

**DISTRIBUTION, BEHAVIOURAL BIOLOGY, REARING AND
POLLINATION EFFICIENCY OF FIVE STINGLESS BEE SPECIES
(APIDAE: MELIPONINAE) IN KAKAMEGA FOREST, KENYA**

BY

KIATOKO NKOBA

Reg No. I84F/11631/08

A thesis submitted in fulfillment of the requirements for the award of the degree
of Doctor of Philosophy (Ph.D) in Agricultural Entomology in the School of Pure
and Applied Sciences of Kenyatta University

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DECLARATION

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

Kiatoko Nkoba

Department of Zoological Science

Signature:.....

Date:.....

We confirm that the work reported in this thesis was carried out by the candidate under our supervision. We have read and approved this thesis for examination.

Professor J. M. Mueke

Department of Zoological Sciences

Kenyatta University

Signature:.....

Date:.....

Professor K. Suresh Raina

Commercial Insects Programme, *icipe*

African Insect Science for Food and Health

Signature:.....

Date:.....

Dr. Elliud Muli

Department of Biological Sciences

South Eastern University College

(A Constituent College of the University of Nairobi)

Signature:.....

Date:.....

DEDICATION

This thesis is dedicated to The All Mighty God, My parents Prefessor Kiatoko Mangeye Honore and Madame Kialungila Mundengi Cecile, My lovely daughters Kiatoko Makuzayi Emile and Kiatoko Mangeye Pongelle and to my wife Luntonda Buyakala Nicole. Thank you for your love and support.

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LIST OF ABBREVIATIONS

cip: Commercial Insect Programme

GIS: Geographical Information System

Gle: Grassland with *Eucalyptus* tree species

Gli: Grassland with the indigenous tree species

GPS: Global Positioning System

HQ: Head quater

icipe: International Centre of Insect Physiology and Ecology

IITA: International Institute of Tropical Agriculture

Inf: Indigenous forest

Isv: Homesteads at Isiekuti

Ivv: Homesteads at Ivihiga

KIFCON: Kenya Indigenous Forest Conservation Programme

Mif: Mixed indigenous forest

N: Number of sample

NN: Average Nearest neighbour Distance

R: Nearest Neighbor Index

R_e : Expected Nearest neighbour Distance

R_o : Observed Nearest neighbour Distance

R_1 : Minor axis

R_2 : Major axis

S: Surface or Area

vs: Versus

DEFINITION OF MELIPONICULTURE TERMS

Batumen: A thick layer of hard cerumen, often incorporating various other materials, that encloses and protects the entire nest cavity of stingless bees¹.

Brood: Term for young developing bees in the eggs, larva and pupa stage¹.

Brood cell: The individual cerumen capsule constructed by stingless bees in which the egg is laid and the young bee develops¹.

Cerumen: The brown building material used by stingless bees consisting of a mixture of wax, secreted by young bees, and tree resins collected by the foragers¹.

Colony and nest: In the main 'Colony' and 'Nest' refer to the bees and their brood collectively¹.

Hive: An artificial structure made for housing bees (including the colony within-when occupied)¹.

Involocrum: An insulating sheath of thin cerumen (often multi-layered) enclosing the brood area of the stingless bees nest¹.

Propolis: A (usually) dark coloured resin collected and used by honey bees and stingless bees to seal up cracks and to secure hive components¹.

Pillar: Structures inside the nests which connect the brood combs with one another and also support all other structures within the batumen. Pillars are disposed vertically and connectives are disposed more or less horizontally²

¹ Klumpp, J. (2007)

² Wille, A. (1983)

ABSTRACT

The study on distribution, behavioral biology, rearing and pollination efficiency of five stingless bee species (Apidae: Meliponinae) in Kakamega forest, Kenya showed that; species diversity was higher at Ivihiga compared to Isiekuti sites. Within habitats, species diversity varied and the indigenous and the mixed indigenous forests had more species diversity than the other habitats. The number of nesting habitats and also the chance of getting a nest of a particular species within nesting habitats varied among the stingless bee species. The nest pattern was under-dispersed for almost all the species nesting in the indigenous forest; mixed indigenous forest and grassland with the indigenous tree species. The nesting pattern for *M. ferruginea* (reddish brown) and *H. gribodoi* changed from an under-dispersed and over-dispersed pattern, respectively, to a clumped nest pattern when nesting in the homesteads. Within interspecific species nesting in the same habitat and within the nesting habitats of conspecific species; a difference was observed in the average nearest neighbour distance separating their nest entrances. Three different nesting sites were identified overall species (tree, underground, residential houses). Nest aggregation was observed within four bee species (except *M. lendliana*) and the average less minimum nearest neighbour distance between nests aggregated on a single substrate varied within conspecific and interspecific aggregation. Daily temperature and humidity influenced the bee flight activities out for foraging or grooming; with temperatures below 22°C and relative humidity above 70% being not ideal for the five bee species to start or maintain their out going flight activity. *M. ferruginea* (reddish brown) and *H. gribodoi* were the only species which bite to defend their nests from intruders. *M. ferruginea* (reddish brown) and *M. lendliana* were the only species which completely seal the open entrance of their nests at night. Acceptance of the designed hive types varied across bee species. All types of hives designed for *M. ferruginea* (reddish brown) were accepted at a probability of 0.63 and above. *M. ferruginea* (black) showed preferences for the two non compartmented hive designs and OATH hive type at a probability of 0,63 and above. *H. gribodoi* accepted only the *icipi* 1 hive model compared to the two designs of compartmented hives. *M. lendliana* preferred nesting only in the hives made out of clay as opposed to wooden hives. Three colonies of *M. bocandei* were successfully reared in the *icipi* 1M hive design. The average annual honey production under domestication varied among the five bee species and was higher in the *M. bocandei* species (3.13 ± 0.21 litres). The hive splitting method was more successful in propagation of colonies of three *Meliponula* species. Natural enemies recorded in this study included mainly parasites, predators and disturbers. Lastly, flowers of green pepper pollinated by *H. gribodoi* produced heavier fruits with superior number of seeds and the seeds were bigger compared to those produced by self-pollinated flowers or flowers pollinated by feral insects.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background information

Pressure on the Kakamega forest is still growing due to the continually increasing human population and high poverty levels (Raina *et al.*, 2009). Studies have reported on the role this forest plays in satisfying daily needs and income generating for rural families (Kokwaro, 1988; KIFCON, 1994; Raina *et al.*, 2009). The impact of anthropogenic activities on the disturbance of the indigenous Kakamega forest habitat has also been reported. Tsingalia and Kassily (2009) have reported on factors that have caused the apparition of grasslands in the Kakamega forest landscape. Conservation and the sustainable use of the tropical rainforest resources continue to receive increasing attention to preserve their biodiversity. The rainforest ecosystem is vulnerable to over-utilisation and exploitation due to the sensivity of its complex and highly diverse ecosystems (Althof, 2005). It is commonly reported that the disturbance of the natural indigenous forest causes a massive extinction wave or changes in the species diversity of stingless bees (Boontop *et al.*, 2008).

Studies on meliponine bees (stingless bees) community are largely based on the Neotropical regions (Roubik, 1989; Ricketts, 2004). In these regions, studies have considered the impact of natural forest habitat disturbances on the meliponine bees community (Hubbel and Johnson, 1977; Chaves and Aguilar, 2004; Jongjitvimol *et al.*, 2005; Boontop *et al.*, 2008). Winfree *et al.* (2007) studied the impact of anthropogenic land use on the conservation of meliponine bee species.

The nest structure, nesting habit and foraging behaviour of stingless bee species in Neotropical region have also been reported in the literature. Significant advanced studies have also been made in the Neotropical region on the importance of native eusocial stingless bee species as pollinators of flowers on cultivated and wild plants (Roubik, 1995; Slaa *et al.*, 2006). The ability to produce honey has also been studied for different stingless bee species in Neotropical region (Camargo and Posey, 1990; Crane, 1992; Richards, 1993; Kazuhiro, 2004; Cortopassi-Laurino *et al.*, 2006).

According to Cortopassi-Laurino *et al.* (2006) and Kajobe (2007), studies on Afro tropical stingless bee species have not been significantly advanced. Most of the studies on stingless bees in the African continent have been focused on some aspects such as, inventories and taxonomies of Afrotropical meliponine bees (Cockerell, 1933, 1934, 1935; Benoist, 1944; Pauly, 1998; Eardly, 2004). Descriptions of the nest structure of some Subterranean and trees nester stingless bee species (Araujo, 1955a, 1955b, 1963; Darchen and Pain, 1966; Darchen, 1970, 1971a, 1981) have been documented. The ethology, biology and ecology of some African *Trigona* species (Bassindale, 1954; Smith, 1854; Darchen and Louis, 1961; Darchen, 1966, 1969, 1971b, 1972) have also been described. The influence of environmental factors on the foraging behaviours of *Hypotrigona pothieri* (Trigonini) (Lobreau-Callen *et al.*, 1990; Lobreau-Callen *et al.*, 1994) have been studied. Cortopassi-Laurino *et al.* (2006) have reported on the use of traditional harvesting method (cutting down tress, scraping the nest) by African rural communities to obtained honey from colonies in the wild.

Recently, Kajobe (2007) reported on the habitat selection and competition for resources by some stingless bee species at Bwindi Impenetrable National Park in South-Western Uganda.

In Kenya, the literature available on stingless bees found in Kakamega forest shows that only few studies have been carried out. Raina *et al.* (2009) reported on the species namely, *Meliponula bocandei* (Spinola, 1853), *M. ferruginea* (reddish brown) (Lepelletier, 1841), *Meliponula lendliana* (Friese, 1900), *Hypotrigona gribodoi* (Magretti, 1884), and *M. ferruginea* (black) (Smith, 1854) being hunted in the wild for their honey by the Luhya rural communities. The contribution of honey from stingless bees in the local small-scale economy for income generation in the Luhya rural communities have been reported (Raina *et al.*, 2009). On the other hand, some stingless bee species found in the Kakamega forest have been identified to different taxonomy levels (Eardly 2004; Gikungu, 2006) and the physio-chemical composition and antibacterial activity of honey produced by four stingless bee species (*Meliponula bocandei*, *M. ferruginea* (black), *Meliponula lendliana* and *Hypotrigona gribodoi*) has been analyzed (Macharia, 2008).

It is apparent that in the African continent and particularly in Kenya there is limited data on the impact of the disturbance of the natural habitat on the native stingless bee species. The nesting range, foraging behaviour and potential natural enemies for different stingless bee species in dissimilar ecological zones are not well known. Furthermore, their ability to be domesticated in artificial hives, their potentiality for honey production and influence on crop yield if used as pollinators

have not been investigated. Farmers in Kakamega forest are aware of the existence of stingless bees as producers of medicinal honey but their conservation and management for honey production and as pollinators are unknown.

Hence, this study was undertaken to explore the diversity and spatial distribution of nesting colonies of the five cited stingless bee species along a successive gradient of three different habitats (forest, grassland, homesteads) at two different sites (Isiekuti, Ivihiga) of the Kakamega forest. The study also investigated nesting and foraging behaviour of the bees. Furthermore, the methods for propagating and maintaining the stingless bee colonies throughout the year in different hives for honey production were developed and evaluated. The study also documented the natural enemies which affect the nested colonies and the pollination potential of *H. gribodoi* to improve fruit quality of green pepper.

CHAPTER TWO: LITERATURE REVIEW

2.1 Stingless bees

"Stingless bees" sometimes referred to as stingless honey bees are a group of bees which have been described worldwide and are grouped with the common honey bees, carpenter bees, orchid bees and bumblebees to the Apidae family (Hymenoptera: Apoidea) (Michener, 1974; Meyer, 2005). On contrary to *Apis* honey bees, the sting in stingless bees is atrophied in size and does not sting; but they will defend themselves by biting when their nest is disturbed (Willie, 1983). In addition, a few in the genus *Oxytrigona* and several other species of the genus *Trigona* have mandibular secretions that cause painful blisters (Slaa *et al.*, 2006).

2.1.1 Origins and historical perspective

Stingless bees are considered to have originated in South America where meliponiculture is extensively practiced (Eardly, 2004). Africa has been suggested to be the center of stingless bees' origin; which then have dispersed to other tropical and subtropical parts of the world (Velthuis, 1997). The suggestion is based on paleontological and biogeographical data and is also supported by the fact that their primitive species with a well-developed sting system are found in Africa (Willie, 1983). Before the discovery and the conquest of the American continent by Europeans, the rearing of stingless bees was an important part of the commercial and alimentary customs of many indigenous cultures of Central and South America (Cortopassi-Laurino *et al.*, 2006).

Since the sixteenth century when the Spanish conquerors arrived in Mexico, the ancient Maya people in Yucatan held stingless bees in high regard and considered them an integral part of social and religious life (Cortopassi-Laurino *et al.*, 2006). At that time, stingless bees provided a vital small-scale economy due to their honey (used as medicine, sweetener and for mead), their waxes and resins (Cortopassi-Laurino *et al.*, 2006). The stingless bee honey was an important medicinal product (López, 1552) and was also used to pay taxes to the Aztecs, and Inca (Roubik, 2000). In Brazil, stingless bees play an important role in the ethno biology of the Kayapó (Camargo and Posey, 1990).

In view of the growing pressure on the environment and the associated loss of honeybees, attention has focused on meliponiculture as an eco-friendly agro-based venture. Stingless bees are known to be one of the key contributors in providing ecosystem support services vital to the survival of several forest plant and crop species through pollination (Richards, 1993; Roubik, 1995; Heard, 1999).

2.1.2 Geographical distribution and diversity

Stingless bees are reported to be found throughout most of the tropical and subtropical regions of the World (Velthuis, 1997; Michener, 2000) such as Australia, Africa, Southeast Asia, and in some parts of South America (Mexico, Brazil, Peru, Venezuela, Paraguay, Bolivia and Costa Rica) (Roubik, 1989). The different species range from the ecologically diverse habitats such as humid

rainforests and dry savannas (Williams, 1996). They are active all the year round, but are less active in cooler weather periods. It has been reported that it is impossible for them to survive naturally throughout the cold season in countries with temperate climate (Kazuhiro, 2004). Although about 800 species of stingless bees have been described worldwide (Williams, 1996), only 300 species have been categorized as highly eusocial bees living in colonies (Michener, 1974; Meyer, 2005) and these species are not distributed equally within the tropics.

According to Roubik (1989), local and regional diversities are high in the Neotropics, where up to 60 meliponine species can be found locally in a single forest. Cortopassi-Laurino *et al.* (2006) reported less than 45 species of stingless bees in Asia community. In Australia, Klumpp (2007) reported approximately 12 species of stingless bees while Eardley (2004) indicated that in Africa there are over 20 species of stingless bees, which comprise six African genera: *Cleptotrigona*, *Liotrigona*, *Hypotrigona*, *Dactylurina*, *Meliponula* and *Plebeina*. The exact number of species of stingless bees in Africa is not known and research work needs to be carried out on their biology and behaviour (Njoya, 2009).

Stingless bees (Sub family Meliponinae) are divided into two tribes: Meliponini and the Trigonini which has a large number of genera and sub-genera (Sommeijer, 1999). The tribe Meliponini comprises 23 genera and 18 Sub-genera, which consist of 374 recognized species (Michener, 2000).

2.1.3 Nesting and nest structure

Depending on species, stingless bees usually build their nests in hollow trunks, tree branches, or rock crevices and also they have been observed in wall cavities, old rubbish bins, water meters and storage drums (Pyper, 2001). In stingless bees, there is a great variety of forms and sizes of nest constructions (Henigman, 1975). The nest is complex in architecture and usually is made up of five parts; brood comb, involucre, store pots, batumen and an entrance (Kazuhiro, 2004). Honey and pollen are stored in pots quite different from the brood cells and deposited within the nest (Roubik 1989). Food storage allows colonies to survive for months without incoming food (Roubik 1989). Entrance of the nest is a simple hole normally built of wax and mud (Sakagami and Zucchi, 1967; Kazuhiro, 2004).

In the construction of the storage pots, brood comb and involucre, most species use cerumen which is a mixture of wax and plant resin. Some species such as *Leurotrigona muelleri* and *Trigonisca* spp use pure wax. The stingless bees that build exposed nests (such as *Trigona spinipes*) use leaves and other vegetation parts mixed with resin. *Partamona* spp use mud and sometimes faeces in the construction of their semi-exposed nests. The species that construct nests in the underground cavities isolate them using a series of membranes of batumen which is made of resin, mud and wax (Sakagami and Zucchi, 1967).

2.1.4 Colony establishment and life span

In eusocial stingless bees, each colony has a single queen (the mother), together with workers (the daughters) and, at certain stages in the colony cycle, there are also drones (males) (Michener, 2000; Pyper, 2001). New nests are established via swarms, in contrast to honey bees where swarming involves instantaneous departure of the old mother queen and a large mass of workers. In stingless bees, scout bees locate a new nest site and workers from the old colony gradually construct a new nest at the secondary location (nest material and food is first translocated). Later the nest is then joined by a newly-mated queen, at which point many workers take up permanent residence; brood cell construction and ovipositions are initiated in the new nest (Michener, 1974; Dollin, 2001). Several weeks or even months can pass for the new established nest to become completely independent from the mother nest (Wille 1983). Once safety is established, individual meliponine colonies are known to survive for 10 to 26 years (Roubik, 1989).

2.1.5 Honey production

Stingless bees have a system of mass-provisioning of honey unlike the honey bees which are progressive provisioners (Sommeijer, 1999). Honey production in stingless bees is species specific and the quantity produced by each potential species is low compared to that which is produced by honey bees (Pyper, 2001). The average yearly production of honey in most stingless bee species is nearly below one or two liters per colony, but some species are reported to produce more than that (Cortopassi-Laurino *et al.*, 2006).

The stingless bee species *Meliponula bocandei* is reported to produce 10-15 kg of honey in a season in Angola (Murray, 2005). Honey production by *M. subnitida* is nearly 6.0 litres per year in few of its colonies during the rainy season in Brazil (Cortopassi-Laurino *et al.*, 2006). The quantity of honey production in a stingless bee species also depends on the quality of the environment, competition with other bee species for floral resources and bee management (Cortopassi-Laurino *et al.*, 2006). All stingless bee species have their unique and characteristic honey which is a highly valued medicinal honey compared to that of *Apis*-honey bees.

2.1.6 Stingless bees and crops pollination

The issue of maintaining colonies of stingless bees as pollinators to increase crop yield is relatively new (Roubik, 1995; Slaa *et al.*, 2006). The importance and effectiveness of stingless bees as crop pollinators is lacking for most plant species (Heard, 1999). According to Slaa *et al.* (2006), the pollination effectiveness of a specific stingless bee species depends on the crop species. The social bees of the genus *Meliponula* is reported to be of interest because these bees are able to perform 'buzz-pollination' (Cortopassi-Laurino *et al.*, 2007).

In Japan, pollination tests of stingless bees (*Trigona carbonaria* and *Scaptotrigona bipunctata*) and by honeybees (*Apis mellifera*) on white clover, tomatoes, cucumbers, eggplants, paprika and red peppers in greenhouse and apiary areas showed that stingless bees pollinate just as honeybees, factoring in the difference among the crops (Kazuhiro, 2004). In Brazil, pollination tests on tomatoes (variety Rodas; long-life hybrid) in greenhouses under plastic using the

Neotropical stingless bee *Meliponula quadrifasciata* (Lepeletier) compared to manual pollination showed that the maximum fruit diameter, fruit weight and roundness were not significantly different between the treatments, but the fruits visited by *M. quadrifasciata* had 10.8% less seeds (dry mass) than those produced by the manual pollination (Del Sarto *et al.*, 2005).

Santos *et al.* (2004) compared the effectiveness of pollination of tomatoes by *M. quadrifasciata* and *A. mellifera* and found that tomatoes were bigger, heavier and had more seeds following pollination by *M. quadrifasciata* compared to those pollinated by *A. mellifera*. Malagodi-Braga and Kleinert (2004) have reported that *Tetragonisca angustula* was found to be an effective pollinator of strawberry (Oso Grande' cultivar) in greenhouses. Santos (2004) reported that *Scaptotrigona depilis* and *Nannotrigona testaceicornis* were effective pollinators of cucumber under greenhouse, resulting in a higher fruit production, higher fruit weight and a higher percentage of perfect fruits compared to the control where no pollinators were present.

In Mexico (Yucatan), González-Acereto *et al.* (2006) have reported that pollination efficiency of tomato (*Lycopersicon esculentum*) and habanero pepper (*Capsicum chinense*) in the greenhouses by *Nannotrigona perilampoides*, showed that this species of stingless bee could be used as an alternative pollinator to *A. mellifera* or bumble bees (*Bombus spp.*). Evaluation of *Meliponula beecheii* for pollination of habanero pepper in enclosures showed that it was efficient at pollinating this crop (González-Acereto *et al.*, 2006).

For the *Persea americana* crop (Avocado); Slaa *et al.* (2006) reported that some stingless bees species in the genera *Trigona* and *Plebeia* are efficient pollinators of this crop. Ish-Am *et al.* (1999) reported that eight to ten species of stingless bees were effective pollinators of avocado, together with the Mexican honey wasp. Can-Alonso *et al.* (2005) found that *A. mellifera* and *Trigona nigra* carried comparable amounts of avocado pollen grains on their bodies. In Indonesia, Klein *et al.* (2003) reported that four *Trigona* species, contribute a lot to the pollination of coffee (*Coffea arabica* and *C. canephora*) and *Trigona (Lepidotrigona) terminate* was found to be the most efficient pollinator.

Heard (1999) reported that some stingless bees species from the genera *Trigona* are effective pollinators of *Cocos nucifera* (Coconut), *Mangifera indica* (Mango), *Sechium edule* (Chayote), *Averrhoa carambola* (Carambola) and *Macadamia integrifolia* (Macadamia). The vanilla plants *Vanilla planifolia* is hand pollinated; and in Mexico one stingless bee species “abeja de monte” of the genera *Melipona*, is reported to be the only natural insect primarily presumed capable of pollinating blossoms of this crop (Irvine and Delfel, 1961; Herbst, 2001). Dino Martins in his report (FAO, 2008) revealed that *V. planifolia* in western regions of Uganda are being pollinated naturally by *Meliponula* sp.

2.1.7 Natural enemies of stingless bees

The most significant enemies of stingless bees reported in the literature are ants, phorid flies, termites, parasitic stingless bees (*Lestrimelitta* and *Cleptotrigona*)

toads, lizards and birds (Roubik, 1995). In Africa, primates such as chimpanzees try to obtain food from stingless bee nests by inserting flexible sticks in nest entrances (Kajobe 2007).

2.1.8 Economic potential of stingless bees

Since the sixteenth century to date, meliponiculture in the Neotropic region is among the many subsistence farming activities that assists the poor to generate income and conserve their bee biodiversity in the wild (Cortopassi-Laurino *et al.*, 2006). Stingless beekeeping provides incomes to the rural poor through honey, cerumen (mixture of wax and plant resin) and resins production.

The stingless bees honey is sold in market places at a higher price than honey from *Apis* bees (Sanford, 1997). The commercialization of stingless bee honey in small jars indicating stingless bee species of origin has become cost effective than the honey of *Apis* and cerumen is also marketable for making traditional artifacts (Cortopassi-Laurino *et al.*, 2006).

In addition, the role of stingless bees as providers of ecosystem services vital to the survival of several forest plants and crop species through pollination has also become a source of income generation activity. In Australia, stingless beekeepers offer stingless bee services for crop pollination, usually as their secondary financial activity (Cortopassi-Laurino *et al.*, 2006).

2.2 Stingless bees in Kenya

The species richness and abundance of stingless bees all over Kenya are still not known due to shortage of bee taxonomists in East Africa, lack of adequate bee surveys, mis-identified or un-updated taxonomic identities of the bee collections in East African museums and institutions (Gikungu, 2006).

In Arabuko Sokoke forest along the Indian Ocean, the stingless bee species *Dactylurina schimdti*, *Hypotrigona gribodoi* and *Meliponula ferruginea* have been reported to occur in this forest habitat (Raina *et al.*, 2006; Macharia *et al.*, 2007). In Mwingi woodland, the stingless bee species *H. gribodoi* and *Hypotrigona araujo* have been reported to be present in the area (Raina *et al.*, 2006; Macharia *et al.*, 2007). In the Kakamega forest, *Meliponula bocandei*, *M. ferruginea* (reddish brown), *Meliponula lendliana*, *H. gribodoi* *M. ferruginea* (black) and *Plebeina hildebrandti* are the stingless bee species hunted for honey by the local rural community living adjacent to the forest (Raina *et al.*, 2006). Gikungu (2006) in her study on bees of the Kakamega forest recorded the following stingless bee species *H. gribodoi*, *Liotrigona bottegoi*, *Meliponula erythra*, *Meliponula denoiti*, *M. bocandei* and *M. lendliana*.

Farmers in Kenya are aware of the existence of stingless bees as sources of medicinal honey but there is lack of research about their conservation and management for honey production and efficiency as pollinators of crops (Raina *et al.*, 2006).

2.3 Importance of the Kakamega Forest

Kakamega forest is located in the western part of Kenya between latitudes 0°10' and 0° 21' north and longitudes 34° 47' and 34° 58' east. The forest is the easternmost remnant of the rainforest found in the Democratic Republic of Congo and much of West Africa. Kakamega forest is the only surviving rainforest in Kenya (Kokwaro, 1988), its natural vegetation is tropical rainforest covering approximately an area of 265 km² (Muriuki and Tsingalia, 1990) and is an island of relatively 'natural' habitat in a sea of human dominated landscape.

According to KIFCON (1994), the Kakamega forest comprises several separate blocks of forest: Kakamega (13,878.2 ha), Bunyala (825.6 ha), Malava (722.8 ha), Kabiri (3,691.3 ha), Lirhanda hill (52.7 ha), Yala river (2895 ha), Isecheno (415 ha), Ikuywa (380 ha) and Buyangu (3,997.5 ha). These blocks of the forest are not homogenous habitats and depending on the blocks; four different types of forest habitats can be found: forest habitats with only indigenous species (indigenous forest); forest habitats with a mixture of indigenous and exotic species (mixed indigenous forest); forest habitats with only exotic hard wood species (hardwood plantation) and forest habitats with only exotic soft wood species (softwood plantation) (Plate 2.1). Exotic species are mainly Pines, Black Wattle and Eucalyptus. These exotic species have been promoted due to their fast maturation and therefore give quicker returns on investment.

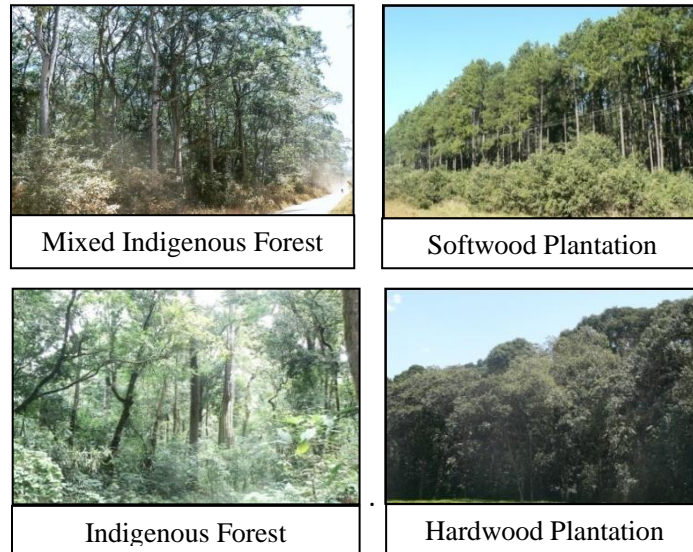


Plate 2.1: Types of forest habitat in the Kakamega forest at Isecheno forest block

There are about 150 species of woody trees, 90 species of dicotyledonous herbs, 80 species of monocotyledonous herbs, of which about 60 are orchids, and a further 62 species of ferns, totaling to about 380 identified species of vascular plants (KIFCON, 1994). About 168 medicinal plant species (21 herbs, 4 lianas, 14 climbers, 45 shrubs, 82 trees and 1 creeper) spanning in 74 plant families have been recorded in the Kakamega forest, and are frequently used as traditional medicine by the local Luhya community (Nyunja *et al.*, 2009). Moreover, Kakamega forest has a diverse fauna exhibiting a high degree of endemism and rarity, and it is estimated that 10 to 20% of the fauna in general may be endemic (KIFCON, 1994). Over 350 species of birds reside in the forest (Bennun and Njoroge, 1999). Among the diverse animal species are: several antelopes like the Red Duiker (*Cephalophus natalensis*) or the Bushbuck (*Tragelaphus scriptus*), snakes, three species of squirrel, bats and several species of primates (Muriuki and Tsingalia 1990). The forest also has a high diversity of insects and is

particularly known for its butterfly diversity that is estimated to be about 400 species (Emmel and Warren, 1993). About 243 bee species of 36 genera including some stingless bees have been reported to be present in this forest (Gikungu, 2006; Raina *et al.*, 2006).

The Kakamega forest plays an important role in satisfying daily needs and income generation activities for rural families mostly for fuel energy, grass (Plate 2.2), wild mushroom, medicinal plants, construction poles, timber and honey from wild bees (Kokwaro, 1988; KIFCON, 1994; Raina *et al.*, 2006).

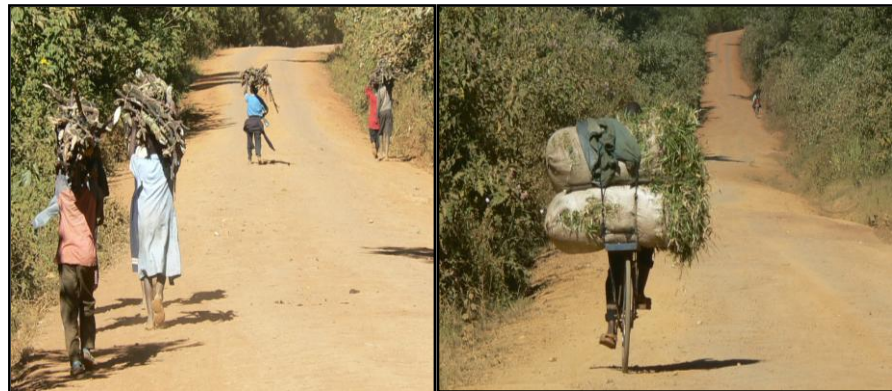


Plate 2.2: Firewood for fuel energy and the grass for feeding cattle at home collected in the Kakamega forest by local community surrounding the forest

Numerous grasslands are found in or surrounding the forest; and vary in structure and composition. Some of these grasslands have scattered trees, some have termite mounds while others are devoid of trees, yet others combine these characteristics in various degrees (Tsingalia, 1988, 1990). The grasslands surrounded by villages are utilized by the local community for grazing their cattle (Plate 2.3).



Plate 2.3: Grazing of cattle by the local community in the Kakamega forest

2.4 Problems statement

Sustainable management of the forest involves maintaining the benefits that come to humans from nature and its components (Cork and Shelton, 2000). The term “Ecosystem services” has been coined to describe benefits, which include life support, the underpinning of agricultural production, and provision of the basis for the fulfillment of human life (Daily, 1997; Mooney and Ehrlich, 1997; Westman, 1977). Increased population in the rural communities living adjacent to the Kakamega forest has led to village extensions, overexploitation and excision of the forest, thus threatening biodiversity therein and also the livelihoods of the people living around the forest.

Stingless bees are found in the Kakamega forest and rural communities continue to harvest their honey for subsistence and also as traditional medicine because they believe that it has high medicinal value (Raina *et al.*, 2006). With the increase in deforestation and the use of traditional honey harvesting method from tree cavities in which the colonies are established, the number of wild stingless

bee colonies is declining rapidly in the Kakamega forest (Raina *et al.*, 2009). This is mainly due to the destruction of colonies and their habitats (forage sources and nesting sites). Consequently, honey harvest and pollination services by stingless bees on the cultivated and wild plants are thought to be decreasing in and around the Kakamega rainforest (Raina *et al.*, 2009). One of the solutions to this depletion problem of stingless bee biodiversity would be the introduction of economic incentives that integrate conservation with economic development of the rural people (Munthali and Mughogho, 1992; Raina, 2000). It is possible that stingless bee species found in the Kakamega forest can offer opportunities as sources of alternative income for communities living around the forest, especially through honey production and for crops pollination (Raina *et al.*, 2006).

2.5 Justification

There are two groups of highly eusocial insects in the world: Apinae bees and Meliponinae bees. Apinae group includes the Western honeybee *A. mellifera*, and the Oriental honeybee *A. cerana* which are well-known as beekeeping species that provide honey and also pollinate crops (Kazuhiro, 2004). Recent problems of diseases emergency such as Varroa mites and the Small Hive Beetle have led to a loss of wild and managed honeybees (Ghazoul, 2005; Villanueva *et al.*, 2005). Also the defensive behaviour of the African honeybees poses handling danger to farmers (Watanabe, 1994; Eardley, 2004; Ghazoul, 2005; Villanueva *et al.*, 2005). Hence, due to these pressures mounting on honey beekeeping, concerns have been oriented to the management of stingless bees. Stingless bees are reported to be

one of the key contributors in providing ecosystem support services vital to the survival of several forest and crop plant species through pollination (Richards, 1993; Cunha *et al.*, 2002). They have also been reported to provide honey which many people use as food, medicine, raw materials for various industries and increased government revenue through levies and taxes (Kihwele *et al.*, 1999). Nevertheless, in Africa and particularly in Kenya studies on the diversity of stingless bees within habitat types and their management in manmade cavities plus as pollinator are scanty (Cortopassi-Laurino *et al.*, 2006). Stingless bees honey is obtained from hunting wild colonies and harvesting methods involve habitat destruction through tree cutting, digging out underground nests and scrapping out the whole nest (Raina *et al.*, 2006). There is therefore need to involve the local communities in the utilization and conservation of their indigenous biodiversity of stingless bees.

There is therefore need to carry out studies on proper identification of stingless bees which have minimal restrictive nesting behaviour in manmade cavities. The best methods for propagating and maintaining the identified bee species throughout the year will promote honey production. Furthermore, the influence of biotic and abiotic factors on the nesting and foraging behaviour of each identified bee species is an important study. For crop pollination, the identification of the stingless bee species which have docile temperaments, non-destructive foraging behaviour on flowers and adequate flight ranges will promote crop production (Cunha *et al.*, 2002; Cortopassi-Laurino *et al.*, 2006; Raina *et al.*, 2009).

This research work was undertaken to explore the species distribution of some stingless bees along successive habitats (forest, grassland, homesteads) in the Kakamega forest. The study also investigated the nesting and foraging behaviour and the potentiality of the species for meliponiculture plus their potential natural enemies in domestication. The effectiveness of *H. gribodoi* as pollinators of green pepper was also studied. The knowledge gained from this study will be useful for the conservation of the biodiversity of stingless bees in Kakamega forest and for sustainable meliponiculture in favor of communities living adjacent to the forest.

2.6 Research questions

- a) What is the diversity and nest abundances of five stingless bee species within three habitats at two sites in Kakamega forest?
- b) What is the pattern of the nest distribution of five stingless bee species and the average nearest neighbour distance within intra and inter specific species nest in three habitats at two sites in Kakamega forest?
- c) Do the stingless bee species have the same nesting behaviour?
- d) Do the stingless bee species use the same defensive mechanisms and in-and out colony activity at their nest entrance tube?
- e) Do the stingless bee species accept artificial hives in the wild and which is the appropriate rearing box for honey production?
- f) Which is the most potential stingless bee species within five species in Kakamega forest for honey production?

- g) Which is the appropriate technique to multiply the bee species with the highest potential for honey production and for supplemental feeding during the dearth period?
- h) Which are the natural enemies affecting the nested colonies of five stingless bee species in hive boxes around the Kakamega forest?
- i) Does *H. gribodoi* have the potential to be utilized as a pollinator to improve fruit quality of green pepper?

2.7 Hypotheses

- a) The diversity and nest abundance of the five stingless bee species do not vary within different habitats in Kakamega forest.
- b) There are no differences in pattern distribution and distances between conspecific and heterospecific nest in various nesting habitat.
- c) The nesting behaviour of the five stingless bee species do not vary among the stingless bee species.
- d) The five stingless bee species have the same defensive mechanism and in-and-out colony activity at their nest entrance tubes.
- e) The five stingless bee species in Kakamega forest are not viable for meliponiculture and produce the same amount of honey under domestication.
- f) There are no natural enemies affecting colonies of the five stingless bee species nested in hive boxes around the Kakamega forest.
- g) The stingless bee species *H. gribodoi* found in Kakamega forest is not an effective pollinator of green pepper *Capsicum annum*.

2.8 Objectives of the study

2.8.1 General objective

To determine the distribution, behavioural biology, rearing techniques and pollination efficiency of five stingless bee species (Apidae: Meliponinae) in Kakamega forest, Kenya.

2.8.2 Specific objectives

1. To determine the diversity of five stingless bee species in some habitats in Kakamega forest.
2. To determine the distribution pattern and distances between nests of five stingless bee species in the Kakamega forest and vicinal habitats.
3. To investigate the nesting behaviour of the five stingless bee species in the environment of Kakamega forest.
4. To investigate the defensive mechanism and in-and-out colony activity at the nest entrance tube of the five stingless bee species.
5. To determine the appropriate rearing techniques and the potential for honey production of the five stingless bee species.
6. To document the natural enemies affecting colonies of the five stingless bee species nested in hive boxes in the Kakamega forest.
7. To evaluate the effectiveness of *H. gribodoi* to improve fruit quality of green pepper *Capsicum annum* under small scale farming system in Kakamega forest.

CHAPTER THREE: DIVERSITY OF STINGLESS BEES WITHIN HABITATS IN KAKAMEGA FOREST

3.1 Introduction

In ecology conservation it is important to understand how disturbance of natural habitats affect community diversity of organisms in a particular area. Ecologically, habitat characteristics are important in regulating diversity of species and population size as plants and animals are highly dependent on the quality of their habitats (MacArthur and MacArthur, 1961). Disturbances of natural habitats affect diversity of vertebrates and invertebrates by altering their habitats, such as nesting or housing on which organisms depend (Oldroyd *et al.*, 1994; Lindenmayer *et al.*, 1997). A study carried out by Winfree *et al.* (2007), revealed that some anthropogenic habitats may offer more opportunities for conservation of a species than it has been previously thought. Knowledge of the abundance of a species across a gradient of successive different habitats gives greater indication on how the population of the species is affected due to changes in the habitat.

Studies on stingless bees have indicated that these bees are strongly associated with native forest habitats for nesting (Roubik, 1989; Ricketts, 2004; Brosi *et al.*, 2007; Brosi *et al.*, 2008). Stingless bees are reported to be one of those terrestrial invertebrates mostly affected by forest deterioration in the tropics due to anthropogenic disturbance (Hubbell and Johnson, 1977; Boontop *et al.*, 2008). According to Ricketts (2004) and Brosi *et al.* (2007, 2008); when meliponine bees

are abundantly found nesting in human-dominated land near the forest, this may be an indication of a high degree of disturbance of the neighbouring native forest habitat.

In Kenya, the Kakamega forest is the only surviving tropical rainforest and most of its natural indigenous forest has been disturbed through anthropogenic activities. Nowadays, this forest stands as an island comprising different types of forest habitats ranging from natural indigenous forest to heterogeneous forest such as mixed indigenous forest, hardwood and softwood plantations. Modification in the type of the original forest habitat may have affected the diversity of the stingless bee species compared to the natural indigenous forest. The Kakamega forest also comprises several grasslands which differ in structure and composition. Some of these grasslands have scattered trees, some have termite mounds, others are devoid of trees, yet others combine these characteristics in various degrees (Tsingalia, 1988, 1990). These grasslands are found either in or surround the forest and some are particularly surrounded by villages (Tsingalia and Kassily, 2009); which have provided Meliponinae bees an ideal habitat for nesting.

In order to provide information to support the development of conservation plans for natural areas or recovery plans for the threatened or endangered stingless bee species, this study was undertaken to determine the species richness, evenness, diversity and nest occurrence of five stingless bee species over different habitats.

Furthermore, the degree of similarity in species composition and fauna within habitats; and the degree of similarity in habitat preferences within the five species were studied.

3.2 Materials and methods

3.2.1 Study area

The study was carried out in two sites namely Isiekuti at Muhutu Location, Shavirenga Division and Ivihiga at Ivihiga Location, Ileho Division in the Kakamega forest (Plate 3.1) where the icipe commercial insect programme carries out its projects.

The Kakamega forest at Isiekuti (Isv) is a mixed indigenous forest (Mif) and the grassland is a habitat with dispersed indigenous tree species (Gli). At Ivihiga (Ivv), the Kakamega forest is an indigenous forest (Inf) and the grassland is a habitat with dispersed *Eucalyptus* tree species (Gie). The sites were chosen due to their long history in the traditional harvesting of the stingless bee honey for medicine and traditional rituals.

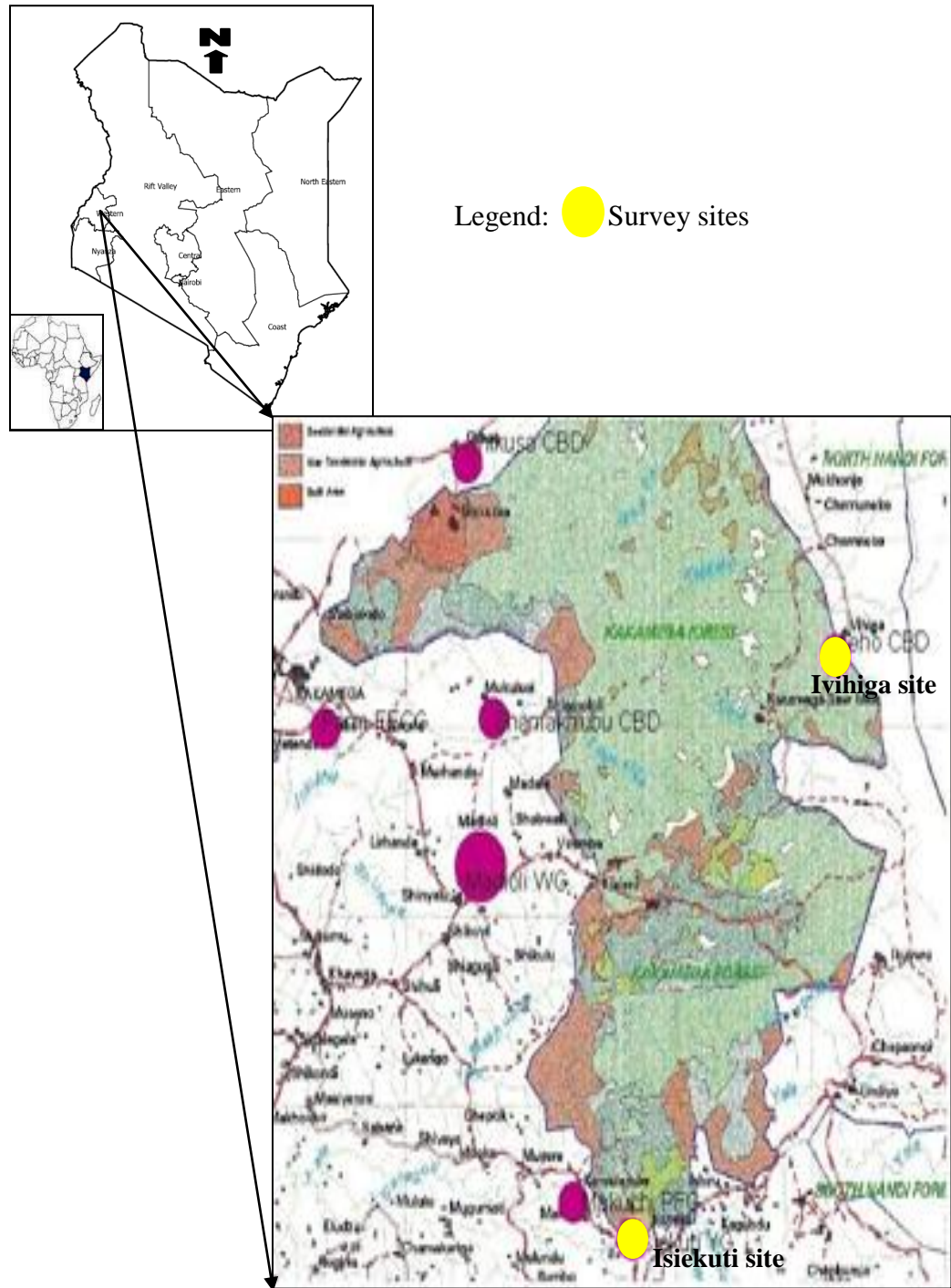


Plate 3.1: Map of Kenya locating the Kakamega forest in the Western province and map of Kakamega forest (ICIPE, 2001) showing the two study sites

3.2.2 Sampling methods

In each of the two study sites, nesting colonies of the five species of the stingless bees namely *Meliponula bocandei* (Spinola, 1853), *M. ferruginea* (reddish brown) (Lepeletier, 1841), *Meliponula lendliana* (Friese, 1900), *Hypotrigona gribodoi* (Magretti, 1884), and *M. ferruginea* (black) (Smith, 1854) (Plate 3.2) were surveyed following a successive gradient of the three different habitats namely homesteads, grassland and forest. Line transect methods were used in the population survey in each habitat (Krebs, 1999; Jongjitvimol *et al.*, 2005; Otieno *et al.* 2008). A total of thirty line transects of 500 metres long and 20 metres apart on a base line, were followed in 30 ha area in each three successive habitats to investigate for nesting colonies.

Field surveys were carried out during the sunny days in order to facilitate viewing of forager bees flying in and out of the nests. Nests inspections were carried out on every substrate likely to have nests such as living and dead trees, termite mounds, residential houses and the ground. For living or dead trees higher than six meters, a binocular spectrum (Olympus, porro prism standard binocular) was used to detect the presence of nesting colonies (Eltz *et al.*, 2003; Mbahin, 2008).

When a nest was found, bees flying in and out of the nest were collected using a sweepnet net and the species identified and recorded (Kajobe, 2007). The specimens from different nests were preserved in 70% alcohol in separate vials and coded for further identification to confirm the species identity. The number of stingless bee species and their nests observed per transect in the different habitats

were recorded. Sampling of the species was carried out from June 2009 to February 2010.



Meliponula ferruginea (black)



Meliponula ferruginea (reddish brown)



Meliponula bocandei



Hypotrigona gribodoi



Meliponula lendliana

Plate 3.2: The five stingless bee species studied in the present research work

3.2.3 Data analysis

3.2.3.1 Species richness within the two sites and six habitats

Species richness (S) is defined as the number of species occurring within a specific sampled area (Barbour *et al.*, 1999; Kindt and Coe, 2005). The species richness was analyzed based on the species accumulation curves, which were calculated separately for each site and habitat as total species richness versus

pooled nest abundance and pooled transect respectively (Kindt and Coe, 2005; Winfree *et al.*, 2007). Biodiversity R software loaded in R statistical software version 2.1.1 was used to perform and portray the species accumulation curves (Kindt and Coe, 2005).

3.2.3.2 Species evenness and diversity within the two sites and six habitats

Evenness in species composition or equitability is defined as the relative abundance with which each species is represented in an area (Barbour *et al.*, 1999). Species diversity is measured as a combination of species richness and species evenness (Barbour *et al.*, 1999). To compare the evenness and diversity of species within the two sites and three habitats in the two sites; Evenness index (J) was calculated while Rényi evenness and diversity profiles were performed using Biodiversity R software loaded in R statistical software version 2.1.1 (Kindt and Coe, 2005; Ayuke *et al.*, 2009).

