Pupa (*Apis mellifera* L.) Rearing Conditions to Improve Queen Weight at Emergence

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Abstract   Queen quality depends on increased body weight at emergence, which is strongly affected by ecological factors during brood rearing; however, much remains undefined in terms of improving queen weight at emergence. We initially reared 12-20 h-old larvae in queenright (QR) and queenless (QL) colonies at temperatures of 28 ± 2ºC and $70\pm6\%$ relative humidity (RH) and 29 ± 3ºC and $65\pm8\%$ RH respectively, and later transferred 50% of the pupae into an incubator (34.7 ± 0.42ºC and $80\pm5\%$ RH). Queen weight at emergence was observed to be the highest in the QR and QL colonies of the incubator (217.36 ± 5.51 mg and 199.61 ± 4.24 mg, respectively) compared to that of QR and QL colonies (199.45 ± 3.60 mg and 182.94 ± 3.35 mg respectively) in the hives. Queen weight at emergence differed significantly among rearing groups ($P<0.0001$), whereas the difference in the emergence rate was insignificant. We observed that rearing queen bees initially from QR colonies and pupae in an incubator at an optimum and more stable temperature could be an advantage in improving queen weight at emergence. Further studies are needed to investigate their acceptance, mating, and reproductive success rates.

Keywords   Queen quality, Weight at emergence, Pupa, Ecological factor, Honey bee queens

INTRODUCTION

Queen bees are the only reproductive females and are the most important individuals in honey bee colonies. They are known for their responsibility in ensuring colony survival by laying eggs that develop into individuals of other castes. The quality of queen bees is beneficial for beekeepers in the production of hive products. While poor-quality drones are equally responsible, poor-quality queens remain the principal reason for colony failure (Kairo *et al*., 2016, 2017). Studies have reported poor-quality queens as the primary problem in a number of beekeeping operations, which rank among the top reasons for colony failure (Vanengelsdorp *et al*., 2009, 2013). Queen quality depends on several characteristics (body size, weight, thorax width, wing length, number of ovarioles, and spermatheca diameter) that have been used to describe reproductive success. Certain morphological characteristics (thoracic width, wing length, and weight) have also been used to quantify the quality of newly emerged queens (Tarpy *et al*., 2000, 2011; Delaney *et al*., 2011; De Souza *et al*., 2013). Additionally, queen size, weight, and thorax width are positively correlated with higher sperm counts and laying efficiency (Kahya *et al*., 2008; Delaney *et al*., 2011). Queen weight is also positively correlated with ovary weight, size, number of ovarioles, diameter of the spermatheca, and the number of stored spermatozoa (Kahya *et al*., 2008). These characteristics have been identified as being essential to mating success (Delaney *et al*., 2011; Tarpy *et al*., 2011), the queens' ability to be attractive to drones during mating flights (Gilley *et al*., 2003; Rangel *et al*., 2016),

and increased body weight at emergence, which is strongly linked to reproductive fitness among queens (Dedej *et al*., 1998; Kahya *et al*., 2008; Tarpy *et al*., 2011). Queen weight could be influenced by both environmental and human-induced factors, especially larval and pupal rearing conditions which consequently affect reproductive success. Queen weight is determined by larval rearing conditions, such as royal jelly diet (Hartfelder *et al*., 2015) and temperature (DeGrandi-Hoffman *et al*., 1993). Increased temperature during honey bee brood capping can negatively affect emerged adult bees and, consequently, mating success. Temperature is a prominent environmental and ecological factor that affects insect growth and development (Seeley and Heinrich, 1981; Shi *et al*., 2014; Kobori and Hanboosong, 2017). Many deformations in insect biology, such as body size (Atkinson, 1994), mating (Kuang *et al*., 2010) and metabolism (Wang *et al*., 2016; Qian *et al*., 2017) are associated with changes in temperature. For instance, in the pupal stage, bees raised at 34.5°C were found to have the highest numbers of microglomeruli in the olfactory lobes compared to those raised below or above this temperature (Groh, 2004). Due to the importance of temperature in brood development, most social insects, including honey bees, have begun to regulate nest temperature (Heinrich, 1993; Stabentheiner *et al*., 2010). However, maintaining normal brood development is costly for honey bees, as considerable energy is spent in regulating brood temperature within the range of 32−36°C (Seeley and Heinrich, 1981; Kronenberg and Heller, 1982). Therefore, the most important output in selecting and maintaining good brood rearing conditions is to increase queen weight at emergence for better reproductive success. According to De Souza *et al*. (2013) and Rangel *et al*. (2013), queens weighing over 200 mg headed colonies with higher brood populations, stored more pollen, and had greater population growth than those weighing less than 180 mg at emergence. Therefore, queen weight is the most reliable parameter for measuring queen reproductive quality (Amiri *et al*., 2017).

