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Increased Mass-Rearing of Queens in High Royal-Jelly-Producing Honey Bee Colonies (*Apis mellifera ligustica*) Generates Smaller Queens with Comparable Fecundity

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Abstract: The mass rearing of high-quality queen bees is an essential beekeeping practice for producing new queens to maintain colony productivity. A strain of high royal-jelly-producing bees (RJBs; *Apis mellifera ligustica*) in China exhibits high potential for the rapid mass rearing of queens. To explore the potential changes in the quality of mass-reared queens, a set of morphometric traits and the sealed brood area were compared between the queens reared from 64 and 320 queen cells in RJB colonies. The increase in the queen cell number was found to induce a slightly but significantly reduced body weight and smaller wing length and thorax width in the reared queens at emergence. However, the ovariole number and sealed brood area, an indicator of the queen fecundity, were not observed to be significantly influenced. With respect to body weight and ovariole number, all the reared queens satisfied the current criteria for high-quality queens. Our findings provide evidence for the efficient mass production of high-quality queens using RJB colonies.

Keywords: queen cell number; queen quality; queen rearing; royal jelly; ovariole number; fecundity

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1. Introduction

As a pivotal colony member of honey bees (*Apis mellifera*), the queen is specialized in egg laying and pheromone releasing to maintain colony stability and function [1,2]. A poor-quality queen is generally regarded as one of the key factors contributing to colony loss, which remains an urgent global issue in modern apiculture [3,4]. In commercial beekeeping, old queens are periodically replaced with newly raised queens because colony reproduction and productivity decline dramatically as queens age [5,6]. Notably, this requeening typically occurs once or twice a year [7,8]. Considering the total of 100 million honey bee colonies across the globe according to Food and Agriculture Organization (FAO, https://www.fao.org/faostat; accessed on 10 January 2024), there is thus a considerable demand for mass rearing of high-quality queens.

Artificial queen rearing is a vital beekeeping practice to regularly requeen honey bee colonies. The key step in traditional procedures for queen rearing involves grafting young larvae from worker cells to plastic queen cells that are then introduced into honey bee colonies [7]. These larvae are provisioned by nurse bees with copious amounts of royal jelly, a glandular secretion of nurse bees and exclusive nourishment for queens [9], until the cells are capped. When the queens are to emerge, the queen cells are moved to queenless hives or mating nuclei.

During the rearing process, a wide variety of factors, such as genotype [7,10], queen cell size [11,12], age of the grafted larvae [13,14], and supplementary feeding [13,15], have been reported to affect the morphometric and reproductive traits of the queens. Among them, body weight, an integrative measure of size and physiological status, is regarded as

the most informative indicator of queen quality [1]. Other measurements, e.g., wing length and number of ovarioles, which are fundamental units of queen ovaries for egg producing, are widely employed to assess queen quality [15–17].

Mass rearing of queen bees could be ideally facilitated by increasing the queen cell number per colony. The queen cell number has varied in previous studies, and no more than 60 larvae are usually grafted for queen rearing [13,16–19]. It has been reported that the grafting of different numbers of larvae (30, 45, and 60) in *A. m. anatoliaca* colonies significantly impacts the body length and head width of the reared queens [20]. However, comparative studies that establish the optimum number of queen cells per colony for high-quality queen rearing are still lacking.

A strain of high royal-jelly-producing bees (RJBs) selectively bred from Italian bees (*A. m. ligustica*) in China has an increased reproductive investment in queens. This is reflected in the higher larval acceptance rate and royal jelly provisioning in RJBs than in other honey bee races during royal jelly production [21–23]. Notably, the larval acceptance rate is over 90% even with 320 queen cells per colony [24]. The remarkable performance of RJBs makes it possible to rapidly rear a large number of queens. It should be noted, however, that the introduction of 320 queen cells could lower the royal jelly quantity and alter its chemical constituent levels compared to the introduction of 64 queen cells [24]. It is yet unknown whether these changes could influence the quality of the reared queens.

To bridge this knowledge gap, we measured a series of morphometric traits including the body weight, wing length, thorax width, head width, and ovariole number of the queens reared from 64 and 320 queen cells per RJB colony. We also compared the sealed brood area of the colonies headed by these queens. Our study provides insights into the mass production of high-quality queens using RJBs.

