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Effect of queen cell size on morphometric characteristics of queen honey bees (*Apis mellifera ligustica*)

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ABSTRACT

Rearing techniques are important to determine a successful honey bee production. Queen cell cup size may affect the acceptance rate of grafted larvae and queen's size, which in turn may influence the quality of the colonies. The present study compared the effect of different queen cell cup sizes (8.0 vs 9.0 mm diameter) on morphometric characteristics of queen honey bees. Sixty-five larvae were grafted to each treatment. Larvae transfer was carried out five times, in June and August 2019. Head, thorax and abdomen width of the newly emerged queens were measured using an electronic calliper, and the weight of each of the three segments was recorded using a precision scale. All morphometric traits measured on the accepted larvae were significantly ($p < .001$) higher in queens raised in larger cell cups, except for head width. Principal Component Analysis on morphometric traits shows higher values on PC1 (58.4% of explained variance) for queens raised in larger cell cups. Highest loadings were found for variables related to weight of the three parts. Among the many factors affecting queen's quality, cell cup size seems to have a positive effect on queens' body parts weights.

HIGHLIGHTS

- Increasing the standard diameter of conventional cell cups allows rearing heavier and larger honey bee queens.
- The grafting period affects morphometric parameters, with higher size and body weight in the last decade of June.

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Honey bee; queen quality; virgin queen; cell cup size; queen morphometric traits

Introduction

The quality of honey bee queens is fundamental to determine a successful honey bee production and for economically successful beekeeping (Büchler et al. 2013). In fact, the health and productivity of the queen significantly affect the state of a colony (Fine et al. 2018). Queens' quality is determined by measuring its physical characteristics such as weight, thorax and head width, number of ovarioles, spermathecal size, number of stored spermatozoa. Genetic and environmental factors affect the queen's quality (Büchler et al. 2013) and include breed, age and number of grafted larvae (Mahbobi et al. 2012; Ozbakir 2021), rearing season and feeding (Cengiz et al. 2019). It is therefore important to develop suitable honey bee queen rearing techniques. For instance, cell cup size where queen larvae are reared may affect the

quality and the reproductive ability of the newborn queens (Wu et al. 2018). The recommended queen cell cup size ranges from 8.0 to 9.0 mm in diameter at the rim (Büchler et al. 2013). Using smaller cell cup sizes (7.0 mm, 7.5 mm, 8.0 mm, 8.5 mm), Adgaba et al. (2019) did not find significant differences in the acceptance rate of grafted larvae in *Apis mellifera jemenitica*. On the other hand, different cell cup sizes (9.4 mm, 9.6 mm, 9.8 mm, 10.0 mm) significantly affected body weight, thorax length and thorax width of *A. m. ligustica* queens at emergence (Wu et al. 2018). Based on their studies on the paper wasp *Polistes metricus*, Wright et al. (2019) showed that larger queens gave rise to larger colonies than smaller queens and suggest that queen morphology can predict colony size in social insects, and can therefore be considered as a proxy of queen fitness and quality.

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This consideration is supported by the work carried out by Honěk (1993), who investigated the relationship between female body size and potential fecundity in several insect species, including Hymenoptera, and found a significant effect of body weight on fertility parameters (fecundity rate and ovariole number). Queens' body weight at emergence was found positively correlated to characteristics of reproductive organs such as ovaries and number of ovarioles, diameter of the spermatheca, and number of stored spermatozoa (Delaney et al. 2011; Rangel et al. 2013; Hatjina et al. 2014; Arslan et al. 2021). According to others Authors (Hatjina et al. 2014), however, weight or other body size measures are not good predictors of the number of ovarioles. Instead, it seems that queen weight is positively correlated with queen's attractiveness to worker bees and larger body mass improves queen's acceptance into another colony (Amiri et al. 2017).

