8	0	4	54
	Wadborough	1B	Sep-21	221	42	3	0	176	100	7	81	80
	Wadborough	G/W	Jul-21	283	283	0	0	0	0	0	0	0
	Wadborough	W/Y	Jul-21	178	178	0	0	0	0	0	0	0
	Wadborough	10W	Sep-21	373	325	0	48	0	0	0	0	13
	Wadborough	9W	Sep-21	369	363	4	2	0	0	1	1	1
	North Piddle	7	Oct-21	488	285	13	177	13	7	4	5	39
	Wadborough	9W	Oct-21	367	355	7	5	0	0	2	2	1

Table S2. Raw data for sugar shake tests performed on colonies in the queen swap experiment.

Colony queen	Apiary location	Colony ID	Weight (g)	Mites	No. of bees
Wadborough queen colonies	North Piddle	16W	111	23	722
	Wadborough	81W	109	119	709
	Wadborough	86	112	58	728
	Wadborough	30W	117	58	761
	Wadborough	73W	128	52	832
	Wadborough	8B	101	30	657
	Wadborough	52W	103	20	670
Colonsay queen colonies	Wadborough	1B	112	464	728
	Wadborough	3B	134	261	871
	Wadborough	10W	93	225	605
	Wadborough	2B	113	183	735
	Wadborough	5G	110	181	715
	North Piddle	7W	113	185	735

Table S3. Mite fall raw data for colonies in the queen swap experiment treated with Api-bioxal.

Colony queen	Colony ID	Seams of bees treated	*Varroa* drop 33 hrs after trickling
Wadborough queen colonies	8B	8	694
	30W	6	234
	73W	10	844
	81W	9	952
	86W	8	607
Colonsay queen colonies	1B	7	1400
	2B	9	1440
	3B	9	1136
	5B	8	1335
	9W	7	67

Table S4. Raw data for the recapping and infestation levels of UK apiaries.

Apiary location	Colony ID	Collection date	Total cells (n)	Non-infested recapped	Infested normal	Infested recapped	Infested recapped %	Non-infested recapped %	Total recapped (%)	Total infested (%)
Beckford	1B	Sep-20	463	15	7	8	53	3	5	3
Beckford	19R	Sep-20	318	183	1	83	99	37	45	14
Beckford	20B	Sep-20	218	25	18	4	18	10	11	8
Beckford	41B	Sep-20	385	33	6	14	70	8	11	5
Beckford	56G	Sep-20	334	23	19	19	50	6	11	10
Beckford	60B	Sep-20	294	20	27	29	52	6	13	15
Besford Bridge	10W	Sep-21	192	72	2	4	67	27	28	2
Besford Bridge	21B	Sep-20	200	145	0	3	100	42	43	1
Besford Bridge	21W	Sep-21	274	60	6	23	79	18	23	8
Besford Bridge	57B	Sep-20	246	104	19	25	57	30	33	11
Besford Bridge	73G	Sep-20	250	106	6	99	94	30	44	23
Besford Bridge	9B	Sep-20	101	0	6	1	14	0	1	6
Besford Bridge	9B	Sep-20	193	2	27	0	0	1	1	12
Besford Bridge	9B	Sep-20	284	0	27	1	4	0	0	9
Bishampton	N7	Sep-20	178	93	0	40	100	34	43	13
Bishampton	NN1	Sep-20	177	56	15	43	74	24	34	20
Bishampton	NN4	Sep-20	179	9	30	3	9	5	5	15
Bishampton	‘P blue'	Sep-20	175	41	34	51	60	19	31	28
Bredon's Norton	1W	Sep-20	257	2	17	1	6	1	1	6
Bredon's Norton	18W	Sep-21	336	8	4	1	20	2	3	1
Bredon's Norton	19W	Sep-21	292	154	0	1	100	35	35	0
Bredon's Norton	20B	Sep-21	407	71	24	12	33	15	16	7
Bredon's Norton	56W	Sep-21	306	169	1	4	80	36	36	1
Bredon's Norton	60W	Sep-21	237	24	17	17	50	9	14	12
Bredon's Norton	66W	Sep-21	243	6	24	24	50	2	10	16
Bredon's Norton	66W	Jul-21	275	51	1	0	0	16	16	0