Rényi evenness profiles are reported to be direct methods of comparing evenness (Ricotta, 2003). The evenness profiles are interpreted as follows: a site or habitat of larger evenness in species composition will have an evenness profile that is everywhere above the evenness profile of another site or habitat of lower evenness; intersecting evenness profiles means that no ranking in evenness can be provided between the intersecting profile of the concerned sites or habitats (Kindt and Coe, 2005).

To assist in ranking the evenness distribution profile of both sites and six habitats; in cases of bias in their profiles (intersecting profile); their Evenness index (J) was calculated (Kindt and Coe, 2005; Ayuke *et al.*, 2009). A site or habitat with a highest value of evenness index than other sites or habitats indicate a highest evenly distribution of its species composition (Kindt and Coe, 2005).

Rényi diversity profiles are curves that provide information on richness and evenness, but do not provide information on the proportions of each species (Tóthmérész, 1995; Kindt and Coe, 2005). According to Kindt and Coe (2005), the interpretation of the Rényi diversity profile is as follows: the starting position at the left-hand side of the profile is an indication of the species richness. Profiles that start at a higher level have higher richness; the shape of the profile is an indication of the evenness. A horizontal profile indicates that all species have the same evenness. The less horizontal a profile is, the less evenly species are distributed; site with the highest profile is the more diverse. If the profiles intersect, it is not possible to order the sites from lowest to highest diversity. It is possible that one site has larger species richness, but lower species evenness. If evenness is the same for the sites that you are comparing, then differences in richness will correspond to differences in diversity. If the richness is the same, then differences in evenness will correspond to differences in diversity (Kindt and Coe, 2005).

The Rényi diversity profile provides some specific details on the corresponding site (Kindt and Coe, 2005): the profile values of H-alpha (x) for each site or habitat at alpha = 0 provide information on its species richness (species richness = $\exp(x)$); the profile value of H-alpha (x) for each site or habitat at alpha = 1 is its Shannon diversity index; the profile value of H-alpha (x) for each site or habitat at alpha = 2 is the logarithm of its reciprocal Simpson diversity index; the profile value of H-alpha (x) for each site or habitat at alpha = infinity provides its information on the proportion of the most abundant species (proportion of the most dominant species = $1/\exp(x)$).

3.2.3.3 Species composition and rank abundance of nests overall and within the two sites

Species composition refers to the different species recorded in a specific sampled area and the proportion abundance of their population (Hwang *et al.*, 2009; Rueda *et al.*, 2010). The proportion abundance ($F_{\%}$) of a species (A) in a specific habitat (Z); is an expression of the following formula proposed by Canard and Poinso (2004): $F_{A\%}(Z) = (N_A(Z) / \sum N_i(Z)) * 100$. Where $N_A(Z)$ is the population number recorded for a species A in an area Z, and $\sum N_i(Z)$ is the sum of population of each different species recorded in the area Z.

In order to study the community structures of the stingless bee species overall and within sites; the proportion abundance of nests for each species was ranked in a decreasing order. The rank abundance is the ordering of the recorded species

according to the frequency of occurrence of their population in a sampled area (Mitchell, 2004). Analysis of rank is one approach to substantiate the numerical structure of organisms in a given community (Mitchell, 2004; Hwang *et al.*, 2009). Calculation of the proportion abundance of nests for each stingless bee species recorded in a specific habitat and their ranking from highest abundance to lowest abundance overall the site and across both sites were performed using Biodiversity R software loaded in R statistical software version 2.1.1 (Ayuke *et al.*, 2009).

3.2.3.4 Species composition within habitats of the two sites

The species composition within habitats was reported based on: (i) the species fauna recorded nesting in each specific habitat, (ii) the total nest discovered overall stingless bee species per 30 hectare area in each habitat (Boontop *et al.*, 2008), (iii) the nest density as the mean number of nests observed overall the stingless bee species per 500 metres line transect in each habitat (Barbour *et al.*, 1999), (iv) the relative proportion of nests of each species in the different habitats reported as the frequency of one species in a specific habitat as a percentage of the total nests recorded for all stingless bee species in the entire surveyed area of both sites (Barbour *et al.*, 1999; Canard and Poinso, 2004) and (v) the dominant species in each habitat reported as the species that whose relative nest abundance numerically predominates (Boontop *et al.*, 2008).

Statistical software R version 2.14.0 was used for statistical analysis on the mean nest density overall the stingless bee species within habitats (R Development Core Team, 2005). Generalized linear model (binomial distribution model with logit link) was used to model data on the mean density of the nest overall stingless bee species on 500 metres transect. Differences in the mean density of the nests overall the stingless bee species was compared using Tukey's test (R Development Core Team, 2005).

The relative proportion (RF%) of the nests for a stingless bee species (A) in a habitat (Z) was calculated in excel and plotted on a graph (Boontop *et al.*, 2008). The following formula proposed by Canard and Poinso (2004) was used: $RF_{A\%}(Z) = (N_A(Z) / \sum N_i(Z_j)) * 100$. Where $N_A(Z)$ is the population number recorded for a species A in an area Z, and $\sum N_i(Z_j)$ is the sum of population recorded for each species i in the different surveyed areas Z_j .

3.2.3.5 Predicted probabilities of the presence of a nest of a stingless bee species within habitats

The predicted probability of the presence of a nest of a stingless bee species in a specific habitat was reported as the chance of that species to be presently nesting in the habitat. The calculated value vary from 0 (species A exclusively absent in the zone Z) to 1 (species A exclusively present in the zone Z) and is an indicator of the preferential habitat of a species (Canard and Poinso, 2004).

In this study case, the expression of the predicted probability of presence (P) of nesting colonies of a species (A) in a habitat (Z) was modified from the following formula proposed by Canard and Poinso (2004): $P(A) = \frac{F_A(Z)}{F_A(Z)+F_A(Z')+F_A(Z'')...}$; where P(A) is the presence of the species A in a zone Z, $F_A(Z)$ is the frequency of the species A in a zone Z, and $F_A(Z)$, $F_A(Z')$, $F_A(Z'')$ are the frequencies of the species A in the different zone Z^i . The used modified formula was expressed as follows: $P(A) = \frac{\sum T_{iA}(Z)}{T_Z}$; where $\sum T_{iA}(Z)$ is the sum of cotation (0,1) given to each surveyed line transect (T_i) in the habitat (Z) which indicate the presence (1) or absence (0) of a nest of the species A in the line transect and T_Z being the total number of line transect surveyed in the habitat Z. Generalized linear model (binomial distribution model with logit link) was used to model data on the predicted probability of a presence ($P_A(Z)$) of a nest of a stingless bee species (A) in a habitat (Z). Differences in mean predicted probability within habitats were compared using Tukey's test. The analysis was performed in R statistical software version 2.14.0 (R Development Core Team, 2005).

The calculated value of the predicted probability of presence of a species A in a habitats Z was then used to classify arbitrary the habitat for each stingless bee species in one of the five following categories (Canard and Poinso, 2004): accidental habitat for the species ($P_A(Z) < 0,1$), accessory habitat for the species ($0,1 \leq P_A(Z) < 0,2$), preferential habitat for the species ($0,2 \leq P_A(Z) < 0,9$), elective habitat for the species ($0,9 \leq P_A(Z) < 1$) and exclusive habitat for the species ($P_A(Z) = 1$).

3.2.3.6 Degree of similarity in species fauna within habitat

The degree of similarity in the species fauna within habitat was based on the calculation of their Sørensen's similarity coefficient (C_s) and Jaccard similarity coefficient (C_j) between paired habitats (Viana *et al.*, 1997; Rasmussen, 2009). The Sorensen's and Jaccard similarity coefficient are a statistics used for comparing similarity between qualitative data of two samples; paired habitat with highest index values indicated highest similarity in their species fauna (Looman and Campbell, 1960). The degree of similarity in species fauna was calculated using the following formula of the Sorensen's similarity coefficient (C_s) and Jaccard similarity coefficient (C_j): $C_s = \frac{2C}{A+B}$ and $C_j = \frac{C}{(A+B-C)}$; where A and B are the species numbers in samples A and B, respectively, and C is the number of species shared by the two samples (Looman and Campbell, 1960).

3.2.3.7 Similarity in species composition within habitat and habitat preferences within stingless bee species

The clustering analysis was applied to the species quantitative data to compile similarity in species composition within the five types of habitats and similarity in habitat preferences within the five stingless bee species (Kindt and Coe, 2005; Boontop *et al.*, 2008). To calculate the similarity in the stingless bee species composition between habitats and in the habitat preferences within the stingless bee species; the Bray-Curtis distance was used in the clustering method (Kindt and Coe, 2005). To reduce the influence of strongly dominant species; data was first transformed by $\log(n+1)$ to standardize the species matrix (Kindt and Coe,

2005). The information of the Bray-Curtis distance in the dissimilarity matrices was portrayed in a dendrogram by Unweighed Pair-Wise Group Average (UPWGA). The cluster analysis was performed using Biodiversity R software loaded in R statistical software version 2.1.1 (Kindt and Coe, 2005).

3.3 Results

3.3.1 Stingless bee species and local names

In the communities adjacent to the Kakamega forest, stingless bees or meliponine bees are locally known as *Tsikalasanga*. The communities categorized the five species according to some features such as, general body color associated to particular color or spots on the bee body, nesting site and bee size (Table 3.1).

The stingless bee *H. gribodoi* is locally called *Vuyuyi*; hence categorized as the smallest stingless bee, black pale in color, found nesting in branch/wall crevices and the storage pots are not connected by pillars. *M. bocandei* is locally called *Ikora* and is known as the biggest stingless bee species, black in color with brownish thorax, found nesting in tree cavities in the forest and the storage pots are not connected by pillars. *M. ferruginea* (reddish brown) species is a medium size bee, called *Inasasa* in the local language and is generally black pale in color with a reddish brown and black stripe abdomen and found nesting in tree cavities, underground and wall crevices and the storage pots are usually connected by pillars.

The *M. ferruginea* (black) species, called *Iwere*, locally is medium in size and black in color with a brown black abdomen. The colonies are found nesting in tree cavities and storage pots are not connected by pillars. The stingless bee *M. lendliana* is locally called *Vusutsi*; black in color with a yellow stripe on legs, found nesting underground and the storage pots are not connected by pillars.

Table 3.1: Body profile, nesting behaviour and colony structure of various stingless bee species (local names in Luhya language)

Scientific names	Local names	Bee color	Nesting site	Storage pots
Stingless bees	Tsikhalasanga	-	-	-
<i>H. gribodoi</i>	Vuyuyi	Black pale	Tree, wall	Storage pots not connected by pillars
<i>M. bocandei</i>	Ikora	Black and brownish thorax	Tree	Storage pots not connected by pillars
<i>M. ferruginea</i> (reddish brown)	Inasasa	Black and reddish brown abdomen	Tree, wall, Underground	Storage pot connected by pillars
<i>M. ferruginea</i> (black)	Iwere	Black and brown black abdomen	Tree	Storage pot not connected by pillars
<i>M. lendliana</i>	Vusutsi	Black with a yellow stripe on legs	Underground	Storage pot not connected by pillars

3.3.2 Species richness within the two sites

A total of 1030 nests of five meliponine species were located from both Ivihiga and Isiekuti sites. The highest number of nests was recorded at Isiekuti (56.5%) compared to Ivihiga (43.5%) and each of the five stingless bee species was recorded in each site as indicated by the species accumulative curve in figure 3.1.

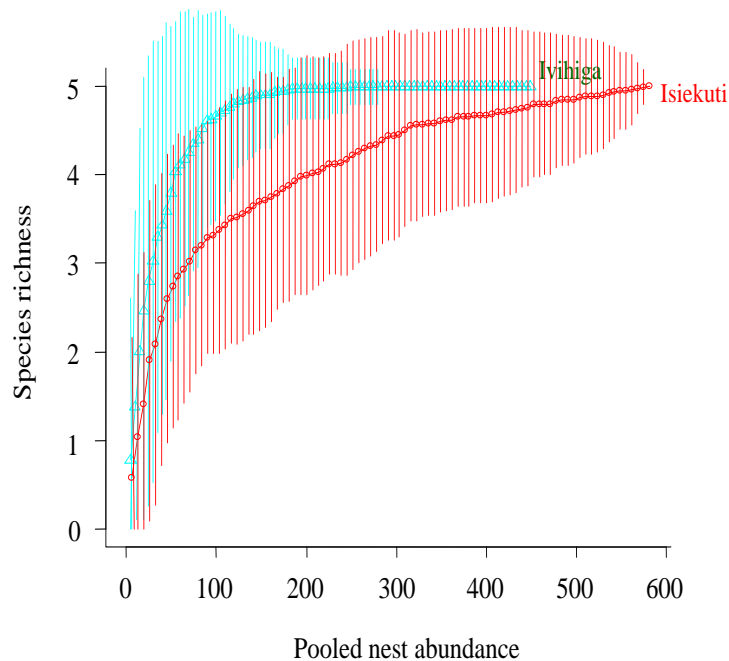


Figure 3.1: Species accumulation curves comparing the total number of species richness and nest abundance between Ivihiga and Isiekuti sites

3.3.3 Species richness within the six habitats

Within the six habitats, the highest number of nests was recorded in homesteads at Isiekuti (54.2%) and at Ivihiga (39.0%), followed by the indigenous forest (4.5%), the mixed indigenous forest (1.5%), the grassland with indigenous tree species (0.8%) and the grassland with *Eucalyptus* sp trees (0.0%) (Figure 3.2). The species richness within the habitats varied from 0 to 5 as indicated by the species accumulative curve in figure 3.2. All the five stingless bee species were recorded nesting in the indigenous forest at Ivihiga while the lowest number of species (2) was recorded nesting in the homesteads at Isiekuti and Ivihiga sites, respectively. None of the five stingless bee species was found nesting in the grassland with *Eucalyptus* sp trees.

A moderate number of 3 species was recorded nesting in the grassland with the indigenous tree species at Isiekuti. The mixed indigenous forest at Isiekuti accounted for 4 species nesting in this habitat.

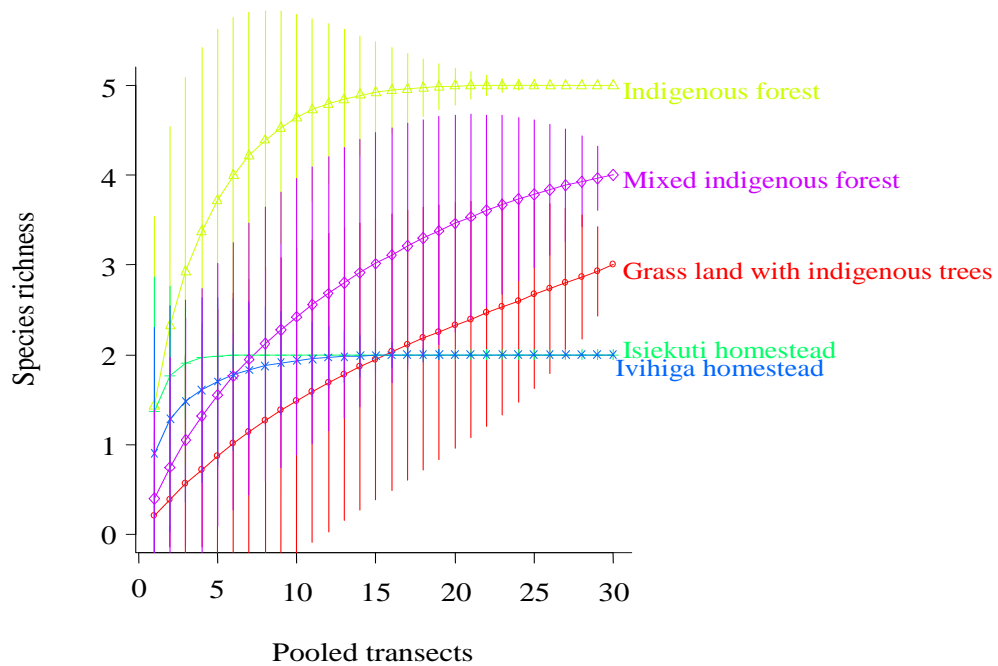


Figure 3.2: Species accumulation curves comparing total species richness and total transect between habitats at Ivihiga and Isiekuti sites

3.3.4 Evenness in species composition within the two sites

The species composition of the recorded stingless bee species was unevenly distributed in both Isiekuti and Ivihiga sites as indicated by their Rényi evenness profile that declines from left to right (Figure 3.3). Due to the bias in their profiles indicated by the crossings observed for their evenness profiles curves, no rank ordering in the evenness distribution of their species composition could be brought out by the Rényi evenness profile between both sites.

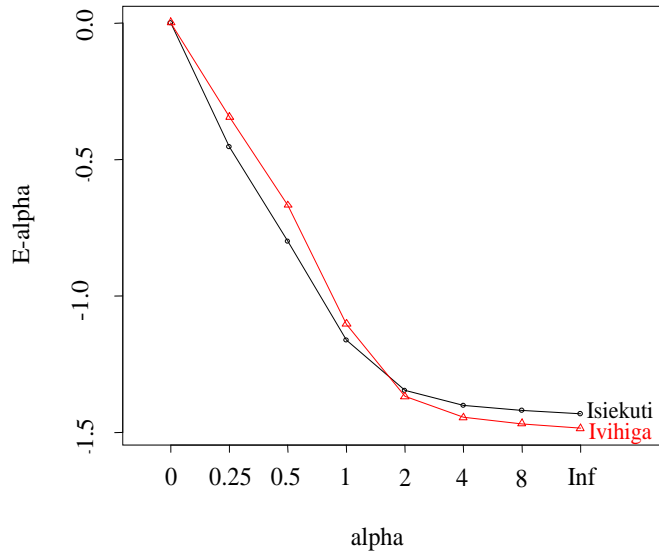


Figure 3.3: Rényi evenness profiles indicating evenness distribution in species composition within Ivihiga and Isiekuti sites

However, the values of the calculated evenness index in figure 3.4 of both sites indicated that species compositions were most evenly distributed at Ivihiga compared to Isiekuti site.

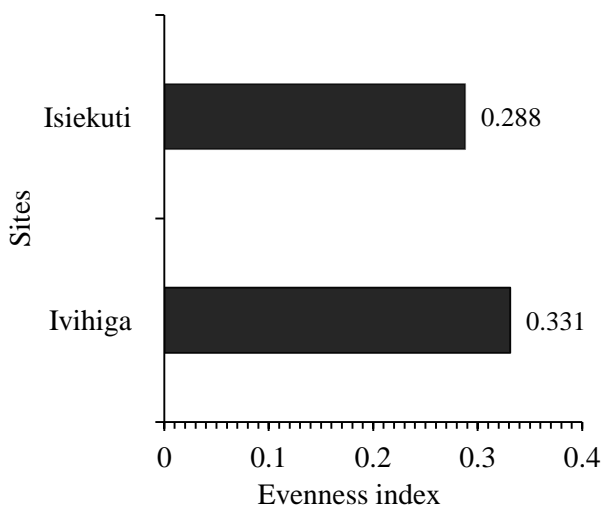


Figure 3.4: Evenness index (J) in stingless bee species composition between Isiekuti and Ivihiga sites

3.3.5 Evenness in species composition within the five habitats

The species composition was unevenly distributed in each of the five habitats as indicated by their Rényi evenness profile that declines from left to right (Figure 3.5). Due to the bias in their profiles indicated by the numerous crossings observed between their evenness profiles curves; no rank ordering in the evenness distribution of their species composition could be brought out between the five habitats.

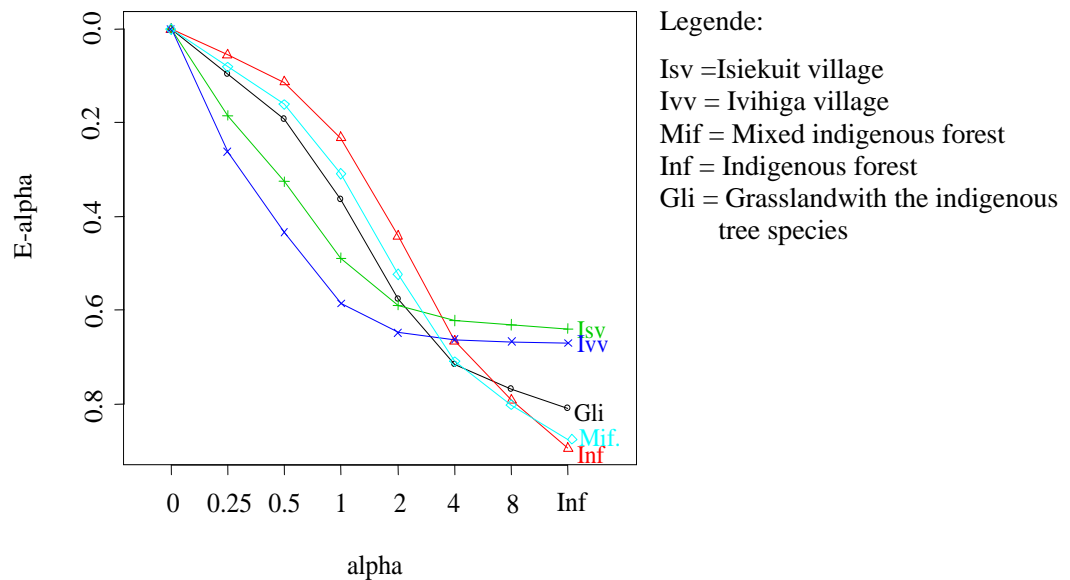


Figure 3.5: Rényi evenness profiles indicating evenness distribution in species composition within the five habitats

The calculated evenness index (J) shown in figure 3.6 for each habitat indicated that species composition was evenly distributed in the indigenous forest and less evenly distributed in the homesteads of both sites. The ordering of the habitats from the most evenly distributed to the less evenly distributed in their species composition was as follows: indigenous forest (0.79), mixed indigenous forest

(0.73), grassland with the indigenous tree species (0.69), homesteads at Isiekuti (0.61) and homesteads at Ivihiga 0.56).

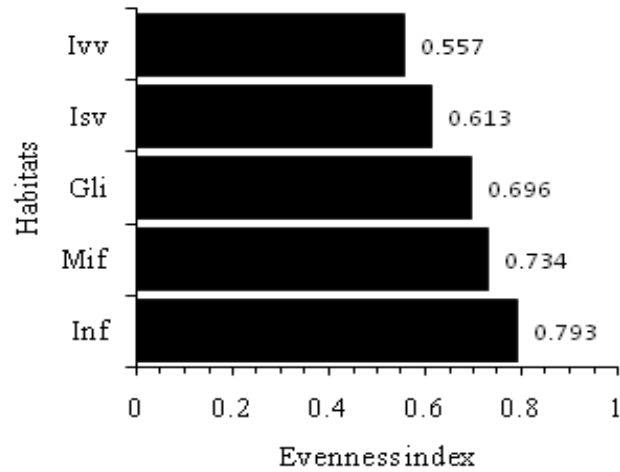


Figure 3.6: Evenness index (J) in the bee species composition within five habitats

3.3.6 Diversity within the two sites and five habitats

The Rényi diversity profile within both sites indicated that species diversity was highest at Ivihiga than Isiekuti (Figure 3.7).

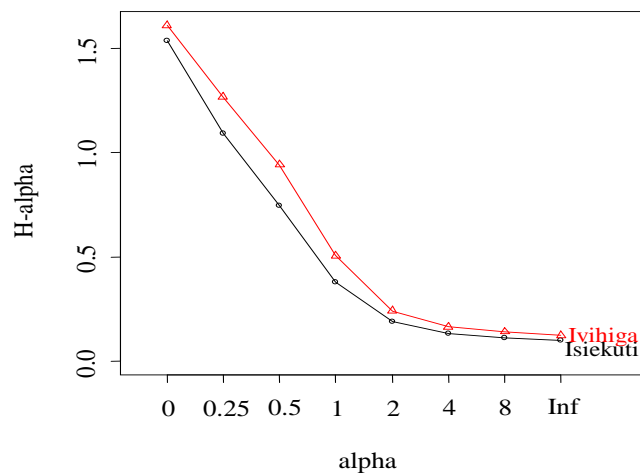


Figure 3.7: Rényi diversity profiles indicating differences in diversity between Ivihiga and Isiekuti sites

The Rényi diversity profile in figure 3.8 indicates that the indigenous forest was the most diverse in the stingless bee species, while the homesteads at Ivihiga site were the least diverse. The rank ordering of the habitats from the highest diverse to the lowest diverse was as follows: indigenous forest, mixed indigenous forest, grassland with the indigenous tree species, homesteads at Isiekuti and homesteads at Ivihiga.

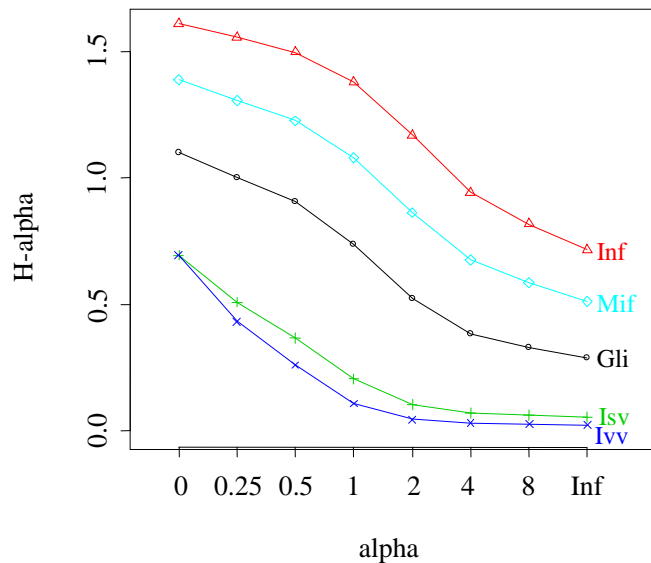


Figure 3.8: Rényi diversity profiles indicating differences in diversity within the five habitats

3.3.7 Species composition and rank abundance of the nest overall sites

The species composition in regard to the nest abundance of each stingless bee species and the rank ordering of their nest abundance in the entire survey area is indicated in table 3.2. Nests of *H. gribodoi* were dominant in the entire area; accounting for 90% of the total nests recorded overall species. The least abundant species in the entire area was *M. lendliana*; which accounted for 0.9% of total nest

recorded overall species. The rank ordering of the nest abundance recorded within the five stingless bee species was as follows: *H. gribodoi* (90%), *M. ferruginea*-reddish brown (5%), *M. ferruginea*-black (2.3%), *M. bocandei* (1.8%) and *M. lendliana* (0.9%).

Table 3.2: Species composition and the rank abundance of nests of the five stingless bee species

Stingless bees species	Abundance	Proportion (%)	Accumulated Frequency	Rank
<i>H. gribodoi</i>	927	90	90.0	1
<i>M. ferruginea</i> (reddish brown)	52	5	95.0	2
<i>M. ferruginea</i> (black)	24	2.3	97.4	3
<i>M. bocandei</i>	18	1.8	99.1	4
<i>M. lendliana</i>	9	0.9	100	5

3.3.8 Species composition and the rank abundance of nest within sites

The species composition of the five stingless bee species varied within sites as indicated in tables 3.3 and 3.4, respectively. Nests of *H. gribodoi* were dominant at Isiekuti and Ivihiga sites accounting for 91.2% and 88.4% of the total nests recorded overall species in each site respectively. *M. lendliana* (1.3%) and *M. ferruginea*-black (0.2%) were the least abundant species nesting at Ivihiga and Isiekuti sites, respectively.

The rank ordering of the nest abundance recorded of the five stingless bee species in each site was as follows: *H. gribodoi* (88.4%), *M. ferruginea*-black (5.1%), *M. ferruginea*-reddish brown (3.1%), *M. bocandei* (2.0%), *M. lendliana* (1.3%) and

H. gribodoi (91.2%), *M. ferruginea*-reddish brown (6.5%), *M. bocandei* (1.5%), *M. lendliana* (0.5%), *M. ferruginea*-black (0.2%) at Ivihiga and Isiekuti respectively.

Table 3.3: Species composition and the rank abundance of nests of the five stingless bee species at Ivihiga site

Stingless bee species	Abundance	Proportion (%)	Accumulated Frequency	Rank
<i>H. gribodoi</i>	397	88.4	88.4	1
<i>M. ferruginea</i> (black)	23	5.1	93.5	2
<i>M. ferruginea</i> (reddish brown)	14	3.1	96.7	3
<i>M. bocandei</i>	9	2.0	98.7	4
<i>M. lendliana</i>	6	1.3	100	5

Table 3.4: Species composition and the rank abundance of nests of the five stingless bee species at Isiekuti site

Stingless bee species	Abundance	Proportion (%)	Accumulated Frequency	Rank
<i>H. gribodoi</i>	530	91.2	91.2	1
<i>M. ferruginea</i> (reddish brown)	38	6.5	97.8	2
<i>M. bocandei</i>	9	1.5	99.3	3
<i>M. lendliana</i>	3	0.5	99.8	4
<i>M. ferruginea</i> (black)	1	0.2	100	5

3.3.9 Species fauna and nest abundance within habitat

Nests of all the five studied stingless bee species were recorded in the indigenous forest but *M. ferruginea* (black) was the only species whose nest was not recorded in the mixed indigenous forest. The nests of *M. ferruginea* (black), *M. ferruginea*

(reddish brown) and *M. lendliana* were observed in the grassland with the indigenous tree species; while *H. gribodoi* and *M. ferruginea* (reddish brown) were the only species whose nests were present in the homesteads at Isiekuti and Ivihiga sites respectively. No nests of any of the five studied species were recorded in the grassland with *Eucalyptus* sp trees.

The total number of the stingless bee species nests recorded varied from one habitat to another; ranging from 0 to 558 nests. Nests were relatively more abundant in the homesteads at Isiekuti (54.2%) and Ivihiga (39.0%) sites; and less abundant in the mixed indigenous forest (1.5%) as well as in the grassland with the indigenous tree species (0.8%).

The density in terms of the mean number of nests overall the stingless bee species recorded per 500 metres line transect within habitats was significantly different (F value = 26.21; df = 5, 174; P < 0.001) (Table 3.5). The mean number of nests was higher in homesteads and was not significantly different between the homesteads at Isiekuti (18.6 ± 3.93) and the Ivihiga (13.4 ± 2.85) sites; but was significantly different compared to the three other habitats. The lowest mean number of nests was recorded in the grasslands with the indigenous tree species (0.3 ± 0.11) and in the mixed indigenous forest (0.5 ± 0.17). No significant difference was observed in the mean nest density between these two habitats. The indigenous forest (1.6 ± 0.397) was a divergent habitat as its mean nest number was significantly different compared to the other four habitats.

Table 3.5: Overall stingless bee species fauna, nest abundance per 30 ha area and mean (\pm SD) number of nests per 500 m line transect within six habitats

Habitat	Species	Nests/30 ha	Mean* (\pm SE)
Inf	<i>M. ferruginea</i> (reddish brown), <i>H. gribodoi</i> , <i>M. ferruginea</i> (black), <i>M. bocandei</i> , <i>M. lendliana</i>	47	1.6 \pm 0.4 ^c
Ivv	<i>M. ferruginea</i> (reddish brown), <i>H. gribodoi</i>	402	13.4 \pm 2.9 ^a
Isv	<i>M. ferruginea</i> (reddish brown), <i>H. gribodoi</i>	558	18.6 \pm 3.9 ^a
Mif	<i>M. ferruginea</i> (reddish brown), <i>H. gribodoi</i> , <i>M. lendliana</i> <i>M. bocandei</i>	15	0.5 \pm 0.2 ^b
Gli	<i>M. ferruginea</i> (reddish brown), <i>M. ferruginea</i> (black), <i>M. lendliana</i>	8	0.3 \pm 0.1 ^b
Gle ¹	-	-	-

¹This habitat was not included in the analysis due to the fact that no nest of the five stingless bee species was recorded in it.*High significant difference between habitats at P <0.001 and values followed by the same letters within rows are not significantly different at P<0.001.

3.3.10 Species composition and dominant species within the habitats

The relative nest abundance and the dominant species varied from one habitat to another (Figure 3.9). The relative nest abundance between species nesting in the same habitat indicated that: (i) *H. gribodoi* nests were dominant in the homestead at Isiekuti (51.4%) and in the Ivihiga (38.2%) sites. (ii) Nests of *M. ferruginea* (reddish brown) were dominant in the grassland with the indigenous tree species (0.6%). (iii) Nests of *M. ferruginea* (black) were dominant in the indigenous forest (2.2%) and (iv) the *M. bocandei* nests were dominant in the mixed indigenous forest (0.9%). The species *M. lendliana* was divergent as its relative nest abundance was not dominant in any of the five habitats.

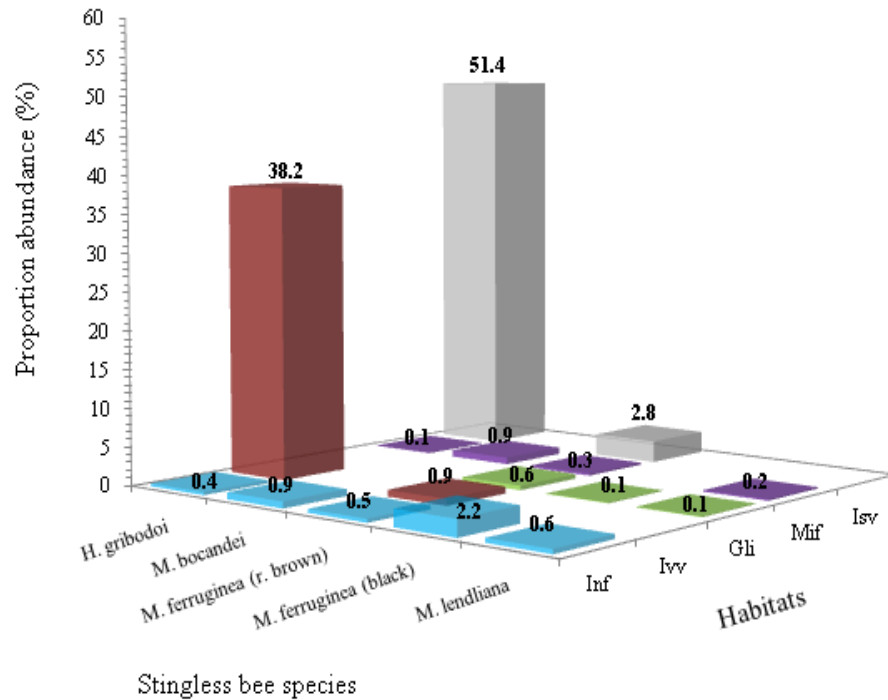


Figure 3.9: Relative nest abundance of *M. lendliana*, *H. gribodoi*, *M. ferruginea* (black), *M. bocandei* and *M. ferruginea* (reddish brown) across habitats

3.3.11 Predicted probabilities for nest presence of each stingless bee species within habitats

The predicted probability estimated as the chance of a stingless bee species to be presently nesting in a specific habitat is summarized in table 3.6. A highly significant difference ($\chi^2 = 21.3$; $df = 4, 145$; $P < 0.001$) was observed within habitats where *M. ferruginea* (reddish brown) was recorded nesting. The chance of getting a nest of *M. ferruginea* (reddish brown) was highest (0.53 ± 0.09) and highly significantly different in the homesteads at Isiekuti compared to the other four habitats. No significant differences were observed in the chance of getting a nest of *M. ferruginea* (reddish brown) within the four other habitats. The homesteads at Isiekuti appeared to be a preferential habitat for the nesting of *M.*

ferruginea (reddish brown) ($0,2 \leq P_{ferruginea \text{ (reddish brown)}} < 0,9$), while the Mixed indigenous forest appeared to be an accidental habitat ($P_{ferruginea \text{ (reddish brown)}} < 0,1$) for the nesting of this bee species. The three other habitats appeared to be an accessory habitat ($0,1 \leq P_{ferruginea \text{ (reddish brown)}} < 0,2$) for the nesting of *M. ferruginea* (reddish brown).

The chance of getting a nest of *M. bocandei* showed no significant difference ($\chi^2 = 0.34$; $df = 1, 58$; $P > 0.001$) within the two habitats where this species was recorded nesting. The indigenous and the mixed indigenous forests appeared to be the preferential habitats for *M. bocandei* ($0,2 \leq P_{bocandei} < 0,9$).

For the *M. ferruginea* (black) species, a highly significant difference ($\chi^2 = 28.18$; $df = 1, 58$; $P < 0.001$) was observed between the two habitats where it was recorded nesting. There was a highest chance of getting *M. ferruginea* (black) nests in the indigenous forest (0.63 ± 0.088) at Ivihiga compared to the grassland with the indigenous trees (0.03 ± 0.033) at the Isiekuti site. The indigenous forest appears to be a preferential habitat for *M. ferruginea* (black) ($0,2 \leq P_{ferruginea \text{ (black)}} < 0,9$), while the grassland with the indigenous tree species appeared to be an accidental habitat ($P_{ferruginea \text{ (black)}} < 0,1$) for this bee species.

A significant difference ($\chi^2 = 5.03$; $df = 2, 87$; $P < 0.001$) was observed between the three habitats where *M. lendliana* was recorded nesting. The chance of getting a nest of *M. lendliana* was highest in the indigenous forest (0.20 ± 0.073) and there

was a significant difference from the mixed indigenous forest (0.07 ± 0.045) and the grassland with the indigenous tree species (0.03 ± 0.032) at Isiekuti. The indigenous forest appeared to be a preferential habitat for *M. lendliana* ($0,2 \leq P_{lendliana} < 0,9$), while the mixed indigenous forest and the grassland with the indigenous tree species appeared to be an accidental habitat ($P_{lendliana} < 0,1$) for this bee species.

A highly significant difference ($\chi^2 = 67.63$; $df = 3, 116$; $P < 0.001$) was observed between the chance of getting *H. gribodoi* nest between the four habitats where this species was recorded nesting. Homesteads at Isiekuti (0.83 ± 0.068) and Ivihiga (0.70 ± 0.834) sites had the highest chance of getting a nest of this species. No significant difference was observed between the homesteads of both sites compared to the other two habitats where this species was recorded nesting. No significant difference was observed between the different forest habitats. Homesteads at both sites appeared to be a preferential habitat for *H. gribodoi* ($0,2 \leq P_{gribodoi} < 0,9$), while the different forest habitats appeared to be an accessory ($0,1 \leq P_{gribodoi} < 0,2$) and accidental habitats ($P_{gribodoi} < 0,1$) for this species.

Table 3.6: Predicted probability of a presence of a nest for each stingless bee species within habitats ($P_A(Z)$)

Bee species (A)	Habitats (Z)	$P_A(Z)^*$	Pattern of $P_A(Z)$	Habitat category
<i>M. ferruginea</i> (r. brown)	Indigenous forest	0.17 ± 0.068^{abc}	$0,1 \leq P_A(Z) < 0,2$	Accessory
	Ivihiga village	0.20 ± 0.073^{ade}	$0,2 \leq P_A(Z) < 0,9$	Preferential
	Grass land with indigenous trees	0.13 ± 0.062^{bdf}	$0,1 \leq P_A(Z) < 0,2$	Accessory
	Mixed indigenous forest	0.07 ± 0.046^{cef}	$P_A(Z) < 0,1$	Accidental
	Isiekuti village	0.53 ± 0.09^g	$0,2 \leq P_A(Z) < 0,9$	Preferential
<i>H. gribodoi</i>	Isiekuti village	0.83 ± 0.068^a	$0,2 \leq P_A(Z) < 0,9$	Preferential
	Ivihiga village	0.70 ± 0.834^a	$0,2 \leq P_A(Z) < 0,9$	Preferential
	Indigenous forest	0.13 ± 0.062^b	$0,1 \leq P_A(Z) < 0,2$	Accessory
	Mixed indigenous forest	0.03 ± 0.032^b	$P_A(Z) < 0,1$	Accidental
<i>M. lendliana</i>	Indigenous forest	0.20 ± 0.073^a	$0,2 \leq P_A(Z) < 0,9$	Preferential
	Mixed indigenous forest	0.07 ± 0.045^b	$P_A(Z) < 0,1$	Accidental
	Grass land with indigenous trees	0.03 ± 0.032^b	$P_A(Z) < 0,1$	Accidental
<i>M. bocandei</i>	Indigenous forest	0.30 ± 0.083^a	$0,2 \leq P_A(Z) < 0,9$	Preferential
	Mixed indigenous forest	0.23 ± 0.077^a	$0,2 \leq P_A(Z) < 0,9$	Preferential
<i>M. ferruginea</i> (black)	Indigenous forest	0.63 ± 0.088^a	$0,2 \leq P_A(Z) < 0,9$	Preferential
	Grassland with indigenous trees	0.03 ± 0.033^b	$P_A(Z) < 0,1$	Accidental

*Values followed by the same letter within nesting habitats of a specific bee species are not significantly different at $P < 0.001$.

3.3.12 Degree of similarity in the stingless bee species fauna within habitats

The results revealed that the species fauna of stingless bees recorded between homesteads in the neighbourhood of the indigenous forest at the Ivihiga site and the mixed indigenous forest at the Isiekuti site was exclusively similar (Table 3.7).

The species fauna between the indigenous forest at the Ivihiga site and the mixed indigenous forest at Isiekuti site; were almost similar (Coefficient of Sørensen 0.9; Coefficient of Jaccard 0.8). The *M. ferruginea* (black) species was the taxon that differentiated the stingless bee fauna of the indigenous forest to the mixed indigenous forest.

The grassland with the indigenous tree species versus the homesteads of both sites were the less similar habitats sharing the same species fauna of the nesting stingless bee (Coefficient of Sørensen 0.4; Coefficient of Jaccard 0.2). *M. ferruginea* (black), *H. gribodoi* and *M. lendliana* species were the taxons that differentiated the stingless bee fauna of the grassland with the indigenous tree species and the homesteads of both sites.

Table 3.7: Similarity coefficient comparing similarity in the stingless bee species fauna within pairwise habitats

Paired site	Coefficient of Sørensen (C _s)	Coefficient of Jaccard (C _j)
Isv x Ivv	1.0	1.0
Inf x Mif	0.9	0.8
Inf x Gli	0.8	0.6
Mif x Ivv	0.7	0.5
Mif x Isv	0.7	0.5
Inf x Isv	0.6	0.4
Inf x Ivv	0.6	0.4
Mif x Gli	0.6	0.4
Gli x Isv	0.4	0.2
Gli x Ivv	0.4	0.2

3.3.13 Degree of similarity in stingless bee species composition within habitats

The habitats which appear to be similar in species composition in their recorded stingless bee species are illustrated by the Unweighed Pair-Wise Group Average (UPWGA) cluster in figure 3.10. The cluster analysis at 0.40 dissimilarity level resulted in having three groupings of memberships within the habitats. The grassland with the indigenous tree species was not clustered to any other habitat at 0.40 dissimilarity level. A similarity in species composition was observed between the homesteads at Isiekuti and the Ivihiga sites where they were joined at a Bray Curtis ecological distance of 0.077. The indigenous forest and the mixed indigenous forest were also grouped as similar habitats in their species composition at a Bray Curtis ecological distance of 0.33.

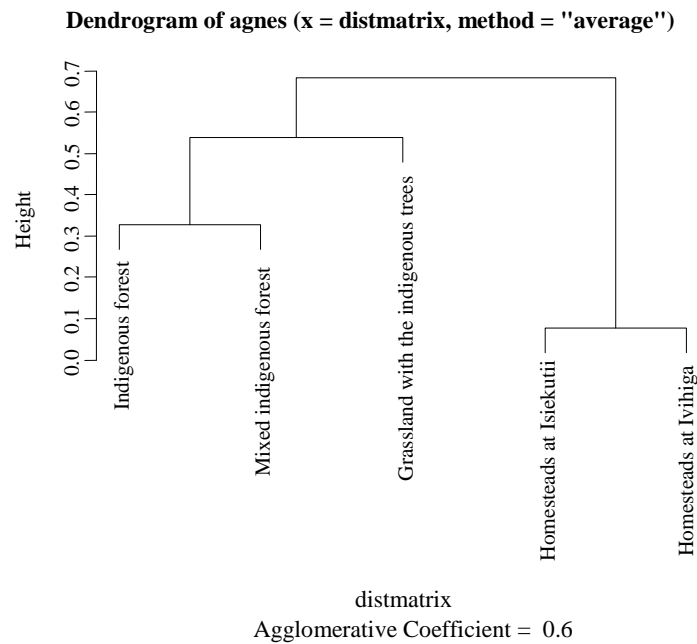


Figure 3.10: Dendrogram of UPWGA clustering habitats which are similar in species composition

3.3.14 Similarity in habitat preferences within stingless bee species

The stingless bee species which appear to have similar habitat preferences are illustrated by Unweighed Pair-Wise Group Average (UPWGA) cluster in figure 3.11. The cluster analysis at 0.38 dissimilarity level resulted in having three groupings of memberships within the five stingless bee species. A similarity in habitat preferences was observed between *M. bocandei* and *M. lendliana* species; where they were joined at a Bray Curtis ecological distance of 0.27. The stingless bee *M. ferruginea* (reddish brown) and *H. gribodoi* species were also similar in habitat preferences for nesting at a Bray Curtis ecological distance of 0.37. The *M. ferruginea* (black) species was the divergent species which was not clustered to any other species at below 0.38 dissimilarity level.

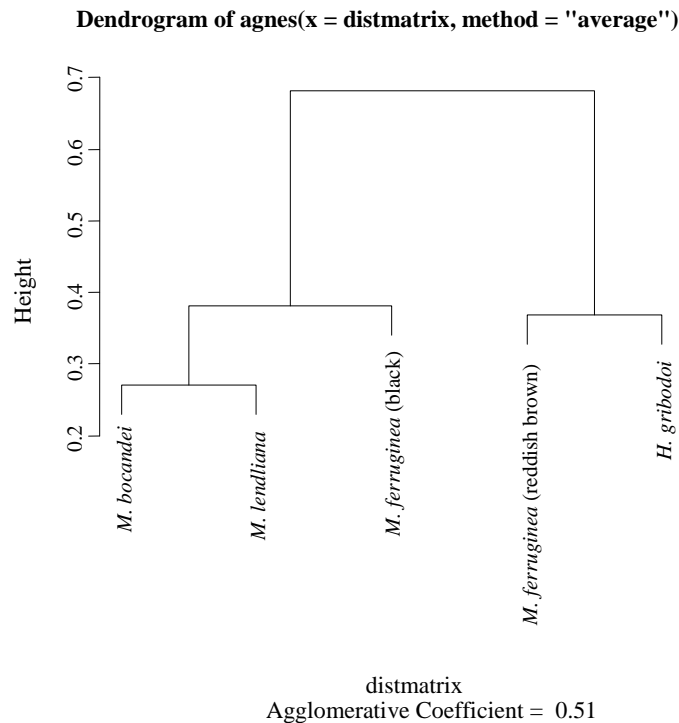


Figure 3.11: Dendrogram of UPWGA clustering species which are similar in habitats preferences

3.4 Discussion

The lowest number of species richness, evenness and diversity in the habitat was recorded in the homesteads of both sites. No nests of the five studied species were observed in the grassland with *Eucalyptus* sp trees. This may indicate that, forest regeneration by introducing *Eucalyptus* sp trees species might negatively affect the community of the studied stingless bee species. Parallel studies carried out in the Atlantic Rain Forest, in Brazil; reported that stingless bee species richness was abundant in the structured forest, followed by the depleted forest; while the rejuvenated forest contained the smallest numbers of species (Batista *et al.*, 2003). Boontop *et al.* (2008) also reported that a variation in species richness and diversity was recorded among four types of forest habitats at Kanchanaburi Province, in Thailand. In this current study, the indication of the highest number of five species more evenly distributed and more diverse in the indigenous forest confirm previous studies that reported that meliponine bees are strongly associated with natural native forest habitats for nesting and food (Roubik, 1989; Ricketts, 2004; Brosi *et al.*, 2007; Brosi *et al.*, 2008). According to Roubik (1989), this association with forest is not surprising given that most of meliponine bees are tree-cavity nesters and therefore rely on tropical forests for nesting habitats. The results obtained from the current studies are also in agreement with Bommarco *et al.* (2010) who observed that natural native habitat loss poses a major threat to biodiversity as it leads to clear shifts in the species richness and the composition of wild bee communities.

