ORIGINAL ARTICLE

Royal jelly production in East Africa: performance potential of the honey bees, *Apis mellifera scutellata* and *Apis mellifera monticola* in Kenya

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SUMMARY

This is the first study to evaluate the royal jelly production potential of two honey bee races in Kenya, *Apis mellifera monticola* and *Apis mellifera scutellata*. No significant differences were observed in cell acceptance rates and royal jelly yields between the two races. However, the age of larvae at grafting, supplementary feeding and time between grafting and harvest had significant effects on cell acceptance rates and royal jelly yields.

Keywords: honey bees, *Apis mellifera scutellata*, *Apis mellifera monticola*, royal jelly, cell acceptance, royal jelly yields, Kenya

INTRODUCTION

Beekeeping is an important component of agriculture, rural employment, human nutrition and economic development (Verma, 1990; Richard, 1999; Crane, 1999; Raina, 2000; Raina, 2004). Among hive products, interest is growing in the use of royal jelly as a human dietary supplement and additive in cosmetics (Crane, 1999). Royal jelly is a yellowish-white, creamy, acidic material with slightly pungent odour and taste and is secreted by the hypopharyngeal and mandibular glands of young worker honey bees. It is fed to queens throughout their larval and adult stages, and also to young worker and drone larvae (Lercker, 1982; Howe *et al*., 1985; Knecht & Kaaz, 1990), and it plays a major role in caste differentiation (Beetsma, 1979; Elton, 1992). However, royal jelly is fed directly to queen larvae and adults as it is secreted; it is not stored, and for this reason has not been a traditional beekeeping product. Although commercial production of royal jelly is decades old in some parts of the world, it is a relatively new venture for East African beekeepers. Due to the traditional mode of beekeeping, queen rearing techniques are yet to be adopted by a majority of the local beekeepers in East Africa and thus production of royal jelly (whose production relies on artificial queen rearing) for commercial economic purposes is poorly developed.

This study was carried out to assess the production potential of two honey bee races in Kenya, *Apis mellifera scutellata* and *A. m. monticola*., for commercial production of royal jelly. Additionally, we aimed to determine the effects of age of larvae grafted, supplementary feeding and duration of harvesting time after grafting on royal jelly production.

MATERIALS AND METHODS

Experimental colonies were established in 10-frame Langstroth hives. Royal jelly was collected from queen rearing colonies following the procedures of Laidlaw & Eckert (1962), Okada & Obata (1962), and Guanhuang (1990) during the months of October to December. Floral resources were mainly sunflower (*Ocimum* sp.) and bottle brush (*Callistemon citrinus*). Prior to grafting, the inner surfaces of commercial plastic queen cups (9 mm diameter and 10 mm height) were brushed with honey and given to experimental colonies so that the bees would clean and prepare them for larvae. Grafting frames were prepared to hold cell bars of 30 queen cups each. Three days after grafting, grafting frames with queen cells containing royal jelly were removed from the cell-rearing hive. The ring of wax at the opening of each cell was removed to expose the queen larva and royal jelly. Each larva was removed and the royal jelly extracted with a royal jelly spoon. To compare bee races, six queenright colonies (three of *A. m. scutellata* and three of *A. m. monticola*.) in 10-frame Langstroth hives were established in an experimental apiary at the International Center of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. Larvae of the same age were grafted into queen cups and royal jelly collected following the procedures of Laidlaw & Eckert (1962), Okada & Obata (1962), and Guanhuang (1990). In each colony a total of 24 grafts were placed, with 30 cells per graft. Thus, a total of 2160 cells were made available to each race during the experimental period. The average percentage of cells accepted and royal jelly yields for each race were pooled at each harvest and recorded for 12 weeks. Harvesting was done twice a week to give a total of 24 harvests. Percentages were arcsin transformed before data analysis. Student's *t* test was used to compare races for cell acceptance and royal jelly yields.

