VARROA-SPECIFIC HYGIENIC BEHAVIOUR AND POPULATION ABUNDANCE OF *Varroa destructor* IN COLONIES OF *Apis mellifera scutellata* IN KARURA FOREST, KENYA

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DECLARATION

Declaration by Candidate

This thesis is my original work and has not been presented for a degree or for any other award in any other University.

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DEDICATION

This thesis is dedicated to my father, Mr. Jackson Mibei and mother, Mrs. Esther Mibei both who laid foundation for my education. I am grateful to my beloved wife, Cherono Hellen Doren and our children; Emannuel Kipruto, Victoria Chepchumba, Simeon Kipchirchir and Titus Kibet for their moral support during the entire duration of my studies. You have always been a source of my inspiration in all my endevours.

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ACRONYMS AND ABBREVIATIONS

ABPV	Acute Bee paralysis Virus
AFB	American Foulbrood
AIC	Akaike Information Criterion
AmFV	*Apis mellifera* Filamentous Virus
ASAL	Arid and Semi-Arid Lands
BMR	Brood and Mite Removed
BQCV	Black Queen Cell Virus
CBPV	Chronic Bee Paralysis Virus
CCD	Colony Collapse Disorder
CCO	Cell Cap Opened
DWV	Deformed Wing Virus
EFB	European Foulbrood
GDP	Gross Domestic Product
GHB	General Hygienic Behaviour
GLMM	Generalized Linear Mixed Model
GOK	Government of Kenya
HSD	Honest Significant Difference
IAPV	Israel Acute Paralysis Virus
icipe	International Centre of Insect physiology and Ecology
KBV	Kashmir Bee Virus
LSV	Lake Sinai Virus
MRBR	Mite Removed and Brood cell Recapped

PC	Principal Component
PCA	Principal Component Analysis
PVC	Poly-Vinyl Chloride
SBV	Sac Brood Virus
UBC	Untouched Brood Cell
VDV-1	*Varroa destructor* Virus 1
VSH	*Varroa* Specific Hygiene

DEFINITION OF TERMS

Absconding: It is a situation whereby the whole honeybee colony leaves the hive to find a new habitat probably due to unfavourable environmental conditions, lack of food, and attack by pests or diseases.

Africanized honeybee: Is a hybrid of the European honeybee and the African honeybee species.

Brood: Young ones of honeybees.

Caste system: Is categorization of bees into ranks.

Colony: Is a family unit consisting of a queen, workers and drones living inside a hive.

Foundress mite: Is a female mite that is ready to reproduce.

General hygienic behaviour: Is the ability of honeybees to detect, uncap and remove the brood cells with sick, parasitized or dead bees.

Phoresy: This is a situation whereby one organism (for example, mite) travels on the body of another organism (honeybee), especially when not reproducing.

Propolis: is a resinous mixture that honeybees produce by mixing saliva and beeswax with exudate gathered from tree buds and sap flows.

Robbing: Is a term used to describe honeybees that are invading another colony resulting to stripping off its stored food (honey).

Sexual dimorphism: is distinct differences in size or appearance between sexes of an animal in addition to sexual organs themselves.

Swarming: Is a situation whereby a large group of worker bees leaves the colony with the old queen to a new site leaving behind the virgin queen and few worker bees. It is a natural means by which honeybee colonies are created.

***Varroa*-specific hygienic behaviour:** Is the ability of honeybees to detect, uncap and remove *Varroa* mite-infested brood.

ABSTRACT

The ectoparasitic mite *Varroa destructor* is one of the parasite globally reported affecting honeybee health and causing high colony losses. Of notable importance is the association of the mite with viruses and their transmission to honeybees which causes great harm to bees. Kenyan beekeepers have reported that bee populations have been on decline in recent years and therefore the need for research to establish whether *Varroa destructor* is negatively affecting honeybee survival and development. The objectives of this study were to evaluate *Varroa*-specific hygienic behaviour of *Apis mellifera scutellata*, assess population abundance of *V. destructor* and determine the effects of *V. destructor* on local honeybee *A. m. scutellata.* The study was conducted at International Centre of Insect Physiology and Ecology (*icipe*) research apiaries located in Karura forest, Nairobi County. Thirty colonies were randomly selected and monitored from April to November 2016. Data collection on *Varroa*-specific hygienic behaviour was done from ten colonies and the response of *A. m. scutellata* to mite introduction were evaluated at intervals of 72 hours for a period of three months. The pre-pupae worker brood cells were uncapped and 10, 8 and 5 adult female phoretic mites were introduced repeatedly per colony and brood cells recapped. Assessing population abundance and the effects of *V. destructor* on colony size and productivity of *Apis mellifera scutellata* were done on twenty colonies. For each experimental colony, infestation of *V. destructor* on adult bees was measured twice a month using sugar shake method. Mite infestation in worker brood cells was assessed fortnightly by uncapping 200 purple eyed pupae and adult mites found were counted and recorded. Quantifying the amount of brood, adult bees and colony stores (pollen, nectar and honey) was done once every month. The data on *Varroa*-specific hygienic behaviour of *Apis mellifera scutellata*, population abundance of *V. destructor* and effects of *V. destructor* on *Apis mellifera scutellata* were analyzed using Generalized Linear Mixed Model and the means separated using Tukey's HSD at P value = 0.05 (5% significance level). The mean percentage of untouched brood cells was significantly high in control experiments (80%, n = 579) compared to manipulated brood cells in which mites had been introduced (12.5%, n = 110) (P = <0.001). There were significant differences between the different densities of mites introduced and percentage response of *Apis mellifera scutellata* in untouched brood cells (UBC, P = <0.001) and where mites were removed and brood cells recapped (MRBR, P = <0.001). The population abundance of *V. destructor* varied within the months of study and was generally characterized by low mite infestation levels. The mites collected within the first four months of study (April, May, June and July) were significantly lower than those collected within the last four months (August, September, October and November) (P = <0.001). Colony stores also varied throughout the study period with the month of July recording the lowest mean numbers of nectar (38.8 ± 12.5 cm^2), pollen (33.8 ± 8.8 cm^2) and honey (45 ± 10.5 cm^2). The number of adult bee population was positively correlated with overall *V. destructor* population with significant difference (P = 0.0014). The amount of honey was positively correlated with overall *V. destructor* population with significant difference (P = 0.03). In spite of the presence of the parasitic *V. destructor* in bee colonies, all the colonies appeared healthy. Therefore, control measures should be put in place by the government in order to curb any increase in infestation levels of *V. destructor* and maintain the apparent healthy status of honeybees in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Beekeeping is an important component of economic development, agricultural production, rural employment and human nutrition. It improves the livelihood of millions of household in Sub Saharan Africa through sale of bee products such as honey, beeswax, bee pollen, propolis, royal jelly and bee venom (Gidey and Mekonen, 2010). Honey is a sweet, viscous food substance which is used by most communities for treating wounds, healing skin conditions and boosting human energy. Propolis is used by bees to seal crevices in hives and has human medicinal properties. Royal jelly and bee venom are used in making medicinal drugs whereas beeswax is used in candle making. Bee pollen is commonly used as a dietary supplement by humans as it is a protein rich commodity (Raina, 2000).

Beekeeping has led to improved crop production and biodiversity sustenance through honeybee pollination services (Sande *et al*., 2009). It is estimated that approximately 70% of all food crop species (including wild flora) are dependent on insect mediated pollination (Klein *et al*., 2007; Potts *et al*., 2010). In Africa, native honeybees *Apis mellifera* Linnaeus of several different sub-species pollinate 40-70% of indigenous plants, some of which are important in providing nutrient and medicinal rich fruits, vegetables and nuts (Allsopp, 2004). In light of this increasing appreciation of the role of honeybees in food security, it is important to understand the performance of honeybee colonies and how this might be affected by pests and diseases.

In Kenya, beekeeping is majorly practiced in arid and semi-arid (ASAL) areas such as Baringo, West Pokot, Machakos, Makueni, Turkana, Embu and Kitui due to the abundance of bee flora in these regions. Beekeeping plays an important role as a source of income through honey production for small scale farmers. The country's potential for apiculture development is estimated at over 100,000 metric tonnes of honey and 10,000 metric tonnes of beeswax (GOK, 2008; Muli *et al*., 2014), but only about one fifth of this potential is being exploited (Kiptarus and Asiko, 2014). This economic contribution by honeybees may not be realized due to various biotic and abiotic factors affecting honeybee health worldwide such as diseases, parasites, poor beekeeping practices, use of pesticides, climate change, and loss of habitats for foraging (Genersch *et al*., 2010; Guzman-Novoa *et al*., 2010). Honeybees are attacked by several pests such as wax moth *Galleria mellonella* Linnaeus, small hive beetle *Aethina tumida* Murray, large hive beetle *Oplostomus fuligineus* Oliv, bee louse *Braula coeca* Nitzsch, tracheal mite *Acarapis woodi* Rennie, *Varroa* mite *Varroa destructor* Anderson and Trueman, and diseases such as American foulbrood (AFB), European foulbrood (EFB), Nosema and several viral diseases (Mumoki *et al*., 2014; Mcmenamin and Genersch, 2015; Pirk *et al*., 2015). The haemophagous mite *Varroa destructor* is considered one of the major ectoparasite of honeybees of great economic importance to beekeepers worldwide. This study investigated the *Varroa*-specific hygienic (VSH) behavior of *Apis mellifera scutellata*; the seasonal populations of *V. destructor* and the impact of *V. destructor* on colony size and productivity of *Apis mellifera scutellata* bees in Karura Forest, Nairobi County, Kenya.

1.2 Problem statement

The loss of honeybee colonies in recent years is a global phenomenon and Kenya is not exceptional. Honeybees are under a threat due to the decline in the abundance and diversity of flowers, continuous exposure to agrochemicals, poor nutrition, poor beekeeping practices, loss of habitats for foraging, climate change and spread of novel parasites and diseases. The detection of the *Varroa destructor*, an ectoparasitic mite in Kenya in 2009 (Frazier *et al*., 2009), a country which has been known to be free of damaging honeybee pests and diseases, constitutes the most serious threat to the beekeeping industry and agricultural production. One of the major effects of beekeeping on the health of the European honeybee *Apis mellifera mellifera* Linnaeus has been the introduction of this ectoparasitic mite with colony losses of 20% to 40% being reported by beekeepers (Francis *et al*., 2013). In Kenya, beekeepers have reported a reduction in the number of beehives that are being colonized, small sizes of swarms have been observed, and decline in honey production (Muli *et al*., 2014). The effect of *Varroa destructor* in reduction of colony size and productivity is unknown. Hence, studying this recently acquired, exotic ectoparasitic mite is necessary to give some light in fully understanding its seasonal dynamics and effects on Kenyan honeybee colonies since it has become the main pathogenic threat facing honeybee colony survival and development worldwide.

It is also noted that one of the *V. destructor* control strategies at present involves the use of miticides to keep managed honeybee colonies alive. Owing to the frequent use of these chemicals, hive products are contaminated in way of chemical residues that are potentially harmful to human health. The mites have also been shown to develop resistance against the miticides overtime (Irungu *et al*., 2016). It is therefore important to investigate whether *Apis*

mellifera scutellata exhibit *Varroa*-specific hygienic behaviour which is a defense mechanism against *V. destructor* infestations.

1.3 Justification of the study

The ectoparasitic mite *Varroa destructor* globally identified as causing honeybee colony losses has been reported in most beekeeping regions of Kenya. It is therefore necessary to assess population abundance and investigate its role if any; on the decline in honey production. Furthermore, *Apis mellifera scutellata* is known to uncap and remove dead or diseased brood (general hygienic behaviour) but their ability to detect, uncap, remove the mites and recap brood cells (*Varroa*-specific hygienic behaviour) in colonies has not been investigated. Furthermore, this East African honeybee has been reported to survive *V. destructor* parasitism, requiring no chemical treatment even when coexisting with other pathogens responsible for the losses of colonies (Muli *et al*., 2014). It is unknown whether resistance (the ability to limit the fitness of the mite) in *A. m. scutellata* is associated with VSH behaviour. This study evaluated the ability of *Apis mellifera scutellata* to detect the presence of *Varroa* mites in live brood. *Varroa*-specific hygienic behavior is a hereditary trait of honeybee *Apis mellifera* L. which supports resistance to *Varroa destructor*.

1.4 Hypotheses

i. *Apis mellifera scutellata* colonies do not exhibit *Varroa*-specific hygienic behaviour.

ii. The population abundance of *Varroa destructor* in *Apis mellifera scutellata* colonies do not change with time.

iii. *Varroa destructor* has no significant negative effect on the colony size and productivity of *Apis mellifera scutellata* colonies.

1.5 General objective

To investigate *Varroa*-specific hygienic behaviour of *Apis mellifera scutellata*, the seasonal populations of *Varroa destructor* and the negative effects of *Varroa destructor* in *A. m. scutellata* colonies in Karura forest, Nairobi County, Kenya.

1.5.1 Specific objectives

i. To evaluate *Varroa*-specific hygienic behaviour in *Apis mellifera scutellata* colonies.

ii. To assess temporal changes in the population abundance of *V. destructor* within colonies of *Apis mellifera scutellata*.

iii. To determine the effect of *V. destructor* on colony size and productivity of *Apis mellifera scutellata* colonies.

1.6 Significance of the study

Majority of people in Kenya live in rural areas, therefore commercial beekeeping provide source of income and creates job opportunities for the rural communities. This has led to alleviation of poverty, improved living standards and also contributed significantly to

conservation of forest resources. The reportage of threatening honeybee pests and diseases in other parts of the world raises a major concern on the status of bee health in Kenya. Therefore, there is need to determine the interaction between Kenyan honeybees with the recently introduced invasive pests and develop sustainable management solutions. Breeding hygienic colonies is one of the management practices, therefore this study will investigate *Varroa*-specific hygienic behaviour with an ultimate goal of breeding for the trait in African honeybees. Stronger colonies will be developed through breeding which will ensure continuous production of honeybee products that contributes to the Gross Domestic Product (GDP) of the country.

CHAPTER TWO

LITERATURE REVIEW

2.1 Races of honeybees

Honeybees belong to Order Hymenoptera, Super-family Apoidae, Family Apidae and Genus Apis. Apid bees make intricate nests and live in complex societies(Ruttner *et al*., 1978). Genus *Apis* has five main species; the giant honey bee *Apis dorsata* Fabricius, the Asian honeybee *Apis cerana* Fabricius, the little honeybee *Apis florea* Fabricius, the Indian honeybee *Apis laboriosa* Smith and the common honeybee *Apis mellifera* Linnaeus (Sakagami *et al*., 1980). Four of these species; *A. dorsata*, *A. laboriosa*, *A. cerana*, and *A. florea* are among nine honeybee species mostly inhabiting the Asian continents (Chantawannakul *et al*., 2016) while *A. mellifera* inhabit Europe and Africa continent. *Apis mellifera* L. is categorized into; the African, the European, and the Oriental (Asian) species (Ruttner *et al*., 1978).

Honeybees are well represented in Africa, being found almost throughout the continent and represented by different geographical races or subspecies (Hepburn and Radloff, 1998). Honeybees are mainly categorized using their various morphological features, and currently using molecular techniques (Meixner *et al*., 2013). Eleven honeybee sub-species have been identified in Africa and Madagascar; *Apis mellifera intermissa* Maa (North Africa), *Apis mellifera sahariensis* Baldensperger (Morocco desert oases of Northwest Africa), *Apis mellifera lamarckii* Cockerell (Egypt), *Apis mellifera jemenitica* Ruttner sometimes called *Apis mellifera nubi* (Somalia), *Apis mellifera bandasii* Mogga (Sudan), *Apis mellifera monticola* Smith (High altitude areas of East Africa), *Apis mellifera litorea* Smith (low

elevations of East Africa), *Apis mellifera adansonii* Latreille (Nigeria), *Apis mellifera scutellata* Lepeletier (East, Central and West Africa), *Apis mellifera capensis* Eschscholtz (South Africa), *Apis mellifera unicolor* Latreille (the only sub species found in Madagascar) and *Apis mellifera simensis* Meixner (Ethiopia) (Hepburn and Radloff, 1998; Meixner *et al*., 2011; Al-Ghamdi *et al*., 2013).

