

# Critical Note on Incubation of Capped Queen Cells and Need for Research

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## The Use of Incubators in Queen Rearing

The high demand for honey bee queens has led commercial queen breeders to use incubators instead of the traditional method of incubating capped queen cells inside colonies. The incubators offer advantages such as temperature control and synchronized hatching. Meanwhile, the artificially incubated queens are apparently healthy and can mate and lay eggs in a similar way to those incubated naturally. However, the majority of the Egyptian beekeepers have reported faster failure of artificially incubated queens compared to those incubated naturally. This article aims to highlight this issue and suggest areas for further research.

The traditional approach involves incubating capped queen cells in queen-less colonies. The temperature around capped cells fluctuates by  $<10^{\circ}\text{C}$ , with a preferred range of  $32.3\text{--}34.9^{\circ}\text{C}$  for queen emergence (Degrandi-Hoffman et al., 1993a). The history of using incubators in commercial queen rearing is not well documented. Incubators were initially used in scientific experiments for artificial queen rearing and other laboratory experiments (e.g. Nobusawa, 1970). Over time, this technique has been adopted by commercial queen breeders to control and simplify the queen rearing process due to the high queen demand.

Incubators have been used in scientific experiments for laboratory rearing of queens (Crailsheim et al., 2013), and to investigate the effects of different temperature gradients on queens. In this regard, Spivak et al. (1992) showed that the artificial incubation of black and yellow queen lines at  $30.5$ ,  $33.5$ , and  $35.5^{\circ}\text{C}$  resulted in faster development of black queens than yellow ones, with induction of yellow coloration in both lines at  $35.5^{\circ}\text{C}$  than at lower temperatures. DeGrandi-Hoffman et al. (1993b) found variations in queen colors after

artificial incubation at different temperature gradients ( $31.1$ ,  $32.8$ , or  $34.4^{\circ}\text{C}$ ), with lighter color mostly observed with increasing temperature. However, naturally incubated queens also sometimes had similar colors to those artificially incubated at  $34.4^{\circ}\text{C}$ . Chuda-Mickiewicz and Samborski (2015) found that incubation temperature affected the development time of queen pupae, with longer time at  $32^{\circ}\text{C}$  than at  $34.5^{\circ}\text{C}$ , but without effects on queen quality.

Practically, incubators have several advantages in queen rearing such as controlling temperature, facilitating queen observation until emergence, and enabling the collection of queens that emerge within similar time periods. However, incubators cannot guarantee the hatching of all capped cells, as queens may still make efforts to uncap cells to hatch and some queens may emerge with deformations (Abou-Shaara & Staron, 2018). Both artificially and naturally incubated queens can hatch normally (Figure 1). A study found that artificially incubated queens at  $34.7^{\circ}\text{C}$  and 80% RH had a higher weight than naturally incubated queens in queen-right/queen-less colonies by  $16.67\text{--}17.91$  mg (Akongte et al., 2022), supporting the advantages of artificial incubation. However, the subsequent effects of artificial incubation on queen performance after emergence remain unknown and require further research.

