Original Article

DRONE AND WORKER BROOD WAS UNEXPECTEDLY WELL HEATED BOTH IN STANDARD-CELL AND SMALL-CELL COMB COLONIES

Krzysztof Olszewski[1]*
Grzegorz Borsuk[1]*
Piotr Dziechciarz[1]
Łukasz Wójcik[1]
Jerzy Paleolog[2]*

[1] Institute of Biological Basis of Animal Production, Faculty of Biology, Animal Sciences and Bioeconomy, University of Life Sciences, Akademicka 13, 20-950, Lublin, Poland
[2] Department of Department of Zoology and Animal Ecology, Faculty of Biology, Animal Sciences and Bioeconomy, University of Life Sciences, Akademicka 13, 20-950, Lublin, Poland

*corresponding authors: krzysztof.olszewski@up.lublin.pl, grzegorz.borsuk@up.lublin.pl, jerzy.paleolog@up.lublin.pl
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Abstract

Temperatures of worker- and drone-brood rearing in various hive locations were compared in both colonies kept on small-cell combs (4.90 mm) (SMC) and standard-cell combs (5.50 mm) (STC) in two seasons. Temperatures close to the worker-brood comb placed near the rightmost storage-comb were lower than those near the worker brood in the nest centre but equal to those near the outskirt drone-brood comb (34.37-35.24°C) regardless of the month and the comb-cell size. Temperatures of the brood rearing in the SMC did not differ from those in the STC, independently on the location (center-periphery) and the brood type (drone-worker). Occasionally, they were even higher in the STC near the peripheral drone-brood comb and in the nest centre. We concluded that the drones which are involved in colony reproduction could affect its thermoregulation. The peripheral drone brood can be heated just as well as the worker brood, if the colony is strong enough and has the proper drone-worker ratio. Therefore, it is doubtful whether a higher temperature near the worker brood in the SMC limit the development of the *V. destructor* population. A lower temperature may not be a factor in encouraging *V. destructor* females to prefer trap-drone-combs for reproduction in the SMC. Strong field colonies may be especially prone to such behaviour. Therefore, temperature cannot be considered a mechanism of effective *Varroa* control in SMC.

Keywords: comb cell size, drone-brood, honeybee, nest, rearing temperature, *Varroa destructor*

INTRODUCTION

The ability to maintain a constant temperature in the nest allows a honeybee colony to be independent from changing external environmental conditions. The inside temperature ranges from 33°C to 36°C in nest centres close to the worker brood (Kleinhenz et al., 2003) during the brood rearing period, and drone brood rearing occurs at a lower temperature ranged from 30°C to 34°C (Le Conte & Arnold, 1988). On the other hand, the temperature of 32.6°C is believed to be optimal for the reproduction of *Varroa destructor* females and therefore, according to a popular opinion, *Varroa* females prefer the drone brood for rearing (Le Conte & Arnold, 1988; Le Conte, Arnold, & Desenfant, 1990). In this context, lower temperatures prevailed on the periphery of the colony nest (Winston, 1987), where beekeepers place trap-drone-combs to better attract *V. destructor* females and consequently remove them together with the capped drone-brood.
Drones are mainly present during the period of colony reproduction, while workers are involved in colony reproduction and survival

and also constitute the colony "body", and so are always present in a beehive. Li et al. (2016) assumed that drone-brood rearing conditions are less regulated than in the case of the worker brood, and thus the heating is assumed to be worse. This assumption contradicts findings by Koeniger (1978) who had revealed that the drone brood attracted much more workers than the worker brood. Thus workers may take greater care of the drone brood and consequently heat it better. The first purpose of this study was to clarify this contradiction. We expect that our findings would be useful to better develop natural methods for *Varroa* mite control.