Honeybee subspecies *Apis mellifera scutellata*, *Apis mellifera monticola* and *Apis mellifera litorea* are commonly found in Kenya (Raina and Kimbu, 2005; Muli *et al*., 2014). *Apis mellifera scutellata* is found in Central and Eastern part of Kenya. It has a medium size body which is covered in fuzz and the abdomen ringed with black stripes (Hepburn and Radloff, 1998) (Plate 2.1a). It resembles European honeybee race *Apis mellifera mellifera* Linnaeus, though slightly smaller, respond quickly and aggressively to humans and intruders disturbance (Raina and Kimbu, 2005). *Apis mellifera monticola* is found in high altitude areas around Mt. Elgon and Mt. Kenya. It is darker in colour, larger in size, gentle and less migratory as compared to *Apis mellifera scutellata* (Plate 2.1b). *Apis mellifera litorea* has a small size body and found in altitude below 500 metres above sea level majorly along the coastal regions (Muli *et al*., 2014) (Plate 2.1c).

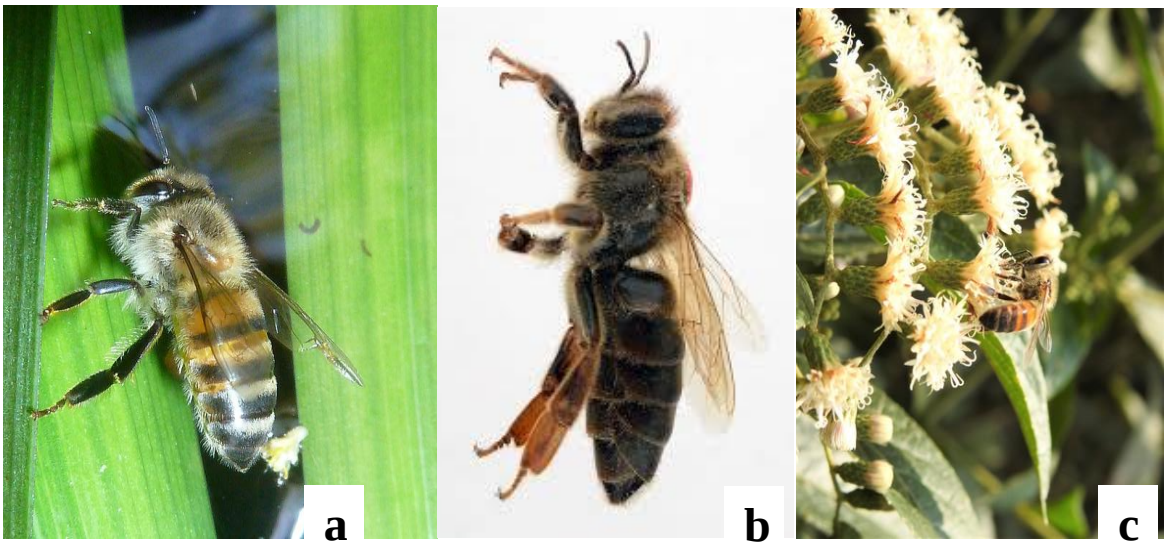

Plate 2.1: Subspecies of *Apis mellifera* bees in Kenya. **(a)** *Apis mellifera scutellata* **(b)** *Apis mellifera monticola* **(c)** *Apis mellifera litorea*

(Modified from https://subspecies of *Apis mellifera*.en.wikipedia.org)

2.2 Honeybee caste system

Honeybees are social insects living in colonies with overlapping generations, cooperative care of brood and reproductive division of labour. A honeybee colony is a family unit of related and closely interacting individuals that form a highly complex society. The colony comprises of three kinds of adult honeybees; female worker bees which can number between 15,000 to 50,000 depending on the time of year, a few hundred drones (male honeybees) and one reproducing female queen (Locke, 2012).

Atypical honeybee measures 12 to 23 mm in length. Figure 2.1 below shows a worker bee, drone and a queen bee.

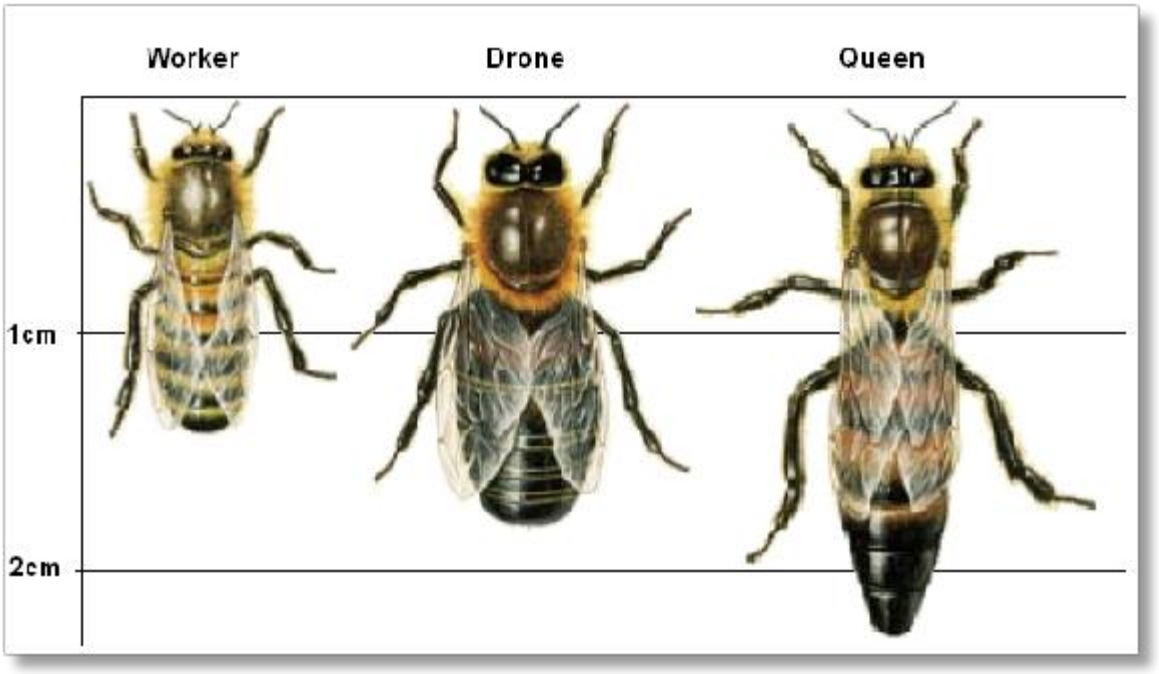

Figure 2.1: Relative sizes of honeybee castes © 2006 Encyclopedia Britannica, Inc.

During its life cycle, a honeybee undergoes complete metamorphosis with the egg, larva, pupa, and adult developmental stages (Figure 2.2). The developmental time for the worker bee is twenty one (21) days, drone twenty four (24) days and the queen is sixteen (16) days (Locke, 2012). The queen bee is the only fertile female individual of the colony. Drones mate with the queen and after the task they die (Fathian *et al*., 2007). After mating in the drone congregation areas, the queen returns to the colony and starts laying eggs singly in each comb cell. Workers and queen hatch from fertilized (diploid) eggs and drones from unfertilized (haploid) egg. The emergence of an adult honeybee into a queen, a drone or a worker bee depends on food supplied to larvae. Royal jelly is the main diet given to larvae throughout their development time when a colony requires a queen. Worker bees are fed on royal jelly for the first three days, then on bee bread and honey for the remaining days. Drone

larvae are fed on royal jelly in the first three days of their larval development, and then diet is changed into modified worker jelly which contains a larger quantity of pollen and nectar (Brodschneider and Crailsheim, 2010).

Foraging worker bees collect pollen, which supplies dietary protein. They also collect nectar which is used to make honey and surplus stored to be a source of food during periods of scarcity. Nurse bees carry out the tasks within the colony such as cell cleaning, cell capping, tending brood, comb building and food storage, whereas mature adult worker bees collect food and protect the hive against colony intruders (Moore *et al*., 1987).

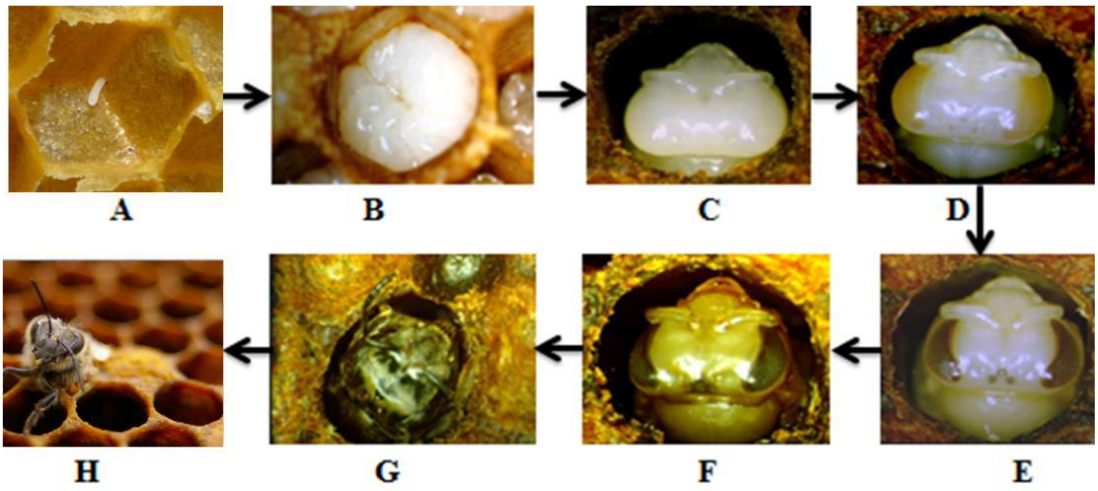

Figure 2.2: Developmental stages of the honeybee *Apis mellifera*. **A-** Egg laid at the bottom of a brood cell; **B**- Larva; **C**- White eyed pupa; **D**- Pink eyed pupa; **E**- Dark eyed pupa; **F**- Dark body pupa; **G**- Pre-emerge adult; **H**- Adult

(Modified from https://resistantbees.com/blog/?page_id=1757)

2.3 History, taxonomy and distribution of *Varroa destructor*

Varroa destructor commonly referred to as *Varroa* mite belongs to Order Mesostigmata, Family Varroidae (Anderson and Trueman, 2000). It was first identified by A.C. Oudemans in Indonesia parasitizing Eastern honeybee *Apis cerana* Fabricius (Oudemans, 1904). *Varroa destructor* is considered a serious parasite of honeybees causing colony losses worldwide (Neumann and Carreck, 2010). It parasitizes different races/sub species of honeybees, through feeding on haemolymph and fat body of different developmental stages ranging from larvae to adults (Rosenkranz *et al*., 2010; Ramsey *et al*., 2019).

The genus *Varroa* comprises four species of obligate ectoparasitic mites; *Varroa jacobsoni* (Oudemans, 1904) which is a native ectoparasitic mite of the Eastern honeybee *Apis cerana* Fabricius, *Varroa underwoodi* (Delfinado-Baker and Aggarwal, 1987) parasitizing Eastern honeybee *Apis nigrocincta* Smith, *Varroa rindereri* (De Guzman and Delfinado-Baker, 1996) parasitizing Eastern honeybee *Apis koschevnikovi* Enderlein and *Varroa destructor* (Anderson and Trueman, 2000) parasitizing European honeybee *Apis mellifera*.

After its identification by Oudemans (1904), *V. destructor* spread to different countries of Asian continent and later it was detected in United States of America (USA) in 1987 (Wenner and Bushing, 1996), North Africa in 1979 (Crane, 1979), South Africa in 1997 (Allsopp *et al*., 1997). The mite has spread to many countries of the world and has become nearly cosmopolitan in distribution (Figure 2.3) (Ellis and Nalen, 2016). The mite spread through commercial transportation of bees, the migratory activities of beekeepers, bees robbing infested colonies, drifting between adjacent colonies and swarms flying long distances (Sumpter and Martin, 2004). The mite was reported in Kenya in 2009 (Frazier *et*

al., 2009). *Varroa destructor* has also been identified on insects that feed on flowers *Bombus pennsylvanicus* (Hymenoptera: Apidae), *Palpada vinetorum* (Diptera: Syrphidae) and on *Phanaeus vindex* (Coleoptera: Scarabaeidae) (Kevan *et al*., 1991).

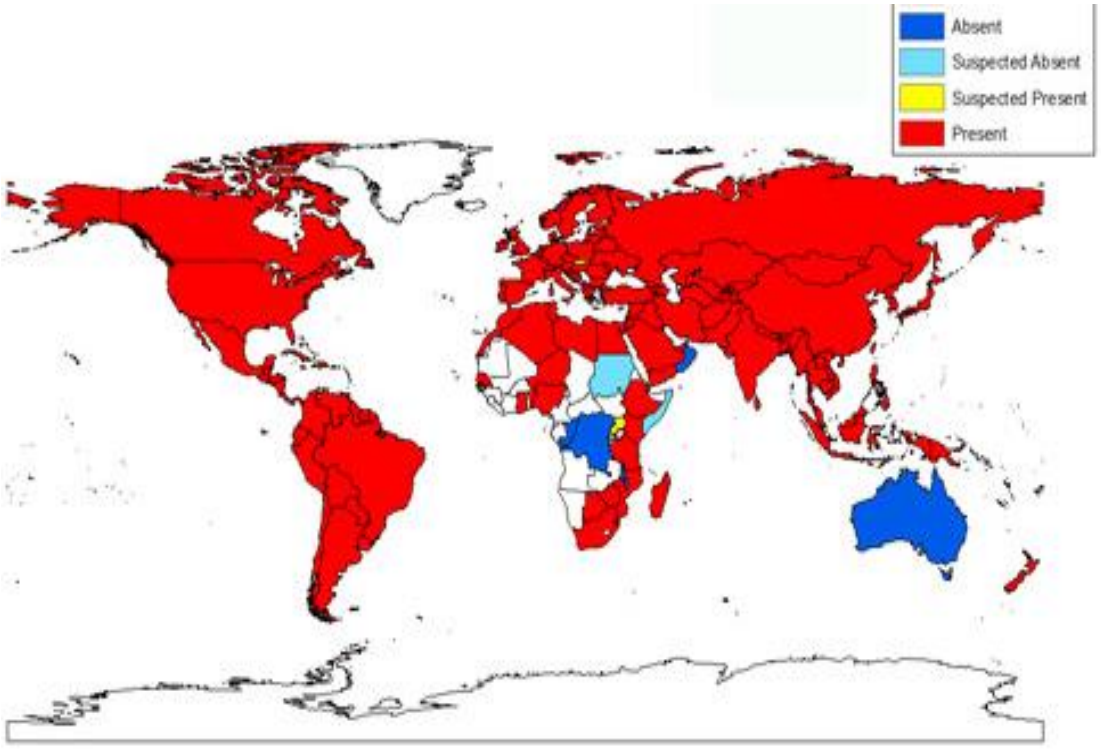

Figure 2.3: Global distribution of *Varroa destructor*
(Adapted from Ellis and Nalen, 2016)

2.4 Morphological features of *Varroa destructor*

Varroa destructor show distinct sexual dimorphism and have bodies divided into two regions; the gnathosoma and idiosoma (Rosenkranz *et al*., 2010). Gnathosoma is located anteriorly forming the piercing and sucking mouthparts and has two sensory pedipalps and two chelicerae. The chelicerae of the male are modified for sperm transfer; thus males can

only feed through a punctured hole made by the adult female on the honeybee larvae (Kanbar and Engels, 2003). Idiosoma is the largest part, transversely oval in shape and has sclerotized dorsal and ventral shields (Plate 2.2). The adult female *Varroa* mites have a flattened oval shape body with strong short legs, reddish brown in colour and are about 1.1 mm long x 1.6 mm wide). Adult males are yellowish-white in colour with long legs and a rounded spherical body measuring about 0.8 mm long x 0.7 mm wide (Martin, 2001; Rosenkranz *et al*., 2010).