Other environmental factors, such as pollen quality and availability, may have significant effects on the quality and health of honey bees. As pollen composition may show seasonal variations in terms of protein and lipid concentrations (Di Pasquale et al. 2016; Al-Kahtani et al. 2020, 2021), we may hypothesise that there may be a significant effect of the interaction between rearing techniques, i.e., cell cup size, and the time of grafting on quality parameters of honey bee queens.

The present study aims to investigate the effect of larger cell cups, and its interaction with the grafting season, on morphometric traits of queen bees, which may potentially affect queens' quality, of *A. m. ligustica*.

Materials and methods

Study site

This study was carried out in the 2019 bee-summer season in an apiary located at 200 m asl on Lake Como (Lombardia region, Italy; 45°55'12"N, 09°19'13"E) and managed by a beekeeper specialised in the breeding of selected queens for sale.

Queen rearing and morphometric measures

One-hundred and thirty *A. m. ligustica* virgin queens were reared using the wet grafting method in which one-day-old larvae were grafted into queen cells in a starter hive and transferred in a queenright finisher colony after one day. At 8–9 days of age, sealed queen

cells were placed individually into plastic cages in an incubator until emergence.

The grafting and sampling of the queen cells was carried out on a weekly basis during the month of June and in the last week of August; therefore, the grafting was performed on 7th June, 15th June, 21st June, 29th June and 23rd August.

The starter hive was crowded with mostly young, healthy and well-fed bees and consisted of five frames arranged so that the cell bar frame with cups for the grafted larvae was in the centre and was surrounded with food for workers.

The queens were allocated to two different cell cup types: small cells ($n=65$) and large cells ($n=65$). Small cells were commercially available and made of plastic with an inner diameter of 8.0 mm. Large cells were made of beeswax and were obtained by the beekeeper using a metal mold consisting of dowels with rounded, smooth ends with a diameter of 9.0 mm; the mold was soaked in cold water, then dipped into melted wax several times to build a thick layer and then twisted to remove the cup when cool. After the grafting procedure in the apiary, the cups with larvae were attached to two cell bars, 13 small cups on one cell bar and 13 large cups on the other.

As soon as they emerged from the pupal stage, 110 virgin queen bees were collected and immediately frozen at -20°C in order to prevent weight loss and dehydration, and stored at this temperature until measurements.

The head, the thorax and the abdomen of each queen were weighted on an analytical scale recording. The following metrics were taken manually using a digital calliper: the width of the head was assessed measuring the distance from the two compound eyes; the width of the thorax was recorded measuring the distance between the two tegulae, which are the scales on the mesothorax that overlap the root of the forewing; the width of the abdomen was assessed measuring the width of the first abdominal tergite in its wider part.

Statistical analysis

Normality of data was assessed using Shapiro–Wilk test. Relationships between measurements and queen cell type were first assessed through a principal component analysis (PCA) in order to reduce the dimensionality of the data set containing the four measures into components that account for the maximal amount of total variance in the observed variables. Results are presented as loadings and scores