Knowledge of the nest abundance of different species across a gradient of successive different habitats gives indication on how a population of the species is affected due to changes in habitat. The results obtained from the current study revealed that the nest abundance of each specific stingless bee species varied within the two sites. The mean number of nests of the stingless bee species recorded per 500 metres line transect was heterogenous within habitats; and both homesteads had the highest mean number of nests. This indicated that homesteads in both sites might have more available nesting places for one or more species. According to Ricketts (2004), Brosi *et al.* (2007, 2008), meliponine species will even nest in human-dominated habitats neighbouring their natural forest habitats that have experienced high degrees of disturbance. This attribute might contribute to an increase in nest biomass of one or more species by offering them more available nesting sites in the area. Winfree *et al.* (2007) reported that anthropogenic land use may be compatible with the conservation of many, but not all bee species. This is relevant in the present study with *H. gribodoi* and *M. ferruginea* (reddish brown) being the only species that nested in the homesteads of both sites; and whose predicted probability of getting their nests was higher in the homesteads than in the other habitats.

The results also revealed that the nesting habitat and the nest abundance varied within the five species. Similar results were reported by Nates-Parra *et al.* (2008) who observed that habitats have an important impact on the natural composition of the stingless bee community structure, a fact which is reflected in the nest

abundance. According to Nates-Parra *et al.* (2008), the nest density and abundance per landscape was heterogeneous within three landscape environments (secondary forest, agro-ecosystems and urban areas) in the Colombian llanos piedmont. Similar results were also reported by Samejima *et al.* (2004), who found that some species were abundant in the primary forests, whereas other species were abundant in the disturbed forests. It was also revealed in this present study that, *M. ferruginea* (reddish brown) and *H. gribodoi* nested in more habitats than the other species. The presence of nesting colonies of *M. ferruginea* (reddish brown) and *H. gribodoi* in more habitats indicates that these bee species might be cosmopolitan compared to the other four species and might easily adapt to nest in any type of disturbed habitat.

The chance of getting a nesting colony of each specific stingless bee species in a 500 metres line transect also varied within the nesting habitats. This variation indicates that each species had a habitat and nesting site preference and a difference in the availability of nesting site might occur within the studied habitats. According to Velthuis (1997) and Pyper (2001), each species of stingless bees has a preference for specific nesting sites and substratum. Limitation in the availability of nesting sites and substratum in a specific habitat has been reported to be one of those factors which affect nest biomass of Meliponine bees (Hubbell and Johnson, 1977). Human disturbances such as, forest destruction, habitat modification through village implantation and forest regeneration through the introduction of exotic tree species in tropical areas are reported among those factors that destroy stingless bees habitats by limiting the availability of their

nesting sites and food which cause disappearance or reduction of key species and in some cases extremely low abundances (Samejima *et al.*, 2004; Boontop *et al.*, 2008).

At the Isiekuti site, only one nest of *M. ferruginea* (black) species was recorded and the nest was found in the grassland with the indigenous tree species in a tree cavity of *Spathodea campanulate*. In the indigenous forest, the nests of *M. ferruginea* (black) were mostly recorded in tree cavities of *Diospyras abyssinica*. This tree was not found in the mixed indigenous forest and the grassland with the indigenous tree species at the Isiekuti site during the survey. On the other hand, *Spathodea campanulate* was not observed in the mixed indigenous and the indigenous forest; but was frequently recorded in the villages. Nevertheless, not a single nest of the other studied stingless bee species was found in this tree species. According to Boontop *et al.* (2008), management practices such as deforestation and forest regeneration through introduction of exotic trees species have been shown to be among one of the factors destroying bees habitats and these cause disappearance or reduction of the key species in the tropical areas. With regard to similarity in habitats preferences for nesting, the stingless bee *M. ferruginea* (black) was the most divergent species at 0.38 dissimilarity level of their Bray Curtis ecological distance. This result indicates that the indigenous forest is the mostly preferred nesting habitat for *M. ferruginea* (black) in the Kakamega forest. Qualitative and quantitative changes in diversity and nest abundance of the five stingless bee species in the Kakamega forest occur when transiting from a natural

forest habitat to an anthropogenic habitat, as the natural native forest had a diverse component of bees. There are taxon-specific responses to habitat change and, in this study; there is clear value to conserve native indigenous forest, due to the ecological and economical importance of meliponine bees. Anthropogenic management such as establishment of villages neighbouring the Kakamega forest facilitated the nesting of *M. ferruginea* (reddish brown) and *H. gribodoi*. This anthropogenic management contributes positively to increase nest biomass of these two bee species by providing them more available nesting sites in the area. It was possible that some nests escaped detection or that nests at canopy-level were undersampled. However, the results which were obtained collaborate with other studies that have found contrasting responses from different meliponine bee groups to anthropogenic land use habitat. Conserving meliponine bees, which are important for pollination of crops will require conservation of the native indigenous forest (Brosi *et al.*, 2008) as well as their domestication in artificial cavities.

CHAPTER FOUR: SPATIAL DISTRIBUTION PATTERN AND DISTANCE BETWEEN NESTS OF STINGLESS BEE SPECIES IN KAKAMEGA FOREST

4.1 Introduction

Visualization and analysis of occupancy pattern of individuals or groups of organisms in the wild are basic to ecological research (Byers, 1992); and provide vital information for interpreting spatial distribution of populations of organisms (Kuno, 1991). New technologies like geographical information system (GIS) and geoprocessing tools have been developed; and are mostly used in the production of maps or spatial analysis (Pfeiffer and Morris, 1994; Mbahin, 2008). Spatial analysis involves the analysis of data representing geographical features which have a location attribute such as absolute location (coordinates) or relative positioning (distance). Some common method of spatial analysis include: the description of the pattern of occupancy of organisms in a specific area; the distribution of organisms across elevation gradients; the nearest neighbour distance from a reference point to an organism or between conspecific or heterospecific organisms and detection of the degree of clumping in a particular environment (Taylor, 1984; Rossbacher, 1986; Byers, 1992; Slaa, 200a; Baddeley, 2008).

The dispersion pattern of stingless bee species nests in Neotropical habitats has been fairly well documented (Michener, 1979; Camargo and Posey, 1990; Roubik, 1990, 1992). On the African continent, there is paucity of geographical

information such as pattern dispersion of stingless bee nests within habitats, records of elevation gradients where nests occur and nearest neighbour distance between nests of conspecific and interspecific stingless bee species with regard to the habitats. In the Kakamega forest, information on the nests occupancy pattern of stingless bees within habitat, records of elevation gradients where nests occur and average of distances within nesting colonies of stingless bee species are unknown.

This study was aimed to provide an insight of the elevation gradients where stingless bee nests were recorded, their dispersion pattern across habitats and assess the degree of nest clustering in species with clumped nest pattern. Furthermore, the average nearest neighbour distance between nests entrances of intra and inter specific stingless bee species across habitats was investigated.

4.2 Materials and methods

4.2.1 Sampling procedures

In each habitat, a 30 hectare area was surveyed in which thirty line transects of 500 metres long and 20 metres apart on a base line were followed and geographical position (longitude, latitude, altitude) of nesting colonies for each stingless bees species found were recorded. The geographical position of the nest was recorded using a Global Positioning System (GPS) hand set receiver (model GARMIN eTrex Legend[®] HCx) and was taken at the location of the nest entrance (Plate 4.1). For intra and inter species whose records indicated a similar location (geographical position) in nesting; their identity was recorded and the distance

separating their nest entrance was additionally recorded using either a vernier caliper or tape measure (Plate 4.2). Each recorded nest was coded for easy recognition in the output of ArcGIS software. Sampling on the species was carried out from June 2009 to February 2010.



Plate 4.1: Recording the geographical position of a nesting colony of *M. lendliana* in the indigenous forest at Ileho



Plate 4.2: Two different species of stingless bee nesting on the same location in a house wall in homesteads at Isiekuti site

4.2.2 Data analysis

4.2.2.1 Frequency of nests across the recorded altitudinal gradient

The frequency in which nests of each of the five stingless bee species were located across the recorded elevation gradient overall habitats was compiled in a table by range/class of 100 meters according to the method by Kajobe (2007).

4.2.2.2 Pattern dispersion

The pattern dispersion of nests in a specific habitat was described for the stingless bee species whose nests were recorded for at least four nests according to the method by Slaa (2006a). Spatial statistics tools in ArcGis version 9.3 were used to calculate the nearest neighbour index (R) and the associated Z score and p-value to describe the spatial distribution of species occurrence.

The nearest neighbour statistics compare the field data distribution to a random distribution (expected nearest neighbour distance) with the same number of samples. The nearest neighbour index (R) is expressed as the ratio of the observed average nearest neighbour distance (R_o) divided by the expected nearest neighbour distance (R_e). The Z scores are measures of standard deviation away from the mean and the p-value is the probability that you have falsely rejected the null hypothesis. Z score and p-value are measures of statistical significance, which tell you whether or not to reject the null hypothesis. Euclidean distance (the straight-line distance between two points) was used in the calculation of distances and projected data (WGS 1984 UTM Zone 37S.prj) to get accurate measurements.

Rejection the null hypothesis is based on a subjective judgment taking into account the degree of risk the experimenter is willing to accept for being wrong. This degree of risk is often given in terms of critical values and/or confidence levels. For nearest neighbour index, the null hypothesis states that features are randomly distributed ($R = 1$). If the nearest neighbour index is less than 1, it implies the dispersal pattern is clustering and if this index is greater than 1, the trend is toward uniform.

4.2.2.3 Degree of clustering

The General G tool in ArcGis version 9.3 was used to calculate the high/low value of the General G statistic (observed and expected), associated Z score and p-values for a given stingless bee species nesting in a clumped pattern. A high index value indicates that high values are clustered for the specific species within a studied habitat. A low index value indicates that low values tend to cluster. The Z score value (measure of statistical significance) is calculated to help determine if the index value is significant, so whether or not to reject the null hypothesis, "there is no apparent spatial clustering of the values".

When the Z score is large (or small) enough such that it falls outside of the desired significance, the null hypothesis is rejected; then sign of the Z score becomes important. If the value is positive, it means that high values are clustered together. If the value is negative, it means that low values are clustered together. A Z score near zero indicates no apparent clustering within the study area.

4.2.2.4 Average distance between nest entrances

Nest spacing between conspecific and interspecific stingless bee species was calculated using the nearest neighbour distances. The nearest neighbour distance (NN) was assessed for species that accounted for at least 3 nests in a specific habitat according to Hubbell and Johnson (1977) and Slaa (2006a). The Hawth's analysis tools in ArcGis version 9.3 were used to output a table that listed the distance within nests of each intra and inter stingless bee species that is closest to another in a specific habitat. The average nearest neighbour distance within nests of intra and inter stingless bee species in a habitat was calculated in Excel by averaging the distances of all the observed nearest neighbour (R_o) nest locations. The average nearest neighbour distance within nests of intraspecies across different habitats and within nests of interspecies in the same habitat was analysed by ANOVA, with unequal variance and unbalanced model. Differences in mean nearest neighbour distance were compared using Welch Turkey test. The analysis was performed in R software version 2.14.0.

4.3 Results

4.3.1 Altitudinal distribution of nests overall habitats

Nesting colonies of all the five stingless bee species were observed within the two altitude ranges of $1500\text{m} \leq 1600\text{m}$ and $1600\text{m} \leq 1700\text{m}$ in the Kakamega forest. However, a higher number of 998 nests of all the five stingless bee species were found at the elevation gradient ranging from 1600 to 1700 meters (Table 4.1).

Table 4.1: Abundance of nesting colonies of five stingless bee species across two classes of elevation gradients recorded overall habitats at Ivihiga and Isiekuti sites in the Kakamega forest

Stingless bee	Total nest	Altitude range (m)	
		1500 ≤ 1600	1600 ≤ 1700
<i>H. gribodoi</i>	927	12	915
<i>M. ferruginea</i> ¹	24	1	23
<i>M. ferruginea</i> ²	52	11	41
<i>M. bocandei</i>	18	6	12
<i>M. lendliana</i>	9	2	7
Total nest	1030	32	998
Total species	5	5	5

¹Morpho species black, ² Morpho species reddish brown

4.3.2 Pattern dispersion of nests

In the indigenous forest and homesteads in both sites, all the recorded stingless bee species had the number of nests required to determine their pattern dispersion. On the other hand, in the mixed indigenous forest and the grassland with indigenous tree species, *M. bocandei* and *M. ferruginea* (reddish brown), respectively were the only species that had sufficient nest numbers to determine their spatial patterns. Nesting colonies of *M. bocandei* were uniformly dispersed within the two types of forest habitats; as well as the nesting colonies of *M. ferruginea* (reddish brown) in the indigenous forest and the grassland with indigenous tree species (Table 4.2).

Meliponula bocandei species had nests more uniformly spaced in the indigenous forest (1.7) compared to those in the mixed indigenous forest (1.4); while *M. ferruginea* (reddish brown) nests were more uniformly spaced in the indigenous forest (2.2) compared to the ones in the grassland with indigenous tree species (2.1).

As regards to all species nesting in the indigenous forest, an uniform dispersion patterns were observed for *M. lendliana*, *M. ferruginea* (reddish brown), *M. bocandei* and *M. ferruginea* (black); whereas *H. gribodoi* nests were randomly dispersed. The rank ordering of species with uniform dispersed nest patterns in the indigenous forest from the most uniformly nest distribution to the less uniformly distributed according to their nearest neighbour index was as follows: *M. lendliana* (2.6), *M. ferruginea*-reddish brown (2.2), *M. bocandei* (1.7) and *M. ferruginea*-black (1.3) (Table 4.2).

However, changes from an under-dispersed and over-dispersed nest patterns to a clumped nest pattern were observed for *M. ferruginea* (reddish brown) and *H. gribodoi*, respectively when colonies are nesting in homesteads. In the homesteads of both sites, a clustered nesting pattern was observed between *M. ferruginea* (reddish brown) and *H. gribodoi*. The species *H. gribodoi* had the lowest nearest neighbour index thus indicating that in this species, nests spacing were more clumped compared to those of *M. ferruginea* (reddish brown) (Table 4.2).

Table 4.2: Pattern dispersion of nests among intraspecific stingless bees in a habitat

Habitat	Stingless bee	R	Z score*	P-value	Pattern
Mif	<i>M. bocandei</i> (9)	1.4	2.07	0.038	Dispersed
Gli	<i>M. ferruginea</i> ² (6)	2.1	4.96	0.000001	Dispersed
Inf	<i>M. ferruginea</i> ² (5)	2.2	5.16	0.0000	Dispersed
	<i>M. ferruginea</i> ¹ (23)	1.3	2.81	0.0050	Dispersed
	<i>M. bocandei</i> (9)	1.7	3.91	0.0001	Dispersed
	<i>H. gribodoi</i> (4)	1.0	0.13	0.89	Random
	<i>M. lendliana</i> (6)	2.6	7.44	0.0000	Dispersed
Isv	<i>M. ferruginea</i> ² (29)	0.6	-3.77	0.0002	Clustered
	<i>H. gribodoi</i> (529)	0.3	-32.25	0.0000	Clustered
Ivv	<i>M. ferruginea</i> ² (9)	0.8	-3.89	0.0001	Clustered
	<i>H. gribodoi</i> (393)	0.4	-33.61	0.000	Clustered

*Confidence level at 95%; ¹Morpho species black; ² Morpho species reddish brown. Mif= Mixed indigenous forest, Gli = Grassland with the indigenous trees, Inf = Indigenous forest, Isv = Isiekuti village and Ivv = Ivihiga village. Values in the parentheses indicates the total number of nests recorded for the stingless bee species.

4.3.3 Degree of clustering within species with clumped nests pattern

The results indicated that within both species nesting in the homesteads, a low degree of clustering was observed in the clustered nest pattern of *M. ferruginea* (reddish brown), while a high degree of clustering was observed in the clustered nest pattern of *H. gribodoi* (Table 4.3). This indicates that nests of *H. gribodoi* were found more aggregated and colonies nested more closely in the homesteads compared to the nesting colonies of *M. ferruginea* (reddish brown). Within the homesteads of both sites, the degree of nest clustering of *M. ferruginea* (reddish brown) and *H. gribodoi* was respectively low at the Ivihiga site compared to the Isiekuti site.

Table 4.3: Degree of clustering between clumped nests pattern of *M. ferruginea* (reddish brown) and *H. gribodoi* in homesteads at both sites

Homestead	Stingless bee	General G index	Z score*	P-value	Degree of clustering
Isiekuti	<i>M. ferruginea</i>	0.3	-2.9	0.0037	Low
	<i>H. gribodoi</i>	1.0	15.95	0.0000	High
Ivihiga	<i>M. ferruginea</i>	0.2	-1.8	0.0021	Low
	<i>H. gribodoi</i>	0.7	12.47	0.0000	High

* Confidence level at 95%.

4.3.4 Average distance between intraspecific species

The average distance separating nests of intraspecific stingless bee species differed highly within their nesting habitat and between interspecific species nesting in the same habitat (Table 4.4). The average distance within *M. bocandei* nests was significantly different within the indigenous and the mixed indigenous forests ($F = 7.63$; $N = 120$; $df = 1$; $p < 0.05$). Nesting colonies of *M. bocandei* although uniformly dispersed were closer to one another in the mixed indigenous forest (58 ± 7.06 meters) compared to the indigenous forest (132 ± 25.8 meters).

The average distance of nests within nesting habitats was also significantly different for *M. ferruginea* (reddish brown) ($F = 4.15$; $N = 5$; $df = 4$; $p < 0.05$) and *H. gribodoi* ($F = 5.23$; $N = 3$; $df = 2$; $p < 0.05$). The average distance of *M. ferruginea* (reddish brown) nests was significantly different and lowest in the homesteads at Isiekuti and in the mixed indigenous forest compared to those of the indigenous forest. The average distance between *H. gribodoi* nests was

significantly lower at Isiekuti homesteads compared to the homesteads at Ivihiga and the indigenous forest.

Within interspecies nesting in the same habitat, it was observed in the indigenous forest that there was a significant difference in the average distances of nest in the conspecific species ($F = 5.52$; $N = 5$; $df = 4$; $p < 0.05$). The average distance between *M. ferruginea* (black) nests was not significantly different from those observed for *H. gribodoi* and *M. bocandei*; whereas, a significant difference and less distance were found between *M. ferruginea* (black) nests compared to *M. ferruginea* (reddish brown) and *M. lendliana* nests. The average distance between nests was similar within *M. ferruginea* (reddish brown), *M. lendliana*, *H. gribodoi* and *M. bocandei* species.

Significant differences in the average distances were also recorded within nests of species nesting in the homesteads at Isiekuti ($F = 10.08$; $N = 2$; $df = 1$; $p < 0.05$) and in homesteads at Ivihiga ($F = 13.23$; $N = 2$; $df = 1$; $p < 0.05$). The average distance between *M. ferruginea* (reddish brown) nests was higher than that of *H. gribodoi* in both homesteads. In the mixed indigenous forest, significant differences in the average distances were recorded within nests of species ($F = 7.12$; $N = 2$; $df = 1$; $p < 0.05$). The average distance between nests was higher in the *M. bocandei* species compared to *M. ferruginea* (reddish brown).

Table 4.4: Average nearest neighbour distance (NN) between intraspecific nests of each stingless bee species in the habitats

Bee species	Average Nearest Neighbor Distance (NN± SE) (m)				
	Inf*	Ivv*	Isv*	Mif*	Gli
<i>M. ferruginea</i> ^{2*}	175.8±55.3 ^{aA} (5)	68.5±6.7 ^{bA} (9)	40.7±11.9 ^{cA} (29)	15.9±14.1 ^{cA} (3)	120.3±42.6 ^{ab} (6)
<i>H. gribodoi</i> *	138.4±59.3 ^{aA} _B (4)	40.1±3.7 ^{bB} (393)	2.7±0.2 ^{cB} (529)	-	-
<i>M. bocandei</i> *	132±25.8 ^{aAB} (9)	-	-	57.9±7.06 ^{bB} (9)	-
<i>M. lendliana</i>	169±19.4 ^A (6)	-	-	-	-
<i>M. ferruginea</i> ¹	74.6±7.3 ^B (23)	-	-	-	-

¹Morpho species black; ² Morpho species reddish brown; Values in parentheses indicate the number of all nearest neighbour's nest locations for the species in the particular habitat; *High significant difference at $P < 0.05$ for the same species within its nesting habitats and within species nesting in the same habitat. Values in a row with a same minuscule letters are not significantly different at $P < 0.05$; while values in a column with the same capital letters are not significantly different at $P < 0.05$.

4.3.5 Average distance between interspecific species

Within interspecies nesting in the indigenous forest, a significant difference in their average distance was observed within pairwise group ($F = 2.72$; $N = 10$; $df = 9$; $p < 0.05$). The nest spacing of all species paired to *M. ferruginea* (black) was significantly different compared to the distance between nests of *M. ferruginea* (black) versus *H. gribodoi*. The colonies of *H. gribodoi* and *M. ferruginea* (black) discovered in the indigenous forest nested further from one another than *M. ferruginea* (black) paired to the other three species (Table 4.5). In regard to both species that nested in homesteads, the average distance within interspecific nest of *M. ferruginea* (reddish brown) and *H. gribodoi* was significantly different between homesteads of both sites ($F = 26.31$; $N = 3$; $df = 2$; $p < 0.05$).

In the homesteads at Isiekuti the interspecies nests of *M. ferruginea* (reddish brown) and *H. gribodoi* were closer to each other (66.9 ± 1.9 meters) compared to the average nearest neighbour distance of their interspecies nests in the homesteads at Ivihiga (88.7 ± 2.3 meters) (Table 4.5). The average distance of interspecific nests of *M. bocandei* vs *M. ferruginea* (reddish brown) was not significantly different within the indigenous and the mixed indigenous forests where nests of both species were recorded ($F = 0.01$; $N = 2$; $df = 1$; $p > 0.05$).

Table 4.5: Average nearest neighbour distance (NN) between nests of paired stingless bee species in the different nesting habitats

Paired species	Average nearest neighbour distance (NN \pm SE) (m)			
	Indigenous Forest	Isiekuti homestead	Ivihiga homestead	Mixed indigenous forest
A vs B	102.4 \pm 27.6(9)	66.9 \pm 1.9(558)	88.7 \pm 2.3 (402)	-
A vs C	120.4 \pm 17.4(14)	-	-	123.6 \pm 26.7(12)
A vs D	123.4 \pm 20.1(11)	-	-	-
A vs E	110.5 \pm 9.9(28)	-	-	-
E vs C	86.8 \pm 6.9(32)	-	-	-
E vs D	105.3 \pm 9.6(29)	-	-	-
E vs B	185.3 \pm 23.6(27)	-	-	-
C vs D	117.1 \pm 12.7(15)	-	-	-
C vs B	165.3 \pm 32.9(13)	-	-	-
D vs B	141.4 \pm 22.4(10)	-	-	-

Values in parentheses indicate the number of all nearest neighbour's nest locations for the paired species in the particular habitat. A = *M. ferruginea* (reddish brown), B = *H. gribodoi*, C = *M. bocandei*, D = *M. lendliana* and E = *M. ferruginea* (black)

4.4 Discussion

Based on altitudinal distribution and pattern dispersion, 96.9% of the nests of the five stingless bee species were found at an altitude ranging from 1600 m to 1700 m. According to Kajobe (2007), the elevation gradient of Bwindi forest at Uganda ranges from 1160m to 2607m; and about 68.4% of nest overall six stingless bee species (all our five studied species included) were found at an altitude ranging from 1600 m to 2600 m. However, Kajobe (2007) reported not having found *M. ferruginea* (reddish brown) and *H. gribodoi* nests above 1900 m altitude compared to the *M. bocandei* and *M. ferruginea* (black) nests.

The nearest neighbour index revealed that nest pattern was uniform for almost all the species that accounted for at least 4 nests in a habitat. An exception was the nest pattern dispersion which was observed for *H. gribodoi*. In the indigenous forest, *H. gribodoi* nests were randomly dispersed; and clumped in both the homesteads for *M. ferruginea* (reddish brown) and *H. gribodoi*. The nest patterns of *M. ferruginea* (reddish brown) and *H. gribodoi* changes from a uniform and random pattern, respectively, to a clumped nest pattern when nesting in the homesteads. According to Jongjitvimol *et al.* (2005), the pattern of nest dispersion in stingless bees is species specific (that specific pattern must give a benefit to the species), and differences in nest dispersion may result from diversity in ecosystems. Slaa (2006a) reported that nest dispersion in stingless bees is also related to features linked to their competition for food or mechanism of territorial behaviour. The observed changes in the nest pattern of *M. ferruginea* (reddish

brown) and *H. gribodoi* might be influenced by differences in field distribution of the available nesting sites of the species, with the homesteads offering more nesting sites compared to the other habitats. Additionally, there might be a low competition in food foraging between the two species and low conspecific and interspecific aggressive interaction mechanisms when they nest closer to one another. These might explain why nests of these two bee species were clumped in the homesteads and mostly associated in intraspecific or interspecific nest aggregation on a single substrate (wall).

In the homesteads, *H. gribodoi* nested up to 9.0 ± 0.71 colonies aggregated (41 nests max.) on a single wall façade; whereas, *M. ferruginea* (reddish brown) nested up to 1.8 ± 0.15 colonies aggregated (3 nests max.) on a single wall façade. Moreover, the occurrence of conspecific and interspecific aggregated nests of both species was very high along the surveyed transects in the homesteads. Similar results were reported by Slaa (2006a) within three non territorial and non aggressive foraging Neotropical stingless bee species *Nannotrigona testaceicornis*, *Tetragonisca angustula* and *Scaptotrigona pectoralis*. According to Slaa (2006a), whereas nests of *S. pectoralis* were randomly dispersed; nests of *Nannotrigona testaceicornis* and *Tetragonisca angustula* were occurring aggregated and conspecific nests were located only 18 m from each other. Slaa (2006a) also reported that all the four colonies of *N. testaceicornis* were located in a single tree; while those of *T. angustula* were found significantly aggregated. With regard to all species nesting in the indigenous forest, nest patterns of almost

all the species except for *H. gribodoi* (randomly dispersed) were uniformly dispersed. This might suggest that there is competition for food or mechanisms of aggressive interactions (territoriality) probably exist within the four species. Similar results were reported by Kajobe (2007) who observed that nests of all the studied *Meliponula* species were scattered all over the Bwindi forest; and suggested that there was little or no interspecific and intraspecific nesting cooperation within the species.

Nest clustering was stronger in *H. gribodoi* compared to that of *M. ferruginea* (reddish brown) species. This was evident because the number of nests of *H. gribodoi* on a single wall façade was far higher than that of *M. ferruginea* (reddish brown). Additionally, the aggregated *H. gribodoi* nests occurred most frequently on a single substrate on the surveyed transect compared to that of the *M. ferruginea* (reddish brown) species. According to Roubik (1989) the amount of inbreeding that can be tolerated by a bee species might influence the dispersal of daughter colonies. This probably explains why the occurrence of the aggregated colonies of *H. gribodoi* on a single substrate was more abundant compared to *M. ferruginea* (reddish brown).

The average distance within conspecific and interspecific species whose nest pattern was uniformly dispersed and those whose nest was clumped differed highly within their nesting habitat and between interspecies nesting in the same habitat. *H. gribodoi* nested very close to one another compared to *M. ferruginea*

(reddish brown). Additionally, the average distance within intraspecific and interspecific nests of *H. gribodoi* and *M. ferruginea* (reddish brown) were lowest in the homesteads at the Isiekuti site compared to the Ivihiga site. This might be due to differences in the density of houses and the occurrences of preferable nesting substratum (walls) on houses in both sites. The number of houses recorded in a transect of 500 meters was higher at the Isiekuti site compared to the Ivihiga site; and this might have influenced the difference observed in the average distance of intraspecific and interspecific nests of both species between both sites. Moreover, the type of substratum (walls) on houses that was preferred by *H. gribodoi* (muddy wall not smoothed) and *M. ferruginea*-reddish brown (muddy wall smoothed with a mixture of cow dung and mud) for nesting occurred more frequently in the homesteads at Isiekuti than at Ivihiga site.

Colonies of *M. bocandei* and *M. ferruginea* (reddish brown) nested closer to another in the mixed indigenous forest compared to other nesting habitats where their nest pattern was uniformly dispersed. This might indicate that a low competition in food foraging existed between conspecific colonies nesting in the mixed indigenous forest. The average distance between interspecific nest of *M. bocandei* vs *M. ferruginea* (reddish brown) was not significantly different within the indigenous and the mixed indigenous forest. There was a similarity in the availability and the dispersion of nesting sites for both species in the two different forest habitats. In the indigenous forest, distance within conspecific and interspecific nests was not significantly different for almost all the single and pair species. This indicates that there was almost no cooperation in the nesting sites

within the species. Similar observation was reported by Kajobe (2007) who found that nests of species from the genus *Meliponula* at the Bwindi forest in Uganda were scattered; and suggested that there was a little or no interspecific and intraspecific nesting cooperation within the *Meliponula* species.

CHAPTER FIVE: NESTING BEHAVIOUR OF FIVE STINGLESS BEE SPECIES IN KAKAMEGA FOREST, WESTERN KENYA

5.1 Introduction

Eusocial stingless bees (Hymenoptera, Apidae, Meliponini) often build nests to shelter their populous colonies (Batista *et al.*, 2003). Stingless bees nesting sites and substrata are reported to differ from one species to another (Wille and Michener, 1973; Hubell and Johnson, 1977; Sakagami, 1982; Roubik, 1989; Pyper, 2001). Stingless bees nest structure has species-specific characteristics (Michener, 1974; Sakagami, 1982). There is a great variety of forms and sizes of the nest entrance and cavity (Henigman, 1975) and height of the nest (Sakagami and Zucchi, 1967; Kazuhiro, 2004). Moreover, studies have reported that in their native forest habitats, meliponine bees have preferences to some tree species to shelter their nests (Ricketts, 2004; Brosi *et al.*, 2007; Brosi *et al.*, 2008).

In Kenya, the stingless bee species *Meliponula bocandei* (Spinola, 1853), *Meliponula ferruginea*-reddish brown (Lepelletier, 1841), *Meliponula ferruginea*-black (Smith, 1854), *Meliponula lendliana* (Friese, 1900) and *Hypotrigona gribodoi* (Magretti, 1884) have been described as highly eusocial bee species living in perennial colonies in the Kakamega forest (Raina *et al.*, 2006). However, data on their nesting behaviour which is basic to any biological and ecological study is still scarce. Generally, species specific data on stingless bee species of Kakamega forest such as their nesting site, nesting substrata, host plant range of the specific stingless bee species and the tendency to nest on agglomeration on a

single substrate is largely unknown. Furthermore, data on the form and size of the nest entrances and height of the nests in regard to the nesting sites is not available for each stingless bee species. This information is important because the attributes of the nests are useful in taxonomic studies especially in equatorial tropical Africa where little has been studied (Kajobe and Roubik, 2006). It does also contribute to the ecological information about the species, thus is a critical guidance in environmental management for conservation of the stingless bee species and in studies on hiving the bee species.

This chapter aimed to investigate the nesting sites and nesting substrata of five stingless bee species in the Kakamega forest. The tree species and types of human residential houses selected for nesting by each stingless bee species were also investigated. Furthermore, the type of nests aggregation within species, height and depth of the nest on nesting sites plus the shapes and sizes of the nest entrances for each stingless bee species were also investigated

5.2 Materials and methods

5.2.1 Nest searching

Nests of the five stingless bee species were searched in the indigenous forest, mixed indigenous forest, grassland with the indigenous tree species and homestead at Isiekuti and Ivihiga study sites. Nests were located in each habitat in three ways: by standardized nest survey along thirty line transects (500 metres long and 20 metres apart on a base line), reporting of nest presence by the local

communities sensitized about the ongoing research studies and by chance during field trips by inspecting places likely to have nests (Eltz *et al.*, 2001). Sampling was carried out from May 2009 to January 2011.

5.2.2 Nesting sites, substratum and host plant range

The places where a nest of a stingless bee species was found (tree, underground, human house) and the substrata which sheltered the nest were recorded according to the method described by Eltz *et al.* (2003) and Danaraddi *et al.* (2009). On trees, the recorded nesting substratum (trunk, branch) and its characteristic (live or dry) was either live trunk, dry trunk, live branch and dry branch. The nesting tree was identified by its local name by the local honey hunters residing around the forest. The tree was also identified upto species level by the Kenya Forest Service (KFS) field assistants at the Isiecheno Forest Station. Samples (leaves, fruits, flowers and photographs) which could not be identified by the KFS field assistants were taken to the University of Nairobi herbarium for identification. Nests found in human houses, the nesting substratum was recorded according to the type of construction material used to build the wall (cement or mud). In the case of muddy wall houses, the nesting substratum was recorded according to the wall pattern (smoothened/ not smoothened) and the material used to smoothen the wall: (1) smoothened wall using mud or using mixture of mud and cow dung and (2) wall not smoothened and not smoked or wall not smoothened but smoked. The underground nests were also recorded in regard to factor originally responsible for the underground hollow where the nest was sheltered (termite mound, abandoned

rat nests, hollows in the ground made by roots, hollows between rocks, unknown). Rényi diversity profiles was performed as described in section 3.2.3.3 of chapter three to compare the diversity of nesting sites and of nesting substratum within stingless bee species. The number of nests and nest site richness was analyzed to determine if a relationship existed between the two parameters. The number of nesting sites and nesting substrata richness was also analyzed to determine if a relationship existed between the two parameters. Furthermore, the number of nests and nesting tree species recorded among the stingless bee species were also analyzed to determine if a relationship existed between the two parameters. The proportion of nests of each stingless bee species that was recorded across the different nesting sites, nesting substrata and nesting tree species were summarized.

The evenness distribution of nests recorded for each stingless bee species within their identified nesting tree species was compared using Rényi evenness profiles as described in section 3.2.3.2. The degree of similarity in nesting tree species within tree nester stingless bee species was calculated and compared based on their Sørensen's similarity coefficient (C_s) and Jaccard similarity coefficient (C_j) between paired bee species as described in section 3.2.3.7.

5.2.3 Nest agglomeration

Stingless bee species and their tendency to form a type of nest aggregation on a single substrate (tree, wall facade, termite hill) was assessed using frequency table according to Eltz *et al.* (2003). The average number of nests of each stingless bee

species that was counted in a type of aggregation and the less minimum distance between nest entrances of conspecific (Plate 5.1) and interspecific (Plate 5.2) aggregated species was also recorded. A tape measure or Vernier caliper was used to measure the distance between nest entrances of the aggregated stingless bee species.

One-way analysis of variance (ANOVA) was used to analyze data on the average number of nests of each stingless bee species that was counted in a conspecific and interspecific nest aggregation; as well as, the average less minimum nearest neighbour distance separating nest entrance between each aggregated species. Bartlett Welch Tukey's test was used to compare differences in mean number of nests within stingless bee species in a conspecific and interspecific nest aggregation respectively and the mean less minimum distance separating nest entrances of each stingless bee species recorded in a conspecific and interspecific nest aggregation respectively. The analysis was performed using SAS software version 9.2.

The different stingless bee species that were recorded associated in interspecific nest aggregation and the average number of aggregated nests of each stingless bee species that was counted within the nesting site and substrata recorded on human houses were reported.

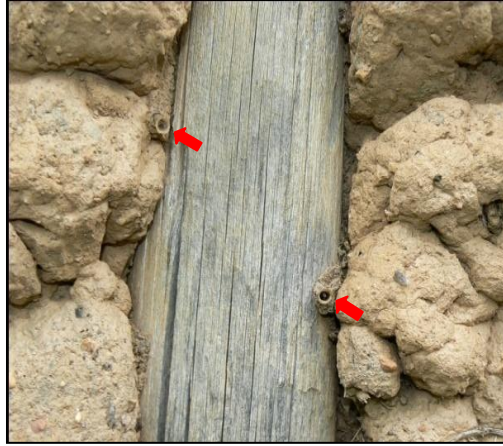


Plate 5.1: Conspecific nest aggregation of *H. gribodoi* in a house wall not smoothed



Plate 5.2: Interspecific nest aggregation in a house wall between *M. ferruginea*-reddish brown (left) and *H. gribodoi* (right).

5.2.4 Height of the nests

The height of the nests on nesting sites was recorded for each stingless bee species according to Eltz *et al.* (2003), Kajobe (2007) and Danaraddi *et al.* (2009).

The heights of the nest on trees and walls of houses were measured from the ground surface to the nest entrance tube and the depth of the underground nests

was measured from the ground surface to the upper nest part. A tape measure was used to measure the height or the depth of the nest.

The mean height or depth of the nest of each stingless bee species on their different nesting sites was reported. One-way analysis of variance (ANOVA) was used to analyze data on the mean height or depth of nests of each stingless bee species on the same nesting site. Bartlett Welch Tukey's test was used to compare differences in mean height or depth of the nests. The analysis was performed using SAS software version 9.2.

5.2.5 Shape and surface of the open entrance tube of the nest

The shape of the nest entrance at the open entrance tube was recorded and its surface was calculated. The shape of the open entrance tube was determined by calculating the ratio (R_2/R_1) from the measurement of the minor axis (R_1) and major axis (R_2). The open entrance tube with a ratio equal to 1 ($R_1 = R_2$) was attributed a circular shape and that with ratio greater than 1 ($R_1 \neq R_2$) attributed an oval shape.

A Vernier caliper was used to take measurements of the minor axis (R_1) and major axis (R_2) of the entrance tube. The cross sectional area (cm^2) of the open entrance tube was calculated by using the geometric formula for each shape recorded. The area of a circular open entrance tube was calculated by the geometric formula, $S = \pi.(R_1/2)^2$ and by $S = \pi.(R_1/2).(R_2/2)$ for an oval open

entrance tube shape (Couvillon *et al.*, 2008; Helmenstine, 2011). The value of π used in the calculation of the surface was equal to 3.14. The form of the nest entrance and the surface (area) of the nest entrance within form of nest entrance of each stingless bee species were reported.

One-way analysis of variance (ANOVA) was used to analyze data. Bartlett Welch Tukey's test was used to compare differences in mean surface of the nest entrance. The analysis was performed using SAS software version 9.2.

5.3 Results

5.3.1 Diversity of nesting sites and relationship between the number of nests and nest site richness

The nesting sites recorded were diverse within the five stingless bee species and the highest diversity was recorded in *M. ferruginea* (reddish brown) followed by *H. gribodoi* species. The *M. ferruginea* (reddish brown) species was recorded nesting in three sites; while *H. gribodoi* was recorded nesting in two sites. The *M. ferruginea* (black), *M. bocandei* and *M. lendliana* species were observed nesting only in a single nesting site (Figure 5.1).

The number of nesting sites recorded between the stingless bee species were not linearly related on the number of the identified nests ($R^2 = 0.083$; $N = 5$; $p = 0.64 > 0,05$) (Figure 5.2).

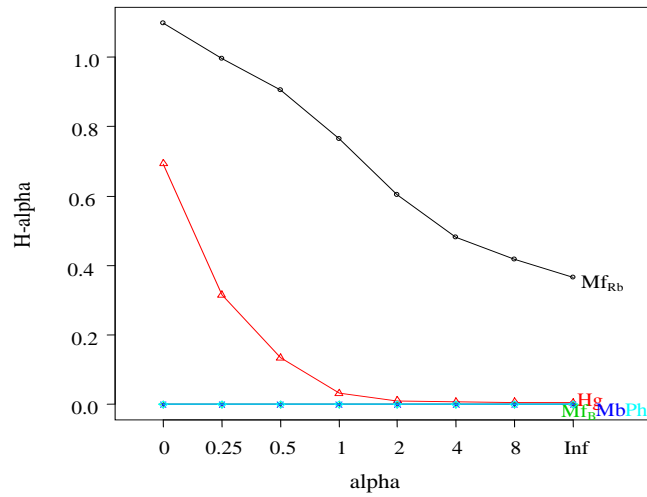


Figure 5.1: Rényi diversity profiles indicating differences in the diversity of nesting sites within the five stingless bee species. Hg = *H. gribodoi*, Mf_B = *M. ferruginea* (black), Mb = *M. bocandei*, Ph = *M. lendliana* and Mf_{Rb}=*M. ferruginea* (reddish brown)

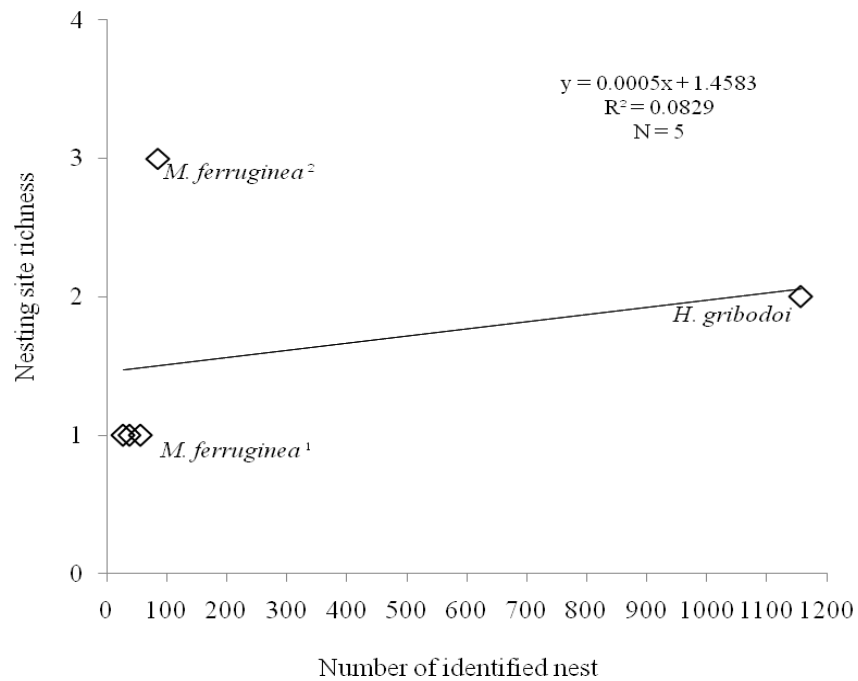


Figure 5.2: Relationship between the number of nests and the nesting sites richness of each stingless bee species

5.3.2 Nesting sites and nest abundance of each stingless bee species

A total of 1360 nests of the stingless bee species were observed and recorded. The recorded number of nesting colonies was higher in the houses (89%) compared to those on the trees (8.8%) and the underground (2.2%) (Table 5.1). Four stingless bee species were recorded nesting in trees cavities; while two species were recorded nesting in the houses and the underground respectively. *M. ferruginea* (reddish brown) was the only stingless bee species whose nests were found in all the three nesting sites. Nests of *H. gribodoi* were observed in the trees and wall cavities; while those of *M. lendliana* species were only observed in the underground hollows (Plate 5.3) and only in trees cavities for both species *M. ferruginea-black* (Plate 5.4) and *M. bocandei*. *M. ferruginea-reddish brown* (69.4% of nests) and *H. gribodoi* (99.5% of nest) seemed to prefer hollows in walls of houses for nesting compared to the hollows in the other nesting sites.



Plate 5.3: Nest of *M. lendliana* recorded in a hollow underground in the Kakamega forest



Plate 5.4: Nest of *M. ferruginea* (black) recorded in a tree cavity at the grassland with dispersed indigenous tree species vicinal to the mixed indigenous forest at Isiekuti site

Table 5.1: Nesting sites recorded for each stingless bee species, the proportion of nests recorded for each bee species per nesting sites and number of bee species per nesting site.

Bee species	(N)	Proportion of nest within nesting sites			Number of nesting sites
		Trees	Human house	Underground	
<i>M. ferruginea</i> ²	85.0	24.7	69.4	5.9	3
<i>H. gribodoi</i>	1157.0	0.5	99.5	-	2
<i>M. lendliana</i>	25.0	-	-	100	1
<i>M. bocandei</i>	37.0	100	-	-	1
<i>M. ferruginea</i> ¹	56.0	100	-	-	1
Total nests	100 (1360)	8.8 (120)	89 (1210)	2.2 (30)	-
Total species	5	4	2	2	-

¹Morpho species black, ² Morpho species Red brown; Values in the parentheses are the overall number of stingless bee species nests recorded

5.3.3 Diversity of nesting substratum and relationship between the numbers of nesting substratum and nest site richness

The recorded nesting substratum was diverse within the five stingless bee species and the highest diversity of the nesting substratum was recorded in *M. ferruginea* (reddish brown) species accounting up to 10 different substrata across the three nesting sites (Figure 5.3). The number of the nesting substrata was strongly linearly related to the number of the nesting sites in a stingless bee species ($R^2 = 0.94$; $N = 5$; $p = 0.03 > 0,01$) (Figure 5.4). Species that accounted for three and two nesting sites had more nesting substrata than the species which accounted for only one nesting site. However, within the three species that accounted for only one nesting site; it was observed that species which nested only in tree cavities accounted for more nesting substrata than the *M. lendliana* species which nested only in the underground hollow.

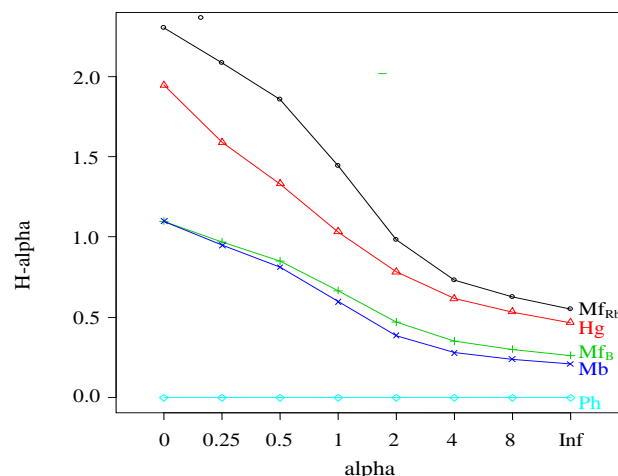


Figure 5.3: Rényi diversity profiles indicating differences in the diversity of nesting substrata within the five stingless bee species. Mf_{Rb} = *M. ferruginea* (reddish brown), Hg = *H. gribodoi*, Mf_B = *M. ferruginea* (black), Mb = *M. bocandei* and Ph = *M. lendliana*

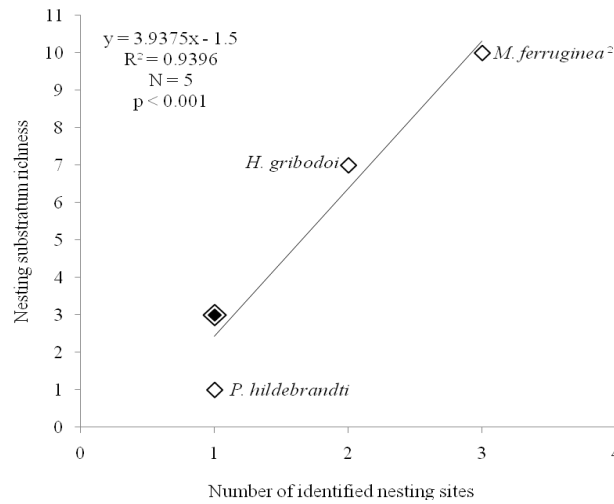


Figure 5.4: Relationship between the number of nesting sites and nesting substratum richness of each stingless bee species

5.3.4 Nesting substratum and nest abundance of each stingless bee species

A total of 13 different nesting substrata were recorded from the three nesting sites across the different stingless bee species (Table 5.2). It was observed that an underground hollow under plant roots was the only preferred nesting substratum for *M. lendliana*; while *M. ferruginea* (reddish brown) nested in the abandoned termite nests, rats nests or between rocks. For the four bee species that were recorded nesting in tree cavities, hollows in live tree parts were their most preferred nesting substratum compared to the hollows on dead (dry) trees.