2. Materials and Methods

2.1. Queen Bee Rearing

RJB colonies were maintained at the Institute of Apicultural Research, Chinese Academy of Agricultural Sciences in Beijing, China. Each colony was divided by a queen excluder into a queenless super chamber for production and a queenright chamber with two separated queens for propagation. Six colonies with 10 combs of adult bees each, similar brood patterns and stored food, were selected. They were randomly divided into two groups (three colonies for each) to rear queens by introducing 64 or 320 queen cells per colony. In brief, the queen in a source colony was restricted for 5 h to lay eggs in worker cells. Newly hatched larvae (within 24 h of hatching) from the eggs were grafted into the queen cells mounted on bars, and thereafter the bars were put into the queenless super chamber of the colony [25]. Ten days after the grafting, the capped queen cells were transferred to an incubator (34 °C, 60% humidity) until emergence.

2.2. External Morphometric Trait Measurement

Body weight of the virgin queens at emergence (n = 101 for each group) was measured with a digital balance scale (0.1 mg accuracy; Mettler-Toledo, Giessen, Germany). For each group, 30 queens were immediately frozen at −40 °C until subsequent measurements. Wing length, thorax width, and head width of the frozen queens (Figure 1A) were determined as described elsewhere [26]. Briefly, the queen was pinned to a paraffin surface of a dissection dish. The right forewing was detached from the body and laid beside the queen. The queens were photographed with a digital microscope (LEICA, Wetzlar, Germany), and the images were used for morphological measurements with ImageJ v. 1.54e (National Institutes of Health, Bethesda, MD, USA). Each image contained a reference scale to determine the pixel/mm ratio.