## The Reported Problems with Artificially Incubated Queens

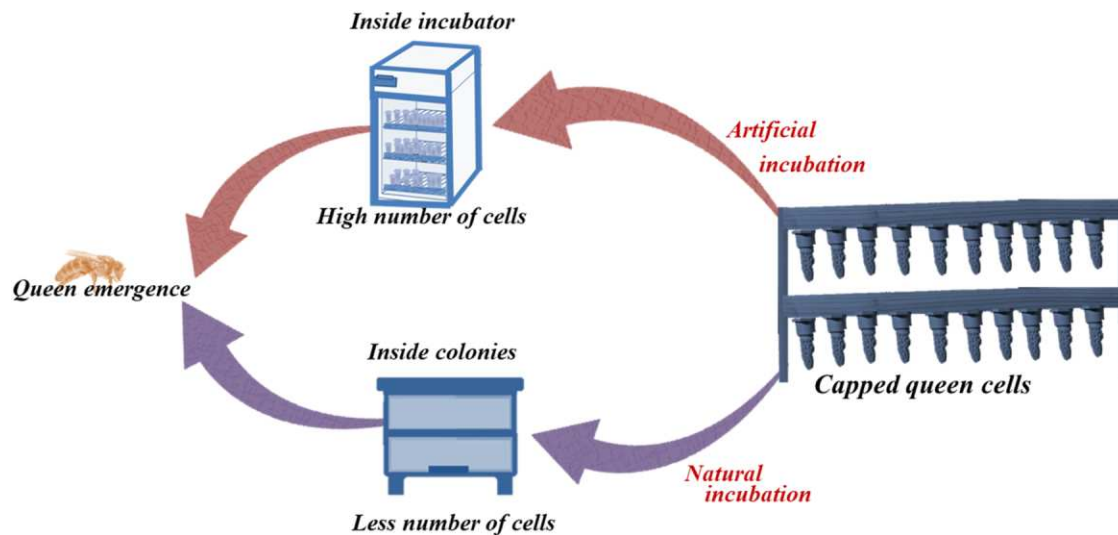
Local beekeepers from different regions in Egypt have reported lower quality of artificially incubated queens compared to naturally incubated ones. They claim that artificially incubated queens have a shorter lifespan, with lower colony development and overwintering ability.

Additionally, colonies tend to replace these queens more frequently than naturally incubated ones. It appears that the incubation method affects mating quality, egg-laying ability, and the ability of queens to dominate colonies. Some commercial queen breeders do not use scientific-grade incubators but instead rely on low-cost commercially available ones made with lower quality materials. Local beekeepers are hesitant to purchase artificially incubated queens and inquire about incubation methods. Although this opinion has not been scientifically tested using a variety of analysis methods, it highlights the need for future studies to focus on the incubation method instead of overlooking it.

## The Potential Problems during Artificial Incubation of Queen Cells

The problems regarding the low quality of artificially incubated queens could be attributed to the potential fluctuations in temperature, relative humidity, and carbon dioxide levels during the incubation period of 7–9 days. While the calibration of incubators can be done by using a thermometer to measure internal temperature, the fluctuations in temperature during the incubation period of capped cells are not almost tracked. Additionally, relative humidity is mostly overlooked and only a water container is generally used as a source of humidity inside the incubator.

Comprehensive studies on naturally and artificially incubated queens should be conducted by researchers in various countries. These studies should compare queens on both morphological and physiological levels, followed by field studies to track their performance in colonies over a year or more. Short and long-term behavioral disruptions should also be tracked. Additionally, different incubation techniques and incubator



**Figure 1.** Incubation methods of capped queen cells.

brands should be compared, with a focus on precise recording of temperature, relative humidity, and CO<sub>2</sub> fluctuations during the incubation period of capped cells. These efforts will greatly aid in the commercial breeding of queens to determine the best incubation practices. Comparisons between honey bee subspecies and hybrids are also important as they may respond differently to incubation methods. The reported problems could be linked to a combination of factors, including the efficacy of the incubation instrument, honey bee subspecies/hybrids, and queen rearing method. Therefore, survey studies using questionnaires are recommended to gather feedback from beekeepers and commercial queen breeders.

The exposure of queens to temperature extremes during package transportation does not have a major impact on subsequent queen failure in their colonies (Withrow et al., 2019). In fact, queen failure can occur due to various stressors, such as pesticide exposure during development (Williams et al., 2015), low sperm viability in queens (Pettis et al., 2016), and disease infection (Amiri et al., 2017). However, artificially incubated queens may fail more frequently than naturally incubated ones in the same apiary, indicating the impact of incubation methods on queens' resilience to stressors during their lifespan after emergence. Therefore, there is a need for comparative studies on the interactions between incubation methods and stressors on queen quality during both pre- and post-emergence periods.

