THE QUALITY OF HONEY BEE QUEENS FROM QUEEN CELLS INCUBATED AT DIFFERENT TEMPERATURES

Article · December 2015

CITATIONS
11

READS
8,218

2 authors:

Bożena Chuda-Mickiewicz
West Pomeranian University of Technology, Szczecin
22 PUBLICATIONS **222** CITATIONS
SEE PROFILE

Jerzy Samborski
West Pomeranian University of Technology, Szczecin
13 PUBLICATIONS **130** CITATIONS
SEE PROFILE

ACTA SCIENTIARUM POLONORUM
ISSN 1644-0714
ISSN 2300-6145 (online)
www.asp.zut.edu.pl
Acta Sci. Pol. Zootechnica 14(4) 2015, 25–32

THE QUALITY OF HONEY BEE QUEENS FROM QUEEN CELLS INCUBATED AT DIFFERENT TEMPERATURES

Bożena Chuda-Mickiewicz, Jerzy Samborski

West Pomeranian University of Technology, Szczecin, Poland

Abstract. The effect of capped queen cell incubation temperature on the quality of honey bee queens was examined. It was shown that the period of pre-imaginal development in the queen bees from queen cells incubated at 32°C was longer by 1 day and 3 hours when compared to those being incubated at 34.5°C, for which this period amounted to 16 days and 1 hour. On the other hand, the quality of queens from cells incubated at 32°C and 34.5°C was similar, they did not differ in body weight, spermathecal volume, ovariole number in both ovaries, or onset of oviposition.

Key words: *Apis mellifera*, queen, rearing, quality

INTRODUCTION

The strength of honey bee colony and its productivity are determined to a large extent by the quality of queen, being the resultant of her physical features i.e. number of ovarioles in ovaries and size of spermathecal [Woyke 1971, Woyke et al. 1974, Tarpy et al. 2000, Hatjina et al. 2014]. The quality of queens being reared in honey bee colonies depends on many factors: age of brood being used for their rearing, time of season, biological status and strength of colony, and number of queens being reared [Tarpy et al. 2000, Koc and Karacaoğlu 2004, Skowronek et al. 2004, Mohammad et al. 2011]. According to Eskov [1990], good-quality queens develop from queen cells being incubated at 33–35°C; when their development takes place at a temperature below 33°C or above 36°C, they have ovaries

Corresponding author: Bożena Chuda-Mickiewicz, Department of Zoology and Apiculture, West Pomeranian University of Technology, Szczecin, Doktora Judyma 20, 71-466 Szczecin, Poland, e-mail: bozena.chuda-mickiewicz@zut.edu.pl

with a smaller number of ovarioles. In the hive, worker bees maintain a diverse temperature, the highest is 32–36°C in the broodnest centre within the brood being reared, whereas a lower one on its periphery [Seeley and Heinrich 1981, Chuda-Mickiewicz 1994, Kleinhenz et al. 2003, Jones et al. 2004]. Because bees build queen cells both on the comb periphery and in its central part, the queens developing in them are not reared under identical thermal conditions [Degrandi-Hoffman et al. 1993].

The aim of our study was to evaluate and compare the length of preimaginal development period, robustness and onset of oviposition queens developing during the prepupal stage and the pupal stage at different temperature.

MATERIAL AND METHODS

The study was conducted in summer (June–July) 2012 and 2014 at the Department of Zoology and Apiculture, West Pomeranian University of Technology, Szczecin. The study material consisted of sister queens of *Apis mellifera carnica* being reared in a queenless colony from one-day larvae [Ruttner and Ruttner 1983, Büchler et al. 2013] from one rearing series. On day nine of queen development, capped queen cells were separated into two groups and placed in an incubator with different temperature – group 1 at 32°C (M32), and group 2 at 34.5°C (M34.5). Starting from day 15 of queen development, queens emerging was controlled every six hours. Immediately after emerging, queens were weighed (on a RADWAG WXD 200/2000 laboratory balance for weighing the moving animals, with accuracy to 1 mg) and introduced into plastic mailing cages provided with candy with 8–10 bees. Then, they were placed in an incubator at 24°C. In the first year of the study (2012), sexually mature queens were killed at the age of 5–6 days [Koeniger 1986] and the reproductive system was removed [Dade 2009]. The diameter of spermatheca and the number of ovarioles in both ovaries were determined by a modified method of Woyke [1971]. Spermathecae were transferred onto a microscope glass slide. After removing the tracheae, the photographs of spermathecae were taken using a Zeiss Primo Star microscope with a digital camera (at 20× magnification) and their diameter was measured by means of ScopePhoto software to calculate the spermathecal volume. Ovaries were preserved in a 4% formaldehyde solution for making histological slides of their cross-sections. The ovariole number was determined on the photographs of histological slides of ovary cross-sections taken under a Zeiss Primo Star microscope with ScopePhoto software. In the second year of the study (2014), one day after emergence, queens were introduced in cages into mini-plus mating nuclei, being settled with approximately 2000 bees. The nuclei entrances were secured with a queen excluder. At the age of seven days, queens – after weighing (on a