Meliponula ferruginea (reddish brown) and *H. gribodoi*, which had nesting preferences in human houses showed differences in preferences of the nesting substrata on the house (Plate 5.5). *Meliponula ferruginea* (reddish brown) nesting

colonies were most abundantly recorded (57.6 %) in muddy walls smoothed using a mixture of mud and cow dung in the homesteads; while nests of *H. gribodoi* were most abundantly recorded (62.7 %) in the muddy unsmoothed walls (Plate 5.6).

Table 5.2: Nest abundance (%) recorded for each stingless bee species across the different nesting substratum

Nesting site	Substratum	Proportion of the nest within the nesting substratum				
		<i>Mf_{Rb}</i>	<i>Hg</i>	<i>Mf_B</i>	<i>Mb</i>	<i>Ph</i>
Tree	Hollow in live trunk	17.6	0	76.8	81.1	0
	Hollow in live branch	4.7	0.4	17.8	13.5	0
	Hollow in dry trunk	2.4	0	5.4	5.4	0
	Hollow in dry branch	0	0.1	0	0	0
Human house	Wall built with brick	2.4	1.0	0	0	0
	Wall built with mud and smoothed with mud+cow dung	57.6	9.9	0	0	0
	Wall built with mud and smoothed with mud	7.1	23.2	0	0	0
	Wall built with mud and not smoothed	2.4	62.7	0	0	0
	Roof frame made with pole from trees	0	2.7	0	0	0
Underground	Hollow between rocks	1.2	0	0	0	0
	Hollow in abandoned termite nest	3.5	0	0	0	0
	Hollow in abandoned rat nest	1.2	0	0	0	0
	Hollow under plant roots	0	0	0	0	100

Mf_{Rb} = *M. ferruginea* (reddish brown), *Hg* = *H. gribodoi*, *Mb* = *M. bocandei*, *Mf_B* = *M. ferruginea* (black) and *Ph* = *M. lendliana*



Plate 5.5: Four different wall substrata recorded in human house at homestead. a) Bricked cemented wall; b) Muddy wall smoothed with mud; c) Muddy wall not smoothed and d) Muddy wall smoothed with mixture of mud and cow dung

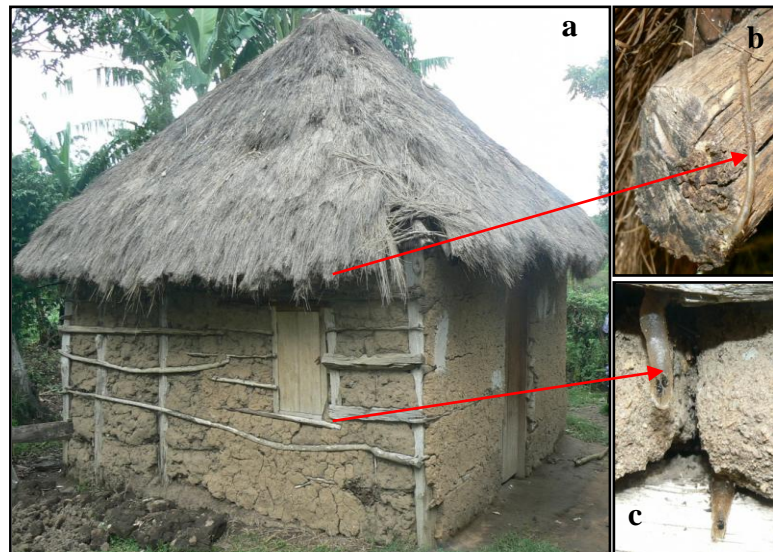


Plate 5.6: Nesting substrata of *H. gribodoi* on human houses in homesteads at the Kakamega forest. a) House with muddy wall not smoothed. b) A colony nesting on a roof frame (pods) of a house with muddy wall. c) Two different colonies of *H. gribodoi* nesting in the muddy wall not smoothed

5.3.5 Botanical taxonomy and Luhya names of each identified host plant

A total of twenty tree species belonging to fourteen different tree families were identified as host plants of the four stingless bee species (Table 5.3). *Meliponula lendliana* was the only bee species whose nests were not found on the trees.

Table 5.3: The host plants used by the four stingless bee species as nesting sites in the Kakamega forest

Host plants (Scientific name)	Local names (Luhya)	Family names
<i>Antiaris toxicaria</i>	Mulundu	Moraceae
<i>Zanthoxylum macrophylla</i>	Shikhuma	Rutaceae
<i>Croton megalocarpus</i>	Musine	Euphorbiaceae
<i>Diospyras abyssinica</i>	Lusui	Ebenaceae
<i>Celtis mildbraedii</i>	Shunza	Ulmaceae
<i>Markhamia lutea</i>	Lusiola	Bignoniaceae
<i>Spathodea campanulate</i>	Mdhulia	Bignoniaceae
<i>Olea capensis</i>	Mutukuyo	Oleaceae
<i>Albizia gummifera</i>	Mukhunzulu	Mimosoideae
<i>Harungana madagascariensis</i>	Musila	Clusiaceae
<i>Cardia africana</i>	Mukamari	Boraginaceae
<i>Sapium ellipticum</i>	Musasa	Euphorbiaceae
<i>Celtis africana</i>	Mweywe	Ulmaceae
<i>Trichilea emetica</i>	Munyama	Meliaceae
<i>Syzygium guineense</i>	Musioma	Myrtaceae
<i>Croton sylvaticus</i>	Musutsu muna muliru	Euphorbiaceae
<i>Ficus exasperate, Ficus lutea,</i>	Museno, Mukavakava,	Moraceae
<i>Ficus thonningii</i>	Mutoto	
<i>Aningeria altissima</i>	Mukangu	Sapotaceae
<i>Prunus africana</i>	Mwililita	Rosaceae
<i>Eucalyptus sp</i>	Ikambi	Myrtaceae

5.3.6 Relationship between the number of nest trees and their species richness

The number of nests for a stingless bee species found on trees was linearly related ($R^2 = 0.86$; $N = 4$; $p = 0.123 > 0,05$) to the nesting trees species richness among the stingless bee species (Figure 5.5). *Meliponula ferruginea* (black) followed by *M. bocandei* and *M. ferruginea* (reddish brown) bee species had a relatively high number of nesting tree species compared to the *H. gribodoi* species. *Meliponula ferruginea* (black) nested in up to 11 tree species for the fifty six nests recorded on the trees; *M. bocandei* nested in up to 10 tree species for the thirty seven nests recorded on the trees; while *M. ferruginea* (reddish brown) nested in up to 9 tree species for the twenty one nests recorded on the trees. The lowest number of nesting tree species was recorded in the *H. gribodoi* (3 species of trees) species.

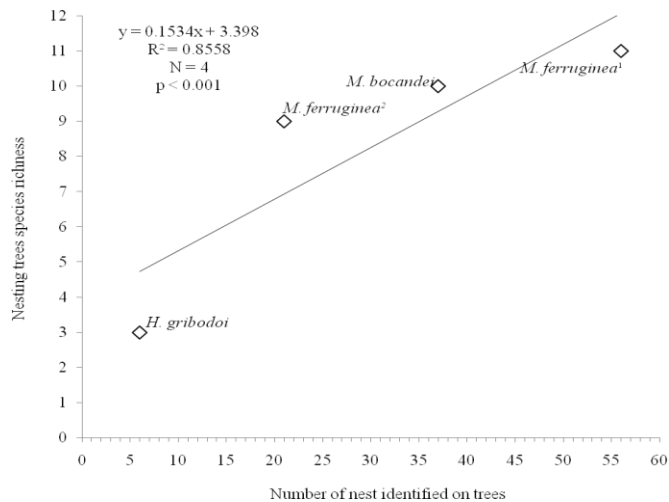


Figure 5.5: Relationship between the number of nests found on trees for each stingless bee species and their tree species richness

5.3.7 Nest abundance and evenness per tree species

A total of 20 different tree species were found used for nesting by the four stingless bee species (Table 5.4). Nine nesting tree species were recorded for *M. ferruginea* (reddish brown). The nests were abundantly recorded on *Diospyras abyssinica* (23.8%), *Antiaris toxicaria* (19.05%) and dry *Eucalyptus* (19.05%). *Meliponula bocandei* nested on ten tree species and nests were abundantly recorded on *Antiaris toxicaria* (27.0%), *Croton megalocarpus* (21.6%) and *Syzygium guineense* (13.5%). The nests of *M. ferruginea* (black) were recorded on eleven tree species; and *Diospyras abyssinica* accounted for the highest number of nests (58.9%). For the *H. gribodoi* stingless bee species, three of the six recorded nests were located on *Celtis africana* and two were recorded on *Diospyras abyssinica*.

The number of stingless bee species nesting on the same tree species varied from one to four. Nests of all the four stingless bee species were found on the *D. abyssinica*. The *C. Africana* tree species was nested by *H. gribodoi*, *M. bocandei* and *M. ferruginea* (black). Eight species served as nesting hosts for the three groups of paired stingless bee species: *Spathodea campanulate*, *Trichilea emetica* and *Croton sylvaticus* were all nested by *M. ferruginea* (black) and *M. ferruginea* (reddish brown); *Zanthoxylum macrophylla* and *Olea capensis* were used as nesting tree by *M. ferruginea* (black) and *M. bocandei*; *Antiaris toxicaria*, *Aningeria altissima* and *Eucalyptus sp* (dry tree) served as *M. bocandei* and *M. ferruginea* (reddish brown) nesting trees.

The other ten tree species (50%) were used as nesting trees by a single species of stingless bee among the four bee species.

The proportion of nests distribution across their different nesting tree species was uneven in each of the four bee species. However, nest distribution across the nesting tree species was less evenly distributed in *M. ferruginea* (black) species and more evenly distributed in *H. gribodoi* species; followed by *M. ferruginea* (reddish brown) and *M. bocandei* species.

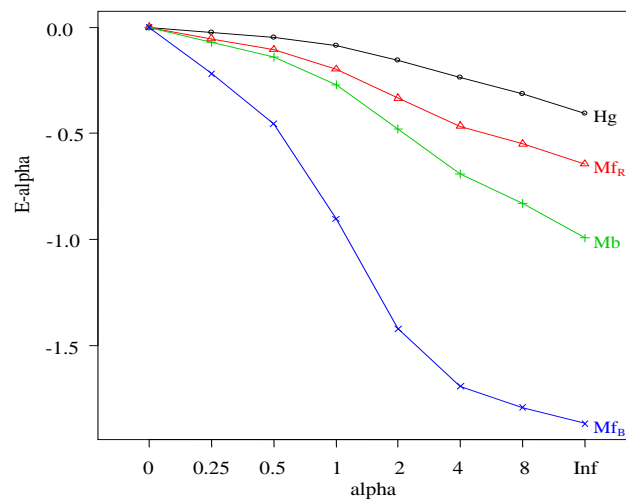


Figure 5.6: Rényi evenness profiles comparing evenness distribution of nest of each stingless bee species along their nesting tree species. Mf_{Rb} = *M. ferruginea* (reddish brown), Hg = *H. gribodoi*, Mf_B = *M. ferruginea* (black) and Mb = *M. bocandei*

Table 5.4: Number of nests for each bee species and the stingless bee species across the different nesting tree species

Nesting tree species (Scientific name)	Nest abundance per bee species				Total bee species
	<i>Hg</i>	<i>Mf_{Rb}</i>	<i>Mb</i>	<i>Mf_B</i>	
<i>Antiaris toxicaria</i>	0	4	10	0	2
<i>Z. macrophylla</i>	0	0	2	1	2
<i>Croton megalocarpus</i>	0	0	8	0	1
<i>Diospyras abyssinica</i>	2	5	2	33	4
<i>F. thonningii</i> x <i>C. mildbraedii</i>	0	2	0	0	1
<i>Markhamia lutea</i>	1	0	0	0	1
<i>Spathodea campanulate</i>	0	1	0	1	2
<i>Olea capensis</i>	0	0	2	1	2
<i>Albizia gummifera</i>	0	0	0	1	1
<i>H. madagascariensis</i>	0	0	0	2	1
<i>Cardia africana</i>	0	0	3	0	1
<i>Sapium ellipticum</i>	0	0	0	1	1
<i>Celtis africana</i>	3	0	3	7	3
<i>Trichilea emetica</i>	0	2	0	1	2
<i>Syzygium guineense</i>	0	0	5	0	1
<i>Croton sylvaticus</i>	0	1	0	3	2
<i>F. exasperate</i> x <i>F. lutea</i> x <i>F.thonningii</i>	0	1	0	0	1
<i>Aningeria altissima</i>	0	1	1	0	2
<i>Prunus africana</i>	0	0	0	5	1
<i>Eucalyptus sp</i>	0	4	1	0	2
Number of identified nests	6	21	37	56	-
Number of tree species	3	9	10	11	-

Mf_{Rb} = *M. ferruginea* (reddish brown), *Hg* = *Hypotrigona gribodoi*, *Mb* = *M. bocandei* and *Mf_B* = *M. ferruginea* (black)

5.3.8 Similarity in the nesting tree species between stingless bee species

The results obtained from this study revealed that none of the pairwise comparisons between the stingless bee species had a Coefficient of Sørensen above 0.50 and the Coefficient of Jaccard above 0.30 (Table 5.5). This indicates that fewer numbers of similar nesting tree species were shared within the stingless bee species. The highest Coefficient of Sørensen and Jaccard were recorded between pairwise species *M. ferruginea* (reddish brown) versus *M. bocandei*, *M. ferruginea* (reddish brown) versus *M. ferruginea* (black) and *M. bocandei* versus *M. ferruginea* (black). The lowest Coefficient of Sørensen and Jaccard were recorded between *M. ferruginea* (reddish brown) versus *H. gribodoi*.

Table 5.5: Similarity coefficient in nesting tree species between the stingless bee species

Paired bee species	Coefficient of Sørensen (C _s)	Coefficient of Jaccard (C _j)
Mf _{Rb} x Hg	0.17	0.09
Hg x Mf _B	0.29	0.17
Hg x Mb	0.31	0.18
Mf _{Rb} x Mb	0.42	0.27
Mb x Mf _B	0.38	0.24
Mf _{Rb} x Mf _B	0.40	0.25

Mf_{Rb} = *M. ferruginea* (reddish brown), Hg = *Hypotrigona gribodoi*, Mb = *M. bocandei* and Mf_B = *M. ferruginea* (black)

5.3.9 Nest aggregation within the stingless bee species

From the one thousand three hundred sixty (1360) stingless bee nests recorded overall the five species; 90.6% of the nests were recorded aggregating on a single

substrate (tree, wall facade). The observed nest aggregation was of two types: conspecific and interspecific aggregation. Conspecific nest aggregation accounted for 78.9% of the total nests recorded in all the four stingless bee species; while interspecific nest aggregation accounted for 11.7% of the total nests recorded overall stingless bee species (Table 5.6). *Meliponula lendliana* was the only stingless bee species which did not nest in aggregation. The ordering of the proportion abundances of the non-aggregated nests within the five stingless bee species was as follows: *M. lendliana* (100%), *M. ferruginea*-black (60.7%), *M. bocandei* (54.1%), *M. ferruginea*-reddish brown (32.9%) and *H. gribodoi* (1.8%).

Nests of *H. gribodoi* were most abundantly recorded in conspecific aggregation (89.0%); while nests of *M. ferruginea* (reddish brown) were most abundantly found in interspecific aggregation (45.9%) (Table 5.6). Nests of *M. ferruginea* (black) and *M. bocandei* were most abundantly found to be non-aggregated (60.7% and 54.1%, respectively). Between the different stingless bee species, the proportion of the recorded conspecific aggregated nests was high in *H. gribodoi* species (89.0%) and similarly, less in *M. ferruginea* (black) (28.6%), *M. bocandei* (24.3%) and *M. ferruginea* (reddish brown) (21.2%). The proportion of the interspecific aggregated nests was higher in *M. ferruginea* (reddish brown) (45.9%); and similarly less in *H. gribodoi* (9.2%) and *M. ferruginea* (black) (10.7%).

Table 5.6: Type of nest aggregation in stingless bee species

bee species	Nests (N)	Proportion of nest within the type of aggregation		
		Non- aggregation	Conspecific aggregation	Interspecific aggregation
<i>M. ferruginea</i> ²	85.0	32.9 (28.0)	21.2 (18.0)	45.9 (39.0)
<i>H. gribodoi</i>	1157.0	1.8 (21.0)	89.0 (1030.0)	9.2 (106.0)
<i>M. lendliana</i>	25.0	100 (25.0)	0.0 (0.0)	0.0 (0.0)
<i>M. bocandei</i>	37.0	54.1 (20.0)	24.3 (9.0)	21.6 (8.0)
<i>M. ferruginea</i> ¹	56.0	60.7 (34.0)	28.6 (16.0)	10.7 (6.0)
Total nest	100 (1360)	9.4 (128)	78.9 (1073)	11.7 (159)
Total species	5	5	4	4

¹Morpho species black, ²Morpho species Reddish brown. Values in the parentheses are the number of nest discovered of each stingless bee species in a specific type of nests aggregation

5.3.10 Number of nests and less minimum distance within nest entrances in an aggregation

A total of 183 sets of the aggregated nests were recorded in the four stingless bee species (Table 5.7). The highest number of a set of a conspecific aggregated nest was recorded in *H. gribodoi* (103 sets) while the lowest was in *M. bocandei* (4 sets). In the interspecific nest aggregation, the highest number of a set of the aggregated nests involving a specific stingless bee species was recorded in *M. ferruginea* (reddish brown) (25 sets) followed by *H. gribodoi* (24 sets). The average number of nests recorded in the conspecific aggregation was highly significantly different between the stingless bee species ($F = 28.15$; $N = 122$; $df = 3$; $p < 0.001$). Comparison of the mean number of nests indicated that the nests recorded in the conspecific aggregation were significantly highest in *H. gribodoi* (10.0 ± 0.79) while it was less between the three other stingless bee species.

In the interspecific nest aggregation, the average number of nests of each stingless bee species associated in the aggregation was significantly different ($F = 4.11$; $N = 61$; $df = 3$; $p < 0.001$). The average number of nests associated with interspecific aggregation was significantly higher in *H. gribodoi* (4.4 ± 1.04) and less in the other three stingless bee species. Moreover, the average of the less minimum distance recorded between nest entrances of each stingless bee species was highly significantly different in the conspecific aggregated nests ($F = 22.42$; $N = 122$; $df = 3$; $p < 0.001$) and significantly different in the interspecific aggregated nest ($F = 87.26$; $N = 61$; $df = 3$; $p < 0.001$). In the conspecific nest aggregation, nest entrances of *H. gribodoi* were significantly closer to one another (0.74 ± 0.06 meters); while nest entrances of *M. bocandei* were more distantly spaced to one another (6.3 ± 0.80 meters). The average of the less minimum distance between nest entrances recorded for each species in the interspecific nest aggregation was not significantly different between *M. ferruginea* (reddish brown) (0.9 ± 0.19 meters) versus *H. gribodoi* (0.8 ± 0.17 meters) and *M. ferruginea* (black) (4.5 ± 0.22 meters) versus *M. bocandei* (4.3 ± 0.25 meters). The species *M. ferruginea* (reddish brown) and *H. gribodoi* nested more closely to an associated interspecies compared to *M. ferruginea* (black) and *M. bocandei*. Two forms of interspecific nest aggregation were observed among the four stingless bee species: colonies nesting up to two different bee species and those colonies nesting up to three different bee species. The *M. ferruginea* (black) species in the interspecies aggregation was recorded only associated to the nest of *M. bocandei*.

For *M. ferruginea* (reddish brown), the interspecific aggregated nests were mostly recorded with a second species (38/39) than with the other three species (1/39). The interspecies aggregated nests of *M. ferruginea* (reddish brown) were mostly recorded aggregated with the nests of *H. gribodoi* (37/39); than with *M. bocandei* (1/39) or *H. gribodoi* x *M. bocandei* (1/39). The *H. gribodoi* species was mostly recorded in interspecies aggregation nesting with *M. ferruginea* (reddish brown) (105/106) than with *M. bocandei* x *M. ferruginea* (reddish brown). The interspecies aggregated nests of *M. bocandei* were mostly recorded aggregating with those of *M. ferruginea* (black) (6/8); than with *M. ferruginea* (reddish brown) (1/8) and *H. gribodoi* x *M. ferruginea* (reddish brown) (1/8) nests.

Table 5.7: Average number of nests and less minimum distance between nest entrances of species in an intraspecific and interspecific aggregation

Bee species	Type of nest aggregation						Associated species
	Intraspecific aggregation			Interspecific aggregation			
	N	Nest number ¹ ± SE	Min.NN ² ± SE(m)	N	Nest number ¹ ± SE	Min.NN ² ± SE(m)	
A	8	2.3 ± 0.16 ^a (3)	1.7 ± 0.49 ^a (0.48)	25	1.6 ± 0.15 ^a (3)	0.9 ± 0.19 ^a (0.045)	B (37) ^I , C (1) ^I , BxC (1) ^{II}
B	103	10.0 ± 0.79 ^b (41)	0.74 ± 0.06 ^b (0.07)	24	4.4 ± 1.04 ^b (19)	0.8 ± 0.17 ^a (0.045)	A (105) ^I , AxC (1) ^{II}
C	4	2.3 ± 0.25 ^a (3)	6.3 ± 0.80 ^c (4.7)	7	1.14 ± 0.14 ^a (2)	4.3 ± 0.25 ^b (3.1)	A (1) ^I , D (6) ^I , AxB (1) ^{II}
D	7	2.3 ± 0.18 ^a (3)	3.7 ± 0.55 ^d (2.75)	5	1.2 ± 0.20 ^a (2)	4.5 ± 0.22 ^b (3.85)	C (6) ^I

A = *M. ferruginea* (reddish brown), B = *H. gribodoi*, C = *M. bocandei* and D = *M. ferruginea* (black); ^IValue in the parentheses indicates the highest number of nest observed aggregated for the particular bee species within the recorded sets of aggregation, ²Value in the parentheses indicates the lowest distance observed between nest entrance for the particular bee species within the recorded sets of aggregation; ^ITwo different species are aggregated together, ^{II}Three different species are aggregated together, Values in a column with the same letters are not significantly different at P<0.001.

5.3.11 Number of nests aggregated on nesting site and substratum in human houses

Out of the three nesting sites recorded, nest aggregation within the four stingless bee species was not found in the underground nests. Additionally, *M. bocandei* and *M. ferruginea* (black) aggregated nests were not recorded on human houses as the two species were not found nesting in this site. A total of 28 sets of the aggregated nests were recorded on trees while 155 sets of aggregated nests were found on human houses (Table 5.8). The average number of aggregated nests of each stingless bee species which were recorded on trees seemed to be similar within the four stingless bee species. Between the two species whose nests were recorded aggregated on tree and human houses; the average number of nests of *H. gribodoi* seemed to be higher in the human houses (9.0 ± 0.71) and less in the trees (1.0 ± 0.00). The average number of *M. ferruginea* (reddish brown) nests which were recorded in an aggregation seemed to be similar in the trees (1.50 ± 0.29) and in the human houses (1.80 ± 0.15).

Table 5.8: Average number of nests counted for a stingless bee species in an aggregation

Nesting sites	N	Mean number of nest (\pm SE)			
		Mf _{Rb}	Hg	Mb	Mf _B
Trees	28	1.50 ± 0.29 (4)	1.0 ± 0.00 (1)	1.55 ± 0.21 (11)	1.83 ± 0.21 (12)
Human houses	155	1.80 ± 0.15 (29)	9.0 ± 0.71 (126)	-	-

Values in the parentheses indicate the number of set of aggregated nests counted in each species of stingless bees within three nesting sites; Mf_{Rb} = *M. ferruginea* (reddish brown), Hg = *Hypotrigena gribodoi*, Mb = *M. bocandei* and Mf_B = *M. ferruginea* (black).

In regard to the type of nesting substratum on human houses, the average number of *H. gribodoi* nests counted in an aggregation seemed to be higher in muddy unsmoothend walls (19 ± 1.11 nests) compared to the other substratum on human houses (below 6 nests) (Table 5.9). For *M. ferruginea* (reddish brown), the average number of nests counted in an aggregation was similarly higher in muddy walls smoothed with mud (2.0 ± 0.0) and muddy walls smoothend with a mixture of mud and cow dung (1.80 ± 0.17).

Table 5.9: Average number of *H. gribodoi* and *M. ferruginea* (reddish brown) nests counted in an aggregation within different nesting substrata identified on human houses

Human houses substratum	Mean number of nest (\pm SE)	
	Mf _{Rb}	Hg
Wall built with brick	-	2.0 ± 0.0 (2)
Wall built with mud and smoothed with mixture of mud and cow dung	1.80 ± 0.17 (24)	3.7 ± 0.44 (30)
Wall built with mud and smoothed with mud	2.0 ± 0.0 (3)	5.91 ± 0.35 (45)
Wall built with mud and not smoothed	1.0 ± 0.0 (2)	19.0 ± 1.11 (38)
Roof frame built with pole from trees	-	2.73 ± 0.43 (11)

Value in the parentheses indicates the number of sets of aggregated nests recorded for a stingless bee species within nesting substratum in human houses; Mf_{Rb} = *M. ferruginea* (reddish brown) and Hg = *Hypotrigona gribodoi*.

5.3.12 Height/depth of the nest within nesting site

The average height of the nests within stingless bee species on the same nesting site revealed some degree of nest height/depth partitioning. A highly significant difference in the height of the nests was observed within species that nested on trees ($F = 12.93$; $N = 120$; $df = 3$; $p < 0.001$). The species with big body sizes *M.*

bocandei selected higher heights on trees for nesting (31.1 ± 1.79 m) (Table 5.10). The small body sized *H. gribodoi* nested at the same height on trees (18.8 ± 1.65) as the medium body sized *M. ferruginea* (black) (21.9 ± 1.05) and *M. ferruginea* (reddish brown) (15.0 ± 2.09) respectively.

The depth of the nests of the two species that nested underground was highly significantly different ($F = 28.63$; $N = 30$; $df = 1$; $p < 0.001$). *M. ferruginea* (reddish brown) seemed to select underground hollows located at low depth (0.5 ± 0.05 m); while *M. lendliana* preferred underground hollows located at high depth (1.1 ± 0.9 m). Between the two species recorded nesting in human houses; no significant difference was indicated in their nest height ($F = 0.16$; $N = 153$; $df = 1$; $p > 0.001$). *M. ferruginea* (reddish brown) and *H. gribodoi* seemed to select a similar height on human houses for nesting.

Table 5.10: Average height/depth of the nest in the wild within stingless bee species that nested on the same nesting site

Bee species	N	Height/depth of the nest on nesting site (m)		
		Trees	House wall	Underground
<i>M. ferruginea</i> ²	85	15.0 ± 2.09^a (21)	1.4 ± 0.08^a (59)	0.5 ± 0.05^a (5)
<i>H. gribodoi</i>	100	18.8 ± 1.65^{ac} (6)	1.3 ± 0.07^a (94)	-
<i>M. lendliana</i>	25	-	-	1.1 ± 0.9^b (25)
<i>M. bocandei</i>	37	31.1 ± 1.79^b (37)	-	-
<i>M. ferruginea</i> ¹	56	21.9 ± 1.05^c (56)	-	-

¹Morpho species black, ²Morpho species Red brown; Values in a column with the same letters are not significantly different at $P < 0.001$

5.3.13 Shape and surface of the open entrance tube of the nest

Two main shapes (circular and oval) of the open entrance tubes of the nests were constructed by each stingless bee species. The area of oval nest entrance ($F = 92.85$; $N = 99$; $df = 4$; $p < 0.0001$) and circular nest entrance ($F = 27.95$; $N = 84$; $df = 4$; $p < 0.0001$) were highly significantly different within the stingless bee species. Oval nest entrance was bigger in the big body size bee *M. bocandei* (1.2 ± 0.09); compared to the other four stingless bee species (Table 5.11). However, the area of circular nest entrance was not significantly different between paired species *M. bocandei* (1.2 ± 0.25) x *M. ferruginea* (black) (0.9 ± 0.16) and *M. ferruginea* (black) (0.9 ± 0.16) x *M. lendliana* (0.7 ± 0.07). Difference in area of both form of nest entrance was indicated between two morph species of *M. ferruginea*. The area of both forms of nest entrance was bigger in the morph species Black compared to the morph species Reddish brown.

Table 5.11: Form and surface of the nest entrance of each stingless bee species

Bee species	N	Shape	Surface (cm ²)	
			Oval	Circular
<i>M. ferruginea</i> ²	43	Circular (21), Oval (22)	0.3 ± 0.02^a	0.4 ± 0.05^a
<i>H. gribodoi</i>	52	Circular (40), Oval (12)	0.1 ± 0.01^a	0.1 ± 0.01^b
<i>M. lendliana</i>	25	Circular (17), Oval (8)	0.3 ± 0.07^a	0.7 ± 0.07^c
<i>M. bocandei</i>	22	Circular (3), Oval (19)	1.2 ± 0.09^b	1.2 ± 0.25^d
<i>M. ferruginea</i> ¹	41	Circular (3), Oval (38)	0.8 ± 0.04^c	0.9 ± 0.16^{cd}

¹Morph species black, ²Morph species red brown. Values in the parentheses indicate the number of nests recorded

5.4 Discussion

The nesting sites and substratum varied within the five stingless bee species and the different species nested either on trees, underground or in walls of residential houses. *Meliponula ferruginea* (reddish brown) and *H. gribodoi* had the highest number of nesting sites and substrata compared to the other three species. The flexibility in nesting sites and nesting substrata observed for *M. ferruginea* (reddish brown) and *H. gribodoi* might offer them the ability to be less affected by habitat loss compared to the other three species. Pyper (2001), Velthuis (1997) and Sakagami (1982) reported that nesting sites and substratum are species specific within stingless bee species.

Among tree nesting species, *M. ferruginea* (reddish brown), *M. bocandei* and *M. ferruginea* (black) preferred nesting in tree trunks; while *H. gribodoi* preferred nesting in tree branches. Michener (2000) reported that species that make combs of brood cells must nest in substantial tree cavities mostly found in trunks; while those that place brood cells in clusters can take advantage of small and irregular cavities in branches. Similar results were recorded that is, the comb builders *M. ferruginea*-reddish brown, *M. ferruginea*-black and *M. bocandei* nested in tree trunks compared to *H. gribodoi* which places brood in clusters nested in tree branches. Moreover, nests among the tree nesting species were mostly observed on live tree parts compared to dead tree parts. Similar results were also reported by Antonini (2002) in a Brazilian savanna area where most nests of *M. quadrifasciata* was found in living trees.

Eltz *et al.*, (2003) also similarly recorded most nests of 12 *Trigona* species in living trees at lowland dipterocarp forest in Sabah, Borneo, Malaysia. Roubik (1989) stated that some Meliponinae which have colonies with prolonged longevity and low swarming frequency will select nesting on live trees because they give good protection against predators and parasites for many years. Martins *et al.* (2004) also stated that, most stingless bees preferred living trees to nest in; probably to escape devastation of the tree cavity by termites.

Meliponula ferruginea (reddish brown) and *M. lendliana* were two species that nested underground and had a difference in the underground substrata preferences. The difference within the two species was due to the fact that; (1) *M. ferruginea* (reddish brown) had the ability to extend parts of its nests (storage pots) in the other adjoining cavities regardless of their shapes and small volume in contrast to *M. lendliana* species. Furthermore, *M. ferruginea* (reddish brown) was able to fix its nests on any material present in the cavity in contrast to *M. lendliana*, whose nests were always fixed on plant roots present in the underground hollows.

Between the two species that nested in walls of human residential houses, *M. ferruginea* (reddish brown) and *H. gribodoi* had differences in their preference for nesting substratum. The preferences of *H. gribodoi* to nest in the unsmoothed mud walls was because of the abundant cracks present on this wall type which facilitated them accessibility to hollows formed as these walls dried.

The preferences of *M. ferruginea* (reddish brown) to nest in hollows of mudwalls smoothed using a mixture of mud and cow dung was due to the availability of appropriate cavity sizes which may not often be available in bricks and cement walls. Moreover, the smoothing of this wall type give a good compactness to the external wall structure than the mud wall smoothed with mud. Such wall compactness is better in keeping off predators such as ants and phorid flies to enter in the nest through cracks on wall; and also limit the colony abundant use of propolis to seal cracks than they could be if nesting in unsmoothed mud walls.

On the other hand, the number of nesting sites for *M. bocandei*, *M. ferruginea* (reddish brown) and *H. gribodoi* in the Kakamega forest environment differed from the records of Kajobe (2007) in Bwindi forest and vicinal habitats at Uganda. Kajobe (2007) never recorded nests of *H. gribodoi* on trees but only on walls of human residential houses. *Meliponula ferruginea* (reddish brown) nests were only found in tree hollows and not in the underground or in hollows on walls of human residential houses. Nests of *M. bocandei* were found only in tree hollows in habitats (northern sector) at lower gradient (1160-1800m) and only in the underground hollows in habitats (southern sector) at relatively higher elevational gradients (1900-2607m). Differences in the nesting sites recorded within *M. bocandei*, *H. gribodoi* and *M. ferruginea* (reddish brown) in the Kakamega forest compared to Bwindi forest could have been due to: (1) differences in the nesting sites of *M. bocandei* might be due to differences in elevation gradient between Bwindi forest (1160-2607m) and the Kakamega forest (1500-1700m); (2) the small body and colony size of *H. gribodoi* along with its

habit to nest in small cavities in branches in the canopy make difficult to detect their nests in an undisturbed forest such as Bwindi forest and (3) the degree of forest disturbances might be high in the Kakamega forest than in the Bwindi forest and this might have caused *M. ferruginea* (reddish brown) to shelter in human houses vicinal to Kakamega forest. These arguments are supported by Ricketts (2004) and Brosi *et al.* (2007; 2008); who reported that some meliponines bees can shelter in the human-dominated land neighbouring native forest habitats that have experienced a high degree of disturbance. Eltz *et al.* (2003) reported that colonies of small body size bees that nest in small cavities in branches of canopy trees are usually undetected due to lack of canopy access in forest that have not experienced a high degree of disturbance.

The number of host plants used as nesting site varied within the four tree nester stingless bee species. Differences in number of nesting tree species within stingless bee species was also reported by Kajobe (2007) in Bwindi forest at Uganda. In Kakamega forest, the tree species *Diospyras abyssinica* was nested by all the four tree nester species; while *Diospyras abyssinica* and *Celtis africana* were nested only by three stingless bee species. Similarly, Martins *et al.* (2004) encountered that only two tree species in the Brazilian Caatinga was used as same nesting host plant by 75% of their studied stingless bee species; and only one tree species namely *C. pyramidalis* was nested by all the seven stingless bee species. We suggest that in a community of stingless bees nesting in a habitat, only a few tree species share the same nesting host plant. In Kakamega forest environment, trees from the family Euphorbiaceae were the mostly abundant tree family nested

by the stingless bee species. Eltz *et al.* (2003), found that tree species from the family Euphorbiaceae was ranked in fifth position of predominant nesting tree species of stingless bees; in lowland Dipterocarp forest in Sabah in Malaysia. There was a little selectivity within the four Meliponine species in the Kakamega forest environment to prefer nesting in a specific tree. Results obtained corroborate with Slaa (2003; 2006b), who reported that stingless bees generally show little selectivity for tree species for nesting. Similar tendency of the Meliponine bee to prefer nesting on a specific tree was also reported by Antonini (2002) in a Brazilian savanna area, where *Meliponula quadrifasciata*; showed an active nesting tree species selection on *Caryocar brasiliense* tree. In Bwindi forest in Uganda, Kajobe (2007) reported that *M. ferruginea* (reddish brown) seemed to have some selectivity preferences for *Parinari excelsia*. Hubbell & Johnson (1977) and Roubik (1989) reported that many stingless bees are opportunists in their use of tree cavities for nesting. However, the fact that the four Meliponine species in Kakamega forest environment were found nesting in many different tree species confirms that these bees are opportunist in selecting the tree species for nesting.

A variation in the tendency to non-aggregate and to form conspecific or heterospecific nest aggregation was observed within the five Meliponine species in the Kakamega forest environment. Out of the three nesting sites recorded, intraspecific and interspecific nest aggregation was not found within the Meliponine species that nested underground. Similarly, variation in the tendency to form a type of nest aggregation within stingless bee species was reported by

Eltz *et al.* (2003) in the lowland dipterocarp forest in Sabah of Malaysia. The highest average number of aggregated nests on a single tree in the Kakamega forest environment was in the *M. ferruginea*-black species (1.83 ± 0.21) and was closely similar to *Trigona* species per nest tree (1.94 nests) reported by Eltz *et al.* (2003). The higher average numbers of nests of *H. gribodoi* present in a single aggregation on walls of human residential houses in homesteads compared to trees in the Kakamega forest indicate that walls of human residential houses offer to this species more available cavities suitable for nesting. Similar observation of *H. gribodoi* to abundantly aggregate their nests on human houses was also reported by Kajobe (2007) in homesteads vicinal to Bwindi forest at Uganda. The difference in the number of nests aggregated on a single substrate within *H. gribodoi* and the three other studied stingless bee species might also confirm and support affirmation of Chinh *et al.* (2005) that, colony reproducing tendency of a species might influence the occurrence of aggregations of their nests in a single substrate in cases where suitable cavities for nesting occur. We suggest that the *H. gribodoi* species have a higher swarming frequency (reproduction rate) compared to the four studied species from the genus *Meliponula*. These explain the higher number of total nests and set of aggregated nests of *H. gribodoi* recorded on substrate with more available cavities suitable for nesting.

A partitioning in nest height/depth was observed in the Kakamega forest within species that nested on trees and species that nested underground, respectively. Within tree nesting species, *M. bocandei* selected the highest height for nesting. Roubik and Aluja (1983) reported that an average height of 30m on trees seems to be the normal average height limit in highly eusocial bee species to build their

nest. Eltz *et al.* (2003) observed that the highest average height of the nest on trees within 12 species of *Trigona* in lowland dipterocarp forest at Malaysia was 25.7m for an unknown identified species of *Trigona*. Kajobe (2007) observed a mean height on trees of 16.3m for *M. bocandei*, 9.4m for *M. ferruginea* (black) and 8.3m for *M. ferruginea* (reddish brown) in Bwindi forest in Uganda. Among the underground nesting species in the Kakamega forest, it was relevant that *M. ferruginea* (reddish brown) species seemed to select the underground hollows located near the soil surface; while *M. lendliana* preferred the underground hollows located deeper from the soil surface. The differences in nest depth within the two species might result from difference in nest thermoregulation requirement. Thus, *M. lendliana* might require more low temperature than *M. ferruginea* (reddish brown), and this probably explains why the latter nests closer to the soil surface.

CHAPTER SIX: FLIGHT AND DEFENSIVE MECHANISMS OF FIVE STINGLESS BEE SPECIES

6.1 Introduction

In the course of forager bees trafficking in their nests, the bee colony in some species might strike a balance between the trafficking of forager bees and nest intrusion by natural enemies. Thus, to defend their nests against intruders, the nest entrance of an insect colony should be small or even closed (Couvillon *et al.*, 2008). Although to permit foraging and to allow easy passage of forager traffick, the entrance should be larger and open (Couvillon *et al.*, 2008). However, to deter intruders under high levels of foraging, bees trafficking back into the nest; there might be in some meliponine bees a trade-off between the size of the nest entrance, the number of bees trafficking back into the nest and the number of guard bees positioning at the open entrance of the nest to reduce the likelihood of being invaded by natural enemies (Couvillon *et al.*, 2008).

Several studies have determined foraging resources of meliponine bees in their natural habitats. However, few studies have focused on how climatic conditions influence flight traffick of different species of stingless bees during foraging hours. Studies have also focused on the predation risk of stingless bees by specific predators during their route to the foraging site (Wcislo and Schatz, 2002; Rao *et al.*, 2008). Nevertheless very few studies have considered defensive mechanisms of the bee species at the nest entrance during and after foraging hours.

The sizes of the open entrance tube of their nests have also been considered in such studied. Various mechanisms are thought to have evolved to enhance colony survival (Schmid-Hempel, 1998). Mechanical defense is a preliminary common means by which adult social insects protect their nests against specific intruders (Ayasse and Paxton, 2002). Stingless bees species defend their nests against intruders using a variety of mechanical defensive strategies. Closure of the nest entrance (Roubik, 2006) and the placing of sticky resin around the entrance tube (Wittmann, 1985) are considered as protective building behaviour. The positioning of guard bees at the open entrance tube of the nest and spitting attacks are referred to as “defensive reactions” (Wittmann, 1985). The use of these mechanicals defensive behaviour varies according to the bee species.

Thus, the aim of this study was to describe how the flight activity of the five meliponine bee species in terms of bees trafficking out of the nest is influenced by abiotic factors such as temperature and relative humidity, during different hours of the day at abundant food resource in the habitat environments. The five bee species were also compared regarding their protective building behaviour at night when there is no foraging activity. The defensive reactions of *M. bocandei*, *M. ferruginea* (reddish brown), *M. ferruginea* (black), *H. gribodoi* and *M. lendliana* bee species when their nest was disturbed by a human and the number of visible guard bees positioned at the open entrance of the nest was also studied.

6.2 Materials and methods

6.2.1 Data collection

Different colonies of five stingless bee species which nested in the hives at the experimental meliponary at Ivihiga site were used to collect the data. Data collection was carried out only on species that accounted for at least three colonies in the hives according to the method described by Couvillon *et al.* (2008). The hive entrance hole which was drilled in each hive was modified with wax and propolis by each meliponine species to suit their own species-specific nest entrance size (Plate 6.1).

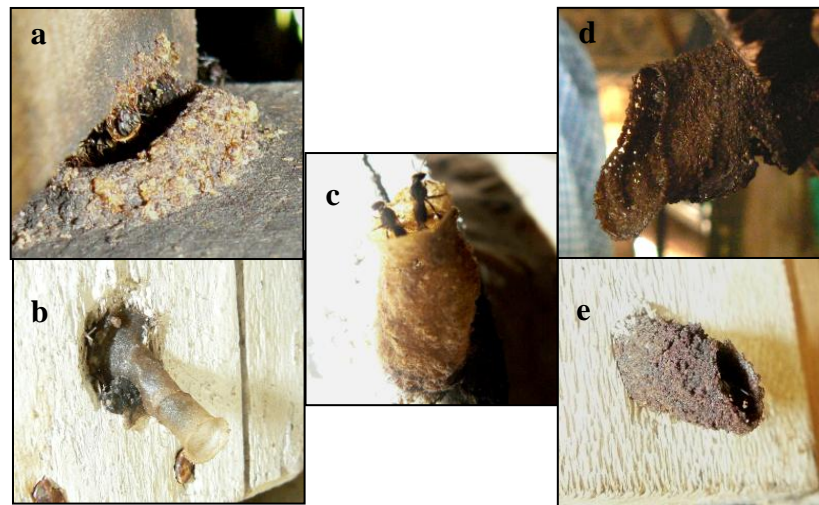


Plate 6.1: Species specific nest entrances of the five stingless bee species built in hives. a) *M. bocandei*, b) *H. gribodoi*, c) *M. lendliana*, d) *M. ferruginea* (black) and e) *M. ferruginea* (reddish brown)

Recommended measurements regarding the shape of the opening of the nest entrance tube were taken and were used to calculate their surface which was calculated using appropriate formulae as described in section 5.2.5 in Chapter

Five. The number of bees flying out of the hive (external flight activity) during twenty-five minutes during ambient temperature and relative humidity conditions was recorded at thirty five minutes intervals beginning from 6:15 am to 17:45 pm. The data for each bee species were recorded from two hives in April 2011 during the blossom of most trees in the area. The number of bees trafficking into the nest during three consecutive minutes and the number of guard bees positioned at the open of the nest entrance at the start of every sampling during different hours from 7:00 am to 16:00 pm were also recorded. The data was collected from three replicates with similar size of the opening of the nest entrance tube and similar colony size for each bee species, where a colony constitutes a replicate.

The number of guard bees which positioned at the open entrance tube during peak foraging hours was recorded and in turn related to the size of the entrance tube of their nest according to the method described by Couvillon *et al.* (2008). In all the five studied species, guard bees were easily recognized by their posture blocking the open entrance tube of the nest and only moving back to allow forager nest mates to leave or enter the nest according to the method described by Couvillon *et al.* (2008). The bees entering into the hive consecutively during three minutes were counted five times for each nest studied among the bee species according to the method described by (Couvillon *et al.*, 2008). The number bees entering into the hive was related to that of the guard bees which were posted at the nest entrance and the size of the open entrance tube of the nest in the meliponary. Data on these parameters were collected during sunny days and during the hours when

their foraging activities were intense (April, 2011) during the blossom of most trees in the area.

The inspection of the hives was carried out during the night hours to record which protective building behaviour was used by each bee species to protect the open entrance tube of their nest. The response of the guard bees to a person who approached their nest entrance or disturbed the hive was also observed and recorded for each bee species according to the method described by Couvillon *et al.* (2008).

6.2.2 Data analysis

The average number of bees in each species which flew out of the hive per minute and the ambient temperature and relative humidity conditions during the different intervals of daily hours were plotted in a graph. The relationship between the open entrance size and the number of bees trafficking back into the nest, between the open entrance size and the number of guard bees at the nest entrance and those between the number of guard bees and the number of bees trafficking back into the nest were evaluated using correlation by way of R statistic software version 2.14.0. The average number of guard bees posted at the open entrance tube for each bee species was recorded. The average number of visible guard bees positioned at the open entrance of nest was categorized into three groups namely few guards (1-2), several guards (3-5) and many guards (6 or more) according to Couvillon *et al.* (2008).

The different species were also categorized into three groups depending on how they protect the open entrance tube of their nest during the night, thus three categories namely open, gluing and sealing.

The defensive behaviour of the guard bees of each species to a person who approached their nest entrance or disturbed the hive was also categorized into three groups namely timid, mildly defensive and aggressive. Timid species were species with guard bees that retreated into the nest or closed the nest entrance when disturbed by humans, while mildly defensive species were those with guard bees that flew out and attacked human intruders by biting when their nests were disturbed. Aggressive species were those that attacked a human intruder even if the intruder merely stood the nest (Couvillon *et al.*, 2008).

6.3 Results

6.3.1 Influence of temperature and humidity on the number of bees trafficking out of the nest

a) *Meliponula ferruginea* (reddish brown)

The proportion of bees of the *M. ferruginea* (reddish brown) species which flew out of their nests varied considerably during the different hours depending on the prevailing temperature and humidity conditions of the day (Figure 6.1). Flight activity of *M. ferruginea* (reddish brown) species commenced around 6:15 am in the morning when relative humidity and temperature were moderate, 52% and 22°C, respectively. The bees of *M. ferruginea* (reddish brown) species showed

two peaks in their flight activity across the day; with the first peak occurring around 9:00 in the morning when the temperature and humidity were 30°C and 30%, respectively and the second peak around 15:00 in the afternoon (32°C, 40%). When the levels of the temperature reached below 22°C and the levels of humidity reached above 70%, the flight activity of *M. ferruginea* (reddish brown) species reduced considerably.