Bredon's Norton	67W	Jul-21	302	62	1	2	67	17	17	1
Bredon's Norton	68W	Sep-21	386	239	4	44	92	38	42	7
Bredon's Norton	68W	Jul-21	322	31	3	1	25	9	9	1
Charlton	38G	Sep-20	263	14	77	14	15	5	8	25
Charlton	49G	Sep-20	208	1	97	0	0	0	0	32
Church Lench	Hive 5	Oct-21	268	14	102	23	18	5	9	31
Church Lench	Hive 7	Oct-21	237	103	8	93	92	30	44	23
Croome	12B	Sep-20	192	156	0	36	100	45	50	9
Croome	12B	Sep-20	285	237	0	37	100	45	49	7
Croome	15W	Sep-21	247	0	64	0	0	0	0	21
Croome	15Y	Sep-20	157	125	0	14	100	44	47	5
Croome	15Y	Sep-20	127	106	0	16	100	45	49	6
Croome	24W	Sep-21	211	15	27	23	46	7	14	18
Croome	4G	Sep-20	260	22	36	5	12	8	8	13
Croome	4G	Sep-20	260	34	37	15	29	12	14	15
Croome	54G	Sep-20	314	43	63	41	39	12	18	23
Croome	54W	Sep-21	246	1	5	0	0	0	0	2
Croome	55G	Sep-20	293	4	89	6	6	1	3	24
Croome	57W	Sep-21	196	12	3	0	0	6	6	1
Croome	92W	Sep-21	246	123	0	2	100	33	34	1
Harvington	3(H)	Oct-21	127	85	0	14	100	40	44	6
Harvington	4(H)	Oct-21	320	1	170	1	1	0	0	35
near Harvington (Salford Lodge)	12	Sep-20	341	92	0	9	100	21	23	2
near Harvington (Salford Lodge)	3	Sep-20	308	11	0	2	100	3	4	1
near Harvington (Salford Lodge)	15	Sep-20	357	60	0	0	0	14	14	0
North Piddle	18B	Sep-20	139	8	35	49	58	5	25	36
North Piddle	61G	Sep-20	190	4	54	55	50	2	19	36
Pershore College	2B	Sep-20	218	83	1	5	83	28	29	2

Pershore College	EBKA 2B	Sep-20	211	50	11	8	42	19	21	7
Pershore College	EBKA 2B	Sep-21	290	22	20	6	23	7	8	8
Pershore College	EBKA 3B	Sep-21	203	24	19	38	67	11	22	20
Pershore College	EBKA 3B	Sep-20	287	37	14	10	42	11	14	7
Pershore College	EBKA 4B	Sep-20	242	3	11	1	8	1	2	5
Pershore College	EBKA 4W	Sep-21	336	4	47	0	0	1	1	12
Pershore College	EBKA 5B	Sep-21	200	117	0	12	100	37	39	4
Pershore College	2B	Sep-21	528	394	0	21	100	43	44	2
Pershore College	70B	Sep-20	250	32	9	4	31	11	12	4
Pershore College	82W	Sep-21	282	4	48	4	8	1	2	15
University of Salford	IOM1	Sep-20	389	4	32	2	6	1	1	8
University of Salford	IOM1	Apr-21	171	2	3	0	0	1	1	2
University of Salford	IOM2	Aug-20	259	0	20	0	0	0	0	7
University of Salford	IOM2	Sep-20	312	4	56	0	0	1	1	15
University of Salford	IOM3	Aug-20	290	30	53	12	18	9	11	17
University of Salford	IOM3	Aug-20	194	7	3	0	0	3	3	1
University of Salford	NW2	Aug-20	205	18	18	18	50	8	14	14
University of Salford	NW2	Sep-20	131	0	92	5	5	0	2	43
University of Salford	NW2	Sep-20	151	6	9	0	0	4	4	5
University of Salford	NW3	Aug-20	344	25	4	2	33	7	7	2
University of Salford	NW3	Apr-21	234	0	3	0	0	0	0	1