RADWAG WXD 200/2000 laboratory balance, with accuracy to 1 mg) – were instrumentally inseminated once with 8 µl of semen [Cobey et al. 2013, Woyke 1960] collected from drones aged 14–16 days of the same subspecies [Woyke and Jasiński 1978]. On the second day after instrumental insemination, queens were anaesthetised with CO2 for 3 minutes [Woyke et al. 2001]. The onset of oviposition by queens was determined by examining the mating nuclei every second day. In total, 30 queens were evaluated in 2012, and 36 ones in 2014, i.e. 16 queens in 2012 and 16 ones in 2014 in group M32 and 14 in 2012 and 20 in 2014 in group M34.5, respectively.

The figures obtained in the experiment were analysed statistically using Statistica PL v.9 computer software. The percentages of queens emerged from queen cells and those undertaking oviposition were analysed between both groups by the test for differences between two structure indicators. Differences between the body weight of queens, volume of their spermathecae, and the number of ovarioles were evaluated with the Student's t-test. The coefficients of correlation between the physical features of queens being examined were calculated. Differences in the waiting time for starting oviposition by queen bees was analysed with a χ^2 test.

RESULTS

Different temperature of capped queen cell incubation (32 and 34.5°C) had no effect on queen emerging. In total, the percentage of emerged queens for the first and the second year of the study was 88.9% in group M32 (32 queens emerged from 36 queen cells) and 89.2% in group M34.5 (34 queens emerged from 37 queen cells). The difference observed in queens emerging between groups was not significant (P = 0.969). Temperature during the incubation of capped queen cells had, however, an effect on the length of preimaginal development period in queens. In group M32, this period was significantly longer, on average by 27 hours (1 day and 3 hours) than in group M34.5, where it amounted on average (±SD) to 367 ±8 hours (16 days and 1 hour) (P = 0.000). On the other hand, the body weight of queens immediately after emerging was similar in both groups and did not differ significantly (P = 0.909) (Table 1). When evaluating the reproductive system of queens (in the first year of the study), no significant differences were found in the spermathecal volume (P = 0.358) or the ovariole number in both ovaries (P = 0.143) (Table 1). No significant correlation was observed between the body weight of queens and the spermathecal volume (r = 0.06, P = 0.731) and the ovariole number (r = 0.25, P = 0.328), or between the spermathecal volume and the ovariole number (r = 0.12, P = 0.640), either.

Table 1. Means (±SD) for the values of honey bee queen features being analysed
Tabela 1. Średnie (±SD) wartości badanych cech matek

Feature analysed – Badana cecha	Group – Grupa	
	M32	M34.5
Body weight immediately after emerging, mg Masa ciała bezpośrednio po wygryzieniu, mg	214.9 ±39 a	214.1 ±17 a
Spermathecal volume, µL Objętość zbiorniczka nasiennego, µL	0.82 ±0.06 a	0.84 ±0.07 a
Ovariole number in both ovaries Liczba rurek jajnikowych w obu jajnikach	349 ±21 a	333 ±23 a
Body weight prior to instrumental insemination, mg Masa ciała przed unasienieniem, mg	185.1 ±11 b	176.3 ±15 a

Values in rows with different letters differ significantly at $P \leq 0.05$.
Wartości w wierszach oznaczone różnymi literami różnią się istotnie przy $P \leq 0{,}05$.