Problems with fighting *V. destructor* with chemicals and care for the quality of honeybee by-products encourage bee-scientists to develop non-chemical methods for parasite control. Message & Goncalves (1995) and Piccirillo & De Jong (2003) suggested that such a non-chemical method was keeping colonies on the small-cell combs (cell size: 4.9 mm) because there was not enough room for the optimal reproduction of female *Varroa* (Martin & Kryger, 2002). Kober (2003) suggested that a higher temperature for brood rearing in small-cell combs compared to that in standard-cell combs (cell size 5.4 mm) might handicap mite reproduction within small cells. This higher temperature would result in a shorter duration of worker sealed-brood development, which makes it more difficult for *Varroa* females to complete a full reproductive cycle (Siuda & Wilde, 1996; Rosenkranz, 1999; Bąk, Siuda, & Wilde, 2012). Consequently, a higher temperature for worker-brood rearing in small-cell colonies has been hypothesized to limit the development of the *V. destructor* population. In such colonies, *Varroa* mites' preference for the drone-brood might be increased as well because of the greater difference between the temperatures of the worker- and drone brood combs. An important hypothesis resulting from these considerations is that a higher temperature for worker-brood rearing in colonies kept on small-cell combs encourages *V. destructor* females to prefer the drone-brood for reproduction in the small-cell colonies which makes the drone-brood combs more efficient as mite-traps (the greater temperature differences between these two brood types). However, the above hypotheses are true only when the brood rearing temperatures in the small-cell and the standard-cell combs differ significantly and when temperatures near the peripheral drone-brood comb and the nest worker-brood combs greatly differ in the colonies kept on the small-cell combs from those in the standard-comb ones. The second purpose of this study was to clarify these issues, which also would be helpful to better develop natural methods for *Varroa* mites control.

In previous studies, rearing temperatures of worker broods and the drone broods were compared in different parts of the nest, but these two types were placed either in the same comb or in the adjacent combs (Levin & Collison, 1990; Li et al., 2016). However, such an approach could cause temperatures at adjacent locations to influence each other and their potential difference could thus be adulterated. Therefore, in this experiment, the temperature for rearing a drone brood located on the periphery of the nest was compared with the temperatures for rearing a worker-brood located both in the centers and on the peripheries of the nest. So, the distances between these two brood types were marked. To our knowledge, the effect of comb-cell size on temperatures for rearing the worker-brood versus the drone-brood has not yet been investigated in such fully developed colonies under the field conditions.

Summarizing, the aims of this study were to compare the temperatures for rearing the worker- and drone-brood in colonies kept on both small-cell and standard-cell combs and to examine how these temperatures depend on the nest location of these two brood types. We also decided to face a hypothesis that the drone-brood rearing conditions are regulated less carefully than in the case of the worker brood placed on the edges of the nest in this context.

MATERIAL AND METHODS

Ten strong colonies of similar strength and structure headed by naturally mated Buckfast sister-queens were established for further studies which were performed in May and June in two subsequent apiculture seasons (2013-2014) in Lublin, Poland (51.224039N – 22.634649E). These queens were the certified Buckfasts by Landesverband Niedersächsischer Buckfastimker e.V. (Germany). According to our former observations, workers from such queens stored hardly any honey in their nests when their strong-colonies had enough space in the honey supers, which was important during the studies on the bee brood in the nests. Workers populated one brood chamber (10 frames; 435 x 300 mm) and one honey super (10 frames; 435 x 150 mm) at the beginning of May on the first day of the experiment, and one brood chamber and three honey supers in the last decade of May and in June. Such colonies can be considered strong. The honey supers were separated from the brood chambers by queen excluders.

Five colonies were kept on the standard-cell combs (STC) and the other five on the small-cell combs (SMC). The brood chamber was arranged in each colony in such a way that one drone-brood comb was located as the leftmost comb of the nest (facing the hive entrance), the next eight combs were the worker-brood combs fully populated by workers and the worker-brood at various development stages, and the last rightmost storage-comb contained honey and beebread (Fig. 1). The vast majority of drone brood was concentrated in the brood comb and only a few dozen drone cells were scattered on the outskirts of some worker combs. Several generations of workers had been reared in each of the worker-brood combs before, whereas each of the drone-brood combs had been built by the colonies without the use of wax foundation and the queens laid eggs therein immediately before the onset of the experiment. Drone combs were always fully occupied by the drone brood, except for June in the second season, when due to a lack of nectar and pollen only 30% of the drone comb surfaces were occupied by the scattered drone-brood.

Electronic temperature data loggers (Comet S0141, Czech Republic) were used (one logger in each colony). Each logger was equipped with four measurement probes ranging from -30°C to +80°C, accuracy ±0.2°C, which allowed the prevailing temperature to be measured in the different parts of the nest and outside the beehive. The first probe (Fig. 1), which touched the drone-brood comb, recorded the temperature in the bee space between the drone-brood comb and the adjacent worker-brood comb. The second probe measured the temperature in the centre of the nest between the worker brood combs, touching one of them. The third probe recorded the temperature on the right side of the nest in the bee space between the worker brood-comb and the adjacent rightmost storage-comb touching the worker-brood comb. The fourth probe measured the outside temperature on the ground level under the beehive. The temperatures in the nest were measured continuously from the beginning of May to the end of June every four hours during two con-