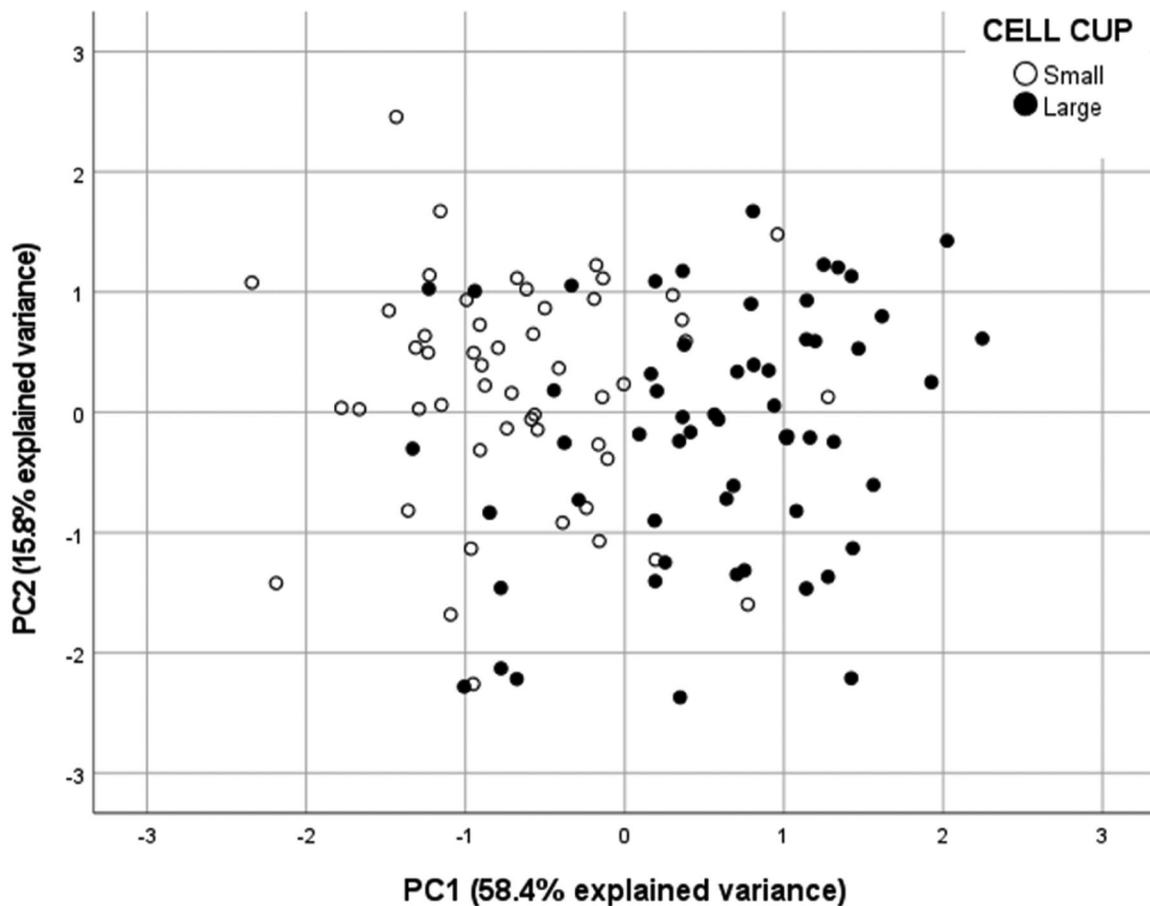


Figure 1. Scatter plot showing the distribution of queen bees on the first two Principal Components.

computed on the components accounting for the largest proportion of variance.

All morphometric measurements fulfilled the normality assumption and therefore were analysed using a linear model including the fixed factors of cup type (2 levels: small or large), grafting date (5 levels: on 7th June, 15th June, 21st June, 29th June and 23rd August) and the interaction between these two factors. Least square means were separated by pair-wise *t*-test.

Statistical differences were declared at $p < .05$. All statistical analyses were performed using SAS[®] 9.4 program (SAS Institute, 2013).

Results

The first two Principal Components (PC) explained 74.2% of variance (PC1: 58.4%; PC2: 15.8%). Queens raised in large cell cups clustered mainly on the right side of PC1 (Figure 1), characterised by larger body size and weight. All the considered variables presented high positive loadings on PC1, with particularly high loadings of variables related to the weight of the three segments and head width showing the lowest loading; all morphometric traits were highly correlated (Figure 2).

The analysis of variance confirmed PCA results, showing that queen bees raised in large cell cups were larger and heavier than those raised in small cups, with significant differences for all morphometric traits (Table 1).