Varroa mites live within the dark honeybee nest, mostly inside capped brood cells. They have special appendages called peretrime between the third and fourth pairs of legs that help them breathe inside capped brood cells (Richard *et al*., 1990). *Varroa* mites are found predominantly between the inter-segmental membrane of the abdomen because these regions are more easily penetrated (Kanbar and Engels, 2003). The mite causes wounding, access the haemolymph and can introduce foreign compounds to the haemolymph of the infested honeybee (Koleoglu *et al*., 2017).

The whole body is covered with sensory hairs which have receptors that detect changes in temperature, moisture, chemical stimuli and enables the parasite to locate the brood cells in the colony (Dillier *et al*., 2006). The front legs are not commonly used for movement but used for sensing its surrounding environment.

Plate 2.2: Adult *Varroa* mite
(Modified from https://lifestages of bee mite:idtools.org)

2.5 Biology and life history of *Varroa destructor*

Varroa destructor has two distinct life stages namely phoretic phase spent attached on the adult honeybees and a reproductive phase occurring in capped brood cells during development of immature stages (Rosenkranz *et al*., 2010). During the phoretic phase, the mites suck haemolymph and fat body from adult bees (Rosenkranz *et al*., 2010; Ramsey *et al*., 2019), mostly nurses probably because they carry them to the brood cells for reproduction (Del Piccolo *et al*., 2010). The duration of phoretic phase is variable depending on the season and mites' chance to find preferred brood cell to invade (Boot *et al*., 1994). This phoretic period is 5 to 11 days when brood is present or can be as long as five months during the winter when no brood is present in honeybee colonies (Ellis and Nalen, 2016). *Varroa* mites that enter brood cells without passing phoretic phase could still reproduce, though at a reduced reproduction rate (Beetsma and Zonneveld, 1992). *Varroa destructor* favours distinct attachment sites, particularly the 3rd and 4th ventro-lateral abdominal tergites (Bowen-Walker *et al*., 1997). Adult female *Varroa* mites can recognize potential host honeybees in close proximity and can jump occasionally to attach themselves phoretically

to their new hosts. The phoretic phase is important for horizontal transfer of mites to other colonies by drifting honeybee foragers or when worker bees rob weak colonies that are dying due to *V. destructor* infestations (DeGrandi-Hoffman *et al*., 2016).

The reproductive phase occurs in the capped brood cells and consists of four developmental stages; the egg, two nymph stages (protonymph and deutonymph) and the adult (Donze and Guerin, 1994; Rosenkranz *et al*., 2010). Chemical cues from the larvae of honeybee attracts foundress mites 15 to 20 hours before cell capping and it enters brood cells of 5^{th} honeybee instar larvae (Le Conte *et al*., 1989; Aumeier *et al*., 2002). When *V. destructor* enters brood food, it is activated and oviposition of the mite starts after food is used up by the honeybee larva (Rosenkranz *et al*., 2010). The mite feeds on the fat body of honeybee larva (Ramsey *et al*., 2019) and lays the first egg approximately 60 hours after cell capping which is unfertile and this develops into a haploid male. The other five or six eggs are laid at 30 hour intervals are fertile and develops into diploid females (Ifantidis, 1988; Ellis and Nalen, 2016). The developmental time from egg to adult is about 6.6 days in males; egg (30hours), protonymph (52 hours), deutonymph (72 hours). The development time for females is about 5.8 days; egg (20-24 hours), protonymph (30 hours), deutonymph (75-80 hours). Males are clearly smaller than females in all developmental stages (Ifantidis, 1988; Donze and Guerin, 1994; Martin, 2001; Rosenkranz *et al*., 2010) (Plate 2.3).

Varroa mites reach sexual maturity during the last molting stage. Males reach sexual maturity earlier and stay at the faecal accumulation site, waiting for the first adult female which molts to adulthood some 20 hours later and as soon as females arrives mating takes place (Donze *et al*., 1996). When adult bees emerge from capped brood cells, adult daughter mites also leave the brood cell and can be found later within the phoretic mite population

under the abdominal segments of adult honeybees (Garrido *et al*., 2003). Male and nymphal stages of the mite are only found within the capped brood cells and die shortly when the adult honeybee emerges due to dehydration (Moore *et al*., 1987).

Varroa destructor depends entirely on its host biology for survival, growth and development (Rosenkranz *et al.,* 2010). The mite depends on brood to reproduce and therefore cannot reproduce during brood less periods and depending on the post capping duration of the worker brood cells, only one to three adult female mites can reach maturity (Rosenkranz *et al*., 2010).

Reproduction of *V. destructor* is affected by several factors which includes; type of reproductive host, cell size, temperature and relative humidity. The reproductive rate of *Varroa* mites in worker brood is lower; give rise to 2 daughter mites as compared to drone brood which produce 3 daughter mites (Fries *et al*., 1994). Maggi *et al*. (2010) found that the fertility of mites was lower in brood cells with smaller diameter as compared to brood cells with larger diameter. *Varroa* mites that are introduced into brood cells that have been capped for over 14 hours do not reproduce probably due to absence of odour from fifth instar larvae which are used as signals by mites to activate their ovaries (Garrido and Rosenkranz, 2004).

Temperature affect the physiology of the mites. In an experiment carried out under laboratory conditions, mites reproduced at 34.5°C whereas no offspring was observed at 31.5°C (Chiesa *et al*., 1989). High humidity between 79% and 85% has been shown to reduce reproduction of *Varroa* mites whereas optimum reproduction occurs at relative humidity ranging from 55% to 70% (Kraus and Velthuis, 1997).

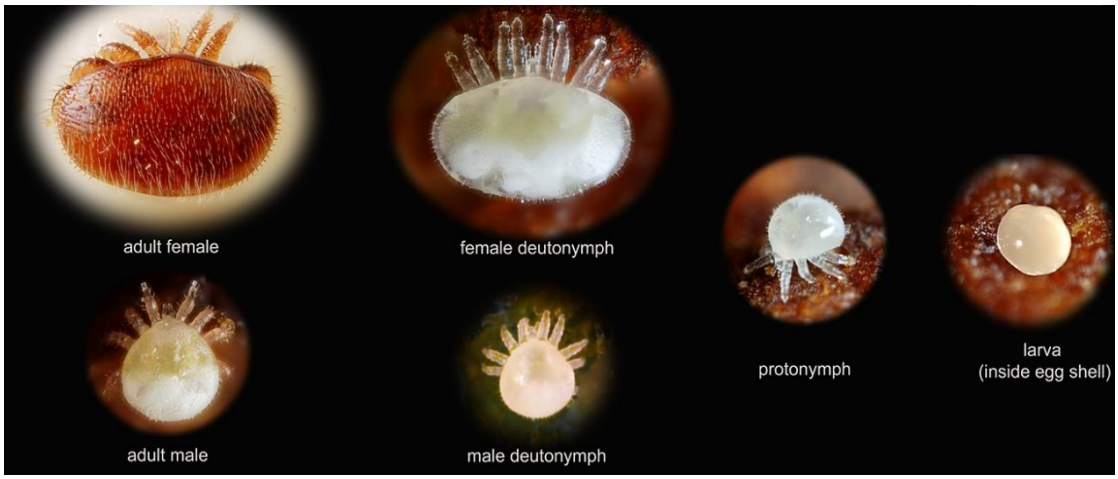

Plate 2.3: Different stages of *Varroa destructor*
Modified from https://lifestages of Beemite:idtools.org

2.6 Factors affecting population abundance of *Varroa destructor*

The population growth of *V. destructor* is highly variable and is affected by several factors of the host and those of the mite itself (Fries *et al*., 1994; Fries *et al*., 2003).

Factors that influence population abundance of the mite are; the size of initial mite population, mite invasion rate and the reproductive capacity of the mite. When colonies are established with low *Varroa* mite populations, it takes more than a year before high population is reached (DeGrandi-Hoffman and Curry, 2004). The rate of invasion increases with increase in the number of suitable brood cells and is slower when the distance between the mite on the honeybee and the brood cell is large (Boot *et al*., 1994). The reproductive capacity of the *Varroa* mite varies among the various honeybee races. There is reduced fertility rate on *A. mellifera* of African origin and in Africanized honeybee compared to *A. mellifera* of European origin and this has been considered an important factor for coexistence between parasite and the host (Carneiro *et al*., 2007; Nganso *et al*., 2018).

Features of the host influencing population abundance of the mite include; race and strain of honeybee, ratio of brood to adult honeybees, brood availability, brood attractiveness, the duration of post capping stage, absconding, swarming and level of behavioural defense mechanism. The race and strain of honeybee influences population abundance of *Varroa destructor*, for example, *Apis cerana* F. has lower infestation levels due to exclusive reproduction of the mites in the drone brood and a higher hygienic removal of the infested worker brood by hygienic bees (Peng *et al*., 1987). European honeybees are found to be more heavily infested than Africanized honeybees when reared under same ecological conditions (Guzman-Novoa *et al*., 1996).

The ratio of brood to adult honeybees affect mite population when the surface area of suitable brood cells increases, more bees will come close to brood and the phoretic mites have more opportunities to leave the adult honeybee and enter brood cells (Boot *et al*., 1994; Sammataro *et al*., 2000).

The availability of bee brood provides better reproductive conditions for the *V. destructor* females. Drone brood is highly preferred by the mites than worker brood (Fuchs, 1990). They produce higher quantities of chemical cues (methyl palmitate) for longer periods which are involved in *V. destructor* attractiveness (Le Conte *et al*., 1989; Calderone and Lin, 2001). The brood of honeybees of European origin have been shown to be more attractive than brood of Africanized honeybees (Guzman-Novoa *et al*., 1996, 1999).

The development of *Varroa destructor* can be limited by post-capping duration. A longer post-capping period of the brood cell increases the number of adult mature daughter mites.

Africanized honeybees as well as African honeybee races have a significant shorter period of post-capping than European honeybee races (Rosenkranz, 1999; Nganso *et al*., 2018).

Swarming and high rates of absconding are promising strategies that reduces the population of the mite in honeybee colonies. The incidence of swarming typically causes a loss of 40–70% of the adult worker bee population which is followed by a broodless period of several weeks when reproduction of the mite is interrupted (Wilde *et al*., 2005).

Honeybees exhibit behavioural defense mechanisms such as hygienic and grooming behaviours. Defense mechanisms when expressed at high levels in honeybee colonies, mite reproduction is suppressed and reduces population of *Varroa* mites overtime (Spivak and Gilliam, 1993; Boecking and Spivak, 1999; Nganso *et al*., 2017).

Environmental factors such as climate and nectar flow, influence population of the mites (Moretto *et al*., 1991). When nectar is deficient, robbing activities of honey and drifting of foragers are high resulting to increased mite population in strong honeybee colonies **(**Kralj and Fuchs, 2006). However this depends on the density of *Varroa* mites in honeybee colonies and the proportion of foragers with mites (Seeley and Smith, 2015; DeGrandi-Hoffman *et al*., 2016).

Population abundance of the mite also varies depending on the regions due to different environmental conditions (Calderon *et al*., 2010). There is high population abundance of mites in temperate regions as compared to tropical regions which have lower mite population growth (Moretto *et al*., 1991; Medina- Flores *et al*., 2014). This low population growth of mites in tropical regions is unexpected because honeybee brood is available throughout the year and therefore mite reproduction is not interrupted as it is during the winter under temperate climatic conditions. Several extensive survey studies have been carried out in

temperate regions to monitor mite infestation levels over several seasons. High *V. destructor* infestation levels were reported and beekeepers were experiencing high colony losses (Webster *et al*., 2000; Calderon and van Veen, 2008; Genersch *et al*., 2010; van Dooremalen *et al*., 2012). Previously in West Africa, population dynamics of *Varroa destructor* was assessed in *Apis mellifera intermissa* (Adjlane *et al*., 2015), but no study has been done in sub-Saharan Africa. Studies of this host-parasite interactions in tropical zones are rare, but important, especially since this subspecies is able to survive parasitization without acaricide treatment by the beekeepers.

2.7 Effects of *Varroa destructor* on honeybees

Varroa destructor has been the greatest cause of colony losses especially to honeybees of European origin through increased mortality of brood and reduction in the longevity of adult worker bees (Rosenkranz *et al*., 2010). *Varroa* mite feeds on adult honeybee and immature stages injuring the bees physically, reduce their protein content and interfere with development of tissues and organs (Bowen-Walker and Gunn, 2001). Interference of brood development and reduced lifespan of adult bees negatively affect the colony population of honeybees and over time lead to loss of colonies (van Dooremalen *et al*., 2012). Prior to collapse, colonies sustaining high *Varroa* mite levels also exhibit reduced honey production (Erickson *et al*., 1998; Emsen *et al*., 2014). Previous studies have shown that colonies can die from *Varroa* mite infestation if left untreated within a period of 1 to 3 years and the damage caused to honeybees is related to the level of *Varroa* mite infestations in colonies (Martin, 1998; Calderon and van Veen, 2008).

Varroa destructor also serves as a vector for various honeybee viruses. There are about 23 viruses transmitted by *V. destructor* to honeybees (McMenamin and Genersch, 2015). Nine viruses have been reported in many African countries; Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Israel Acute Paralysis Virus (IAPV), *Apis mellifera* Filamentous Virus (AmFV), *Varroa destructor* Virus 1 (VDV-1), Chronic Bee Paralysis Virus (CBPV), Lake Sinai Virus (LSV) and Sac Brood Virus (SBV) (Chen and Siede, 2007; Muli *et al*., 2014; Mumoki *et al*., 2014). These many viruses transmitted by the ectoparasitic mite contribute to morphological deformities, reduced vigour and longevity of honeybees (Bowen-Walker *et al*., 1999; Shen *et al*., 2005; Mondet *et al*., 2014). The most common honeybee virus is DWV whose clinical symptoms in adult honeybee include deformed wings, body discolouration and shortened body size (Gisder *et al*., 2009).

Varroa destructor rarely kills adult honeybees, but causes reduction in body weight (Annoscia *et al*., 2012) and may promote accelerated maturation leading to early and inefficient foraging activity of honeybees (Downey *et al*., 2000). Foragers have reduced flight durations and homing abilities with those that are highly infested not returning to their colonies due to impaired orientation abilities (Kralj and Fuchs, 2006; Kralj *et al*., 2007). *Varroa* mite has also been implicated for Colony Collapse Disorder (CCD), a condition in which adult honeybees disappear from the colonies leaving only the queen and few immature honeybees (Le Conte *et al*., 2010).

The damage caused by *V. destructor* depends on infestation levels. A worker bee when infested results in an average loss of body weight of between 7-10% (Bowen Walker and Gunn, 2001; Annoscia *et al*., 2012). When drones are highly infested, they lose 11–19% of

their body weight (Duay *et al*., 2003), leading to decreased flight activities in drone congregation areas and reduced mating abilities (Duay *et al*., 2002; Annoscia *et al*., 2015). Studies have also shown that when mite infestation levels are > 5%, the parasite can severely affect honey production in honeybee colonies (Medina-Flores *et al.,* 2011). In tropical regions, Kenya included, brood rearing takes place year-round in honeybee colonies, *V. destructor* reproduction therefore occurs throughout the year increasing its population in honeybee colonies which might significantly reduce honeybee population and, eventually decline in honey production.

2.8 Behavioural defense mechanisms facilitating mite mortality

Honeybees have well developed behavioural defense mechanisms that reduce the effects of *Varroa destructor*. These mechanisms are grooming and hygienic behaviours.