The mean body weight (±SD) of queens just before instrumental insemination (the second year of the study) in group M32 was by 8.89 mg greater than in group M34.5 (Table 1) and this difference was significant ($P = 0.050$). Out of all instrumentally inseminated queens, 87.5% ones in group M32 and 95% ones in group M34.5 started oviposition. The difference observed in the percentage of egg laying queens between groups was not significant ($P = 0.418$). The waiting time for starting oviposition by queens was similar in both groups and amounted on average (±SD) to 10.4 ±1.82 days in group M32 and 10.3 ±3.00 days in group M34.5, not differing significantly ($P = 0.797$). On the other hand, the modal value for the number of days from instrumental insemination to the onset of oviposition in group M32 was by 5 days smaller than in group M34.5, where it amounted to 14 days.

DISCUSSION

Our study showed that the temperature of queen cell incubation had no effect on the rearing efficiency; the percentage of emerged queens was similar in both groups. On the other hand, it had an effect on the length of preimaginal development period; it was longer, on average by 27 hours, at a temperature lower by 2.5°C (group M32). Eeskov [1990] and Degrandi-Hoffman et al. [1993] also observed a prolonged preimaginal development period by 12 to 35 hours when lowering the temperature of queen cells incubation from 34°C to 32.8 and 31°C. The body weight of queens immediately after emerging in both groups (M32 and M34.5) corresponded to average body weight of Carniolan queens being given in literature – 199 to 226 mg [Skowronek et al. 2004, Bieńkowska et al. 2009, Hatjina et al. 2014]. The spermathecal volume in the queens of group M32 and group

M34.5 was comparable with the results reported in the study by Bieńkowska et al. [2009]. Greater spermathecal volume (0.89 and 0.94 μL) in Carniolan queens was reported by Woyke et al. [1974] and Hatjina et al. [2014]. The average ovariole number being found in this study in the queens of both groups was larger by 13 to 166 than that observed in the study by Woyke et al. [1974] and Hatjina et al. [2014]. No correlation was found between the body weight of queens and the spermathecal volume and the ovariole number. Our findings are consistent with those of Corbella and Gencalves [1982], who did not found any correlation between the body weight on the day of emergence and the spermatheca size either. Similarly, Corbella and Gencalves [1982], Hatch et al. [1999] and Jockson et at. [2011] did not observe any correlation between the body weight of queens and the ovariole number. Szabo et al. [1987], Kahya et al. [2008] and Bieńkowska et al. [2009] obtained the results conflicting with our findings and those of the authors cited above. Woyke [1971] also showed a positive correlation between the spermatheca size and the ovariole number, which does not match our findings. The lack of correlation between the physical features of queens in this study was probably affected by equal age of larvae being used for rearing the queens. Woyke [1971] found this correlation comparing jointly the queens being reared from eggs, one-day, two-day and three-day larvae. A significant difference in the body weight of queens between groups on the day of queen instrumental insemination showed in our study had no effect on the onset of oviposition by them. The percentage of queens laying eggs in both groups exceeded by 3 to 10% that being obtained by Mackensen [1947], Kaftanoglu and Peng [1982], Moritz and Kühnert [1984], Kühnert et al. [1989], Chuda-Mickiewicz and Prabucki [1993] and Czekońska and Chuda-Mickiewicz [2007]. On the other hand, the average waiting time for starting oviposition was longer by 1.5 to 2.7 days when compared with the results obtained by the researchers anaesthetising queens with CO_2 for 3 minutes two days before or after instrumental insemination [Kaftanoglu and Peng 1982, Moritz and Kühnert 1984, Woyke et at. 2001, 2008, Skowronek et al. 2002, Czekońska and Chuda-Mickiewicz 2007, Chuda-Mickiewicz et al. 2012].

CONCLUSION

The temperature difference of 2.5°C during the period of capped queen cells incubation had an effect on the length of queen bee preimaginal development period but did not affect the robustness of queens or the waiting time for starting oviposition by them.

REFERENCES

Bieńkowska, M., Panasiuk, B., Gerula, D., Węgrzynowicz, P. (2009). Weight of honeybee queens and its effect on the quality of instrumentally inseminated queens. Proceedings of the 4th International Apicultural Congress, Montpellier, France, 15–20 September 2009, p. 135.

Büchler, R., Andonov, S., Bienefeld, K., Costa, C., Hatjina, F., Kezic, N., Kryger, P., Spivak, M., Uzunov, A., Wilde, J. (2013). Standard methods for rearing and selection *Apis mellifera* queens. J. Apic. Res., 52(1),1–9. DOI 10.3896/IBRA.1.52.1.07.

Corbella, E., Gencalves, L.S. (1982). Relationship between weight at emergence, number of ovarioles and spermathecal volume of Africanized honeybee queens *Apis mellifera*. Rev. Brasil. Genet., 5(4), 835–840.

