BREEDING OF THE HONEY BEE (*APIS MELLIFERA L.*) AND ITS POTENTIAL FOR ROYAL JELLY PRODUCTION IN KENYA

BY

ELLIUD M. MAUNDU

B. Ed. (Sc.), M. Sc, Kenyatta University.

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Maundu, Eliud M.
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This thesis is my original work and has not been presented for a degree in any other University

Signature _____ Date 2nd Nov. 2004

Elliud M. Muli

DECLARATION BY SUPERVISORS

This thesis has been submitted for examination with our approval as supervisors

Signature _____ Date 8/11/04

Prof. Jones M. Mueke
Department of Zoology
Kenyatta University
P. O. Box 43844
Nairobi, Kenya

Signature _____ Date 2nd NOV. '04

Dr. Suresh K. Raina
Commercial Insects Program
The International Center of Insect
Physiology and Ecology (ICIPE)
P. O. Box 30772
Nairobi, Kenya

DEDICATION

This thesis is dedicated to my wife Jane, my daughter Anita and my late brother Justus Mwanzia

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ABSTRACT

Royal jelly is a creamy, milky white, strongly acidic and highly nitrogenous substance secreted by the hypopharyngeal and mandibular glands of worker honey bees at the ages of 5 – 15 days of age. The jelly is fed to queens through out their life and also to drone and worker larvae of less than 3 days old. The principal constituents of royal jelly are water, protein, lipids, sugars and mineral salts. Due to differences in climate, *Apis mellifera* races have evolved in response to the local environmental conditions, with each race acquiring a different potential for production of honey and other hive products and even pollination of crops. In Kenya, new generation commercial hive products like royal jelly, propolis and pollen are insignificantly produced or utilized due to lack of knowledge on production skills, awareness and undeveloped marketing systems and also due to the nature of hives used.

The aim of this study was to develop a royal jelly production system and breed for high honey production and reduced defensiveness using the East African honey bees. Royal jelly production was done in queenright colonies following the procedure of Laidlaw and Eckert, (1962). Defensive behavior was evaluated following the procedure of Stort (1974) and Collins *et al.,* (1984). The bred queens were mated by instrumental insemination and their honey production and defensive behavior compared to that of colonies headed by non-selected queens.

There were no significant differences between *Apis mellifera scutellata* and *Apis mellifera monticola* in the cell reception rates and mean royal jelly yields. Grafting larvae at the age of 24 hours significantly increased queen cell reception and royal jelly yields compared to those of larvae grafted at the ages of 36; 48 and 60 hours. Supplementary feeding significantly increased colony cell reception, mean royal jelly yields per queen cell and colony royal jelly yields. Harvesting royal jelly 2 days after grafting resulted into a higher number of harvested cells compared to harvesting in a 3-day cycle. However, cells harvested 3 days after grafting yielded more royal jelly per queen cup compared to that produced by cells harvested 2 days after grafting. However, royal jelly yields were not significantly different in the 2 and 3-day cycle. The major components in the Kenyan royal jelly were found to be moisture, lipids, proteins, sugars and ash and their composition was comparable to that reported in literature.

Colonies of *Apis mellifera scutellata* were found to have variations in both defensive behaviour and honey production. A negative and significant correlation for SN and T1S was recorded. Variations were noted in honey production with annual honey yields of 27 – 48 kilograms per colony. There were no significant differences in sting number (SN), time to first sting (T1S) and honey production between colonies headed by selected queens and those headed by unselected queens. Colonies headed by selected queens had significantly higher queen cup acceptance rates and royal jelly yields compared to those headed by unselected queens.

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

The majority of the world's poor people live in rural areas and farming is the livelihood of these 900 million people (Sawadogo, 2002). Since the human population is ever increasing, food production must continue to increase as well in order to satisfy the nutritional requirements of this ever-increasing population. This has led to extensive agriculture resulting in irreversible environmental degradation and consequent loss of biodiversity as more acreage of forests is being cleared annually to pave way for cultivation of crops. Thus, environmental degradation – both a cause and a consequence of poverty – is becoming increasingly severe in rural areas, exacerbating poverty. Environmental degradation threatens rural livelihoods and the economies of most developing countries that are based on natural resources. Over the past half-century more than a quarter of the world's 8.7 billion hectares of agricultural lands, pastures, forests and woodlands have been degraded. It has become very clear that there are close links between rural poverty and environmental degradation, and that the underlying problems must be tackled in an integrated way by protecting and expanding the environment and natural resources on which the rural poor depend (Uwe, 2002).

This degradation, however, can be slowed down by the introduction of economic incentives that integrate conservation with economic enterprises of the rural people (Munthali and Mughogo, 1992). Bee keeping is one such enterprise. It has a strong

local tradition in many African tribes, thus the technology is already available and what is needed is an improvement on the methods already in use in the local bee-human relationship. Bee keeping provides rural people in developing countries with sources of income and nutrition and as such has potential for enhancing rural livelihoods (Raina, 2000). It is especially suitable for arid and semi-arid areas that are unsuitable for other agricultural activities (Mbae, 1999) or where food crops are likely to fail due to unpredictable rainfall. Farmers located in such areas can produce a crop of honey to supplement other agricultural activities and build more security into their farming business enterprises. The greatest economic value of honeybees comes from pollination of fruits, seeds and berries both in agriculture and in nature, hence bees are responsible for maintenance of a great part of plant biodiversity. In addition, honeybees are incontestably the most valuable insect pollinators in agriculture throughout the world, as they are manageable, available in large numbers, forage on flowers of a wide range of plants and are efficient in exploiting their immediate environment for resources (nectar and pollen) (Westerkemp, 1991). As such, honeybees lead to potentially increased yield from food crops as a result of pollination (Coleman, 2000).

Thus farmers could keep bees alongside their other farming activities and boost their crop production through pollination, especially of legumes and fruits, as well as get honey crop. Bee keeping is a sustainable form of agriculture which provides economic reasons for the retention of native habitats and utilizes the locally available resources some of which may be wasted if bees are not kept, for example nectar and pollen from flowering plants (Richard, 1999).

Kenya's honey production potential is estimated at 100,000 metric tones with an equivalent of 10,000 metric tones of beeswax. However, only around 25,000 metric tones with an equivalent of 2,000 tons of beeswax are realized, (FAO, 1998), because most of the highly productive areas are not exploited while over 90% of those practicing bee keeping use traditional methods (Mbae, 1999). Constraints associated with traditional bee keeping include low productivity per hive, traditional hives have fixed combs and thus do not allow for any meaningful colony manipulation practices, lower quality products (e.g example honey mixed with brood and/or pollen) and the restriction to produce only honey and wax. Other products like royal jelly, propolis, and pollen are insignificantly produced due to lack of awareness, nature of hives used, lack of knowledge on production techniques and undeveloped local marketing systems. Moreover, by the 1950s royal jelly had become a hive product of exploitation and has continued to maintain a place in the world market as a specialized dietary supplement for human consumption and for the cosmetic industry (Crane, 1999). China is the world's largest royal jelly producer with a production of more than one million kilograms annually (Chen *et al.,* 2002).

This study was designed to set up a royal jelly production system using the East African bee races, *Apis mellifera scutellata* and *Apis mellifera monticolla* and carry out a breeding programme in an attempt to explore the possibility of developing local honeybee strains with higher honey/royal jelly production and reduced defensive behaviour. This is the first study of its kind in East Africa and will be a reference point for royal jelly production and honey bee breeding in other parts of Africa where beekeeping has a potential as an income generating enterprise.

1.2 Literature Review

1.2.1 Origin and distribution of honey bees

All honey bees fall in only one genus, *Apis*, which apparently is tropical in origin, most likely India and southeast Asia, and until recently included four traditional species: the western honey bee, *Apis mellifera* (L.), the giant honey bee, *Apis dorsata*, the eastern honey bee, *Apis cerana* Fabr., and the little honey bee, *Apis florea* Fabr. These honey bee species are characterized by a remarkable conformity in a number of morphological and physiological characters (Koeniger, 1976). *Apis laboriosa,* the world's largest honey bee, and closely related to *Apis dorsata,* was reported by Sakagami *et al.,* (1980) as a new species. According to Ruttner (1988), the proposition to recognize this taxon as another species is based on the following three major arguments: (1) considerable quantitative differences supposed to be surpassing those found within one of the known *Apis* species, (2) presumably sympatric occurrence, and (3) ecological divergence.

A. mellifera is divided into three distinct groups, i.e, the African, the European, and the Oriental (Ruttner 1975; Ruttner *et al.*, 1978). The African races are (i) *Apis mellifera intermissa* Bettel-Reepen. This is a North African race found north of the Sahara from Libya to Morocco. It is a small, dark bee, is highly aggressive and swarms frequently (Ruttner, 1975) (ii) *Apis mellifera lamarckii* Cockrell. This is the Egyptian bee found in North East Africa, chiefly in Egypt and Sudan along the Nile Valley. It rears numerous queens and swarms frequently (von Frisch, 1967) (iii) *Apis mellifera capensis* is restricted to the southwest coast of the Republic of South Africa especially around Cape town (Anderson, 1961) (iv) *Apis mellifera unicolor*

Latreille (Ruttner, 1975b) is the honey bee species found in Madagascar (v) *Apis mellifera jemenitica* Ruttner (1975b) found in parts of Ethiopia, Eritrea and Somali (vi) The East African bees are found throughout much of E. Africa, in the savannahs of Central Africa, equatorial East Africa and most of South Africa. It is a small highly defensive bee, swarms and absconds frequently, and is able to nest in a broad range of sites from cavities to open nests. There are three races in East Africa. *Apis mellifera litorea* is found below 500 metres above sea level - principally along the coastal regions stretching from Somali to Mozambique. It is more prone to stinging and is less migratory, probably due to the somewhat more predictable rainfall available in its habitat. *Apis mellifera scutellata* is found above the coastal escarpment and is common throughout most of E. Africa. It frequently swarms and absconds and is highly defensive (Ruttner, 1981). *Apis mellifera monticola* is found 2000 - 3000 metres above sea level (Dietz and Krell, 1986). It is less migratory probably due to rainfall stability (Ruttner, 1975; Smith, 1961).

1.2.2 Royal jelly

Royal jelly is a creamy, milky white, strongly acidic and highly nitrogenous substance secreted by hypopharyngeal and mandibular glands of worker bees normally 5 – 15 days of age. The jelly is fed to queens throughout their larval and adult stages, and also to young worker and drone larvae (Howe *et al.*, 1985). It has a pungent odour and a characteristic sour flavour (Lercker *et al.,* 1992). The principal constituents of royal jelly are water, protein, lipids, sugars and mineral salts (Echigo *et al.*, 1986; AOAC, 1984).

Water makes up 66.05 % of fresh royal jelly, but by dry weight, proteins and sugars are by far the largest fractions (50.2% and 20 – 39% respectively) (Chen Yaochun, 1993). Of the nitrogenous substances, proteins average 73.9% (Otani *et al.*, 1985), free amino acids average 2.3% and peptides 0.16% (Takaneka, 1984). All amino acids essential for humans are present and a total of 29 amino acids and derivatives have been identified, the most important being aspartic acid and glutamic acid (Howe *et al.*, 1985). The free amino acids are proline and lysine (Takaneka, 1987). An insulin-like substance has been identified (Kramer *et al.*, 1982). The sugars consist mainly of fructose and glucose with fructose being more prevelant and together account for 90% of the total sugars. Other sugars present in much lower quantities are maltose, melibiose, trehalose and ribose (Lercker *et al.*, 1992). The lipid content is unique and accounts for 3.5% of the dry weight (Lercker *et al.*, 1984). The lipid fraction consists of 80-90% by dry weight of free fatty acids with unusual and uncommon structures. They are mostly short chain (8-10 carbon atoms) hydroxy fatty acids or dicarboxylic acids, in contrast to the fatty acids with 14-20 carbon atoms commonly found in animal and plant materials. The principal acid is 10-Hydroxy-2- decanoic acid (Blum *et al.*, 1959). It is these fatty acids that are responsible for most of the recorded biological properties of royal jelly (Schmidt and Buchmann, 1992). In addition to the free fatty acids, the lipid fraction contains some neutral lipids and sterols (Lercker *et al.*, 1992). The total ash content of royal jelly is about 1% of fresh weight or 2-3% of dry weight. The major mineral salts are, in descending order: Potassium, Calcium, Sodium, Zinc, Iron, Copper and Manganese (Benfenati *et al.*, 1986). Vitamins found in royal jelly include Thiamine (B1), Riboflavin (B2), Pantothenic acid (B5), and Ascorbic acid (Vitamin C) (Vecchi *et al.*, 1988; Cerna, 1963; Evans *et al.*, 1937). Other substances identified in royal jelly

include several nucleotides as free bases (adenosine and uridine), the phosphates ADP and ATP (Marko *et* al., 1964) and acetylcholine (Henschler, 1954).

Royal jelly is produced from colonies maintained for this purpose and are either queenless or queenright (Laidlaw and Eckert, 1962). Young worker larvae are grafted into artificial queen cell cups on wooden bars that are placed adjacent to combs of unsealed brood. Started (grafted) cells are harvested on day three when royal jelly is at maximum (150 grams) (Okada and Obata, 1962). Larvae are then removed using a forceps and discarded. What remains is the royal jelly that is removed using a royal jelly spoon or soft suction tube (Vacuum apparatus). Collected jelly can be refrigerated in airtight containers for several months.

Royal jelly has been shown to have antibacterial activity against gram-positive and gram negative bacteria like *Escherichia coli, Staphilococcus aureus* and *Bacillus subtilis* (Xiao Jingwei *et al.*, 1996; Abdalla *et al.*, 1995; Blum *et al.*, 1959) and it has demonstrated antifungal acivity against dermatophytes *in vivo* (Nassis *et al.*, 1998). Due to its antibacterial and antifungal properties, royal jelly is used in the cosmetic industry (Yatsunani and Echigo, 1985). It is also marketed as a 'healthfood' supplement and is used in production of food and beverages (Takaneka and Echigo, 1983). Since it does not inhibit the growth of *Lactobacillus bulgarius* and *L. plantarum,* royal jelly can be added to fermented dairy products such as yoghurt (Namaichaikool and Sanquandeekul, 1993). It has been promoted as a 'miracle food' medicine and humans have used it for centuries (Wetherill, 1852). The world's royal jelly production is estimated at 1,000 metric tones with China and Japan being the largest producer and consumer respectively (Chen Yaochun, 1993; Crane, 1999).

