Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2636
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v37i1.24006

Morphometric measurements of Africanized honeybee queens kept in an incubator or in queen banking

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ABSTRACT. This study aimed to estimate live weight, the length and the width of wing and abdomen and the length, the width and the height of thorax in newly emerged Africanized honeybee queens up to 192 hours and their influence on final quality regarding appropriate storage techniques in queen banking or incubator and to verify the possibility of using these parameters as selection criteria in honeybee breeding programs. Queen honeybees were produced in summer, autumn and winter. The queen production method used throughout the experiments was adapted from Doolittle, and 68 the queens were weighed at emergence. Data were analyzed in R software by regression analysis of queen weight at emergence according to life time after emergence, in two types of storage. The weight of queens emerged in queen banking and incubator was significantly different ($p < 0.05$), with mean values of 165.61 and 157.04 mg, respectively. Queens kept in an incubator and in queen banking showed no significant differences ($p > 0.05$) in wing length, abdomen length and thorax height. The weight of queen at emergence can be used as a selection criterion in honeybee breeding programs.

Keywords: queen size, emergence, honeybee breeding, queen production.

Medidas morfométricas de rainhas africanizadas mantidas em estufa ou colmeia banco de rainhas

RESUMO. O objetivo do trabalho foi estimar peso vivo, comprimento e largura de asa e abdome e comprimento, largura e altura do tórax de rainhas *Apis mellifera* africanizadas recém-emergidas até 192h e sua influência na qualidade final com relação às técnicas apropriadas de armazenamento em banco de rainhas ou estufa e verificar se estes parâmetros podem ser utilizados como critério de seleção. Foram produzidas nas seguintes estações do ano: verão, outono e inverno. O método utilizado durante todo o experimento foi o adaptado de Doolitlle para a produção de rainhas e 68 rainhas foram pesadas à emersão. Os dados foram analisados no software R pela análise de regressão do peso da rainha à emergência em função do tempo de vida após a emersão, nos dois tipos de alojamento. Para peso, os efeitos da colmeia banco de rainhas e estufa foram significativos ($p < 0,05$), médias de 165,61 e 157,04 mg, respectivamente. As rainhas mantidas em estufa e colmeia banco de rainhas não apresentaram diferenças em relação ao comprimento da asa, ao comprimento do abdome e à altura do tórax. O peso de rainhas à emergência mantidas em colmeia banco de rainhas foi maior que o peso das rainhas mantidas em estufa. O peso das rainhas à emergência pode ser utilizado como critério de seleção.

Palavras-chave: tamanho de rainhas, emersão, melhoramento de abelhas, produção de rainhas.

Introduction

Some characteristics are little influenced by the environment but highly correlated with honey production, and can assist in selecting colonies with greater production capacity for more efficient analysis. Kahya et al. (2008) suggested that queen weight might be one of these characteristics and the queen can transmit this characteristic via drone.

The haplo-diploid sex determination system in *Apis mellifera* enables the transference of 100% of the genetic material of the drone from its mother to its offspring, because it is originated from an egg without fertilization (Cobey, 2007). Tarpy et al. (2000) reported a potential selection for newly emerged queens, as there is variation in the reproductive potential.

Queen reproductive potential is correlated with weight at emergence (Gilley et al., 2003; Tarpy et al., 2000). On the other hand, Kahya et al. (2008) found no significant correlation between queen weight at

emergence and number of ovarioles. Harano et al. (2007) reported that even when the ovaries are poorly developed, the abdomen of queens with potential for high production of eggs is heavy, which can adversely affect long mating flights, so that the temporary weight loss becomes a benefit to the flight.

The thorax is the locomotor center of insects, grouping the legs and wings containing the muscles that move them. Furthermore, natural fertilization occurs in flight and thus depends on wings to perform this movement (Moritz & Southwick, 1992).