Cobey, S.W., Tarpy, D.R., Woyke, J. (2013). Standard methods for instrumental insemination of *Apis mellifera* queens. J. Apic. Res., 52(4). DOI 103896/IBRA.1.524.09.

Chuda-Mickiewicz, B. (1994). Wpływ temperatury otoczenia na zachowanie kłębu zimującej rodziny [Ambient temperature effect on wintering bee colony cluster behaviour]. Rozprawy nr 162, AR Szczecin [in Polish].

Chuda-Mickiewicz, B., Prabucki, J. (1993). Podejmowanie czerwienia przez inseminowane matki pszczele przetrzymywane w skrzynkach w asyście swobodnie oblatujących się pszczół [Undertaking the oviposition by honey bee queens kept in cages with non-free flaying bees]. Pszczel. Zesz. Nauk., 37, 23–31 [in Polish].

Chuda-Mickiewicz, B., Czekońska, K., Samborski, J., Rostecki, P. (2012). Success rates for instrumental insemination of carbon dioxide and nitrogen anaesthetised honey bee (*Apis mellifera*) queens. J. Apic. Res., 51(1),74–77.

Czekońska, K., Chuda-Mickiewicz, B. (2007). Effect of induced defecation on the effectiveness of insemination in honey bee (*Apis mellifera*) queens. J. Apic. Res., 46(4), 242–244.

Dade, H.A. (2009). Dissection of the queen, In: Anatomy and dissection of the honeybee. Int. Bee Res. Assoc., 137–140.

Degrandi-Hoffman, G., Spivak, M., Martin, J.H. (1993). Role of thermoregulation by nestmates on the development time of honey bee (Hymenoptera: Apidae) queens. Ann. Entomol. Soc. Amer., 86(2), 165–172.

Eskov, E.K. (1990). Termofaktor. V: Ekologija medonosnoj pceli [The thermal factor. In: Ecology of honey-bee], Moscow, Rasogropomizdat, 39–96.[in Russian]

Hatjina, F., Bieńkowska, B., Charistos, L., Chlebo, R., Costa, C., Dražić, M.M, Filipi, J., Gregorc, A., Ivanova, E.N., Kezić, N., Kopernicky, J., Kryger, P., Lodesani, M., Lokar, V., Mladenovic, M., Panasiuk, B., Petrov, P.P, Rašić, S., Smodis Skerl, M.J., Vejsnæs, F., Wilde, J. (2014). 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., 53(2), 337–367. DOI10.3896/IBRA.1.53.02.

Hatch, S., Tarpy, D.R., Fletcher, D.J. (1999). Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality. Insect. Soc., 46(4), 372–377.

Jockson, J.T., Tarpy, D.R., Fahrbach, S.E. (2011). Histological estimations of ovariole number in honey bee queens, *Apis mellifera*, reveal lack of correlation with other queen quality measures. J. Insect Sci., 11, 82. DOI 10.1673/031.011.8201.

Jones, J.C., Myerscough, M.R., Graham, S., Oldroyd, B.P. (2004). Honey bee nest thermoregulation: diversity promotes stability. Science, 305, 402–404.

Kahya, Y., Gencer, H.V., Woyke, J. (2008). Weight at emergence of honey bee (*Apis mellifera caucasica*) queens and its effect on live weights at pre and post mating periods. J. Apic. Res., 47(2),118–125.

Kaftanoglu, O., Peng, Y.S. (1982). Effect of insemination on the initiation of oviposition in the queen honey bee. J. Apic. Res., 21, 3–6.

Kleinhenz, M., Bujok, B., Fuchs, S., Tautz, J. (2003). Hot bees in empty broodnest cell: heating from within. J. Exper. Biol., 206, 4217–4231.

Koc, A.U., Karacaoğlu, M. (2004). Effects of rearing season on the quality of queen honeybee (*Apis mellifera*) raised under the conditions of Aegean region. Mellifera, 4(7), 34–37.

Koeniger, G. (1986). Reproduction and mating behaviour. In Rniderer T.E. (ed). Bee genetics and breeding. Academic Press Inc., 255–280.

Kühnert, M., Carrik, M.J., Allan, L.F. (1989). Use of homogenized drone semen in a breeding program in Western Australia. Apidologie, 20, 371–381.