## Conclusion

This article emphasizes the importance of conducting comprehensive studies on incubation techniques for capped cells until emergence. The failure of queens in their colonies, despite successful mating and egg-laying, may be linked to the incubation method. The irregular levels of temperature, relative humidity, and CO<sub>2</sub> could potentially contribute to the disadvantages of using artificial incubation of queens, which should be taken into consideration by researchers. Since there is only one queen in each colony, it is crucial to ensure the use of the best practices to rear queens, especially on a commercial level. This article neither recommends nor discourages the use of artificial incubation, but rather raises awareness about its potential effects on queen quality.

## Disclosure Statement

No potential conflict of interest was reported by the author(s).

## References

- Abou-Shaara, H. F., & Staron, M. (2018). Notes on queen cells and abnormal development of honey bee queens. *Bee World*, 95(4), 128–129. <https://doi.org/10.1080/0005772X.2018.1522836>
- Akongte, P. N., Frunze, O., Kim, D., Kang, E. J., Park, B. S., Kim, K. M., & Choi, Y. S. (2022). Pupa (*Apis mellifera* L.) Rearing conditions to improve queen weight at emergence. *Journal of Apiculture*, 37(4), 365–371. <https://doi.org/10.17519/apiculture.2022.11.37.4.365>
- Amiri, E., Strand, M. K., Rueppell, O., & Tarpy, D. R. (2017). Queen quality and the impact of honey bee diseases on queen health: Potential for interactions between two major threats to colony health. *Insects*, 8(2), 48. <https://doi.org/10.3390/insects8020048>
- Chuda-Mickiewicz, B., & Samborski, J. (2015). The quality of honey bee queens from queen cells incubated at different

temperatures. *Acta Scientiarum Polonorum. Zootechnica*, 14(4), 25–32.

Crailsheim, K., Brodschneider, R., Aupinel, P., Behrens, D., Genersch, E., Vollmann, J., & Riessberger-Gallé, U. (2013). Standard methods for artificial rearing of *Apis mellifera* larvae. *Journal of Apicultural Research*, 52(1), 1–16. <https://doi.org/10.3896/IBRA.1.52.1.05>

DeGrandi-Hoffman, G., Spivak, M., & Martin, J. (1993b). The influence of temperature on cuticular color of honeybee (*Apis mellifera* L.) queens. *Apidologie*, 24(2), 101–105. <https://doi.org/10.1051/apido:19930203>

Degrandi-Hoffman, G., Spivak, M., & Martin, J. H. (1993a). Role of thermoregulation by nestmates on the development time of honey bee (Hymenoptera: Apidae) queens. *Annals of the Entomological Society of America*, 86(2), 165–172. <https://doi.org/10.1093/aesa/86.2.165>

Nobusawa, C. (1970). Artificial rearing of queen honeybees in an incubator. *Saisyu to Siku*, 32(8), 269–271.

Pettis, J. S., Rice, N., Joselow, K., vanEngelsdorp, D., & Chaimanee, V. (2016). Colony failure linked to low sperm viability in honey bee (*Apis mellifera*) queens and an exploration of potential causative factors. *PLoS One*, 11(2), e0147220. <https://doi.org/10.1371/journal.pone.0147220>

Spivak, M., Zeltzer, A., Degrandi-Hoffman, G., & Martin, J. H. (1992). Influence of temperature on rate of development and color patterns of queen honey bees (Hymenoptera: Apidae). *Environmental Entomology*, 21(2), 364–370. <https://doi.org/10.1093/ee/21.2.364>

Williams, G. R., Troxler, A., Retschnig, G., Roth, K., Yañez, O., Shutler, D., Neumann, P., & Gauthier, L. (2015). Neonicotinoid pesticides severely affect honey bee queens. *Scientific Reports*, 5(1), 14621. <https://doi.org/10.1038/srep14621>

Withrow, J. M., Pettis, J. S., & Tarpy, D. R. (2019). Effects of temperature during package transportation on queen establishment and survival in honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, 112(3), 1043–1049. <https://doi.org/10.1093/jeet/toz003>

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