Faquinello et al. (2011) associated the morphometric characteristics of newly emerged queens with royal jelly production in mini-hives and found potential selection for production, based on the abdomen width. Costa-Maia et al. (2011) estimated genetic and phenotypic parameters, via Bayesian inference, for weight and morphometric measurements of wing and abdomen in newly emerged Africanized honeybee queens and registered potential selection in all characteristics separately. Akyol et al. (2008) reported that queen weight is the most important among morphometric characteristics assessed given the positive correlation between queen weight and the brood area of the colony, the number of sperm and the diameter of spermatheca. In addition to this correlation, Akyol et al. (2006) observed a variation between morphological and physiological characteristics between subspecies and ecotypes.

Therefore, this study aimed to estimate queen weight at emergence and morphometric characteristics of the wing, abdomen and thorax of Africanized honeybee queens regarding storage techniques after emergence in queen banking or incubator.

Material and methods

Matrixes colonies that supply larvae located at the apiary in the farm at Universidade Estadual de Maringá were used to produce queens in summer, autumn and winter. Queens were produced by the method adapted from Doolittle (1889) throughout the experiments. On the day of larvae grafting, the unsealed brood comb was taken to the Laboratory of Queen Production at appropriate temperature (34 ± 2ºC) and humidity (60 ± 10%) to maintain the quality of the larvae until the time of grafting.

The grafted larvae were subsequently placed in mini-hives consisting of two overlapping nuclei separated by a queen excluder screen. The lower nuclei was composed of five combs and the upper, of four combs and a frame with cell cups spaced on cell bars (Mouro & Toledo, 2004). This cup bar frame consisted of 28 acrylic cups, 14 at the upper bar and 14 at the lower bar. During the experimental period, every 12 days, mini-hives were managed following method proposed by Faquinello et al. (2011), and received sugar syrup at a ratio of 2: 1 sugar and water.

On the tenth day after the grafting, bars with accepted cell cups were removed from the mini-hives and taken to the Laboratory of Queen Production. To house them, we prepared 20 mL-glass vials containing a strip of paper, so that when a new queen emerged it could move and reduce the risk of injury. Each glass vial was identified as by bar and mini-hive. Later, the vials with cell cups were taken to the incubator at an average temperature of 34ºC and 60% humidity. The technique of providing queen food (made of powdered sugar and honey) inside the vial for maintenance was not used because it could interfere with the queen weight at emergence.

Newly emerged queens were anesthetized with CO_2 for determining measures of body weight (mg) on a precision balance AL500 MARTE from 0.001 to 500 g, the length and width of the right forewing and abdomen (cm), the length, width and height of the thorax (cm) by using a caliper accurate to 0.01 mm. The length and width measurements were taken from the right forewing, and the abdomen length was measured at a relaxed position in width to the third segment.

Queens were then placed in an introduction cage and the variation in weight was monitored every hour for six hours per day after emergence to check its variation. Morphometric measurements were taken again at the last weight assessment for possible variations within this period due to dehydration after emergence.

Queens were stored in the bacteriological incubator 002CB or in the queen banking, 28 in the incubator and 40 in the queen banking. In the incubator, each queen was housed in a JZsBZs™ cage and received four nurse worker honeybees and queen candy. The queen banking consisted of a populous colony with a queen excluder screen in mini-hive system with young worker honeybees ensuring the care and maintenance of virgin queens. Weight and morphometric measurements of the queens stored in both systems were monitored once a day over an eight day-period, i.e. until the reproductive viability of the queen.

We used the Bayesian inference, implemented in R software Development Core Team (2011). Data were tested for the effect of life time of the queen on

the characteristics measured by a regression analysis. For this, we assumed a mixed model, considering the effects of animal and mini-hive as random effects, and the life time of the queen and storage as fixed effects.