University of Salford	SQ	Aug-20	214	18	9	0	0	8	7	4
University of Salford	SQ	Aug-20	97	6	4	3	43	6	8	6
University of Salford	SQ	Sep-20	248	9	11	15	58	4	8	9
Wadborough	46B		166	12	55	31	36	7	16	33
Wadborough	75G	Sep-20	342	149	5	11	69	30	32	3
Wadborough	75G	Sep-20	340	146	1	9	90	30	31	2
Wishaw	Hive 1		160	0	3	0	0	0	0	2
Wishaw	Hive 3		79	7	9	3	25	8	10	12
Wishaw	Hive 4		285	5	0	0	0	2	2	0
Wishaw	Nuc (hive 3 queen)		102	1	27	6	18	1	5	24
Little Aston	2		221	34	2	5	71	36	37	3
Netherton	32B	Sep-20	253	50	3	1	25	20	20	2
Weston Subedge	Hive 2		303	34	0	0	0	11	11	0

Table S5. Raw data for the average recapped diameter of non-infested and infested cells.

Apiary location	Colony ID	Average recapped diameter non-infested (mm)	Average recapped diameter infested (mm)
Beckford	1B	1.7	1.5
Beckford	19R	2.8	4.2
Beckford	20B	2.8	2.8
Beckford	41B	1.3	2.4
Beckford	56G	1.2	1.3
Beckford	60B	1.3	1.8
Besford Bridge	10W	2.5	1.0
Besford Bridge	21B	2.8	4.0
Besford Bridge	21W	2.2	3.0
Besford Bridge	57B	2.2	2.6
Besford Bridge	73G	2.8	3.5
Besford Bridge	9B		5.0
Besford Bridge	9B	4.5	
Besford Bridge	9B		2.0
Bishampton	N7	2.2	3.7
Bishampton	NN1	2.6	3.2
Bishampton	NN4	2.1	3.3
Bishampton	‘P. Blue’	2.0	2.6
Bredon's Norton	1W	1.5	1.0
Bredon's Norton	18W	1.3	2.0
Bredon's Norton	19W	2.0	
Bredon's Norton	20B	2.5	4.2
Bredon's Norton	56W	3.0	3.8
Bredon's Norton	60W	2.7	3.0
Bredon's Norton	66W	1.8	
Bredon's Norton	66W	2.1	

Bredon's Norton	67W	1.6	2.0
Bredon's Norton	68W	2.4	3.4
Bredon's Norton	68W	1.4	1.0
Charlton	38G	2.5	3.2
Charlton	49G	1.0	
Church Lench	Hive 5	1.6	1.7
Church Lench	Hive 7	3.0	4.0
Croome	12B	3.1	4.3
Croome	12B	2.3	3.4
Croome	15Y	2.5	3.4
Croome	15Y	3.6	3.7
Croome	24W	2.9	
Croome	4G	2.9	3.2
Croome	4G	2.6	2.7
Croome	54G	1.7	2.5
Croome	55G	1.5	1.3
Croome	92W	2.4	3.5
Harvington	Colony 3H	2.7	3.7
Harvington	Colony 4H		
Harvington (Salford Lodge)	12	1.6	2.1
Harvington (Salford Lodge)	3	2.0	4.0
Harvington (Salford Lodge)	15	1.8	
Little Aston	2	2.1	2.4
Netherton	32B	1.9	3.0
North Piddle	18B	1.5	2.6
North Piddle	61G	2.3	2.5
Pershore College	2B	2.3	2.8
Pershore College	EBKA 2B	2.5	2.9