Figure 3 shows that grafting date also had a significant effect on thorax width and weight ($p < .05$) and on abdomen width and weight ($p < .001$ and $p < .01$, respectively). In particular, pairwise comparisons for thorax weight highlighted a significant difference between the highest value, recorded on 21st June, and the lowest value, recorded on 23rd August ($p < .01$). Abdomen weight presented significantly higher values on 21st June compared to the one on 23rd August ($p < .01$). Abdomen width was significantly higher on 23rd August compared to 15th, 21st and 29th June ($p < .01$, $p < .01$ and $p < .001$, respectively). Significant differences were also found between the highest thorax width recorded on 7th June and the measures taken on 15th, 21st and 29th June ($p < .01$), but not between 7th June and 23rd August, when thorax width started to increase again (Figure 3). For abdomen width, significant differences were found between the value recorded on 23rd August and the three lower values

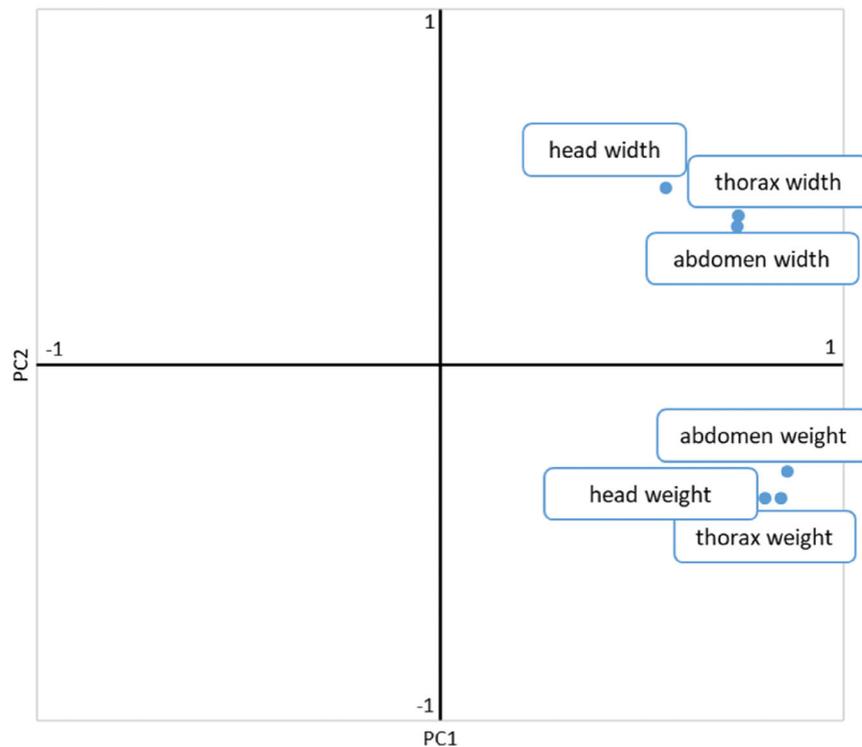


Figure 2. Scatter plot showing the distribution of the loadings of morphometric traits on the first two Principal Components.

Table 1. Least square means (\pm s.e.) of morphometric traits of queen bees raised in small and large cell cups and probability of pair-wise comparison.

Morphometric trait	Small cell cups <i>n</i> = 52	Large cell cups <i>n</i> = 58	<i>p</i>
Head width, mm	3.7446 \pm 0.0361	3.8487 \pm 0.0342	.039
Thorax width, mm	4.3470 \pm 0.0360	4.5532 \pm 0.0341	<.0001
Abdomen width, mm	4.4800 \pm 0.0367	4.8136 \pm 0.0348	<.0001
Head weight, g	0.0095 \pm 0.0004	0.0127 \pm 0.0003	<.0001
Thorax weight, g	0.0644 \pm 0.0016	0.0803 \pm 0.0015	<.0001
Abdomen weight, g	0.0705 \pm 0.0017	0.0895 \pm 0.0016	<.0001
Total weight, g	0.1443 \pm 0.0034	0.1826 \pm 0.0032	<.001