The development of control systems to improve queen quality by improving their weight at emergence has been a driving factor for researchers and beekeepers (Amiri *et al*., 2017). Although the weight of first-instar larvae (larval age) plays an important role in addressing queen quality, much remains undefined in improving queen weight at emergence. In this study, we aimed to investigate the effects of pupal rearing conditions on queen weight at emergence, pupal mortality, and emergence rates in queenright and queenless colonies.

MATERIALS AND METHODS

This study was conducted in an apiary at the honey bee breeding laboratory at the Department of Agricultural Biology, National Institute of Agricultural Science (NIAS), Republic of Korea.

1. Colony selection and grafting of larvae

The larvae in a single strong colony (six combs, good brood, approximately 80% worker bee population, and good performance) bred in 2021 were selected and used in this study. Two empty built combs of honey bees (*Apis mellifera* L.) were marked and inserted into the selected queenright colony for the queen to deposit eggs. After 24 h, the presence of eggs was checked and recorded as day 1. Within a period of 3 days when eggs are expected to hatch into larvae, the combs were removed to transfer the first-instar (12−20 h-old) larvae into artificial queen cell cups (Doolittle, 1915). Queen cell cups were attached to rearing frames, and ≤20 h-old larvae were transferred into each cup containing one drop (5 mL syringe) of diluted royal jelly in water at a of ratio 1 : 1 (v/v) using the Chinese grafting tool (Fig. 1a). Prior to queen rearing, four strong colonies of *A. mellifera* were sorted and digital thermosensors (ONSET, HOBO ext temp/RH logger, UX100-023A) were inserted to monitor hive temperature and relative humidity (RH) over 3 days. Colonies (one queenright and one queenless) with similar conditions (temperature and relative humidity) were selected for queen rearing under the same environmental conditions. No adaptations were made to regulate the hive temperature.

2. Larvae rearing and transfer of pupae into queen rearing cages

After selecting the rearing colonies, the queen bee in the queenright colony was excluded using a queen excluder (Fig. 1b). Rearing frames with grafted larvae were placed into each rearing colony and fed sugar syrup (pow-
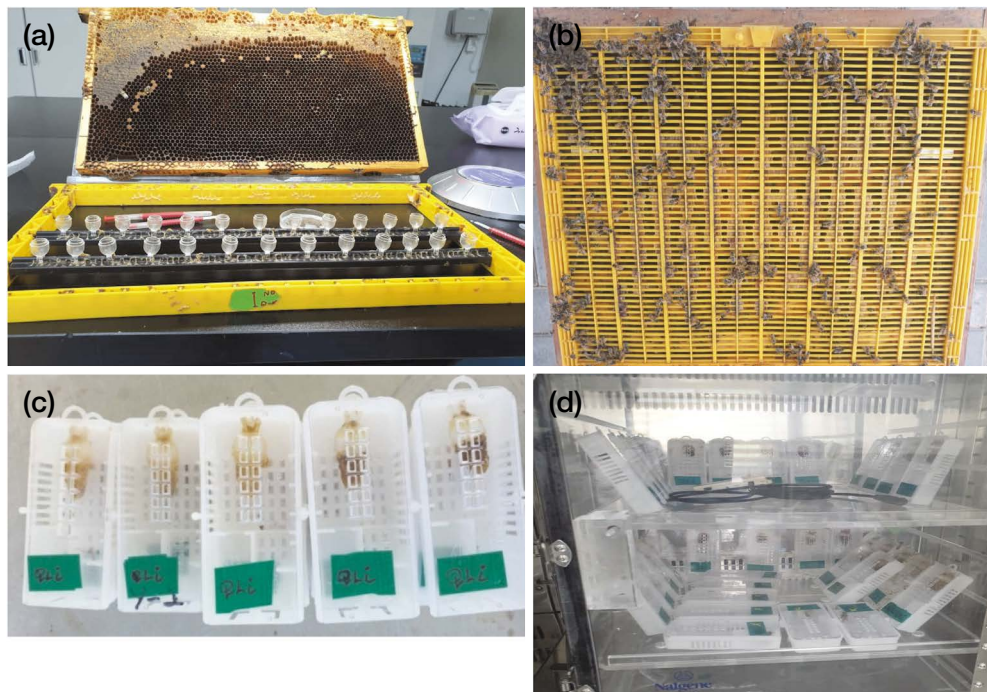
Fig. 1. The rearing process. a: Queen cell cups attached to rearing frames for grafting, b: Queen excluded in a QR colony, c: Capped queen cells in queen cages, d: pupae in a desiccator placed in an incubator.