Pershore College	EBKA 2B	1.4	2.3
Pershore College	EBKA 3B	1.9	2.6
Pershore College	EBKA 3B	2.0	2.0
Pershore College	EBKA 4B	2.0	1.0
Pershore College	EBKA 4W	1.3	
Pershore College	EBKA 5B	3.0	2.0
Pershore College	2B	3.3	4.5
Pershore College	70B	2.3	3.5
Pershore College	82W	1.8	4.0
University of Salford	IOM1	1.8	2.0
University of Salford	IOM1	2.0	
University of Salford	IOM2	1.0	
University of Salford	IOM3	1.5	2.3
University of Salford	IOM3	2.6	
University of Salford	NW2	2.3	3.0
University of Salford	NW2		2.2
University of Salford	NW2	1.3	
University of Salford	NW3	1.2	2.0
University of Salford	Salford	2.0	
University of Salford	Salford	2.3	2.3
University of Salford	Salford	3.5	3.9
Wadborough	46B	2.1	2.9
Wadborough	75G	2.1	3.5
Wadborough	75G	2.0	3.4
Weston Subedge	Hive 2	1.4	
Wishaw	Hive 3	2.3	2.7
Wishaw	Nuc (Hive 3 queen)	3.0	1.5
Croome	8B	1.7	2.4

Croome	84W	1.8	
North Piddle	16W	2.7	2.0
North Piddle	7	1.4	1.5
Wadborough	30B		
Wadborough	30B	2.3	2.6
Wadborough	30W	1.0	
Wadborough	52B	1.0	
Wadborough	52W		2.0
Wadborough	52W	2.4	
Wadborough	67W	1.9	
Wadborough	74G	1.5	
Wadborough	74W	3.1	
Wadborough	8B		2.5
Wadborough	81W	1.9	2.2
Wadborough	84W	2.3	1.0
Wadborough	9 W	2.0	
Wadborough	9W	1.3	
Wadborough	1B	1.0	1.7
Wadborough	2B	2.1	
Wadborough	3B	2.7	
Wadborough	3B	1.9	2.0
Wadborough	3B	1.5	
Wadborough	3B	2.0	
Wadborough	5B		1.5

Table S6. Raw data for the recapping levels of mite non-reproductive and mite reproductive cells.

Apiary location	Colony ID	Total cells	Mite non-reproductive cells	Recapped mite non-reproductive cells	Mite reproductive cells	Recapped mite reproductive cells
Besford Bridge	21B	200	1	1	2	2
Besford Bridge	10W	192	1	0	5	4
Bishampton	NN4	170	1	0	32	3
Bishampton	NN1	177	8	5	50	38
Bishampton	'P blue'	175	9	4	76	47
Bredon's Norton	1W	257	2	0	16	1
Bredon's Norton	66W	275	0	0	1	0
Bredon's Norton	67W	302	0	0	3	2
Bredon's Norton	68W	322	0	0	4	1
Bredon's Norton	56W	306	1	0	4	4
Bredon's Norton	66W	219	4	0	20	0
Bredon's Norton	18W	339	1	0	4	1
Bredon's Norton	19W	292	0	0	1	1
Charlton	38G	263	11	1	80	13
Church Lench	Hive 5	268	11	2	114	21
Croome	92W	246	0	0	2	2
Croome	54W	246	0	0	5	0
Harvington	3	308	0	0	2	2
Harvington	12	341	1	1	8	8
Harvington	15	357	0	0	0	0

Harvington	3H	127	4	4	10	10
Little Aston	Hive 2	221	2	1	5	4
Netherton	32B	253	1	0	3	1
North Piddle	18B	138	4	1	79	48
North Piddle	61G	190	72	6	37	37
North Piddle	16W	240	11	0	30	1
Pershore College	2B	218	2	2	4	3
Pershore College	70B	250	3	0	10	4
Pershore College	2B EBKA	211	6	1	13	7
Pershore college	4W EBKA	242	3	0	9	1
Pershore College	4W EBKA	336	6	0	41	0
Pershore College	5W EBKA	200	2	2	10	10
Pershore College	2B EBKA	290	1	0	25	6
Pershore College	68	386	9	7	39	37
Pershore College	3B EBKA	203	3	2	54	36
University of Salford	IOM3	194	0	0	3	0
University of Salford	NW2	151	5	0	4	0
Wadborough	8B	202	2	1	7	1
Wadborough	30B	309	0	0	2	0
Wadborough	30W	189	1	0	5	0
Wadborough	30B	307	8	1	27	17