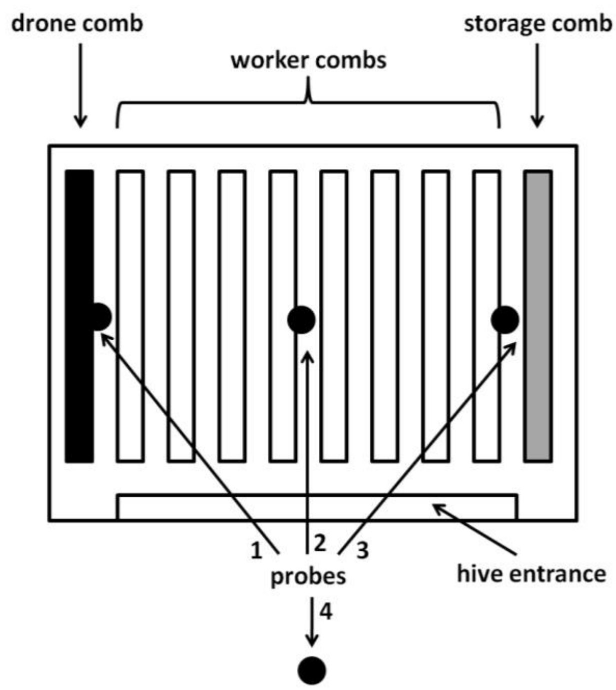

Fig. 1. Comb arrangement scheme in a colony brood-chamber and the distribution measurement probes in bee spaces between the combs.

secutive beekeeping seasons. Consequently, the data base obtained from each of the four probes was six measurements a day x 61 days x five colonies x two groups x two seasons = 7320 measurements and in total for four probes 29280 measurements.

Statistical analysis

Eight one-way ANOVA procedures accompanied by Tukey's post hoc test were performed (Statistica 13.3) to assess the significance of the differences between temperatures recorded in three parts of the bee nest (Fig. 1 - probe 1, 2, 3), within each group (STC and SMC) nested in month (May and June) and season (2013 and 2014). Then twelve one-way ANOVA procedures were performed to assess the significance of the differences between temperatures recorded in the STC and SMC groups, within each part of the nest separately (Fig. 1 - probe 1, 2, 3), nested within month (May and June), and seasons (2013 and 2014).

RESULTS

Regardless of the measurement term (month, season) and the comb-cell size (STC, SMC), the temperature for rearing of the worker brood on the combs adjacent to the rightmost storage comb (Fig. 2 and 3) were lower than those on the worker-brood combs located in the centre of the nest. The temperature for rearing the worker brood on the combs adjacent to the rightmost storage comb were the same, sometimes with the slight tendency to be lower, as the temperatures of the drone-brood rearing on the peripheral leftmost drone-brood combs. Temperature for rearing the worker-brood recorded in the nest centre did not differ from the temperatures for rearing the drone-brood on the

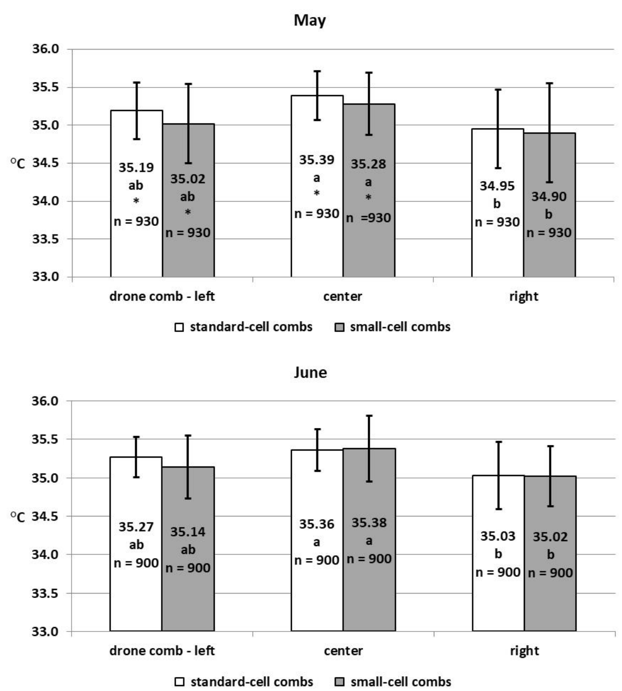

Fig. 2. Temperatures (means) in different parts of a bee nest in colonies kept on small-cell and standard-cell combs in the first season.
a, b - the differences between different parts of the nest within each group (small and standard-cell colonies) are significant at $p \leq 0.05$; * - the differences within different parts of the nest, between each group (small and standard-cell colonies) are significant at $p \leq 0.05$ test; - vertical lines attached to the bars illustrate the values of standard deviations.