Recent reports indicate that more than one million kilograms of royal jelly are produced annually in China alone (Chen *et al.,* 2002)

1.2.3 Mating behaviour of honey bee queens

Honey bee queens mate on average with between about 7 and 17 different males (Adams *et al.,* 1977; Laidlaw *et al.,*1956; Palmer and Oldroyd, 2000). Different methods in different populations have resulted in different estimates. These matings take place while drones and queens are in flight and within 5 – 10 days after the virgin queen emerges as an adult. Each queen takes one or a series of mating flights; the series may extend over several days (Nolan, 1932; Triasko, 1951; Woyke, 1962). Drones orient to the queen using both visual and chemical cues (Gary and Marston, 1971). Mounting and copulation are rapid and spectacular, with the drones literally exploding their semen into the genital orifice of the queen. Within a split second the drone grasps the queen with all six legs and everts the endophallus into the queen's open sting chamber. Males mate just once, depositing 6 – 10 million spermatozoa into the oviducts of the queen and then die. Pairings of drones and queens are probably random, there seems to be no apparent assorting of males (Page and Metcalf, 1988). However, males do seem to 'prefer' to mate with a queen that already is showing a mating sign, evidence that she has already mated at least once on that particular flight (Koeniger, 1990).

Queens return to the hive after the mating flight where a total of about 4 – 7 million (Mackensen and Roberts, 1948; Woyke, 1964) of the sperm deposited by the drones in the oviducts of the queen migrate by primarily active processes (Ruttner and Koeniger, 1971) into the spermatheca over a period of about 40 hours. The sperm

that enter the spermatheca are fairly mixed well, representative of the drones, and last the egg-laying life of the queen (Woyke, 1983).

1.2.4 Honey bee breeding successes

It is suggested that selective breeding coupled with sound bee management practices especially in areas where bee keeping has a large unexploited potential, is likely to offer prospects for increasing world hive production (Crane, 1984; Raina, 2000; Shi, 2001).

Honeybee stock improvement has been achieved in areas such as resistance to American foulbrood (AFB), a disease caused by a spore forming bacterium, *Bacillus larvae*, (Cale and Rothenbuhler, 1976); resistance to the protozoan *Nosema apis* (Rinderer *et al.*,1983); resistance to tracheal mite, *Acarapis woodi*, (Page & Gary, 1990; Cobey, 1997); reduced larval developmental period as a mechanism for reducing infestation levels by the parasitic mite *Varroa destructor* (Harbo, 1992); pollen hoarding (Nye & Mackensen, 1965; Hellmich *et al.*, 1985; Calderone & Page, 1988; Gordon *et al.*, 1995); defensive behaviour (Boch and Rothenbuhler, 1974; Collins *et al.*, 1984) and improved honey production (Szabo & Lefkovitch, 1987)

1.3 Justification

Environmental degradation – both a cause and a consequence of poverty – is becoming increasingly severe in rural areas, exacerbating poverty. This degradation threatens rural livelihoods and the economies of most developing countries that are based on natural resources. All our actions ultimately have consequences on the quality and quantity of natural resources on the planet. It has become quite clear that there are close links between rural poverty and environmental degradation, and that the underlying problems must be tackled in an integrated way by protecting and expanding the environment and natural resources on which the rural poor depend. Thus, management of natural resources is the frontline of the struggle for more sustainable and equitable development. In order to diversify incomes, farmers are being encouraged to look into ways and means of using natural resources, which have either been ignored or under-utilized in the past, in a sustainable way. Consequently, beekeeping is being promoted as a sustainable form of agriculture with the potential to conserve the environment and reverse environmental degradation (through conservation of forests and associated animal and plant species) and generate income for the rural masses (through direct sales of hive products and indirectly due to benefits of pollination on food crops). It is hoped that an improved apiculture industry will generate employment and subsidiary income, reduce destruction of honeybees and other bee species and their nesting sites through deforestation and indiscriminate use of insecticides in modern agriculture and increase food production as a result of the presence of efficient and sufficient pollinators.

High heritabilities have been reported for traits such as honey production, stinging behaviour (Collins, 1986), pollen collecting and hoarding (Nye and Mackensen, 1965; Gordon *et al.*, 1995), and resistance to diseases like American foulbrood (Rothenbuhler, *et al.*, 1968) among other traits and honeybees have been selected and bred for such traits. However, there are no reports on similar studies using the East Africa honeybee races. On the basis of the breeding success accomplished with European honeybees so far, it is assumed that it would be possible to achieve similar results using East African honeybees in the area of improved honey and royal jelly production and reduced defensive behaviour.

Royal jelly is used, especially in dietetics and cosmetics and has become a commercial product in many countries (Takaneka and Echigo, 1983). Its production relies on artificial queen raising based on transfer of young worker larvae to artificial queen cups, a process called grafting. However, in Kenya it is insignificantly produced due to lack of awareness and production skills (Mbae, 1999). Commercial production of royal jelly could be an avenue for local beekeepers to diversify and improve their income from beekeeping activities. However, if incomes of the rural poor are to be improved, there is need for production-oriented research for other hive products apart from honey such as royal jelly and bee propolis. Eventually, the results of such research should get to the end-users (farmers) through training and extension programs. This study was designed to accomplish this by developing the knowledge and production system using the indigenous honeybee races.

1.4 Objectives

1.4.1 General objective

To set up a royal jelly production system using the local honeybee races *A.m. scutellata* and *A.m. monticola* and to selectively breed for queens with high honey and royal jelly production and less defensive behaviour

1.4.2 Specific objectives

1. To determine the amounts of royal jelly produced by colonies of *A. m. scutellata* and *A.m monticola*
2. To determine the effect of age of larvae at grafting on the amounts of royal jelly produced by colonies of *A. m. scutellata* and *A.m monticola*
3. To determine the effect of supplementary feeding on the amounts of royal jelly produced by colonies of *A. m. scutellata* and *A.m monticola*
4. To determine the effect of harvesting time after grafting on the amounts of royal jelly produced by colonies of *A. m. scutellata* and *A.m monticola*
5. To analyse the chemical composition of Kenyan royal jelly
6. To selectively breed queens for improved honey and royal jelly production and reduced defensive behaviour using *A. m. scutellata*

CHAPTER TWO

GENERAL MATERIALS AND METHODS

2.1 Honey bee colony management for royal jelly and honey production

Experimental colonies were kept in the 10-frame Langstroth hives (Figure 2.2). A plastic queen excluder (Plate 2.1) was placed between the brood chamber and the super chamber (honey/royal jelly production area) to confine the queen to the brood chamber and exclude her from the super chamber during the royal jelly and honey production seasons. Royal jelly production colonies were continuously supplied with empty worker combs for egg laying to maintain a high population of young nurse bees.

2.2 Evaluation and selection of breeder queens

The characteristics considered during the selection were honey production (foraging ability of the workers) and defensive behaviour.

Estimation of honey yields was done as described below. During honey harvesting the number of combs with honey for each colony was counted and recorded. For comparison of productivity, the following values were determined:

i). Apiary average = Total apiary honey yield ÷ Total no. of colonies

ii). Colony production as % of (i) above = Yield of colony ÷ Apiary average

X 100

Honey production by colonies (ii above) for two seasons was then used to rank the colonies. Generally, colonies with a higher than the calculated average apiary production are considered to be good honey producers (Ruttner, 1988).

Defensive behaviour was evaluated using the Stort method (1974) modified by Collins *et al* (1982). A dark leather ball was used as a stinging target, and the variables number of stings and time to first sting were recorded. Colonies with the highest combined scores for the two characteristics were used as breeders (sources of queens and drones).

2.3 Queen rearing

Honeybees rear queens under three circumstances, when about to swarm, when an old queen is failing (supersedure) and when through accidents or natural causes, the colony suddenly becomes queenless (emergency). Beekeepers raise queens by stimulating these circumstances. However, dietary differences leading to caste differentiation occur as soon as the egg hatches and it is recommended that young larvae about 24 hours old or less are ideal for grafting (i.e the transfer of young larvae from worker cells into artificial queen cells) (Morse and Hooper, 1985). Queen rearing by grafting larvae is the popular method used to select and breed honeybees with percentage acceptance of larvae being more than 80% (Pothichot *et* al., 1989; Wongsiri *et* al., 1990). The procedure below was used to rear queens.

i) Wax queen cells preparation: A forming stick made of hardwood (about 7 cms long, tapering from approximately a 1.0 cm diameter at a point of 1.3 cm from the

tip to a diameter of 0. 65 to 0.8 cms at the tip and with a smooth surface and rounded end) was prepared (Plate 2.2). The forming stick was dipped into clean, cold water and the excess water shaken off and then dipped into wax melted in a tray to a depth of about 1cm. The stick was then quickly withdrawn and held above the tray for a few seconds to allow the wax to solidify. This was repeated 2-3 times, after which the stick was submerged into the cold water to solidify the wax. The same procedure was followed for each cup. The wax cups were then attached to wooden bars that fitted between the end bars of a modified frame. Since the end bars do not touch the sides of the hive it becomes easy to remove the frame (no propolis at the sides). A space of 4 centimetres was left between the cell bars and between the lower cell bar and the frame bottom bar. Grafting frames were each modified to hold at least two to three cell bars. To attach the cell cups to the bars, a bar was laid on a flat surface, and some melted wax was spread along the upper side of the bar. A wooden base for each cup was then pressed into the barely melted wax and spaced about 2 cms from each other. The wooden bases give each wax cup a solid base for easier handling (Plate 2.3).

ii). Queen breeder hives: Colonies with desired traits were used as the sources of grafting larvae. An empty dark worker comb was placed in the central part of the brood box of the selected colonies each day for the queen to deposit eggs. Combs were removed on daily basis, marked and transferred to another part of the brood box and a new comb given to the breeder hives. Larvae began to hatch on the third day and at the age of 8-24 hours were considered ready for grafting.

ii). Grafting: Before grafting cell cups were availed to the nursing colonies for 30 minutes for cleaning and familiarization; larvae from breeder combs were transferred into the queen cups from their worker cells using a Chinese grafting tool (Plate 2.4). The cell bars with grafted cells were then placed in starter-finisher colonies. These are queen-right, populous and healthy colonies whose queens were confined in the brood-box with a queen excluder. Heavy feeding with sugar syrup was administered until the queen cells were capped, around the fourth to fifth day after grafting

v). Ripe queen cells (Plate 2.5) were then removed from the cell builders on the tenth day, separated from the cell bar and placed singly in small colonies commonly referred to as nuclear boxes or 'nucs' (Plate 2.6). The nucs were established with a few bees (2000 - 3000) on two (2) frames of capped brood and two (2) frames of honey and pollen plus all the bees adhering on the frames. Extra bees were shaken from frames of the parent colony into the nucs to increase their worker bees population. Queens emerged on the 12^{th} –13^{th} day and remained in the nucs till maturity (were ready for mating about seven days after emerging). A piece of queen excluder was placed at the nuc-hive entrance to prevent the queen from flying out.

2.4 Rearing of drones

Drones have a longer developmental period than queens and their rearing was started fourty days to the time they were needed or around two weeks before queen rearing was started. The procedure outlined below was followed:

i). A drone comb was placed at the center of the brood rearing unit of the selected drone-mother colony. Five days later the comb was inspected for laying and a new comb supplied. The laid on comb, now with eggs and some larvae was transferred to the super of strong queenright colonies for feeding and development. Combs with open brood and pollen were placed adjacent to the drone combs to attract young bees for proper nourishment of the developing drones. The drone nursing colonies were also fed on sugar syrup constantly.

ii). The drone combs were capped on the 10^{th} day after the eggs were laid and remained in the sealed/capped state for 14 days. After the drone combs were capped they were removed from the nurse colonies and established in separate colonies of their own (5-frame nucs stocked with brood and bees from the nurse colony).

iii). The colonies with drones were moved about 200 metres away from all other colonies for isolation. Isolation prevents drifting i.e entry of stray drones. Honey/sugar syrup and pollen was amply supplied to the colonies holding the drones.

2.5 Instrumental/Artificial insemination

The queens and drones were sexually mature from 7 and 10-14 days after hatching respectively.

i). Semen was taken from sexually mature drones obtained from the selected mother colonies. The drones were stimulated to ejaculate by squeezing their abdomen.

Semen at the tip of the endophallus was then collected with the tip of a syringe. About 8 drones were used to inseminate one queen.

ii). The queen was placed in a queen cage on the inseminator (Figure 2.3) and anaesthetized with carbon dioxide gas. The ventral and sting hooks were inserted to open the sting chamber and semen directly inserted into the oviducts (i.e behind the valve fold).

iii). Ovary development and oviposition were stimulated by carbon dioxide narcosis given to the queens one day before, during and one day after insemination.

iv). The inseminated queens were then caged and introduced into their respective nuc-boxes and supplied with empty combs in which to lay eggs. Queens were checked for egg laying after one week.

2.6 Data analysis

Statistical analysis of results was carried out using Analysis of Variance (ANOVA) and t-test. The effect of age of larvae and supplementary feeding and harvesting time after grafting on royal jelly yields and cell reception was analysed by ANOVA. Significant means were separated using Tukey's test. A t-test was used to compare royal jelly production by the two honeybee races, defensive behavior, royal jelly and honey production by colonies headed by selected and unselected queens. The Friedman test was used to rank colonies for stinging behavior. Pearson correlation was used to determine the relationship between the number of stings and time to first sting.