2.8.1 Grooming behaviour

Grooming behaviour is a behavioural mechanism in which honeybee workers clean themselves and other nest mates leading to removal of mites from honeybees' bodies. Grooming behaviour includes auto grooming and allo grooming performed by worker bees not infested by *Varroa* mites (Peng *et al*., 1987). Auto-grooming enables bees to remove foreign particles, ectoparasites, dust and pollen from their own bodies. It involves biting and licking with their mouthparts as well as movement of mesothoracic legs (Boecking and Spivak, 1999).

Allo grooming involves several nestmates acting collaboratively. The infested worker bee attracts other bees by vibrating its body laterally. The other nestmates will approach the infested honeybee, which will stretch the wings and legs and raise up its body. The nestmates examine the body for the presence of mites using antennae and remove them with their mandibles. In the process of removing mites from the honeybee's body, mites can be damaged, eventually leading to reduced mite infestations in honeybee colonies (Nganso *et al*., 2017).

2.8.2 Hygienic behaviour of honeybees

Hygienic behaviour is a specific type of nest hygiene in which dead or diseased bees are removed from colonies. Currently, two types of hygienic behaviour are known; General hygienic behaviour (GHB) and *Varroa*-specific hygienic (VSH) behaviour. General hygienic behaviour of a worker bee is defined as the ability to detect, uncap and remove the cells with dead, parasitized, diseased or infected brood (Rothenbuhler, 1964; Arathi *et al*., 2000; Harbo and Harris, 2005). It is an inherited activity and given the limited number of immune effector genes in honeybees (Evans *et al*., 2006), GHB is considered an important behavioural defense mechanism playing a key role in maintaining health of honeybee colonies (Wilson-Rich *et al*., 2009). General hygienic behaviour is determined largely by two behavioural components, the uncapping of brood cells and the removal of dead brood (Rothenbuhler, 1964). It is a multi-step process involving several hygienic bees and is determined by several genes (Lapidge *et al*., 2002). Its expression depends on environmental factors and colony strength and has been shown to reduce during periods of nectar scarcity

(Momot and Rothenbuhler, 1971) and weak colonies (those with small populations) displays reduced hygienic response (Spivak and Gilliam, 1993).

Varroa-specific hygienic (VSH) behaviour is the ability of honeybees to detect, uncap and remove *Varroa* mite-infested brood, particularly the mites that are actively laying eggs. *Varroa*-specific hygienic behaviour is more effective towards *V. destructor* infestation and leads to higher infertility of *Varroa* mites in honeybee colonies (Harbo and Harris, 2005; Ibrahim and Spivak, 2006).

The distinction between *Varroa*-specific hygienic (VSH) behaviour and general hygienic behaviour (GHB) is in the detection stimulus of the adult honeybees, which for VSH behaviour seems to be as a result of high mite infestations and its indirect effects such as increased pupal viral transmission or deformities in emerging bees (Mondet *et al*., 2014). Additionally, general hygienic behaviour is commonly associated with removal of dead or diseased brood and fewer mites removal whereas *Varroa*-specific hygienic behaviour is characterized by a higher removal rate of mites and manipulation of brood cell contents (Danka *et al*., 2011). *Varroa*-infested brood produce uniquely identifiable cues that are used by VSH-performing bees to identify with high specificity which brood cells to target and remove the mites (Mondet *et al*., 2015).

Varroa-specific hygienic behaviour is heritable and highly variable and hence has been used in programs to breed mite tolerant strains of European honeybees. This has resulted in diverse honeybee populations that are desirable for beekeeping (Guerra *et al*., 2000; Vandame *et al*., 2002; Ibrahim and Spivak, 2006). *Varroa*-specific hygienic behaviour

involves a series of complex reactions during opening and recapping of mite infested brood cells (Villegas and Villa, 2006).

Varroa-specific hygienic behaviour is performed by worker bees with hereditary distinct specialized hygienic characteristics. Highly hygienic honeybee colonies are more responsive to anomalies in infested pupae (Masterman *et al*., 2001), and they remove them faster than non-hygienic colonies. *Varroa* mite escapes or is removed by hygienic honeybees before recapping of brood cells (Aumeier *et al*., 2000; Aumeier and Rosenkranz, 2001). The removal of *V. destructor* from the brood cells leads to reduced reproduction of the parasite, extended phoretic period on honeybees and even the death of the ectoparasitic mite (Fries *et al*., 1994; Harris *et al*., 2010).

During short term experiments, *Varroa*-specific hygienic bees have proved to reduce mite population in honeybee colonies (Harbo and Harris, 2009; Villa *et al*., 2009) and can keep mite populations at low infestation levels thus delaying the need for chemical treatments (Delaplane *et al*., 2005). The use of miticides such as Apistan strips, ApiGuard to control this parasite leaves chemical residues in bee products (Szczesna *et al*., 2009). Therefore keeping honeybees having higher degree of hygienic behaviour is necessary as a natural method of minimizing the level of infestation by parasites and diseases in honeybee colonies (Palacio *et al*., 2000). *Apis mellifera scutellata* is known to exhibit general hygienic behaviour (Fries and Raina, 2003), a factor contributing to resistance of honeybees to *Varroa* mite (Frazier *et al*., 2009). However, its ability to detect and remove *Varroa* mites in live brood is unknown. Identification of this behavioural defense mechanism is beneficial in selection and breeding of resistant *A. m. scutellata* bees.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted at the International Centre of Insect Physiology and Ecology (*icipe*) research apiaries in Karura forest between April and November 2016. The forest is located in Nairobi County, Kenya at 1.2333^0 S, 36.8333^0 E and altitude of about 1,663 metres above sea level (Figure 3.1).

Karura forest is one of the largest urban gazetted forests in the world averaging 1041 hectares (Parita and Irandu, 2015). The vegetation is characteristic of African savannas, predominantly trees and shrubs. Trees include both exotic and indigenous trees. Exotic trees cover 632 hectares and include *Eucalyptus camuldensis* Dehnh, *Eucalyptus saligna* Smith, *Grevillea robusta* Cunn, *Cypressus lusitanica* Mill. Indigenous trees cover 260 hectares and include *Markhamia lutea* Benth, *Croton megalocarpus* Musine, *Warburgia ugandensis* Warbug. A number of shrubs are found which have local medicinal use. They include *Strychnos henningsii* Gilg, *Vangueria madagascariensis* Gmel, *Solanum incanum* Linn, *Rhus natalensis* Bernh. These plants produce flowers and are therefore source of nectar and pollen for honeybees.

The average daily temperature ranges between 18-23ºC with minimum temperature ranging between 9-12°C and the maximum temperature is 25ºC. The forest has two wet seasons, April to June (long rains) and October to December (short rains). The other months are sunny and dry except for July which is usually cool and cloudy. The average mean annual rainfall is 930 mm ranging from 350 mm to 1250 mm during dry and wet seasons respectively.

Karura forest is protected by Kenyan government and human activities such as deforestation, charcoal burning, illegal logging are prohibited.

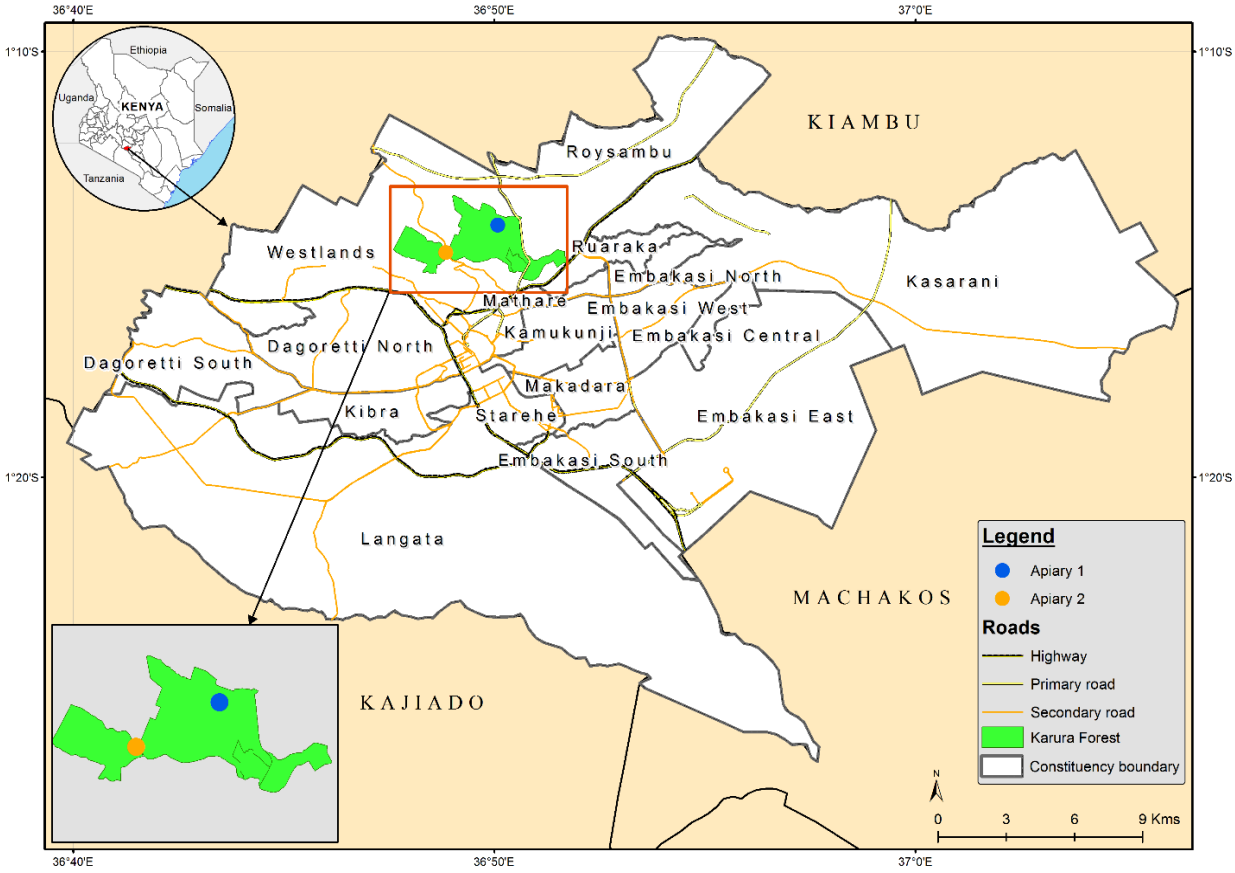

Figure 3.1: Map of Nairobi County showing location of Karura forest and apiary sites
Source: *icipe* Geo-Information Unit

3.2 Establishment of honeybee colonies

Study colonies were already established by the *icipe* Bee Health Unit. Thirty colonies were randomly selected in two different apiary sites within Karura forest; twenty colonies in Apiary 1 and ten in Apiary 2 (Plate 3.1). Apiary 1 was located at 1.23442^0 S and 36.8347^0 E

while Apiary 2 was located at 1.24525^0 S and 36.81416^0 E. Colonies were housed in standard frame Langstroth hives containing 8 to 10 brood combs and arranged on stands dipped in plastic containers with used motor oil to prevent ants from climbing and entering the hives and causing absconding of honeybees (Kinati *et al*., 2012). The hives hosted *A. m. scutellata* bees (Raina and Kimbu, 2005; Muli *et al*., 2014).

Plate 3.1: Honeybee colonies in the study sites **(a)** Apiary 1 **(b)** Apiary 2

3.3 Evaluation of *Varroa*-specific hygienic behaviour in *Apis mellifera scutellata* colonies

Experiments on *Varroa*-specific hygienic behaviour in *Apis mellifera scutellata* colonies was carried out in two steps described below:

3.3.1 Selection of hygienic colonies

Twenty colonies were randomly selected in the apiary and were tested for general hygienic behaviour (GHB) using pin killing brood assay. It is a common assay that involves artificial

killing of brood using sterilized pin in order to measure the willingness of the hygienic bees to remove dead brood (Palacio *et al*., 2010).

A brood frame containing capped worker brood was selected and removed from each colony, honeybees were shaken off and a few brood cells were uncapped to confirm the developmental stage (purple eyed pupa). Each brood frame was placed on a flat surface and an open-ended Poly-vinyl chloride (PVC) pipe (7.62 cm in diameter) was pressed into a frame containing purple eyed pupae. This area corresponds to approximately 207 brood cells of naturally drawn African honeybee comb (Muli *et al*., 2014). The brood were then pin killed using a sterilized needle and each brood frame was returned into its colony. After 24 hours, percent general hygienic behaviour (GHB) of a colony was calculated as follows:

$$\% \text{ GHB} = \left(\frac{\text{N}_{rem}}{\text{N}_{tot} - \text{N}_{uncap}}\right) x\ 100$$

Where;

N_{rem} = number of fully or partially removed pupae

N_{tot} = total number of cells (in *A. m. scutellata* this area corresponds to 207 cells)

N_{uncap} = number originally uncapped or empty cells

Colonies that removed >80% of the pin killed worker brood cells were considered hygienic (Pereira *et al*., 2013) and were used to evaluate *Varroa*-specific hygienic behaviour (Plates 3.2a, b, c, d).

Sugar roll assay was also carried out in these colonies to quantify the number of *Varroa* mites and correlated with general hygienic behaviour. About 300 adult bees were collected

from brood nest of each colony using a standardized cup and put into a glass jar with a removal lid made of a 2 mm wire mesh. Two teaspoons of powdered icing sugar were added and the jar was shaken to dislodge the mites from the bees (Macedo *et al*., 2002). The jar was then inverted over a piece of white paper to collect the fallen *Varroa* mites (Dietemann *et al*., 2013).

Plate 3.2a: Comb frame of compact brood

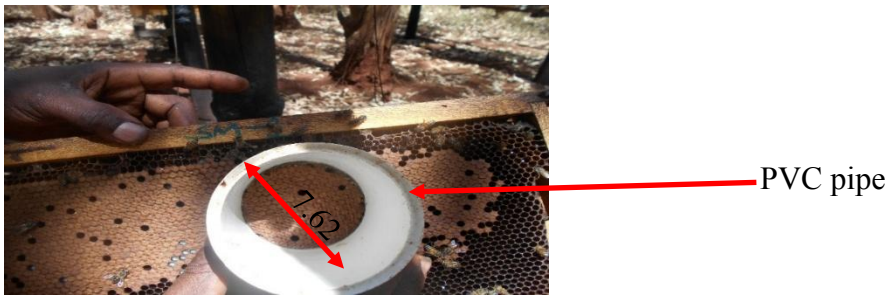

Plate 3.2b: Using a PVC pipe to mark the diameter of the brood cells

Plate 3.2c: Pin killed brood cells before inserting into the colony

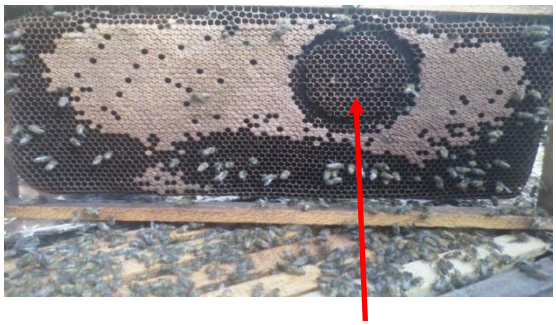

Plate 3.2d: The colony 'cleaned' most of the pin killed brood cells after 24 hours and is classified as 'hygienic'

3.3.2 Evaluation of *Varroa*-specific hygienic behaviour in selected hygienic colonies

Varroa-specific hygienic behaviour was determined using the method of Aumeier *et al*. (2000). Frames containing worker brood cells with eggs were identified in each hygienic colony, marked and followed up until capping (Plate 3.3). A frame of worker brood cells, 24 hours after cell capping was removed from each of the ten selected hygienic colonies. Worker brood cells were opened at one edge using sterilized forceps with ethanol. On each experimental day, closely neighbouring brood cells were singly introduced with ten mites (10) in the first colony, eight (8) mites in the second colony and five (5) mites in the third colony using a tip of a sterilized needle. The same procedure was repeated for the other remaining seven colonies. All target mites were placed on the ventral side of the pre-pupa without harming the host and the cells recapped with a drop of melted beeswax. At the end of the study period, each colony had been singly introduced with a total of 69 female phoretic mites. The same number of brood cells per colony (10, 8 and 5) were sham manipulated (opened and recapped without inserting mites) on the other side of the same brood frame where the mites were introduced. Immediately after handling, combs were returned into their original colonies. Cells were marked and identified by use of a transparent plastic sheet for

subsequent recording of the number of brood cells that were emptied by honeybees. Seventy-two hours later, the manipulated cells were carefully examined and recorded as follows: Untouched Brood Cell (UBC), Cell Cap Opened (CCO), Brood and Mite Removed (BMR), Mite Removed and Brood cell Recapped (MRBR).