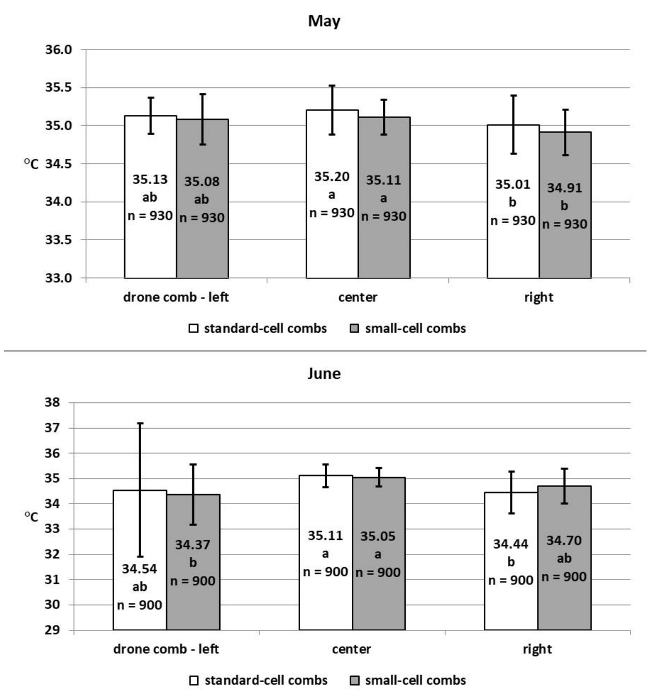

Fig. 3. Temperatures (means) in different parts of a bee nest in colonies kept on small-cell and standard-cell combs in the second season.
a, b - the differences between different parts of the nest within each group (small and standard-cell colonies) are significant at $p \leq 0.05$; - vertical lines attached to the bars illustrate the values of standard deviations.

Table 1.
Temperatures outside of the beehives in May and June in the first and second seasons

Seasons	May mean ± SD (n = 1860)	June mean ± SD (n = 1800)
first season	14.22 ± 4.54 [a]	17.33 ± 3.79 [b]
second season	15.01 ± 3.33 [a]	18.15 ± 2.59 [b]

SD – standard deviation;
a, b – differences between months were significant when compared within each season for $p \leq 0.05$. Differences between the seasons were not significant when compared within each month (May p = 0.802, June p = 0.824).

peripheral drone brood combs and usually exceeded 35°C. SMC was the exception in June of the second season in (Fig. 3).
Temperatures for the worker brood rearing recorded both in the nest centres and on the combs adjacent to storage rightmost combs did not differ in SMC and STC, with the exception of the nest centres in May of the first season, when unexpectedly higher temperatures were recorded in STC (Fig. 2 and 3). The temperatures for the drone brood rearing on the leftmost drone comb were the same in SMC and STS, again with the exception of May of the first season when the temperatures were higher in STC. So, the cell size did not affect rearing temperatures of the two assayed brood types in the majority of cases. Monthly temperatures outside beehives (Fig. 1, probe 4) did not differ between seasons while seasonally temperatures were lower in May than in June (Tab. 1).

DISCUSSION

We have revealed that workers heated the leftmost drone-brood comb at 35°C and higher as well, as the worker brood located in the nest center and tended to heat this drone-brood comb even better than the worker brood comb located on the nest outskirts but isolated from the external environment by the storage combs (Fig. 1 and Fig. 2). This is a novelty since it contradicts the common theory that temperature for rearing the drone brood located on the nest outskirts is lower (Winston, 1987; Le Conte & Arnold, 1988; Le Conte, Arnold, & Desenfant, 1990). Concluding, temperatures on the nest outskirts does not have to be lower when a drone brood is there.
Comparing temperatures of the drone and worker brood rearing, Levin & Collison (1990) discovered that the temperature closet to the worker brood, even though not located on the nest, was higher than the temperature recorded close to the drone brood in the nest centre. This could fit our results, although in the study by Levin & Collison (1990) these two brood types were located on the same comb. Li et al. (2016) compared temperatures of rearing two worker-brood combs and two drone-brood combs adjacent to each other in the three colonies' nest centres and then reported that the temperature for the worker-brood rearing was higher than that recorded in the case of the drone-brood in two colonies, but unfortunately the opposite was the case in the third one. However, we suspect that these discrepancies could have occurred due to methodological reasons. Levin & Collison (1990) and Li et al. (2016) placed both brood types close to one another, while in our studies we placed them in distant parts of a colony. Perhaps workers in our studies did not have to deal with such an expressive necessity to decide which type of brood to heat better. This phenomenon can be explained through the results of the laboratory experiment conducted by Koeniger (1978), who found the capped drone-brood to be much more attractive to worker bees than the capped worker-brood when placed directly next to each other.

