Grafting worker larvae to obtain uniform *Apis mellifera* queen bees in support of the evaluation and identification of forage plant species

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Abstract. The productivity of bee colonies is greatly influenced by the performance of the queen in production. This research aims to obtain uniform *Apis mellifera* queen bees, both in terms of age and morphometrics, which will be developed into colonies to evaluate quality and identify bee forage plant species in grazing areas. The study was conducted in two different locations: Sukorame Village, Tumpang Subdistrict, Malang Regency, and Pajaran Village, Poncokusumo Subdistrict, Malang Regency. Pollen sample analysis was carried out at the Genetech Saraswanti Indo Surabaya Laboratory. The research method involved field observations and direct experiments, focusing on the research locations and grafting techniques. Data collected included both primary and secondary data, and data analysis was performed using unpaired t-tests to compare data from the two different research locations. The research results indicate that the difference in location has no significant effect (P>0.05) on the *Apis mellifera* queen bee production using the grafting method in terms of the measured morphological variables. The average abdomen length in Sukorame and Pajaran locations was 9.08±1.65 mm and 9.11±1.37 mm, respectively. The average abdomen width in Sukorame and Pajaran locations was 4.99±0.41 mm and 4.96±0.36 mm, respectively. The average abdomen height in Sukorame and Pajaran locations was 4.18±0.51 mm and 4.35±0.57 mm, respectively. The difference in location with the grafting method also had no significant effect (P>0.05) on the proboscis length, with average values of 1.30±0.39 mm for Sukorame and 1.45±0.43 mm for Pajaran locations. The use of different locations in the production of *Apis mellifera* queen bees using the grafting method provides consistent performance in terms of abdomen length, abdomen width, abdomen height, and proboscis length.

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1 Introduction

The diversity of vegetation in Indonesia, supported by its tropical climate, has the potential to provide flowers throughout the year. This can be utilized for honeybee cultivation because these plants contain pollen and nectar that can be utilized as food for bees to sustain their lives. Similar to other animal species, bees also require energy from food throughout their lives. Honeybee cultivation can be considered an alternative business to enhance income, especially for communities around forested areas [1].

Honey bees are insects that offer numerous benefits, with their products being highly beneficial to health. The products derived from bee cultivation include honey, pollen, beeswax, propolis, royal jelly, and queen bees. The *Apis mellifera* bee is one of the most cultivated honey bee species in Indonesia due to its high honey production, ranging from 35 to 50 kg per colony per year, and its docile nature.

The queen bee plays a crucial role in the success of honey bee cultivation. The queen bee can produce larvae that develop into new queen bees, worker bees, and drones. If the queen bee becomes less productive, replacement becomes necessary. The technique used in the queen bee replacement process involves grafting or transplanting worker bee larvae because both are fertile, allowing for the quick and abundant production of new queens.

Given this background, research is needed in different grazing locations with varying plant support capacities as sources of quality nutrition. This is essential for obtaining uniform and optimal morphometric performance in *Apis mellifera* queen bees. The objective of this research is to determine the effects of forage quality from plants in different locations on the production of *Apis mellifera* queen bees using the grafting method, specifically assessing the morphological quality of the bees, including abdomen length, abdomen width, abdomen height, and proboscis length.

2 Materials and Methods

2.1 Research time and location

This research was conducted for 45 days, starting from May to June 2023, at the Honeybee Farm 'PT. Kembang Joyo Sriwijaya,' located in two different places: Sukorame Village, Tumpang Subdistrict, with an elevation of 536 meters above sea level, and Pajaran Village, Poncokusumo Subdistrict, Malang Regency, also at an elevation of 536 meters above sea level. The distance between location 1 and location 2 is 3.3 km. Pollen samples were analyzed at the Saraswanti Indo Genetech Laboratory in Surabaya. The environmental conditions at location 1 are much more enclosed compared to location 2, which will affect the sunlight entering the grazing area, thereby impacting temperature and humidity conditions at the research sites.

2.2 Research materials

The materials used in this research were 30 one-day-old *Apis mellifera* bee larvae obtained from the honeybee farm PT. Kembang Joyo. The larvae were collected from comb cells containing honeybee eggs by drawing a square line. This method was employed to identify the larvae of the desired age for grafting.

2.3 Research method

The research method employed involved direct field observation and experimentation. This approach included observing the research locations and techniques used in grafting, as well as collecting data by gathering all necessary information for the study. The collected data comprised both primary and secondary data. Primary data were obtained from temperature, humidity, and vegetation in two different locations. Pollen samples were collected for proximate analysis, and morphological observations of grafted queen bees were conducted. A comparison was then made between the locations, which are in Sukorame Village at an elevation of 536 meters above sea level and Pajaran Village at an elevation of 535 meters above sea level, with a distance of 3.3 km. Temperature, humidity, and pollen sample checks were performed every three days during monitoring. Secondary data were obtained from rainfall data provided by BMKG Malang and vegetation data obtained from BPS Malang.