To investigate the effects of larval age on royal jelly yields, grafting frames were each modified to hold four bars of queen cups. Each bar received larvae of one of four ages at grafting: 24 h, 36 h, 48 h and 60 h. Four cell-rearing colonies (*A. m. scutellata*.) were used, each colony receiving one grafting frame containing larvae of the four ages. A dedicated colony (queen) was used to provide a simultaneous and abundant source of larvae of different age classes. In the days leading up to grafting the queen was successively caged on single combs in which she could lay eggs. The combs were then labelled and returned to the colony until their larvae were needed. The effect of position in the colony was controlled by ensuring that each age class was represented at every possible position in the grafting frame. The arrangement of larvae in the four production colonies was as follows, from top to bottom: Colony 1 – 24 h, 36 h, 48 h and 60 h; Colony 2

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– 60 h, 48 h, 36 h, and 24 h; Colony 3 – 36 h, 24 h, 60 h and 48 h; and Colony 4 – 48 h, 60 h, 24 h and 36 h. Harvesting was done 72 h after grafting. Fifteen grafts were carried out in each colony. The number of cells accepted and royal jelly yields per treatment were recorded. Royal jelly yields of each larval stage were pooled at each harvest. Percentages were arcsin transformed before ANOVA was done to test for differences in cell acceptance and royal jelly yields between larvae grafted at different ages. Separation of means was done using Tukey's test.

To determine the effect of supplementary feeding, six cell-rearing colonies of *A. m. scutellata* were divided into three groups of two colonies each. The three groups were randomly assigned different feeding regimes of sugar syrup (1 : 1.5 ratio of sugar to water) or water control. Group 1 was fed 15 h prior to receiving grafts (18:00 h the day before); Group 2 was fed during the 72-h production period, and Group 3 (controls) was given 200 ml of water during the production period. Each colony received 14 grafts over an experimental period lasting two months (November to December). In each colony, 20 cells were grafted each time; a total of 40 cells were obtained for each treatment per grafting session. In each colony, 14 grafts were done, giving a total of 28 observations for each treatment. Harvesting was done 72 h after grafting. The number of cells accepted and royal jelly yields were recorded. Percentages were arcsin transformed before data analysis. The Kruskal-Wallis test was used to identify differences in cell acceptance and royal jelly yields among the treatments by rank. Means were separated by non-parametric multiple comparison.

To investigate the effect of duration of harvesting time after grafting, eight production colonies of *A. m. scutellata*. were divided into two groups (Group 1 and 2). The grafting procedures used were as in the previous experiments. Royal jelly in Group 1 colonies was harvested two days after grafting, while Group 2 was harvested three days after grafting. In both cases, re-grafting was done immediately after harvesting. Thus, 30 grafts were done in each colony for the 2-day cycle, and 20 grafts in each colony for the 3-day cycle in a period of 60 days. The royal jelly yields and number of cells in each treatment were pooled for each week and recorded for eight weeks. Student's *t* test was used to determine differences in royal jelly yields and cell acceptance rates.

RESULTS

Royal jelly production potential

Colonies of *A. m. scutellata* and *A. m. monticola* produced a total of 502.2 and 526.1 g royal jelly, respectively, an average of 167 g and 175 g per colony over the study period. *A. m. monticola* produced slightly higher amounts of royal jelly compared to *A. m. scutellata*.. However, the production in royal jelly yields was not significantly different (t = 0.32; df = 23; P = 0.05). A total of 720 cells per colony was grafted over the study period. *A. m. monticola* colonies had higher cell acceptance rates, with 1585 cells accepted out of a total of 2160 grafted cells (73%) compared to 1496 cells (69%) in *A. m. scutellata*. However, cell acceptance rates were not significantly different (t = 0.09, df = 23, P = 0.05; table 1).

Effect of larval age

Cell acceptance rates were highest in the 24-h-old larvae (74.5%) and least in 48- and 60-h-old larvae (35%) (table 2). There was a significant difference in acceptance rates of 24-h-old larvae compared to 36-, 48- and 60-h-old larvae and those of 36 hr-old larvae compared to 48- and 60-h-old larvae However, cell acceptance rates of 48- and 60-h-old larvae were not significantly different (F = 13.2; df = 14; P< 0.05). Royal jelly yields decreased with an increase in larval age at grafting (r = –0.83). The average royal jelly per queen cup was highest in 24-h-old larvae (419.5 mg) and least in queen cups grafted with 48- or 60-h-old larvae (181.5 mg). Royal jelly yields from larvae grafted at the age of 24 h were significantly different from those of larvae grafted at the ages of 36, 48 and 60 h. Royal jelly yields from 36-h-old larvae were also significantly different from the yields of 48- and 60-h-old larvae whilst there was no significant difference in yields from 48- and 60-h-old larvae (F = 5.23, df = 14 , P = 0.05, table 2).