Results and discussion

The length of the right forewing, length of the abdomen and height of the thorax were not statistically different between newly emerged Africanized honeybee queens kept in an incubator and queen banking, and their overall mean values were 9.60, 9.43 and 4.71 mm, respectively. The analysis of morphometric characteristics, especially of Africanized honeybees is little studied preventing the comparison of results. Many data are discussed on the basis of the existing literature on European honeybees.

There was no significant effect for the wing length, but the grafting as a whole, from the transport of combs containing the larvae to be grafted to the ideal conditions of the incubator, where cell cups are put for the emergence had influence on this characteristic. It is recommended a monitoring so that this effect is always minimized, or to establish a more appropriate technique and time of year for rearing queens (Faquinello et al., 2011). The average length of the forewing was greater than that registered by Tarpy et al. (2000) of 9.33 mm. Figure 1 illustrates the forewing length of Africanized honeybee queen at emergence in relation to the life time for the two types of storage.

Over time after emergence, the forewing length increased linearly until reproductive viability. The study of wing size in virgin queens is of great importance because the process of natural fertilization in conventional colonies or nuclei occurs during flight and depends on this for the success of fertilization (Moritz & Southwick, 1992).

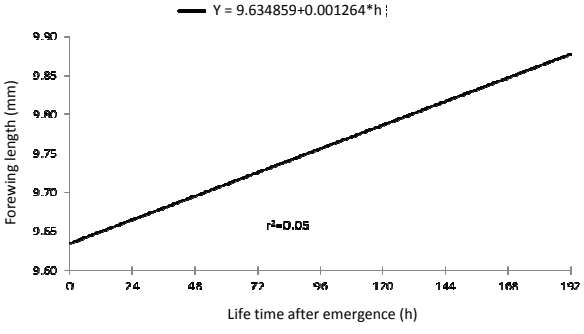

Figure 1. Forewing length (mm) of Africanized honeybee queens in relation to life time (h) after emergence, stored in queen banking or incubator.

As to the abdomen width, there was no effect, but Delaney et al. (2011), working with insemination, found a positive correlation for the abdomen width. Bogomolov et al. (2013) stated that queens with larger abdomen have greater success at the instrumental insemination.

Queens instrumentally or naturally inseminated showed a complete filling of the spermatheca, leading to a positive correlation between the queen weight, the brood area of the colony and the number of sperm in the spermatheca (Akyol et al., 2008). While the brood area raises, the number of adult individuals rises too, this leads to a higher effective production of the colony, aiming an increase in the effectiveness of all honeybee products and by-products.

In Figure 2 is shown the abdomen length of Africanized honeybee queens at emergence in relation to life time for the two types of storage. There was a reduction in the abdomen length during the first hours after the emergence indicating that the queen must be weighed immediately after emergence. Approximately 96 hours after emergence, the length of the abdomen increased due to the large weight loss in the early hours and, soon after, the weight increased again.

Approximately 72 hours following emergence, queens gained weight due to the care of workers in the two types of storage and subsequently they lost weight again due to the advanced age of up to eight days. Evidently, the search for heavier (longer) queens must also consider that there is a limit, since Hayworth et al. (2009) reported that the heavier queens tend to decrease the number of flights, the average duration of flights and hence the total time spent in flight.

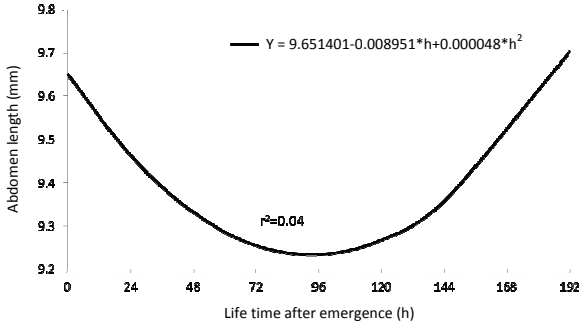

Figure 2. Abdomen length (mm) of Africanized honeybee queens in relation to life time (h) after emergence, stored in queen banking or incubator.