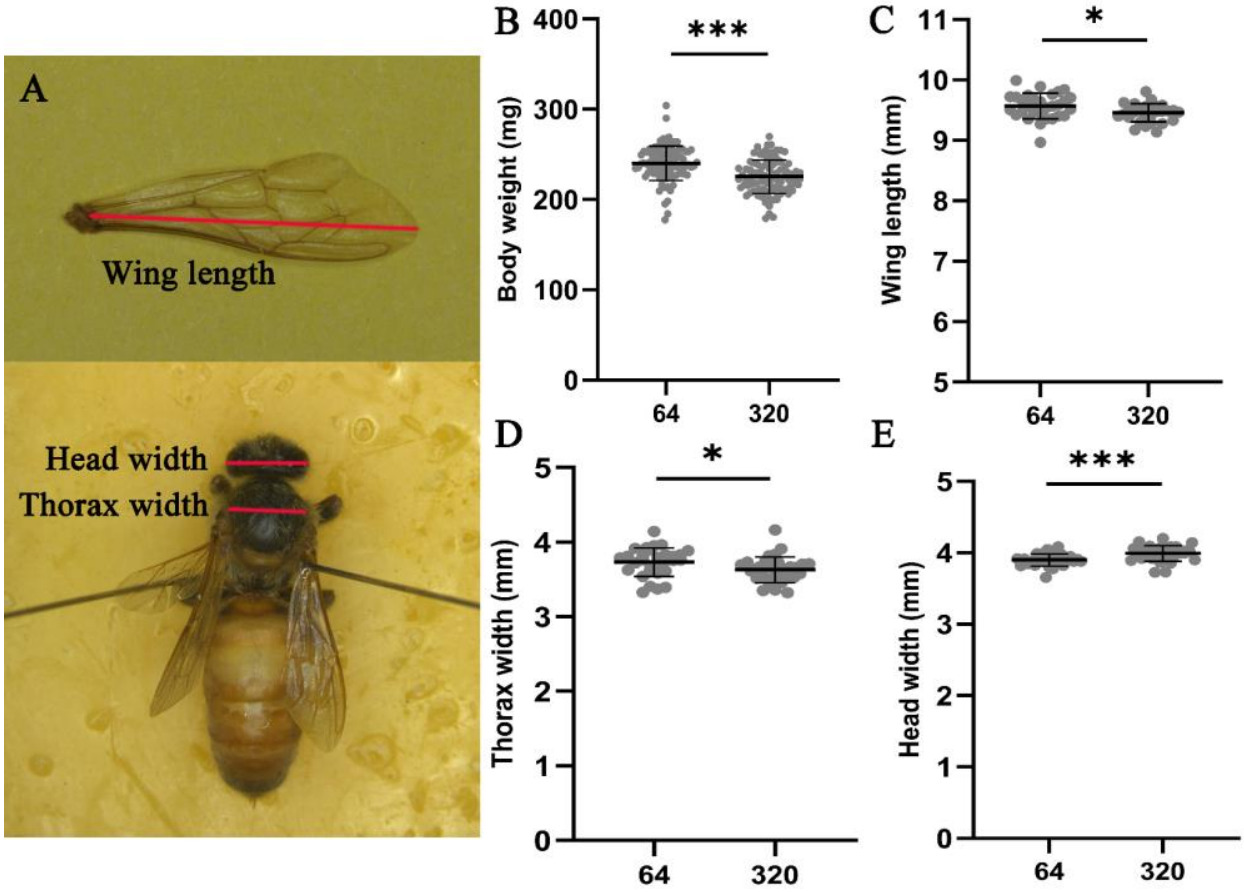

Figure 1. Morphometric traits of the reared queen bees. Example of wing length, thorax width, and head width of the queens at emergence for morphological measures (**A**). The rise in queen cell numbers from 64 to 320 reduced the body weight (**B**), wing length (**C**), and thorax width (**D**), but increased the head width (**E**). * $p < 0.05$; *** $p < 0.001$.

2.3. Ovariole Number Measurement

For each group, 30 of the newly emerged queens were sampled to measure ovariole number. Briefly, these queens were restricted in queen cages placed in a queenless colony for 6 days to acquire feeding from worker bees, since it is easier to separate and count the ovariole number of queens at this age [27]. The queens were frozen and stored at −40 °C. The procedures for ovariole number counting were performed as previously described, with modifications [28]. Briefly, the queens were fixed in a wax-based dissection dish, and the abdomen was dissected (Figure 2A). The left ovarian tissue was selected and immediately immersed in 4% paraformaldehyde fixative (Coolaber, Beijing, China) at room temperature for 1 h, followed by immersion in 70% ethanol (Macklin, Shanghai, China) three times each for 15 min. The next procedures included dehydration, diaphanization, and infiltration. Thereafter, the ovarian tissue was placed in base molds (1.5 × 1.5 cm) and embedded in paraffin for 1 h. Histological sections with a thickness of 5 µm were created using a manual rotating microtome (LEICA, Wetzlar, Germany). The cut sections were subjected to wax removal, rehydration, and staining with hematoxylin and eosin (Solarbio, Beijing, China). The images of the sections were captured with a microscope (LEICA, Wetzlar, Germany), and the number of ovarioles was counted manually.