Mackensen, O. (1947). Effect of carbon dioxide on initial oviposition of artificial inseminated and virgin queen bees. J. Econ. Entomol., 40, 344–349.

Mohammad, A., All-Fattah, A., Mazeed, A.M., Al-Hady, N.A. (2011). Quality and quantity of honeybee queens as affected by the number and distribution of queen cells within queen rearing colonies. J. Apic. Sci., 55(2), 31–43.

Moritz, R.F.A., Kühnert, M. (1984). Seasonal effects on artificial insemination of honeybee queens (*Apis mellifera* L.). Apidologie, 15(2), 223–231.

Ruttner, H., Ruttner, F. (1983). Reliable rearing methods. In: Queen rearing – biological basis and technical instruction. Apimondia Publishing House, Bucharest, 179–277.

Seeley, T.D., Heinrich, B. (1981). Regulation of temperature in nests of social bee. In: Henrich B. (ed) Insect thermoregulation. Wiley, New York, 159–234.

Skowronek, W., Kruk, C., Kłopot, J. (2002). Factors affecting oviposition of artificially inseminated honeybee queens. J. Apic. Res., 46(2), 85–95.

Skowronek, W., Bieńkowska, M., Kruk, C. (2004). Changes in body weight of honeybee queens during their maturation. J. Apic. Sci., 48(2), 61–68.

Szabo, T.I., Mills, P.F., Heikel, D.T. (1987). Effects of honeybee queen weight and air temperature on the initiation of oviposition. J. Apic. Res., 26(2), 73–78.

Tarpy, D.R., Hatch, S., Fletcher, D.J.C. (2000). The influence of queen age and quality during queen replacement in honeybee colonies. Anim. Behav., 59, 97–101.

Woyke, J. (1960). Naturalne i sztuczne unasienianie matek pszczelich [Natural and instrumental insemination of queen bees]. Pszczel. Zesz. Nauk., 4(3–4), 183–275 [in Polish].

Woyke, J. (1971). Correlation between the age at which honeybee brood was grafted, characteristics of the resultant queens, and result of insemination. J. Apic. Res., 10(1), 45–55.

Woyke, J., Jasiński, Z., Smagowska, B. (1974). Porównanie organów rozrodczych oraz efektów sztucznego unasieniania i naturalnego unasieniania pszczół różnych ras i ich mieszańców [Comparison of reproductive organs and effects of artificial and natural insemination in honey bees of different races and hybrids]. Pszczel. Zesz. Nauk., 18, 53–74 [in Polish].

Woyke, J., Jasiński, Z. (1978). Influence of age of drones on the results of instrumental insemination of honeybee queens. Apidologie, 9(3), 203–212.
Woyke, J., Fliszkiewicz, Z., Jasiński, Z. (2001). Prevention of natural mating of instrumentally inseminated queen honeybees by proper method of instrumental insemination. J. Apic. Sci., 45, 101–114.
Woyke, J., Jasiński, Z., Prabucki, J., Wilde, J., Chuda-Mickiewicz, B., Siuda, M., Madras-Majewska, B., Bratkowski, J., Jojczyk, A. (2008). Onset of oviposition by honey bee queens, mated either naturally or by various instrumental insemination methods, fits a lognormal distribution. J. Apic. Res., 47(1), 1–9.

JAKOŚĆ MATEK PSZCZELICH Z MATECZNIKÓW INKUBOWANYCH W ZRÓŻNICOWANEJ TEMPERATURZE

Streszczenie. Badano wpływ temperatury inkubacji zasklepionych mateczników na jakość matek pszczoły miodnej. Wykazano, że długość okresu rozwoju preimaginalnego matek z mateczników inkubowanych w temperaturze 32°C była dłuższa o 1 dzień i 3 godziny w porównaniu do inkubowanych w temperaturze 34,5°C, których wynosiła 16 dni i 1 godzinę. Jakość zaś matek z mateczników inkubowanych w 32 i 3,5°C była podobna, nie różniły się masą ciała, objętością zbiorniczka nasiennego, liczbą rurek jajnikowych w obu jajnikach i rozpoczynaniem czerwienia.

Słowa kluczowe: *Apis mellifera*, matka pszczela, wychów, jakość

Accepted for print: 10.10.2015

For citation: Chuda-Mickiewicz, B., Samborski, J. (2015). The quality of honey bee queens from queen cells incubated at different temperatures. Acta Sci. Pol. Zootechnica, 14(4), 25–32.