Supplementary feeding

Feeding sugar syrup to colonies producing royal jelly significantly increased royal jelly yields. Royal jelly yields from colonies fed 15 h prior to receiving grafts (18:00 h the day before grafting) (6.3 g/colony/graft) and those fed during the production period (6.1 g/colony/graft) were significantly different from the control colonies (2.9 g/colony/graft) (χ^2 = 18.5; df = 2; P< 0.05; table 3). There were significant differences in cell acceptance rates in the colonies fed 15 h prior to receiving grafts (61.7%) and those fed during the production period (62.1%) compared to controls (44.2%) (χ^2 =15.5; df = 2; P< 0.05; table 3).

Duration of harvesting time after grafting

A total of 2130 cell cups was harvested from the 2-day cycle compared to 1414 cell cups from the 3-day cycle in a period of two months (60 days). This difference in total number of cells harvested translated into higher numeric production of royal jelly (503.4 g) in the 2-day harvesting cycle compared to that produced in the 3-day cycle (494.2 g). However, royal jelly yields were not significantly different (t = 0.32, df = 7, P = 0.05; table 4). There was a tendency for the 3-day cycle to have higher royal jelly yields per cup (349.5 mg) than the 2-day cycle (236.3 mg).

DISCUSSION

In order to increase and diversify income from beekeeping, technologies for production of other hive products need to be developed, introduced to, and popularized among the local beekeepers. This research on royal jelly production using East African honey bee races is the first of its kind. The results show that there were no significant differences between *A. m. scutellata* and *A. m. monticola* honey bee races in cell acceptance and royal jelly yields. The races gave satisfactory royal jelly yields (*c.* 7 g per colony/harvest), comparable to those reported by Crane (1999) for colonies in Canada. Based on results of this study, annual colony production is estimated at 300–500 g compared to 3–6 kg in Canada (Crane, 1999), 4.5 kg in Taiwan (Fert, 1988), 7.7 kg in China (Chen *et al*., 2002), and 300 g in Vietnam (Apiservices, 2001). This range of published values reflects many variables such as bee subspecies, climatic differences, floral resources

TABLE 1. Comparison of royal jelly (RJ) production between *Apis mellifera scutellata* and *A. m. monticola* colonies; *n* for all means = 24.

Race	Mean ± s.e. cell acceptance (%)	Mean ± s.e. RJ/colony/graft (g)	Total ± s.e. RJ (g)	RJ value (US$)
A. m. scutellata.	56.3 ± 3.3 a	6.9 ± 0.5 a	502.2 ± 2.4 a	60.3
A. m monticola	57.6 ± 3.3 a	7.3 ± 0.5 a	541.8 ± 2.5 a	65.0

Means within column are not significantly different (P > 0.05) by Student's t test

TABLE 2. Queen cell acceptance rates and royal jelly (RJ) yields from larvae grafted at different ages; *n* for all means = 15.

Age of larvae (h)	Mean ± s.e. queen cell acceptance (%)	Mean ± s.e. RJ/cup (mg)
24	74.5 ± 3.3 a	419.5 ± 25.6 a
36	58.5 ± 4.8 b	356.8 ± 41.9 b
48	42.5 ± 5.6 c	284.5 ± 17.2 c
60	35.0 ± 3.1 c	181.5 ± 13.3 c

Different letters within column indicate significant ($P > 0.05$) differences by Tukey's test

TABLE 3. Effect of supplementary feeding with sugar syrup on royal jelly (RJ) production by *Apis mellifera scutellata* colonies; *n* for all means = 28.

Treatment	Mean ± s.e. cell acceptance (%)	Mean ± s.e. average RJ yields/colony/graft (g)
Control (water)	44.2 ± 2.5 a	2.9 ± 1.4 a
Fed syrup 15 hr prior to receiving grafts	61.7 ± 1.8 b	6.3 ± 0.5 b
Fed syrup during production	62.1 ± 2.1 b	6.1 ± 1.2 b

Different letters within column indicate significant ($P > 0.05$) differences; cell acceptance means were separated by non-parametric multiple comparison

and management practices of beekeepers. It is not possible to build up populations of *A. m. scutellata* in the way it is possible with European honey bees (Shi, 2001). This implies that comparatively fewer queen cells can be tended to by *A. m. scutellata* colonies at a particular time.