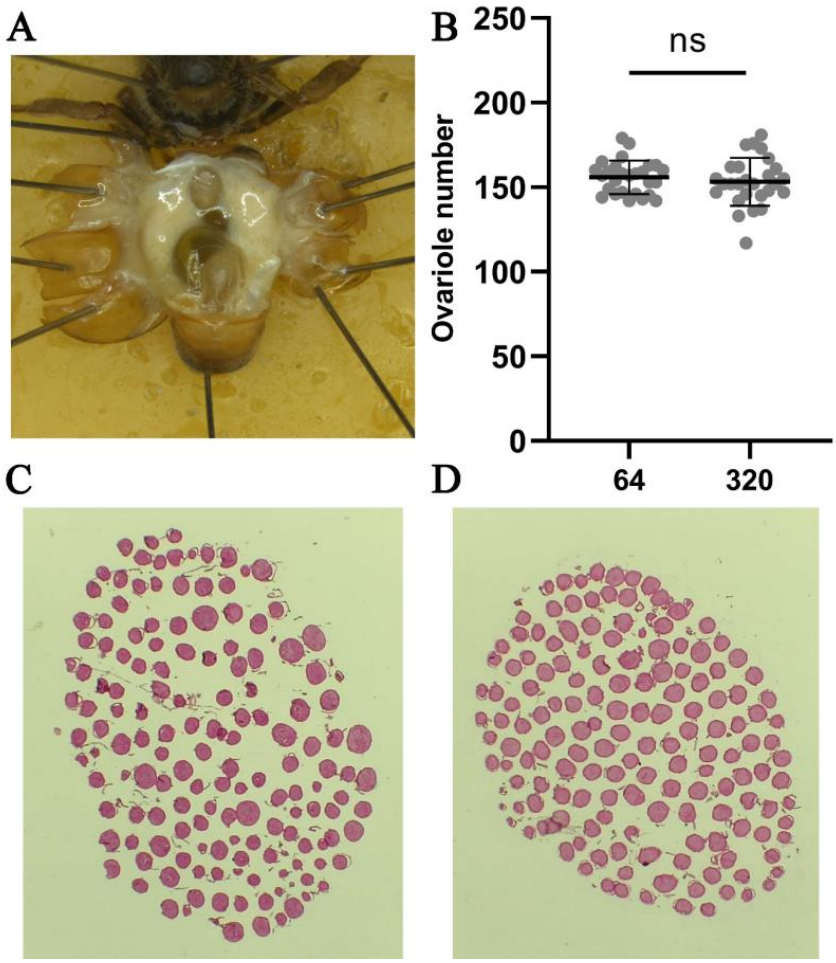

Figure 2. Ovariole number of the reared queen bees. Ovarian tissues of a queen after abdominal dissection (**A**). The ovariole number (**B**) remained unchanged with the increase in queen cell number. ns, not significant. Images of the ovary transverse sections of the queens reared from 64 (**C**) and 320 (**D**) queen cells.

2.4. Sealed Brood Area Measurement

The newly emerged queens were introduced into queenless colonies, which had three combs with mostly young adult bees and sufficient food. The queens were allowed to mate naturally. Seven and six colonies headed by the queens reared from 64 and 320 queen cells, respectively, were used to determine the sealed brood area. Three rounds of the measurement were performed consecutively with an interval of 12 days since the sealing period of *A. mellifera* worker bees is 12 days. The pictures of the sealed brood area were analyzed using ImageJ 1.54e (National Institutes of Health, Bethesda, MD, USA), following a previous study [29].

2.5. Statistical Analysis

Statistical differences were determined at $p < 0.05$ with GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA). Significant differences between two groups were determined using Student's *t*-test. Significant differences among multiple groups were assessed via analysis of variance (ANOVA) followed by Tukey's post hoc test. Quantitative data are presented as means ± standard error of mean (SEM).

3. Results

3.1. Morphometric Traits of the Reared Queens

To test the effect of queen cell number on the morphometric traits of queens, we measured the body weight, wing length, thorax width, and head width of the queens at emergence and the number of ovarioles in 6-day-old queens. The body weight of the queens reared from 64 queen cells per colony was 240.07 ± 18.96 mg, significantly higher than that from 320 queen cells (225.34 ± 18.44 mg; $p < 0.001$) (Figure 1B). The queens reared from 64 queen cells had significantly larger wing length (Figure 1C) and thorax width (Figure 1D) than those reared from 320 queen cells ($p = 0.021$ and $p = 0.035$, respectively). By contrast, the head width was significantly smaller for the queens reared from 64 queen cells ($p < 0.001$) (Figure 1E). In addition, stained transverse sections of the ovary of the reared queens are shown in Figure 2C,D. No significant difference was found in the ovariole number between the queens reared from 64 (155.86 ± 9.86) and 320 queen cells (153.19 ± 14.17; $p = 0.43$) (Figure 2B).

3.2. Sealed Brood Areas

To explore the potential changes in the fecundity of queens in response to different queen cell numbers, the sealed brood areas of the colonies headed by the reared queens were measured. The sealed brood areas ranged from 3101.53 ± 435.39 cm^2 to 3821.35 ± 741.16 cm^2 and from 2923.74 ± 768.65 cm^2 to 3241.31 ± 478.72 cm^2 for the queens reared from 64 and 320 queen cells, respectively. At each observation date, the measurements were not found to be influenced significantly by the queen cell number ($p > 0.05$ for all). Moreover, a significant effect of the observation date on the sealed brood area was found for the queens reared from 64 queen cells ($p = 0.040$) but not for those from 320 queen cells ($p = 0.634$) (Figure 3). Specifically, the values obtained from the first observation date were significantly larger than those from the other dates.