dered sugar dissolved in water at a ratio of 1 : 1, v/v). Digital thermosensors placed in each colony showed that the average temperature and RH for queenright (QR) and queenless (QL) colonies were $28 \pm 1.87°C$ and $70 \pm 6\%$ RH, and $29 \pm 2.28°C$ and $65 \pm 8\%$ RH, respectively. The percentage of accepted larvae was verified 2 days after grafting to estimate the number of pupae to be transferred to the rearing cages. Equal numbers of capped queen cells were transferred into queen rearing cages and labeled as QR and QL (Fig. 1c). Additionally, the cages to be placed in the incubator and hives were differentiated with letters i and h, respectively (Fig. 1c). The height of each capped queen cell was measured before transfer to the rearing cages. Pupae were reared in QR, QL, and an incubator. A desiccator set at $34.7 \pm 0.42°C$ and $80 \pm 5\%$ RH and placed in an incubator was used to rear the pupae (Fig. 1d).

3. Experimental setup

Freshly hatched larvae (≤ 20 h-old) were grafted and reared in QR and QL colonies as described above. The number of larvae that developed into pre-pupae and pupae was recorded. A total of 150 pupae were extracted from initial rearing frames (50% each from QR and QL colonies) and inserted into queen rearing cages (Fig. 1c). The pupal rearing conditions were described as QR, QL, and incubator. Fifty queen cages containing pupae were inserted; however, QR and QL colonies provided 25 pupae each to measure up to the 50 pupae reared in the incubator. The experimental units were monitored twice a day (9:30 am and 5:30 pm) to record the date, number, and weight of the emerged queens. The queen emergence rate was recorded within 48 h, from the late hours of day 15 to the early hours of day 17. This was described as day 1 (early hours of day 15), day 2 (early and late hours of day 16), and day 3 (early hours of day 17). Pupae that did not develop and emerge into adults were removed and uncapped to observe their states. The number of emerging adults was used to calculate the pupal mortality (Cui *et al*., 2018). Queen weight (mg) at emergence was recorded, and weighed queen bees were reinserted into their cages with equal amounts of artificial food and placed in their respective rearing media. Queen bees were fed once every 3 days for 32 days. The queen mortality rate was recorded daily for 32 days after emergence.

4. Data analysis

The data were characterized using Descriptive Statistics. One-way analysis of variance (ANOVA) was used to compare the means of more than two treatments. When a significant difference was detected through ANOVA, we conducted multiple pairwise comparisons of means among treatments using the Newman-Keuls test. Pearson's correlation was used to evaluate relationships between parameters, while the two-sample Student's t-test was used to compare the means of two treatments. XLSTAT statistical software version 2007.8.04 was used to conduct the analysis with levels of significance set at 5%.

RESULTS AND DISCUSSION

1. Effects of rearing conditions on pupal mortality rate

The number of emerged queens and pupal mortalities under the different rearing conditions were recorded (Table 1).