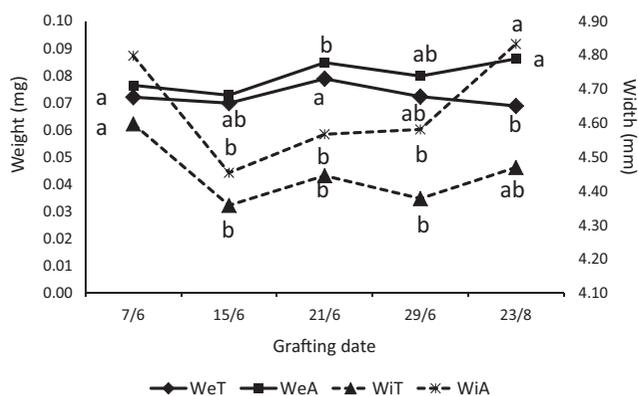


Figure 3. Thorax weight (WeT), abdomen weight (WeA), thorax width (WiT), abdomen width (WiA) of honey bee queens grafted on different dates (7th, 15th, 21st, 29th June and 23rd August). Within each variable, different letters indicate statistically significant differences ($p < .05$).

on 15th, 21st and 29th June ($p < .001$) and also between 7th June and all other dates in June ($p < .001$ for all three comparisons).

For thorax weight ($p < .05$) and head width ($p < .01$), a significant interaction was found between cell cup size and grafting date (Figure 4). The thorax weight of queens reared in larger cups (WeT_L) was always statistically higher ($p < .001$) than that of queens in small cups, with the exception of June 29th. For head width, the only significant difference between small and large cell cups was recorded on 7th June ($p < .001$; Figure 4). Even if not statistically significant ($p = .085$), an effect of the interaction between cell cup size and the time of grafting on body weight is also detectable (Table 2).

Discussion

The queen bee is the fulcrum of the entire colony, since it maintains a constant balance within the super-organism and has an irreplaceable role in the survival of the colony. Colonies headed by a high quality queen show better performance characters than those headed by a low quality queen: low swarming tendency, high hygienic behaviour, high brood production, high honey and pollen yield and resistance to disease (Hatjina et al. 2014). The qualitative potential of a queen bee is strongly conditioned by the

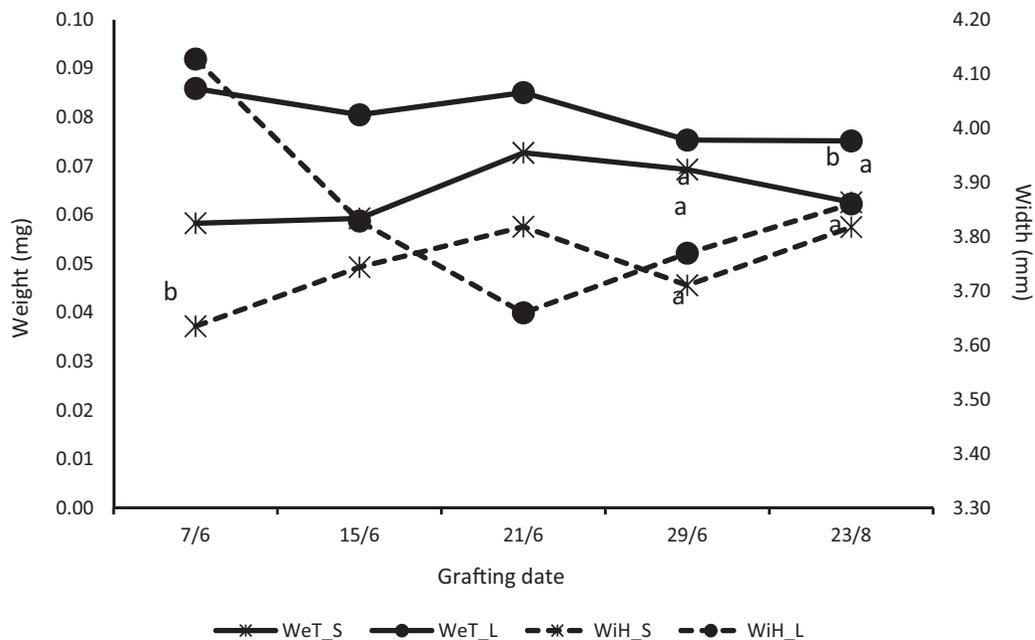


Figure 4. Interaction between cell cup size (small, S vs large, L) and grafting date (7th, 14th, 21st, 29th June and 23rd August) on thorax weight (WeT) and head width (WiH). Within each variable and each date, different letters indicate statistically significant differences ($p < .05$).