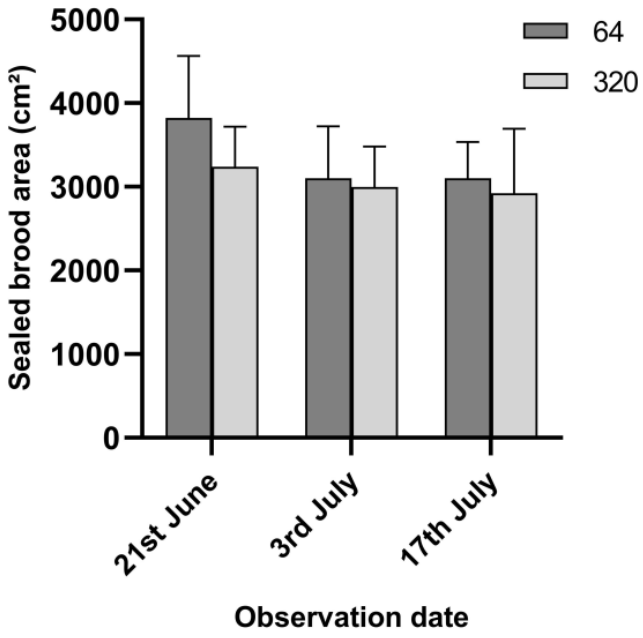

Figure 3. The sealed brood areas in the colonies headed by the reared queens. At each observation date, the sealed brood areas were not affected by queen cell numbers. Observation date had a significant effect on the sealed brood areas for the queens reared from 64 queen cells.

4. Discussion

The quality of queens is one of the important factors affecting the stability and development of honey bee colonies [30]. The purpose of this study was to investigate the effect of differences in the number of grafted larvae used for queen rearing on the morphometric

traits and fecundity of the reared RJB queens. Our main findings demonstrate that the increase in the queen cell number from 64 to 320 could result in smaller-sized queens but had no significant impact on queen fecundity.

The morphological characteristics associated with queen size are regarded as critical parameters for evaluating the quality of honey bee queens. Among them, the weight of the queen at emergence is a widely used indicator, which is required to be a minimum of 190 mg for high-quality queens in Bulgaria and Italy [31]. Heavier queens with a birth weight of more than 200 mg could generate faster population growth than queens weighing less than 180 mg [32]. In this study, we observed a slight (6.14%) but significant reduction in the body weight of the queens reared from 320 queen cells (225.34 mg) relative to those from 64 queen cells (240.07 mg). Accordingly, the associated morphological characteristics including wing length and thorax width were found to be shortened. The correlations of the two parameters and queen weight have been reported [20]. Nevertheless, the head width showed an opposite trend, which was wider for the queens reared from 320 queen cells in our study. Although the potential reasons for this discrepancy are unclear, a similar inconsistent changing tendency between head width and other features has been reported previously. For example, no significant difference was found in the head width between queens with different body weights [20,30]. The validity of head width for queen quality evaluation needs to be examined in future studies. Overall, our findings indicate that slightly smaller queens could be reared with increased queen cell numbers, but they still fulfill the weight requirement of high-quality queens.

The measurements of reproduction-related characteristics were also included to assess the quality of the queens reared in our study. First, we obtained a similar number of ovarioles for the RJB queens reared from 64 (155.86 ovarioles) and 320 queen cells (153.19 ovarioles). The threshold for ovariole number per ovary is 130 for high-quality queens in Greece [31], and an average of 149 ovarioles has been proposed for *A. m. carnica* in Slovenia [33]. Although the criteria concerning the ovariole number for high-quality RJB queens have not been specified, an average of 154.77 ovarioles for such queens reared from 44 grafted larvae has been reported [34]. Second, we compared the sealed brood area, which acts as a direct criterion for the evaluation of queen fecundity. We found no significant difference in the sealed brood area between the colonies headed by the queens reared from 64 and 320 queen cells. The measurements reported in this study are higher than those reported in previous studies [35,36]. Moreover, the observed fluctuations in the sealed brood area with time were likely due to weather conditions and/or food supplies. Taken together, these two measurements provide further evidence of the similar high quality of our queens reared in both conditions, although more measurements such as honey production are desirable.

The differential morphometric traits mentioned above are probably attributable to royal jelly, the exclusive food of queen bees. Our former study revealed not only lower levels of fatty acids including 10-hydroxy-2-decenoic acid (10-HDA) in royal jelly but also reduced royal jelly amount per queen cell with the rise from 64 to 320 queen cells per colony [24]. To date, the role of 10-HDA in honey bee development and caste determination has been explored. It has been reported that 10-HDA possesses histone deacetylase inhibitor activity that might epigenetically regulate queen development [37]. Increased 10-HDA content in larval diet reduces the weight of newly emerged workers [38]. Alternatively, the observed difference in royal jelly quantity due to queen cell number [24] constitutes another possible reason since in vitro diet quantity influences queen development [39]. Moreover, more residual royal jelly in the queen cells after queen emergence is recognized as a characteristic of high-quality emerged queens [10,40]. Hence, the observed qualitative and/or quantitative differences in royal jelly are possibly, at least in part, responsible for the changes in the size-related features of our raised queens. It is clear that more work is required to determine the causative factors.