Figure 3 shows the thorax height of the Africanized honeybee queen at emergence in relation to life time for the two types of storage, demonstrating the lack of significant effect. The thorax height decreased to approximately 130 hours after emergence, due to the

proximity of reproductive viability, once after the mating flight queens remain in the hive. However, during swarming periods queens are essential because they fly with the workers in search of a new place to nest. Delaney et al. (2011) reported that queens with larger thorax are prone to mate with a higher number of drones and also found a positive correlation between the thorax width with mating frequency and number of sperm stored.

Africanized honeybee queen weight at emergence in relation to life time for the two types of storage is presented in Figure 4. The weight of the queens housed in queen banking was higher ($p < 0.05$) than those stored in incubator; the mean values were 165.61 and 157.04 mg respectively. The live weight of virgin queens can be influenced by the time of the grafting, the type of the grafting and the mini-hive used for rearing larvae, indicating that there is potential for development (Padilha et al., 2013). The type of storage for the honeybee queens after emergence is part of the care for the efficient reproduction, since there is a waiting time required for their introduction in their respective colony.

Figure 3. Thorax height (mm) of Africanized honeybee queens in relation to life time (h) after emergence, stored in queen banking or incubator.

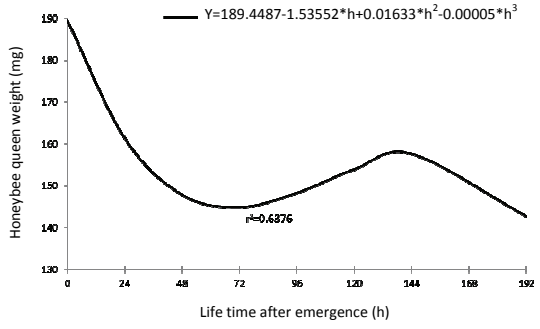

Figure 4. Weight (mg) of Africanized honeybee queens in relation to life time (h) after emergence, stored in queen banking or incubator.

Gilley et al. (2003) and Kahya et al. (2008) verified an association between reproductive quality of the queen with weight at emergence and Akyol et al. (2008) concluded that heavier queens had higher mating rates and started laying eggs earlier than lighter queens. As a result, queen weight is studied as a selection criterion in honeybee breeding programs (Souza et al., 2013) due to high heritability. Rangel et al. (2013) observed that a honeybee colony can be evaluated as the extended phenotype of its queen, and thus the selection working mostly at the colony level may be equivalent at the individual level. Tarpy et al. (2012) did not notice a correlation between queen body size and mating success.

Regarding weight, the average weight of honeybee queens at emergence was lower than that obtained by Tarpy et al. (2000) in European honeybee queens, in which the body weight was 188.00 mg. However, with Africanized honeybee queens, Souza et al. (2013) found values of 180 to 200 mg, while Tarpy et al. (2011; 2012) reported values of 206.6 and 218.7 mg for European honeybee queens. Even with lower weight of European honeybee queens, they showed a more hygienic behavior, once their infestation index by *Varroa destructor* is lower compared with the European queen (Guerra Júnior et al., 2010).

Furthermore, it is noteworthy that the highest weight loss occurred in the first hours after the emergence indicating that the queen must be weighed immediately after emergence to obtain accurate data. Nevertheless et al. (2004) reported that the body weight of queens should be determined two days after emergence, because previous measurements can cause an error due to weight variation. The variation in the queen weight in relation to the life time was efficiently explained by the fitted model ($r^2 = 0.64$).

Africanized honeybee colonies are characterized by producing cell cups all year round, regardless of the age of the queen, both in times of food scarcity and abundance, with a weekly average of four cell cups (Toledo et al., 2012). This can be explained by the low number of drones to fertilize this queen. This fact is very important because if the beekeeper allow their colonies to replace their queens alone, they usually produce new queens from older larvae, resulting in the production of low-quality queens (Tarpy et al., 2011), which are produced with a higher frequency during the year, and cause the production of these colonies to remain low.