Wadborough	52W	250	1	0	5	1
Wadborough	1B	130	1	0	6	0
Wadborough	3B	129	0	0	2	0
Wadborough	67W	341	0	0	3	0
Wadborough	10W	373	9	0	39	0
Wadborough	9W	367	1	0	4	0
Wishaw Apiary	Hive 1	160	0	0	3	0
Wishaw Apiary	Hive 3	78	2	1	9	2
Wishaw Apiary	Nuc (Hive 3 queen)	102	7	0	26	6

APPENDIX 2: BBKA News Article 2021

Varroa-Resistance: A Team Update

By Georgiana Webb, Isobel Grindrod and Stephen Martin, University of Salford

Catch up on the latest developments at Salford with news from Georgiana Webb (Georgi), a new MPhil student part-funded by the BBKA, and from Isobel Grindrod (Izzy) and Stephen Martin. Varroas' days could be numbered.

Georgi's news

During my time at the University of Salford, I completed a degree in Wildlife Conservation with Zoo Biology, gaining a 1st class. My final year dissertation topic revealed a passion I never knew I had: 'bee-research'. I was previously unaware of the many problems that honey bees encounter. My dissertation was entitled 'Selection for Hygienic Behaviour in Honey Bees (*Apis mellifera*): A Meta-Analysis', and I focused on honey bees' normal hygienic behaviour of removing dead brood. The conclusion from data I gathered from 21 scientific research papers was that selectively-bred hygienic colonies are superior to non-selected colonies when performing hygienic behaviour. This suggested that hygienic behaviour can be selected for and it was likely to be due to the earlier detection and removal of dead pupae by hygienic bees. I was hooked, and applied to do a two-year MPhil degree on honey bees that started February 2021.

This year, I have been very busy in the laboratory measuring the recapping rates of many colonies, both experimental and control, in our current 'queen-swap' experiment. Recapping is an important behaviour that appears to be linked to decreased mite reproduction and increased colony survival.[1] The queen-swap experiment is designed to understand if the recapping trait is genetic or a learnt behaviour; we hope to have an answer this winter. It is important, as it will help beekeepers to understand if mite-resistant colonies need to be split or if propagation of locally-mated queens will be sufficient to ensure that recapping and thus mite-resistant traits can be passed on.

I am also saving mite offspring and pupae that are both infested and non-infested for future chemical analysis. This analysis aims to identify where the signal for bees to perform hygienic behaviour emanates from; it could be the mites, the pupae or even an entirely different source. It will also allow us to see which key compounds we can detect in the UK honey bee population. To date, several compounds have been identified by groups in the USA, Italy and France, with the French team having the most compelling data. Finally, I am working with a new graduate, Alex Vatentine, who, last winter, conducted a survey into the treatment habits of British beekeepers. Together, we are writing a scientific publication as the data Alex collected is very interesting. We hope to publish the results this winter if all goes well.

I have been fortunate enough, despite COVID, to have already had several beekeeping experiences, finding queens and even witnessing a swarm; we subsequently collected the bees off a nearby branch and carefully transferred them to a hive. I believe if you question everything, you can often discover topics that may need more consideration and therefore further research and I think this is important because we do not know all of the answers. Therefore, I hope to contribute to the vital research on mite-resistant honey bees and helping beekeepers reduce or eventually stop mite treatments while completing my Master's degree.

Izzy and Georgi getting ready to collect their first swarm.

An important update from Izzy

I started my three-year PhD in October 2019 and am funded by Bee Diseases Insurance Ltd (BDI) with all my studies focused on trying to understand *Varroa*-resistant honey bees. I am pleased to say that after almost two years of hard work, Stephen and I have completed a major part of my research programme. This comprises three key parts of work that have resulted in:

- The *BBKA News Special Issue* on *Natural Varroa-Resistant Honey Bees: Biology, Testing and Propagation.*[2]
- An eight-minute instructional video showing beekeepers how to measure recapping and mite removal behaviours.[3]
- A major, high-impact scientific publication bringing together data from over sixty previous studies conducted over the past

forty years to propose a simple framework that explains how *Varroa*-resistance arises in the *A. mellifera* population across many continents.[4] This paper is free to download and print.