Our findings have practical implications for the mass rearing of high-quality queens. For a non-RJB colony, only a limited number of worker larvae, e.g., 15–34 [13,16–19], are

grafted for queen rearing. In contrast, the increased reproductive investment of RJBs enables the mass production of high-quality queens from 320 queen cells, as shown in our study. Given the fact that beekeepers have a strong preference for larger queens [7,26], additional strategies should be employed to further increase the body size of queens. While this objective can be achieved by reducing the queen cell number alone, the analyzed reproductive traits were not found to be improved in our study. Thus, more breeding techniques should be incorporated, e.g., grafting newly hatched larvae from larger eggs laid by the released mother queen that have been forbidden from laying for 4 days [12] and transferring eggs for queen rearing [18].

5. Conclusions

The increase in queen cell number from 64 to 320 per RJB colony could reduce the queen size and associated morphological features rather than the ovariole number and fecundity. In regards to the body weight and ovariole number, all the reared queens fulfilled the current criteria for high-quality queens. Queen breeders can make full use of the enhanced female reproductive investment of RJB colonies for the mass rearing of queens. To obtain the larger queens favored by beekeepers, however, the queen cell number per colony should be reduced.

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References

1. Amiri, E.; Strand, M.; Rueppell, O.; Tarpy, D. Queen quality and the impact of honey bee diseases on queen health: Potential for interactions between two major threats to colony health. *Insects* **2017**, *8*, e48. [CrossRef] [PubMed]
2. Abou-Shaara, H.F.; Adgaba, N.; Al-Ghamdi, A.A. Current knowledge about behaviors of honey bee queens with highlighting of the importance future studies. *J. Basic Appl. Zool.* **2021**, *82*, 37. [CrossRef]
3. Vanengelsdorp, D.; Hayes, J.; Underwood, R.M.; Pettis, J. A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. *PLoS ONE* **2008**, *3*, e4071. [CrossRef] [PubMed]
4. Vanengelsdorp, D.; Tarpy, D.R.; Lengerich, E.J.; Pettis, J.S. Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States. *Prev. Vet. Med.* **2013**, *108*, 225–233. [CrossRef] [PubMed]
5. Hauser, H.; Lensky, Y. The effect of the age of the honey bee (*Apis mellifera* L.) queen on worker population, swarming and honey yields in a subtropical climate. *Apidologie* **1994**, *25*, 566–578. [CrossRef]
6. Simeunovic, P.; Stevanovic, J.; Cirkovic, D.; Radojicic, S.; Lakic, N.; Stanisic, L.; Stanimirovic, Z. *Nosema ceranaeand* queen age influence the reproduction and productivity of the honey bee colony. *J. Apic. Res.* **2015**, *53*, 545–554. [CrossRef]
7. Büchler, R.; Andonov, S.; Bienefeld, K.; Costa, C.; Hatjina, F.; Kezic, N.; Kryger, P.; Spivak, M.; Uzunov, A.; Wilde, J. Standard methods for rearing and selection of *Apis mellifera* queens. *J. Apic. Res.* **2013**, *52*, 1–30. [CrossRef]
8. Tang, J.; Ji, C.; Shi, W.; Su, S.; Xue, Y.; Xu, J.; Chen, X.; Zhao, Y.; Chen, C. Survey results of honey bee colony losses in winter in China (2009–2021). *Insects* **2023**, *14*, e554. [CrossRef]
9. Fujita, T.; Kozuka-Hata, H.; Ao-Kondo, H.; Kunieda, T.; Oyama, M.; Kubo, T. Proteomic analysis of the royal jelly and characterization of the functions of its derivation glands in the honeybee. *J. Proteome Res.* **2012**, *12*, 404–411. [CrossRef]
10. Yu, L.; Shi, X.; He, X.; Zeng, Z.; Yan, W.; Wu, X. High-quality queens produce high-quality offspring queens. *Insects* **2022**, *13*, e486. [CrossRef] [PubMed]