Plate 3.3: A comb frame containing eggs

3.4 Assessing population abundance of *Varroa destructor* in *Apis mellifera scutellata* colonies

Twenty colonies naturally infested by *V. destructor* were randomly selected and monitored to determine mite infestation levels for a period of eight months using four different methods: Use of sticky boards, Sugar shake (roll) method, Quantifying *V. destructor* in capped worker brood and Colony size estimation.

3.4.1 Use of sticky boards

Twenty colonies were equipped with screened *V. destructor* bottom boards (460 x 360 x 5 mm) on which sticky *Varroa* monitoring sheets were inserted to collect fallen *Varroa* mites (representing natural mite fall) (Branco *et al*., 2006). After every seven days, *V. destructor* monitoring sheets in all colonies were removed and the fallen and stuck mites counted. Average daily *Varroa* mite fall was obtained using the following formulae by Calderon and Van Veen (2008).

$$\text{Daily } Varroa \text{ mite fall} = \frac{\text{Number of fallen mites}}{\text{Number of days (7)}}$$

After collecting fallen mites, sticky boards were cleaned, smeared with ointment (odorless petroleum jelly) and then placed back into their respective colonies.

3.4.2 Sugar shake method

Varroa destructor infestation rate on adult bees in the twenty colonies was measured twice a month using sugar shake (roll) method. About 300 adult bees were collected from the brood nest of each colony using a standardized cup (Plate 3.4) and transferred into a glass jar with a removable lid made of a 2mm wire mesh. Two teaspoons of powdered icing sugar were added and the jar gently shaken for about two minutes to dislodge the mites from the bees (Macedo *et al*., 2002), then inverted over a piece of white paper to collect falling *Varroa* mites. The bees were placed back into the colony immediately after handling. Mites were counted to establish levels of infestation per 100 adult bees using the following formulae by Dietemann *et al*. (2013).

$$\text{Level of } Varroa \text{ infestation} = \left(\frac{\text{Number of } Varroa \text{ mites}}{300 \text{ adult bees}}\right) x\ 100$$

The population of *Varroa* mites on adult bees in each colony was calculated as follows:

$$Varroa \text{ mite population} = \text{Number of adult bees } x\ \left(\frac{\text{Number of } Varroa \text{ mites}}{300 \text{ adult bees}}\right)$$

Plate 3.4: Collecting about 300 adult bees using standardized cup

3.4.3 Quantifying *Varroa destructor* infestation in capped worker brood cells

Varroa destructor infestation in worker brood cells were assessed fortnightly by uncapping 200 capped worker brood cells at the 'purple-eye' stage of development from randomly selected brood combs in each colony. A sterilized scalpel was used to cut open capped worker brood cells from each colony and investigated in the laboratory. The brood cells were opened with a scalpel and the pupae in the brood cells were removed using a pair of forceps.

Each brood cell was carefully opened and examined for the presence of adult *Varroa* mites. The level of infestation per 100 capped worker brood cells was calculated using the following formulae by Dietemann *et al*. (2013).

$$\text{Level of } Varroa \text{ infestation} = \left(\frac{\text{Number of } Varroa \text{ mites}}{200}\right) x\ 100$$

The population of *Varroa* mites in capped worker brood cells in each colony was calculated as follows:

$$Varroa \text{ mite Population} = \text{Number of capped brood } x\ \left(\frac{\text{Number of } Varroa \text{ mites}}{200}\right)$$

Total *V. destructor* population for each colony was estimated by adding the number of *Varroa* mites on adult bees and number of *Varroa* mites in capped worker brood cells.

3.4.4 Colony size estimation

Colony size was estimated once a month by measuring the number of capped worker brood and adult bees. The number of capped worker brood in each colony was estimated using a transparent polythene grid measuring 40 cm x 20 cm divided into squares of 5 cm x 5 cm (25 cm^2) that was placed over all brood frames (Plate 3.5). The average number of capped worker brood cells in comb area of 25 cm^2 was 100 ± 4.02 (n = 10). The total brood unit areas occupied by the brood was estimated (Delaplane *et al*., 2013) and multiplied by 100 to obtain the number of capped worker brood cells in each colony.

Adult bee population was calculated using Liebefeld colony size estimation method (Gerig, 1983) that involves using a transparent polythene grid divided into 8 squares, each of 1 dm^2 (100 cm^2). A colony was opened and combs of bees sequentially removed and the transparent polythene grid was placed over each side of the comb frame and the number of squares fully occupied by bees were counted and recorded. A precise estimate of the number of adult *A. m. scutellata* that completely filled 1 dm^2 (100 cm^2) was done by taking photograph of frame fully occupied by bees with a transparent grid (Plate 3.6a, b) and this was used to estimate the total number of honeybees in the colonies (Strauss *et al*., 2015). The numbers of fully occupied squares on both sides of the brood frames as well as on the lids and walls of the hives were counted and multiplied by 160 to obtain the number of honeybees present in each colony.

Temperature and relative humidity were measured on a daily basis throughout the study period using a portable digital data logger (Shenzhen Flus Technology Company Limited).

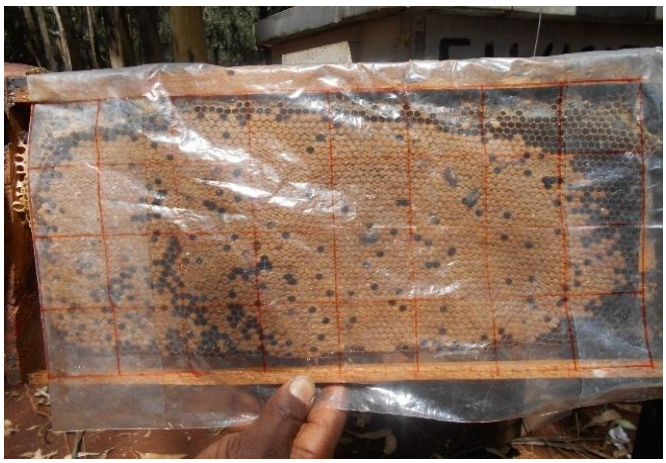

Plate 3.5: Estimating the number of worker brood cells using a transparent polythene grid.

Plate 3.6a: A frame of honeybees

Plate 3.6b: Using a transparent polythene sheet to estimate the number of adult honeybees in 1 dm^2

3.5 Effects of *Varroa destructor* on colony size and productivity of *Apis mellifera scutellata* colonies

Twenty colonies naturally infested with *V. destructor* were monitored to assess its effect on *Apis mellifera scutellata* for a period of eight months. Sugar roll assay and uncapping of 200 purple eyed pupae in each colony was done twice a month to estimate infestation rates of *V.*

destructor on adult bees and in capped worker brood cells respectively. Adult honeybee population in each colony was estimated every month using Liebefeld colony size estimation method (Gerig, 1983; Strauss *et al*., 2015). The total number of open and capped worker brood was estimated using a transparent polythene grid divided into squares of 5 cm x 5 cm. Comb frames from each colony were removed and the transparent polythene grid was placed on top of each frame to estimate the number of squares occupied by brood (Delaplane *et al*., 2013). The total population of *Varroa* mites in each colony was determined by adding population of *V. destructor* in capped worker brood cells to population of *V. destructor* on adult honeybees (Dietemann *et al*., 2013). The surface area of the colony stores (pollen, nectar and honey) was estimated using the same method as for brood estimation (Refer to subsection 3.4.4).

Presence of adult honeybees with wing malformations, capped dead brood in experimental colonies was also assessed and the number recorded.

3.6 Data analysis

The data on *Varroa*-specific hygienic behaviour of *Apis mellifera scutellata* was analyzed using Generalized Linear Mixed Model (GLMM) and the means separated using Tukey's HSD at P value = 0.05. GLMM was used along with the logit link function to account for non-normal distribution of the data for; analysis of effects of mites' introduction and the controls; the effect of the category of *Varroa*-specific hygienic behaviour and the number of mites introduced and the time point of manipulation within each of the categories of *Varroa*-specific hygienic behaviour. Colony was used as a random factor in each of the models. The

response variable was the percentage of brood cells within a category of a specific hygienic behaviour. For comparing treatment and control, category UBC (untouched Brood cells) was only used. For treatment experiments, all categories were used; UBC (Untouched Brood Cells), CCO (Cells Cap Opened), MBR (Mite and Brood Removed), and MRBR (Mite Removed and Brood Recapped).

A null model was set up, which was tested against the full model using an Analysis of Variance (ANOVA) and the Akaike Information Criterion (AIC). When the full model was significantly different from the null model and had a higher explanatory power (ΔAIC of >2), stepwise removal of interactions or factors was done for model simplification. Simplified models were tested against former, more complex models by means of ANOVA and AIC. Spearman rank correlation was used to analyze the correlation between number of *Varroa* mites and general hygienic behaviour.

The data on population abundance of *V. destructor* was subjected to descriptive statistics. The mean monthly number of mites on adult bees, the mean monthly number of mites in capped worker brood cells, the mean monthly natural mite falls, the mean monthly total mite population and the mean monthly surface area of the colony stores (pollen, nectar and honey) were calculated. GLMM analysis was carried out to test whether there were significant differences between the months of study for the population of *Varroa destructor* and in the number of colony stores.

The data on the effect of *V. destructor* on colony size and productivity of *Apis mellifera scutellata* was analyzed using Generalized Linear Mixed Models. GLMM was used to calculate multiple regression models using binomial family and the logit link function with

the *glmer* function of *lme4* (Bates *et al*., 2015). In testing what influenced the overall bee population in honeybee colonies, Principal component analysis (PCA) was used to reduce the complexity of the three types of measures affecting population of bees (*Varroa* on adult bees, *Varroa* in capped brood and amount of honey), and also to break up correlations. First PC (PC1) usually explains a lot of the overall variance therefore was used as the response variable in GLMM with month and colony as random factors.

In testing factors affecting the *Varroa* mite population, the three independent measures for *Varroa* mite population (*Varroa* mites on adult bees, *Varroa* mites on sticky boards and *Varroa* mites in capped brood) were used to derive some combined measure by using a PCA to extract principal components (PC). To test for factors affecting the resources in the colony, the three independent measures (nectar, pollen and honey) were used to derive some combined measure by using PCA to extract principal components. For all statistical tests, R version 3.3.1 was used (R Core Team, 2016).

CHAPTER FOUR

RESULTS

4.1 *Varroa*-specific hygienic behaviour in *Apis mellifera scutellata* colonies

Twenty-four hours after pin killing the worker brood cells, percentage of general hygienic behaviour (GHB) varied between 57.14% and 95.14% in the twenty experimental colonies. Twelve colonies (sm7, sm6, sm2, sm15, sm1, sm8, sm11, sm12, sm3, sm20, sm14 and sm13) corresponding to 60% of the experimental colonies showed general hygienic behaviour of >80% and were considered hygienic. Eight colonies (sm19, sm10, sm17, sm16, sm18, sm5, sm9 and sm4) corresponding to 40% of the experimental colonies showed general hygienic behaviour of <80% and were considered non-hygienic (Figure 4.1).

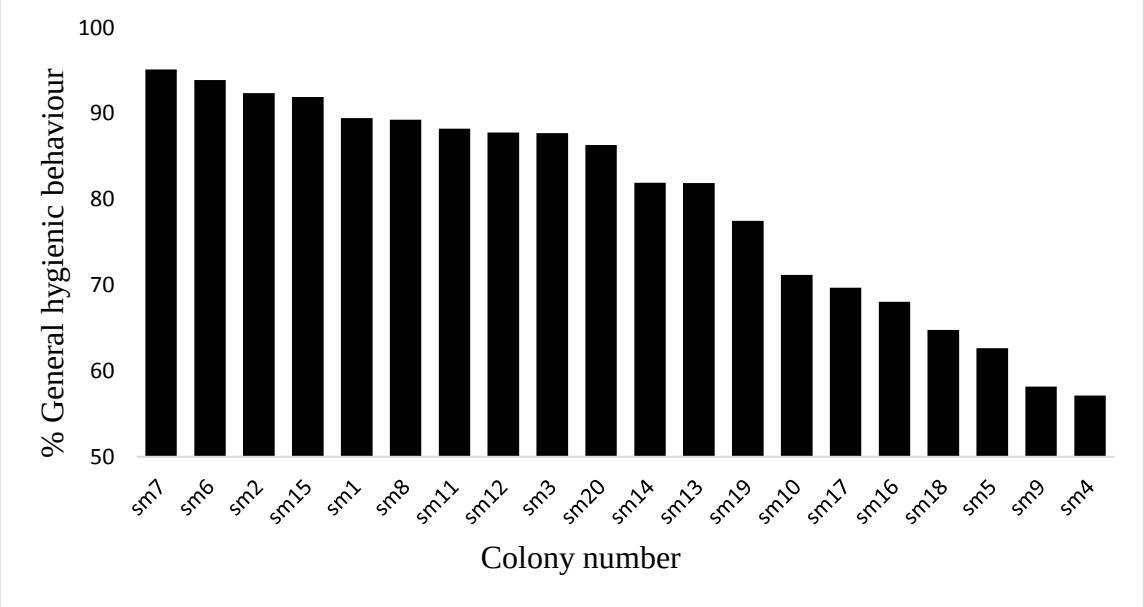

Figure 4.1: Percentage general hygienic behaviour (GHB) of *Apis mellifera scutellata* in the twenty colonies using pin killing method

Sugar roll assay that was carried out in these colonies showed negative correlation between the general hygienic behaviour and number of *Varroa* mites (r (20) = -0.86, P = <0.001) (Figure 4.2).

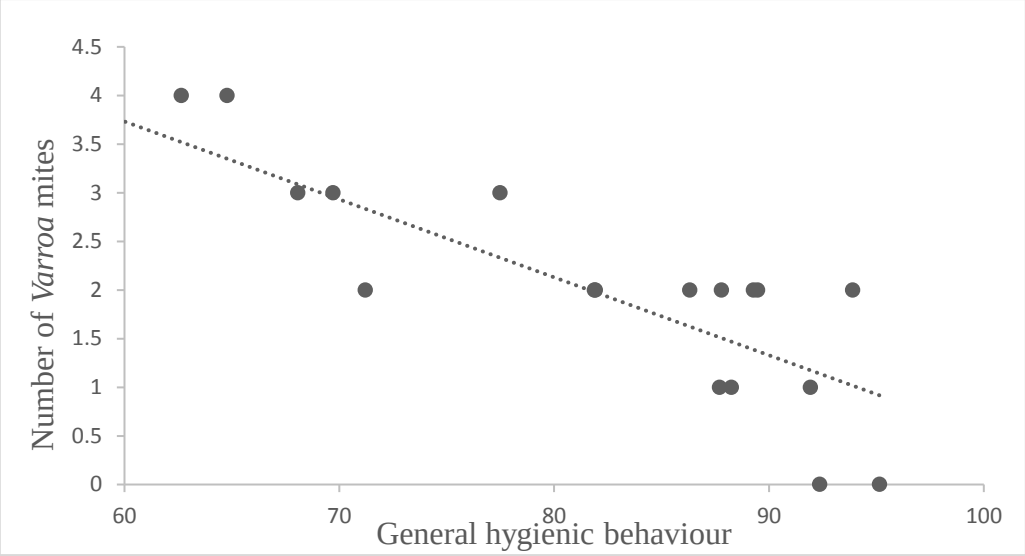

Figure 4.2: Correlation between number of *Varroa* mites and general hygienic behaviour

There were significant differences in the mean percentage of control cells (brood cells without mites) and manipulated cells (brood cells in which mites had been introduced) (P = <0.001). The percentage of untouched brood cells (brood cells not manipulated by bees in any way) by *Apis mellifera scutellata* was high in control cells (80%, n = 579) compared to manipulated cells (12.5%, n = 110) (Figure 4.3).