Figure 2.1: Map of Kenya showing field study sites

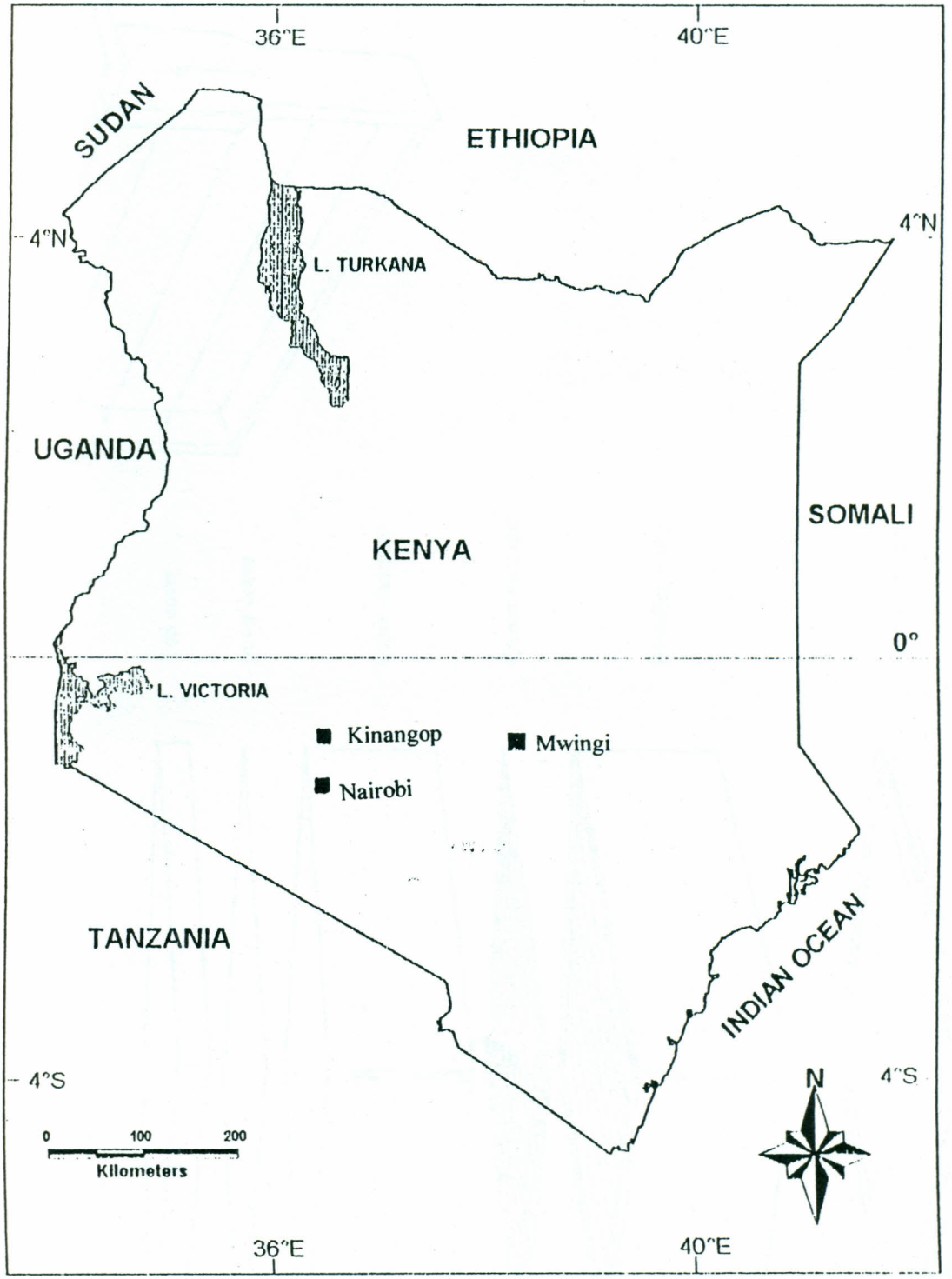

Figure 2.2: Parts of the Langstroth hive

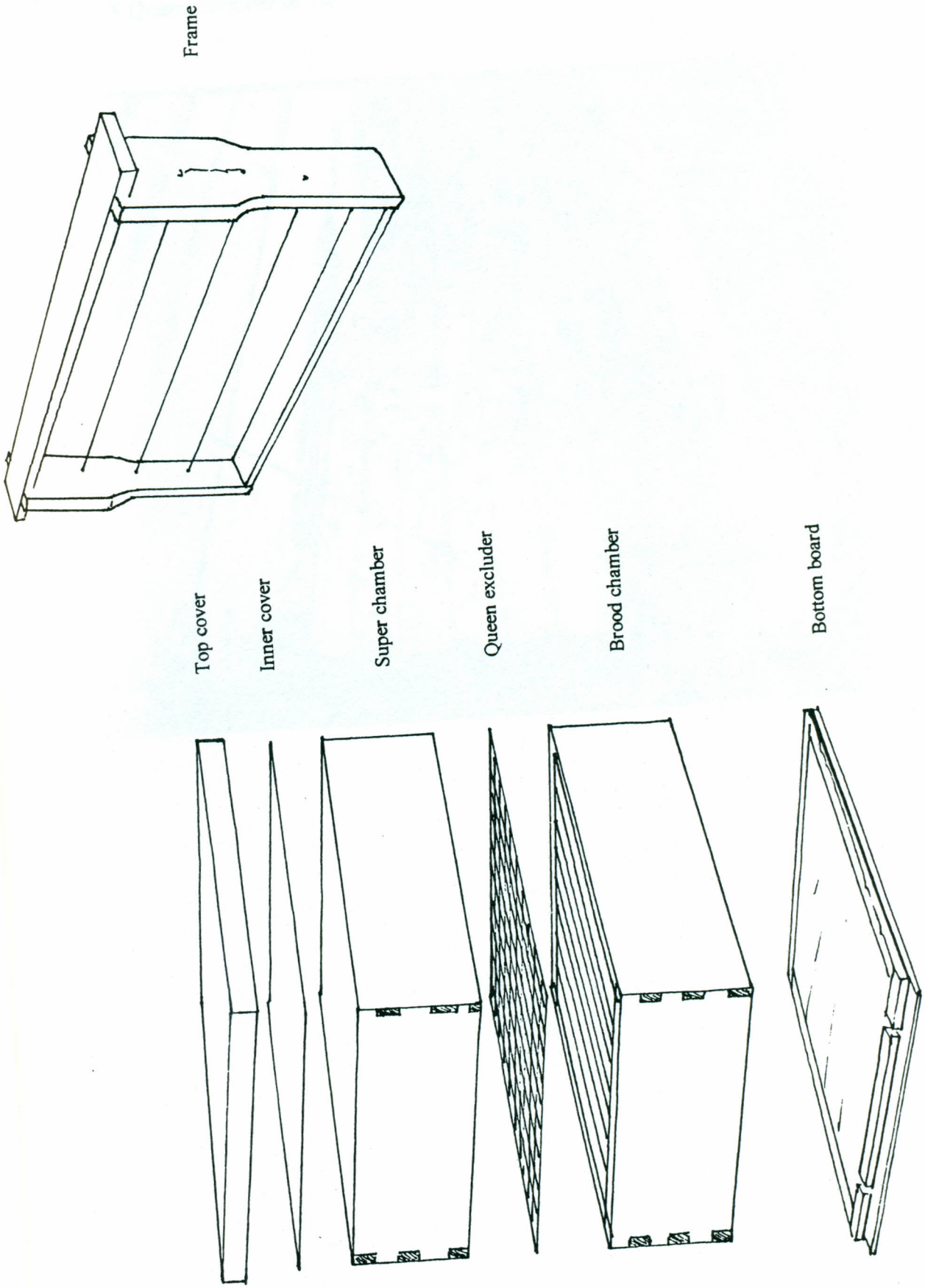

Plate 2.1: A Queen excluder in use

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Plate 2.2: Making wax queen cells using a forming stick

Plate 2.3: **Wax queen cells attached onto a cell bar**

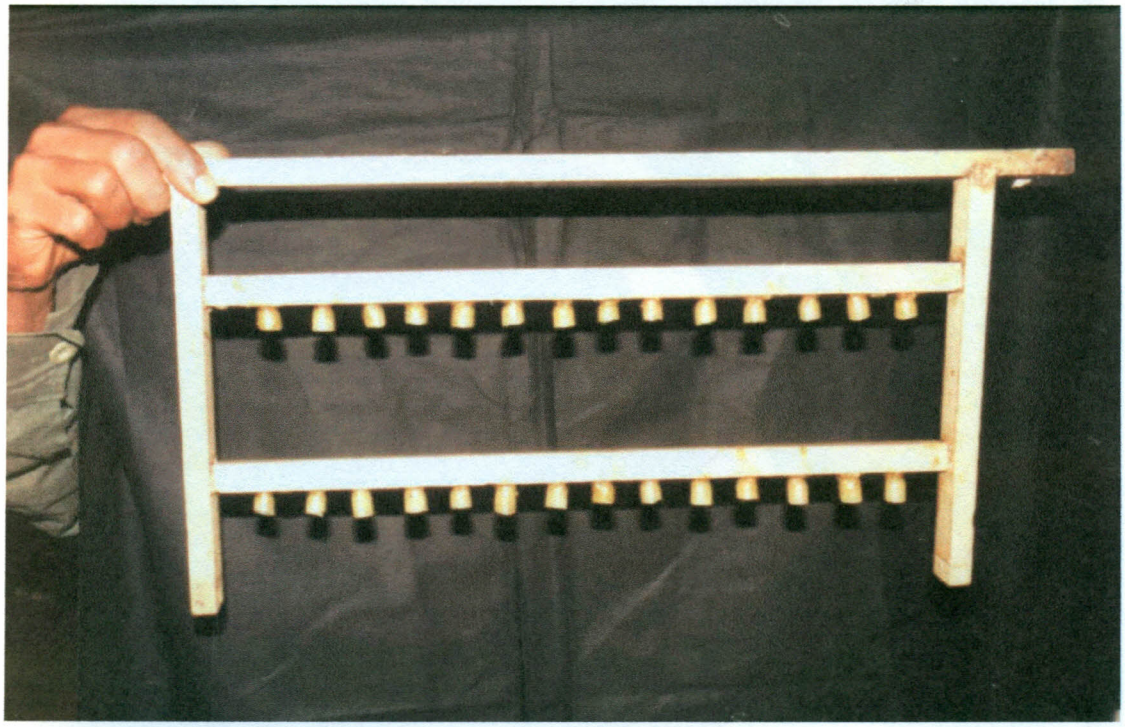

Plate 2.4: A Chinese grafting tool in use during the grafting process

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Plate 2.5: Capped queen cells (with queens developing inside them) on a grafting frame

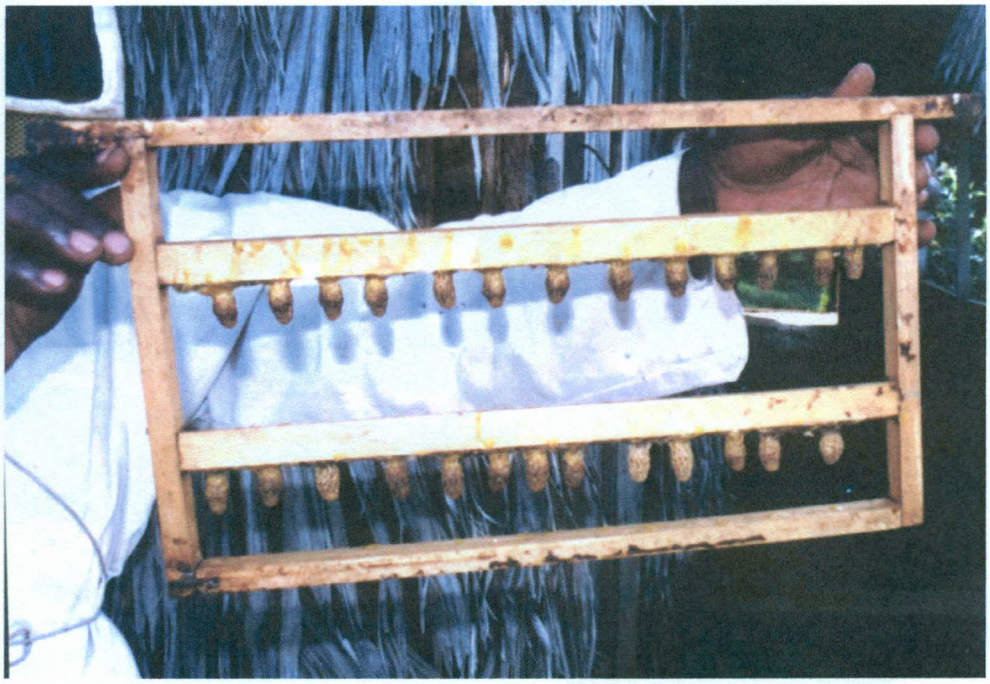
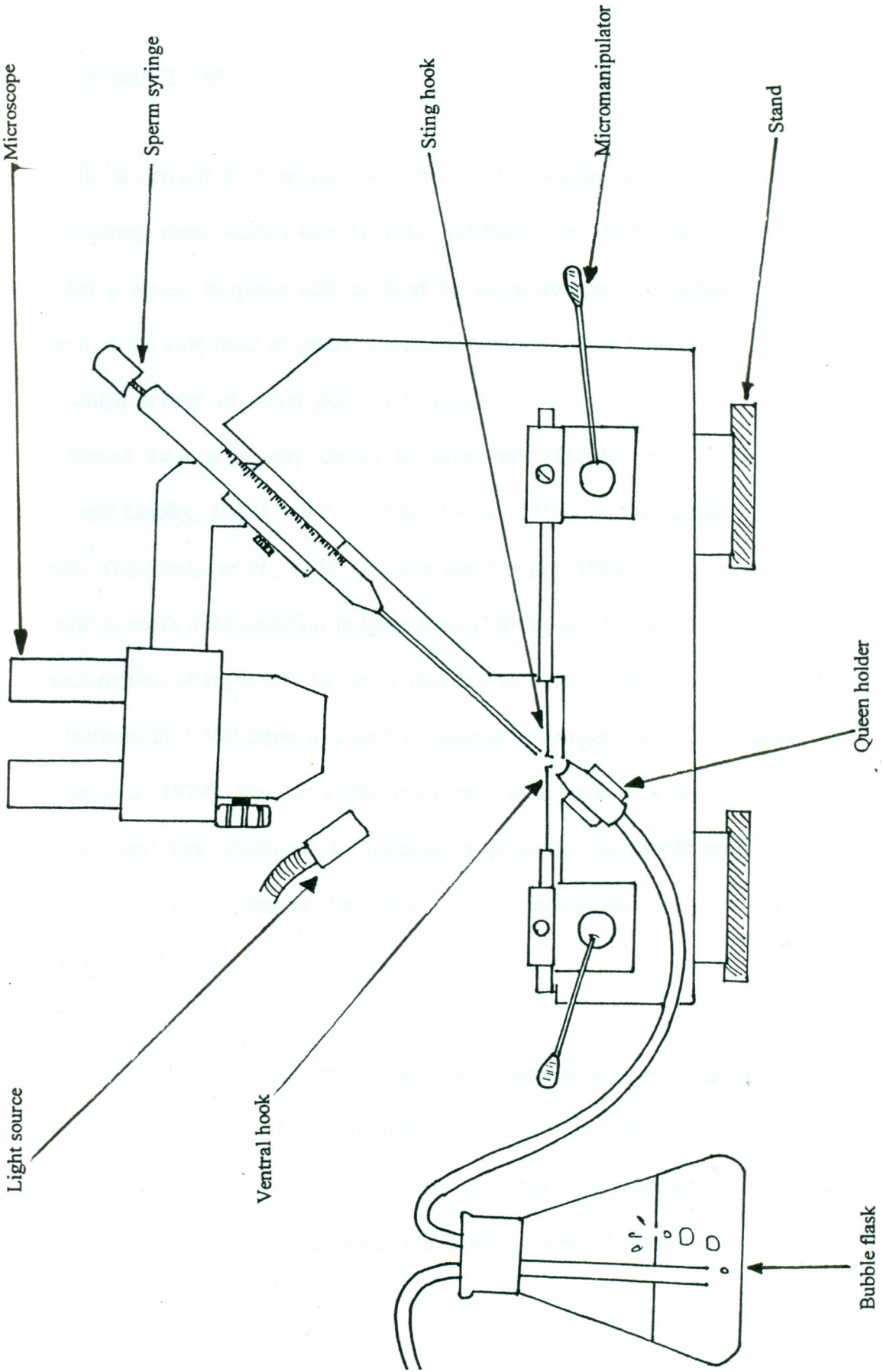