11. Mattiello, S.; Rizzi, R.; Cattaneo, M.; Martino, P.A.; Mortarino, M. Effect of queen cell size on morphometric characteristics of queen honey bees (*Apis mellifera ligustica*). *Ital. J. Anim. Sci.* **2022**, *21*, 532–538. [CrossRef]
12. Wu, X.; Zhou, L.; Zou, C.; Zeng, Z. Effects of queen cell size and caging days of mother queen on rearing young honey bee queens *Apis mellifera* L. *J. Apic. Sci.* **2018**, *62*, 215–222. [CrossRef]
13. Mahbobi, A.; Farshineh-Adl, M.; Woyke, J.; Abbasi, S. Effects of the age of grafted larvae and the effects of supplemental feeding on some morphological characteristics of Iranian queen honey bees (*Apis mellifera meda* Skorikov, 1929). *J. Apic. Sci.* **2012**, *56*, 93–98. [CrossRef]
14. Woyke, J. Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. *J. Apic. Res.* **2015**, *10*, 45–55. [CrossRef]
15. De Souza, D.A.; Huang, M.H.; Tarpy, D.R. Experimental improvement of honey bee (*Apis mellifera*) queen quality through nutritional and hormonal supplementation. *Apidologie* **2018**, *50*, 14–27. [CrossRef]
16. Gilley, D.C.; Tarpy, D.R.; Land, B.B. Effect of queen quality on interactions between workers and dueling queens in honeybee (*Apis mellifera* L.) colonies. *Behav. Ecol. Sociobiol.* **2003**, *55*, 190–196. [CrossRef]
17. Njeru, L.K.; Raina, S.K.; Kutima, H.L.; Salifu, D.; Cham, D.T.; Kimani, J.N.A.A.; Muli, E.M. Effect of larval age and supplemental feeding on morphometrics and oviposition in honey bee queen (*Apis mellifera scutellata*, Hymenoptera: Apidae) in Karura forest, Kenya. *J. Apic. Res.* **2018**, *56*, 183–189. [CrossRef]
18. Cengiz, M.M.; Yazici, K.; Arslan, S. The effect of the supplemental feeding of queen rearing colonies on the reproductive characteristics of queen bees (*Apis mellifera* L.) reared from egg and different old of larvae. *Kafkas Univ. Vet. Fak. Derg.* **2019**, *25*, 849–855.
19. Dolasevic, S.; Stevanovic, J.; Aleksic, N.; Glavinic, U.; Deletic, N.; Mladenovic, M.; Stanimirovic, Z. The effect of diet types on some quality characteristics of artificially reared *Apis mellifera* queens. *J. Apic. Res.* **2019**, *59*, 115–123. [CrossRef]
20. Okuyan, S.; Akyol, E. The effects of age and number of grafted larvae on some physical characteristics of queen bees and acceptance rate of queen bee cell. *Turk. J. Agric. Food Sci. Technol.* **2018**, *6*, 1556–1561. [CrossRef]
21. Zhang, X.; Hu, H.; Han, B.; Wei, Q.; Meng, L.; Wu, F.; Fang, Y.; Feng, M.; Ma, C.; Rueppell, O.; et al. The neuroproteomic basis of enhanced perception and processing of brood signals that trigger increased reproductive investment in honeybee (*Apis mellifera*) workers. *Mol. Cell. Proteom.* **2020**, *19*, 1632–1648. [CrossRef] [PubMed]
22. Wu, F.; Ma, C.; Han, B.; Meng, L.; Hu, H.; Fang, Y.; Feng, M.; Zhang, X.; Rueppell, O.; Li, J. Behavioural, physiological and molecular changes in alloparental caregivers may be responsible for selection response for female reproductive investment in honey bees. *Mol. Ecol.* **2019**, *28*, 4212–4227. [CrossRef] [PubMed]
23. Ma, C.; Zhang, L.C.; Feng, M.; Fang, Y.; Hu, H.; Han, B.; Meng, L.F.; Li, J.K. Metabolic profiling unravels the effects of enhanced output and harvesting time on royal jelly quality. *Food Res. Int.* **2021**, *139*, e109974. [CrossRef]
24. Ma, C.; Ahmat, B.; Li, J. Effect of queen cell numbers on royal jelly production and quality. *Curr. Res. Food Sci.* **2022**, *5*, 1818–1825. [CrossRef] [PubMed]