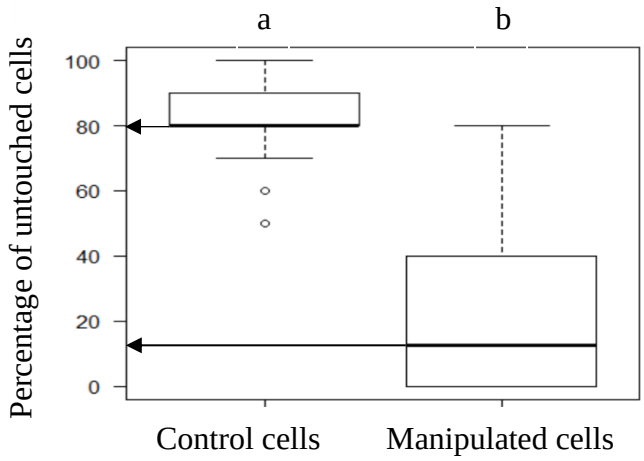

Figure 4.3: Percentage of untouched brood cells by *Apis mellifera scutellata* in manipulated cells and control cells

Assessment of the comb brood frame 72 hours after introduction of *Varroa* mites showed that *Apis mellifera scutellata* bees responded to manipulated brood cells, and there were recordings of Untouched Brood Cells (UBC), Cell Caps Opened (CCO), Brood and Mite Removed (BMR), Mite Removed and Brood cell Recapped (MRBR) (Plate 4.1a,b).

Plate 4.1 a: A comb brood frame containing eight cells introduced with *Varroa* mites and recapped with melted beeswax

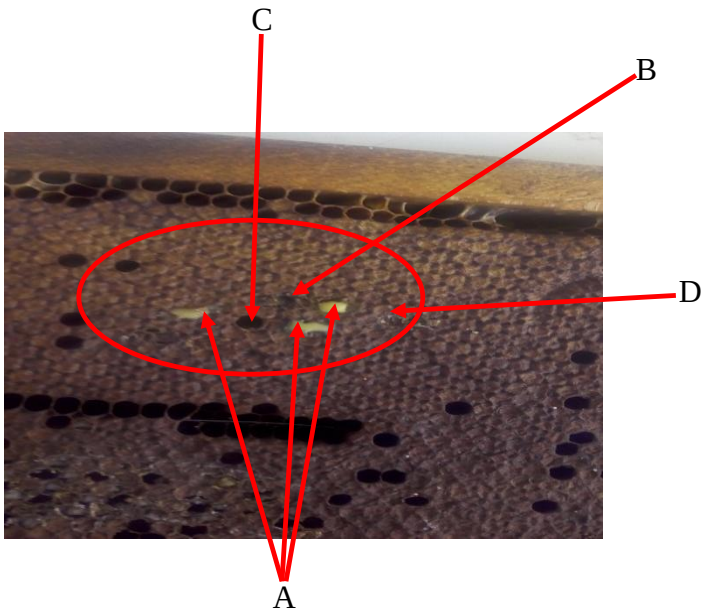

Plate 4.1b: Assessment of the comb brood frame 72 hours after introduction of *Varroa* mites. Three Untouched Brood Cells (UBC) indicated by letter **A**, one cell with Cell Cap Opened (CCO) indicated by letter **B**, one cell where Brood and Mite was Removed (BMR) indicated by letter **C** and one cell where Mite was Removed and Brood cell Recapped (MRBR) indicated by letter **D**

Of the 690 brood cells (69 brood cells per colony) with artificially introduced mites, *A. m. scutellata* bees were able to uncap, remove the mite and recap 25.8% of the brood cells (178 of 690 manipulated cells) and removed 33.5% of the infested pupae together with the mite (231 of 690 manipulated cells). There were significant differences in the mean percentage response of *Apis mellifera scutellata* and brood cells of three VSH behavioural components (UBC, P = <0.001, BMR, P = 0.0125 and MRBR, P = <0.001). There were no significant differences in the mean percentage response of manipulated brood cells by *A. m. scutellata* and those where cell caps were opened (CCO, P = 0.213). There were no significant differences in the mean percentage response of manipulated brood cells by *A. m. scutellata*

and number of days of mites introduction into brood cells; UBC (P = 0.327), CCO (P = 0.735), BMR (P = 0.586), MRBR (P = 0.211) (Table 4.1).

Table 4.1: Mean percentage response of *Apis mellifera scutellata* to manipulated brood cells expressed at different VSH behavioural parameters

VSH behaviour	UBC	CCO	BMR	MRBR
Mean percentage (%) response of *A. m. scutellata*	15.9	24.8	33.5	25.8
	(n=110)	(n=171)	(n=231)	(n=178)
P value	<2e-16***	0.213	0.0125*	5.82e-09***
No. of days	90	90	90	90
P value	0.327	0.735	0.5858	0.211

Number of brood cells responded to by *A.m. scutellata* is shown in the parenthesis

There were significant differences between the number of mites introduced at different VSH behavioural parameters (UBC, CCO, BMR and MRBR) and percentage response of *A. m. scutellata* (Figure 4.4). Results for A (Untouched Brood Cells) indicated that when 5 mites were introduced 40% of the brood cells were untouched by *A. m. scutellata* and this was significantly higher (P = <0.001), than when 8 mites (12.5%) and 10 mites (0%) were introduced which showed no significant differences (P = 0.07). Results for B (Cells Cap Opened) showed that when 5 mites were introduced 20% of the cells cap were opened by *A. m. scutellata* which was not significantly different from when 8 mites (25%) and 10 mites (30%) were introduced which also showed no significant differences (P = 0.213). Results for C (Brood and Mite Removed) showed that when 5 mites were introduced 20% of the

brood and mite were removed by *A. m. scutellata* which was not significantly different from when 8 mites (37.5%) and 10 mites (40%) were introduced (P = >0.05). Results for D (Mite Removed and Brood cells Recapped) indicated that when 5 mites were introduced 0% of the mites were removed and brood cells recapped by *A. m. scutellata* and this was significantly lower (P = <0.001) than when 8 mites (25%) and 10 mites (35%) were introduced which showed no significant differences (P = 0.44).

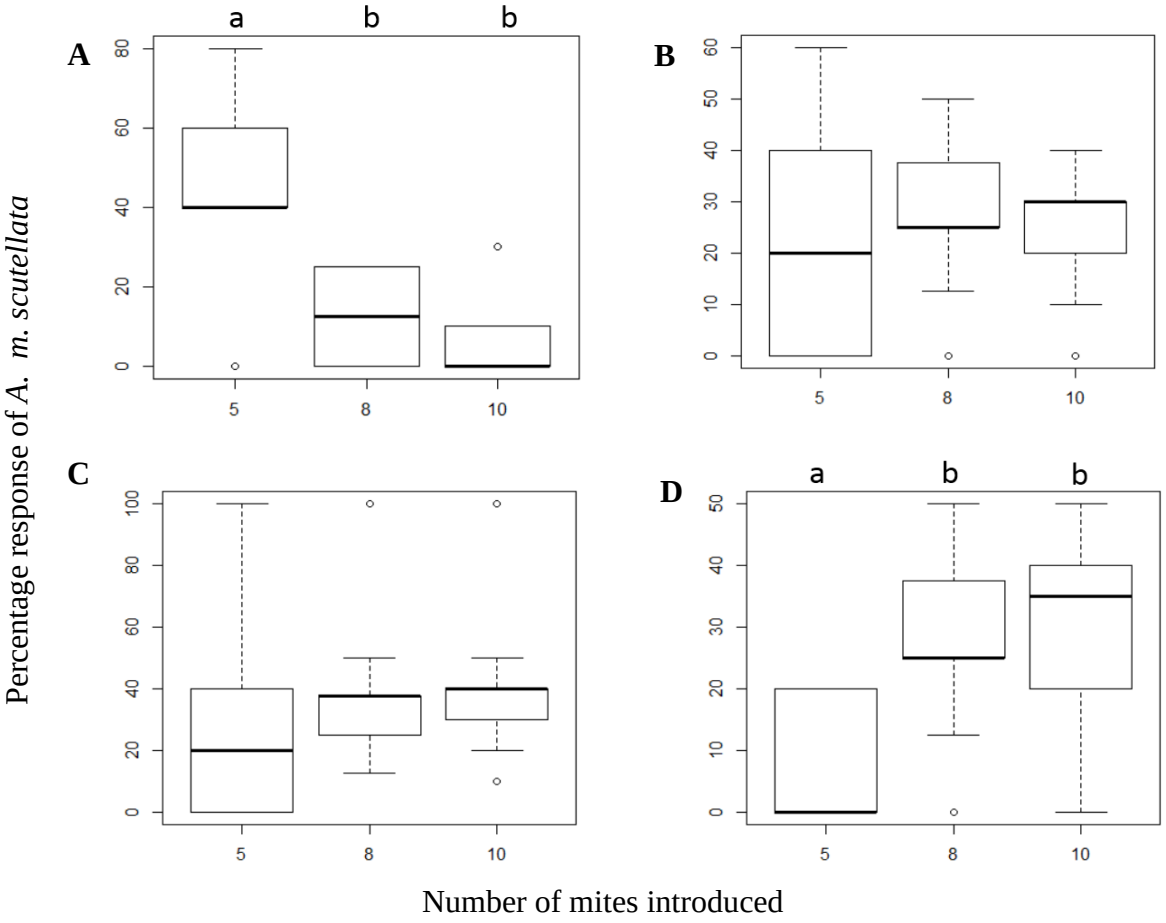

Figure 4.4: Percentage response of *Apis mellifera scutellata* to manipulated cells introduced with different densities of *Varroa destructor*. **A** = Untouched Brood cells, **B** = Cells Cap Opened, **C** = Brood and Mite Removed, **D** = Mite Removed and Brood cells Recapped. Different letters in **A** and **D** indicates significant differences after Tukey post hoc test

4.2 Abundance of *V. destructor* in *Apis mellifera scutellata* colonies

There were significant differences in the mean total number of *Varroa* mites collected on sticky boards between the first four months of study (April, May, June, July) and the last four months of study (August, September, October, November). The mites collected within the first four months were significantly lower than those collected within the last four months ($P = <0.001$). The mean monthly mite fall varied with the month of November recording the highest number of mites (27.9 ± 4.4) and July the lowest number of mites (3.3 ± 0.3).

There were significant differences in the mean number of *Varroa* mites on adult bees between the first four months of study and the last four months of study. The mites collected within the first four months were significantly lower than those collected within the last four months ($P = <0.001$). The mean monthly number of *Varroa* mites on adult bees ranged between 10.8 ± 2.3 and 32.6 ± 8.3 with August recording highest number of mites (32.6 ± 8.3) and July the lowest number of mites (10.8 ± 2.3). There were no significant differences in the mean number of *Varroa* mites in capped brood between the months of study ($P > 0.05$). The mean monthly number of *Varroa* mites in brood ranged between 16.1 ± 2.3 and 32.6 ± 6 mites with October recording the highest number of mites (32.6 ± 4.19) and July the lowest number of mites (16.1 ± 4.6). There were significant differences in the mean number of total *Varroa* mites between the first four months of study and the last four months of study. The mites collected within the first four months were significantly lower than those collected within the last four months ($P = <0.001$, Table 4.2).

Table 4.2: Mean monthly number of *Varroa* mites (± SE) on sticky boards, on adult bees, in capped brood and total *Varroa* mites over the study period (April-November, 2016)

Month	Number of colonies	*Varroa* on sticky boards	*Varroa* on adult bees	*Varroa* in capped brood	Total *Varroa* mites
April	20	5.9 ± 0.4a	17.6 ± 2.4a	21.3 ± 2.3a	38.9 ± 3.3a
May	20	5.4 ± 1.1a	18.9 ± 3.9a	24 ± 2.8a	43 ± 5.2a
June	20	5.8 ± 1.1a	18.4 ± 2.5a	26 ± 3.9a	44.4 ± 4.6a
July	20	3.3 ± 0.3a	10.8 ± 2.3a	16.1 ± 4.6a	26.9 ± 4.7a
Aug	13	21.3 ± 4.1b	32.6 ± 8.3b	27.8 ± 2.6a	59.6 ± 9.9b
Sept	13	23.1 ± 6.2b	31.5 ± 8.3b	30.1 ± 5.7a	59.2 ± 8.8b
Oct	13	25.4 ± 3.5b	29.7 ± 5.8b	32.6 ± 6a	59.8 ± 7.9b
Nov	13	27.9 ± 4.4b	23.4 ± 4.6b	25.6 ± 4.2a	56 ± 5.3b

Means within the same column followed by the same letter are not significantly different at P = 0.05 (GLMM, P = <0.001)

There were no significant differences in the mean number of total brood between the months of study (P = >0.05). The mean number of brood ranged between 3323 ± 323 and 5500 ± 521 cells. The average number of capped worker brood cells in the comb area of 25 cm^2 was 100 ± 4.02 (n = 10). There was high reduction of total brood from 5,500 ± 716 cells in the month of June to 3,730 ± 597 cells in the month of July. The mean number of adult bees ranged between 6,486 ± 637 and 7,354 ± 722. Results showed that the average number of adult bees on 1 dm^2 was 160 ± 7.53 (n = 10). The colonies had the highest number of adult bees in April. During May and June, the number of adult bees decreased with its lowest numbers in July. There were significant differences in the mean number of pollen between

the first four months of study (April, May, June, July) and the last four months of study (August, September, October, November). The amount of pollen recorded within the first four months were significantly lower than those collected within the last four months (P = <0.001). The mean monthly surface area occupied by pollen ranged between 33.8 ± 8.8 cm^2 and 205.8 ± 10.9 cm^2. The mean monthly surface area occupied by nectar ranged between 38.8 ± 12.5 cm^2 and 292.3 ± 57.4 cm^2. The mean monthly surface area of combs occupied by honey ranged between 45 ± 10.5 cm^2 and 419.2 ± 71.2cm^2. There were significant differences in the mean number of honey between the first four months of study and the last four months of study (P = <0.001). Compared to other months, July recorded the lowest mean surface area occupied by pollen, nectar and honey.

The climatic factors that were measured during the months of study were temperature and relative humidity. The mean monthly temperature ranged between 16.1^oC and 21.2^oC while the mean monthly relative humidity ranged between 62.9% and 79% (Table 4.3).