Pupal mortality was higher in the incubator (10%) than in the QR (2%) and QL (0%) colonies, with emergence rates of 90%, 98% and 100%, respectively (Table 1). The high pupal mortality in the incubator can be attributed to rapid temperature changes. The larvae were initially reared under lower temperatures (above 5°C) than those in the incubator. The results of our study are consistent with those of other studies that have reported the sensitivity of insects to changes in temperature, which affects their growth and development (Li *et al*., 2017; Cui *et al*., 2018). Zero pupal mortality in the QL colony might be due to the absence of the queen, which leads to worker bees focusing on raising new queens. Other studies on larval acceptance rate showed a higher percentage of acceptance in queenless colonies than queenright colonies (Cengiz *et al*., 2009).

2. Effects of rearing condition on queen weight at emergence and rate of emergence

The length of the sealed queen cells and queen weights at emergence were measured based on different rearing conditions (Table 2).

The mean lengths of sealed queen cells were higher in QL (27.04 ± 0.42 mm) than in QR (26.24 ± 0.29 mm) colonies although no significant difference was observed. In our study, the difference in the length of sealed queen cells between the QL and QR colonies was not significant compared with that in similar studies (Cengiz *et al*., 2009). The relationship between the lengths of sealed queen cells and queen weight at emergence from QR and

Table 1. Mortality rate of honey bees under different rearing conditions

Rearing condition	Temperature/RH	Number of pupae reared	Number emerged	Pupa mortality (%)	Emergence rate (%)
QR	28 ± 1.87°C/70 ± 6% RH	50	49	2	98
QL	29 ± 2.28°C/65 ± 8% RH	50	50	0	100
Incubator	34.7 ± 0.42°C/80 ± 5% RH	50	45	10	90

QR, queenright colony; QL, queenless colony; RH, relative humidity.

Table 2. Lengths of sealed queen cell and queen weights at emergence under different rearing conditions

Rearing condition		N	Length of sealed queen cell (mm, mean ± SE)	N	Emergence weight (mg, mean ± SE)
Hive	QR	50	26.24 ± 0.29a	49	199.45 ± 3.60a
	QL	50	26.24 ± 0.29a	50	182.94 ± 3.35b
Incubator	QR	25	−	22	217.36 ± 5.51c
	QL	25	−	23	199.61 ± 4.24a

Means within columns followed by different letters by category definition indicate significant difference at $P < 0.01$, $\alpha = 0.05$.

QL colonies was investigated using Pearson correlation. The results showed no correlations in QR ($R^2 = 0.111$) and QL ($R^2 = 0.263$) colonies. The length of sealed queen cells did not significantly affect the weight of queen bees at emergence. In other studies, queen-worker differentiation was affected by queen cell size (Shi *et al*., 2011) whereas Wu *et al*. (2018) reported that queen weight at emergence increased with larger diameter queen cells and had better performance.

The weights of queen bees at emergence from QR and QL colonies differed significantly when reared in hives ($P < 0.001$) and in the incubator ($P < 0.01$). However, it is important to understand the variation in queen weight between QR and QL colonies, to better select starter colonies. The emergence weight of 198.20 ± 8.74 mg obtained in QR colonies (Cengiz *et al*., 2009) and 178.42 ± 2.05 mg obtained in QL colonies (Dodologlu *et al*., 2004) were similar to our findings. However, our value of 182.94 ± 3.35 mg in QL colony was lower than 199.07 ± 7.55 mg reported by Cengiz *et al*. (2009). Additionally, a significant difference was recorded in queen weight at emergence among the three pupa rearing groups ($df = 2$, M.S = 7936.875, F = 13.21, $P < 0.0001$). Queen weight at emergence was highest in the QR and QL colonies of the incubator, followed by QR and QL colonies (Table 2). The temperature of the incubator, which was maintained at a consistently 34.7 ± 0.42°C could influence brood rearing condition and consequently queen weight at emergence because good brood rearing demands an optimum temperature of 32−35°C. The increased body weight of emerged queens in an incubator initially reared from both QR and QL colonies could be associated with pupa rearing temperature, as Kobori and Hanboosong (2017) reported that growth and development in insects is highly affected by temperature. Worker bees spend considerable energy and time in regulating brood rearing temperature (Kronenberg and Heller, 1982). Consequently, optimal brood rearing temperature may not be attained at all periods as workers have other duties to perform in the colony. Many aspects of insect biology, including body size, are affected by changes in temperature (Atkinson, 1994). Cao *et al*. (2012) reported that insect molting and temperature are closely related. Pupae transferred from the QR colony to the incubator emerged to queen bees with higher body weight (217.36 ± 5.5 mg) (Table 2). Some studies on queen quality have con-