25. Altaye, S.Z.; Meng, L.; Li, J. Molecular insights into the enhanced performance of royal jelly secretion by a stock of honeybee (*Apis mellifera ligustica*) selected for increasing royal jelly production. *Apidologie* **2019**, *50*, 436–453. [CrossRef]
26. Facchini, E.; De Iorio, M.G.; Turri, F.; Pizzi, F.; Laurino, D.; Porporato, M.; Rizzi, R.; Pagnacco, G. Investigating genetic and phenotypic variability of queen bees: Morphological and reproductive traits. *Animals* **2021**, *11*, e3054. [CrossRef] [PubMed]
27. Remolina, S.C.; Hughes, K.A. Evolution and mechanisms of long life and high fertility in queen honey bees. *Age* **2008**, *30*, 177–185. [CrossRef]
28. Raulino-Domanski, F.; Potrich, M.; Freitas, P.F.; Abdalla, F.C.; Martins, E.N.; Lourenco, D.A.L.; Costa-Maia, F.M. Optimized histological preparation of ovary for ovariole counting in Africanized honey bee queens (Hymenoptera: Apidae). *J. Insect Sci.* **2019**, *19*, 12. [CrossRef]
29. Yoshiyama, M.; Kimura, K.; Saitoh, K.; Iwata, H. Measuring colony development in honey bees by simple digital image analysis. *J. Apic. Res.* **2015**, *50*, 170–172. [CrossRef]
30. Rangel, J.; Keller, J.J.; Tarpy, D.R. The effects of honey bee (*Apis mellifera* L.) queen reproductive potential on colony growth. *Insect Soc.* **2012**, *60*, 65–73. [CrossRef]
31. Hatjina, F.; Bieńkowska, M.; Charistos, L.; Chlebo, R.; Costa, C.; Dražić, M.M.; Filipi, J.; Gregorc, A.; Ivanova, E.N.; Kezić, N.; et al. A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. *J. Apic. Res.* **2015**, *53*, 337–363. [CrossRef]
32. De Souza, D.A.; Bezzera-Laure, M.A.F.; Francoy, T.M.; Gonçalves, L.S. Experimental evaluation of the reproductive quality of Africanized queen bees (*Apis mellifera*) on the basis of body weight at emergence. *Genet. Mol. Res.* **2013**, *12*, 5382–5391. [CrossRef]
33. Gregorc, A.; Smodiš Škerl, M.I. Characteristics of honey bee (*Apis mellifera carnica*, Pollman 1879) queens reared in Slovenian commercial breeding stations. *J. Apic. Sci.* **2015**, *59*, 5–12. [CrossRef]
34. Zhang, B.; Wu, X.; Liao, C.; He, X.; Yan, W.; Zeng, Z. Research and application of honeybee non-grafting larvae technology. *Sci. Agric. Sin.* **2018**, *51*, 4387–4394.
35. Lamontagne-Drolet, M.; Samson-Robert, O.; Giovenazzo, P.; Fournier, V. The impacts of two protein supplements on commercial honey bee (*Apis mellifera* L.) colonies. *J. Apic. Res.* **2019**, *58*, 800–813. [CrossRef]
36. Khan, K.A.; Ghramh, H.A.; Ahmad, Z. Honey bee (*Apis mellifera jemenitica*) colony performance and queen fecundity in response to different nutritional practices. *Saudi J. Biol. Sci.* **2022**, *29*, 3151–3156. [CrossRef]

37. Spannhoff, A.; Kim, Y.K.; Raynal, N.J.M.; Gharibyan, V.; Su, M.B.; Zhou, Y.Y.; Li, J.; Castellano, S.; Sbardella, G.; Issa, J.P.J.; et al. Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. *EMBO Rep.* **2011**, *12*, 238–243. [CrossRef] [PubMed]
38. Wang, W.X.; Tian, L.Q.; Huang, Q.; Wu, X.B.; Zeng, Z.J. Effects of 10-Hydroxy-2-decenoic acid on the development of honey bee (*Apis mellifera*) larvae. *J. Apic. Res.* **2014**, *53*, 171–176. [CrossRef]
39. Slater, G.P.; Yocum, G.D.; Bowsher, J.H. Diet quantity influences caste determination in honeybees (*Apis mellifera*). *Proc. R. Soc. B-Biol. Sci.* **2020**, *287*, e614. [CrossRef]
40. Yi, Y.; Liu, Y.B.; Barron, A.B.; Zeng, Z.J.; Tarpy, D. Transcriptomic, morphological, and developmental comparison of adult honey bee queens (*Apis mellifera*) reared from eggs or worker larvae of differing ages. *J. Econ. Entomol.* **2020**, *113*, 2581–2587. [CrossRef]