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Acceptance of light and dark combs in Apis mellifera hybrid colonies

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ABSTRACT

This research was carried out to evaluate the acceptance of light and dark comb for brood rearing and/or food storage at the center and sides of the hive by hybrid workers from Africanized, Caucasian, Italian and Carniolan queen sisters. Honeybees used the light and dark combs for brood rearing and food storage in central position, and the hybrids did not presented preference for either light or dark comb introduced in the hive. At the center and sides, the dark comb had larger storage area for both honey - 186.60 and 242.00 cm² and pollen - 67.30 and 118.10 cm² than the light comb - 53.30 and 126.90 cm² for honey storage area and 13.90 and 14.70 cm² for pollen storage area, respectively. When storing food, nectar and pollen, honeybees prefer dark combs; but the occupied area by honey in the lateral position was larger in light combs than dark combs.

KEYWORDS Brood rearing, food storage, hybrid honeybee, honeybee behavior.

Introduction

In the colony, wax produced by honeybees is used for building combs, which serve to store pollen and honey, and to the development of eggs, larvae and pupae (Brighenti, Carvalho, Carvalho & Brighenti, 2005; Zovaro, 2007), and its structure and odors are also important for communication (Yang, Tan, Radloff, Phiancharoen & Hepburn, 2010). Honeycomb is vital to the colony, for both the development of queen offspring, to restore and expand the workforce, and to store and keep food (Pratt, 2004). Worker honeybees require six to seven kg honey for the production of 1 kg wax, (Nogueira-Couto & Couto, 2006). Thus, the production of wax to build new combs is a limiting factor for honey production, due to the time spent and food consumed and the lost opportunity of nectar flow (Pratt, 2004).

Combs are successively reused and the accumulation of wax, propolis and debris from ecdises during development makes alveoli smaller and combs darker. A new comb after the first brood increases by 25% its weight, after the fifth brood, the weight doubles, at the fifteenth brood, the weight triples, and after the thirtieth brood, the weight increases fourfold (Zovaro, 2007).

The durability of dark comb is longer thus the honey storage is more advantageous and efficient in this type of comb during the honey extraction (Rinderer & Baxter, 1980; Rocha, Lara, Cecchetti, & Pacheco, 2010) and honeybee foraging is faster on larger amounts of dark combs available (Rinderer & Baxter, 1980). This is because, in general, the empty comb acts as a stimulus to the harvesting behavior and storage, and indirectly, as a stimulus for the secretion of wax (Hepburn & Magnuson, 1988). However, this trend is highly variable among the colonies (Rinderer & Baxter, 1980).

For the colony, use of old combs results in the emergence of smaller and fewer honeybees (Berry & Delaplane, 2001; Piccirillo & De Jong, 2004). Furthermore, it may have a negative influence on the honey quality (Rocha et al., 2010) and increase diseases in the colony (Piccirillo & De Jong, 2004). Otherwise, Berry and Delaplane (2001) reported that survival of honeybees was greater when originating from old combs.

Pratt (2004), Buchwald, Breed, Greenberg & Otis (2006), Hepburn, Radloff, Duangphakdee & Phaincharoen (2009), Yang et al. (2010) and Kather, Drijfhout & Martin (2011) conducted behavioral studies comparing the use of comb in different honeybee species and in relation to nesting, composition, cell size, mechanical properties and flexibility. In this way, the present study evaluated the acceptance of dark and light combs by Africanized, Caucasian, Italian and Carniolan hybrid honeybees for brood rearing and/or food storage at the center and sides of the hive.

Material and Methods

Twenty-two Langstroth hives were used, of which five received Africanized honeybee queens, five, Caucasian queens, six, Italian queens and six, Carniolan queens. Africanized and Italian queens were obtained from the Genetics Department, Ribeirão Preto Medical School, University of São Paulo, and the Caucasian and Carniolan queens were obtained from the Apiary Pascon in Rio Claro, state of São Paulo, Brazil.

Virgin queens marked in the thorax were introduced into the colonies that were homogenized as to the brood and food areas and previously orphaned. In each hybrid studied, queens were sisters, mated in the air, possibly with Africanized drones, giving rise to hybrid worker daughters. We waited for 60 days for the replacement of workers by the hybrid progeny of the introduced queens, to start the observations.

Evaluating the acceptance of dark and light combs, areas of

occupied combs were periodically mapped as to the brood rearing and food storage, using the adapted method from AlTikrity, Hillmann, Benton & Clarke Jr (1971). All colonies received energetic supply based on water and sugar at a ratio of 1:1. At first, in each hive we placed an empty dark and an empty light comb, initially at the central position. These combs were taken without honeybees to the lab and mapped, separately, for their occupied area by brood - egg-larva and pupa of workers and drones; and by food - honey and pollen. The combs were put into a support whose sides had taut wire forming square of 2 cm. From the number of squares counted for every variable, we estimated the occupied area in cm^2, multiplying by four ($2x2=4cm^2$).

Subsequently, we introduced other empty dark and light combs in a lateral position on either side of the hive and periodically mapped. For each treatment, in half of the hives, the dark comb was on one side, and in the other half, on the other side, to avoid effects of position and environmental conditions. We mapped the combs seven times at intervals of about eight days, regarding their brood and food areas.

Preliminary tests were performed with all data, separately, with Bartley test using the software BART to indicate the best transformation of data, if necessary, to achieve more homogeneity and the lower coefficient of variation. The Bartley test indicates, by means of a chi square, significant differences between the variances of the samples (Beiguelman, 1988).

The statistical analysis was performed with data transformed to $X^{0.20}$ in a two-factor (2 X 4) arrangement; the type of comb - light or dark was the first factor and the hybrid, the second. Analyses of variance were run regarding the number of squares counted in each mapping. These values were multiplied by four to obtain the result of the area in cm^2. We adopted the significance level of 5% and used Tukey's test for comparison of means.