cluded that it is advantageous to raise new queens from queenright colonies, although queens emerging from queenless colonies could develop higher body weights than queenright colonies (Dodologlu *et al*., 2004; Cengiz *et al*., 2009). Thermoregulation in maintaining optimal temperature in brood-rearing honey bee colonies may not be stable, while a stable temperature is needed for survival and good development to increase adult quality (Jones *et al*., 2005). This could be attributed to the energy required for thermoregulation (Stabentheiner *et al*., 2010) as workers also spend some energy to forage to help the colonies meet its requirements. The present study suggests that QR colonies can be used as initial rearing colonies, while pupae were transferred to an incubator set at an optimum temperature.

The number of queen bees that emerged was recorded based on the time of emergence (Fig. 2). The emergence rate of queen bees differed significantly ($df = 2$, M.S = 1089.0, F = 75.977, $P < 0.0001$). However, the emergence rate on days 1 and 3 did not show any significant variation (Fig. 2). More queen bees emerged in the incubator (nine bees) on the third day than in QR (three bees) and QL (three bees) colonies reared in hives. The life cycle of the queen bees from the egg stage to adulthood at emergence was 16 days. The deviations in the emergence periods under different rearing conditions were insignificant, and the queen survival rate within 32 days after emergence was high in QL, incubator, and QR colonies. The low survival rate in QR could be due to the presence of the old queen, which discouraged workers
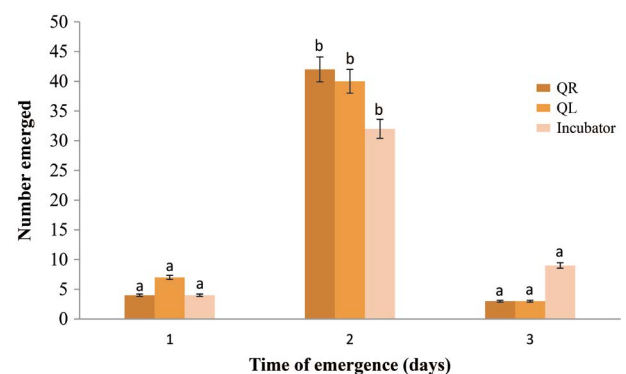

Fig. 2. Effects of rearing conditions on emergence rate. Time of emergence was categorized as: day 1 (late hours of day 15); day 2 (early and late hours of day 16); day 3 (early hours of day 17). Means with different letters are significantly different at $P < 0.0001$, $\alpha = 0.05$. QR, queenright colony; QL, queenless colony.

to pay attention to new queens. This is consistent with the results reported by Al-Fattah *et al.* (2016), who found that queen bee survival rate was significantly higher in queenless colonies (77.4%) than in queenright colonies (68.0%). They further discussed that the reversal effect on the survival rate of stored queens was due to the presence of originally mated free-laying queens around the caged virgin queens in the colony. In other studies, increasing the degree of queenlessness led to a decrease in the number of rejected queens (Szabo, 1977; Shawer, 1981).

CONCLUSION

Queen weight at emergence has been documented as an essential factor for improving queen quality, and is affected by both environmental and human-induced factors. In this study, queen weight at emergence was the highest in the queenright and queenless colonies in an incubator compared to the queenright and queenless colonies in the hive. Therefore, rearing queen bees initially from queenright colonies and pupae in an incubator at an optimum and stable temperature could aid in improving queen weight at emergence. However, further studies are needed to investigate their acceptance, mating, and reproductive success rates.

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