The colony strength should also be considered. We have used very strong colonies; the drone-brood : worker-brood ratio amounted to 1:8 (12.5% of drone brood). Conversely, Li et al. (2016) assayed weaker, six-comb colonies that contained two worker-brood and two drone-brood combs in their nests; the drone-brood : the worker-brood ratio was 50% in their study. Seeley & Morse (1976) claimed that under typical field conditions the percentage of the drone-brood should be around 13-17% of the total colony brood. Czekońska, Chuda-Mickiewicz, & Samborski (2015) pointed out that the number of drones does not have to exceed 10% of the workers in a well-functioning colony. Therefore, in the investigations conducted by Li et al. (2016), an untypical proportion of the drone brood in combination with the low colony strength did not encourage their workers to care for the drone brood properly, because building up the colony strength (worker brood) was their first priority. Concluding, our results showed that a colony can afford to properly heat the drone brood only when it is strong enough, no matter in which part of the colony this brood is placed. It is believed that the nest worker bees preferred to take care of the drone brood because their colony could increase its reproductive success in this way, which is in agreement with the main goal of evolution (Koeniger, 1978; Li et al., 2016). Drones are indispensable for the successful spreading of the colony genes. Our study showed that a colony is able to fulfil this evolutional goal only when it is strong enough and has a proper drone-worker ratio (compare to Czekońska, Chuda-Mickiewicz, & Samborski, 2015).

Contrary to Kober's (2003) assumptions, our study showed that small-cell colonies did not have to maintain higher nest temperature than standard-cell colonies regardless of the within-colony comb location, outside temperatures (Tab. 1) and the brood type (Fig. 2 and Fig. 3). Sometimes the temperature on standard-cell combs was even higher.

Our results show that there is a need to further investigate the influence of the drone-brood presence on thermoregulation of honeybee colonies (compare: Kovac, Stabentheiner, & Brodschneider, 2009), as the colony temperature was regulated by the workers but the drone brood could influence this process. If a colony is strong enough and has a proper drone-worker structure, even a peripheral drone-brood comb can be heated as well as a worker brood in the nest centre. Rosenkranz, Aumeier, & Ziegelmann, (2010) hypothesised that a higher worker-brood rearing temperature encourages *V. destructor* females to prefer the drone-brood for reproduction, but our research has shown that this is not always the case as the rearing temperature for these two brood types may be the same. This needs the further studies, in which an action of the brood pheromones, i.e. chemical orientation (Calderone & Lin, 2001), might also need to be considered. Recording temperatures within brood cells is also recommended in such studies. It is doubtful whether the higher worker brood rearing temperature in small-cell colonies would limit the development of *V. destructor* population there, as we have found that such a difference might do not always exists. This might be the case in strong field colonies with a proper worker-drone structure, that was used in our studies. Therefore, keeping bees in small-cell-comb colonies cannot be considered an effective auxiliary method of controlling *V. destructor* infestations, as temperature not always is a main factor interferes the mite reproduction there. Consequently, this disputes the opinion that a greater temperature difference between nest worker-brood-combs and peripheral drone-brood-combs makes the latter more efficient as mite traps in small-cell colonies, since we have shown that such a difference may not exist.

Finally, one issue requires an additional comment. The temperature standard deviations usually did not exceed 0.5°C, irrespectively of the brood type (Fig. 2 and Fig. 3), which was probably caused by the high strength of the colonies used in our study. The temperature measured at the drone-brood comb in June of the second season was an exception as standard deviations reached 2.63°C in the colonies kept on standard-cell combs and 1.20°C in the colonies kept on small-cell combs. Also, most exceptions from the aforementioned regularities and trends occurred in this period of the second season. We assume that because of a temporary lack of nectar and pollen flow, drone brood rearing significantly decreased in, and therefore, only scattered small drone-broods aggregated in the drone-brood combs.

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