2.4 Research procedure

2.4.1 Research preparation

- Conducting a survey of location and plant vegetation.
- Preparing equipment: smoker, thermometer, bar frames, queen cells, grafting tools.
- Selecting colonies and comb nests as the source of larvae.
- Marking comb nests containing eggs using thread.
- Preparing builder colonies (Cell builders).

2.4.2 Research implementation

- Pollen Sampling Stage
  1. Installing a pollen trap at the entrance and exit of bee colonies every 3 days during the research.
  2. Placing a collecting tray at the bottom of the entrance for pollen storage. Storing collected pollen in a refrigerated cabinet.

- Larva Grafting Stage
  1. Preparing tools and materials
  2. Selecting combs with larvae aged less than 24 days that have been marked.
  3. Retrieving Queen cells installed on bar frames and left undisturbed for 1 day in the cell builder.
  4. Applying royal jelly to the queen cell using a brush.
  5. Transferring larvae from worker bee cells to queen cells using a grafting tool.
  6. Inserting the grafted queen cell into the cell builder
- Larva Inspection Stage
  1. Observing grafted larvae the next day.
  2. Subsequent observations conducted every 3 days.
  3. Performing queen pupa caging once they are covered, on the 13th day

- Future Queen Bee Rearing Stage

1. Preparing a queen cage based on the success of grafting
2. Gradually transferring the queen cell to the queen cage.
3. Placing the queen cage in the hatching colony until the new queen hatches
4. Harvesting the new queen

- Morphology Measurement of Queen Bees

1. Placing the newly hatched queen in a container sprayed with alcohol.
2. Separating each part of the queen's body after it has fainted or died.
3. Placing body parts on millimeter paper, followed by photography.
4. Observing the photographed body parts using Photoshop.
5. Measuring the morphology according to the predetermined variables.

2.5 Measured variables

The research method employed involved direct field observation and experimentation. This approach included observing the research locations and techniques used in grafting, as well as collecting data by gathering all necessary information for the study. The collected data comprised both primary and secondary data. Primary data were obtained from temperature, humidity, and vegetation in two different locations. Pollen samples were collected for proximate analysis, and morphological observations of grafted queen bees were conducted. A comparison was then made between the locations, which are in Sukorame Village at an elevation of 536 meters above sea level and Pajaran Village at an elevation of 535 meters above sea level, with a distance of 3.3 km. Temperature, humidity, and pollen sample checks were performed every three days during monitoring. Secondary data were obtained from rainfall data provided by BMKG Malang and vegetation data obtained from BPS Malang.

2.5.1 Abdomen length

The separated abdomen is then measured in length by placing it on a millimeter block paper. Subsequently, photographs are taken from the ventral side, and the images are imported into the Photoshop application for the abdomen length measurement stage, involving the conversion from pixel units to millimeter units [2]. The measurement of abdomen length starts from point A to point B, as illustrated in Figure 1.

Source: Personal Documentation (2023)

Fig. 1. Abdomen Length Measurement

2.5.2 Abdomen width

The separated abdomen is then measured in width by placing it on a millimeter block paper. Subsequently, photographs are taken from the dorsal side, and the images are imported into the Photoshop application for the abdomen width measurement stage, involving the conversion from pixel units to millimeter units [2]. The measurement of abdomen width starts from point A to point B, as illustrated in Figure 2.

Source: Personal Documentation (2023)

Fig. 2. Abdomen Width Measurement

2.5.3 Abdomen height

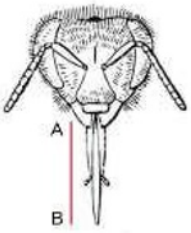
The separated abdomen is then measured for its height by placing it on a millimeter block paper. Subsequently, photographs are taken from the ventral side, and the images are imported into the Photoshop application for the abdomen height measurement stage, involving the conversion from pixel units to millimeter units [2]. The measurement of abdomen height starts from point A to point B, as illustrated in Figure 3.

Source: Personal Documentation (2023)

Fig. 3. Abdomen Height Measurement

2.5.4 Proboscis length

The separated head is then measured for its proboscis length by placing it on a millimeter block paper. Subsequently, photographs are taken of the rostral part, and the images are imported into the Photoshop application for the proboscis length measurement stage, involving the conversion from pixel units to millimeter units [2]. The measurement of proboscis length starts from point A to point B, as illustrated in Figure 4.