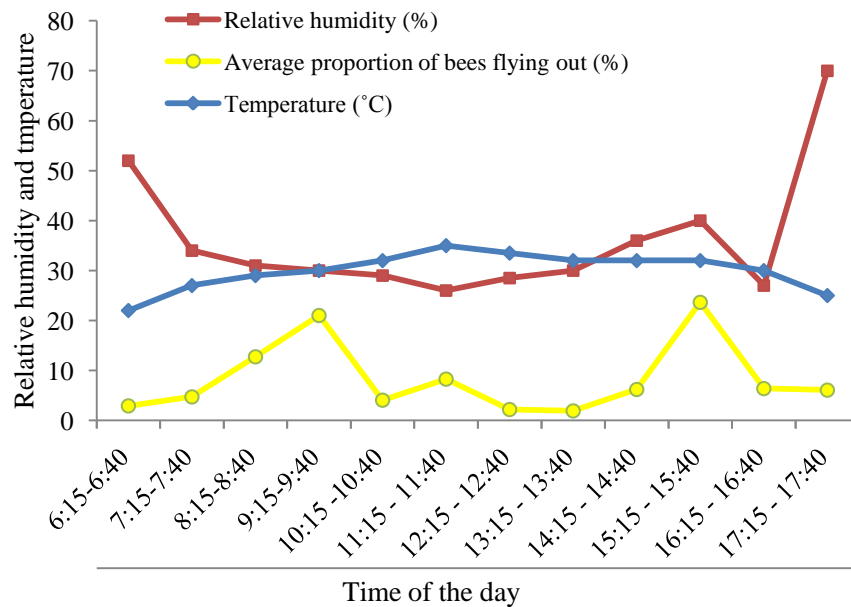


Figure 6.1: Relationship between temperature, relative humidity and proportion of *Meliponula ferruginea* (reddish brown) bee species flying out of the nest

b) *Meliponula ferruginea* (black)

The flight activity of *M. ferruginea* (black) species also commenced at around 6:15 in the morning when relative humidity and temperature were moderate, 52% and 22°C, respectively (Figure 6.2). The bees of *M. ferruginea* (black) species showed three peaks in their flight activity across the day; with the first peak

occurring before noon at around 9:00 am when the temperature and humidity were 30°C and 30%, respectively; while the second and third peaks occurred at around 14:00 (32°C, 36%) and 16:00 (30°C, 27%) in the afternoons. When temperature levels reached below 22°C and the humidity levels reached above 70%, the flight activity of *M. ferruginea* (black) species also reduced considerably.

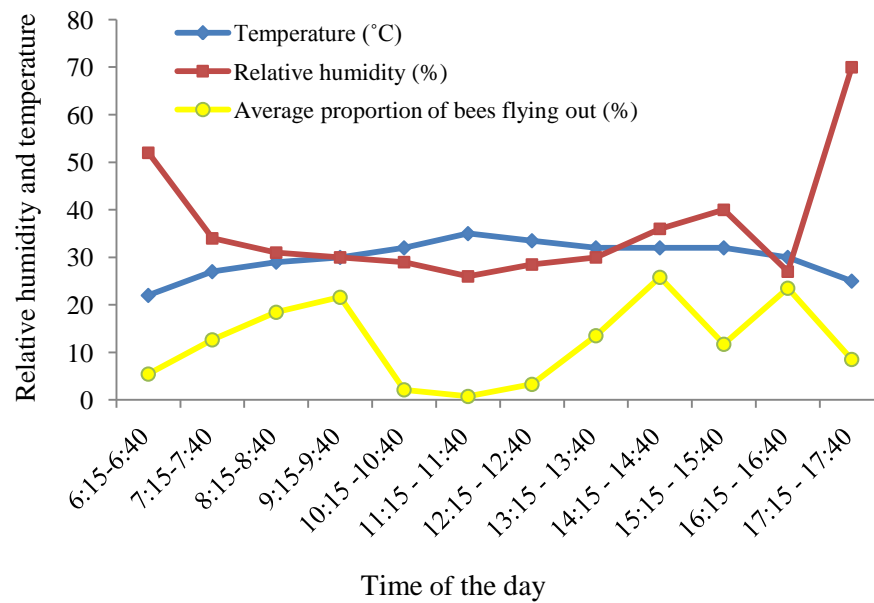


Figure 6.2: Relationship between temperature, relative humidity and proportion of *Meliponula ferruginea* (black) bee species flying out of the nest

c) *Meliponula lendliana*

The bees of *M. lendliana* species started intensive flight activity (peak) from around 6:15 am in the morning when relative humidity and temperature were also moderate, 52% and 22°C, respectively (Figure 6.3). Their flight activity decreased before noon as the temperature increased to 35°C while the relative humidity

decreased to 26%. During the afternoon hours, flight activities of the bees started to increase as the ambient temperature decreased and the relative humidity increased and the flight activity reached a peak at around 15:00 pm when temperature and humidity levels reached, 32°C and 40% respectively.

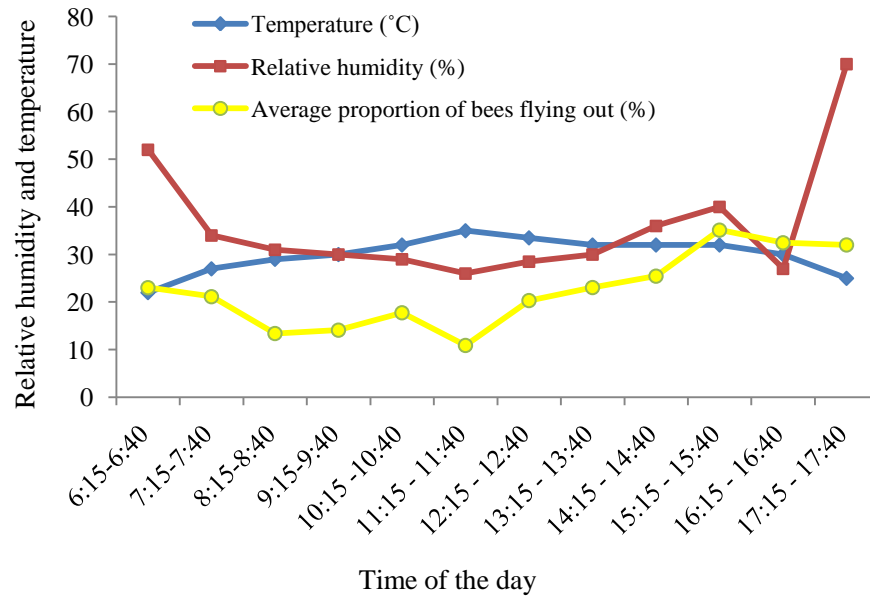


Figure 6.3: Relationship between temperature, relative humidity and proportion of *Meliponula lendliana* bee species flying out of the nest

d) *Hypotrigona gribodoi*

The relative proportion of bees of the *H. gribodoi* species which flew out of their nests also varied considerably during the different hours and ambient temperature and humidity conditions of the day (Figure 6.4). The *Hypotrigona gribodoi* bee species started their flight activity around 7:15 in the morning when relative humidity and temperature reached, 34% and 27°C, respectively, and showed two peaks in their flight activity across the day. Their flight activity increased before

noon while the peak flight activity was reached around 9:00 am when the temperatures level reached 30°C and when the humidity level reached 30%.

During the afternoon hours, the *H. gribodoi* flight activity started to increase as the ambient temperature decreased and the relative humidity increased and the flight activity reaching the second peak at around 15:00 pm when the temperature and humidity levels were 32°C and 40% respectively.

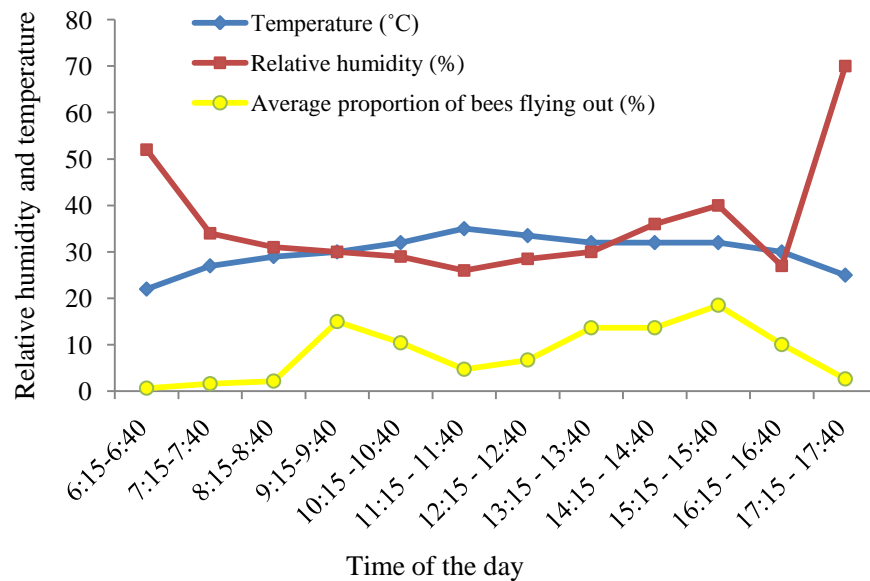


Figure 6.4: Relationship between temperature, relative humidity and proportion of *Hypotrigena gribodoi* bee species flying out of the nest

e) *Meliponula bocandei*

The *M. bocandei* bee species started their flight activity late in the morning around 9:15 when relative humidity and temperature reached, 30% and 30°C, respectively (Figure 6.5). The *M. bocandei* bee species showed two peaks in their flight activity across the day. Their flight activity increased before noon while the

peak flight activity was attained around 11:00 am when temperature level was 35°C and relative humidity level at 26%. As the temperature decreased and the relative humidity increased during afternoon hours; the *M. bocandei* flight activity decreased. During the afternoon hours, the *M. bocandei* bees flight activity decreased as the ambient temperature levels decreased and the relative humidity levels increased. However, a second flight activity peak occurred during the afternoon hours at around 15:00 pm when the temperature and humidity levels reached, 32°C and 40% respectively.

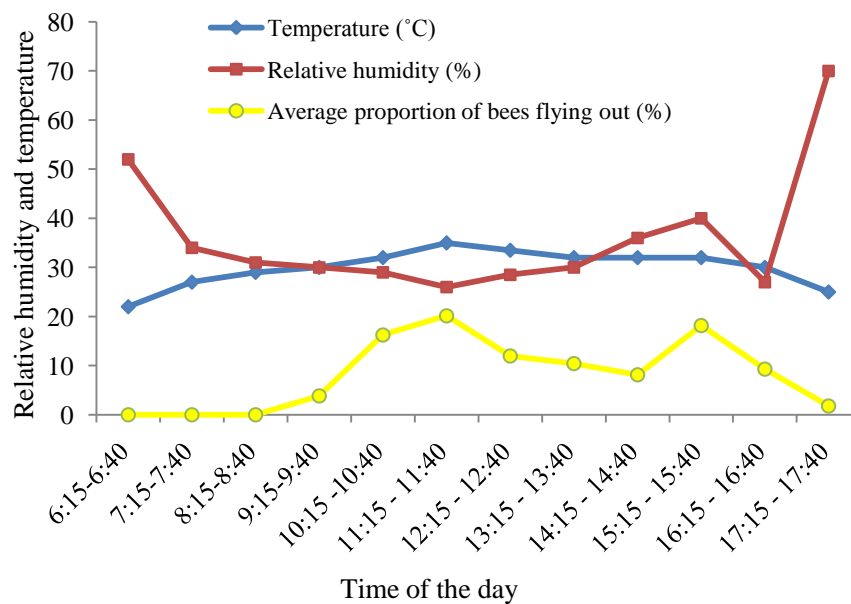


Figure 6.5: Relationship between temperature, relative humidity and proportion of *Meliponula bocandei* bee species flying out of the nest

6.3.2 Classification of the five bee species in relation to their protective night behaviour

The manner of protecting the open entrance of the nests at night hours varied within the five stingless bee species. The presence of a protective barrier at the open entrance of the nest to guard against intrusion during the night was found in three bee species namely, *M. bocandei*, *M. lendliana* and *M. ferruginea*-reddish brown. In the *H. gribodoi* and *M. ferruginea* (black) species, the open entrances of nests were never closed during the night hours (Table 6.1).

Table 6.1: Classification of the five bee species based on how they protect the open entrance tube of their nests during the night

Bee species	N*	Category
<i>M. ferruginea</i> (black)	18	Open
<i>H. gribodoi</i>	10	Open
<i>M. bocandei</i>	3	Gluing
<i>M. lendliana</i>	10	Sealing
<i>M. ferruginea</i> (reddish brown)	37	Sealing

*Number of hives inspected per colonies

The *M. bocandei* species protected the open entrance of their nests during the night hours by reducing their sizes with sticky resin deposited surrounding the opening of the nest entrance tubes. The *M. lendliana* and *M. ferruginea* (reddish brown) species were the only species among the five studied that completely sealed open entrances of their nests at night (Plate 6.2).

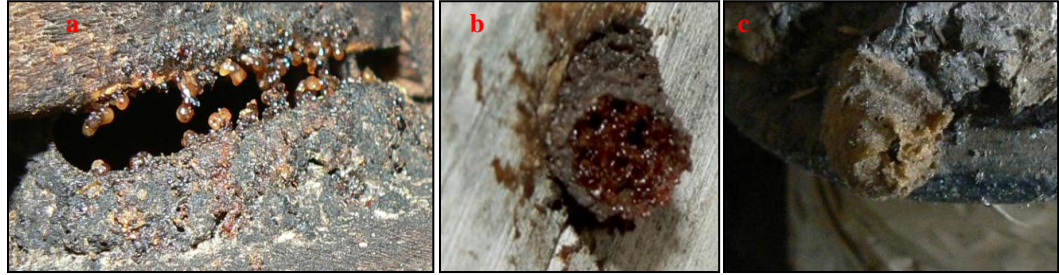


Plate 6.2: Protective building behaviour at the open entrance tube of the nest during night among three stingless bee species. a) *M. bocandei*, b) *M. ferruginea* (reddish brown) and c) *M. lendliana*

6.3.3 Classification of the bee species on the average number of guard bees placed at the opening entrance tube of the nest during peaks foraging activity

In all the five studied species, the guard bees were always stationed inside the funnel at the open entrance of the nest (Plate 6.3).

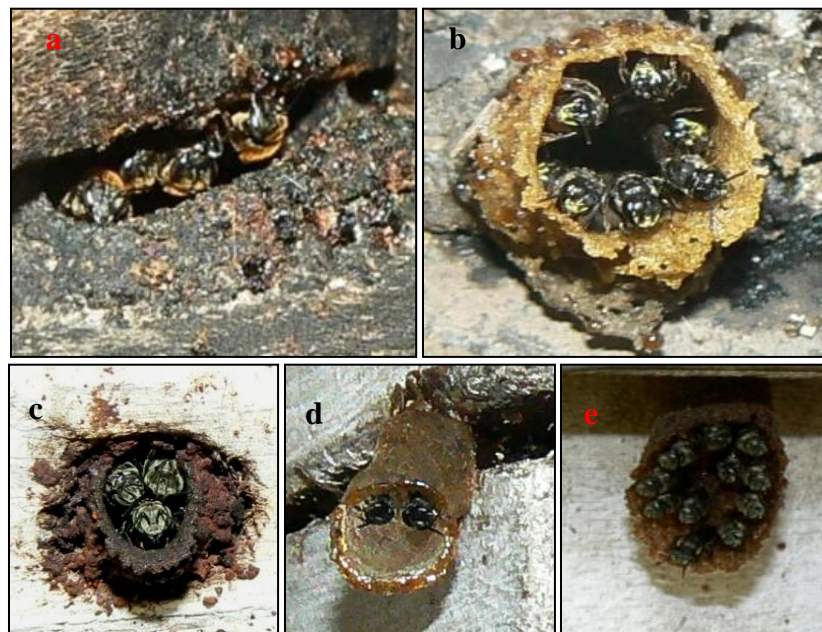


Plate 6.3: Positioning of the guard bees at the open entrance of the nest. a) *M. bocandei*, b) *M. lendliana*, c) *M. ferruginea* (reddish brown), d) *H. gribodoi* and e) *M. ferruginea* (black)

The average number of guard bees placed at the opening entrance tube of the nest during peaks foraging activity varied from two to nine within the five bee species. Their average number were higher in the *M. ferruginea*-black (8.6 ± 0.36) followed by the *M. lendliana* species (6.1 ± 0.48). These two latter species are categorized as bees having many guard bees positioning at the open entrance of their nest. The *M. bocandei* and *M. ferruginea* (reddish brown) bee species had several guard bees placed at the nest entrance (4 ± 0.87 and 3 ± 0.25 , respectively); while the *H. gribodoi* species had few guard bees (1.9 ± 0.48) (Table 6.2).

Table 6.2: Classification of the five stingless bee species in regard to the number of guard bees visible at the open tube of the nest entrance of each species

Bee species	N*	Average number of guards (\pm SE)	Range	Category
<i>M. ferruginea</i> ¹	18	8.6 ± 0.36	5-14	Many guards
<i>M. lendliana</i>	10	6.1 ± 0.48	1-8	Many guards
<i>M. bocandei</i>	3	4 ± 0.87	1-5	Several guards
<i>M. ferruginea</i> ²	37	3 ± 0.25	1-4	Several guards
<i>H. gribodoi</i>	10	1.9 ± 0.48	1-3	Few guards

*Number of hives inspected per colonies; ¹*M. ferruginea* (black); ²*M. ferruginea* (reddish brown)

6.3.4 Classification of the bee species based on their level of aggression towards human presence

Defence of the colony against human disturbance was observed only in the *M. ferruginea* (reddish brown) and *H. gribodoi* species (Table 6.3). The two stingless bee species were both mildly defensive, biting humans only when their nest was

disturbed. The three other stingless bee species were found to be timid with guard bees that retreated into the nest when they were disturbed by a human.

Table 6.3: Classification of the five stingless bee species into categories in regard to the defensive behaviour of guard bees visible at the entrance of each species

Bee species	N*	Defensivity
<i>M. ferruginea</i> (black)	18	Timid (retreat inside the nest)
<i>M. bocandei</i>	3	Timid (retreat inside the nest)
<i>M. lendliana</i>	10	Timid (closes the entrance)
<i>H. gribodoi</i>	10	Mildly defensive (bites)
<i>M. ferruginea</i> (reddish brown)	37	Mildly defensive (bites)

*Number of hives inspected per colonies

6.3.5 Relationship between area of the open entrance tube and number of guard bees posted

In all the five bee species there was an increase in the number of the guard bees posted at the open entrance of their nests as the area of the open of the nest entrance tube increased (Figure 6.6). However, the relationship was positively stronger in the *M. ferruginea*-black ($R^2 = 0.90$), *H. gribodoi* (0.89), *M. lendliana* (0.80) and *M. bocandei* (0.76) species whose correlation coefficients were higher compared to the *M. ferruginea*-reddish brown (0.48) bee species.

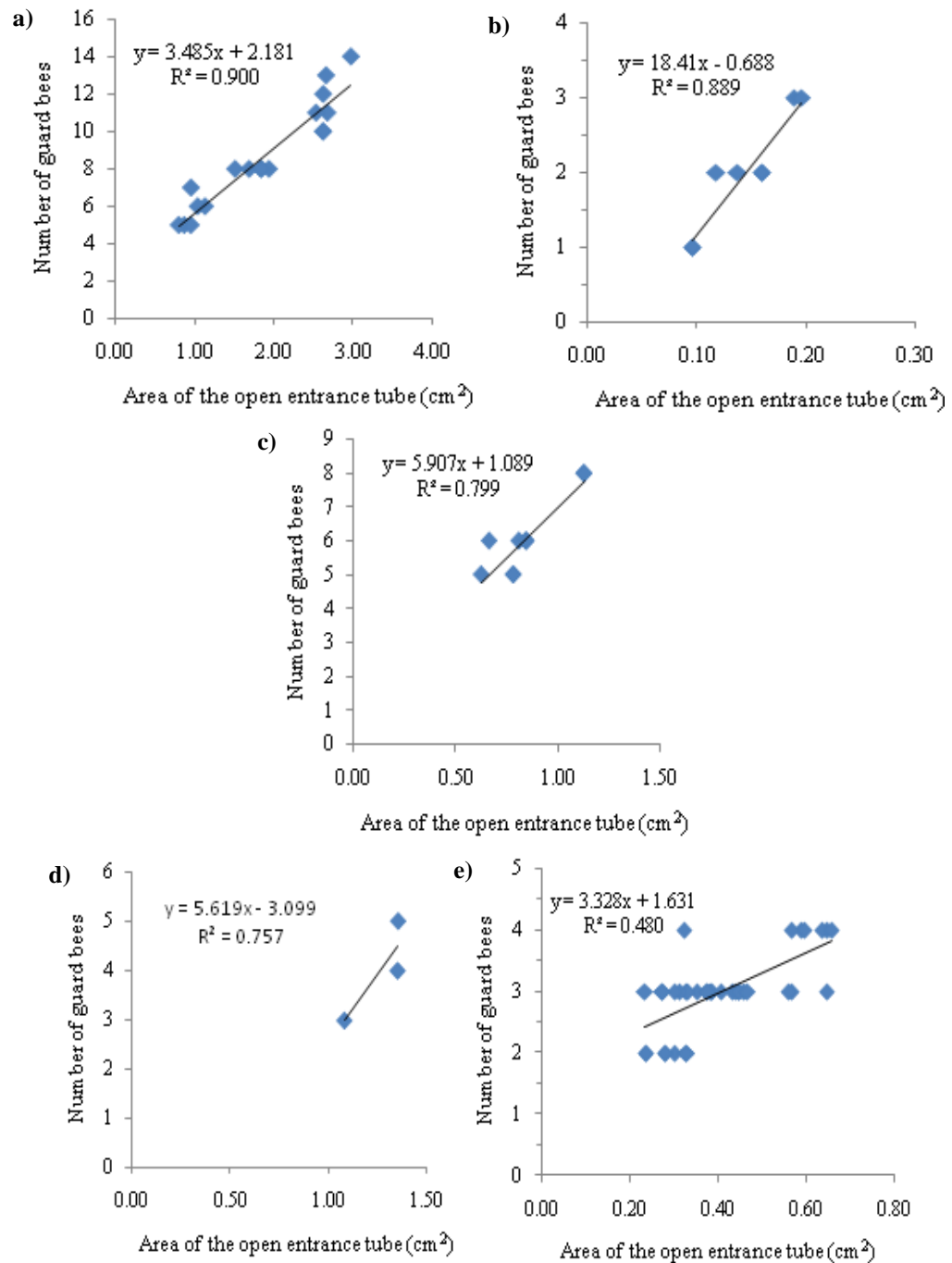


Figure 6.6: The relationship between the areas of the open entrance tubes of the nests and the number of guard bees posted at the nest entrance a) *Meliponula ferruginea* (black), b) *H. gribodoi*, c) *M. lendliana*, d) *M. bocandei* and e) *Meliponula ferruginea* (reddish brown)

6.3.6 Relationship between the numbers of guard bees posted at the open entrance tube and the number of bees trafficking into the nest

The number of guard bees posted at the nest entrance in the same hive varied during the different hours of the day (Plate 6.4). A strong positive correlation between the numbers of guard bees posted at the open entrance tube and the number of bees trafficking into the nest was observed in four bee species, except in *M. ferruginea* (reddish brown) whose correlation was not strong (Figure 6.7).



Plate 6.4: Variation in the number of guard bees posted at the nest entrance of the same hive during different intensity of foraging traffick. a) *M. lendliana*, b) *M. ferruginea* (black), c) *M. bocandei*, d) *H. gribodoi* and e) *M. ferruginea* (reddish brown)

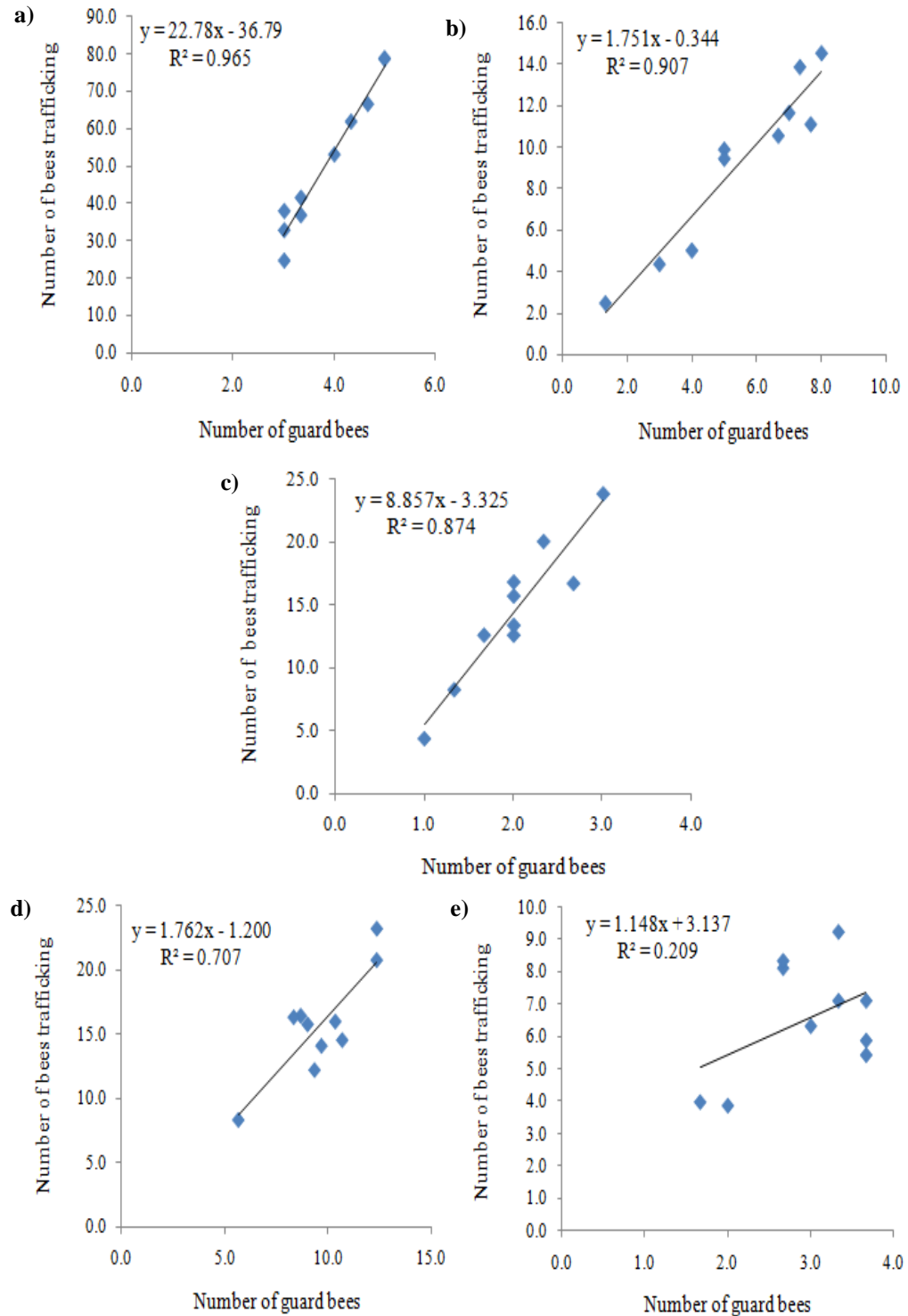


Figure 6.7: The relationship between the number of guard bees posted at the nest entrance and the number of bees trafficking into the nest a) *M. bocandei*, b) *M. lendliana*, c) *H. gribodoi*, d) *Meliponula ferruginea* (black) and e) *Meliponula ferruginea* (reddish brown)

6.3.7 Relationship between the number of bees trafficking into the nest and surface area of the open entrance of the nest

In all the five bee species, there was an increase in the number of bees trafficking into the nests as the area of the open entrance tube increased (Figure 6.8). A strong positive correlation between the numbers of bees trafficking into the nest and surface area of the open entrance tube was observed in two bee species namely, *M. ferruginea*-black ($R^2 = 0.90$) and *M. bocandei* (0.81). In the *H. gribodoi*, *Meliponula ferruginea* (reddish brown) and *M. lendliana* species this correlation was not strong.

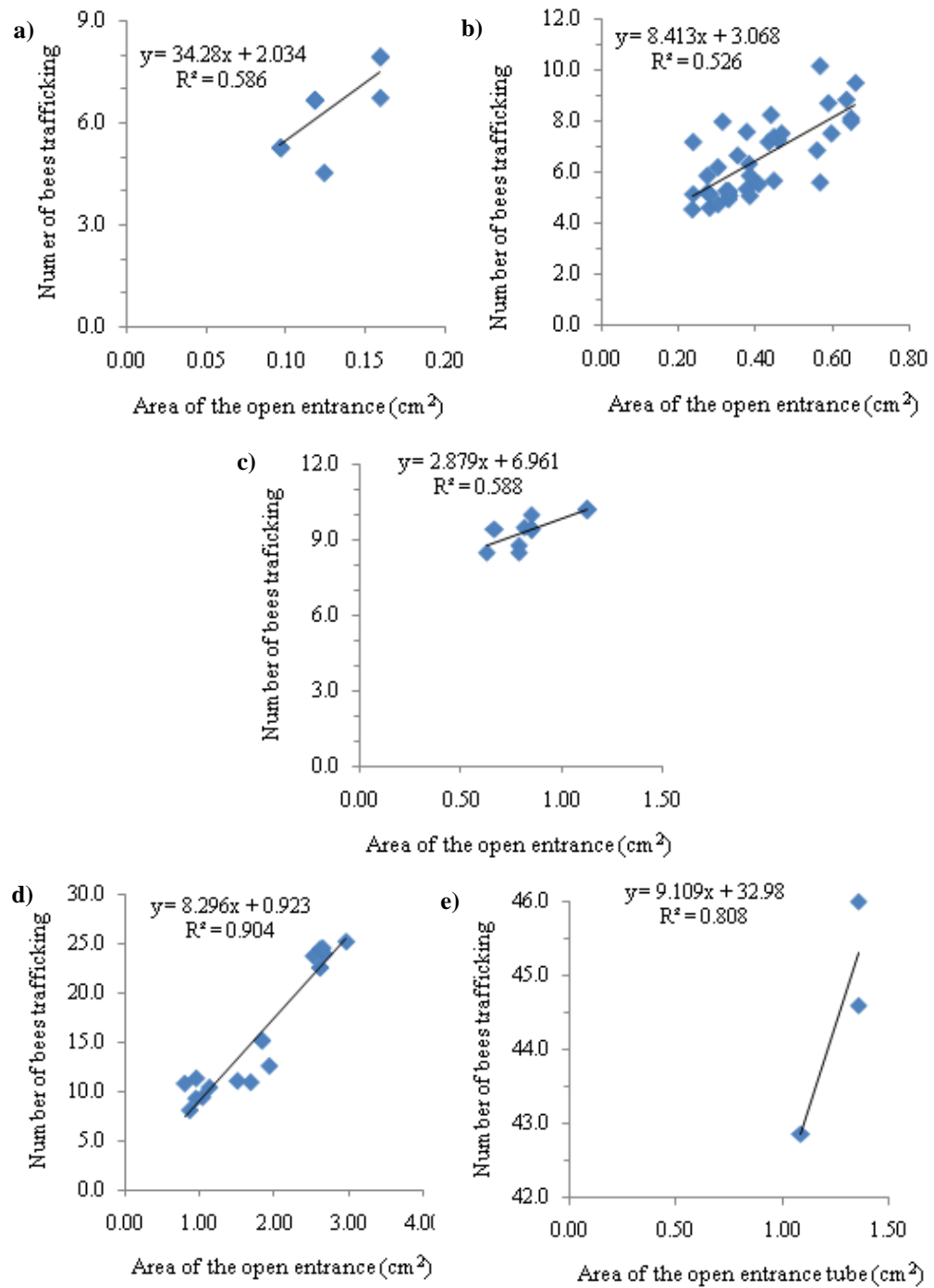


Figure 6.8: Relationship between the dimensions of the open of the nests entrance and the number of bees trafficking into the nest a) *H. gribodoi*, b) *Meliponula ferruginea* (reddish brown), c) *M. lendliana*, d) *Meliponula ferruginea* (black) and e) *M. bocandei*

6.4 Discussion

Daily temperature and humidity influenced the proportion of bees trafficking out of the nest along the different hours of the day in each of the five species. The optimum temperature for almost all the five stingless bee species to keep on flying out of the nests was above 22°C and relative humidity of below 70%. Similar results were reported by Hilário *et al.* (2000) where most *Melipona* species began their flight activity when the optimal levels of relative humidity and temperature are between 70% - 90% and approximately 20 °C respectively. In the Kakamega forest environment, the first peak of flight activity in almost all the five species was at around 9:00 in the morning. According to Roubik (1989), most of the eusocial bees show first foraging activity peaks during morning hours. Thus, it can be suggested that when food is available, flight activities of meliponine bees in the Kakamega forest are influenced by the ambiente temperature and relative humidity conditions.

A positive correlation between the number of bees trafficking into the nests per minute and the dimension of the open entrance tube of the nest was observed in all the five species. Similarly more guard bees were posted at the nest entrance as the area of the open nest entrance and the number of bees trafficking into the nest increased. Variation in the number of guard bees posted at the entrance tube of the nest was associated to the level of bee trafficks into the nest of all the five bee species. The posting of guard bees inside the funnel around the inner entrance was recorded in all the five stingless bee species. It therefore was suggested that, the positioning of the guard bees inside the funnel around the inner entrance and the

numbers of guard bees posted at the nest entrance are one of the mechanical defensive behaviour which was utilized by the five Meliponine bees in the Kakamega forest to defend their nests from intruders. Positive correlation between the size of the entrance tube of the nest and the number of bees traffick into the nests had also been reported in Neotropical stingless bee species (Couvillon *et al.*, 2008). Variation in the number of guard bees posted at the nest entrance has also been reported between Neotropical stingless bee species (Couvillon *et al.*, 2008). Furthermore, in Neotropical species higher forager traffick was associated with more guard bees positioning at the nest entrance and those guard bees were more aggressive (Couvillon *et al.*, 2008). Neotropical bee species of the genus *Nannotrigona*, *Tetragonisca*, *Scaptotrigona*, *Tetragona* and *Trigona* post six or more guard bees at their nest entrance (Couvillon *et al.*, 2008). The positioning of the guard bees inside the funnel around the inner entrance have also been reported in twenty-six Neotropical stingless bee species (Couvillon *et al.*, 2008). *H. gribodoi* and *M. ferruginea* (reddish brown) were the only defensive bees (mildly defensive) within the five species that bite hands and face of humans when their nests were disturbed. Once an assault begun, other worker bees were recruited from inside the nest to join in the attack. Similar mildly defensive behaviour had also been reported in *Tetragonisca angustula* a Neotropical stingless bee species (Couvillon *et al.*, 2008). Nevertheless, within the bee species classified as timid, there might have an effective defensive mechanisms against parasites or predators that attempt to intrude into their nests.

CHAPTER SEVEN: REARING OF THE STINGLESS BEE SPECIES IN DIFFERENT HIVE DESIGNS

7.1 Introduction

Stingless bees provided a vital small-scale economy due to their honey, their cerumen and resins (Cortopassi-Laurino *et al.*, 2006). Honey production in stingless bees is species specific, they all have their unique and characteristic honey which is a highly valued medicinal honey compared to that of *Apis*-honey bees (Pyper, 2001; Cortopassi-Laurino *et al.*, 2006). Stingless beekeeping is also reported in Neotropical region as an important activity among others and its management contributes to conserve their biodiversity in the wild (Cortopassi-Laurino *et al.*, 2006). Meliponine colonies life span is reported to be longer in domestication where an individual colony can live up to 61 years compared to natural colonies in the wild (Murillo, 1984).

The domestication of stingless bee in Africa is not as advanced as in the Neotropical regions and also is not comparable to the advances seen in the honeybee beekeeping (Cortopassi-Laurino *et al.*, 2006). In Kenya, projects on bee rearing have exclusively focussed on *Apis* honey bee and are promoted in rural communities as a sustainable development activity to improve livelihoods. Nevertheless, in Kakamega forest, western Kenya, the stingless bee species *M. bocandei*, *M. ferruginea* (reddish brown), *M. ferruginea* (black), *H. gribodoi* and *M. lendliana* are mostly hunted in the wild for honey which is used for subsistence, traditional medicine and rituals by the Luhya communities adjacent

to the forest (Raina *et al.*, 2006). This practice has led to increased deforestation through trees cutting and decline of the wild population of these stingless bee species due to destruction of their habitats. Preserving and utilizing of these stingless bee species in Kakamega forest through meliponiculture will contribute a great deal to the conservation of the species in their natural habitats, generate income through honey, resins and cerumen production and provide effective crop pollination in small scale farming systems. Considerable evidence, from experiments and non manipulative field studies on stingless bees, shows that not all species are equally important in providing honey for human welfare (Cunha *et al.*, 2002). Studies should include identification of species with minimal restrictive nesting habits (adaptability to artificial hives) and investigating the best methods for maintaining and propagating colonies throughout the year (Cunha *et al.*, 2002; Cortopassi-Laurino *et al.*, 2006).

This study investigated which stingless bee species had minimal restrictive nesting habits in the artificial hives and the appropriate hive design for each species. The potential of each species for honey production in meliponiculture and the best method to use to propagate colonies was also investigated. In addition, two types of feeders were tested to assess their use in meliponiculture to sustain colonies in hives during drought periods. Furthermore, the study also assessed if worker bees of a queenless colony can accept into their nest a new queen, a laying queen taken from another nest of their own species.

7.2 Materials and methods

7.2.1 Acceptance of different hive designs by bee species

Different designs of hives which included those already in use to rear Neotropical species and those developed by icipe were tested on each of the five stingless bee species. These hives included horizontal and vertical designed hives either with or without compartments (Appendices 1.1-1.14). The horizontal hive has storage pots and brood disposed following an horizontal axis; while in vertical hive the storage pots and brood are disposed following vertical axis. The non compartmented hive has a single chamber which served as brood and food storage; while compartmented hive had separated chambers for the brood and food storage.

Three hive designs namely icipe 1Hg, icipe 2 Hg and icipe 3Hg were tested for the *H. gribodoi* stingless bees. Six hive designs namely Utrecht University Tobago Hive (UTOB) (Sommeijer, 1999), Original Australian Trigona Hive (OATH) (Russell and Zobel, 2008), icipe 1M, icipe 2M, icipe 5M, and icipe 6M were tested for the *M. ferruginea* (black) species. Eight hive designs namely UTOB (Sommeijer, 1999), standard OATH (Russell and Zobel, 2008), icipe 2M, icipe 3M, icipe 4M, icipe 5M, icipe 6M and icipe 7M were tested for *M. ferruginea* (reddish brown). Two designs of the wooden hives (UTOB, icipe 2M) and clay pot hive (OATH, “*Iyambova*”) were tested on *M. lendliana*. Each hive design was replicated eight times for the different stingless bee species. The dimensions of the different hives were as follows: icipe 1H (26 cm x 6 cm x 5 cm); icipe 2H (Brood chamber: 10 cm x 7 cm x 12 cm, Honey chamber: 10 cm x

7 cm x 18 cm); icipe 3H (Brood chamber: 20 cm x 10 cm x 10 cm, Honey chamber: 20 cm x 10 cm x 5 cm); icipe 1M (55 cm x 14 cm x 13 cm); icipe 2M (30 cm x 15 cm x 22 cm); icipe 3M (Brood chamber: 28 cm x 10 cm x 10 cm, Honey chamber: 28 cm x 10 cm x 8 cm); icipe 4M (Brood chamber: 12 cm x 12 cm x 14 cm, Honey chamber: 12 cm x 12 cm x 25 cm); icipe 5M (Brood chamber: 15 cm x 15 cm x 22 cm, Honey chamber: 25.5 cm x 15 cm x 7 cm); icipe 6M (Brood chamber: 18 cm x 18 cm x 20 cm, Honey chamber: 18 cm x 18 cm x 5 cm); icipe 7M (Brood chamber: 18 cm x 13 cm x 14 cm, Honey chamber: 16 cm x 13 cm x 7.5 cm); OATH (Brood chamber: 18 cm x 18 cm x 20 cm, Honey chamber: 18 cm x 18 cm x 5 cm); UTOB (Brood chamber: 12 cm x 12 cm x 25 cm, Honey chamber: 42 cm x 12 cm x 7.5 cm); and Iyambova pot (18 cm of diameter).

Establishment of a colony in an experimental hive was realized by transferring into the hive the whole brood and adult bees from the wild nest. So as not to harvest most colonies identified in both studied sites; 60 % of the required colonies were harvested from sites which were not included in this study. The hives were kept in a shed constructed to conserve the hive from rain and sun (Plate 7.1).

To assess the estimated probability of acceptance of each designed hive by a stingless bee species each of the nested hive was scored as follows: (i) a hive that contained an established colony for one year since its transferred date was considered as an accepted hive and was scored as 1 and (ii) a no accepted hive

(absconded/died) was considered as a rejected hive and was scored as zero (0). Compartmented hives were considered as best designed hive because of the manipulation facilities they offer which is mainly the possibilities to harvest honey in the storage pots without disturbing the brood or contaminating them with honey. Data collection of this study was carried out from June 2009 to August 2010.



Plate 7.1: *icipe* experimental meliponary station implanted at Isiekuti village

7.2.2 Honey production per stingless bee species

To assess honey production by the five stingless bee species; the average quantity of honey (litres) per year that was stored in pots was harvested from colonies nesting in hives. For colonies nesting in a non compartmented designed hive, honey stored in pots was collected by cutting off the honey pots surrounding the involucrem of the nest in hive (Plate 7.2 a, c). Whereas for colonies nesting in a compartmented designed hive, honey stored in pots was collected by cutting off the honey pots present in the storage chamber of the hive (Plate 7.2b). Extracted honey pots from each hive were squeezed separately and the flowing honey was

sieved to remove all particles, including pot scrappings. A one liter measuring cup with precise graduation of 50 ml was used to quantify the harvested honey per colony of each bee species.



Plate 7.2: Harvesting honey from domesticated colony of (a) *M. ferruginea* (black) nested in a icipe-1M hive; (b) *M. ferruginea* (reddish brown) in the storage chamber of a UTOB hive and (c) *M. ferruginea* (black) nested in a icipe-2M hive

7.2.3 Methods for propagating colonies

Three different artificial methods which are reported to be applicable for colony multiplication were tested to assess their estimated probability of success on the species that produced at least one litre of honey or above in hives. The three methods included the hive splitting method (Klumpp, 2007), Education method (Dollin, 2001) and the standard brood transfer method (Roubik, 1995) (Plate 7.3). The hive splitting method consisted to use a designed hive constructed with a brood chamber that can be manually divided into two equal parts (A_1 , A_2).

The Education method involve the use of a hive that contains a strong established colony and connect it to an empty hive by a pipe which allows the bees to pass from one hive to the other hive. In this method, the nest entrance of the colony is located at the empty hive. The standard brood transfer method involves splitting the brood of a nest of a strong colony into two equal parts and introducing each of them in an empty hive. Each of the methods was replicated eight times for the two morpho species of *M. ferruginea*. To assess the effectiveness of the hive splitting and the standard brood transfer methods, the number of queenless colonies which had established their nests for the period of one year and had newly emerged queens were considered as successful and were scored as one (1); while all queenless colonies resulting from both splitting methods that later absconded or died were considered as unsuccessful and were scored as zero (0). For the Education method, all empty hives that later had a new established nest were considered as successful and were scored as one (1); while all hives without newly established nests were considered as unsuccessful and were scored as zero (0). Data collection was carried out from June 2010 to August 2011.



Plate 7.3: (a) UTOB hive with a splitting brood chamber (A₁, A₂); (b) Education method, A₁ is a hive containing a strong colony and A₂ is an empty hive and (c) Transfer in two hives a part of brood obtained by splitted a strong nest

7.2.4 Queen exchange

This experiment was conducted to assess if the worker bees of a queenless colony can accept in their nest a new queen introduced from another colony. Five colonies of the bee species that produced at least one liter of honey or above in a hive were used and kept queenless for two days by removing their respective queen. Each queen was marked with red color on the dorsal side of the thorax using red nail polish to easily recognize in the transferred nest. Queens were kept separately for two days in small nucleus (small number of bees) extracted from their original nests. On the third day, each queen was put in a queen cage and introduced in one of the queenless nests where she did not belong. Data was recorded as a score, with one (1) equal to successful and zero (0) equal unsuccessful for the applied method per stingless bee species.

7.2.5 Acceptance of two feeder designs

The experiment was also carried out on the stingless bee species that produced at least one liter of honey or above in a hive. Two types of designed feeders were tested on the bee colonies. This included a feeder installed inside the bee hive and a feeder installed outside the bee hive but connected to the plastic entrance tube of the nest (Plate 7.4). A solution made of 75% sugar diluted in 25% of water was used as an artificial nectar source to test the two potential ways of feeding the bees during the dry periods. The assessment was carried out by scoring each hive in which each of the designed feeders was tested. Feeders that the bees were coming in to collect the sugary solution was considered as accepted and scored as

one (1); while feeders that the bees refused to resource on by sealing with propolis all access into the feeders was considered as rejected and scored as zero (0). The experimentation was replicated on five colonies of each bee species and was carried out in December 2010 during the dry season when nectar sources are scarce.



Plate 7.4: (a) feeder installed inside the bee hive and (b) a feeder installed outside the bee hive but connected to the plastic entrance tube of the nest

7.2.6 Data analysis

A Generalized linear model (binomial distribution model with logit link) was used to model the binary data (0,1) collected on the estimated probabilities of acceptance of the different hive designed hive per bee species, the estimated probabilities of acceptance of the different methods of propagating colonies, success of queen exchange method in a bee species and the estimated probabilities of acceptance of the designed feeders. Probabilities of success were estimated using the fitted binomial model. Analysis of variance (ANOVA) was used to compare annual quantity of honey (litres) produced by the stingless bee species.

Differences in estimated probabilities for the binomial model and differences in means for the analysis of variance were compared using Tukey's HSD test respectively (R Development Core Team, 2005). The analyses were implemented in R software version 2.14.0.

7.3 Results

7.3.1 Acceptance of hive designs by *Hypotrigona gribodoi*

The probability of acceptance by *H. gribodoi* varied significantly among the three hive designs ($\chi^2 = 17.99$; $df = 2$; $P < 0.001$). *Hypotrigona gribodoi* colonies accepted nesting mostly in the icipe 1H hive which design is a non-compartmented hive and completely refused to maintain their colony in the two types of compartmented hive (icipe 2H and icipe 3H) designed for the species (Table 7.1).

Table 7.1: Estimated probability at which each of the three designed hive was accepted by *H. gribodoi*

Hive design	N	Probability \pm SE
icipe 1H	8	0.75 \pm 0.15 ^a
icipe 2H	8	0.00 \pm 0.00 ^b
icipe 3H	8	0.00 \pm 0.00 ^b

Values within rows followed by a same letters are not significantly different at $P < 0.00$.

7.3.2 Acceptance of hive designs by *Meliponula ferruginea* (black)

Meliponula ferruginea (black) species responded differently to the various hive designs ($\chi^2 = 18.04$; $df = 5$; $P < 0.001$). The icipe 5M hive design was completely

unaccepted by *M. ferruginea* (black) species (Table 7.2). The designed icipe 1M, icipe 2M and OATH hives were the most accepted compared to the icipe 6M and UTOB designed hives. *M. ferruginea* (black) colonies nested in the compartmented hive namely, icipe 6M and UTOB were unable to maintain food stores and brood in the separated food and brood chambers of the hives. The colony maintained their food stores in the same chamber where the brood was placed. In the non-compartmented hive design icipe 2M, *M. ferruginea* (black) colonies totally occupied the hive chamber and the food stores surrounded the brood (Plate 7.5).