Table 2. Least square means (\pm s.e.) of body weight of queen bees raised in small and large cell cups for each grafting date and probability of pair-wise comparison.

Grafting date	Small cell cups, gr $n = 52$	Large cell cups, gr $n = 58$	p
7th June	0.1303 ± 0.0073	0.1880 ± 0.0076	<.0001
15th June	0.1306 ± 0.0080	0.1762 ± 0.0070	<.0001
21st June	0.1569 ± 0.0076	0.1932 ± 0.0067	.0005
29th June	0.1551 ± 0.0073	0.1722 ± 0.0070	.0011
23rd August	0.1489 ± 0.0073	0.1835 ± 0.073	.0924

adopted technique and by the materials and tools used during the entire production process of a queen bee (Büchler et al. 2013; Hatjina et al. 2014).

The objective of this study was to obtain queen bees of increased quality, which present a morphologically higher weight and larger size than queens raised using normal protocols. Our results suggest that this objective was conceivable by controlling and increasing the standard diameter of the conventional cup by 1 mm. Among the measures taken, weight appears to have a greater relevance on the variability compared to length and width measurements. The overall results showed that the virgin queen bees reared in large wax cups have a higher body weight and a significantly wider size of the three parts than those raised in conventional small cups. These results are in agreement with Wu et al. (2018) who reported a significant increase of some morphological parameters of emerging *Apis mellifera ligustica* queens, such as body weight, and thorax length and width, depending on cup diameter (9.4; 9.6; 9.8; 10 mm). The

Authors attributed the larger size of queens in larger cells to the greater availability of space that allows greater development of the body and organs. Besides, the body weight of queens reared in larger cells during our study is in agreement with the findings from a survey performed by Kumar and Mall (2016) on the body weight of newly emerged *Apis mellifera ligustica* queens.

The morphometric parameters of the body parts of queens raised in the two types of cell cups showed consistent differences throughout almost the whole study, from the beginning of June until the end of August. However, some variability was observed during the trial, especially for thorax and abdomen width and weight, with higher weights recorded in the last decade of June and higher widths at the beginning and at the end of the study. In both groups of queens, the body weight was also appreciably higher in the last decade of June. Such overall variability is likely due to differences in pollen quality across the time, especially as regards the protein content that affects the development of the hypopharyngeal glands responsible for the production of royal jelly by nurse bees (Di Pasquale et al. 2016). These latter Authors highlighted that the protein content reaches maximum values in June in western France, while in Greece (Liolios et al. 2015) and Saudi Arabia (Al-Kahtani et al. 2020) the highest values were observed between March and May. In addition, the production of royal jelly depends upon the availability of pollen

or pollen substitutes, and colonies with greater access to feeding sources are able to rear larger numbers of high-quality queens (Anton and Grozinger 2020).

In the future, further experiments could be conducted, by collecting data from queens reared in cups of different diameters, with the aim of confirming the results obtained in the present investigation. Besides, it could be verified whether the morphological improvement recorded in the present study translates into a greater production of eggs in the period of maximum development and, consequently, into a higher number of bees in the colony.

Conclusions

A positive effect on queens' quality, especially in terms of a higher weight, was obtained by increasing cell cup size. This opens the way to further studies to investigate if queens raised in larger cell cups may also show any improvements of their performances in terms of colony size, production and health.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

Raw data were generated at the Department of Veterinary Medicine and Animal Science, University of Milano. Derived data supporting the findings of this study are available from the corresponding author on request.

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