This mix of scientific and outreach work is designed to help both beekeepers and scientists understand, measure and propagate *Varroa*-resistant honey bees in the UK. For the first time we have a simple framework that indicates how mite-resistance may have evolved in honey bees. Over time, knowledge gaps will be filled and hypotheses will be tested. The framework should allow the beekeeper to see how the various traits, often long-associated with mite-resistant colonies, link together. Although, the basic mechanism is shown in the *BBKA News Special Issue*,[2] anyone interested in the full details should read the paper.[4] Originally the paper was concise and somewhat difficult to understand. However, after several rounds of reviewer comments it has become a much more detailed and involved piece of writing. I have had to develop a thick-skin dealing with some reviewer comments, but in the end, it was all worth it. My next task is to continue working with the queen-swap experiment for the rest of the year.

A brief overview from Stephen

I have been in *Varroa* research for many decades now and this is the first time I can see a path to the end of the *Varroa* problem for UK beekeepers. A small number of beekeepers have already had over a decade of mite-free treatment. Also, an increasing number are switching to reduced or even no treatments to control the mite populations and instead allowing their bees to adapt to the mite. This is possible in the UK since we do not typically move our colonies long-distances or keep large colony numbers.

I originally studied mite-resistance of Africanised honey bees in Mexico back in the 1990s. At that time, we understood that this trait was restricted to just Africanised bees. However, as time went by, other isolated populations started to appear in many countries. Despite these populations being studied, and lots of ideas being proposed, no clear evidence was forthcoming. The breakthrough came when a Scandinavian PhD student, Melissa Oddie, found that 'recapping behaviour' was elevated consistently in five mite-resistant populations throughout Europe, relative to five nearby non-resistant populations. This was my 'light-bulb moment' since this was the first consistent behavioural data I had seen linked with resistance. I quickly was able to confirm Melissa's original findings during trips to Brazil and South Africa,[4] (see Martin *et al.*, 2019 for the full story[5]). My BDI/BBKA-funded postgraduate student, George Hawkins, then confirmed the link between increased recapping and resistance in the UK.[6] Despite some initial scepticism by bee scientists, Izzy got to work, first explaining the potential reasons behind the increase in 'recapping of non-infested cells'[1] and then progressed to her '*magnum opus*', bringing together forty years of past *Varroa*-resistance research to provide the first comprehensive mechanism of mite-resistance in honey bees.[4] The bottom line is that any type of honey bee population e.g. strain, colour etc, kept in any environment, by whatever method the beekeeper choses, is capable of developing *Varroa*-resistance if given the chance.

Before we start to advise the best way to achieve this, our aim is to have a sound and detailed understanding of the mechanism of mite-resistance, as this allows all the advice we give to be evidence-based. The work my team is doing is going a long way to achieving that goal. For the first time in decades, I am confident that we will see *Varroa* treatments eventually phased out in the UK. Feral populations have a major role to play in this because they are typically the first colonies to become resistant since the selective forces are greatest in these colonies.

The team at Salford will continue to focus on helping beekeepers in their fight against the mite and, to that end, we will try to ensure

A diagram indicating how the key publications link together along with data from around the world to produce the evidenced-based *BBKA News Special Issue* on *Varroa*-resistance and then the instructional video. Unusually, we produced the *Special Issue* in parallel with the final research paper and, due to the importance of the topic for beekeepers, we published the *Special Issue* before the paper since academic publishing can be a long process.

the studies we publish are all open access publications, allowing any beekeeper, to download for free, read and make up their own mind. In the near future we aim to publish the treatment survey Alex conducted earlier this year, finish the queen-swap experiment and continue our work on mite-resistance both in the UK and elsewhere. Finally, I must thank all BDI/BBKA beekeepers, as it is their funding that helps this research to be conducted and the next generation of bee scientists to emerge.

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