Plate 2.6: A nuc-colony

Figure 2.3: The honeybee queen insemination apparatus



CHAPTER THREE

FACTORS AFFECTING ROYAL JELLY PRODUCTION

3.1 INTRODUCTION

Royal jelly is derived from secretions of both the hypopharyngeal and mandibular glands of young nurse worker bees (Lensky and Rakoover, 1983; Knecht and Kaaz, 1990) that is placed in queen cells as food for larval queens. It is called royal jelly because it is the sole food of queen larvae, in contrast to the food of workers and drones which consist of larval jelly, or brood food (Lercker, 1982; Howe *et al.*, 1985). Brood food is initially similar to royal jelly (Haydak and Vivino, 1950; Asencot and Lensky, 1988), but is modified by day 4 through the addition of honey and pollen (Matskuka *et al.,* 1973; Asencot and Lensky, 1988). Royal jelly plays a major role in caste differentiation in honeybees (Moritz and Southwick, 1992). The high consumption of royal jelly by larvae destined to become queens results in body weight increase by 1,300 times in 6 days compared to workers fed on normal brood food (Beetsma, 1979). By the 1950s royal jelly had become a hive product of exploitation and has continued to maintain a place in the world market as a specialized dietary supplement for human consumption and for the cosmetic industry (Crane, 1999).

However, royal jelly is fed directly to the adult queen honeybee or larvae as it is secreted; it is not stored and it is for this reason that it has not been a traditional beekeeping product. The only situation in which harvesting becomes feasible is during the queen rearing seasons, when the larvae destined to become queen bees

are supplied with an over-abundance of royal jelly. For commercial production purposes, however, beekeepers cannot rely on natural queen rearing which is usually erratic in occurrence and a minimal number of queen cells are nurtured at a particular time. Instead colonies are manipulated to induce conditions similar to those in which queen rearing takes place in nature (swarming and emergency replacement of queens). It is however, worthwhile to note that although the commercial exploitation of royal jelly began in the 1950s, it is a relatively new hive product for East African beekeepers. Queen rearing techniques are yet to be adopted by a majority of the local beekeepers and thus production of royal jelly, whose production relies on artificial queen rearing for commercial economic purpose is least developed.

Any young female larvae, less than 3 days old, can develop into either a queen or a worker bee (Laidlaw, 1992) depending on the quantity and type of food they receive from the nurse bees (Beetsma, 1979; Elton, 1992). The larva has the genetic constitution to respond to the food given it by nurse bees to channel its development toward either a worker or a queen by a route controlled by nurse bees (Laidlaw, 1992). It is such young larvae that are recommended for grafting during royal jelly production.

The conventional method of royal jelly production reported by Okada and Obata (1962) involves grafting larvae and harvesting 72 hours after grafting when it is at maximum. This translates to only 10 grafts or harvests in a working period of 30 days. In this study it was envisaged that harvesting two days after grafting, even

though yielding less royal jelly per cell cup would allow for 15 grafts in the same working period, which could eventually lead to higher yields.

Due to the potential royal jelly has as a marketable hive product and hence an additional source of income for East African beekeepers, this study was designed to evaluate the potential of the local honeybee races *A.m.scutellata* and *A.m. monticolla* for royal jelly production and to determine the effect of age of larvae grafted, supplementary feeding and duration of time taken to harvest after grafting on royal jelly yields.

3.2 MATERIALS AND METHODS

3.2.1 Royal jelly production levels of *Apis mellifera scutellata* and *Apis mellifera monticola*

Six (three for each race) honeybee colonies established in 10-frame Langstroth hives were transferred from Mwingi (1°12'S, 38°16'E) and Kinangop (0°35'S, 36°34'E) to an experimental apiary at the International Center of Insect Physiology and Ecology (ICIPE), Nairobi. This was done in order to evaluate the two races under similar climatic and floral resource conditions. The colonies from Mwingi and Kinangop were *A.m.scutellata* (Plate 3.1) and *A.m. monticolla* (Plate 3.2) respectively (Shi, 2001).

Royal jelly was collected from these queenright colonies following the procedure of Laidlaw and Eckert (1962). Dark empty combs were availed to the queen in a nurse colony to lay eggs on. Using a grafting tool the young worker larvae hatching from

the eggs were transferred (grafted) into artificial plastic queen cell cups at the age of 24 hours. A total of 720 larvae were grafted in each colony during the experimental period. The wooden bar frames holding the queen cups were then placed in the supers in between two combs of young open brood and pollen. Cells were harvested three days (72 hours) after introduction into the production colonies (Okada and Obata, 1962). To harvest, grafting frames with queen cups containing royal jelly were removed from the production colony and the adhering bees gently shaken off. In the grafting room, the number of accepted (nurtured) queen cups were counted and recorded. The wax at the opening of the queen cups was cut, larvae were then removed from the queen cups using a forceps and discarded. The royal jelly was then scooped using a royal jelly spoon (Plate 3.3), collected in airtight glass containers, weighed and stored in a refrigerator.

Plate 3.1: A queen of *Apis mellifera scutellata*

Plate 3.2: A queen of *Apis mellifera monticola*

Plate 3.3: Harvesting royal jelly using a royal jelly spoon

3.2.2 Effect of age of larvae grafted on royal jelly production

The study was carried out at the ICIPE Experimental Apiary, Nairobi Kenya. Experiments on the production of royal jelly was carried out using four queen-right colonies of *Apis mellifera scutellata* colonies following the procedure of Laidlaw and Eckert, (1962).

Grafting frames were prepared to have 4 notches (to hold 4 bars of queen cups) thus a total of 16 bars (4 bars per age group in each colony for 4 colonies) were used. The age at which the larvae were to be grafted were marked on the upper bar of the frame with the comb providing the larvae and the bars holding the queen cups for ease in identification. In each grafting frame, 4 bars of plastic queen cups were fixed. Each experimental colony received 10 larvae per age group to give a total of 40 larvae per graft.

Five colonies with young good-laying queens were used as nurse colonies, to serve as sources of larvae of different ages. Each nurse colony was provided with an old dark worker-comb (Plate 3.4) to lay eggs in. Larvae are easily seen against a dark background making the process of grafting much easier. The preparation of larvae of different ages for grafting was carried out as shown in Table 3.1.

Plate 3.4: A dark old comb for preparing larva for grafting

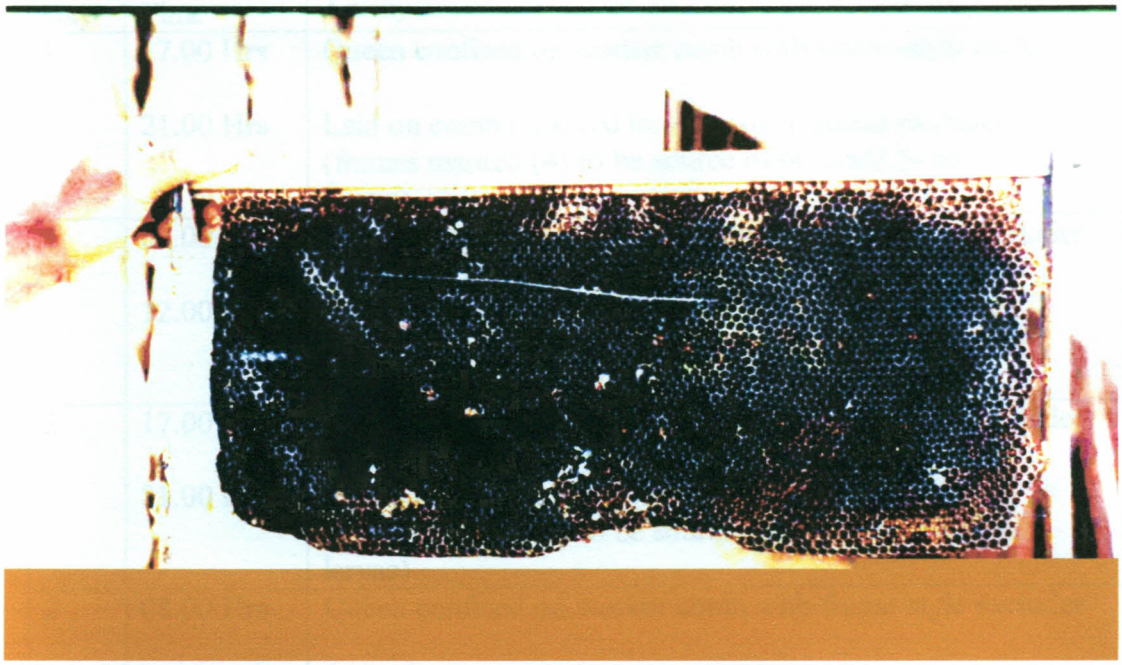


Table 3.1: Procedure for obtaining different age larvae simultaneously for grafting

Day	Time	Activity
1	17.00 Hrs	Queen confined on worker comb with frame-style excluder
	21.00 Hrs	Laid on comb removed to super over queen excluder (frames marked (4) to be source of 60 hrs/2.5- day old larvae)
2	08.00 Hrs	Queen confined on worker comb with frame-style excluder
	12.00 Hrs	Laid on comb removed to super over queen excluder (frames marked (3) to be source of 48 hrs/2.0- day old larvae)
2	17.00 Hrs	Queen confined on worker comb with frame-style excluder
	21.00 Hrs	Laid on comb removed to super over queen excluder (frames marked (2) to be source of 36 hrs/1.5- day old larvae)
3	08.00 Hrs	Queen confined on worker comb with frame-style excluder
	12.00 Hrs	Laid on comb removed to super over queen excluder (frames marked (1) to be source of 24 hrs/1.0- day old larvae)

1. A frame-style queen excluder was used to confine the queen to lay eggs on the supplied combs in order to have an overabundance of same-age larvae. The eggs hatched 72 hours from time of laying.

2. At 08.00 Hrs – 12.00 Hrs on the seventh day after the first comb was given, combs of larvae of different age were obtained at the same time.

Plastic queen cups into which larvae were to be grafted were left in the colonies for 24 hours for cleaning and familiarization. Larvae were grafted at the ages of 24 hrs, 36 hrs, 48 hrs and 60 hrs. Fourteen grafts of 10 larvae each were used for each age group and each colony, thus a total of 560 queen cups were harvested per age group. The arrangement of the larvae in the four colonies was as follows from top to bottom: Colony A - 24 hrs, 36 hrs, 48 hrs and 60 hrs; Colony B – 60 hrs, 48 hrs, 36 hrs, and 24 hrs; Colony C – 36 hrs, 24 hrs, 60 hrs, and 48 hrs; Colony D – 48 hrs, 60 hrs, 24 hrs and 36 hrs. The queen cup arrangement was alternated in each colony after a single graft, aimed at reducing the effect (if any) of position of the grafted cells on the frame on cell reception and royal jelly yields.

Started cells were harvested 72 hours after grafting when royal jelly is at maximum (Okada and Obata, 1962; Abdalla *et al.,* 1995). The frames with queen cups containing royal jelly were removed from the production hives, the wax at the opening of the cup was removed and the cup opened to expose the queen larva and royal jelly. The number of accepted cells in each age group were recorded and the larvae removed from the queen cups with forceps and discarded. For each respective age group, royal jelly in the queen cups was harvested using a royal jelly spoon and stored in airtight glass bottles. Weights of the harvested royal jelly of each age group were taken using an electronic balance (precision of 0.001 grams). The average amount of royal jelly per cell was determined by dividing the total amount of royal jelly by the total number of accepted cells.

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3.2.3 Effect of supplementary feeding on royal jelly amounts

Six honey bee colonies were prepared as previously in Section 3.2.1 The colonies were divided into three groups of two colonies each. The three groups were randomly assigned different feeding schedules of sugar syrup (1 part sugar: 1.5 parts water). Colonies of group 1 were fed at dusk before the day of grafting and those of group 2 were fed during grafting. Colonies of group 3 served as controls. The control colonies were availed with 500 mls of plain water in the plastic bowls. Sugar syrup was availed by placing a plastic bowl with 500mls sugar syrup solution in a shallow super (with all frames removed) above the main super. Sugar syrup was prepared by boiling water, then adding white sugar when the water was still warm to dissolve the sugar faster. The sugar syrup was then fed to the bees after cooling. Before grafting, queen cups were availed to the experimental colonies for 24 hours for cleaning and familiarization. Larvae were prepared by providing an empty dark worker comb to nurse colonies four days before the grafting date (thus grafted larvae had a maximum age of 24 hours). Started cells were harvested 72 hours after grafting as described in Section 3.2.2.