Table 4.3: Mean monthly total number of brood, adult bees, pollen, nectar, honey and weather conditions of study site over the study period (April-November, 2016)

Month	Number of Colonies	Total Brood	Adult bees	Pollen (cm^2)	Nectar (cm^2)	Honey (cm^2)	Temperature (^oC)	Relative Humidity (%)
April	20	5020 ± 521a	7354 ± 722a	158.8 ± 64a	126.7 ± 59.9a	155.8 ± 61.9a	20.1	62.9
May	20	4855 ± 619a	6910 ± 691a	135 ± 76.4a	143.6 ± 66.7a	132.4 ± 70.7a	18.4	68.8
June	20	5500 ± 716a	6704 ± 645a	147.5 ± 49.7a	118.8 ± 46.1a	160 ± 49.6a	18.4	71.5
July	20	3730 ± 597a	6486 ± 637a	33.8 ± 8.8a	38.8 ± 12.5a	45 ± 10.5a	16.1	79
Aug	13	3808 ± 611a	7130 ± 621a	184.6 ± 79.4b	111.5 ± 45.7a	227.2 ± 35.6b	19.7	63.8
Sept	13	3777 ± 548a	7237 ± 532a	187.3 ± 94.7b	150 ± 44.6a	259.6 ± 34.6b	18.3	64.4
Oct	13	3485 ± 406a	7126 ± 452a	198.1 ± 61.6b	250 ± 88.4b	327 ± 102.1b	20.1	65.6
Nov	13	3323 ± 323a	6769 ± 481a	205.8 ± 10.9b	292.3 ± 57.4b	419.2 ± 71.2b	21.2	69.7

Means within the same column followed by the same letter are not significantly different at P = 0.05 (GLMM, P = <0.001)

Varroa mites were found in all the twenty colonies examined and averaged 1.0 ± 0.3 to 2.6 ± 0.6 per sample of adult bees and 2.8 ± 0.5 to 6.4 ± 0.8 per sample of capped worker brood. The total *V. destructor* population in capped worker brood ranged between 9.5 ± 0.3 and 45.5 ± 2.1. It ranged between 3.5± 0.9 and 46 ± 9.7 mites on adult bees. The mean infestation rates/100 adult bees was < 1%. The mean daily mite fall of *Varroa* mites per colony over the study period ranged between 0.1 ± 0.01 and 1 ± 0.2 (Table 4.4).

Table 4.4: Mean monthly total number of *Varroa* mites (± SE) on sampled adult honeybees, in sampled capped brood, and daily mite fall per colony over the study period (April-November, 2016)

Colony	*Varroa* mites per sampled adult honeybees	*Varroa* mites per sampled capped brood	Total *Varroa* mites in capped brood	Mean infestation rates/ 100 adult bees	Total *Varroa* mites on adult bees	Mean daily mite fall
1	1 ± 0.3	3.5 ± 0.6	9.5 ± 0.3	0.2 ± 0.04	11.3 ± 3.1	0.1 ± 0.01
2	2.6 ± 0.6	7.3 ± 1	30.8 ± 5.7	0.4 ± 0.1	46 ± 9.7	1± 0.2
3	1.8 ± 0.5	6 ± 0.5	24.9 ± 5.7	0.3 ± 0.1	24.1 ± 6.2	0.7 ± 0.2
4	2.1 ± 0.3	6.1 ± 0.8	20.9 ± 7	0.4 ± 0.1	38.4 ± 4.9	0.9 ± 0.3
5	1.5 ± 0.2	3.5 ± 0.2	11.5 ± 0.8	0.3 ± 0.03	22.8 ± 3.4	0.1± 0.03
6	1.8 ± 0.3	3.8 ± 0.4	12.8 ± 1	0.3 ± 0.1	34.5 ± 6.9	0.2 ± 0.04
7	1.9 ± 0.2	6.4 ± 0.8	22.9 ± 3.2	0.3 ± 0.03	24.4 ± 2.8	0.8 ± 0.1
8	1.4 ± 0.2	5.5 ± 0.6	22.9 ± 2.9	0.3 ± 0.03	19 ± 2.7	0.6 ± 0.1
9	1.4 ± 0.4	5.4 ± 0.5	32.9 ± 2.3	0.2 ± 0.1	20.6 ± 5.8	0.4 ± 0.1
10	3 ± 0.8	4.8 ± 0.4	12.3 ± 0.9	0.6 ± 0.1	24 ± 5.9	0.2 ± 0.03
11	1.5 ± 0.3	4.5 ± 0.5	22.8 ± 2.4	0.3 ± 0.1	11.9 ± 3.1	0.4 ± 0.1
12	2 ± 0.3	6 ± 0.7	45.5 ± 2.1	0.3 ± 0.1	19.4 ± 2.9	0.3 ± 0.1
13	2.5 ± 0.4	5.3 ± 0.7	30.1 ± 3	0.4 ± 0.1	23.1 ± 4.6	0.5 ± 0.1
14	2.1 ± 0.3	5.4 ± 0.4	18.3 ± 2.4	0.4 ± 0.04	19.3 ± 2.2	0.3 ± 0.1
15	1.5 ± 0.4	2.8 ± 0.5	14 ± 0.3	0.3 ± 0.1	12 ± 3.1	0.1± 0.01
16	2 ± 0.2	5.5 ± 0.7	30.9 ± 2.6	0.3 ± 0.03	20 ± 1.9	0.3 ± 0.1
17	1.6 ± 0.5	3.4 ± 0.3	26.5 ± 1.5	0.3 ± 0.1	13.1 ± 4.3	0.3 ± 0.1
18	1.3 ± 0.4	5 ± 0.8	30 ± 0.9	0.2 ± 0.1	10 ± 3.6	0.2 ± 0.03
19	1.9 ± 0.6	4.9 ± 0.8	28.9 ± 1.9	0.4 ± 0.1	16.4 ± 5.5	0.4 ± 0.1
20	1.3 ± 0.3	5.5 ± 1.1	12.5 ± 0.5	0.3 ± 0.1	3.5 ± 0.9	0.1 ± 0.02

4.3 Effects of *Varroa destructor* on colony size and productivity

Inspection of the individual colonies showed no serious negative effects of *V. destructor* on *A. m. scutellata* colonies. There were no recordings of capped dead brood in the experimental colonies in all the months of this study (Table 4.5).

Table 4.5: Composition of capped dead brood and honeybees with deformed wings over the study period (April-November, 2016)

Month	Capped dead brood	Honeybees with deformed wings
April	X	X
May	X	X
June	X	X
July	X	X
August	X	X
September	X	✓
October	X	✓
November	X	X

✓ implies present

X implies absent

Few cases of honeybees with deformed wings (Plate 4.2) were recorded only in the month of September and October.

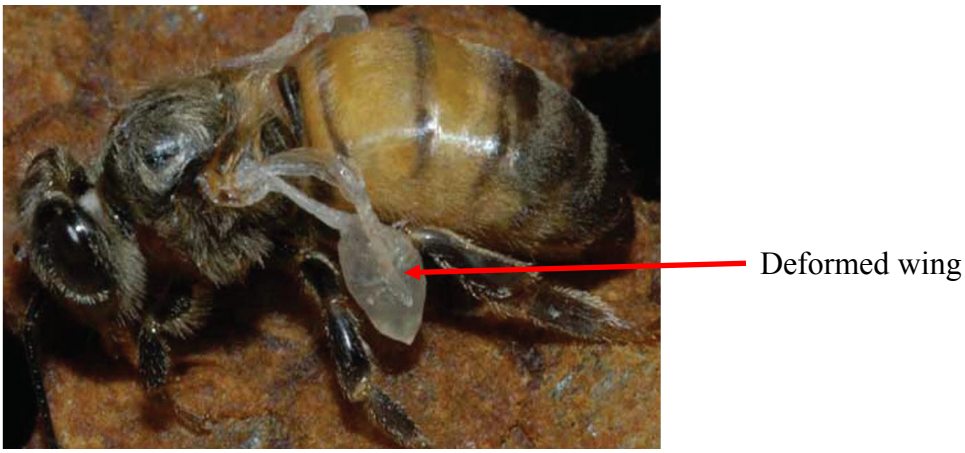

Plate 4.2: Honeybee with deformed wings

There were significant differences in the mean number of capped brood between colonies that absconded and those that remained. The colonies that absconded had significantly lower mean number of capped brood (1638.1 ± 190.9) than those that remained (2994.9 ± 247.8) (P = 0.02, Figure 4.5).

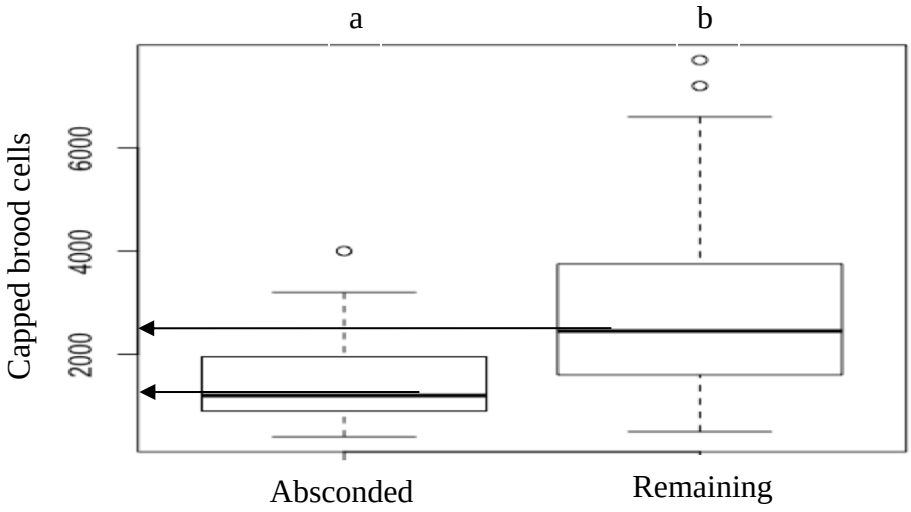

Figure 4.5: Mean numbers of capped brood in the absconded and remaining colonies

The mean number of *Varroa* mite population on adult bees (21.5 ± 1.9) was positively correlated with bee population with significant differences (T = 2.59, P = 0.009), while the mean number of *Varroa* mite population in capped brood (24.8 ± 2.2) was negatively correlated with bee population with significant differences (T = -5.36, P = <0.001). The mean number of honey stores (216.1 ± 18.9) was positively correlated with bee population with significant differences (T = 2.04, P = 0.04). The mean number of adult bee population (6943.2 ± 606.7) was positively correlated with *Varroa* mite population with significant differences (T = 3.19, P = 0.001). The mean number of capped worker brood (2099.2 ± 183.4) was negatively correlated with *Varroa* mite population with significant differences (T = -3.14, P = 0.002). The mean number of pollen (168.8 ± 14.7) was negatively correlated with *Varroa* mite population with no significant differences (T = -1.31, P = 0.19). The mean number of honey stores (216.1 ± 18.9) was positively correlated with *Varroa* mite population with significant differences (T = 2.24, P = 0.03). The mean number of adult bee population (6943.18 ± 606.7) was negatively correlated with colony resources with significant differences (T = -4.37, P = < 0.001) (Table 4.6).

Table 4.6: Mean numbers of different parameters (± SE) affecting the bee population, *V. destructor* population and resources in *A. m. scutellata* colonies

Parameter	Mean	T value	P value
Response variable*:* Bee population (PC1)			
Varroa mite on adult bees	21.5 ± 1.9	2.59	0.009**
Varroa mite in capped brood	24.8 ± 2.2	-5.36	8.17e-08***
Honey	216.1 ± 18.9	2.04	0.04*
Response variable: *Varroa* mite population (PC1)			
Adult bees	6943.2 ± 606.7	3.19	0.001**
Capped brood	2099.2 ± 183.4	-3.14	0.002 **
Pollen	168.8 ± 14.7	-1.31	0.19
Honey	216.1 ± 18.9	2.24	0.03 *
Response variable: Resources (PC1)			
Adult bees	6943.18 ± 606.7	-4.37	1.26e-05***

P values with one asterisk* shows $P < 0.05$, with two asterisks** shows $P < 0.01$, with three asterisks*** shows $P < 0.001$

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Results on selection of hygienic colonies revealed that variation in percentages of general hygienic behaviour (GHB) ranged between 57.14% and 95.14% with overall mean removal efficacy of 79.8% ± 12.54 (mean ± SD). Previous studies by Fries and Raina (2003) reported that the overall mean removal efficacy of pin killed brood cells by African honeybee *Apis mellifera scutellata* was 76.6% ± 34.8. These results show that there are varied and higher rates of general hygienic behaviour in *A. m. scutellata*. The variations can be due to colony size (number of adult bees in the colony) and the effect of environmental factors (nectar flow and strength of honeybee colonies). Weak colonies and lack of incoming nectar have been shown to reduce hygienic behaviour of honeybees (Momot and Rothenbuhler 1971; Spivak and Gilliam, 1993). Expression of hygienic behaviour can also be influenced by interactions between colony resources, amount of brood in the colonies and time of the year (Bigio *et al*., 2013). Furthermore, Wagoner *et al*. (2018) reported that wounded brood from hygienic colonies was highly removed than brood from non-hygienic colonies, regardless of where the brood was obtained.

There was a negative correlation between the general hygienic behaviour and number of *Varroa* mites, an indication that colonies with high levels of hygienic behaviour have lower levels of *Varroa* mites. These results conform to studies done by Muli *et al*. (2014) which reported that colonies with high levels of hygienic behaviour had lower levels of *Varroa*

destructor. Hygienic behaviour decreases the reproduction rate of the mites (Wielewski *et al*., 2012).

Results on *Varroa*-specific hygienic behaviour indicated a more complex combination of behavioural components in *Apis mellifera scutellata* colonies. In this study, honeybees were more responsive to manipulated cells (brood cells with artificially introduced mites) as compared to control cells (brood cells without mites) probably because of the chemical cues produced by *V. destructor* that trigger higher hygienic behaviour in manipulated brood cells with mites as reported by Masterman *et al*. (2001). Results of this study are comparable to those of previous studies that compared Africanized bees and Carniolan bees *Apis mellifera carnica* Pollmann and reported that 10% of the infested honeybee pupae were removed by *Varroa*-specific Africanized hygienic bees and in about one third (25-30%) of the manipulated brood cells the mites were removed by Carniolan bees (Aumeier *et al*., 2000). It was also noted by Aumeier and Rosenkranz (2001), that Africanized honeybees were able to express hygienic behaviour of between 24% and 44% (summing up all behavioural components) in cells artificially infested with dead mites. Guerra *et al*. (2000) also reported that Africanized honeybees expressed hygienic behaviour with a mean of 51% of the total artificially mite infested brood in experiments that compared them with Italian honeybees. Panziera *et al*. (2017) reported that *Varroa*-specific hygienic bees were able to remove up to 40% of mite infested brood cells in naturally selected *Varroa* resistance honeybees of European origin.

Varroa-specific hygienic bees were more responsive to manipulated brood cells singly introduced with ten mites in a patch of closely neighbouring brood cells as compared to those singly introduced with five mites in a patch of closely neighbouring brood cells because the

chemical signals that triggers hygienic behaviour are less concentrated and harder to detect in brood with low infestation rate (Masterman *et al*. 2001; Nazzi and Le Conte *et al*., 2016). Worker bees may have detected the presence of different densities of mites in brood cells using these cues and the response to these chemical signals seem to be additive as highest removal rates were observed for 8 or 10 mites compared to 5 mites singly introduced in closely neighbouring brood cells. Furthermore, it might be that the hygienic bees that uncap and remove mites have a specific internal threshold (Theraulaz *et al*., 1998) for performing that task and under low infestation levels only a minority, those with a low threshold for the odorant cue performs the task. With increasing numbers of mites more workers will become involved, as those having a higher threshold will also perform the task.

The factor time had no effect on the outcome of VSH behaviour indicating that learning might not play any significant role. Presumably, workers perform the tasks of hygienic behaviour only during a restricted time of their lifetime and hence learning is not possible over longer time periods. Furthermore, a colony is genetically diverse hence the activity of individual hygienic bees is not sufficient to meet the increased demand for task performance (Arathi *et al*., 2006). Studies have shown that olfaction plays a role in hygienic behaviour (Masterman *et al*., 2001; Plettner *et al*., 2017) and that the origin of VSH behaviour is at least partly related to shifts in antennal gene expression (Mondet *et al*., 2015), therefore, olfactory cues might have played a critical role in the expression of VSH behaviour in this study.

Varroa-specific hygienic behaviour in this study was estimated in the first 72 hours after introduction, an indication that hygienic bees have the ability to recognize and eliminate mites as such before (severe) damage to the pupae occurs. A study by Frey *et al*. (2013)

showed that within the first few hours after cell capping, honeybee worker larvae provide the signals for the activation of the *Varroa* reproduction and therefore increased chances of mites' removal during this period. Therefore, these results demonstrate that honeybees continuously seek to clean brood even beyond this active brood examination period.