Source: Personal Documentation (2023)

Fig. 4. Proboscis Length Measurement

2.6 Data analysis

The data obtained from the research will be processed using statistical analysis, specifically an unpaired t-test, to compare data from two different research locations: the grazing locations in Sukorame Village, Tumpang Subdistrict, and Pajaran Village, Poncokusumo Subdistrict, Malang Regency.

3 Results and discussion

The variety of vegetation is a crucial factor in the world of beekeeping. This is because the overall species richness of plants has a strongly positive correlation with the abundance of bees in general. Diverse plant species have the potential to provide greater diversity and continuity in food sources such as nectar and pollen throughout the bee activity season [3]. The presence of an adequate supply of nectar and pollen is essential to avoid impacting the productivity of honeybees in producing various products. Insufficient food can weaken the bee colony, ultimately affecting the morphometric quality of the bees themselves. The measurement results from the observation of the impact of different locations on the production of *Apis mellifera* queen bees using the grafting method can be seen in Table 1.

Table 1. The average morphological measurements of *Apis mellifera* queen bees in the research locations.

Parameter	Average ± SD (mm)	
	Location 1	Location 2
Abdomen Length	9.10 ± 1.70	9.17 ± 1.35
Abdomen Width	5.00 ± 0.43	4.86 ± 0.38
Abdomen Height	4.20 ± 0.52	4.36 ± 0.56
Proboscis Length	1.29 ± 0.40	1.47 ± 0.44

3.1 The length of the abdomen of the queen honeybee (*Apis mellifera*)

The results of observing the influence of different locations with the grafting method on the abdomen length of *Apis mellifera* queen bees can be seen in Table 1. Subsequently, after being analyzed using an unpaired t-test, it was found that the different locations and grafting methods did not show a significant difference ($P>0.05$) in the abdomen length of queen bees. The average results of the abdomen length of *Apis mellifera* queen bees are shown in Table 1. The research findings indicate an average abdomen length of 9.10 ± 1.70 mm at location 1 and 9.17 ± 1.35 mm at location 2. These results reveal that the highest average abdomen length of queen bees is found at location 2 compared to location 1. This is due to the prolonged rainy season, during which a bee colony may face difficulties in finding food sources as pollen and nectar become damaged. Consequently, the population may decrease, affecting the process of providing royal jelly to potential queen bees [4]. According to Kuntadi [5], larvae will develop into queen bees if they receive more royal jelly consumption, primarily from the secretion of the mandibular gland. Conversely, larvae receiving a small amount of royal jelly, with a higher content primarily from the hypopharyngeal gland, will develop into worker bees.

The length of a queen bee's body can be influenced by its species. The bee's body serves as a crucial space for vital organs to develop, and one of the ideal factors determining egg

storage and spermatheca capacity can be seen in the body's length. This aligns with Gabka [6], who states that a queen bee's egg-laying ability depends on the capacity of the egg sac (ovarium) and the volume of the sperm sac (spermatheca) in its reproductive organs. The larger the volume of the egg sac, the more egg tubes (ovariols) it contains, thus affecting the quantity of eggs produced. This is further supported by Floris's statement that queen bees have a larger body size than other bee castes due to the developed ovaries functioning for egg-laying. One of the criteria for assessing the quality of a queen bee is the length of the abdomen. This is because one of the main tasks of a queen bee is to produce eggs. If a queen bee has a long, large, and proportional abdomen, it will be capable of accommodating a large quantity of spermatozoa.

3.2 The abdomen width of the queen bee *Apis mellifera*

The results of observing the influence of different locations using the grafting method on the abdomen width of *Apis mellifera* queen bees are presented in Table 1. Subsequently, after being analyzed using an unpaired t-test, it was found that the different locations and grafting methods did not show a significant difference ($P>0.05$) in the abdomen width of queen bees. The average results of the abdomen width of *Apis mellifera* queen bees are shown in Table 1. The research findings indicate an average abdomen width of 5.00 ± 0.43 mm at location 1 and 4.86 ± 0.38 mm at location 2. These results reveal that the highest average abdomen width of queen bees is found at location 1 compared to location 2. This is due to the similar environmental conditions and vegetation variety in the two research locations, which correlates with the average morphological body size. Windarsih, et al [7], stated that environmental conditions such as temperature, humidity, wind speed, altitude, and light intensity can influence the morphometric characteristics of insects. Another factor that can affect it is the number of nurse bees, as a low number of nurse bees may make it impossible to provide royal jelly. Widowati [8] stated that royal jelly is the food for honeybee larvae and queen bees throughout their lives. This is what makes queen bees true egg producers with a larger body size than other bee castes.