3.2.4 Effect of duration of time after grafting on royal jelly amounts

To investigate the effect of duration of time taken to harvest after grafting on royal jelly amounts, eight (8) colonies with eight frames in the super fully covered by bees were randomly selected. Two nurse colonies (to supply young larvae for grafting) with young proficient laying queens were also selected. The eight production colonies were divided into two groups.Grafting procedures were as in previous experiments. Royal jelly in Group 1 colonies was harvested two days (2) after

grafting while colonies in Group 2 were harvested three days (3) after grafting. In both cases, re-grafting was done immediately after harvesting. As such Group 1 colonies were harvested 30 times in a period of 60 days while Group 2 colonies were harvested 20 times in the same period.

3.3 RESULTS

3.3.1 Royal jelly production levels of *Apis mellifera scutellata* and *Apis mellifera monticola*

Royal jelly yields of 501.7 grams and 526.1 grams were realized from the three colonies of *A. m. scutellata* and *A. m. monticola* respectively over a period of three months, an average of 167grams and 175 grams per colony or an equivalent of 6.9 and 7.3 grams per colony in a single harvest respectively. *A. m. monticola* produced slightly higher amounts of royal jelly compared to *A. m. scutellata*. However, the royal jelly yields were not significantly different ($F=5.37;p<0.05$;Table 3.2). The average royal jelly yields per queen cup was 333.4 milligrams for *A. m. scutellata* and 331 milligrams for *A. m. monticola* (Table 3.2). Colonies of *A. m. monticola* showed higher acceptance (cell reception) rates (73%), with 1585 cells accepted out of a total of 2160 total grafted cells (720 in each colony) compared to *A. m. scutellata* with 1496 accepted cells (69%). However, the cell receptive rates were not significantly different ($F=0.26;p< 0.05$;Table 3.2).

Table 3.2: Comparison of royal jelly (RJ) production between *A. m. scutellata* and *A. m. monticola* colonies

Honeybee race	No. of cells grafted	Cell reception (%)	Mean RJ yields/colony /graft (g)	Mean RJ yield/cell (mg)	RJ yield/ colony (g)	Total royal jelly yields (g)
A.m.scutellata	2160	69 ± 4.8a	6.9 ± 0.5 a	333.4 a	167	501.7
A.m monticola	2160	73 ± 4.8a	7.3 ± 0.5 a	331 a	175	526.1

Means followed by the same letter within the same column are not significantly different ($p<0.05$) by t-test.

3.3.2 Effect of age of larvae grafted on royal jelly production

The mean percentage cell reception were highest in the 24 hours-old larvae (74.5%) and least in the 60 hours-old larvae (35%) (Table 3.3). Cell reception of 24 hours-old larvae was significantly higher compared to cell reception of 36, 48 and 60 hours-old larvae. Cell reception of 36 hours-old larvae was also significantly higher from those of 48 and 60 hours-old larvae ($F=13.18;p<0.05$). Cell reception of 48 and 60 hours-old larvae were not significantly different ($F=13.18;p>0.05$).

The total royal jelly yields obtained per colony were low with an increase in the age of larvae grafted. Royal jelly yields were highest in the 24 hours-old larvae and an analysis of variance indicated that larvae grafted at the age of 24 hours yielded significantly higher royal jelly yields compared to those grafted at the age of 36, 48 and 60 hours ($F=5.23;p<0.05$). Royal jelly yields from 36 hours-old larvae were also significantly higher from the yields of 48 and 60 hours-old larvae. However, there was no significant difference in yields between the 48 and 60 hours-old larvae ($F=5.23;p<0.05$). Larvae grafted at the age of 60 hours had the least yields of royal jelly (Table 3.3). The average royal jelly per queen cell also decreased with an increase in larval age and was highest in 24 hours-old larvae (419.5 mg) and least in the queen cups grafted with 60 hours-old larvae (181.5mg) (Table 3.3)

Table 3.3: Queen cell reception rates and royal jelly yields from larvae grafted at the ages of 24,36, 48 and 60 hours.

Age of Larvae	No. of grafted cells	Queen cell reception (%) ± S.E	Royal jelly Yield/ cup (mg)
24	560	74.5 ± 3.3 a	419.5 ± 25.6 a
36	560	58.5 ± 4.8 b	356.8 ± 41.9 b
48	560	42.5 ± 5.6 c	284.5 ± 17.2 c
60	560	35.0 ± 3.1 c	181.5 ± 13.3 c

Means followed by the same letter in the same column are not significantly different ($p<0.05$) by Tukey's Test.

3.3.3 Effect of supplementary feeding on royal jelly amounts

Feeding sugar syrup to royal jelly producing colonies had no significant effect on cell reception. There were also no significant differences in cell reception rates of the grafted larvae in colonies fed at dusk a day before grafting, during grafting and controls (not fed) ($F=2.97;p<0.05$; Table 3.4). The mean cell reception rates were 75%, 75% and 63% respectively (Table 5.1). However, feeding sugar syrup to royal jelly producing colonies significantly increased royal jelly yields. Royal jelly yields from colonies fed at dusk and colonies fed during grafting were significantly higher than those obtained from the control colonies ($F=98.53;p<0.05$;Table 3.4). Colonies fed at dusk before the day of grafting had lower royal jelly amounts (419 mg/cup) compared to the royal jelly amounts from colonies fed during the time of inserting the grafting frame (495mg/cup). However, these amounts were not significantly different ($F=98.53;p< 0.05$;Table 3.4).

Table 3.4: Cell reception and royal jelly (RJ) yields after various feeding schedules with sugar syrup.

Treatment	Number of cells grafted	Mean cell reception (%) ± S.E	Average RJ yields/colony/ graft	Mean RJ yields/cell (mg)
Control	560	63.0 ± 6.2 a	2.9 ± 1.4 a	196.0 a
Fed at dusk	560	75.0 ± 2.5 a	6.3 ± 0.5 b	419.0 b
Fed at grafting	560	75.0 ± 3.3 a	6.1 ± 1.2 b	495.0 b

Means followed by the same letter within the same column are not significantly different ($p<0.05$) by Tukey's test.

3.3.4 Effect of duration of time after grafting on royal jelly amounts

A total of 2,130 cell cups were harvested from Group 1 where harvesting was done two days after grafting and 1,414 cell cups were harvested in Group 2 where harvesting was done after three days in a period of 60 days (Table 3.5). Harvesting after two days and re-grafting immediately after harvesting led to more grafts/harvests hence a higher number of cells compared to the 3-day cycle. Even though higher amounts of royal jelly (503.4 grams) were obtained in the 2-day cycle compared to the 3-day cycle (494.2 grams), a t-test analysis showed that the amounts were not significantly different ($0.76; p<0.05$; Table 3.5). It was also noted that the 3-day cycle had significantly higher royal jelly yields per cell cup (349.5 mgs) compared to 236.3 mgs in the 2-day cycle.

Table 3.5: Royal jelly (RJ) yields harvested in 2 and 3 days after grafting

Harvesting time after grafting	Queen cells harvested	RJ/cell cup (milligrams)	Total RJ yields ± S.E (grams)
2 days	2,130 a	236.3 a	503.4 ± 0.97 a
3 days	1,414 b	349.5 b	494.2 ± 0.97 a

Means followed by the same letter within the same column are not significantly different ($p<0.05$) by t-test.

3.4 DISCUSSION

Several factors are responsible for honeybee colony growth such as the queen's capacity to lay eggs, size and age structure of worker population, availability of nectar and pollen and availability of space for expansion of the brood nest and storage of nectar/honey (Farrar, 1968). Availability of nectar and pollen from flowering plants, commonly called nectar flows, are influenced by seasonal weather patterns. The honeybee colony responds to these changes by either raising more brood when resources of both pollen and nectar are plentiful or raising less brood when the resources are scarce. A reduction in brood rearing inevitably leads to a reduction in colony populations (Otis, 1982; Shi, 2001).

Seasonal weather patterns and beekeeping management practices being constant, the productivity of honeybee colonies entirely depends on the egg-laying performance and pheromone production ability of queens (Laidlaw, 1992). The reproductive ability (combination of the queen's egg-laying ability and the worker bees' nursing ability) and foraging ability of honeybees are genetically controlled (Chen Yaochun, 1993).

Results on the royal jelly production levels of *Apis mellifera scutellata*and *Apis mellifera monticola* indicate that there were no significant differences in queen cells reception and royal jelly yields between the two honey bee races. Since the two races were evaluated under similar environmental conditions and colony management practices, their performance in regard to productivity can only be attributed to the reproductive and foraging abilities of the queens and workers

respectively. As no differences were found in their royal jelly production levels, it is probable that the nursing abilities for brood rearing and queen raising of the two races have not been affected by their geographic adaptations.

Colony royal jelly yields of 167 and 175 grams for *A. m. scutellata* and *A. m. monticola* respectively were attained over a production season of 3 months. Crane (1999) reported that well managed colonies in Canada produced 7 grams per harvest with an average yield of 200 – 250 milligrams per queen cell. She further reported that such colonies produce about 300 – 500 grams within a three-month period. This is almost twice as much as the royal jelly produced by the East African honeybee races investigated in this study. It is possible that these differences in royal jelly production could be due to the differences in races of honeybees used, climatic conditions under which production is done and floral resources available to the colonies at the times of production. It is worthwhile to note that for royal jelly production to be successful, a high population of young nurse workers should be available. However, a high population of young workers causes swarming, (Lensky and Slabezki, 1981) leading to loss of part of the colony population.

The East African honey bee races are known for their high swarming tendencies where about half the workers leave the old nest (Winston, 1987). In addition, Shi (2001) reported that *Apis mellifera scutellata* colonies do not grow into what would be considered large colonies (over 30,000 bees) under European conditions, and as such it is not possible to build up colony populations similar to those possible with the European honeybee races. The lower colony populations means that only a limited number of queen cells can be availed to and nursed by the colonies at a

particular time while colonies with high populations can accept and nurse a higher number of queen cells. This probably with other factors such as floral resources explains why there is a difference in production of royal jelly between the honeybee races.

In Vietnam, one colony of *Apis mellifera* produces an average of 300 grams of royal jelly annually (Apiservices, 2000), an amount which is comparable to that produced by the two East African honeybees. In Syria the total royal jelly production is estimated at 300 kilograms annually (Syrian Bee Center, 2002); in Taiwan, 350 metric tones are produced annually with an average of 4.5 kilograms per colony (Fert Gilles, 1999) while in China more than 1000 metric tones are produced annually with annual colony production of 7.7 kilograms (Chen *et al.,* 2002). It should also be noted that royal jelly yields of as high as 7 grams per harvest per colony were achieved during this study. However, the yields were not steady and fluctuated between grafts. The major challenge in producing royal jelly using the local honeybee races will be to come up with measures on how to build up colony populations and avoid swarming at the same time for steady royal jelly yields.

Currently, there are imported value-added royal jelly products (especially food supplements in form of capsules) on sale in the Kenyan market, an indication of local demand. With time, such value-added products could be produced locally either by the beekeepers themselves or other private entrepreneurs. The Commercial Insects Program at ICIPE is currently 'enhancing' honey quality by adding 2% of royal jelly. This work, being the first report on royal jelly production using the East African honeybees, is expected to stimulate interest among bee researchers and

beekeepers and it is hoped that with time better production techniques and management skills for royal jelly production using the local honeybees will be developed, evaluated and popularized among beekeepers in the region for increasing their income and improving livelihoods.

Results on the effect of age of larvae at grafting showed, that larvae grafted at the age of 24 hours had the highest reception rates and royal jelly yields. Larvae at this age are small but clearly visible, float on a mass of brood food and thus are easily grafted with the least risk of injury, and this probably leads to more acceptance. The older and the larger the larvae become, the less the reception rates. Larvae at the age of 60 hours were large, occupying a large area of the base of the worker cells, and this made it difficult to efficiently graft such larvae. The long periods of time taken to graft these large larvae and the high risk of injury could have led to poorer reception.

Royal jelly yields were also significantly affected by the age of larvae used. On a 72-hour cycle, 24 hours-old larvae had the highest royal jelly yield with an average of 419.5 mg per queen cup whilst larvae grafted at the age of 60 hours had the least royal jelly yields with an average of 181.5 mg per queen cup. Probably larvae at the age of 24 hours, due to their smaller sizes, consumed less of the food (royal jelly) supplied thus accumulating more and consequently had higher royal jelly yields. The larger the larvae were then the more they consumed and thus larvae grafted at the age of 60 hours consumed much of the food supplied and accumulated less.

Young female larvae, less than 3 days old, can develop into either a queen or a worker bee depending on the quantity and type of food they receive from the nurse

bees (Beetsma, 1979; Elton, 1992). The royal jelly production cycle is similar to queen rearing, with the only difference being that the cycle for royal jelly production takes only 3 days as opposed to sixteen days in queen rearing. However, for maximum production it becomes necessary to determine a specific time between the ages of 1 – 3 days at which grafted larvae produce maximum royal jelly yields. There are no reports on royal jelly production using the East African honeybees. Okada and Obata (1962) using the European honey bee (EHB), *Apis mellifera* reported that honey bee worker larvae of 8-24 hours old yielded maximum royal jelly (148 - 281mg/queen cup) when harvested at 72 hours from grafting. Laidlaw, (1992) reported that larvae of the age of 18 – 36 hours are the most ideal for grafting during queen rearing. It is suggested that royal jelly yields should not fall below 200mg/queen cup (Krell, 1996). In the current study, larvae grafted at the ages of 24-60 hours gave yields higher than or equivalent to 200mg/queen cup. However, for optimum yields, it is suggested that larvae be grafted at the age of 24 hours or slightly younger if one has skillfully mastered the grafting process.