Wang et al. (2014) concluded that nurse honeybees controlled the larval growth and the number of ovarioles, by temporarily manipulating

the delivery of food and that the response of larvae to food differs according to the larval stage and genotype. The body mass of the larvae was more sensitive to nutrition during the first (L1) and fourth instar (L4), whereas the number of ovarioles was more sensitive to the food in the fifth instar (L5). Therefore, during all larval stages, the conditions of temperature and humidity must be carefully controlled to obtain higher quality queens.

Besides that, Andi & Ahmadi (2014) recommended supplemental vitamin C at 2,000 mg L^{-1} of syrup in the spring food (1:1, sugar: water) to the colonies, because it increases the brood area, the population of the colony and can help to increase body protein content and the body weight. The intention pointed out here is that not only genetic origin but also management techniques and rearing conditions of the rearing nurse honeybees and/or future queens are critical when seeking higher quality, because these factors are closely related (Oldroyd et al., 2014).

Conclusion

The storage of honeybee queens, either in an incubator or in queen banking, has no influence on the forewing length, abdomen length and thorax height. However, Africanized honeybee queens kept in queen banking emerged with greater weight than those stored in an incubator. Thereby, there is potential selection of Africanized honeybee queens based on their weight, since it is very important to weigh queen soon after its emergence to be efficiently used as a tool in honeybee breeding programs.

Acknowledgements

To CNPq process number 479329/2009-5 and 308283/2011-2 and Araucaria Foundation protocol number 15095 – 422/2009.

References

Akyol, E., Ahinler, N. & Ozkok, D. (2006). Honeybee (*Apis mellifera*) races, ecotypes and their general charecterisctisc in Turkey. *Journal of Animal and Veterinary Advances*, 5(9), 771-774.

Akyol, E., Yeninar, H. & Kaftanoglu, O. (2008). Live weight of queen honey bees (*Apis mellifera* L.) predicts reproductive characteristics. *Journal of the Kansas Entomological Society*, 81(2), 92-100.

Andi, M. A. & Ahmadi, A. (2014). Influence of vitamin C in sugar syrup on brood area, colony population, body weight and protein in honey bees. *International Journal of Biosciences*, 4(6), 32-36.

Bogomolov, K. V., Bordachev, A. V. & Grabski, J. (2013). *Instrumental insemination of queen bees by using modern technical devices* (Vol. 1). Ryazan: Publishing house Ryazan.

Cobey, S. W. (2007). Comparison studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. *Apidologie*, 38(4), 390-410.

Costa-Maia, F. M., Toledo, V. d. A. A. d., Martins, E. N., Lino-Lourenço, D. A., Sereia, M. J., Oliveira, C. A. L. d., Halak, A. L. (2011). Estimates of covariance components for hygienic behavior in Africanized honeybees (*Apis mellifera*). *Revista Brasileira de Zootecnia*, 40(9), 1909-1916.

Delaney, D. A., Keller, J. J., Caren, J. R. & Tarpy, D. R. (2011). The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera L.*). *Apidologie*, 42(1), 1-13.

Doolittle, G. M. (1889). *Scientific queen-rearing as practically applied* (Vol. 1).

Faquinello, P., Toledo, V. A. A., Martins, E. N., Oliveira, C. A. L., Sereia, M. J., Costa-Maia, F. M. & Ruvolo-Takasusuki, M. (2011). Parameters for royal jelly production in Africanized honeybees. *Sociobiology*, 57(3), 495-509.

Gilley, D. C., Tarpy, D. R. & Land, B. B. (2003). Effect of queen quality on interactions between workers and dueling queens in honeybee (*Apis mellifera* L.) colonies. *Behavioral Ecology and Sociobiology*, 55(2), 190-196.