Larvae grafted at the age of 24 h had the highest acceptance rates and royal jelly yields. Larvae at this age were found to be small in size but clearly visible, floating on a mass of brood food. These larvae were easily grafted with the least risk of injury, and this probably contributed to higher cell acceptance rates. It was difficult to freely lift the older and larger larvae into the queen cups. The longer periods of time taken to graft the larger larvae and the higher risk of injury could have led to lower cell acceptance rates. Royal jelly yields were significantly affected by the age of larvae used. Larvae 24-h-old at grafting produced the highest royal jelly yield (419.5 mg per cup) whilst larvae grafted at the age of 60 h had the least (181.5 mg). Okada & Obata (1962), using European honey bees reported that worker larvae 8–24-h-old yielded maximum royal jelly (150 mg/cup) when harvested at 72 h from grafting. In the present study, royal jelly yield for larvae grafted at the age of 24 h averaged 419.5 mg per cup, far higher than that reported by Okada & Obata (1962). It is possible that this difference might be due to differences in floral resources and colony populations during the time the two studies were carried out. Krell (1996) suggested that royal jelly yields per queen cup should not fall below 200 mg. Yields below this indicate that the ratio of cups to colony bee population is too high.

Supplementary feeding significantly improved both cell acceptance and royal jelly yields. These colonies had higher amounts of royal jelly per queen cup (6.3 and 6.1 g/colony) compared to controls (2.9 g/colony). It is thought that supplementary feeding increases secretion of royal jelly by the hypopharyngeal and mandibular glands of young nurse worker bees. Laidlaw (1992) and Morse & Hooper (1985) advocate supplementary feeding of colonies used for queen rearing. The quality of queens is largely due to the nature of food provisioned to the developing queen larvae. Supplementary feeding of queen rearing or royal jelly producing colonies probably increases the secretion of royal jelly in the head glands of the young nurse bees and thus leads to better queens or higher royal jelly yields. Supplementary feeding was also found to be cost effective. In this case, two unfed colonies produced 76.96 g of royal jelly (worth US$9.6 at the market price of US$120 per kg), while two fed colonies produced 165.5 g (worth US$19.9). The cost of sugar consumed by the two colonies was US$1.8; therefore, if we subtract the cost of sugar, fed colonies produced royal jelly worth US$18.1 compared with US$6.9 in the controls.

Colonies harvested two days after grafting produced slightly higher yields of royal jelly than those harvested three days after grafting. It is worth noting that harvesting two days after grafting and immediately re-grafting into the harvested cells resulted into more harvests (30 harvests, compared to 20 harvests when harvesting was done three days after grafting) in the same period of 60 days. Thus the higher number of queen cups harvested in the 2-day cycle resulted in higher royal jelly yields, but the results were not significantly different. However, queen cups harvested three days after grafting yielded more royal jelly per queen cup. It is possible that due to the small size of larvae at day 2, the larval food (royal jelly) supplied to their cells is less compared to that provisioned to the larger larvae at day 3. This probably explains the low royal jelly yields per queen cup in queen

TABLE 4. Effect of duration of harvesting time after grafting on royal jelly (RJ) production by *Apis mellifera scutellata* colonies; *n* for all means = 8.

Harvesting time after grafting	Queen cells harvested	Mean ± s.e.) RJ/cup (mg	Mean ± s.e. total RJ yields (g)	Royal jelly value (US$)
2 days	2130	236.3 ± 10.2 a	503.4 ± 0.97 a	60.4
3 days	1414	349.5 ± 10.2 a	494.2 ± 0.97 a	59.3

Means within column are not significantly different ($P > 0.05$) by Student's *t* test

cups harvested two days after grafting compared to the higher yields of queen cups harvested three days after grafting. These results are similar to those of Okada & Obata (1962) who reported that harvesting cells three days after grafting yielded maximum amounts of royal jelly (150 mg). However, yields of more than 150 mg per queen cup were achieved in this experiment in both the 2-day (236.3 mg) and 3-day (349.5) harvesting cycles. This difference could probably be due to differences in populations of experimental colonies and floral resources available to the bees during the two studies. Harvesting royal jelly on a 2-day cycle could be better if 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 fewer cell cups. The 3-day cycle is less laborious and requires fewer disruptions to the colony, an important advantage with the local *A. m. scutellata* which are highly defensive.

The transformation of Kenyan traditional beekeeping into a modern enterprise is under way and there seems a promising future for production and marketing of high value products such as royal jelly. It should be noted that allergic reactions involving products derived from bees have been documented (Thien *et al*., 1996; Jellin, *et al*., 2004; CARN, 2005). Therefore, bee products sold to the public should bear labels warning of possible adverse reactions. However, with royal jelly prices ranging from US$2.00 per g in Syria (SBC, 2001) to US$0.9 per g in Peru (Llaxacondor, 1997), the incentive is good for increasing royal jelly production in developing countries.

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