Results on the effect of supplementary feeding showed that feeding sugar syrup had no significant effect on the reception of grafted cells but had a remarkable effect on the amounts of royal jelly secreted. Fed colonies had higher amounts of royal jelly per queen cup (495 and 419 mg) compared to the controls (196 mg). It is thought that stimulative feeding increases the secretion of royal jelly by the hypopharyngeal and mandibular glands of young nurse worker bees leading to the production of good quality queens (Morse and Hooper, 1985). The quality of queens is largely due to the nature of food provisioned to the developing queen larvae. Mass consumption of pollen begins when bees are about 1.5 – 2.5 days old (Hagedorn and Moeller, 1967), and reaches a maximum when they are five days old. Within five days after

the emergence the nitrogen content of adult bees increases to 93% in the head, by 76% in the abdomen, and by 37% in the thorax (Haydak, 1934). Simultaneously, their hypopharyngeal glands, fat bodies, and other internal organs develop (Maurizio, 1954). It is the bees of the age of 5 – 15 days that are involved in nursing duties.

Consequently, it is probable that colonies availed with sugar syrup during queen rearing or the royal jelly production recruit most of the foragers to concentrate on collection of pollen as opposed to nectar. This increases the amounts of pollen in their colonies, leading to better development of the nurse bees and hence increased royal jelly secretion. Sucrose has been reported as the ideal carbohydrate supplement for honeybees (Elton, 1992) and that it is superior to other sugars in both acceptance and nutritive value to honeybees (Barker and Lehner, 1974a). Thus the synergistic effect of more pollen and nutritive value of sugar syrup could have led fed colonies to produce more royal jelly.

Stimulative feeding was also found to be cost effective. In this case, for example, two unfed colonies produced 76.96 grams of royal jelly (worth 9.6 US $ at the market price of 120 US $ a kilogram) while the two fed colonies produced 165.5 grams (worth 19.9 US $). The cost of sugar consumed by the two colonies was 1.8 US dollars, therefore if we subtract the cost of sugar, the fed colonies produced royal jelly worth 18.1 US $ compared to 9.6 US $ by the controls.

Results on the effect of duration of time after grafting revealed that harvesting two and three days after grafting had no significant difference on royal jelly amounts. However, harvesting two days after grafting and re-grafting immediately led to more grafts/harvests hence a higher number of cells are harvested compared to the 3-day

cycle within the same time frame. However, cell cups harvested three days after grafting yielded more royal jelly on average. These results are similar to those of Okada and Obata (1962) who reported that harvesting cells 3 days after grafting yielded a maximum amount of royal jelly (150 mgs). However, higher amounts of royal jelly per cell cup than 150gms were achieved in this experiment in both the 2-day and 3-day harvesting regimes.

These results most probably were due to differences in the test colony populations and floral resources available to the bees during the two studies. Harvesting two days after grafting is time consuming and laborious. It is suitable where one has a higher number of colonies to compensate for the lower yields per cell cup. In cases where one is working on fewer colonies, harvesting three days after grafting seems more ideal because one can get higher yields from a less number of cell cups. The 3-day cycle is less laborious and may allow the beekeeper more time for other on-farm activities. Due to the defensive nature of the local honeybees, the 3-day cycle seems to be more ideal as it allows for less colony manipulations (disturbances).

CHAPTER 4

CHEMICAL COMPOSITION OF THE ROYAL JELLY PRODUCED BY KENYAN HONEY BEES

4.1 INTRODUCTION

Royal jelly is a yellowish white, creamy, acidic material with a slightly pungent odour and taste which is secreted from hypopharyngeal and mandibular glands of young worker honeybees and is fed to the queen bees throughout their larval and adult stages and to worker and drone larvae less than three days old (Lercker, 1982). Since the discovery of significant differences in foods given to worker bee larvae and the queen bee larvae, and the discovery that queens' body weights increase by about 1300 times in 6 days due to their diet (Asencot and Lensky, 1976), there has been an increasing interest on the queen bee larvae food (royal jelly) composition. Royal jelly is regarded as the major reason for the considerable morphological and functional differences between queen and worker bees (Beetsma, 1979). It has been used by humans, especially in dietetics and cosmetics, and has become a commercial product in many countries. When a worker emerges the development of her glandular system is extremely dynamic and this complex pattern results into changes in the worker bee's behaviour, related to the tasks she performs over its life time (Lindauer, 1952; Sakagami, 1953; Free, 1965; Michener, 1974; Robinson and Page, 1989). The mandibular secretion of *Apis mellifera* is involved in food preservation and larval nutrition. Under queenright conditions, the mandibular secretion of workers is characterized by 10-hydroxy-decanoic acid (10-HDA) (Plettner *et al.*, 1993). The hydroxy acids found in royal jelly act as antiseptics (Weaver *et al.*, 1968;

Blum *et al.,* 1959). 10-HDA inhibits the germination of pollen, which is important for pollen storage (Winston, 1987), and it is an important larval nutrient that prevents larvae from pupating precociously (Plettner *et al.,* 1997).

By the 1950's royal jelly had become a hive product of exploitation. Royal jelly has won and has maintained a place in the world market, as a specialized dietary supplement for human consumption and the cosmetic industry. It is estimated that about 500 – 600 tones are produced annually (Crane, 1999). The principal constituents of royal jelly are moisture, crude protein, lipids, sugar, and fatty acids, the major fatty acid being 10-Hydroxy-2-decanoic Acid (Chen Yaochun, 1993). It is, however, worthwhile to note that although commercial exploitation of royal jelly began in the 1950s, it is a relatively new hive product for East African beekeepers. As such there are no established identification or quality standards.

Several chemical characterization studies on royal jelly have been reported (Steyn, 1973; Finzi, 1980; Ivanov, 1981), however, there is still no international standard for either its composition or for the necessary analytical procedures to characterize it (Karaali *et al.,* 1988). Since chemical characterization is vital in the control of marketing of natural products for human consumption, the need arose to study the chemical composition of the royal jelly produced by Kenyan honey bees in order to control its quality standard and effectively market this product in the Kenyan and export markets.

4.2 MATERIALS AND METHODS

4.2.1 Royal jelly production

Royal jelly was produced in queenright colonies following the procedures of Okada and Obata, (1962). After 3 days the queen cells containing royal jelly were removed from the grafting frame (Plate 4.1). The wax at the opening of the cell was removed and the cell opened to expose the queen larva and the royal jelly. Each larva was removed and the royal jelly extracted with a royal jelly spoon. All the royal jelly collected from the hives on the same day was pooled and weighed as a pooled wet sample.

The royal jelly not to be immediately assayed for moisture was frozen in a sealed vial. For future analysis, the stored frozen samples were allowed to equilibrate to room temperature and then stirred with a micro-spatula to produce a homogenous mixture.

Plate 4.1: Queen cells containing royal jelly

4.2.2 Moisture content

Fresh samples of royal jelly were weighed in a pre-weighed sample bottle. Sample weights were thus ccalculated as:

Wt. of RJ sample = Wt. of sample bottle + RJ - Wt. of sample bottle

The samples were then freeze dried at -20^0 C and weighed after 12, 24, 36 and 48 hours till a constant weight was reached. The weights of the dry samples were arrived at by averaging constant weights and percentage of water calculated as:

% Water content = Wt. of freeze dried sample ÷ Wt. of fresh sample X 100

4.2.3 Crude protein

Two hundred (200) milligrams of royal jelly samples were each reconstituted in 1 millilitre of distilled water. A BCA assay procedure was followed, thus 1 ml of bicinchoninic acid was added to the sample and mixed. The resultant mixtures were incubated at 37^0 C for 30 minutes after which the samples were cooled at room temperature.

Using a Spectrophotometer (Cecil CE 3041 3000 Series) a standard working curve was prepared by plotting the Net (blank corrected) absorbance at 562 nm against Standard protein concentration. The samples were then run against a corrected blank at 562 nm and protein content calculated as:

% Protein = Concentration of protein ÷ Sample weight X 100

4.2.4 Crude lipids

Three (3) grams of lyophilized royal jelly samples were subjected to the Soxhlet method of extraction. The samples were placed in a porous thimble in an extraction chamber suspended above a solvent flask and condenser. The flask was heated to evaporate the solvent (ethyl ether), which in turn moved up into the condenser – where it was cooled/condensed into a liquid. This liquid trickled down into the extraction chamber, extracting the lipids into the flask.

The organic solvent and the aqueous phase were then allowed to separate (by gravity) after which the aqueous phase was decanted off, and the solvent evaporated. The weight of remaining mass (lipid mass) was recorded.

% Lipids = Mass of lipids ÷ Mass of sample X 100

4.2.5 Total Sugars

Analytical grade sugars used as standards were trimethlsilylated to allow their analysis by Gas chromatography (GC). One milligramme of each standard was dissolved in 100ul of dry pyridine in a 2ml reaction vial. An equal volume of N-methyl-N-trimethylsilyltrifluoroacetamide (MNSTFA) derivatizing reagent was added. The mixture was then placed in an oven and the reaction allowed to run at 60°C for 1hour. The samples were then removed and stored at room temperature prior to analysis. Dry samples of royal jelly extracts were treated similarly.

1ul in ml of each trimethylsilated standard and sample were analyzed. GC analysis was performed on a capillary gas chromatograph Hp model 5890A series II equipped with a splittles capillary injector system FID coupled to Hp3393A series

integrator. The separation was done on a cross-linked methylsilicone capillary column (50 m x 0.2 mm) operating condition were as follows: carrier gas hydrogen; make up gas Ar/ch4; temperature detector 300°C, injector 270°C and Oven 60-280°C at rate 10°C /min with initial and final time of 5min and 20min respectively.

The retention times of the sugars were compared to those of the standards and their concentrations calculated. The amount of sugars was then calculated as

Total amount of sugars ÷ Sample weight (g) X 100:

4.2.6 Ash content

Five samples of royal jelly 5g each were subjected to temperatures up to 600^0 C in a Muffle furnace. For each sample the final weight was recorded and total ash content calculated as:

Total ash content = Final sample weight ÷ Initial sample weight X 100

The average final weight for all the samples was then recorded.

4.3 RESULTS

Table 4.1 summarizes the results of analyses on the chemical composition of the royal jelly. Produced by Kenyan honey bees. The major components of fresh royal jelly are moisture, sugars, proteins and lipids. Moisture content of the samples ranged from 65.5 – 67.5% with an average of 66.24%, thus the dry matter content was at 33.76%. The sugar fraction was at an average of 18.45% of fresh royal jelly and consisted of fructose (58.9%), glucose (21.4%), galactose (17.1%) and sucrose (2.5%). The average crude protein content for the samples was 35.02% of the dry

weight while the average crude lipid content was 18.2% and the ash content was 2.6%.

Table 4.1. Summary on composition of the royal jelly produced by Kenyan honey bees

Constituent	Method	Composition (%)	Proposed limits (Lercker *et al.*, 1992)
Moisture	Freeze-drying	66.24	57 – 70
Dry matter		33.76	30 – 43
Crude protein	BCA- assay procedure	35.02	17.0 – 45.0
Total lipids	Selective extraction with ethyl ether	18.2	3.50 – 19.0
Total sugars	Gas Chromatography	18.2	18.0 – 52.0
Fructose (% of total sugars)		58.9	-
Glucose (% of total sugars)		21.4	-
Galactose (% of total sugars)		17.1	-
Sucrose (% of total sugars)		2.5	-
Ash	Combustion at 600^0C	2.61	2.00 – 3.00
PH	PH meter reading	3.60	-

4.4 DISCUSSION

The composition of the Kenyan royal jelly is comparable to that of other countries as reported by other authors. These authors have reported moisture content of 22.4% on an air-dried sample (Aeppler, 1922), 69.9% (Haydak, 1943), and 45% (Townsend & Lucas, 1940). Karaali *et al,* (1988) reported a dry matter content of 34.68% for Turkish royal jelly, and other literature reports include 33.20 – 37.40% for Bulgarian royal jelly (Ivanov, 1981), 33.10% for Japanese royal jelly (Takaneka, 1981) and 38.40% for Italian royal jelly (Steyn, 1973). The moisture levels obtained in the Kenyan royal jelly (66.24%) is comparable to values reported by these workers and within the limits proposed by Lercker *et al.,* 1992.

The level of sugars in the Kenyan royal jelly was 18.28% (of fresh weight) which is comparable to the results of other researchers. Karaali *et al.,* (1988) reported sugar values of 22.72 – 29.85% for Turkish royal jelly, 28.68% for Japanese royal jelly (Takaneka, 1981), 33.6% for Italian royal jelly (Steyn, 1973) and other values reported range from 9.90 – 1.30% (Ivanov, 1981). The constituent sugars identified in the Kenyan royal jelly were fructose, glucose, sucrose and galactose. Of the identified sugars, fructose and glucose had the highest concentrations of 58.9 and 21.4% respectively followed by galactose (17.1%), and sucrose (2.5%), and as such, simple sugars (monosaccharides) form the bulk of the sugars found in royal jelly. Steyn (1973) and Finzi (1980) also reported that fructose, glucose and sucrose are the major constituent sugars in royal jelly. Asencot and Lensky, (1988) reported that sugars are the components with greatest variations in gross composition mainly because workers add different amounts of sugars to royal jelly depending on the age of the queen larvae. During this study, larvae were grafted at a uniform age of 24

hours to minimize variations in the sugar components in the royal jelly due to differences in larval ages.

The crude protein, lipid and ash fractions of the Kenyan royal jelly is comparable to that reported in literature. Crude protein estimates from literature vary from a minimum of 6.7% (Lercker, *et al.,* 1981) to a maximum of 30.6% on a wet basis and 33.64% to 41.81% for lyophilized royal jelly (Ivanov, 1981). Reported values in literature for the lipid fraction vary from 4.4% (Lercker *et al.,* 1981) to 29.95 – 28.1% for Turkish royal jelly (Karaali *et al.,* 1988) and 13.38 – 16.15% for Bulgarian royal jelly (Ivanov, 1981). Other researchers have also given similar results on the lipid fraction in royal jelly (Lercker, 1982). The latter author reported that lipid content of royal jelly is composed primarily of polar components, with 8 – 10% unsaponifiable material, which consists of various hydrocarbons and sterols. Ash value reported in this study was 2.6%, comparable to values reported in literature, 3% for Turkish royal jelly (Karaali *et al.,* 1988), 2.85 – 3.30% for Bulgarian royal jelly (Ivanov, 1981) and 3.00% for Japanese royal jelly (Takaneka, 1981).