Other fundamental factors that can influence the acceptance of grafted larvae in a colony include the selection of a strong colony, the availability of food reserves, the age of the larvae used, the technique of grafting, and the presence of a queen bee in the colony. Minarti and Akhiroh [9] added that the cup used in the grafting process is one of the factors where the diameter size directly correlates with the development of the potential queen. Therefore, if the queen is formed with the same diameter, it will result in the same abdomen width. Abdomen width can be used to assess productivity in egg-laying. The larger the abdomen of the queen bee, the more productive it is in laying eggs, as a large abdomen allows a queen bee to accommodate a larger spermatheca from the male bees.

3.3 The abdomen height of the queen bee *Apis mellifera*

The observations on the influence of different locations using the grafting method on the abdomen height of *Apis mellifera* queen bees can be seen in Table 1. Furthermore, after being analyzed using an unpaired t-test, it was found that the different locations and grafting methods did not show a significant difference ($P>0.05$) in the abdomen height of queen bees. The average results of the abdomen height of *Apis mellifera* queen bees are shown in Table 1. The research findings indicate an average abdomen height of 4.20 ± 0.52 mm at location 1 and 4.36 ± 0.56 mm at location 2. These results reveal that the highest average abdomen height of queen bees is found at location 2 compared to location 1. This condition can be influenced by the surrounding environmental conditions at the honeybee grazing location. This is reinforced by Budiwijono's [4] opinion that different locations' elevations in the

grazing process can be influenced by microclimate conditions, namely temperature, humidity, wind speed, rainfall, and light intensity. In humid and wet environmental conditions, honeybee colonies may face difficulties in obtaining food in the form of nectar and pollen for their living and colony development needs. Seeing this condition, beekeepers usually provide artificial feed consisting of sugar and water to maintain the survival of a bee colony. However, in general, this condition can lead to a decrease in the number of bee colonies and no harvesting process, making them susceptible to pests and diseases.

The larvae used in this research are 1-day-old larvae because in the process of providing royal jelly, 1-day-old larvae have a longer time frame compared to larvae aged 2 or 3 days. As a result, the proportion of royal jelly obtained by young larvae will be higher than that of older larvae. This is supported by Pavel, stating that differences in body size can be caused by the food provided by nurse bees to the larvae. This statement is reinforced by previous research indicating that changes in feeding patterns will affect larval development into new individuals. Factors influencing abdomen height include the age of the grafted larvae, the provision of royal jelly, and the presence of the old queen in the colony. This aligns with Kuntadi's [5] assertion that grafting techniques using young larvae will produce potential queen bees with higher quality.

3.4 The length of the proboscis of the queen bee *Apis mellifera*

The results of observing the influence of different locations with grafting methods on the proboscis length of *Apis mellifera* queen bees are presented in Table 1. Furthermore, after being analyzed using an unpaired t-test, it shows that the different locations with different grafting methods are not significant ($P>0.05$) regarding the proboscis length of queen bees. The average proboscis length of *Apis mellifera* queen bees can be seen in Table 1. The research results obtained an average proboscis length at location 1 of 1.29 ± 0.40 mm, while at location 2 it was 1.47 ± 0.44 mm. These results indicate that the highest average proboscis length of queen bees is found at location 2 compared to location 1. The differences in overall variability may be caused by differences in the quality of pollen throughout the time, especially related to the protein content that influences the development of the hypopharyngeal gland responsible for royal jelly production by nurse bees. If this requirement is met, it will result in high-quality queens in larger quantities.

In general, a longer proboscis is more suitable for flowers with deep nectar positions, while a shorter proboscis is used for flowers with shallow nectar positions. This indicates that the proboscis size within a bee colony is a population strategy to expand opportunities in obtaining sufficient nectar from various flowers with diverse nectar positions [10]. This differs from the conditions of queen bees because the queen's duties, unlike worker bees, do not involve various tasks during her lifetime due to morpho-functional differences among bee castes, including proboscis length. The proboscis of queen bees is shorter than that of worker bees, making it less suitable for exploring the inside of flowers to collect nectar. While some morphometric measurements of queen bees may be superior to those of worker bees, the proboscis length of worker bees is longer, as they are responsible for collecting food. Based on previous research, it is mentioned that a queen bee has been observed engaging in flower probing activities. This likely occurs during orientation or mating flights.

4 Conclusion

The use of different locations in the production of *Apis mellifera* queen bees using the grafting method provides consistent performance in terms of abdomen length, abdomen width, abdomen height, and proboscis length.

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