Table 7.2: Estimated probability of hive acceptance by *M. ferruginea* (black)

Designed hive	N	Probability \pm SE
icipe 1M	8	0.75 ± 0.15^a
icipe 2M	8	0.75 ± 0.15^a
OATH	8	0.63 ± 0.17^a
icipe 6M	8	0.38 ± 0.17^b
UTOB	8	0.25 ± 0.15^b
icipe 5H	8	0.00 ± 0.00^c

Values within rows followed by a same letters are not significantly different at $P < 0.001$.

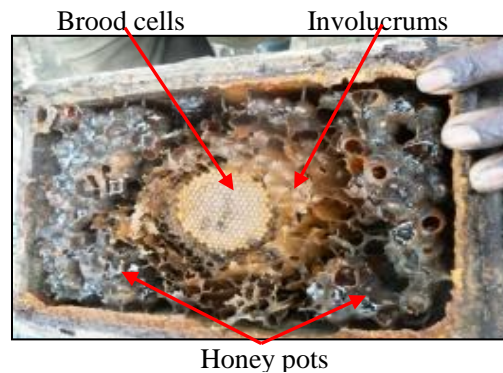


Plate 7.5: A colony of *M. ferruginea* (black) established in an icipe 2M hive design

7.3.3 Acceptance of hive designs by *Meliponula ferruginea* (reddish brown)

Meliponula ferruginea (reddish brown) accepted both non-compartmented and compartmented designed hives. In all the compartmented hive designs, *M. ferruginea* (reddish brown) colonies were able to store food and maintain brood in the respective food and brood chambers of these hives (Plate 7.6). The probability of acceptance to nest in the various hive designs did not vary significantly among the eight designed hives ($\chi^2 = 2.76$; $df = 7$; $P > 0.05$). The estimated probability of acceptance to nest in the various hive designs ranged from 0.63 ± 0.17 to 0.88 ± 0.12 (Table 7.3). The designed icipe 4M and UTOB hives were ranked first in the ordering of average probability of acceptance. The icipe 2M, icipe 3M, icipe 5M and icipe 7M were ranked second; while the icipe-6M and OATH hives both were ranked third in the average estimated probability of acceptance by *M. ferruginea* (reddish brown).

Table 7.3: Estimated probability at which each of the eight designed hive was accepted by *M. ferruginea* (reddish brown)

Designed hive	N	Probability \pm SE
icipe 4M	8	0.88 ± 0.12^a
UTOB	8	0.88 ± 0.12^a
icipe 2M	8	0.75 ± 0.15^a
icipe 3M	8	0.75 ± 0.15^a
icipe 5H	8	0.75 ± 0.15^a
icipe 7M	8	0.75 ± 0.15^a
icipe-6M	8	0.63 ± 0.17^a
OATH	8	0.63 ± 0.17^a

Values within rows followed by a same letters are not significantly different.

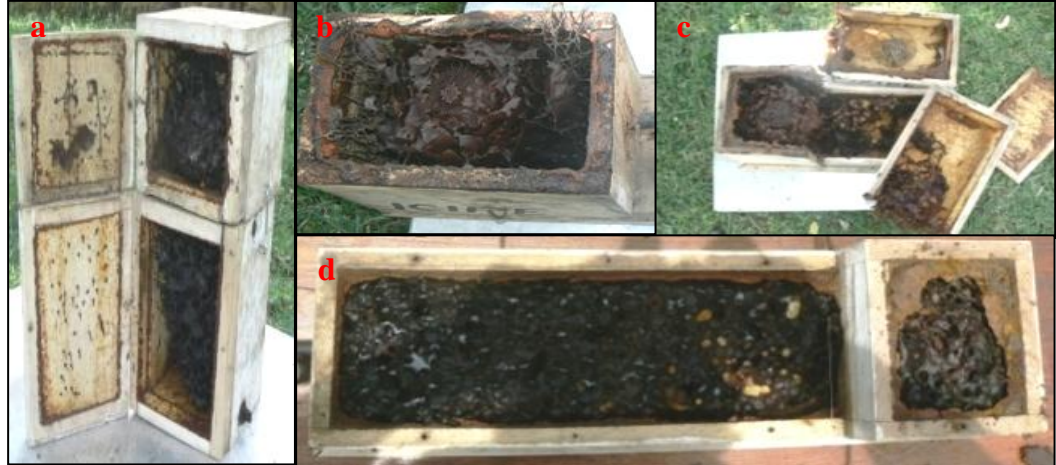


Plate 7.6: Colonies of *M. ferruginea* (reddish brown) established in (a) icipe 4M, (b) icipe 2M, (c) icipe 5M and (d) UTOB hive designs

7.3.4 Acceptance of hive designs by *Meliponula lendliana*

The hive design made of clay and wood were significantly different in their acceptance by *M. lendliana* ($\chi^2 = 16.35$; $df = 3$; $P < 0.001$). None of the hive designs made of wood were accepted by *M. lendliana* species compared to those hive designs which were made of clay. There was no significant difference in the probability of acceptance between the two clay hive designs (Table 7.4). *Meliponula lendliana* colonies nested in the Iyambova clay pot hive extended the nest and occupied the entire hive chamber with food stores (Plate 7.7).

Table 7.4: Estimated probability of hive design acceptance by *M. lendliana*

Designed hive	N	Probability \pm SE
Iyambova clay pot	8	0.63 \pm 0.18 ^a
OATH clay hive	8	0.50 \pm 0.17 ^a
Icipe 2M wooden hive	8	0.00 \pm 0.00 ^b
UTOB wooden hive	8	0.00 \pm 0.00 ^b

Values within rows followed by a same letters are not significantly different at $P < 0.001$.



Plate 7.7: Honey pots of *M. lendliana* nested in the pot *Iyambova*

7.3.5 *Meliponula bocandei*

The species *M. bocandei* was tested for acceptance to nest in more than five designed hives. However, due to prominent nest infestations by small hive beetle and phorid flies larvae in all the hive designs tested for this bee species; only three colonies of *M. bocandei* out of the several wild nests harvested had resulted into the establishment of the colonies in the artificial cavities. These three *M. bocandei* nests were established in the designed icipe 1M hive (Plate 7.8).



Plate 7.8: An established colony of *M. bocandei* in the hive design icipe 1M at which a super-chamber was added for propagating the colony using the splitting hive method. a = Brood and b = Storage pots

7.3.6 Honey production within the five stingless bee species

The quantity of honey produced annually was significantly different among the five bee species ($F = 35.26$; $df = 4$; $P < 0.01$). The average honey production in these hives was higher and significantly different in the big sized bee species *M. bocandei* compared to the two medium sized bee species (*M. ferruginea*-black, *M. ferruginea*-reddish brown) and both small body size bee species (*H. gribodoi*, *M. lendliana*) (Table 7.5).

The average quantity of honey produced annually within the two medium body size bee species was significantly higher in *M. ferruginea* (black) species compared to *M. ferruginea* (reddish brown). The two small sized *H. gribodoi* and *M. lendliana* bee species produced less amount of honey and was significantly different compared to the other three studied bee species. There was no significant difference in the average quantity of honey produced annually by the small body size bee species.

Table 7.5: Average quantity of honey produced annually in hives within the five stingless bee species in a meliponary at the Kakamega forest

Bee species	N	Quantity of honey \pm SE
<i>M. bocandei</i>	3	3.13 ± 0.21^a
<i>M. ferruginea</i> (black)	22	1.37 ± 0.08^b
<i>M. ferruginea</i> (reddish brown)	29	1.05 ± 0.07^c
^c <i>M. lendliana</i>	13	0.04 ± 0.09^d
<i>H. gribodoi</i>	7	0.01 ± 0.14^d

Values within rows followed by a same letters are not significantly different at $P < 0.01$.

7.3.7 Acceptance of colony propagation methods by *M. ferruginea* (reddish brown)

The probability of success of the colony propagation methods on *M. ferruginea* (reddish brown) species varied significantly among the three methods tested ($\chi^2 = 6.99$; $df = 2$; $P < 0.001$). The hive splitting method was more successful for the propagation of *M. ferruginea* (reddish brown) bee colonies compared to the standard brood splitting and education methods. However, no significant difference in the means probability of success was observed within the standard brood splitting and Education method (Table 7.6).

Table 7.6: Estimated probability of each three methods applied to propagate a colony of *M. ferruginea* (reddish brown)

Method of propagation	N	Probability \pm SE
Splitting hive	8	0.88 \pm 0.12 ^a
Standard brood splitting	8	0.63 \pm 0.17 ^{ab}
Education method	8	0.25 \pm 0.15 ^b

Values within rows followed by a same letters are not significantly different at $P < 0.01$.

In the Education method, establishment of nest material in the adjoining hive by the new established colony started with the building of pots to store food (Plate 7.9). The splitting hive method required four steps in the process of joining to an empty honey chamber of the same hive design a colonized brood chamber taken from a mother colony (Plate 7.10)



Plate 7.9: Colony multiplication of *M. ferruginea* (reddish brown) using the Eduction method. A₁ = mother colony and A₂ = newly established colony



Plate 7.10: Colony multiplication of *M. ferruginea* (reddish brown) using the hive splitting method. a) Mother hive whose brood chamber will be separated into two parts (right) and an UTOB hive without brood chamber prepared to receive one of the separated brood chamber of the mother hive; b) Separation of the brood chamber of the mother hive into two separate compartment each containing brood and bees; c) Transferring the super brood chamber of the of the mother hive on the tray and honey chamber of another UTOB hive and d) Mother hive (left) and newly propagated hive (right)

7.3.8 Acceptance of colony propagation methods by *M. ferruginea* (black)

The probability of success of the colony propagation methods on *M. ferruginea* (black) species did not vary significantly among the three methods tested ($\chi^2 = 4.89$; $df = 2$; $P > 0.05$). However, the probability of success of the hive splitting method and standard brood splitting were three times higher than the Education method (Table 7.7).

Table 7.7: Estimated probability of succes within the three applied methods to propagate of *M. ferruginea* (black) colony

Method of propagation	N	Probability \pm SE
Splitting hive	8	0.63 \pm 0.17 ^a
Standard brood splitting	8	0.50 \pm 0.18 ^a
Education method	8	0.13 \pm 0.12 ^a

Values within rows followed by a same letters are not significantly different.

7.3.9 Acceptance of the splitting hive method by *M. bocandei*

The hive splitting method was tested once on a single colony of *M. bocandei* species that had extended the brood cells and storage pots up to the super chamber which was added to the icipe 1M hive design. The splitting was succfull and led to the establishment of a sister colony from the mother colony. Both mother and daughter colonies were not infested by the small hive beetle and phoride flies larvae (Plate 7.11).



Plate 7.11: Colony multiplication of *M. bocandei* using the hive splitting method. a) Mother colony with an upper box (A₂) which was added to extend the nest; b) Split of the hive into two separated compartments (A₁, A₂) each containing brood, food storages and bees and c) Pose of the upper compartment (A₂) on a tray (daughter colony) (right) and joining of another empty box (A₂) to the icipe 1M hive that contained the remaining of the mother nest (left)

7.3.10 Queen exchange

There was 100% success rate in *M. ferruginea* (reddish brown) and *M. ferruginea* (black) stingless bee species where a laying queen of the same kind species was introduced in a queenless colony (Table 7.8).

Table 7.8: Estimated probability of acceptance of an introduced laying queen within the queenless colonies of two bee species

Bee species	N	Probability \pm SE
<i>M.ferruginea</i> ¹	5	1.00 \pm 0.00
<i>M.ferruginea</i> ²	5	1.00 \pm 0.00

7.3.11 Acceptance of two designs of feeder by *M. ferruginea* (reddish brown)

The probability of success of *M. ferruginea* (reddish brown) species did not vary significantly among the two types of the tested feeders ($\chi^2 = 0.48$; $df = 1$; $P > 0.05$). However, the internal feeder had a higher probability of being accepted than the external feeder which was used in this study (Table 7.9).

Table 7.9: Estimated probability at which the two designs of feeders were accepted by *M. ferruginea* (reddish brown) bee species

Type of feeder	N	Probability \pm SE
Internal feeder	5	0.80 \pm 0.18 ^a
External feeder	5	0.60 \pm 0.22 ^a

Values within rows followed by a same letters are not significantly different.

7.3.12 Acceptance of two designs of feeder by *M. ferruginea* (black)

The probability of success of *M. ferruginea* (black) species did not vary significantly among the two tested types of feeders ($\chi^2 = 1.73$; $df = 1$; $P > 0.001$). However, the internal feeder had a higher probability of being accepted than the external feeder which was used in this study (Table 7.10).

Table 7.10: Estimated probability at which the two designs of feeders were accepted by *M. ferruginea* (black) bee species

Type of feeder	N	Probability \pm SE
Internal feeder	5	0.80 \pm 0.18 ^a
External feeder	5	0.40 \pm 0.22 ^a

Values within rows followed by a same letters are not significantly different.

7.4 Discussion

Meliponula lendliana species seemed to have restrictions to nest in hive designs which were constructed with wood compared to those hive designs which were constructed with clay. According to Cortopassi-laurino *et al.* (2006), stingless bee species which nest only in subterranean hollows are difficult to maintain in wooden hives compared to those bee species which construct their nests in aerial cavities. Thus, it can be suggested that the preference of *M. lendliana* species to be reared in hives constructed with clay might be explained by its behaviour to nest only in underground hollows. The *H. gribodoi* species showed restriction to be reared into the two vertical compartmented hives icipe 2H and icipe 3H; and preferred the vertical non-compartmented hives, icipe 1H design. A similar tendency of preference to non-compartmented hives, such as the icipe 1M and icipe 2M was also observed in the *M. ferruginea* (black) species. However, the compartmented hive designs which had a brood chamber connected to one or two honey chambers arranged in a vertical alignment seem to be more accepted by *M. ferruginea* (black) compared to the horizontally compartmented hive designs (UTOB, icipe 5M). Stingless bee species in the wild nest mainly in simple cavities that offer them availability to arrange their brood and food stores in such a way that the brood occupies the central part of the nest cavity surrounded by food stores. Such an arrangement of the nest in artificial cavities is readily possible in a non-compartmented hive design rather than the compartmented hive design (Cortopassi-laurino *et al.*, 2006). This might explain why *H. gribodoi* and *M. ferruginea* (black) species preferred mostly the non-compartmented hives compared to the compartmented hive designs. Additionally, the two species seem

not to be specialized to extend their nests in the available adjacent cavities surrounding the main cavity which is used by the colony to establish the brood.

The tendency to mostly prefer the non-compartmented hive to compartmented hive design was not observed in the *M. ferruginea* (reddish brown) species. The latter species showed a contrast compared to *M. lendliana*, *H. gribodoi* and *M. ferruginea* (black) species in preferring both the compartmented and non-compartmented designed hives which were tested for nesting. This tendency which was observed for *M. ferruginea* (reddish brown) to nest in any designed wooden hives supports our earlier suggested argument for this species to be cosmopolitan in nesting sites. The *M. ferruginea* (reddish brown) species seem to have specialized to nest in any type of cavity compared to the other four bee species. In the wild, *M. ferruginea* (reddish brown) was the only species which was found to have a high number of nesting sites recorded among the five studied bee species. Additionally, the latter species nested in several substratum compared to the other four bee species. Moreover, the species was found in the wild to extend its food storage pots into any available adjacent cavity surrounding the cavity they occupied to establish the brood. Lastly, hived *M. bocandei* species were observed to be easily infested by the small hive beetle and the phorid fly larvae which damaged their nests during the first week. Due to attack by these natural enemies, it was not possible to come up with an estimated probability at which *M. bocandei* species accepted the designed hives. However, two colonies of *M. bocandei* hived in the icipe 1M design escaped infestation by the small hive beetle and the phorid flies larvae. Nevertheless, the two colonies nested in the

icipe 1M hive design accepted this latter design. It is here suggested that nest protection from intruders such as small hive beetles and phorid flies in the newly hived *M. bocandei* colonies is not sufficiently assured by the worker bees when a wild colony is hived in artificial cavities.

The average annual honey production in meliponiculture varied within the five stingless bee species and was related to the body size of the bee species. The big and medium sized bee species from the genus *Meliponula* produced more honey compared to the *M. lendliana* and *H. gribodoi* species. However, *M. bocandei* species which has the biggest body size produced the highest amount of honey compared to the other two medium sized *Meliponula* bee species. Within the two morpho species of *M. ferruginea*, the black morpho species produced more honey compared to the reddish brown morpho species. Differences in the quantity of honey produced annually within Neotropical species are also reported in the literature (Cortopassi-laurino *et al.*, 2006). The average annual production of honey in meliponiculture in Brazil among seven species from the genus *Melipona* varied from one litre to three litres and from one litre to about four litres among three species of the genus *Scaptorigona* (Cortopassi-laurino *et al.*, 2006). In the case of this study, three species from the genus *Meliponula* reared showed good potentiality for honey production comparable to some species promoted in Brazil for honey production through meliponiculture. The species *M. ferruginea* (reddish brown) produced an average quantity of honey (1.05 ± 0.07 litres) closely similar to *Melipona asilvai* and *Trigona angustula* (1.0 litre respectively) in Brazil.

The species *M. ferruginea* (black) produced an average quantity of honey (1.37 ± 0.08 litres) closely to *Scaptotrigona postica* (1.5 litres) in Brazil. The *M. bocandei* species produced an average quantity of honey (3.13 ± 0.21 litres) closely similar to *Melipona rufiventris* (3.0 litre) and *Melipona scutellaris* (3.0 litre); but higher than *Meliponula quadrifasciata* (2.0 litres), *Meliponula fasciculate* (2.4 litres) and *Meliponula subnitida* (2.5 litres) in Brazil.

The two morpho species of *M. ferruginea* responded similarly to the three tested propagation methods in this study. It was found that propagation methods that involved the splitting of the mother brood into two equal parts were more successful compared to the method which involved stimulating the colony to build a new nest in a connected empty hive. Within the two propagation methods which involved the splitting of the mother brood into two equal parts; the hive splitting method was more successful compared to the standard brood splitting method. The success of the hive splitting method compared to the standard brood splitting method might be explained by the fact that the former method results into minimum damage and disturbance of the brood nest. Similar justification was reported by Fajardo and Cervancia (2003) suggesting that usual simple manipulation technics which are applied by farmers such as when harvesting stingless bees honey cause destruction of a large portion of the bee nests which then disturbs the colony. Thus, it will take time and energy for the colony to put back in place the nest material (Fajardo and Cervancia, 2003). Additionally, a layer of membranes protection envelopping the brood (involucrum) had to be disturbed and removed when the brood splitting method was applied; thus, brood

could be easily attacked by parasites whose heavy infestation occasioned the absconding of the nested colonies from such hives.

The introduction of a laying queen into a queen less colony of its kind species was successfully accepted by the two morpho bee species of *M. ferruginea* which were reared in this study. This suggested that replacement of a poor queen in a weak colony by a laying queen taken from an active strong colony can be applied in meliponiculture of these two bee species as a procedure to boost the population of bees in colonies.

The use of a feeder placed inside the hive to feed bees was successful in the two morpho species of *M. ferruginea*. However, it required frequent opening of the hive to refill the container. Due to this constraint, the use of an external feeder still remains the best choice as no opening of the hive is required. Feeding of the bees in meliponiculture had an advantage of sustaining the colonies in times of scarcity of nectar or pollen resources (Quezada-euán *et al.*, 2001); thus the practice assists to greatly reduce annual colony losses during drought periods.

CHAPTER EIGHT: NATURAL ENEMIES AFFECTING MELIPONICULTURE OF FIVE STINGLESS BEE SPECIES IN THE KAKAMEGA FOREST

8.1 Introduction

Social insect colonies usually live in nests, which are often invaded by parasitic species (Breed *et al.*, 2004). The presence of predators in the bee colonies imposes an indirect cost to the colony as they reduce a larger number of foragers which gather food and also because predation increases evasive behaviour which decreases foraging efficiency (Wcislo and Schatz, 2002; Rao *et al.*, 2008). Scanty information is available in the literature on natural enemies that affect stingless bee species in meliponiculture across different regions of the world (Koedam *et al.*, 2009). Adult bees, brood and food provision in the nests of most meliponines, attract a broad range of predators; which in some cases cause destruction of the colony (Roubik, 1989; Hölldobler and Wilson, 1990; Breed *et al.*, 2004; Da Silva and Gil-Santana, 2004). According to Wattanachaiyingcharoen and Jongjitvimo (2007), nests of highly eusocial stingless bees attract several predators because they are rich in food resources and provide many sites for reproduction. Man can be considered as the most serious enemy of the native stingless bees because of destruction of the forests and consequently their nests, thus placing them in extinction risk (Da Silva and Gil-Santana, 2004). Natural enemies of stingless bees include ants, anteaters, birds, lizards, spiders, flies (Diptera, Phoridae), termites and pillage bees (Schwarz, 1948; Nogueira-Neto, 1997; Wattanachaiyingcharoen and Jongjitvimo, 2007).

However, to defend themselves and their nests against natural enemies, Meliponinae species have acquired a variety of defensive strategies (protective building behaviour, defensive reactions); in which the abandoning of nests constitute one of the strategies (Kerr and Lello 1962; Kojima, 1993). Stingless bees do mummify arthropod predator's intruder in the nest by burying it alive in resin; this immobilizes the enemy and suffocates the insect to death by depriving it of air (Pasteels *et al.*, 1983; Roubik, 2006).

In Kakamega forest, in western Kenya, no studies have been carried out so far to document arthropod pests and diseases of the stingless bees, the predators and parasites that affect the nested colonies of these bees. Additionally, there is no data available on the organisms that can cause the absconding of an established stingless bee colony by nesting in a section of the bee hive. The present study investigated arthropods and diseases that affect colonies of *Meliponula bocandei* (Spinola, 1853), *M. ferruginea*-reddish brown (Lepeletier, 1841), *M. ferruginea*-black (Smith, 1854), *H. gribodoi* (Magretti, 1884), and *M. lendliana* (Friese, 1900) under meliponiculture in the Kakamega forest. The study also intended to document eventual organisms that cause absconding of an established stingless bee colony through nesting in a section of the bee hive. The knowledge of diseases, parasites and predators will contribute to successful domestication of the stingless bees and prevent the spread of arthropod enemies and diseases that also affect other domesticated insects such as the honey bee *Apis mellifera*.

8.2 Materials and methods

8.2.1 Sampling methods

Different colonies of the five stingless bee species studied were nested in a meliponary from June 2009 to August 2011 and were used to monitor eventual infestation by diseases, parasites and predators. Hive inspections were carried out every day at the entrance tubes while inside hive inspections were done every week to record the presence of intruders (live or mummified) or abnormalities occurring in the nests. Since newly nested colonies are most vulnerable, inside hive inspections were carried out every three days throughout the first month.

Predators such as birds hunting the bees from outside the hive were trapped using local trap displayed in the meliponary; while other flying or walking organisms such as insects were captured manually or with a sweepnet. Their identification was done at icipe and at the Biodiversity Resource Center-IITA. For the abnormalities observed in any part of the nest, samples were taken and kept separately in vials obturated with cotton and brought to icipe's HQ for identification. The number of grubs of an insect such as small hive beetle that were likely to be encountered in the damaged nests due to their infestations were also recorded. The number of flying predators such as wasps hovering in the meliponary were counted during seven consecutive days along two different intervals of time (6:00-12:00am and 12:00-6:00pm). Similarly, the total number of stingless bees of a particular species that were captured daily in a single hive by specific predators were also recorded.

Daily captures of a particular stingless bee species in a single hive by specific predators were recorded continuously through direct observations under undisturbed hunting. The daily abundance of predators such as wasps hunting in the meliponary were recorded by capturing them by the means of entomological nets. Daily records on the abundance of specific predators in the meliponary and the number of individual stingless bees which they captured were carried out in distinctive days and special care was taken so as not to interfere with the two parameters. Photographs of the developing abnormalities, predators, parasites and disturbers in the nest were taken for documentation.

8.2.2 Data analysis

The organisms from the field census were recorded according to their harmfulness to a colony of the stingless bee species. These were classified into groups namely parasites, predators, disturbers or commensals (Hamida, 1999). The relative proportion of species recorded within the identified categories in the different enemy groups was determined. The number of stingless bee species nested in hives that were infested and damaged by a specific parasite was reported according to the proportion. The type of damage which parasites caused to the affected part of the nest was also reported.

Generalized Linear Model (poisson distribution model with logarithm link) was used to model and analyse the data on the number of bees of a specific species that were captured daily in a single hive or within two ranges of the daily intervals of time by specific predators. The number of specific individual predators that

invaded a single hive or was present hunting daily or within the two ranges of the daily interval of time was also analyzed by Generalized Linear Model (poisson distribution model with logarithm link). Differences in means were compared using Tukey's test. The analysis was performed in R statistical software version 2.14.0 (R Development Core Team, 2005).

8.3 Results

8.3.1 Natural enemies

Different natural enemies were recorded from the nested colonies of the stingless bee species and these included parasites, predators and disturbers and all of them were harmful to the stingless bees (Table 8.1).

In the group of parasites, three species were recorded: species that affected either the pillars or the involucrums and species that infested the brood. Among the predators, nine species were recorded and four categories of colony predation were noted: predators of forager bees, predators of pollen storage in pots, predators of honey storage in pots and robbers of nest constructing materials.

The group of disturbers included three species which interfere with the nested colonies through sheltering either in the hive cavity used for honey and pollen storage and in the small cavities that were present within annexes (hive cover sheets) of a designed hive.

Table 8.1: Different groups of enemies, number of species recorded per group and relative proportion of species recorded according to their category

Enemies groups	Species (N)	Category	Proportion (%)
Parasite	1	Parasite of pillars and involucrums (1)	6.25
		Predator of brood (2)	12.5
		Predator of bees (3)	18.75
Predators	12	Predator of pollen (4)	25
		Predator of honey (2)	12.5
		Predator of nest constructing materials (1)	6.25
		Shelter in hive cavities (2)	12.5
Disturbers	3	Shelter in cavity within hive annex (1)	6.25
Total	16	8	100

8.3.2 Parasites of pillars and involucrums

The pillars and involucrums which are the main components in a stingless bee nest were vulnerable to infestation by a yeast in the three species of stingless bees *M. ferruginea* (reddish brown), *M. ferruginea* (black) and *M. lendliana* (Plates 8.1a, 8.1b and 8.1c). During the two years of study in the experimental meliponary, 20.7% of the nested colonies of *M. ferruginea* (black) and 20.2% of the nested colonies of *M. ferruginea* (reddish brown) and 33.3% of the established colonies of *M. lendliana* in the hives were infested by a yeast. Up to 16.7% of the infested nests of *M. ferruginea* (black) and 17.6% of the infested nests of *M. ferruginea* (reddish brown) and 33.3% of the infested nests of *M. lendliana* absconded due to their nests infestation by a yeast (Table 8.2).

It was observed that pillars and involucrums which were infested by the yeast dried up and became inexploitable by the bee colony to build storage pots (Plates 8.1a and 8.1b). This resulted to reduction of the available cavity inside the hive for the bee colony to store food.

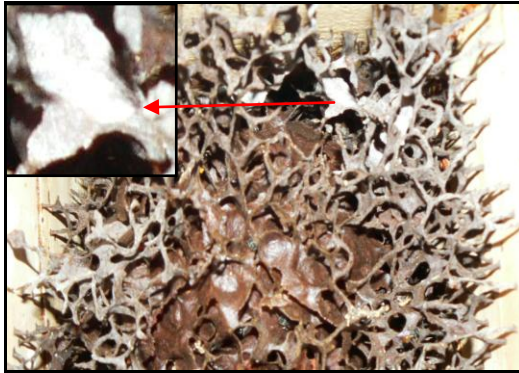


Plate 8.1a: Nest of an established colony of *Meliponula ferruginea* (reddish brown) infested by a yeast (white powdery substance over the nest) in the experimental meliponary at Kakamega forest.

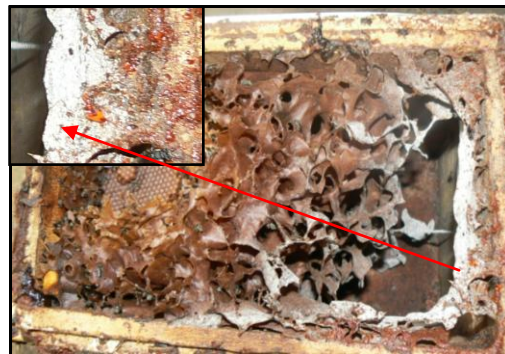


Plate 8.1b: Nest of an established colony of *Meliponula ferruginea* (black) infested by a yeast (white powdery substance over the nest) in the experimental meliponary at Kakamega forest.

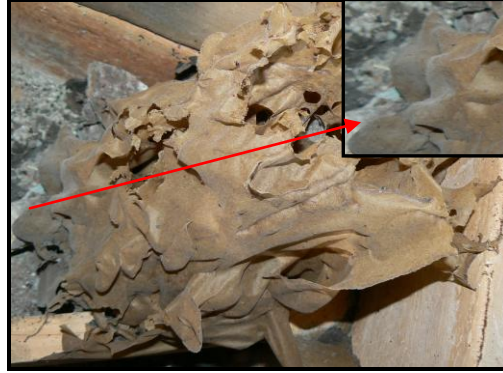


Plate 8.1c: Abandoned nest of an established colony of *Meliponula lendliana* in a pot hive infested by a yeast in the experimental meliponary at Kakamega forest.

Table 8.2: Nest infestation and subsequent damage by a yeast in hives of the five stingless bee species in a meliponary in the Kakamega forest.

Bee species	Number of established colony (N)	Incidence of the yeast	
		Infested (%)	Damaged (%)
<i>M. bocandei</i>	3	-	-
<i>M. ferruginea</i> ¹	29	20.7 (6)	16.7 (1)
<i>M. ferruginea</i> ²	84	20.2 (17)	17.6 (3)
<i>M. lendliana</i>	9	33.3 (3)	33.3 (1)
<i>H. gribodoi</i>	9	-	-

Values in the parentheses are the number of nests recorded

8.3.3 Stingless bees predators

Predators that hunted the domesticated stingless bees in the meliponary at Kakamega forest belonged to five categories of natural enemies namely: predators of the brood, predators of forager bees, predators that rob pollen stored in pots, predators that rob honey stored in pots and those that rob nest constructing materials.

8.3.3.1 Predators of the brood

Brood in the nests of *Meliponula bocandei*, *M. ferruginea* (reddish brown), *M. ferruginea* (black), *M. lendliana* and *Hypotrigona gribodoi* stingless bee species were vulnerable to invasion by *Aethina tumida* Murray larvae and phorid fly *Megaselia scalaris* Loew larvae.

a) Larvae of the small hive beetle *Aethina tumida*

Aethina tumida Murray larvae (Coleoptera: Nitidulidae) hatched from eggs that were laid in the nest by the adult beetle females which intruded into the hive to feed on the pollen stored by the bee colony. In the hives where gaps in joints were sealed with a masking tape; *A. tumida* adults entered into the hives through the nest entrance. The *A. tumida* larvae fed on the brood of the stingless bees until they reached pupal stage. The impact was severe as it resulted to the rotting of the nest, which dried over time. Several individuals of bees died in the nest and the remaining live bees absconded from the hives (Plate 8.2).



Plate 8.2: Larvae of *Aethina tumida* had infested a newly nested colony of *M. bocandei* in a hive box in the experimental meliponary at Kakamega forest

The newly nested and the established colonies were both affected at different rates; but the newly nested colonies were most vulnerable to *A. tumida* larvae infestation (Table 8.3). The highest proportion of nest infestation by *A. tumida* larvae was observed in the newly nested colonies in both the split (92.3%) unsplit (90.5%) nests of *M. bocandei*, followed by the split and unsplit nests of both the newly and the established *M. ferruginea* (black) colonies as well as the newly nested unsplit *M. lendliana* colonies. Nest infestation was low in the newly nested and the established unsplit *M. ferruginea* (reddish brown) colonies. The proportion of the nest damage (colony lost) in regard to the total number of infested colonies was 100% in all the four stingless bee species.

Moreover, the mean number of beetle larvae at their second instar stage of development which was recorded in the infested nests was significantly different between the four stingless bee species ($\chi^2 = 64.35$; $df = 3, 12$; $P < 0.001$) (Figure 8.1); and the infested *M. bocandei* nests had the highest average number of the second instar beetle larvae (346.25 ± 9.30) compared to that of *M. ferruginea* (black) (228.25 ± 7.55), *M. ferruginea* (reddish brown) (223.75 ± 7.48) and *M. lendliana* (200.75 ± 7.08). The average number of *A. tumida* larvae recorded in the damaged nests was not significantly different between the three latter stingless bee species.

Table 8.3: Occurrence of nest infestation and nest damage in the hives of a newly nested colony and established colony of five stingless bee species by *Aethina tumida* larvae in a meliponary established at Kakamega forest

Bee species	Type of nest	Category of colony	N	Incidence	
				Infested (%)	Damaged (%)
<i>M. bocandei</i>	Split	Newly nested	13	92.3 (12)	100 (12)
		Established	1	-	-
	Unsplit	Newly nested	21	90.5 (19)	100 (19)
		Established	2	-	-
<i>M. ferruginea</i> (black)	Split	Newly nested	10	40 (4)	100 (4)
		Established	6	33.3 (2)	100 (2)
	Unsplit	Newly nested	35	28.6 (10)	100 (10)
		Established	23	17.4 (4)	100 (4)
<i>M. ferruginea</i> (reddish brown)	Split	Newly nested	17	11.8 (2)	100 (2)
		Established	11	9.1 (1)	100 (1)
	Unsplit	Newly nested	76	2.6 (2)	100 (2)
		Established	73	1.4 (1)	100 (1)
<i>M. lendliana</i>	Split	Newly nested	-	-	-
		Established	-	-	-
	Unsplit	Newly nested	25	16 (4)	100 (4)
		Established	9	-	-
<i>H. gribodoi</i>	Split	Newly nested	5	-	-
		Established	3	-	-
	Unsplit	Newly nested	28	-	-
		Established	6	-	-

Values in the parentheses are the number of nest recorded

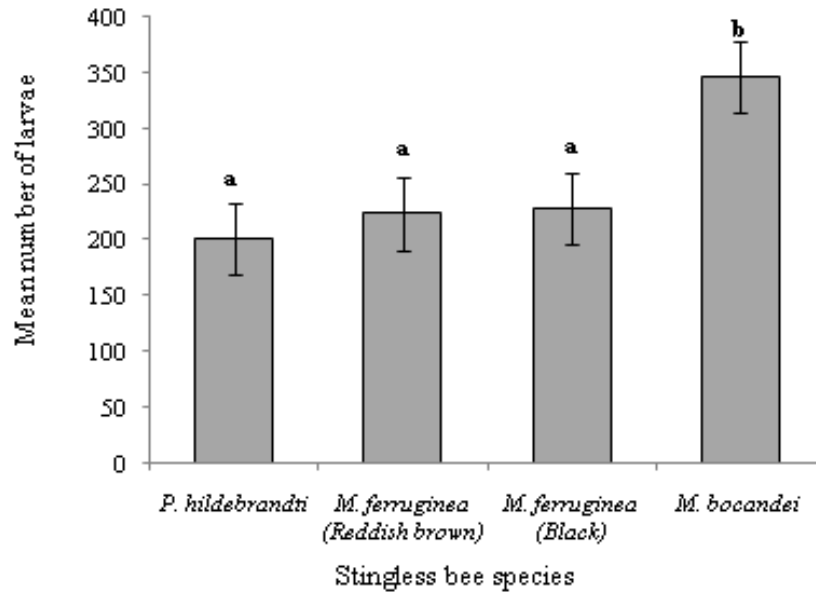


Figure 8.1: Mean number of *Aethina tumida* larvae in an infested nest in the hive among the stingless bee species

b) Larvae of the phorid fly *Megaselia scalaris*

The phorid fly *Megaselia scalaris* Loew Larvae (Diptera: Phoridae) affected the nested colonies of all the five stingless bee species when the adult individuals of *M. scalaris* intruded into the stingless bee hives to feed on food which was stored by the colony. In hives where all gaps were sealed with a masking tape, *M. scalaris* adults entered them through the nest entrance. The *M. scalaris* adult females laid numerous eggs on the substrate of the stingless bee nests such as damaged pollen pots. Larvae fed on the brood of the stingless bees nest until they reached the pupal stage.

Invasions of a stingless bee nests by *M. scalaris* larvae occurred simultaneously with the nest invasion by the small hive beetle, *A. tumida* larvae in the Kakamega forest. The newly nested and the established colonies of the stingless bee species

in the Kakamega forest were both affected at different rates. However the newly nested colonies were the most vulnerable to invasion by *M. scalaris* larvae due to intrusion by the adult phorid fly (Table 8.4). Nest infestation in the hives by *M. scalaris* larvae were higher in the split (92.3%) and unsplit (90.5%) nests of the newly harvested *M. bocandei* colonies compared to the other stingless bee species. However, nest infestation was low in the split and unsplit newly nested and the established *M. ferruginea* (reddish brown) colonies.

Table 8.4: Occurrence of nest infestation and nest damage in the hives of a newly nested and established colony of the five stingless bee species by *Megaselia scalaris* larvae in a meliponary established at Kakamega forest

Bee species	Type of nest	Category of colony	N	Incidence	
				Infested (%)	Damaged (%)
<i>M. bocandei</i>	Split	Newly nested	13	92.3 (12)	100 (12)
		Established	1	-	-
	Unsplit	Newly nested	21	90.5 (19)	100 (19)
		Established	2	-	-
<i>M. ferruginea</i> (black)	Split	Newly nested	10	40 (4)	100 (4)
		Established	6	33.3 (2)	100 (2)
	Unsplit	Newly nested	35	28.6 (10)	100 (10)
		Established	23	17.4 (4)	100 (4)
<i>M. ferruginea</i> (reddish brown)	Split	Newly nested	17	11.8 (2)	100 (2)
		Established	11	9.1 (1)	100 (1)
	Unsplit	Newly nested	76	2.6 (2)	100 (2)
		Established	73	1.4 (1)	100 (1)
<i>M. lendliana</i>	Split	Newly nested	-	-	-
		Established	-	-	-
	Unsplit	Newly nested	25	16 (4)	100 (4)
		Established	9	-	-
<i>H. gribodoi</i>	Split	Newly nested	5	40 (2)	100(2)
		Established	3	-	-
	Unsplit	Newly nested	28	7.1 (2)	100(2)
		Established	6	-	-

Values in the parentheses are the number of nests recorded

8.3.3.2 Hunters of forager bees

Worker bees flying in-and-out of the nest were vulnerable to predators namely, *Philanthus* sp. wasp, the fly catcher bird *Elminia longicauda teresita* Antinori and the ant *Myrmecaria* sp.

a) *Philanthus* sp.

Among the five studied stingless bee species, *M. ferruginea* (black) was a prey to *Philanthus* sp. wasp (Hymenoptera: Crabronidae) which is also referred to as bee wolf. The wasp hunts forager bees flying out and returning into their hives by hovering diagonally closer (up to 7 cm closer) to the nest entrance of the targeted colony (Plate 8.3).

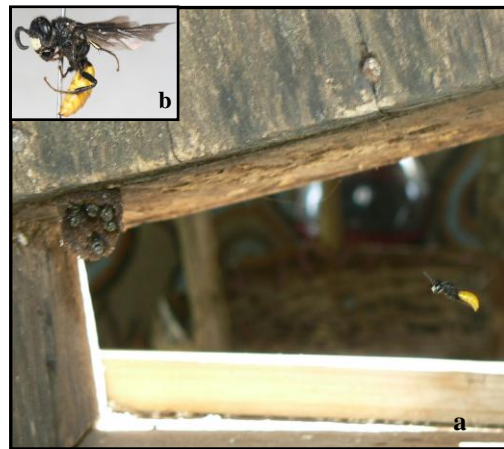


Plate 8.3: a) *Philanthus* sp. hunting *M. ferruginea* (black) at their nest entrance; b) lateral view of a specimen of *Philanthus* sp.

The number of *Philanthus* sp. wasps hovering daily in the meliponary averaged 21.3 ± 0.92 , and no significant difference was observed between the number of individuals hovering before noon (9.6 ± 1.1) and afternoon (11 ± 1.2) ($\chi^2 = 1.51$;

df = 1, 14; P = 0.219) (Table 8.5). The mean number of *M. ferruginea* (black) forager bees captured daily from a single hive by *Philanthus* sp averaged 5.8 ± 0.80 ; a significant difference was observed between the number captured before noon (2.0 ± 0.38) and afternoon (3.8 ± 0.53) ($\chi^2 = 7.68$; df = 1, 8; P = 0.024) (Table 8.5). The highest number of *M. ferruginea* (black) forager bees captured by *Philanthus* sp was observed during afternoon hours.

Table 8.5: Mean number of *M. ferruginea* (black) forager bees captured daily before and after noon by *Philanthus* sp in a single hive and the mean number of *Philanthus* sp hovering daily before and after noon in the meliponary at Kakamega forest

Bee species	Average number of individual (\pm SE)		
	6:00 am-12:00 am	12:00 am- 6:00 pm	Daily
<i>M. ferruginea</i> (black)	2.0 ± 0.38^a	3.8 ± 0.53^b	5.8 ± 0.80
<i>Philanthus</i> sp	9.6 ± 1.10^a	11.6 ± 1.21^a	21.3 ± 0.92

Values within rows followed by a same letters are not significantly different

b) *Elminia longicauda teresita*

Elminia longicauda teresita Antinori (Passeriformes: Monarchidae) is a small African bird commonly known as the African blue fly catcher bird (Plate 8.4) and was commonly found perched in the meliponary; hunting forager stingless bees. *M. ferruginea* (black) and *M. ferruginea* (reddish brown) were the only species which were hunted by this bird. *Elminia longicauda teresita* positioned itself up to one metre away from the nest entrance of a targeted colony.

After capturing the individual forager bee, the bird either flew away immediately with its prey to eat it elsewhere or it ate the prey immediately and hunted the next individual



Plate 8.4: *Elminia longicauda teresita* a common hunter of *M. ferruginea* (black) and *M. ferruginea* (reddish brown) bee species in the meliponary at Kakamega forest

The mean number of *M. ferruginea* (black) and *M. ferruginea* (reddish brown) forager bees captured daily averaged 11.2 ± 1.49 and 4.6 ± 0.96 , respectively. A significant difference was observed between the mean number of the two stingless bee species captured by *E. longicauda teresita* ($\chi^2 = 14.22$; $df = 1, 8$; $P < 0.001$). The mean number of the individuals captured daily by *E. longicauda teresita* was higher in the *M. ferruginea* (black) compared to the *M. ferruginea* (reddish brown) bee species.

A significant difference was also observed between the number of forager bees of *M. ferruginea* (black) (4.6 ± 0.96) and *M. ferruginea* (reddish brown) (1.8 ± 0.59) captured before noon ($\chi^2 = 6.34$; $df = 1, 8$; $P = 0.012$). From 12:00 to 6:00 pm, the

number of bees captured by *E. longicauda teresita* was also significantly different between *M. ferruginea* (black) (6.6 ± 1.15) and *M. ferruginea* (reddish brown) (2.8 ± 0.75) ($\chi^2 = 7.91$; $df = 1, 8$; $P = 0.005$). *E. longicauda teresita* showed preferences for *M. ferruginea* (black) forager bees compared to *M. ferruginea* (reddish brown) along the two different time intervals observed daily (Figure 8.2).

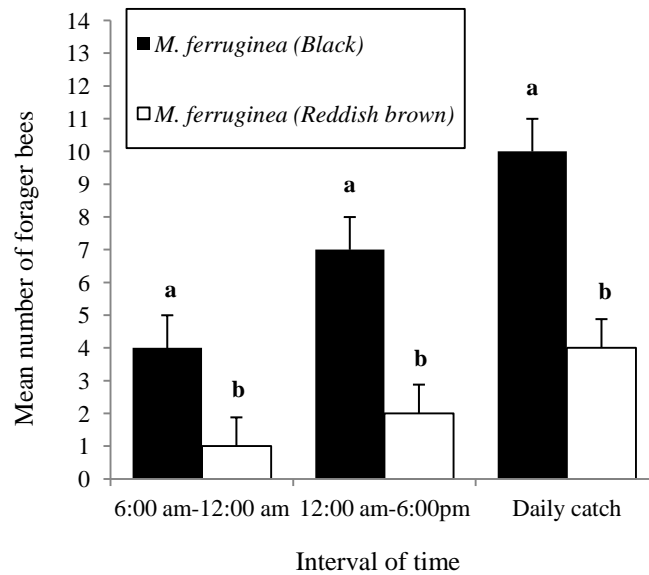


Figure 8.2: Mean number of *M. ferruginea* (black) and *M. ferruginea* (reddish brown) forager bee species captured daily by the African blue fly catcher bird *Elminia longicauda teresita*

c) *Myrmecodia* sp.

Myrmecodia sp. (Hymenoptera: Formicidae) is an African ant species that specifically nests underground and is widely distributed all over the Kakamega forest (Plate 8.5). *Myrmecodia* sp. was the main ant species which was found capturing the stingless bee species in the newly nested hives in which gaps were not completely obtruded. During the harvesting of underground nesting colonies,

Myrmecaria sp. was observed attacking such nests. In the newly nested hives or an established colony, strong invasion of the hive by *Myrmecaria* sp. resulted to the absconding of the nested stingless bee colony.



Plate 8.5: *Myrmecaria* sp. the main ant species that invades stingless bees in hive and captures the bees as prey

8.3.3.3 Predators that rob pollen stored in pots

Three species of Coleoptera namely, *Aethina tumida* Murray, *Rhizoplatys mucronatus* Beauvois, *Tenebroides mauritanicus* Linne and the phorid fly *Megaselia scalaris* Loew were intruders in the stingless bee nests to feed on pollen stored in pots by the colony.

a) Small hive beetle *Aethina tumida*

Aethina tumida Murray adults (Coleoptera: Nitidulidae) (Plate 8.6) commonly found in honey bees hives were also observed as a main predator of food stored in pots (pollen) in the hives of *M. bocandei*, *M. ferruginea* (reddish brown), *M. ferruginea* (black) and *M. lendliana* at Kakamega forest.

In some rare cases, single individuals of *A. tumida* were found mummified in the hives of *M. ferruginea* (reddish brown).



Plate 8.6: Small hive beetle *Aethina tumida* collected inside a stingless bee hive

b) *Rhizoplatys mucronatus*

The Coleoptera *Rhizoplatys mucronatus* Beauvois (Coleoptera: Scarabaeidae) (Plate 8.7) was recorded only in the wild nests of *M. bocandei*, at the Kakamega forest. It was found mummified at the location of pollen pots inside the nest cavity.



Plate 8.7: a) *Rhizoplatys mucronatus* found in a wild nest of *M. bocandei*; b) *Rhizoplatys mucronatus* mummified with resin by *M. bocandei* bees

c) *Tenebroides mauritanicus*

In rare cases, single individuals of *Tenebroides mauritanicus* Linne (Coleoptera: Tenebroididae) (Plate 8.8) were found mummified in hives of *M. ferruginea* (reddish brown) at the location of pollen pots.



Plate 8.8: *Tenebroides mauritanicus* found mummified in a wild nest of *M. ferruginea* (reddish brown)

d) Phorid flies *Megaselia scalaris*

Megaselia scalaris Loew adults (Diptera: Phoridae) (Plate 8.9) were observed to be a main predator of food stored in pots (pollen) in the hives of all the five stingless bee species. The *M. scalaris* adults entered the hives through the nest entrance of the colonies or through the gaps present in the hives. Thus, when the adult individuals of *M. scalaris* intrude into the stingless bee hives the adult females laid numerous eggs on the substrates of the nests (damaged pollen pots). Hatched larvae fed on the brood of the stingless bees nests, and infested stingless bees nest can be damaged in case of severe invasion.