Variations in the content of various components of royal jelly from different countries could be due to different ecological and climatic conditions and the seasons when they were collected, as pointed out by Lercker (1982). Since there are no international standards for either its composition or for the necessary analytical procedures to characterize royal jelly (Karaali, *et al.,* 1988), this could probably explain the many quantitative discrepancies reported for the various components.

CHAPTER 5

SELECTION AND BREEDING OF THE EAST AFRICAN HONEYBEE RACE, *APIS MELLIFERA SCUTELLATA*

5.1 INTRODUCTION

Breeding of organisms by nature is aimed at the survival of the most adaptable and the fittest, as a result time, mutations and selection pressures have resulted in populations of bees called races, somewhat isolated from each other, that excel for various combinations of characteristics (Morse and Hooper, 1985). Such races of bees form the gene pool or genetic base available to bee breeders for stock improvement. However, nature never breeds for performance but only to preserve a particular type, hence the need for beekeepers to select and breed bees suited to their own needs. In bee breeding we are confronted with an array of factors and difficulties simply unknown in animal breeding. For example, in honeybee breeding, we are not concerned with isolated individuals but with a society made up of groups which posses very different hereditary characteristics and which also vary in their strength during the course of the year. The breeding material (queens and drones) give us no indication, with the exception of the fertility of the queens, of the worthwhile factors they are going to bequeth to their progeny. Parents are solely concerned with maintaining and developing the colony. The worker bees alone perform an economic function in the colony and alone manifest the characteristics with which we are concerned in breeding. Other challenges include parthenogenesis (of drones); multiple mating of queens; mating with drones of unknown origins; and

the fact that every drone dies in the act of mating and hence cannot be used for further matings.

Honeybees exhibit large and variable populations, even colonies in the same area may differ in brood rearing, foraging vigour (thus honey production) and defensiveness (Morse and Hooper, 1985). These variations make honeybees to respond well to selection (Collins and Rinderer, 1986). The East African honeybee, *A. m. scutellata* is best known for its defensiveness, absconding and swarming tendencies (Ruttner, 1981). The aim of this study was to explore variations in defensive behaviour and honey/royal jelly production by *A. m. scutellata,* and to breed for queens with reduced defensive behaviour and high honey/royal jelly production.

5.2 MATERIALS AND METHODS

5.2.1 Defensive Behaviour

Fifteen colonies in an apiary site in Mwingi (Nguni) were used, all headed by naturally mated queens. The colonies were installed in Langstroth hives and were standardized at the beginning of the assay in six brood combs and four honey combs, all covered by bees. Defensive behaviour evaluations were done using the method by Stort (1974) and modified by Collins *et al* (1984). Prior to evaluation, each colony was stimulated with 0.30 ml of artificial alarm pheromone (made up of equal proportions of lavender and jasmin oils) in a piece of paper of 2 x 5 cm placed at the hive entrance. Then, a dark leather ball of 5-cm diameter was balanced at 20 cm in front of the colony for one minute (Plate 5.1). Different balls were used for

each colony and they were put in individual bags after the one minute test time, in order to avoid stinging after the test timing. The number of stings on each ball were later counted. Time at first sting (T1S) and sting numbers (SN) were registered for each colony. For each colony, observations were done at 11 AM for 12 days. To avoid stimulating an increase in the number of workers guarding the nest entrance, an interval of 3 days was allowed between successive stinging experiments carried out on the same colony. Colonies were ranked, according to responses obtained for each variable each day. Colonies that reacted first to the stimulus and that left more stings on the ball were positioned in first positions of the rankings. Colony rankings were related with the Friedman test to confirm if colonies were consistent in maintaining their respective positions in the rankings. The Pearson correlation was used to analyze the relationship between the variables sting numbers (SN) and time at first sting (T1S).

Plate 5.1. A ball with stings after evaluation of a colony for defensive behavior

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5.2.2 Honey production

The management of colonies was restricted to provision of enough space for colony growth and protection from pest attack. During honey harvesting the number of combs with honey (Plate 5.2) for each colony were counted, recorded and weighed.

In order to compare productivity of the colonies, apiary average and colony production as percentages of the former were calculated as:

i). Apiary average = Total apiary honey yield ÷ Total no. of colonies

ii). Colony honey production as % of (i) above = Yield of colony ÷ Apiary average X 100

The honey production by colonies (ii above) was then used to rank the colonies (Ruttner, 1988) for two seasons that the colonies were evaluated. The two colonies with the best rankings for the two variables combined (least defensive behaviour and high honey production) were chosen to serve as breeders (sources of queens and drones). Performance of these queens was then evaluated against that of non-selected queens in both defensive behaviour and honey production. The eight daughter queens were introduced into 5-frame nuc hives. Colonies with a similar population and headed by non-selected queens were used as controls. At the time of raising the selected queens, the control colonies were de-queened and allowed to replace their queens. This ensured that all the test colonies were headed by queens of the same age. All the queens were then marked to facilitate easier detection of swarming/supersedure in case it occurred. Evaluation on defensive behaviour started two months after the introduction of the daughter queens. Defensive behaviour and honey production were evaluated as previously in Section 5.2.1.

Plate 5.2. A comb of honey

5.2.3 Royal jelly production

During the study, a breeding effort was also carried out to improve royal jelly production. Three colonies (designated ICIPE RJP1 – 3) were used as parental stock and evaluated for 3 months at the ICIPE experimental apiary. From the colony with the highest production, six daughter queens were raised and mated. The six queens designated ICIPE RJF1a – RJF1f were introduced into production colonies and their royal jelly production evaluated after two months. Larvae were grafted at the age of 24 hours and royal jelly collected after 72 hours, weighed and stored under refrigeration. Colonies were evaluated for 3 months with a total of 24 harvests per colony.

5.3 RESULTS

There were variations in defensive behaviour (sting numbers – SN and time to first sting - T1S) for all colonies in different evaluation days. Colony rankings for each variable were done and the Friedman test detected a tendency of colonies to keep the same position during different sampling days when both sting numbers and time at first sting were considered ($F = 36.12$; $p = 0.001$), that is, some colonies were more defensive than others. Pearson correlation detected a negative and significant correlation between both variables ($p < 0.05$; $r = -0.69$) an indication that colonies that reacted faster to stimulus (low values for T1S) left more stings on the ball (high values for SN).

Honey production also differed among the colonies. Apiary average honey production was 18.8 kgs and 18.1 kgs per colony for the two seasons respectively.

In the first season, eight colonies (C1, C3, C5, C7, C8, C13, C14 and C15) had honey production above the apiary average (18.8 kilograms) while in the second season six colonies (C3, C5, C7, C13, C14 and C15) had above apiary average production (18.1 kilograms) (Table 5.1). Colony rankings were done based on the totals of the percentage averages obtained by each colony for the two seasons. The colony with the highest percentage average was ranked first and that with the least percentage average was ranked last (Table 5.1).

After comparison of the defensive behaviour of colonies headed by the first generation of the selected gentle queens with that of locally caught swarms headed by unselected queens, there were no significant differences in the sting number (SN) ($t = 0.19$; $p < 0.05$). Colonies headed by the selected queens had an average of 27.5 ± 2.8 stings compared to 29.4 ± 2.8 stings for colonies headed by non-selected queens for an evaluation period of six days (Table 5.2). The colonies headed by the selected queens had a higher total honey production (157.1 Kgs) compared to that of colonies headed by non-selected control queens (143.9 Kgs) (Table 5.1), an increase of 4.4%. The average colony honey production for colonies headed by the selected queens was 19.6 ± 1.2 Kgs while that of colonies headed by the non-selected queens was 17.9 ± 1.2 Kgs. However, a t-test did not detect significant difference in honey production between colonies headed by the selected queens and those headed by the non-selected control queens ($t = 1.41$; $p = 0.001$) (Table 5.2).

Before selection, colonies had mean royal jelly yields of 2.9 ± 0.12 grams per harvest. The first generation (F1) colonies had mean yields of 7.5 ± 0.12 grams of royal jelly per harvest (Table 7.3). The colonies headed by selected queens had

significantly higher queen cell acceptance rates and royal jelly yields compared with colonies headed by the non-selected and ($t = 7.70$; $p = 0.001$ and $t = 20.9$; $p = 0.001$ respectively) (Table 5.3)

Table 5.1. Honey production by *A. m. scutellata* colonies for two seasons

	SEASON 1		SEASON 2			
Colony	Honey (Kgs)	Honey production (% of apiary average)	Honey (Kgs)	Honey production (% of apiary average)	Total % of average for 2 seasons	Colony ranking
C1	19.3	102.7	18.0	99.4	202.1	8
C2	18.0	95.7	16.0	88.4	184.1	11
C3	23.5	125.0	24.5	135.4	260.0	1
C4	16.0	85.1	14.0	77.3	162.4	13
C5	20.3	107.9	20.5	113.3	221.2	5
C6	18.0	95.7	17.1	94.5	190.2	10
C7	21.0	111.7	21.0	116.0	227.7	4
C8	19.7	104.8	18.0	99.4	204.2	7
C9	13.5	71.8	13.5	76.6	148.4	14
C10	12.7	67.6	12.0	66.3	133.9	15
C11	18.5	98.4	17.3	95.6	194.0	9
C12	17.0	90.4	15.5	85.6	176.0	12
C13	21.5	114.4	22.5	124.3	238.7	3
C14	19.7	104.8	19.0	104.9	209.7	6
C15	23.0	122.3	23.0	127.1	249.4	2
Total yields (Kgs)	281.7		271.9			
Apiary average	18.8 kgs		18.1 kgs			

Table 5.2. Comparison of sting numbers and honey production by colonies headed by selected and non-selected queens

Queen type	Mean sting numbers (SN)	Mean honey yields/colony (Kgs)
Selected	27.5 ± 2.8 a	19.6 ± 1.2 a
Non-selected	29.4 ± 2.8 a	17.9 ± 1.2 a

Means followed by the same letter within the same column are not significantly different ($p = 0.001$) by t-test

Table 5.3. Comparison of acceptance rates and royal jelly production by colonies headed by selected and non-selected queens

Queen type	% queen cup acceptance rates ± S.E	Mean RJ yield /queen cup (mgs)	Mean RJ yield/colony (g)
Selected	72.9 ± 3.2 a	332.9 a	7.5 ± 0.12 a
Non-selected	37.7 ± 3.2 b	203.2 b	2.9 ± 0.12 b

Means followed by the same letter within the same column are not significantly different ($p = 0.001$) by t-test

5.4 DISCUSSION

The most evident behavioral difference between European honey bees (*Apis mellifera* L.) (EHB) and African honey bees (*Apis mellifera scutellata*) (AHB) is the colony defensive behaviour (Stort, 1975; Collins and Kubasek, 1982; Collins *et al.,* 1982; Gloria *et al.,* 1998). Unlike the EHB colonies, which often mount low or moderate responses to intruders, the response of AHB colonies to disturbance is usually extreme (Sprangler and Sprenkle, 1997). This heightened defensiveness has been attributed to a variety of factors, including greater levels of predation and harvesting of unpredictable resources (Seeley, 1985; Rinderer and Hellmich, 1991). The great variability in colony defensive behaviour found during this study agrees with the results of Brandeburgo (1986); Moritz *et al.,* (1985) and Andere *et al.,* (2000) who noted that individual workers and coordinated groups converge in complex behaviours with different possible responses in regard to colony defense. In a related study, Stort (1975) demonstrated significant differences among colonies in the time it takes a colony to respond to a disturbance, the number of workers responding by stinging, and the distances that workers pursue the observer. During this study it was revealed that colonies tend to keep their position in colony ranking during different days for both variables (SN and T1S), results which agree with those reported by Stort, (1975) and Andere *et al.,* (2000). Similarly, Moritz *et al.,* (1985) revealed that though the sting numbers varied between days, colony rankings were constant in different evaluation days. Breed (1991) reported that there is considerable variation within honey bee races in regard to their defensive behaviour. Collins *et al.,* (1984) suggested that sting number alone is a clear character for use in

the evaluation of colony defensive behaviour. However, in this study, both variables were equally effective as an evaluation tool for defensive behaviour.

Correlation analyses between both variables (SN and T1S) resulted into a negative correlation, an indication that colonies that reacted faster to stimulus (low values for T1S) left more stings on the ball (high values for SN). This is because when a bee stings a target, it leaves its sting in place and the sting continues to emit an alarm pheromone (isopentyl acetate) that excites other bees to sting the 'marked' target (Free, 1961). It is the stings that remain in the target that provide a visual record on the intensity of the attack. These results are similar to those reported by Guzman and Page, (1994). Several authors have reported that though a strong genetic component is present in defensive behaviour during field evaluations, environmental conditions influence this behaviour (Message and Gonclaves, 1972; Collins, 1981; Brandeburgo, 1979; Schneider and Hall, 1997). However, Andere *et al.,* (2000) obtained homogenous results when he considered average responses for both variables, thus the results supported the evidence of genetic control. Similarly, Moritz *et al.,* (1985) emphasized genetic control as being very important in colony defense.

Colonies headed by the selected gentle queens were not different from the locally caught swarms in terms of defensive behaviour. Similar results have been reported by Collins *et al.,* (1996) who reported that the selected gentle line of the Africanized honey bees produced more defensive workers than the local Africanized honey bees in Venezuela. The worker population in a honey bee colony is genetically diverse because the queen mates with up to 17 drones (Adams *et al.,* 1977; Palmer and

Oldroyd, 2000). This results into a complex structure of genetic relationships with several sub-families representative of each drone father within the same colony. These genetic relationships affect behavioural interactions of individuals and the progress of selective breeding programs (Rothenbuhler, 1960). The interactions of individuals of different genotypes confound the ability to predict the response of their breeding population to breeding (Moritz, 1987). Genotypic differences in response thresholds among workers in different sub-families determine which sub-family responds to a disturbance (Robinson and Page, 1995). In the case of defensive behaviour, individuals of a single or two subfamilies may have a high genetic predisposition to defend the hive and as a consequence, make the colony very defensive while the other seven or so subfamilies may be non defensive. The frequency of these subfamilies in a colony might ultimately determine its overall defensive behaviour but will not reflect its true genetic composition, and hence may not be selected as a breeder.