Guerra Júnior, J. C. V., Issa, M. R. C., Carneiro, F. E., Strapazzon, R. & Moretto, G. (2010). RAPD identification of Varroa destructor genotypes in Brazil and other regions of the Americas. *Genetics and Molecular Research*, 9(1), 303-308.

Harano, K.-i., Sasaki, M. & Sasaki, K. (2007). Effects of reproductive state on rhythmicity, locomotor activity and body weight in the European honeybee, Apis mellifera queens (*Hymenoptera, Apini*). *Sociobiology*, 50(1), 189-200.

Hayworth, M. K., Johnson, N. G., Wilhelm, M. E., Gove, R. P., Metheny, J. D. & Rueppell, O. (2009). Added weights lead to reduced flight behavior and mating success in polyandrous honey bee queens (*Apis mellifera*). *Ethology*, 115(7), 698-706.

Kahya, Y., Gençer, H. V. & Woyke, J. (2008). Weight at emergence of honey bee (*Apis mellifera caucasica*) queens and its effect on live weights at the pre and post mating periods. *Journal of Apicultural Research*, 47(2), 118.

Moritz, R. F. & Southwick, E. E. (1992). *Bees as superorganisms: an evolutionary reality* (Vol. 1). Berlin: Springer Verlag.

Mouro, G. F. & Toledo, V. d. A. A. (2004). Evaluation of *Apis mellifera* Carniolan and Africanized honey bees in royal jelly production. *Brazilian Archives of Biology and Technology*, 47(3), 469-476.

Oldroyd, B. P., Allsopp, M. H., Roth, K. M., Remnant, E. J., Drewell, R. A. & Beekman, M. (2014). A parent-of-

origin effect on honeybee worker ovary size. *Proceedings of the Royal Society B: Biological Sciences*, 281(1775), 2013-2388.

Padilha, A. H., Sattler, A., Cobuci, J. A. & McManus, C. M. (2013). Genetic parameters for five traits in Africanized honeybees using Bayesian inference. *Genetics and Molecular Biology*, 36(2), 207-213.

Rangel, J., Keller, J. J. & Tarpy, D. R. (2013). The effects of honey bee (*Apis mellifera* L.) queen reproductive potential on colony growth. *Insectes Sociaux*, 60(1), 65-73.

Skowronek, W., Bieñkowska, M. & Kruk, C. (2004). Changes in body weight of honeybee queens durins their maturation. *Journal of Apicultural Science*, 48(2), 61-68.

Souza, D., Bezzera-Laure, M., Francoy, T. & Gonçalves, L. (2013). Experimental evaluation of the reproductive quality of Africanized queen bees (*Apis mellifera*) on the basis of body weight at emergence. *Genetics and Molecular Research*, 12(4), 5382-5391.

Tarpy, D. R., Hatch, S. & Fletcher, D. J. C. (2000). The influence of queen age and quality during queen replacement in honeybee colonies. *Animal Behaviour*, 59(1), 97-101.

Tarpy, D. R., Keller, J. J., Caren, J. R. & Delaney, D. (2011). Experimentally induced variation in the physical reproductive potential and mating success in honey bee queens. *Insectes Sociaux*, 58(4), 569-574.

Tarpy, D. R., Keller, J. J., Caren, J. R. & Delaney, D. A. (2012). Assessing the' mating health of commercial honey bee queens. *Journal of Economic Entomology*, 105(1), 20-25.

Toledo, V. d. A. A., Nogueira-Couto, R. H., Malheiros, E. B., Faquinello, P. & Sereia, M. J. (2012). Produção de realeiras em colônia híbridas de *Apis mellifera* L. e longevidade de rainhas. *Global Science and Technology*, 5(2), 176-185.

Wang, Y., Kaftanoglu, O., Fondrk, M. K. & Page Jr, R. E. (2014). Nurse bee behaviour manipulates worker honeybee (*Apis mellifera* L.) reproductive development. *Animal Behaviour*, 92, 253-261.

Received on May 30, 2014.
Accepted on October 22, 2014.