Plate 8.9: *Megaselia scalaris* adult phorid fly scuttling around a pot hive containing a newly nested colony of *M. lendliana* in the meliponary

8.3.3.4 Predators that rob honey stored in pots

The ant *Myrmicaria* sp. and the African honey bee *Apis mellifera* Linnaeus were the two predators recorded who robbed honey inside hives of stingless bees.

a) *Myrmicaria* sp.

The ant *Myrmicaria* sp. (Hymenoptera: Formicidae) was observed to be a main predator that robbed stingless bees their honey stored in pots in the hives of the five stingless bee species in the Kakamega forest. Hive invasion of the established stingless bee colony occurred if gaps in hives were not completely sealed after hive inspection or after honey harvesting. Severe hive invasion by *Myrmicaria* sp. resulted in absconding of the established colony (Plate 8.10).



Plate 8.10: *Myrmecaria* sp. robbing honey from pots in a nest of *M. ferruginea* (reddish brown)

b) *Apis mellifera*

The African honey bee *Apis mellifera* Linnaeus (Hymenoptera: Apidae) was a main predator that robbed stingless bees their honey stored in pots in the nests of the five stingless bees at the Kakamega forest (Plate 8.11). Invasion of an established stingless bee colony by *A. mellifera* mostly occurred when harvesting a nest of a stingless bee colony in the wild and in the hives. Honey bees entered hives through gaps, into newly nested colonies or after harvesting of the honey in an established colony.



Plate 8.11: *Apis mellifera* bees robbing honey from honey pots in an open hive of *M. ferruginea* (black)

8.3.3.5 Predators that rob nest constructing materials

The stingless bee *Meliponula lendliana* Friese (Hymenoptera: Apidae) was one of the predators that frequently robbed propolis from *M. bocandei* hives which were put in place to seal gaps in their hives (Plate 8.12).



Plate 8.12: A *Meliponula lendliana* bee robbing propolis from a hive of an established colony of *M. bocandei* in the meliponary

8.3.4 Disturbers

Three different species of insects belonging to the Order Hymenoptera were observed frequenting shelters in the stingless bee hives that contained a newly nested or weak established colony. In most of the cases, their presence in the hives occasioned absconding of the stingless bee colonies. These species included *A. mellifera* Linnaeus (Hymenoptera: Apidae) and two ant species *Lepisiota* sp. (Hymenoptera: Formicidae) and *Camponotus maculatus* Fabricius (Hymenoptera: Formicidae). *Apis mellifera* was found colonizing hive cavities used by the stingless bees to store food (Plate 8.13). On the other hand, colonies of the ant species *Lepisiota* sp. were frequently observed nesting in spaces created by juxtaposed plywood and iron sheets of the cover of icipe 2 hive (Plate 8.14). In contrast, the ant species *C. maculatus* was found nesting in all the small hives designed for *Hypotrigona gribodoi* (Plate 8.15).



Plate 8.13: A colony of *Apis mellifera* nesting inside an icipe 2 designed hive. The hive was previously occupied by a weak colony of *M. ferruginea* (reddish brown) in the meliponary



Plate 8.14: *Lepisiota* sp. one of the ant species that frequently nested in the cover of icipe 2 hive



Plate 8.15: A small colony of *Camponotus maculatus* nesting in the storage chamber of an icipe 3 hive previously occupied by a weak colony of *H. gribodoi*

8.4 Discussion

This study was the first of its kind to record natural enemies that affect domesticated colonies of the stingless bees *M. bocandei*, *M. ferruginea* (reddish brown), *M. ferruginea* (black), *H. gribodoi*, and *M. lendliana* under meliponiculture at Kakamega forest in Western Kenya. The natural enemies recorded in this study included parasites, predators and hive shelter insects. Stingless bees nest infestation by yeast has not been reported in the literature as a pathogen damaging stingless bee nests in Neotropical regions.

The stingless bees nest infestation by the non identified yeast in the Kakamega forest could be occasioned by factors such as nature of the material foraged by the bees to build pillars and involucrum of their nests, hive intruders such as small hive beetle or forager bees of the colony and high temperatures and humidity. The small hive beetle *Aethina tumida* and the phorid flies *Megaselia scalaris* larvae were natural enemies that infested stingless bee brood in hives and their adults feeds on food which was stored in the pots by the bees. Colonies infestation by small hive beetle and phorid flies larvae as well as by the adults are also reported in Neotropical regions. Sommeijer (1999) and González-Acereto *et al.* (2006) reported that, hive invasion by the phorid fly *Pseudohypocera kerteszi* is the most severe danger that faces meliponuculture in South America. Hive intrusion by small hive beetles and phorid flies are also reported in meliponuculture in Australia and heavy infestation of the nest can lead to death of the whole colony (Klumpp, 2007).

Aethina tumida adults were not observed in the nests inside the *H. gribodoi* hives; and this may be explained by the small size of the open tube of their nest entrances ($0.10 \pm 0.01\text{cm}^2$). Couvillon *et al.* (2008) reported that, within stingless bee species, smaller nest entrances are easier to defend from intruders by guard bees of the colony. Such protective mechanism has also been reported in the ant species *Temnothorax curvispinosus* that frequently reduce the size of the nest entrance until it is small enough to be secured from intruders (Visscher, 2007). Additionally, it was observed in this study that, the newly nested and the established colonies of *M. ferruginea* (reddish brown) were less affected by the

two brood natural enemies. This can be explained by the fact that when nested in a new hive, newly introduced colony of *M. ferruginea* (reddish brown) immediately started to secure the nest entrance hole drilled in the new hive, construction of layers of involucrum that protect the brood, grooming, obtrude of gaps in the hive and prolongation of the drilled nest entrance hole with resin. The results obtained from this study suggested that nest protection in hives against intrusion by *A. tumida* and *M. scalaris* adults might be more efficient in *M. ferruginea* (reddish brown) species compared to the three others species whose nests were also invaded by the two brood natural enemies.

Myrmecaria sp., *E. longicauda teresita* and *Philanthus* sp were recorded as the main predators which hunted adult forager stingless bees. The ant *Myrmecaria* sp. was frequently found capturing dead and live worker stingless bees from all the five species that fall on the ground or from the invaded hives. This suggests that *Myrmecaria* sp had no preference for particular stingless bee species. According to Hölldobler and Wilson (1990), ants are among the most dominant and important predators of stingless bees in tropical rainforests as stingless bee colonies provide profitable resources (bees, brood, nectar) for ants. The weaver ant, *Oecophylla smaragdina*, and the fire ant, *Solenopsis geminate*, have been reported as main predators of the stingless bee *Trigona collina* in Thailand (Wattanachaiyingcharoen and Jongjitvimo, 2007). In response to ant predation, some social insects flee and abandon the nest (Wenzel and Pickering, 1991; Kojima, 1993). Similar behaviour was observed in the current studied stingless bee species when their nests were invaded by ants.

Elminia longicauda teresita showed preference for hunting both morpho species of *M. ferruginea* but mainly *M. ferruginea* (black). It is therefore suggested that it might be possible that *E. longicauda teresita* uses chemical cues which are produced by the stingless bee species to locate, track and differentiate *M. ferruginea* (black) and *M. ferruginea* (reddish brown) bees from the other bees. It is further suggest that chemical cues might be similar in both morpho species of *M. ferruginea* and they might be more pronounced in *M. ferruginea* (black), thus a reason why this species was mostly hunted by *E. longicauda teresita*. Predation on species specific stingless bees by birds has also been reported in the Neotropical region. For instance, *Merops leschenalti* (bee-eater), *Cypsiurus balasi-ensis* (asian palm-swift), *Dicrurus macro-cercus* (black drongo) and *D. paradiseus* (greater racket-tailed drongo) bird species have been reported to hunt *T. collina* in Thailand (Wattanachaiyingcharoen and Jongjitvimo, 2007).

Nevertheless, the bee wolf *Philanthus* sp predated specifically on *M. ferruginea* (black) and the wasp was never observed when a colony of *M. ferruginea* (black) was not present in the meliponary at the Kakamega forest. This also suggests that *M. ferruginea* (black) might be a specific prey within the five studied stingless bee species for the beewolf *Philanthus* sp in Kakamega forest. Koedam *et al.* (2009) suggested that wasps use chemical cues from the bee to recognize their suitable prey. The cuticle of a social insect presents a blend of hydrocarbon compounds that carry information indicating colony of origin, sex, caste, age and group-specific task (Koedam *et al.*, 2009). In stingless bees, differences in cuticular hydrocarbons have been found between meliponine bees such as

Melipona bicolor, *Melipona scutellaris*, *Schwarziana quadripunctata* and *Frieseomelitta varia* (Abdalla *et al.*, 2003; Kerr *et al.*, 2004; Nunes *et al.*, 2009a; Nunes *et al.*, 2009b). This difference in chemical cues between meliponine bees might be an element in which *Philanthus* sp also make use of to locate, track and distinguish *M. ferruginea* (black) from other meliponine species in the meliponary at Kakamega forest. Wasp predators of stingless bees are also reported in meliponiculture at Neotropical regions. For example, the solitary wasp *Trachypus boharti* (Hymenoptera, Cabronidae) is reported to specifically prey on males of the stingless bee *Scaptotrigona postica* waiting for virgin queens that initiate their nuptial flight (Koedam *et al.*, 2009). According to Giannotti and Pinto (2001), *T. boharti* wasp exclusively preys on males of *S. postica* and not on the worker bees of this species. According to Koedam *et al.* (2009), there is a distinct and straight forward caste-specific difference in cuticular hydrocarbons between workers and males of *S. postica* which could function as a recognition cue by *T. boharti*.

Apis mellifera and the ant *Myrmecaria* sp were found to be among the group of predators that robbed honey stored in the nests of an established stingless bee colony. Robbery of honey in stingless bee hives usually occurred when gaps in hive were not completely sealed after doing a hive inspection or during honey and colony harvesting. Robbery of stored food such as honey from nests of other Apidae species is reported to be a behavioral pattern commonly found in *A. mellifera* (Bohart, 1970) and in ants (Hölldobler and Wilson, 1990). Ant species such as *Formica rufa*, *Formica sanguinea*, *Formica fusca*, *Lasius niger* are reported to disturb *A. mellifera* colonies in their eagerness to steal honey (Santis

and De Regalia, 1978). According to Bohart (1970), *A. mellifera* bees usually rob from other bees colony during periods of poor natural forage, and sometimes populous colonies completely rob the stores of weaker ones, leaving them to starve.

Robbery of nest building materials by stingless bee was observed in *M. lendliana* where individual forager bees frequently robbed propolis from *M. bocandei* hives without causing damage. Bohart (1970) reported that individual bees of a large assemblage of tropical stingless bees usually try to rob nest building materials and pollen as well as honey from any stingless bee colonies in the vicinity, regardless of the species. However, *Lestrimelitta limao*, a cleptobiotic social stingless bee is well known in Neotropical region as the main predator that invades nests of other stingless bee colonies such as *Trigona angustula* and cause damages during the robbery of the nest constructing material, food supplies and larval provisions (Wittmann, 1985).

The ant *Camponotus maculatus* species attempted to nest at the corners inside the hives which were occupied by very weak colonies of *H. gribodoi*; while *Lepisiota* sp attempted to nest in spaces between the iron sheet and plywood cover board of the icipe 2 hive design. Hamida (1999) reported that some ants establish their nests in bee hives to take advantage of the warm, humid environment, which provides them with optimal nesting conditions. According to the latter author, queen mating nuclei with very small populations of bees are most vulnerable to attack by ants. However, *A. mellifera* attempted to nest in the different designed hives occupied by very weak stingless bee colonies.

CHAPTER NINE: ENHANCEMENT OF FRUIT QUALITY IN GREEN PEPPER THROUGH POLLINATION BY *Hypotrigona gribodoi* IN SMALL-SCALE FARMS IN KAKAMEGA, WESTERN KENYA

9.1 Introduction

The green pepper, *Capsicum annum* L. (Solanaceae) originated from Central America and nowadays it is cultivated worldwide (Pesson and Louveaux, 1984; Cruz *et al.*, 2005). Flowers of this plant are autogamous and the opening of blooms occurs early in the morning during the sunrise (Pesson and Louveaux, 1984; Cruz, 2003). In the open fields, insects such as apoïdes, thrips and ants visit green pepper flowers for their pollen and nectar (Pesson and Louveaux, 1984).

In Kakamega, western region of Kenya, green pepper is one of the main spicy crops cultivated by small scale farmers, mainly for their own consumption and the surplus for market (Kasina *et al.*, 2009). However, the use of a specific pollinator in green pepper farm in western Kenya is unknown and the crop relies entirely on feral pollinators (feral bees and other biotic pollinators) supported by the nearby habitats (Kasina *et al.*, 2009). Feral pollinators that visit flowers of green pepper grown on the farm land in this region gives an economic benefit to the small-holder farmers by improving the quality of the fruits which in turn enhance their prices in the local market (Kasina *et al.*, 2009). Almost 40% of the annual value of farmers represented the net returns derived from feral pollinators; and 99% of this benefit is attributed to pollination by feral bees (Kasina *et al.*, 2009).

The interest in pollination of green pepper has recently been raised worldwide due to the need for adequate pollination levels in an environment such as greenhouses (Cruz *et al.*, 2005), in addition to the concern of the decline of managed and wild honey bees due to diseases (Steffan-Dewenter *et al.*; 2005; Villanueva *et al.*, 2005). Thus, honey bees might not be the ‘be-all’ and ‘end-all’ of crop pollination (Roubik, 1995); there is the need to find alternative pollinators. Stingless bees are frequently reported to be good candidates for future alternatives in commercial pollination (Cunha *et al.*, 2002; Cortopassi-Laurino *et al.*, 2006; Slaa *et al.*, 2006; Cock *et al.*, 2011). Compared to the honey bees, stingless bees display greater diet breadth and range of foraging behaviour (Heard, 1999).

Nevertheless, the evidences of their importance and effectiveness as crop pollinators in setting fruits of several crops are unknown (Cruz *et al.*, 2005). According to Slaa *et al.* (2006), the pollination effectiveness of a specific stingless bee species depends very much on the crop species. The use of stingless bees for crops pollination in the African continent is lacking due to the gaps of research in the domestication of the African stingless bees (Raina *et al.*, 2009). Over twenty species of stingless bees have been described in the African continent (Eardley, 2004); among them *H. gribodoi* being the species reported to be abundantly found nesting in wall crevices of human residential houses (Kajobe, 2007). In Western Kenya, *H. gribodoi* is commonly found foraging on flowers of cultivated crops in homesteads, in contrast to *Meliponula ferruginea* (reddish brown) a species which also nests in wall cavities of human residential houses in Kakamega forest.

The present study investigated the efficiency of *H. gribodoi* to improve, through floral pollination, the quality of green pepper (*Capsicum annum* L.) fruits and seeds set in Kakamega. Parameters were compared with those from unmanaged pollination (feral pollinators) and self-pollination. The evidence of *H. gribodoi* as pollinator of green pepper is likely to contribute to its conservation in the region where it faces threats arising from forest habitat lost through anthropogenic activities.

9.2 Materials and methods

9.2.1 Crop planting and establishment of experimental plots

Seeds of green pepper (*Capsicum annum*) commonly known as California Wonder were used for this experiment and were bought at the Kenya Seed Company. They were planted in April 2011 during the rainy season. Experimental plots (Plate 9.1 a) were located on a farm land neighbouring the Kakamega forest at Isiekuti village. A total of fifteen experimental plots of 4 m² each and 1.5 meter apart were established and six healthy seedlings of the green pepper were planted. In all the plots, plants were arranged in two rows, with a sixty-five centimeter wide aisle between rows and fifty centimeter within plants. In all plots, plants were arranged in two rows, with a sixty-five centimeter wide aisle between rows and fifty centimeter within plants. Double ammonium phosphate (5grams per plants) was applied during planting. Calcium ammonium nitrogen (5 grams per plant) was applied as a top dressing two weeks after transplanting. Appropriate pesticides (Tututhrile) and fungicides (Ritomil, Galben, milbhene) were applied

every two weeks until approximately one month before apparition of flower bug; and the weeding of the plots was done whenever it was necessary. Feral pollinators, *H. gribodoi* and self-pollination treatments were randomly applied and were repeated five times. All plots (repetitions) destined to be pollinated by *H. gribodoi* were covered with a net cage (2.0 m x 2.0 m x 2.5 m) the day a hive containing a nested colony of the bee species was introduced (Plate 9.1b). Only a single hive containing a weak established colony of *H. gribodoi* (approximately less than 200 individuals) was introduced in each plot approximately two weeks before the opening of the first bloom. Plots whose plants were destined to be self-pollinated were covered with a net cage two weeks before the opening of the blooms and no bee colonies were introduced. However, all plots reserved for feral pollinators were partially covered with a net cage up to 45 cm above the canopy of the plants to allow them be pollinate by feral pollinators and create ambient conditions similar to the two previous treatments.



Plate 9.1: Trial on crops pollination: a) experimental plots and b) an experimental plot of green pepper containing a hive with a weak colony of *H. gribodoi*

9.2.2 Data collection

Assessment of pollination of the green pepper within the three treatments was based on the average weight of the first two fruits produced per plant, average number of seeds per fruit, average weight (dry mass) and average size (length, width) of a seed per fruit per plant. Additionally, the mean number of daily visits made by *H. gribodoi* to collect pollen on the same newly opened green pepper flower was also recorded.

The mean number of daily visits by *H. gribodoi* on the same flower of the green pepper under net cages was carried out using the fixed sample method (Dafni, 1992). This method consists of recording the pollinator behaviours on the foraging flowers during a standard period of time on the same number of flowers (Dafni, 1992). The observation was carried out on a single day due to the fact that daily variation in weather condition can result to variations in foraging activities of the stingless bees. Data was collected from 6:30 am to 6:00 pm under a sunny condition, without rains. However, due to variations in bloom opening within plants; data was collected in four different plots under net cages and in each plot; three newly opened flowers on different plants were observed.

The first two fruits produced per plant in each plot from the different treatments were collected as soon as they attained maturity (Plate 9.2) and their weights taken to the nearest gram using a METTLER laboratory scale (0.0001 gram precision). The number of seeds contained in each of these first fruits were counted and recorded.

Those seeds from the different fruits were then dried in the open air under shades and the total weights of seeds based on dry mass were measured and the mean weight of a seed per treatment determined. The length and width was measured (Plate 9.3) on 25 randomly sampled seeds from each batch of the harvested fruit per plant in each of the five plots for the three treatments to determine the average length and width of a seed per treatment. Measurements were taken using a dissecting microscope model Leica with camera incorporated.

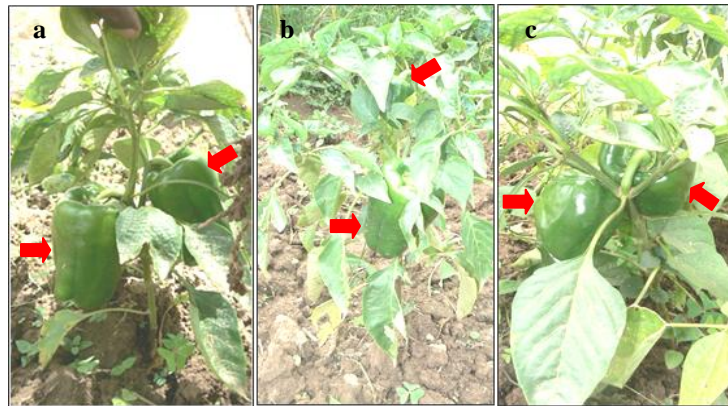


Plate 9.2: a) the two first fruits of green pepper pollinated by *H. gribodoi* under net cage; b) the two first fruits of green pepper pollinated by feral pollinators and c) the two first fruits of green pepper obtained by self-pollination

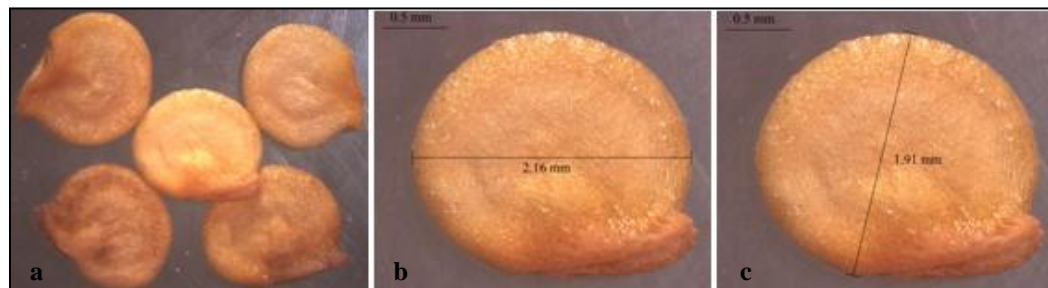


Plate 9.3: Seeds of green pepper a) seeds from a fruit obtained by self-pollinated flower, b) length and c) width. Measurements were taken using a dissecting microscope with camera incorporated

9.2.3 Data analysis

The mean number of daily visits by *H. gribodoi* on the same newly opened green pepper flower was reported as mean daily visit. The average weight of the first two fruits produced per plant and the average number of seeds per fruit within the three treatments were calculated, recorded and compared. The average weight and the size of dry seed per fruit per plant within treatments were calculated, recorded and compared.

ANOVA was used to analyze the data on the means fruits weight per plant and the number of seeds produced per fruit between the three treatments. Tukey test was used to separate means and the level of significance difference was established as $P \leq 0.05$. The statistical analyses were implemented in R software 2.14.0.

9.3 Results

9.3.1 Mean number of daily visits on the same newly opened flower

Forager *H. gribodoi* bee workers visited green pepper plants under the net cage to collect pollen from their flowers (Plate 9.4). The mean number of daily visits by *H. gribodoi* recorded on the same newly opened flower averaged 8.6 ± 0.68 visits. However, on daily basis forager bees of *H. gribodoi* visited the same newly opened flower up to a maximum of 12 times and a minimum of 5 times.



Plate 9.4: A worker bee of *H. gribodoi* foraging pollen on a green pepper flower in one of the plots where a hive of an established colony of this species was introduced in the net cage

9.3.2 Average fruits weight

The average weight of the first two fruits of the green pepper produced varied from 141 ± 5.60 grams for the flowers which were pollinated by *H. gribodoi*, 106 ± 2.89 grams for those flowers which were pollinated by feral pollinators and 90.2 ± 2.05 grams for the self-pollinated flowers (Figure 9.1).

A significant difference was observed between the average weight of the first two fruits produced in the three different treatments ($F = 46.65$; $dl = 2, 12$; $P < 0.001$). Fruits were heavier in the green pepper plants whose flowers were pollinated by *H. gribodoi* and those whose flowers were pollinated by the feral pollinators compared to those fruits which were produced from the self-pollinated flowers. The average weight of the first two fruits was heavier in the green pepper plants whose flowers were pollinated by *H. gribodoi* and was lighter for the green pepper plants whose flowers were pollinated by feral pollinators in the open field.

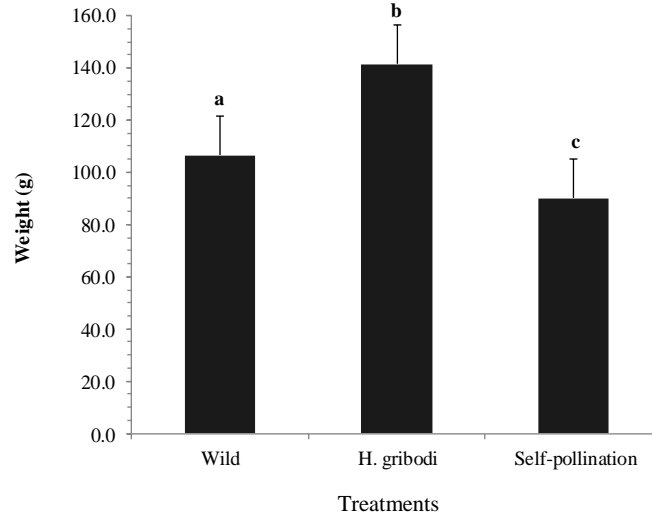


Figure 9.1: Average weight of the first two fruits of green pepper which were produced through pollination by feral pollinators in the open field, by *H. gribodoi* in the net cage and the self-pollinated flowers in the net cage in a small scale farm in the Kakamega forest, western Kenya

9.3.3 Average number of viable seeds per fruit

The average number of seeds set by the first two green pepper fruits ranged from 201 ± 11.06 for the flowers pollinated by *H. gribodoi*, 159.4 ± 14.02 for those which were pollinated by feral pollinators and 109.3 ± 3.94 for the self-pollinated flowers (Figure 9.2).

A significant difference was observed within the average number of seeds set by fruits in the three different treatments ($F = 18.91$; $df = 2, 12$; $P < 0.001$). The average number of seeds set was higher in the green pepper fruits whose flowers were pollinated by *H. gribodoi* and those pollinated by feral pollinators compared to those which were self-pollinated. However, the average number of seeds set in

the first two fruits was higher in the green pepper plant whose flowers were pollinated by *H. gribodoi* and was less in the green pepper plant whose flowers were pollinated by feral pollinators in the open field.

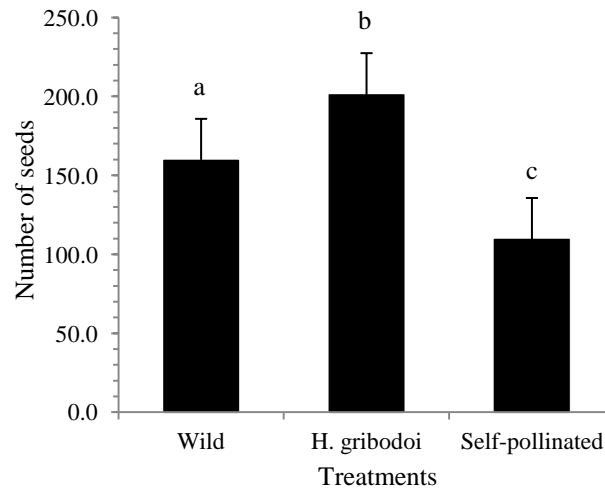


Figure 9.2: Average number of seeds produced in the first two fruits of green pepper through pollination by feral pollinators in the open field, by *H. gribodoi* in the net cage and self-pollinated flowers in the net cage in a small scale in the Kakamega forest, western Kenya

9.3.4 Average weight of seeds (dry mass)

The average weight of a single dry seed per fruit from the first two fruits of green pepper ranged from 5.46 ± 0.09 mg for the flowers which were pollinated by *H. gribodoi*, 4.68 ± 0.04 mg for those pollinated by feral pollinators and 404 ± 0.05 mg for the self-pollinated flowers (Figure 9.3). A significant difference was observed within the average weight of a single seed from the first two fruits which were produced in the three different treatments ($F = 103.92$; $df = 2, 12$; $P < 0.001$). The average weight of a single seed (dry mass) was heavier in the green

pepper plant whose flowers were pollinated by *H. gribodoi* and by feral pollinators compared to the green pepper plants' seeds whose flowers were self-pollinated. Similarly, the average weight of a single dry seed from the fruits produced from flowers pollinated by feral pollinators was lighter compared to those which were pollinated by *H. gribodoi*.

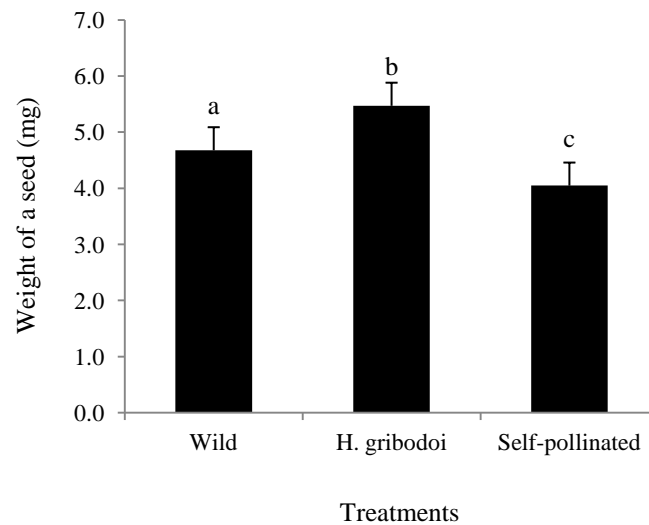


Figure 9.3: Average weight of a seed (dry mass) from the first two fruits of green pepper produced through pollination by feral pollinators in the open field by *H. gribodoi* in the net cage and the self-pollinated flowers in the net cage in a small scale farm in the Kakamega forest, western Kenya

9.3.5 Average length and width of seeds per fruit

The average length and width of a seed from the first two fruits of the green pepper produced varied within the three treatments (Table 9.1). The average length of seeds were significantly different within the fruits which were produced from the flowers pollinated by *H. gribodoi*, feral pollinators and self-pollinated flowers ($F = 163.13$; $df = 2, 12$; $P < 0.001$).

Seeds were longer in the green pepper plants whose flowers were pollinated by *H. gribodoi* (4.3 ± 0.04 mm) and by the feral pollinators in the open field (4.0 ± 0.0 mm). However, seeds from the fruits of green pepper obtained from flowers pollinated by feral pollinators in the open field were shorter (4.0 ± 0.0 mm) compared to those seeds from the flowers pollinated by *H. gribodoi*.

A significant difference was also observed in the average width of the seeds from fruits produced within the three treatments ($F = 111.4$; $df = 2, 12$; $P < 0.001$). The average width of seeds in the fruits produced from green pepper plants whose flowers were pollinated by *H. gribodoi* was higher ($3.69 \pm$ mm) compared to the seeds from the fruits of green pepper plants whose flowers were pollinated by the feral pollinators (3.27 ± 0.0 mm) or were self-pollinated (3.18 ± 0.0 mm). No differences were observed in the mean width within seeds from the fruits of green pepper plants whose flowers were pollinated by feral pollinators or were self-pollinated. As such, seeds contained in the fruits produced by the plants whose flowers were pollinated by *H. gribodoi* were bigger than the seeds in the fruits which were produced by plants whose flowers were either pollinated by feral pollinators or were self-pollinated.

Table 9.1: Average length and width of a seed produced from the first two fruits of green pepper through pollination by feral pollinators in open field, *H. gribodoi* in the net cage and self-pollinated flowers in net cage in a small scale farm in Kakamega forest

Treatments	Number of plots	Average size of a seed	
		Length (mm)	Width (mm)
<i>H. gribodoi</i>	5	4.30 ± 0.029 ^a	3.69 ± 0.042 ^a
Feral pollinators	5	3.98 ± 0.006 ^b	3.28 ± 0.012 ^b
Self-pollination	5	3.85 ± 0.006 ^c	3.18 ± 0.007 ^b

9.4 Discussion

The aim of this study was to compare fruits and seeds quality among green pepper plants grown in plots whose flowers are self-pollinated, pollinated by feral pollinators in the open field and those which were pollinated by *H. gribodoi* stingless bee species. It was observed that the heaviest green pepper fruits were those which were produced from the flowers pollinated by *H. gribodoi* compared to the other two treatments. The highest number of seeds set per fruit was also obtained from the plants whose flowers were pollinated by *H. gribodoi*. The seed set in the fruits from the flowers pollinated by *H. gribodoi* were also heavier (dry mass) and bigger than those which were obtained from the fruits produced from the self-pollinated flowers and those which were pollinated by feral pollinators. Differences in seeds set per fruit due to floral visits by a specific pollinator are reported as a consequence of the quantity of pollen grain deposited on stigmas of flowers which are visited by the pollinators (Serrano and Guerra-Sanz, 2006). Cruz *et al.* (2005) reported that seeds play an important role in fruit setting

processes; and bad developed fruits are the result of an unequal seed distribution inside the fruit. Thus, in a well-pollinated flower, a rapid development of ovary occurs, and the fecundated seeds produce plant growth hormones, leading to a good fruit development (Cruz *et al.*, 2005). These findings suggest that deposition of pollen grains in stigmas might be higher in the green pepper flowers pollinated by *H. gribodoi*; thus resulting in the setting of more seeds of better quality per fruit. As a consequence, fruits produced from the flowers pollinated by *H. gribodoi* were bigger compared to those from the flowers either pollinated by feral pollinators or self-pollinated.

Several studies have shown that some species of stingless bees are efficient pollinators of economically important crops, including sweet pepper. In Mexico, the stingless bee *Nannotrigona perilampoides* species is reported to be a good alternative pollinator (no significant differences in the quality of fruit produced) as *Bombus impatiens* in the pollination of habanero pepper (*Capsicum chinense*, Jacquin) in the greenhouse under tropical climates (Palma *et al.*, 2008). In Australia, pollination of green pepper by *Trigona carbonaria* increased fruit weight by 11% and the number of seeds/fruit by 34% compared to the crops that were self-pollinated (Occhiuzzi, 2000). In Brazil, Cruz *et al.* (2005) suggested that *Melipona subnitida* can be considered an efficient pollinator of sweet pepper and seed growers could use *M. subnitida* to increase seed production of the All Big sweet pepper variety. According to these authors, green pepper crop benefits from pollination by *M. subnitida*, producing fruits significantly heavier and wider, containing a greater number of seeds and of better quality (lower percentage of

malformed fruits) than those which are produced by the self-pollinated flowers. However, Del Sarto *et al.* (2005) reported that tomato fruits whose flowers were pollinated by the stingless bee *Melipona quadrifasciata* contained 10.8% less seeds than the fruits which were obtained from the manually pollinated flowers. These authors have stipulated that the apparent low efficiency of *M. quadrifasciata* pollination was attributed to the overlap of only 30 minutes between the highest bee foraging activity and the highest flower stigma receptivity.

From the results of this study it is evident that the stingless bee *H. gribodoi* species contributes significantly in increasing fruits and seeds quality of green pepper in small scale farming system compared to the feral pollinators in the open field and the self-pollinated flowers in homesteads neighbouring the Kakamega forest. A nested strong colony of *H. gribodoi* in a wooden hive in an established meliponary at homestead vicinal to the Kakamega forest may produce a small amount of honey that averages 200 ml per year. However, the rearing of *H. gribodoi* in rural homesteads will benefit farmers through flower pollination which will improve the quality of the produce for many cultivated crops. Kasina *et al.* (2009) reported that feral pollinators of green pepper in small scale farms in open field at Kakamega forest give farmers more benefit on their annual value (40%) of the net returns compared to those of the self-pollinated green pepper plant. Here, it is suggested that, the uses of *H. gribodoi* in the pollination of green pepper in small scale farming system in Kakamega can benefit the rural farmers by increasing yields due to their better pollination efficiency compared to the feral pollinators.

CHAPTER TEN: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

10.1 General discussion

There was more diversity of the five stingless bee species in the Kakamega forest at Ivihiga site in the perimeter of the indigenous forest than at the Isiekuti site in the perimeter of the mixed indigenous forest. A decrease in diversity of the five stingless bee species was recorded within the six studied habitats; and the indigenous forest was the most diverse in species. The indication of a high diversity of the five bee species at Ivihiga site where the indigenous forest is located and in the indigenous forest within habitats suggests that these species associate more with the indigenous forest for nesting than with the other types of habitats which are found in the Kakamega forest. Roubik (1989) ascribed this type association of meliponine bees with the local native forest habitat to the fact that most species of stingless bees rely on tropical trees for nesting. Brosi *et al.* (2008) also implied that preferable nesting sites and food (nectar and pollen source) of most meliponine bees are mostly available in the native forest habitat. According to Boontop *et al.* (2008), Nates-Parra *et al.* (2008) and Bommarco *et al.* (2010), natural native forest habitat losses are key factors that led to shifts in the diversity of meliponine bee communities. Thus explains why the five meliponine species rely mostly on the native indigenous Kakamega forest and also why the species number and their nest abundance decreased in the disturbed habitats at Kakamega forest.

Meliponula ferruginea (reddish brown) and *H. gribodoi* species had the highest diversity of nesting sites and substrata compared to the three other species. Velthuis (1997) and Pyper (2001) reported that each species of stingless bees has a preference for specific nesting sites and substratum. The flexibility in nesting sites and nesting substratum observed for *M. ferruginea* (reddish brown) and *H. gribodoi* suggest that they are less affected by habitat loss in the Kakamga forest than the other three bee species.

Four meliponine species (*M. bocandei*, *M. ferruginea*-black, *M. ferruginea*-reddish brown and *H. gribodoi*) nested in cavities of indigenous tree species and a total of twenty different indigenous tree species belonging to fourteen different plant families were identified as host plants. Martins *et al.* (2004) reported that stingless bee nests can be encountered in many different tree species. A small number of similar tree species were shared among the four tree nester stingless bee species in Kakamega forest. According to Roubik (1989), many stingless bees are opportunists in their use of tree species for nesting.

The nest pattern was dispersed for almost all the species in the different habitats except for the homesteads where the nesting pattern was clumped for *H. gribodoi* and *M. ferruginea* (reddish brown). Jongjitvimol *et al.* (2005) attributed the differences in nest dispersion of meliponine bees among habitats to diversity in their ecosystems. The dispersion pattern of nests of the five bee species in the different nesting habitats at Kakamega forest might be influenced by the field distribution of their preferable nesting sites. The homesteads might offer more

available and clumped nesting sites to the two nesting species compared to the other habitats. According to Slaa (2006a), features linked to competition for food or mechanism of territoriality behaviour also influence the pattern dispersion of nests in stingless bees in a specific habitat. There might be a low competition in food foraging between *H. gribodoi* and *M. ferruginea* (reddish brown) species and low conspecific and interspecific territorial behaviour when these two species nest closer to one another. The *M. lendliana* species was not found nesting in conspecific or interspecific aggregation. This finding suggested that there may be a tendency of territorial behaviour within the *M. lendliana* species. In *Meliponula bocandei* and *M. ferruginea* (black), the tendency to nest in aggregation and the number of their nests found in aggregation on a single substrate was lower than those of *H. gribodoi* and *M. ferruginea* (reddish brown) species. Moreover, when *M. bocandei* and *M. ferruginea* (black) were found nesting in an aggregation; the average less minimum distance separating their nest entrance to a conspecific and interspecific nest was higher than those aggregated nests between *H. gribodoi* and *M. ferruginea* (reddish brown). These findings suggest that there is a tendency of territorial behaviour in-and-within the *M. bocandei* and *M. ferruginea* (black) species.

The present results support earlier studies by Corbet *et al.* (1993) which suggested that in conditions of food availability, flight activity of meliponine bees are influenced by temperature and humidity conditions. Findings made by Roubik (1989) that in meliponine bees the first peak of flight activity begun in the morning hours before noon have also been supported by the current study. Furthermore, the results also support earlier studies by Couvillon *et al.* (2008)

which suggested that there is a trade-off between numbers of guard bees posted at the nest entrance to defend their nest from intruders and the entrance size as well as the abundance of bees entering into the nest.

The *M. lendliana* species seemed to have restrictions to nest in hive designs constructed with wood compared to those hive designs which are constructed with clay. Cortopassi-laurino *et al.* (2006) reported that stingless bee species that nest only in subterranean hollows are difficult to maintain in hives compared to those bee species which construct their nests in aerial cavities. *H. gribodoi* and *M. ferruginea* (black) species seemed to prefer mostly non-compartmented hive designs as opposed to compartmented hives. According to Cortopassi-laurino *et al.* (2006) non-compartmented hive designs facilitates to meliponine bees the opportunity to arrange their nests in similar way as they do in the wild rather, something not possible in a compartmented hive design. *M. ferruginea* (reddish brown) readily accepted both compartmented and non-compartmented hive designs. This tendency observed for *M. ferruginea* (reddish brown) supports the argument which was suggested earlier for this species to be cosmopolitan in the nesting sites. The average annual honey production by hived colonies of the five stingless bee species was variable. Three bee species from the genus *Meliponula*, *M. bocandei* followed by the *M. ferruginea* (black) and *M. ferruginea* (reddish brown) bee species had a greater potential for honey production compared to the *M. lendliana* and *H. gribodoi* species. Cortopassi-laurino *et al.* (2006) also observed difference in honey production potential among seven stingless bee species from the genus *Melipona* in Brazil.

The small hive beetle *A. tumida* and the phorid fly *M. scalaris* larvae were identified as the major natural enemies that pose a threat to stingless bee nests occasioning destruction and absconding of the colonies from the hives. This finding concurs with observations by Sommeijer (1999), González-Acereto *et al.* (2006) and Klumpp (2007). Nevertheless, in the Kakamega forest these two parasites were not the only natural enemies because predators such as *Formacidae* ants, *Monarchidae* birds and *Crabronidae* wasps were also noted to feed on some of the bee species. Similarly, ants, birds and wasps have also been reported as predators of some stingless bee species by researchers in meliponiculture in Neotropical regions (Wattanachaiyingcharoen and Jongjitvimo, 2007; Koedam *et al.*, 2009).

Hypotrigona gribodoi species contributed significantly to increase fruits and seeds quality of green pepper compared to feral pollinators in the open field and self-pollinated flowers. This finding suggests that when cultivating green pepper crop, colonies of *H. gribodoi* which are reared in the hives can be placed in the farm in order to benefit this crop from pollination thus to improve the quality of the produce. Green pepper fruits and seed quality improvement have been also reported through utilization of stingless bees *Nannotrigona perilampoides* and *Meliponula beecheii* as pollinators (González-Acereto *et al.*, 2006).

10.2 Conclusions

1. The diversity of the five stingless bee species in the Kakamega forest do vary within both sites and diversity is higher at Ivihiga than at the Isiekuti site.

Species diversity and nest abundance of the five stingless bee species change within the six studied habitats of the Kakamega forest. The indigenous forest had the higher species richness and the diversity than the other habitats. The pattern dispersion of nest and distance within conspecific and heterospecific nest are influenced by the habitat in species with ability to nest in homesteads vicinal to the Kakamega forest.

2. Nesting habitats, sites, substratum and host plants are species specific within the five stingless bee species. *Meliponula ferruginea* (reddish brown) and *Hypotrigona gribodoi* have the more diverse nesting habits. *Diospyras abyssinica* is the only tree species which was used for nesting by all the four tree nester stingless bee species (*M. bocandei*, *H. gribodoi*, *M. ferruginea*-black, *M. ferruginea*-reddish brown). *Meliponula lendliana* was the only stingless bee species which did not nest in conspecific and heterospecific aggregation in the Kakamega forest environment.

3. Honey production varied within the five stingless bee species. *Meliponula bocandei*, *M. ferruginea* (black), and *M. ferruginea* (reddish brown) species produced more honey than the other bee species. Therefore they are good honey producers. The five Meliponine species can be domesticated in artificial hives and the hive splitting method is the most appropriate to easily propagate their colonies. Requeening poor colonies with queens from strong and active colonies can be used as a technique to improve colony performance.

4. The defensive mechanism of colonies to a human intruder is species specific within the five stingless bee species; with *H. gribodoi* and *M. ferruginea* (reddish brown) being species defending their nests by biting. *Hypotrigona gribodoi* was an efficient pollinator and it improved fruits and seeds quality of the green pepper *Capsicum annum* under enclosure conditions.

10.3 Recommendations

1. In order to conserve the Meliponine bees in the wild in the Kakamega forest environment, there is need to preserve the natural indigenous forest. The degraded forest zone should be rehabilitated by planting mostly the twenty indigenous tree species which are used by the stingless bee species as nesting sites and not the exotic tree species.
2. The use of *M. bocandei*, *M. ferruginea* (black) and *M. ferruginea* (reddish brown) for honey production and the use of the splitting hive methods on compartmented hive in the rearing protocol are recommended to the small-scale farmers. The extension of meliponiculture as a supplementary income generating activity for the rural communities living adjacent to the Kakamega forest will financially benefit the community. There is need to educate the rural communities on the importance of stingless bees in pollination of forest ecosystems, agricultural and horticultural plant species. These will ultimately result in the conservation of the stingless bees biodiversity and reduce deforestation which is occasioned by cutting down of trees when hunting the bees in their natural habitats.

3. The use of *H. gribodoi* colonies for pollination of green pepper improved fruits size which contained many seeds. There is therefore need to carry out further research to determine the number of hives and the status of the bee colony (number of bees) needed to be introduced in relation to crop acreage.

4. The small hive beetle *Aethina tumida* and phorid fly *M. scalaris* are the main natural enemies of the stingless bees which are under domestication. The challenge here is to innovate adequate protective measures so as to reduce the invasion of *M. bocandei* and *M. ferruginea* (black) nests in hives by these two natural enemies.

5. Further studies are also required to identify the plant resources which provide stingless bees with pollen and nectar throughout the different seasons of the year. This will contribute to the understanding of the foraging behaviours of these five Meliponine bee species in the Kakamega forest environment.

REFERENCES

- Abdalla, F. C.; Jones, G. R.; Morgan, E. D. and da Cruz-Landim, C. (2003).** Comparative study of the cuticular hydrocarbon composition of *Melipona bicolor* Lepeletier, 1836 (Hymenoptera : Meliponini) workers and queens. *Genetics and Molecular Research*, **2**: 191-199.
- Althof von, A. J. (2005).** *Human Impact on Flora and Vegetation of Kakamega Forest, Kenya: Structure, distribution and disturbance of plant communities in an East African rainforest.* PhD thesis, Mathematik/Naturwissenschaften Universität Koblenz-Landau.
- Antonini, Y. (2002).** *Efeitos de variáveis ecológicas na ocorrência de Melipona quadrifasciata (Apidae, Meliponini) em fragmentos urbanos e rurais.* Tese de doutorado, Universidade Federal de Minas Gerais, Belo Horizonte.
- Araujo, V. (1955a).** Notas sobre colonias de Meliponíneos de Angola. *Africa. Dusenía*, **6**: 96-114.
- Araujo, V. (1955b).** Colmeias para ‘abelhas sem ferrão’ ‘MELIPONINI’. *Boletim do Instituto de Angola*, **7**: 9-50.
- Araujo, V. (1963).** Subterranean nests of two African stingless bees (Hymenoptera: Apidae). *Journal of the New York Entomological Society*, **71**: 130-141.
- Ayasse, M. and Paxton, R. J. (2002).** The brood protection in social Insects, pp 117-141. In *Chemoecology of Insect Eggs and Egg Deposition* (Edited by Hilker and Torsten Meiners), 416 pages. Blackwell Verlag gmbh, Berlin.
- Ayuke, F. O.; Karanja, N. K. ; Muya, E. M.; Musombi, B. K.; Mungatu, J. and Nyamasyo, G. H. N. (2009).** Macrofauna diversity and abundance across different land use systems in embu, Kenya. *Tropical and Subtropical Agroecosystems*, **11**: 371-384.
- Baddeley, A. (2008).** *Analysing spatial point patterns in R.* Workshop Notes, version 3. CSIRO and University of Western Australia.
- Barbour, M. G.; Burk, J. H.; Pitts, W. D.; Gilliam, F. S. and Schwartz, M. W. (1999).** *Terrestrial Plant Ecology.* 3rd edition. Benjamin Cummings.
- Bassindale, R. (1954).** The biology of the stingless bee *Trigona* (*Hypotrigona*) *gribodoi* Magretti (Meliponidae). *Proceedings of the Zoological Society, London*, **125**: 49-62.