Results on population of *Varroa destructor* collected on sticky boards representing natural mite fall were generally low between April and July and there was a steady increase from August to November 2016. The increase is as a result of higher number of *Varroa* mites on adult bees in these months. Previous studies reported that high population of *Varroa* mites on adult bees' leads to increased natural mite fall (Calderon and Van veen, 2008). This could be attributed to increased grooming behaviour of honeybees (Guzman-Novoa *et al*., 2012; Nganso *et al*., 2017). It is known that when honeybees are parasitized with *Varroa* mites, they rid themselves of the parasite through vigorous body movement, making the mite to get stressed and their fitness is reduced therefore they fall off (Fries *et al*., 1996; Nganso *et al*., 2017). Honeybees can also detect, grab and bite free moving mites increasing natural mortality in honeybee colonies (Thakur *et al*., 1996). Furthermore, the weather conditions from August to November 2016 were dry and this may have led to increased *Varroa* mite fall. Previous studies reported that live proportion of mite fall increased during periods of hot weather (Webster *et al*. 2000).

The results on population of *V. destructor* in capped worker brood and on adult bees showed low numbers in the month of July and high numbers in the subsequent months (August, September, October and November) of this study. It was also noted that in July, the amount of pollen in honeybee colonies was significantly lower as compared to subsequent months. This conform to previous studies which showed that the low availability of natural flowering

resources around apiaries leads to high infestation levels of *Varroa* mites in subsequent periods in honeybee colonies (Marleza *et al*., 2016). Pollen storage levels have a direct effect on colony population as they are related to rapid colony growth rates through brood production which in turn leads to increased reproduction of the mites (Brodschneider and Crailsheim, 2010).

Results on monthly population of *Varroa* mites in capped worker brood was low in the month of July and coincided with low numbers of total brood in *A. m. scutellata* colonies. Reproduction and population growth of the mites decreases during periods of low honeybee brood (Rosenkranz *et al*., 2010). Low amount of pollen led to less brood and consequently low mite infestations in colonies. There were high numbers of *V. destructor* in the total number of sampled capped worker brood per colony as compared to that of total number of *V. destructor* in sampled adult bees over the study period. In naturally infested colonies more *Varroa* mites are within the capped brood and is closely synchronized with the brood development of the host (Rosenkranz *et al*., 2010). The high proportion in capped brood is as a result of adult mites abandoning adult bees and invading brood cells to complete their reproductive cycle (Boot *et al*., 1994; Nganso *et al*., 2018).

Results on population of *Varroa* mites per colony showed infestation levels of between 1 and 2.6 mites per sample of adult bees per colony over the study period. These results are lower compared to those of previous studies that reported 3 to 108 *Varroa* mites per sample of adult bees per colony in 2009 when *V. destructor* was first detected in Kenya (Frazier *et al*., 2009). The low mite population can be attributed to African honeybees' resistance to *V. destructor* due to factors such as high levels of general hygienic behaviour (Muli *et al*., 2014;

Nganso *et al*., 2017) and high expression of *Varroa*-specific hygienic behaviour that was observed in this study (Cheruiyot *et al*., 2018).

The variability in the monthly *Varroa* mite population that was observed in this study can be attributed to variations in brood availability, the season of the year, the availability of colony resources and emerging bees with different *V. destructor* infestation levels. There was a decrease in the amount of total brood between August and November which lessen the availability of brood for *Varroa* mite infestations, and thereby provided a longer phoretic period for the mites on adult bees. The environment inside the hive is well-controlled by the bees, being stable, particularly the temperature of the brood nest. However, external factors such as humidity and food supply vary considerably according to local and seasonal conditions (Jones *et al*., 2004). The population abundance of *Varroa destructor* was low in the month of July as compared to other months of this study probably because of a lack of resources (pollen, nectar and honey), high levels of hygienic behaviour and unfavourable climatic conditions which resulted in reduction of brood in *A. m. scutellata* colonies and consequently lower levels of mite reproduction. The highest mean relative humidity of 79% and lowest ambient temperature of 16.1°C were recorded in the month of July. The findings that *Varroa* mite abundance decreased as relative humidity increased in the month of July is consistent with previous studies linking high ambient relative humidity and low temperatures to low *Varroa* mite populations (Kraus and Velthuis, 1997; Strauss *et al*., 2015). Furthermore, Nganso *et al*. (2018) reported that high infertility rates as well as low fecundity are key parameters that seem to interact with one another during different seasons to suppress mite reproduction in *A. m. scutellata* colonies.

Seven honeybee colonies absconded during the month of July. Absconding rate in African honeybees is high and can be due to unfavourable climatic conditions, human disturbance and parasitism (Hepburn and Radloff, 1998; Strauss *et al*., 2015). The absconding rate of the examined *A. m. scutellata* bees (7 out of 20 colonies, representing 35%) is similar to those reported previously in Ugandan bees (38–45%) (Chemurot *et al*., 2016) and Ethiopian bees *Apis mellifera simensis* (41.1%) (Gebremedhn *et al*., 2019). This trait negatively affects *Varroa* population dynamics as it creates a brood-free period. Absconding in this study probably occurred due to reduced forage (resources) which appeared to have been influenced by colony size of *Apis mellifera scutellata*. The amount of total brood in July was very low in examined colonies. The adult bee population was negatively associated with colony resources along with weak evidence for pollen availability, which might have been a factor influencing the colony development of *A. m. scutellata*. This conform to previous research which reported that reduced forage rather than parasitism, hive type, or time since colony establishment led to absconding in African honeybees (Mcmenamin *et al*., 2017).

Furthermore, as has been reported in other countries of the world, honeybee populations in Africa are under threat by currently identified novel pathogens, parasites and pests (*Varroa destructor*, American foulbrood, *Nosema ceranae*) as well as habitat loss (Dietemann *et al*., 2009). The high rate of absconding observed during the study period was not as a result of *Varroa* mite infestations but rather the reduced numbers of capped brood led to absconding in *A.m. scutellata* colonies (Cheruiyot *et al*., 2020). These results confirm previous studies which reported that absconding occurred following decreased brood, stored pollen, nectar and honey (Winston *et al*., 1979). The results also conform to previous reportage of African

honeybees showing resilience to introduced parasites and diseases (Muli *et al*., 2014; Mumoki *et al*., 2014).

Varroa mite population in capped worker brood had significant negative influence on honeybee population. Previous studies reported that brood production in honeybee colonies is negatively affected by high *V. destructor* infestations (Fries *et al*., 1994; Murilhas, 2002). The amount of honey was positively associated with *Varroa* mite population, indicating that *Varroa* mites does not lead to reduced honey production in *A. m. scutellata* colonies. This conform to previous studies which reported that *V. destructor* had no effect on honey production by honeybees of European origin (Erickson *et al*., 1998; Emsen *et al*., 2014). In this study, the size of the colony population (amount of brood and the number of adult bees) determined the availability of colony stores in *A. m. scutellata* colonies (pollen, nectar and honey). When the amount of brood and number of adult bees were at lowest levels in the month of July, the amount of pollen, nectar and honey also reduced drastically. The amount of honey significantly increased as the number of adult bees increased. This is because many honeybees get involved in foraging activities of floral resources (nectar) resulting in higher rate of honey production. Unexpectedly, the amount of capped brood was negatively associated with *Varroa* mite population. This is in contrast to previous studies that reported high *Varroa* mite population in capped brood (Harris *et al*., 2003; Adjlane *et al*., 2015). This suggests indirect effects of *V. destructor* which require experimental manipulation on population of brood, adult bees, and amount of colony stores.

There were no cases of capped dead brood though there were few cases of honeybees with deformed wings. The latter coincided with increased levels of *Varroa* mites in the capped brood suggesting that high *Varroa* mite infestations affect emerging adult bees. Previous

studies reported that *Varroa* mite transmits Deformed Wing Virus (DWV) and can trigger its replication in affected bees leading to emergence of honeybees with crippled wings (Gisder *et al*., 2009; Mondet *et al*., 2014). *Varroa destructor* appears to have no negative impact on *A. m. scutellata* colonies in Kenya unlike in the honeybees of European origin where *V. destructor* and honeybee viruses are very prevalent and lead to high colony losses (Genersch *et al*., 2010; Martin *et al*., 2012; Francis *et al*., 2013). In addition, few honey bee viruses have been detected in Kenya (ABPV, BQCV, DWV, IAPV) and the health status of the honeybee colonies are not negatively affected (Muli *et al*., 2014; Mumoki *et al*., 2014; Galbraith *et al*., 2018). This might explain why *A. m. scutellata* population in Kenya is able to survive in the presence of *V. destructor*, without treatment more than ten years since its detection in the country.

Results of this study showed that the mean daily mite fall per colony was < 1 and mean adult infestation level per colony was < 1%, a manifestation of low *Varroa* mite population. This is a significantly low infestation level compared to that reported for Africanized honeybees in Brazil which maintained 3-4 mites/100 adult bees (Rosenkranz, 1999) and in some honeybees of European origin that were selected for resistance to *Varroa* mite (Lattorff *et al*., 2015; Locke, 2016). High expression of hygienic behaviour and lack of chemical control used by beekeepers, is in part, responsible for mite resistance in African honeybees (Strauss *et al*., 2015; Nganso *et al*., 2017; Cheruiyot *et al*., 2018). Other bee health survey studies done in Uganda (Kajobe *et al*., 2010) and South Africa (Strauss *et al*., 2013) indicated that honeybee populations were indeed healthy and *Varroa* mite did not have devastating effects on these African honeybees. In this study there were no negative effects of *V. destructor* on

honey production, a strong indication of resistance and coexistence of a host-parasite balanced relationship.

5.2 Conclusions

i. Apis *mellifera scutellata* was confirmed to exhibit *Varroa*-specific hygienic behaviour and its expression is strongly dependent on the number of *Varroa* mites infesting the colony and is significant at high mite infestation levels.

ii. Infestation levels of *V. destructor* in *A. m. scutellata* colonies varied within the months of study depending on weather conditions (season) and the internal conditions of each colony (availability of brood, number of adult honeybees and amount of colony stores).

iii. *Varroa destructor* did not have negative effects on population and resources of *A. m. scutellata* colonies probably because of the natural resistance of African honeybees to the mite.

iv. The strongest factor that led to absconding of colonies was the number of capped brood cells.

5.3 Recommendations

i. *Varroa*-specific hygienic behaviour is a promising trait for selection and breeding of *Varroa* mite resistant honeybees and therefore it is recommended that Kenyan government; Ministry of Agriculture and Livestock Development do more research on VSH behaviour.

ii. Control measures should be put in place to maintain the apparent healthy status of honeybees in Kenya. Kenyan beekeepers should work with the local honeybee populations and refrain from importing colonies to prevent introduction of other haplotypes of the mite.

iii. Since *V. destructor* is considered a serious threat to beekeeping, it is recommended that proper colony management practices such as use of screened bottom boards to trap falling mites be enhanced and close monitoring of Kenyan honeybee colonies to curb an increase in *Varroa* mite infestations.

5.4 Suggestions for future research

i. Future research should be conducted to determine how infestation by *V. destructor* alters the behaviour of the honeybee brood and which specific chemical cues are involved in triggering VSH behaviour in *Apis mellifera scutellata* colonies.

ii. Future studies should investigate the low reproductive ability of *V. destructor* in African honeybees as most research studies on how *Varroa* mites reproduce in capped brood cells have been done majorly using Africanized honeybees and European honeybees.

iii. More research studies need to be done in other regions of Kenya where beekeeping is practiced in order to assess and compare population abundance of *V. destructor* because of different geographical conditions. This will help ascertain further influences of *V. destructor* on colony size and productivity of honeybee colonies in Kenya.

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APPENDICES

Appendix I: Steps of calculating percentage general hygienic behaviour (GHB) of twenty colonies using pin killing method

Colony	Number of open cells initially	Number of pin killed brood	Number of capped brood after 24 hours	Number of partially uncapped brood	Number of uncapped and cleaned out brood after 24 hours	% General hygienic behaviour
1	36	171	18	11	142	89.47
2	11	196	15	21	160	92.35
3	20	187	23	32	132	87.7
4	25	182	78	12	92	57.14
5	33	174	65	4	105	62.64
6	27	180	11	43	126	93.89
7	22	185	9	10	166	95.14
8	30	177	19	5	153	89.27
9	30	177	74	16	87	58.19
10	16	191	55	12	124	71.2
11	20	187	22	27	138	88.24
12	35	172	21	11	140	87.79
13	25	182	33	12	137	81.87
14	8	199	36	11	152	81.91
15	9	198	16	14	168	91.92
16	41	166	53	11	102	68.07
17	9	198	60	22	116	69.7
18	14	193	68	10	115	64.77
19	56	151	34	21	96	77.48
20	39	168	23	7	138	86.31

Appendix II: Experimental design of *Varroa*-specific hygienic behaviour. At nine different time points, *Varroa* mites were introduced in brood cells containing pupae. Control cells were sham manipulated on the back of the comb. Status of the cells was checked after 72 hours. Introduction of *Varroa* mites occurred approximately every ten days. 10 closely neighbouring brood cells were singly introduced with one mite in the first colony, 8 mites singly introduced in the second colony and 5 mites singly introduced into the third colony. The same procedure was repeated at different time points in all the ten colonies

Colony	**Time point 1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Time point 9**
1	10	8	5	10	8	5	10	8	5
2	8	5	10	8	5	10	8	5	10
3	5	10	8	5	10	8	5	10	8
4	10	8	5	10	8	5	10	8	5
5	8	5	10	8	5	10	8	5	10
6	5	10	8	5	10	8	5	10	8
7	10	8	5	10	8	5	10	8	5
8	8	5	10	8	5	10	8	5	10
9	5	10	8	5	10	8	5	10	8
10	10	8	5	10	8	5	10	8	5

Appendix III: Simplification of GLMMs for *Varroa*-specific hygienic behaviour by means of stepwise removal. The table contains the type of model, the model structure, Akaike Information Criterion (AIK), the P-value derived from ANOVA for model comparison, and the model that has been kept after simplification. Marked in bold letters is the model that was finally identified as the best model. Fixed factors were mites, day, and their interaction term (mites: day), random factor is the colony.

Untouched Brood Cells (UBC)				
Model name	Model structure	AIC	P-value (ANOVA)	Model kept
Null Model	1 + 1|colony	821.38		
Full Model	mites + day + mites: day + 1|colony	726.8	< 2.2e-16	Full model
Reduced Model 1	mites + day + 1|colony	724.8	0.9771	Red. Model 1
Reduced Model 2	**mites + 1|colony**	**723.7**	**0.3276**	**Reduced Model 2**
Cell Caps Opened (CCO)				
Model name	Model structure	AIC	P-value (ANOVA)	Model kept
Null Model	1 + 1|colony	728.67		**Null model**
Full Model	mites + day + mites: day + 1|colony	731.1	0.3158	
Brood and Mite Removed (BMR)				
Model name	Model structure	AIC	P-value (ANOVA)	Model kept
Null Model	1 + 1|colony	827.19		
Full Model	mites + day + mites: day + 1|colony	825.87	0.06242	**Full model**
Mite Removed and Brood cell Recapped (MRBR)				
Model name	Model structure	AIC	P-value (ANOVA) Model kept	
Null Model	1 + 1|colony	764.46		
Full Model	mites + day + mites: day + 1|colony	733.90	5.705e-08	Full Model
Reduced Model 1	mites + day + 1|colony	731.91	0.9173	Red. Model 1
Reduced Model 2	**mites + 1|colony**	**731.46**	**0.2127**	**Reduced Model 2**

Appendix IV: *Varroa destructor* collecting form

DATE	COLONY NUMBER	MITES ON STICKY BOARDS	MITES ON BEES (SUGARSHAKE METHOD)	MITES IN BROOD