Gloria *et al.,* (1998) reported that AHB queens mated to AHB drones resulted into highly defensive workers while AHB queens mated to EHB drones resulted into the least defensive workers. These results indicate that defensive behaviour is a genetically dominant trait and that it might also be influenced by a paternal factor. It is also probable that natural selection has favored the survival of the most defensive colonies of AHB. Also, older bees produce greater amounts of Isopentyl acetate (IPA) alarm pheromone which could cause secondary reactions in other bees during testing (Moritz *et al.,* 1985) and therefore the different ages of the worker bees could also interfere with the interpretation of results on defensive behavior.

Notable variations among colonies in honey production were also noted. Since all colonies were given equal opportunities to perform i.e similar management practices and were all in the same apiary, it is probable that performance of colonies in honey production could be attributed to the foraging vigour of the workers. Yields of up to 270 kilograms of honey per season (an equivalent of 540 Kgs annually since farmers harvest twice in a year) were achieved with 15 colonies. With the current honey prices of Kshs. 100/Kg (US$ 1.27), this indicates that a farmer can earn Kshs 54,000 (US$ 680) from 15 colonies in one year, which is a substantial income for the rural poor, majority of whom live on less than $1 a day. However, this honey production which was achieved with *A. m. scutellata* is low compared to production figures reported by other workers for other races elsewhere. Bradbear, (1999) reported that the European honeybee *Apis mellifera* produces about 20 Kgs of honey per season in Afghanistan, 25 Kgs per season in Peru (Llaxacondor, 1997) and the average production per colony per year in Ottawa, Canada is reported at 66Kgs (CAHR, 1998). The variations in production could probably be due to the races of the bees used, differences in the abundance of bee forage and management practices.

Colonies headed by the selected queens had a higher total honey production compared to those headed by the non-selected queens. However, the results were not significantly different. In some instances, colonies headed by the non-selected queens had higher honey yields than some of the colonies headed by the selected queens. However, of the eight colonies with above average apiary production, five where headed by the selected queens and three by the non-selected queens. It seems likely that the effect of selection is to make production by individual colonies uniform, consequently, the total harvest and average yield in colonies headed by the

selected queens is higher, even if the yields from the best colonies are not much different from the best colonies of the local bees. Honey yields from the selected queens were 4.4% higher compared to the honey yields of unselected queens. William and Essl, (1993) reported that selective breeding for high honey yields would be successful and Sanford, (1992) reported that selectively bred colonies of the European honey bee, *A. mellifera* showed a 10% increase in honey production per year over the unselected colonies.

Selection of young productive queens may have contributed to the increase in royal jelly production by *Apis mellifera scutellata* colonies. In an earlier study, Raina *et al,* (1999) had achieved royal jelly yields of 2.5 grams after harvesting 12 queen cups, an equivalent of 208 milligrams per queen cup using *Apis mellifera monticola* colonies. Generally, overall royal jelly production has been improved by use of specially manufactured royal jelly production plastic queen cups, grafting larvae of the ideal age (24 hour-old larvae), and maintaining 'nurse' colonies to provide larvae. However, this production is still low compared to royal jelly production rates in China. In 1980 the production of royal jelly in China was about 20 grams in three days and annual yield was less than 1 kg per colony and by 1988 production had been increased to 30 grams (Shenglu *et al.,* 1993). Currently, about 150 – 200 grams of royal jelly are produced per colony in three days (Chen *et al.,* 2002). However, the high production in China could be attributed to the honey bee race in use, the Italian honey bee *Apis mellifera ligustica* (Zhang, 1998), differences in abundance of floral resources and experience in royal jelly production. In addition, there have been efforts to breed for high royal jelly producing strains in China since 1980 and to date there are colonies with a production of 7.7 kg per year (Chen *et al.,* 1995). However, it should be mentioned that such colonies are in production through out

the whole year as they are continually shifted from place to place depending on availability of floral resources (migratory beekeeping).

The highly defensive nature of the local honey bee races often deters many people from engaging in beekeeping activities in addition to making colony management difficult (Moritz *et al.*, 1985). In order to make progress in selecting for less defensive strains, studies need to be carried out with queens being inseminated with semen from a single drone from a gentle colony as opposed to mixed semen from several drones Guzman-Novoa and Page (1994) indicated that defensive behaviour might also be influenced by a paternal factor. With single drone inseminations, the workers resulting from such a mating will be from the same drone father and hence will have similar response threshold for colony defense which will be easily recognized as opposed to if several drones were used as this results into several subfamilies and hence workers with different response threshold for colony defense. However, queens inseminated with semen from a single drone will not lay fertilized eggs for long periods and thus cannot support field colonies, but the defensive behaviour of workers from such queens can be satisfactorily evaluated.

Unlike other areas, East Africa is currently free from devastating effects of honey bee diseases like the American Foulbrood and the parasitic mites, *Varroa destructor* (Ingemar *et al.,* 2001). Where diseases and mites occur infected colonies are often treated with antibiotics (Shimanuki, 1997). This makes beekeeping a very expensive affair and for this reason, every effort should be done to ensure that such diseases are not introduced. In addition, the use of chemicals inside the hives may lead to contamination of the hive products, for example Balayannis (2001) detected traces

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of coumaphos (*Perizin* ™) in royal jelly produced under commercial apiaries where the product (coumophas) was sprayed inside hives for control of *Varroa*. Subhuti, (2003) has also reported traces of chloramphenical (0.3 to 34 parts per billion) in honey from China. Chloramphenical has been declared carcinogenic, which makes it an unacceptable substance for use in production of food products where any residue may be left. Also, recent reports indicate a widespread resistance of the AFB pathogen to the most widely used antibiotic formulations (Miyagi *et al.*, 2000). Farmers in areas not affected by AFB and *Varroa* have an added advantage in their beekeeping activities since their hive products are not likely to be contaminated with chemicals that are used for the control of such diseases. This is a factor which if fully exploited could serve as a very significant advantage when it comes to marketing of such products and transformation of their enterprises into organic farming

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

1. There were no significant differences between *Apis mellifera scutellata* and *Apis mellifera monticola* in the cell reception rates and mean royal jelly yields.

2. Grafting larvae at the age of 24 hours significantly increased queen cell reception and royal jelly yields compared to those of larvae grafted at the ages of 36; 48 and 60 hours.

3. Supplementary feeding significantly increased colony cell reception, mean royal jelly yields per queen cell and colony royal jelly yields.

4. Harvesting royal jelly 2 days after grafting resulted into a higher number of harvested cells compared to harvesting in a 3-day cycle. However, cells harvested 3 days after grafting yielded more royal jelly per queen cup compared to that produced by cells harvested 2 days after grafting. However, royal jelly yields were not significantly different in the 2 and 3-day cycle.

5. The major components in the Kenyan royal jelly were found to be moisture, lipids, proteins, sugars and ash and their composition was comparable to that reported in literature.

6. Colonies of *Apis mellifera scutellata* were found to have variations in both defensive behaviour and honey production. A negative and significant

correlation for SN and T1S was recorded. The annual honey production by the colonies ranged from 27 – 48 kilograms per colony.

7. There were no significant differences in sting number (SN), time to first sting (T1S) and honey production between colonies headed by selected queens and those headed by unselected queens.

8. Colonies headed by selected queens had significantly higher queen cup acceptance rates and royal jelly yields compared to those headed by unselected queens.

6.2 RECOMMENDATIONS FOR FUTURE RESEARCH

1. Since production techniques of royal jelly using local honey bee races have been developed during this study, future efforts should be geared towards product development (nutritive supplements and cosmetics products), popularization of its production and developing marketing strategies right from the producers to the consumers. In order to achieve this, supportive studies such as anti-microbial (antibacterial/antifungal) properties of Kenyan royal jelly need to be carried out.

2. Studies to evaluate production methods and potential of local honey bee races to produce other high value hive products like propolis and pollen also need to be carried out.

3. It is also worthwhile to note that the number of beekeepers using modern hives in the country is on the increase. In order to meet the needs of such up coming

commercial beekeepers, the scope of bee breeding programs need to be expanded for the local beekeepers to realize its full potential. Regular replacement of queens (requeening) alone has been shown to have significant effects on colony performance and honey production. However, focus should not only be on queen replacement but also on the improvement of the bee's performance capability through selection and breeding. Breeding programs need to be designed to involve beekeepers in the monitoring and evaluation of stock at the farm level. This will involve evaluation of both potential parental stocks (as sources of breeding stocks) and monitoring of the bred stocks (to confirm superiority in performance).

4. Improved stocks alone cannot offer maximum returns, but they need to be coupled with proper colony management practices. This calls for the continuous training of farmers in honey bee colony management. The Commercial Insects Program at ICIPE, the National Beekeeping Station, several NGOs and private companies are already involved in such trainings. These training activities will need to be increased and expanded to also include training of beekeeping extension officers at certificate, diploma and degree levels.

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APPENDICES

Appendix 1a. Sting number (SN) for *A. m. scutellata* colonies for 12 evaluation days

Day	Colony Number														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	15	15	80	9	14	104	15	86	15	11	96	87	103	94	21
2	12	6	10	7	8	30	6	14	12	3	28	31	21	37	6
3	10	32	22	11	4	32	14	15	11	10	43	31	26	19	9
4	45	28	55	20	34	90	20	3	57	20	34	70	53	31	34
5	13	6	58	7	12	65	21	19	81	61	45	38	23	71	11
6	26	9	18	39	62	35	45	28	61	58	30	37	41	59	17
7	20	3	15	15	27	60	32	39	13	81	29	18	46	31	3
8	10	2	61	6	10	64	26	51	84	61	33	29	60	40	15
9	51	11	145	79	14	173	20	63	113	123	47	34	48	94	16
10	85	9	64	87	7	158	60	22	105	138	19	24	37	46	22
11	94	89	60	38	13	148	21	96	56	16	43	63	49	67	96
12	45	32	40	12	8	78	20	44	11	131	51	27	53	38	9

Appendix 1b. Time to First Sting in seconds (T1S) for 15 *A. m . scutellata* colonies for 12 evaluation days

Day	Colony Number														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	1	2	2	2	4	2	2	3	5	1	1	2	1	4	4
2	4	31	5	7	8	12	49	6	6	59	12	9	29	11	49
3	2	28	1	3	1	2	19	3	2	2	6	10	20	28	43
4	2	1	1	3	1	1	1	1	1	1	9	3	17	25	51
5	5	14	2	6	8	3	3	15	8	5	7	5	7	7	45
6	4	60	3	3	3	5	5	7	3	3	10	5	8	5	33
7	4	4	2	8	4	2	4	12	2	2	12	40	7	22	28
8	1	14	2	2	39	4	5	9	4	1	9	60	3	17	13
9	12	4	3	7	20	1	5	2	5	10	4	10	9	3	9
10	10	15	5	20	30	3	5	40	19	3	14	13	12	6	35
11	4	60	4	3	4	2	6	15	5	2	6	6	11	7	3
12	2	14	3	3	40	5	6	10	5	1	3	1	4	13	6

Appendix 1d. Ranking for *A. m. scutellata* colonies in time to first sting (T1S) for 12 evaluation days. Rankings are in ascending order [(colony with the shortest time to first sting ranked (1) and the longest time to first sting ranked (15) for each sampling day]

Day	Colony Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	RANKING														
1	2.5	7.5	7.5	7.5	13	7.5	7.5	11	15	2.5	2.5	7.5	2.5	13	13
2	1	12	2	5	6	9.5	12.5	3.5	3.5	15	9.5	7	11	8	12.5
3	1.5	12.5	4.5	4.5	1.5	4.5	11	7.5	4.5	4.5	9	10	12	12.5	15
4	9	4.5	4.5	10.5	4.5	4.5	4.5	4.5	4.5	4.5	12	10.5	13	14	15
5	5	13	1	7	11.5	2.5	2.5	14	11.5	5	9	5	9	9	15
6	6	15	3	3	3	8.5	8.5	11	3	3	13	8.5	12	8.5	14
7	6.5	6.5	2.5	10	6.5	2.5	6.5	11.5	2.5	2.5	11.5	15	9	13	14
8	1.5	12	3.5	3.5	11	6.5	8	9.5	6.5	1.5	9.5	13	5	14	15
9	2.5	6.5	4.5	10	15	1	8.5	2.5	8.5	13.5	6.5	13.5	11.5	4.5	11.5
10	5	9	3.5	12	13	1.5	3.5	15	11	1.5	8	7	6	10	14
11	6	15	6	3.5	6	1.5	10	14	8	1.5	10	10	13	12	3.5
12	2	14	4	4	15	7.5	9.5	11	7.5	1	4	12	6	13	9.5
Av. Rank	4.0	10.6	3.9	6.7	8.8	4.8	7.7	9.6	7.2	4.7	8.7	9.9	9.2	10.9	12.7

Appendix 2a) Performance of 3 parental colonies of *Apis mellifera scutellata* in royal jelly production

	Month 1				Month 2				Month 3			
Colony	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12
RJP 1	2.8	5.5	3.8	3.6	7.1	9.8	3.2	4.7	6.1	5.7	7.3	8.9
RJP 2	6.0	5.4	1.2	3.7	4.4	2.1	3.2	1.3	1.3	2.1	3.8	3.5
RJP 3	5.2	2.4	4.2	4.3	2.1	3.2	2.0	2.3	3.4	4.5	5.1	4.3

Appendix 2b). Royal jelly production by *Apis mellifera scutellata* colonies headed by F1 queens bred for high royal jelly production

	Month 1				Month 2				Month 3			
Colony	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12
RJ1a	11.9	29.6	12.6	6.35	17.1	12.4	13.9	11.4	21.7	11.6	16.6	18.6
RJ1b	9.7	26.3	15.7	5.9	21.7	12.4	17.4	10.9	12.3	16.1	13.5	12.3
RJ1c	11.4	18.5	15.9	5.0	12.3	13.3	20.5	19.7	12.7	13.3	15.2	12.3
RJ1d	21.6	15.0	13.6	7.4	12.9	12.7	14.0	13.7	11.0	11.6	16.7	12.6
RJ1e	12.9	13.2	17.0	5.6	17.6	12.7	13.0	19.3	10.5	13.7	12.4	12.7
RJ1f	11.3	11.4	17.2	4.0	13.2	16.3	21.6	13.4	10.8	12.6	12.3	16.6
TOTAL	**319.05**				**360.8**				**329.7**			

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