- Batista, M. A.; Ramalho, M. and Soares, A. E. E. (2003).** Nesting sites and abundance of Meliponini (*Hymenoptera: Apidae*) in heterogeneous habitats of the Atlantic Rain Forest, Bahia, Brazil. *Lundiana*, **4** (1): 19-23.
- Bennun, L. A. and Njoroge, P. K. (1999).** *Important bird Areas in Kenya*. The East Africa Natural History Society, Nairobi Kenya.
- Benoist, R. (1944).** Hyménoptères Mellifères recueillis au Cameroun par MM. P Lepesme, R Paulian & A Villiers. *Bulletin de la Société Entomologique de France*, **49**: 8-13.
- Bohart, G. E. (1970).** The evolution of parasitism among bees. http://digitalcommons.usu.edu/honor_lectures/18
- Bommarco, R.; Biesmeijer, J. C.; Meyer ,B.; Potts, S. G.; Pöyry, J.; Roberts, S. P.; Steffan-Dewenter, I. and Ockinger, E. (2010).** Dispersal capacity and diet breadth modify the response of wild bees to habitat loss. *Proceedings Biological Sciences* **277**: 2075-2082.
- Boontop, Y.; Malaipan, S.; Chareansom, K. and Wiwatwittaya, D. (2008).** Diversity of Stingless Bees (*Apidae: Meliponini*) in Thong Pha Phum District, Kanchanaburi Province, Thailand. *Kasetsart Journal (Natural Science)*, **42**: 444-456.
- Breed, M. D.; Guzman-Novoa, E. and Hunt, G. J. (2004).** Defensive behaviour of honey bees: organization, genetics, and comparisons with other bees. *Annual Review of Entomology*, **49**: 271-298.
- Brosi, B. J.; Daily, G. C. and Ehrlich, P. (2007).** Bee community shifts with landscape context in a tropical countryside. *Ecological Applications*, **17**: 418-430.
- Brosi, B. J.; Daily, G. C.; Shih, T. M.; Oviedo, F. and Duran, G. (2008).** The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology*, **45**: 773-783.
- Byers, J. A. (1992).** Grid cell contour mapping of point densities: bark beetle attacks, fallen pine shoots, and infested trees. *Oikos*, **63**: 233-243.
- Camargo, J. M. F. and Posey, D. A. (1990).** O conhecimento dos Kayapó sobre as abelhas sociais sem ferrão (*Meliponinae, Apidae, Hymenoptera*): notas adicionais. *Boletim Museo Paraense Emilio Goeldi*, **6**: 17-42.
- Can-Alonso, C.; Quezada-Euán, J.J.G.; Xiu-Ancona, P.; Moo-Valle, H.; Valdovinos-Nunez, G.R. and Medina- Peralta, S. (2005).** Pollination of 'criollo' avocados (*Persea americana*) and the behaviour of associated bees in subtropical Mexico, *Journal of Apicultural Research*, **44**: 3-8.

- Canard, A. and Poinso, D. (2004).** Quelques methods statistiques: typiques de l'étude des populations et des peuplements par la method des quadrats. <http://perso.univrennes1.fr/denis.poinso/Bio%20717%20biologie%20des%20populations/rapport%20mars%E9e/poly%20Canard.pdf>
- Corbet, S. A.; Fussel, M.; Ake, R.; Fraser, A.; Gunson, C.; Savage, A. and Smith, K. (1993).** Temperature and pollination activity of social bees. *Ecological Entomology*, **18** (1) : 17-30.
- Chaves, L. S. and Aguilar, I. M. (2004).** Conservation of native trees and the indigenous bees in Costa Rica: food plants and nest tree selectivity; *Tropical Beekeeping: Research and Development for Pollination and Conservation*. Conference 22-25 February 2004 San José, Costa Rica.
- Chinh, T. X; Sommeijer, M. J.; Boot, W. J. and Michener, C. D. (2005).** Nest and colony characteristics of three stingless bee species in vietnam with the first description of the nest of *Lisotrigona carpenteri* (Hymenoptera: Apidae: Meliponini). *Journal of the Kansas Entomological Society*, **78** (4): 363-372.
- Cock, M.J.W.; Biesmeijer, J.C.; Cannon, R.J.C.; Gerard, P.J.; Gillespie, D.; Jimenez, J.J.; Lavelle, P.M. and Raina, S.K. (2011).** Climate change and invertebrate genetic resources for food and agriculture: State of knowledge, risks and opportunities. *FAO Background Study Paper*, **54**: 105p.
- Cockerell, T. D. A. (1933).** Bees from the Belgian Congo. *Revue de Zoologie et de Botanique Africaines*, **23**:18-27.
- Cockerell T. D. A. (1934).** Some African Meliponine bees. *Revue de Zoologie et de Botanique Africaines*, **26**: 46-62.
- Cockerell T. D. A. (1935).** Bees from Eritrea, Northwest Africa. *American Museum Novitates*, **807**: 1-6.
- Cork, S. J. and Shelton, D. (2000).** *The nature and value of Australia's Ecosystem Services: A framework for sustainable environmental solutions*. pp 151-159. In Proceedings of the 3rd Queensland Environmental Conference, Environmental Engineering Society, Queensland.
- Cortopassi-laurino, M.; Imperatriz-fonseca, V. L.; Roubik D. W. ; Dollin, A.; Heard, T.; Aguilar, I.; Venturieri, G. C.; Eardley, C. and Nogueira-neto, P. (2006).** Global meliponiculture: challenges and opportunities. *Apidologie*, **37** (2): 275-292.
- Couvillon, M. J.; Wenseleers, T.; Imperatriz-Fonseca, V. L.; Nogueira-Neto, P. and Ratnieks, F. L. W. (2008).** Comparative study in stingless bees (Meliponini) demonstrates that nest entrance size predicts traffic and defensivity. *Journal of Evolutionary Biology*, **21**: 194-201.

- Crane, E. (1992).** The past and the present status of beekeeping with stingless bees. *Bee world*, **73** (1): 29-42.
- Cunha, R. S.; Saraiva, A. M.; Cugnasca, C. E.; Hirakawa, A. R. and Imperatiz-Fonseca, V.L. (2002).** *WebBee CE A Web-based Information System for Research on Stingless Bees*. pp 676-682. In Proceedings of the World Congress of Computers in Agriculture and Natural Resources.
- Cruz, D. O. (2003).** *Uso e eficiência da abelha jandaíra (Melipona subnitida Ducke) na polinização do pimentão (Capsicum annum L.) sob cultivo protegido*. Dissertação (Mestrado), Universidade Federal do Ceará, Fortaleza.
- Cruz, D. de O.; Freitas, B. M.; Da Silva, L. A.; Sarmiento da Silva, E. M. and Bomfim, I. G. A. (2005).** Pollination efficiency of the stingless bee *Melipona subnitida* on greenhouse sweet pepper. *Pesquisa Agropecuária Brasileira*, **40** (12):1197-1201.
- Dafni, A. (1992).** *Pollination ecology: a practical approach*. Oxford University Press Inc., New York.
- Daily, G. C. (1997).** *Nature's Services: Societal Dependence on Natural Ecosystems*. Island Press, Washington D.C., 392 pp.
- Da Silva, C. A. and Gil-Santana, H.R. (2004).** Predation of *Apiomerus pilipes* (Fabricius) (Hemiptera, Reduviidae, Harpactorinae, Apiomerini) over Meliponinae bees (Hymenoptera, Apidae) in the State of Amazonas, Brazil. *Revista Brasileira de Zoologia*, **21** (4): 769-774.
- Danaraddi, C. S.; Viraktamath, S.; Basavanagoud, K. and Bhat, A. R. S. (2009).** Nesting habits and nest structure of stingless bee, *Trigona iridipennis* Smith at Dharwad, Karnataka. *Karnataka Journal of Agricultural Sciences*, **22** (2): 310-313.
- Darchen, R. and Louis, J. (1961).** Les mélipones et leur élevage (*Melipona-Trigona-Lestremelitta*). *Annales de l'Abeilles*, **4** (I): 39 pp.
- Darchen, R. (1966).** Sur L'éthologie de *Trigona (Dactylurina) staudingeri gribodoi* (Hymenoptera, Apidae). *Biologia Gabonica*, **2**: 37-45.
- Darchen, R. and Pain, J. (1966).** Le nid de *Trigona (Dactylurina) staudingeri gribodoi* (Hymenoptera, Apidae). *Biologia Gabonica*, **2**: 25-35.
- Darchen, R. (1969).** Sur la biologie de *Trigona (Apotrigona) nebulata komiensis* Cock. I. *Biologia Gabonica*, **3**: 151-187.
- Darchen, R. (1970).** Le nid de Deux Nouvelles especes d'abeilles de la Cote d'Ivoire, *Trigona (Axestotrigona) sawadogoi* Darchen et *Trigona (Axestotrigona) eburnensis* Darchen (Hymen., Apidae). *Biologia Gabonica*, **6**: 139-150.

- Darchen, R. (1971a).** *Trigona (Axestotrigona) oyani* Darchen (*Apidae, Trigoninae*), une nouvelle espece d'abeille Africaine. Description du nid inclus dans une fourmilier. *Biologia Gabonica*, **7**: 407-421.
- Darchen, R. (1971b).** Description d'une abeille sociale de tres petite taille *Trigona (Liotrigona) parvula* n.sp. Darchen (*Apidae, Trigoninae*) trouvee dans la savane de Lamto (Cote d'Ivoire). *Biologia Gabonica*, **7**: 403-406.
- Darchen, R. (1972).** Ecologie de quelques trigones (*Trigona* sp.) de la Savane de Lamto (Cote d'Ivoire). *Apidologie*, **3**: 341-367.
- Darchen, R. (1981).** Une nouvelle trigone du Zaire: *Trigona (Axestotrigona) richardsi* [Hym. *Apidae*]. *Bulletin de la Société Entomologique de France*, **86**: 54-56.
- Del Sarto, M. C. L.; Peruquetti, R. C.; Campos, L. A. O. (2005).** Evaluation of the Neotropical Stingless Bee *Melipona quadrifasciata* (*Hymenoptera: Apidae*) as Pollinator of Greenhouse Tomatoes. *Journal of Economic Entomology*, **98** (2): 260-266.
- Dollin, A. (2001).** Natural hive duplication: An alternative method of propagating australian stingless bees. Aussie bee on line, Article **3**: 3p. <http://www.aussiebee.com.au/aussiebeeonline003.pdf>
- Eardly, C. D. (2004).** Taxonomic revision of the African stingless bee (*Apoidea: Apidae: Apinae: Meliponini*). *African Plant Protection*, **10** (2): 63-96.
- Eltz, T.; Bruhl, C. A.; van der Kaars, S. and Linsenmair, K. E. (2001).** Determinants of stingless bee nest density in lowland dipterocarp forest in Sabah, Malaysia. *Oecologia* D01 10. 1007/s 00442-001-0848-6.
- Eltz, T.; Bruhl, C. A.; Imiyabir, Z. and Linsenmair, K. E. (2003).** Nesting and nest trees of stingless bees (*Apidae: Meliponini*) in lowland dipterocarp forest in Sabah, Malaysia, with implication for forest management. *Forest Ecology and Management*, **172**: 301-313.
- Emmel, T. C. and Warren, A .D. (1993).** The butterfly faunas of the Kakamega rain forest and the Masai Mara Savannah in Kenya, East Africa. *Tropical Lepidoptera*, **4**: 66-76.
- Fajardo, A. C. and Cervancia, C. R. (2003).** Simple ways to manage stingless bees. *Bees for Development Journal*, **67**: 3-5.
- FAO (2008).** *Initial survey of good pollination practices: tools for conservation and use of pollination services*, 133p.
- Friese, H. (1900).** Neu Arten der Bienungattungen *Melipona* Ill. und *Trigona* Jar. *Térmezetráji Füzetek*, **23**: 381-394.

- Ghazoul, J. (2005).** Buzziness as usual? Questioning the global pollination crisis. *Ecology and Evolution*, **20** (7): 367-373.
- Giannotti, E. and Pinto, N. P. O. (2001).** *Predação de Machos de Trigona (Scaptotrigona) postica Latreille (Hymenoptera, Apidae, Meliponinae) por Trachypus boharti Rubio (Hymenoptera, Sphecidae, Philanthinae)*. In Anais do XIX Congresso Brasileiro de Etologia, 1-4 novembro (Prezoto F, ed.). Juiz de Fora, Minas Gerais.
- Gikungu, W. M. (2006).** *Bee diversity and some aspects of their ecological interactions with plants in a successional tropical community*. PhD thesis, Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn.
- González-Acereto, J. A.; Quezada-Euán, J. J. G. and Medina-Medina, L. A. (2006).** New perspectives for stingless beekeeping in the Yucatan: results of an integral program to rescue and promote the activity. *Journal of Apicultural Research*, **45** (3): 234-239.
- Hamida B. T. (1999).** *Enemies of bees*. In Colin M. E. (ed.), Ball B.V. (ed.), Kilani M. (ed.); Bee disease diagnosis Zaragoza: CIHEAM-IAMZ (Options Mediterraneennes: Serie B. Etudes et Recherches 25): 147-166(182p).
- Heard, T. A. (1999).** The role of stingless bees in crop pollination. *Annual Review of Entomology*, **44**: 183-206.
- Helmenstine, A. M. (2011).** Surface Area and Volume Formulas: Calculate Surface Area and Volume. <http://www.About.com> Guide.
- Henigman, J. F. (1975).** *The bionomics, economics and management of Neotropical stingless bees (Apidae, Meliponini)*. MSc thesis, Simon Fraser University, Burnaby, British Columbia, Canada.
- Herbst, S.T. (2001).** *The New Food Lover's Companion: Comprehensive Definitions of Nearly 6,000 Food, Drink, and Culinary Terms*. Barron's Cooking Guide. Hauppauge, NY: Barron's Educational Series.
- Hilário, S.D. ; Imperatriz-Fonseca, V.L. and Kleinert, A.M.P. (2000).** Flight activity and colony strength in the stingless bee *Melipona bicolor bicolor* (Apidae, Meliponinae). *Revista Brasileira de Biologia*, **60** (2) : 299-306.
- Hölldobler, B. and Wilson, E. O. (1990).** *The ants*. Harvard Univ. Press; Cambridge, 732 pp.
- Hubbel, S. P. and Johnson, L. K. (1977).** Competition and nest spacing in a tropical stingless bee community. *Ecology*, **58**: 949-963.

- Hwang, J. S.; Souissi, S.; Dahms, H. U.; Tseng, L. C.; Schmitt, F. G. and Chen, Q. C. (2009).** Rank Abundance Allocations as a Tool to Analyze Planktonic Copepod Assemblages off the Danshuei River Estuary (Northern Taiwan); *Zoological Studies*, **48** (1): 49-62.
- Irvine, J. E. and Delfel, N. E. (1961).** Flowering Behavior of Vanilla. *Nature*, **190** (4773): 366 p.
- Ish-Am, G.; Barrientos-Priego, F.; Castañeda-Vildozola, A. and Gazit, S. (1999).** Avocado (*Persea americana* Mill.) pollinators in its region of origin, *Revista Chapingo Serie Horticultura*, **5**: 137-143.
- Jongjitvimol, T.; Boontawon, K.; Wattanachaiyingcharoen, W. and Deowanish, S. (2005).** Nest Dispersion of a Stingless Bee Species, *Trigona collina* Smith, 1857 (*Apidae, Meliponinae*) in a Mixed Deciduous Forest in Thailand. *The Natural History Journal of Chulalongkorn University*, **5** (2): 69-71.
- Kasina, J. M.; Mburu, J.; Kraemer, M.; Holm-Mueller, K. (2009).** Economic Benefit of Crop Pollination by Bees: A Case of Kakamega Small-Holder Farming in Western Kenya. *Journal of Economic Entomology*, **102** (2): 467-473.
- Kajobe, R. and Roubik, D. W. (2006).** Honey-making bee colony abundance and predation by apes and humans in a Uganda Forest Reserve. *Biotropica*, **38** (2): 1-9.
- Kajobe, R. (2007).** Nesting biology of equatorial Afrotropical stingless bees (*Apidae; Meliponini*) in Bwindi Impenetrable National Park, Uganda. *Journal of Apicultural Research and Bee World*, **46** (4): 245-255.
- Kazuhiro, A. (2004).** Attempts to introduce stingless bees for the pollination of crops under greenhouse conditions in Japan. <http://www.agnet.org/library/tb/167/tb167.pdf>
- Kerr, W. E, de Lello, E. (1962).** Sting glands in stingless bees a vestigial character (Hymenoptera: Apidae). *N.Y. Entomological Society*, **70**: 190-214.
- Kerr, W. E.; Jungnickel, H. and Morgan, E. D. (2004).** workers of the stingless bee *Melipona scutellaris* are more similar to males than to queens in their cuticular hydrocarbons. *Apidologie*, **35**: 611-618.
- KIFCON (1994).** *Kenya Indigenous Forest Conservation Programme*. phase 1 Report. Karura Forest Station, Center for Biodiversity, Nairobi.
- Kihwele, D. V. N.; Chiguru, S. K. and Naasi, E. M. (1999).** *Participatory Rural Appraisal in establishing the beekeeping development project in 15 villages of five selected districts: Kibondo, Tabora, Manyoni, Kondoa and Handeni*.

- Kindt, R. and Coe, R. (2005).** *Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies.* Nairobi: World Agroforestry Centre (ICRAF), 196 pp.
- Klein, A. M.; Steffan-Dewenter, I.; Tschardt, T. (2003).** Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society B*, **270**: 955-961.
- Klumpp, J. (2007).** *Australian Stingless Bees: A Guide to Sugarbag Beekeeping.* Earthling Enterprises Pty. Ltd., ISBN 9780975713815, 110pp.
- Koedam, D.; Slaa, E. J.; Biesmeijer, J. C. and Nogueira-Neto, P. (2009).** Unsuccessful attacks dominate a drone-preying wasp's hunting performance near stingless bee nests. *Genetics and Molecular Research*, **8** (2): 690-702.
- Kojima, J. (1993).** A latitudinal gradient in intensity of applying ant-repellent substance to the nest petiole in paper wasps (*Hymenoptera: Vespidae*). *Insectes Sociaux*, **40**: 403-421.
- Kokwaro, J. O. (1988).** Conservation status of Kakamega forest in Kenya. The Eastern most relic of the equatorial rain forest of Africa. *Monographs of Systematic and Botanical Garden*, **25**: 471-489.
- Krebs, C. J. (1999).** *Ecological Methodology* (2nd edition). Benjamin Cummings, Menlo Park, California, 620 pp.
- Kuno, E. (1991).** Sampling and analysis of insect populations. *Annual Review of Entomology*, **36**: 285-304.
- Lepelletier de Saint-Fargeau, A. L. M. (1841).** *Histoire naturelle des insectes. Hyménoptères Suites à Buffon.* Roret 2, 1-680 p. Paris.
- Lindenmayer, D. B.; Cunningham, R. B. and Donnelly, C. F. (1997).** Decay and collapse of trees with hollows in eastern Australian forests: impacts on arboreal marsupials. *Ecological Applications*, **7**: 625-641.
- Lobreau-Callen, D. ; Le Thomas, A. ; Darchen, B. and Darchen, R. (1990).** Quelques facteurs déterminant le comportement de butinage d'*Hypotrigona pothieri* (*Trigonini*) dans la végétation de Côte-d'Ivoire. *Apidologie*, **21**: 69-83.
- Lobreau-Callen, D. ; Darchen, R. and Delage-Darchen, B. (1994).** *Étude comparative du comportement de butinage de deux espèces de Trigona du groupe africain Liotrigona (Hymenoptera, Trigoninae) à partir de la composition pollinique des rations alimentaires des larves.* pp 133-143. In *Trabajos de Palinologia Basica y Aplicada. X. Simposio de Palinologia (A.P.L.E.), Brazil.*

- Looman, J. and Campbell, J. B. (1960).** Adaptation of Sorensen's K (1948) for estimating unit affinities in prairie vegetation. *Ecology*, **41** (3): 409-416.
- López, de G. (1552).** *Historia general de las indias*. pp 169-192. In Lóren J.J., Gómez J.J. (Eds.), *Historia de la Apicultura Española*.
- Mac Arthur, R. H. and Mac Arthur, J. W. (1961).** On bird species diversity. *Ecology*, **42**: 594-598.
- Macharia, J.K.; Raina, S. and Muli, E. (2007).** Stingless bees in Kenya. *Bees for Development Journal*, **83**.
- Macharia, J. K. (2008).** *Status and the potential of stingless bees (Apidae: Meliponinae) for forest conservation and income generation: A case study of Kakamega forest*. MSc thesis, Jomo Kenyatta University of Agriculture and Technology, Kenya.
- Magretti, P. (1884).** Risultati di raccolte imenotterologiche nell'Africa Orientale. *Annali del Museo Civico di Storia Naturale di Genova*, **21**: 523-636.
- Malagodi-Braga, K. S. and Kleinert, A. M. P. (2004).** Could *Tetragonisca angustula* Latreille (Apinae, Meliponini) be used as strawberry pollinator in greenhouses? *Australian Journal of Agricultural Research*, **55**: 771-773.
- Martins, C. F.; Cortopassi-Laurino, M.; Koedam, D. and Imperatriz-Fonseca, V. L. (2004).** The tree species used for nidification by stingless bees in the Brazilian Caatinga (SERIDÓ, PB; JOÃO CÂMARA, RN). *Biota Neotropica*, **4** (2)-BN00104022004
- Mbahin, N. (2008).** *The ecology and economic potential of wild silkmoth Anaphe panda (BOISDUVAL) (Lepidoptera: Thaumetopoeidae) in the Kakamega forest*. PhD thesis, Department of Zoological Science-Kenyatta University, Kenya.
- Meyer, J. K. (2005).** Social Bees. <http://www.earthlife.net/insects/socbees.html>
- Michener, C. D. (1974).** *The social behaviour of the bees: A comparative study*. Harvard University Press; Cambridge, Mass, USA.
- Michener, C. D. (1979).** Biogeography of the Bees. *Annals of the Missouri Botanical Garden*, **66**: 277-347.
- Michener, C. D. (2000).** *The Bees of the World*. Johns Hopkins University Press, Baltimore, Maryland. Xiv, USA.

- Mitchell, J. G. (2004).** *Rank-size analysis and vertical phytoplankton distribution patterns.* pp 257-278. In Handbook of scaling methods in aquatic ecology. In L. Seurong, PG Strutton, eds. Measurement, analysis, simulation. Florida: CRC Press, Boca Raton.
- Mooney, H. A. and Ehrlich, P. R. (1997).** *Ecosystem Services: A Fragmentary History.* pp 11-19. In G. E. Daily, ed. Nature's Services - Societal Dependence on Natural Ecosystems. Island Press, Washington.
- Munthali, S. M. and Mughogho, D. E. C. (1992).** Economic incentives for conservation; An experiment on beekeeping and Saturniidae caterpillar utilization by rural people. *Biodiversity and Conservation*, **1**: 143-154.
- Murillo, R. M. (1984).** Uso y manejo actual de las colonias de *Melipona beechei* Bennett (*Apidae: Meliponini*) en el estado de Tabasco, Mexico. *Biotica*, **9**: 423-428.
- Muriuki, J. H. and Tsingalia, M. H. (1990).** A new population of De Braza's monkey in Kenya. *Oryx*, **24**:157-162.
- Murray, A. (2005).** Stingless bees in Angola. *Bees for Development Journal*, **31**.
- Nates-Parra, G.; Palacios, E. and Parra-H, A. (2008).** Effect of landscape change on the structure of the stingless bee community (*Hymenoptera: Apidae*) in Meta, Colombia. *Revista de Biología Tropical*, **56** (3): 1295-308.
- Njoya, M. T. M. (2009).** *Diversity of Stingless Bees in Bamenda Afromontane Forest-Cameroon: Nest architecture, Behaviour and Labour calendar.* PhD thesis, Institut für Nutzpflanzenwissenschaften und Ressourcenschutz Rheinische Friedrich-Wilhelms-Universität Bonn.
- Nogueira-Neto, P. (1997).** *Vida e Criação de Abelhas Indígenas sem Ferrão.* Editora Nogueirapis, São Paulo.
- Nunes, T. M.; Turatti, I. C. C.; Mateus, S.; Nascimento, F. S.; Lopes, N. P. and Zucchi, R. (2009a).** Cuticular hydrocarbons in the stingless bee *Schwarziana quadripunctata* (*Hymenoptera, Apidae, Meliponini*): differences between colonies, castes and age. *Genetics and Molecular Research*, **8**: 589-595.
- Nunes, T. M.; Turatti, I. C.; Lopes, N. P. and Zucchi, R. (2009b).** Chemical signals in the stingless bee *Frieseomelitta varia* indicate caste, gender, age, and reproductive status. *Journal of Chemical Ecology*, **35**: 1172-1180.
- Nyunja, A. R. O. ; Onyango, J. C. and Erwin, B. (2009).** The Kakamega Forest Medicinal Plant Resources and their Utilization by the Adjacent Luhya Community. *International Journal of Tropical Medecine*, **4** (3): 82-90.

- Occhiuzzi, P. (2000).** *Stingless bees pollinate greenhouse Capsicum, Aussie Bee13 and 15*. Published by Australian Nature Bee Research Centre, North Richmond NSW Australia.
- Oldroyd, B. P., Lawler, S. H., Crozier, R. H. (1994).** Do feral honey bees (*Apis mellifera*) and regent parrots (*Polytelis anthopeplus*) compete for nest sites? *Australian Journal of Ecology*, **19**: 444-450.
- Otieno, N .A.; Le Ru, B. P.; Ong'amo, G. O.; Moyal, P.; Dupas, S.; Calatayud, P. A. and Silvain, J. F. (2008).** Diversity and abundance of wild host plants of lepidopteran stemborers in two agro-ecological zones of Kenya. *International Journal of Biodiversity Science and Management*, **4**: 1-12.
- Palma, G.; Javier, J.; Quezada-Euán, G.; Meléndez-Ramirez, V.; Irigoyen, J.; Valdovinos-Nuñez, G. R.; Rejón, M. (2008).** Comparative Efficiency of *Nannotrigona perilampoides*, *Bombus impatiens* (Hymenoptera: Apoidea), and Mechanical Vibration on Fruit Production of Enclosed Habanero Pepper. *Journal of Economic Entomology*, **101** (1): 132-138.
- Pauly, A. (1998).** Hymenoptera Apoidea du Gabon. Musée Royal de l'Afrique Centrale Tervuren, Belgique. *Annales Sciences Zoologique*, **282**: 1-121.
- Pesson, P. and Louveaux, J. (1984).** *Pollinisation et production végétales*. Editions Quae, INRA, Paris.
- Pfeiffer, D. U and Morris, R. S. (1994).** Spatial analysis techniques in veterinary epidemiology. *The Kenya Veterinarian*, **18** (2): 483-485.
- Pyper, W. (2001).** Six-legged friends. *Ecos*, **107**: 16-17.
- Quezada-Euán, J. J. G; May-Itzá, W. de J. and González-Acereto, J. A. (2001).** Meliponiculture in Mexico: problems and perspective for development. *Bee World*, **82** (4): 160-167.
- R Development Core Team .(2005).** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. <http://www.R-project.org>
- Raina S. K. (2000).** *The Economics of Apiculture and sericulture modules for income generation in Africa*. IBRA Press, ,UK.
- Raina, S. K.; Muli, E.; Nguku, E.; Kioko, E. and Macharia, J. K. (2006).** *Developing incentives for community participation in forest conservation through the use of commercial insects in Kenya*, UNDP-GEF Fourth Technical Project Inception Report, Project ID: KEN/04/G35

- Raina, S.K.; Kioko, E.N.; Gardon, I. and Nyandiga, C. (2009).** *Improving Forest Conservation and Community Livelihoods through Income Generation from Commercial Insect in Three Kenyan Forest.* icipe Science Press, Kenya.
- Rao, D.; Cheng, K. and Herberstein, M. E. (2008).** Stingless bee response to spider webs is dependent on the context of encounter. *Behavioral Ecology and Sociobiology*, **63**: 209-216.
- Rasmussen, C. (2009).** Diversity and Abundance of Orchid Bees (Hymenoptera: Apidae, Euglossini) in a Tropical Rainforest Succession. *Neotropical Entomology*, **38** (1): 66-73.
- Richards, K. W. (1993).** Non - Apis bees as crop pollinators. *Revue Suisse de Zoologie*, **100**: 807-822.
- Ricketts, T. H. (2004).** Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology*, **18**: 1262-1271.
- Ricotta, C. (2003).** On parametric evenness measures. *Journal of Theoretical Biology*, **222**: 189-197.
- Rossbacher, L. A. (1986).** Nearest neighbour analysis: A technique for quantitative evaluation of polygonal ground patterns. *Geografiska Annaler*, **68A** (1-2): 101-105.
- Roubik, D. W. and Aluja, M. (1983).** Flight ranges of *Melipona* and *Trigon* in tropical forest. *Journal of the Kansas Entomological Society*, **56**: 217-222.
- Roubik, W. D. (1989).** *Ecology and Natural History of Tropical Bees*, Cambridge University Press, New York.
- Roubik D.W. (1990).** Biogeographical ecology of *Melipona* (Apidae: Meliponinae), pp. 579–580. In *Social Insects and the Environment* (Edited by G.K. Veeresh, B. Mallik and C.A. Viraktamath). Oxford and IBH Publishing Co., New Delhi.
- Roubik D.W. (1992).** Stingless bees: A guide to Panamanian and Mesoamerican species and of their nests (Hymenoptera: Apidae: Meliponinae), pp. 495-524. In *Insects of Panama and Mesoamerica: Selected Studies* (Edited by D. Quintero and A. Aiello). Oxford University Press, Oxford.
- Roubik, W. D. (1995).** Pollination of cultivated plants in the tropics: Stingless bee colonies for pollination. *FAO Agricultural Services Bulletin*, **118**: 198p.
- Roubik, D. W. (2000).** Pollination system stability in tropical America, *Conservation Biology*, **14**: 1234-1235.

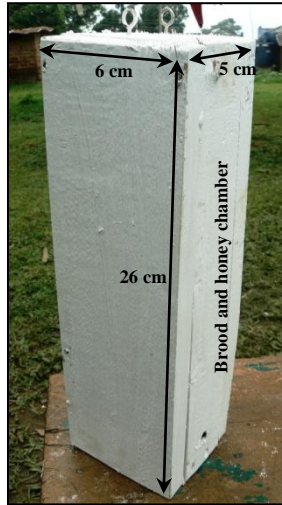
- Roubik, D. W. (2006).** Stingless bee nesting biology. *Apidologie*, **37**: 124-143.
- Rueda, L. M.; Brown, T. L.; Kim, H. C.; Chong, S. T.; Klein, T. A.; Foley, D. H.; Anyamba, A.; Smith, M.; Pak, E. P. and Wilkerson, R. C. (2010).** Species composition, larval habitats, seasonal occurrence and distribution of potential malaria vectors and associated species of Anopheles (*Diptera: Culicidae*) from the Republic of Korea. *Malaria Journal*, **9**: 55.
- Russell, Z. and Janine, Z. (2008).** Australian stingless native bees. <http://www.Zobel.com.au/index/soft-split.html>
- Sakagami, S. F. and Zucchi, R. (1967).** Behaviour Studies of the Stingless Bees, with Special Reference to the Oviposition Process: VI. *Trigona (Tetragona) clavipes*. *Zoology*, **16** (2): 292-313.
- Sakagami, S. F. (1982).** *Stingless Bees*. pp 361-423. In Hermann, H.R. (Ed.). *Social Insects*, Academic Press, New York, USA.
- Samejima, H.; Marzuki, M.; Nagamitsu, T. and Nakasizuka, T. (2004).** The effects of human disturbance on a stingless bee community in a tropical rainforest. *Biological Conservation*, **120**: 577-587.
- Sanford, M.T. (1997).** Eleventh Brazilian Beekeeping Congress Meets in Teresina: Stingless Bee Culture in Brazil. <http://apis.ifas.ufl.edu/papers/teres.htm#14>.
- Santis, L. and De Regalia, J. A. V. S. (1978).** Hormigas recolectoras de polen en colmenas. *Ciencia y Abejas*, **3**: 43-46.
- Santos, S.A.B. dos; Bego, L.R. and Roselino, A.C. (2004).** *Pollination in tomatoes, Lycopersicon esculentum, by Melipona quadrifasciata anthidioides and Apis mellifera (Hymenoptera, Apinae)*, Proc. 8th IBRA Int. Conf. Trop. Bees and VI Encontro sobre Abelhas, 688 p.
- Schmid-Hempel, P. (1998).** *Parasites in Social Insects*. Princeton University Press, Princeton, New Jersey.
- Schwarz, H. F. (1948).** Stingless bees (Meliponidae) of the western hemisphere. *Bulletin of the American Museum of Natural History*, **90**: 1-546.
- Serrano, A. R.; Guerra-Sanz, J. M. (2006).** Quality fruit improvement in sweet pepper culture by bumblebee pollination. *Scientia Horticulturae*, **110** (2): 160-166.
- Slaa, E. J. (2003).** *Foraging ecology of stingless bees: from individual behaviour to community ecology*. PhD thesis, Utrecht university, The Netherlands.

- Slaa, E. J. (2006a).** Spatial nesting patterns in a Neotropical stingless bee community: do bees compete for food? *Proceedings of the Netherlands Entomological Society Meeting*, **17**: 71-78.
- Slaa, E. J. (2006b).** Population dynamics of a stingless bee community in the seasonal dry lowlands of Costa Rica. *Insectes Sociaux*, **53**: 70-79.
- Slaa, E. J.; Sánchez Chaves, L. A.; Malagodi-Braga, K. S.; Hofstede, F. E. (2006).** Stingless bees in applied pollination: practice and perspectives. *Apidologie*, **37** : 293-315.
- Smith, F. (1854).** Notes on the biology and waxes of four species of African Trigona bees (*Hymenoptera: Apidae*). *Proceedings of the Royal Entomological Society (A)* **29**: 62-70.
- Sommeijer, M. J. (1999).** Beekeeping with stingless bees: a new type of hive. *Bee World*, **80** (2): 70-79.
- Spinola, M. (1853).** Compte rendu des hyménoptères in-édités provenant du voyage entomologique de M. Ghiliani dans le Para en 1846. *Memorie della Reale Accademia delle Scienze di Torino*, **13** (2): 92-94.
- Steffan-Dewenter I.; Potts S. G.; Packer L. (2005).** Pollinator diversity and crop pollination services are at risk. *Trends in Ecology and Evolution*, **20**: 651-652.
- Taylor, L. R. (1984).** Assessing and interpreting the spatial distribution of insect populations. *Annual Review of Entomology*, **29**: 321-357.
- Tóthmérész, B. (1995).** Comparison of different methods for diversity ordering. *Journal of Vegetation Science*, **6**: 283-290.
- Tsingalia, H. M. (1988).** *Animals and the Regeneration of a Canopy Tree in an African Tropical Forest*. PhDthesis, Berkeley-University of California.
- Tsingalia, H. M. (1990).** Habitat severity and patterns of species abundance. *African Journal of Ecology*, **28**: 190-220.
- Tsingalia, H. M. and Kassily, F. N. (2009).** The Origins Kakamega Forest Grasslands: A Critical Review. *Journal of Human Ecology*, **27**(2): 129-135.
- Velthuis, H. H. W. (1997).** *The Biology of stingless bees*. Utrecht University press, Utrecht, The Netherlands.
- Viana, B. F.; Kleinert, A. M. P. and Imperatriz-Fonseca, V. L. (1997).** Abundance and Flower Visits of Bees in a Cerrado of Bahia, Tropical Brazil. *Studies on Neotropical Fauna and Environment*, **32**: 212-219.

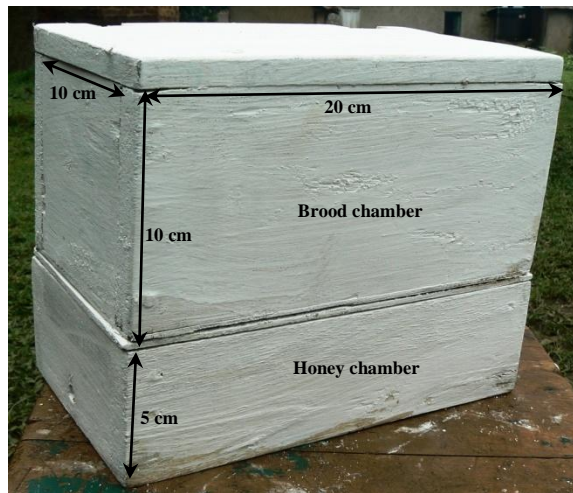
- Villanueva, G. R.; Roubik, W. D. and Colli, U. (2005).** Extinction of *Melipona beecheii* and traditional beekeeping in the Yucatán peninsula, *Bee World* **86**: 35-41.
- Visscher, P. K. (2007).** Group decision making in nest-site selection among social insects. *Annual Review of Entomology*, **52**: 255-275.
- Watanabe, M. (1994).** Pollination worries rise as honey bees decline. *Science*, **265**: 1170.
- Wattanachaiyingcharoen, W. and Jongjitvimo, T. (2007).** First Record of the Predator, *Pahabengkakia piliceps* Miller, 1941 (*Reduviidae, Harpactorinae*) in the Stingless Bee, *Trigona collina* Smith, 1857 (*Apidae, Meliponinae*) in Thailand. *The Natural History Journal of Chulalongkorn University*, **7** (1): 71-74.
- Wcislo, W. T. and Schatz, B. (2002).** Predator recognition and evasive behaviour by sweat bees, *Lasioglossum umbripenne* (Hymenoptera: Halictidae), in response to prédation by ants, *Ectatomma ruidum* (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology*, Springer-Verlag, 10.1007/S00265-002-0564-1.
- Wenzel, J. W. and Pickering, J. (1991).** Cooperative foraging, productivity, and the central limit theorem. *Proceedings of the National Academy of Sciences*, **88**: 36-38.
- Westman, W. E. (1977).** How much are nature's services worth? *Science*, **197**: 960-964.
- Wille, A. and Michener, C. D. (1973).** The nest architecture of stingless bees, with special reference to those of Costa Rica. *Revista de Biología Tropical*, **21**, suplemento 1, Universidad de Costa Rica.
- Wille, A. (1983).** Biology of the stingless bees. *Annual Review of Entomology*, **28**: 41-64.
- Williams, I. H. (1996).** *Aspects of bee diversity and crop pollination in the European Union*. pp 63-80. In Matheson A, Buchmann SL, O'Toole C, Westrich P, Williams IH, eds. *The Conservation of Bees*. New York: Academic Press.
- Winfree, R.; Griswold, T. and Kremen, C. (2007).** Effect of Human Disturbance on Bee Communities in a Forested Ecosystem. *Conservation Biology*, **21** (1): 213-223.
- Wittmann, D. (1985).** Aerial defense of the nest by workers of the stingless bee *Trigona (Tetragonisca) angustula* (Latreille) (Hymenoptera: Apidae). *Behavioral Ecology and Sociobiology*, **16**: 111-114.

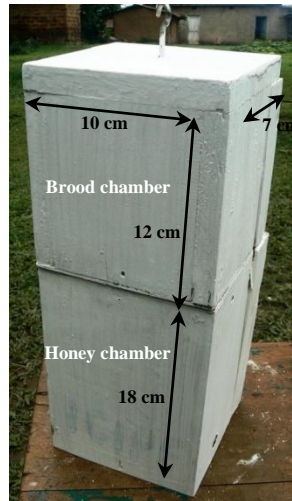
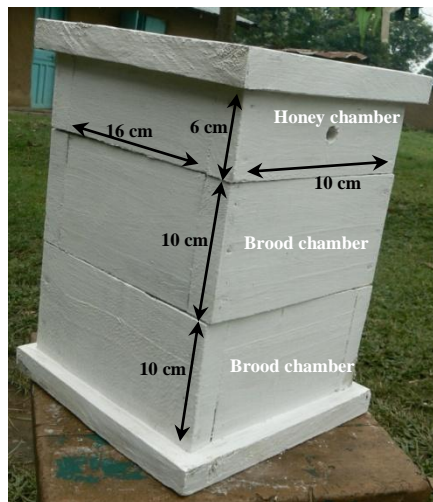
APPENDICES

Appendix 1.1: Vertical non-compartmented hive-icipe 1Hg for the genus *Hypotrigona*

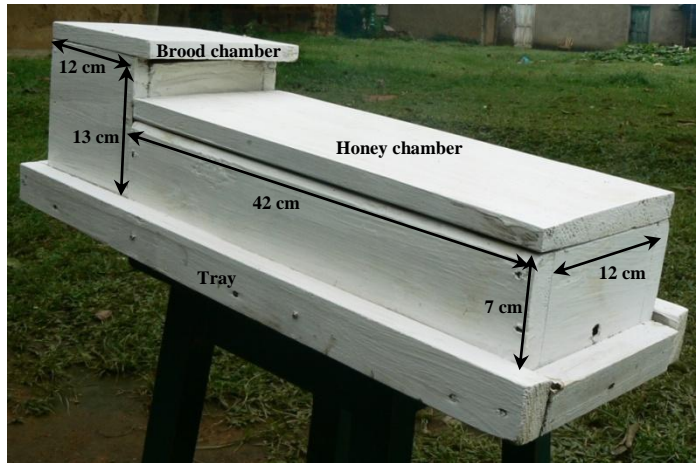


Appendix 1.2: Vertical compartmented hive-icipe 2Hg for the genus *Hypotrigona*

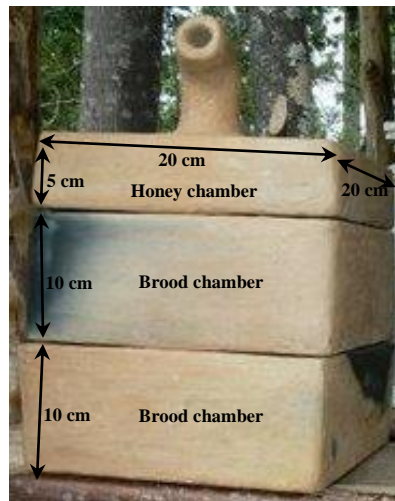


Appendix 1.3: Vertical compartmented hive-icipe 3Hg for the genus *Hypotrigena***Appendix 1.4:** Vertical compartmented hive-Original Australian Trigona Hive (OATH)

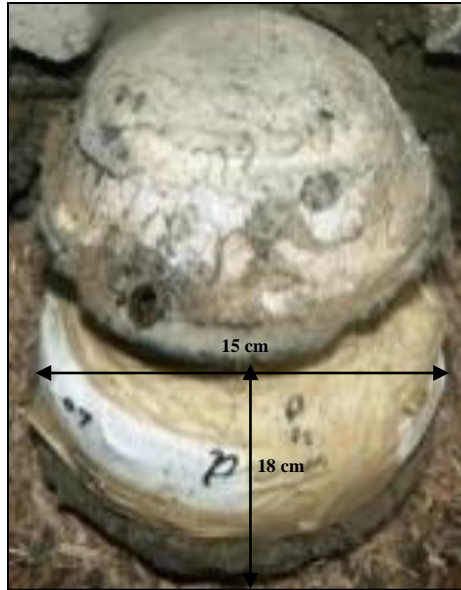
Appendix 1.5: Horizontal compartmented hive-Utrecht University Tobago Hive (UTOB)



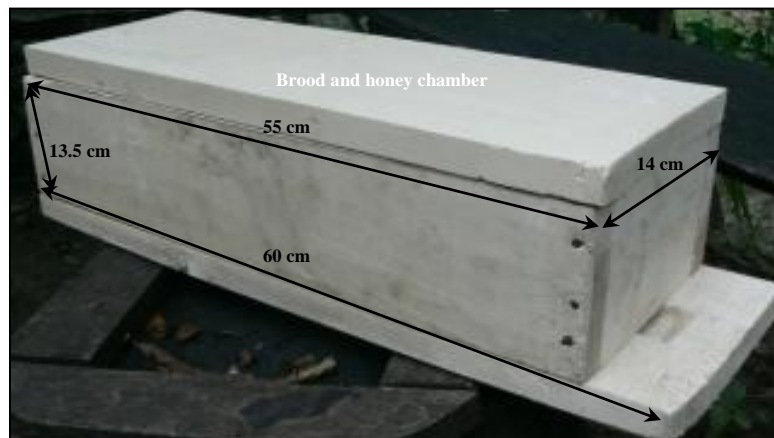
Appendix 1.6: Vertical compartmented clay hive-standard OATH



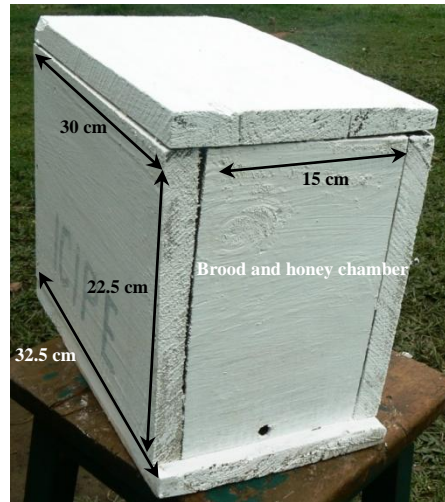
Appendix 1.7: Vertical non-compartmented clay hive “Iyambova” pot for the species *M. lendliana*



Appendix 1.8: Horizontal non-compartmented hive-icipe 1M for the genus *Meliponula*



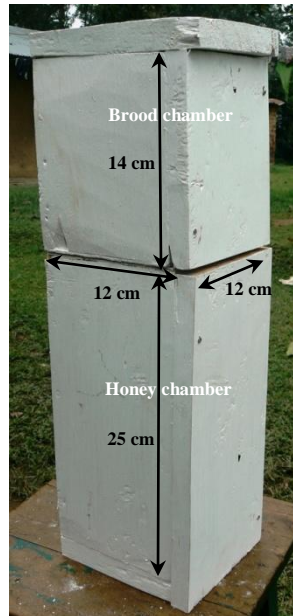
Appendix 1.9: Horizontal non-compartmented hive-icipe 2M for the genus *Meliponula*



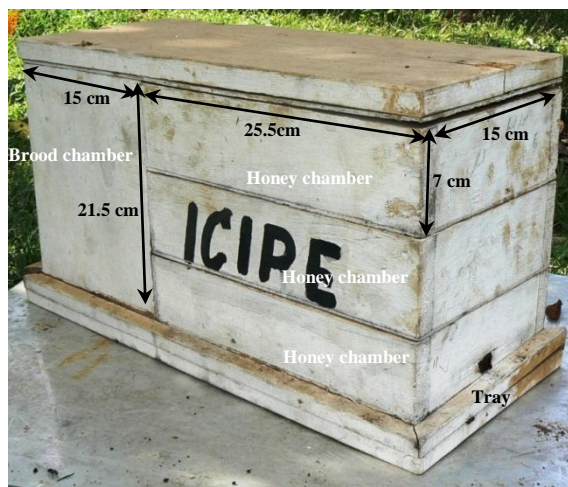
Appendix 1.10: Vertical compartmented hive-icipe 3M for the genus *Meliponula*



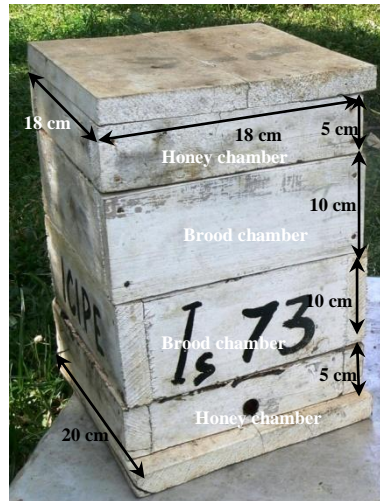
Appendix 1.11: Vertical compartmented hive-icipe 4M for the genus *Meliponula*



Appendix 1.12: Horizontal compartmented hive-icipe 5M for the genus *Meliponula*



Appendix 1.13: Vertical compartmented hive-icipe 6M for the genus *Meliponula*



Appendix 1.14: Horizontal compartmented hive-icipe 7M for the genus *Meliponula*