Results and Discussion

There was no significant interaction between the types of comb - light or dark and hybrids for egg-larva, pupa brood, and total worker brood, pollen, total food and the total occupied area. That is, no hybrid presented preference for the type of comb. The results in cm^2 and in percentage of occupied area by brood and food at the center and sides of the hive, in light and dark combs, by the hybrids analyzed can be seen in Tables 1 and 2.

Table 1. Occupied area (cm^2) by brood and food in central and lateral positions of the hive, in light and dark combs for Africanized, Caucasian, Italian and Carniolan hybrids honeybees

central position of the hive (cm^2)							
	brood (cm^2)			food (cm^2)			Occupied
	egg/ larvae	pupae	Total	honey	pollen	total	area
Light comb	176,70 a	124,20a	300,90 a	53,30 b	13,90 b	67,20 b	368,10 b
Dark comb	255,40 a	154,20 a	409,60 a	186,60 a	67,30 a	253,90 a	663,50 a
Africanized	339,10 a	293,10 a	632,20 a	82,10 a	48,60 a	130,70 a	762,90 a
Caucasian	190,50 a	77,70 a	268,20 a	172,80 a	45,10 a	217,90 a	486,10 a
Italian	142,70 a	81,90 a	224,60 a	78,70 a	19,90 a	98,60 a	323,20 a
Carniolan	191,90 a	104,60 a	296,50 a	146,50 a	48,50 a	195,00 a	491,50 a
lateral position of the hive (cm^2)							
	brood (cm^2)			food (cm^2)			Occupied
	egg/ larvae	pupae	Total	honey	pollen	total	area
Light comb	-	-	-	126,90 b	14,70 b	141,60 b	141,60 b
Dark comb	-	-	-	242,00 a	118,10 a	360,10 a	360,10 a
Africanized	-	-	-	423,50 a	117,30 a	540,80 a	540,80 a
Caucasian	-	-	-	74,90 c	21,00 c	95,90 c	95,90 c
Italian	-	-	-	98,00 b	41,50 b	139,50 b	139,50 b
Carniolan	-	-	-	141,70 b	56,30 b	198,00 b	198,00 b

* Equal capital letters in the same column do not differ by Tukey's test at 5% significance level.

Table 2. Percentage of occupied area by brood and food in central and lateral positions of the hive, in light and dark combs for Africanized, Caucasian, Italian and Carniolan hybrids honeybees

Central position of the hive (%)						
	brood (%)			food (%)		
	egg/ larvae	pupae	total	honey	pollen	total
Light comb	48,00 a	33,74 a	81,74 a	14,48 b	3,78 b	18,26 b
Dark comb	38,49 a	23,24 a	61,73 a	28,12 a	10,14 a	38,26 a
Africanized	44,45 a	38,42 a	82,87 a	10,76 a	6,37 a	17,13 b
Caucasian	39,19 a	15,98 a	55,17 a	35,55 a	9,28 a	44,83 a
Italian	44,15 a	25,34 a	69,49 a	24,35 a	6,16 a	30,51 a
Carniolan	39,04 a	21,28 a	60,32 a	29,81 a	9,87 a	39,68 a
Lateral position of the hive (%)						
	brood (%)			food (%)		
	egg/ larvae	pupae	total	honey	pollen	total
Light comb	-	-	-	89,62 a	10,38 b	100
Dark comb	-	-	-	67,20 b	32,80 a	100
Africanized	-	-	-	78,31 a	21,69 a	100
Caucasian	-	-	-	78,10 a	21,90 a	100
Italian	-	-	-	70,25 a	29,75 a	100
Carniolan	-	-	-	71,57 a	28,43 a	100

* Equal capital letters in the same column do not differ by Tukey's test at 5% significance level.

For the occupied area at the center of the hive with egg-larva brood, pupa and total brood, no significant difference (P> 0.05) was detected between the light and dark combs (Table 1). Likewise, there was no difference (P> 0.05) between hybrids for areas occupied by egg-larva brood, pupa, total worker brood, honey, pollen, total food and total occupied area.

When comparing the colony region - central or side, there was a significant difference (p <0.05) between the light and dark combs, for the food area. At the center and sides, the dark comb presented the largest deposit of honey - 186.60 and 242.00 cm^2 and of pollen - 67.30 and 118.10 cm^2 compared to the light comb - 53.30 and 126.90 cm^2 for honey and 13.90 and 14.70 cm^2 for pollen, respectively (Table 1).

In the case of hybrids, the Africanized, Caucasian, Italian and Carniolan presented no differences in the area occupied by honey and pollen at the center of the nest. However, there were differences for food storage in the lateral region (Table 1). Africanized hybrid honeybees presented the largest occupied area by food - 540.80 cm^2, followed by the Italian and Carniolan hybrids - 139.50 and 198.00 cm^2, respectively. The Caucasian hybrids had the smallest occupied area by food, 95.90 cm^2 (Table 1). For the occupied area by pollen, there were differences (P <0.05) between light - 14.70 cm^2 and 10.38% and dark combs - 118.10 cm^2 and 32.80%; the honeybees, regardless of the hybrid, preferred (P <0.05) dark combs to deposit pollen, at the center and sides of the hive (Tables 1 and 2).

variable, however, Taha, Manosur & Shawer (2010) stated that darker combs have more minerals and the extracted honey also contains more minerals.

In the same way, Berry and Delaplane (2001) observed that new combs promote a greater brood production, but brood survival was higher in old combs. Nevertheless, older combs usually have smaller cells and higher likelihood of disease (Piccirillo & De Jong, 2004). On the hygienic behavior of colonies of hygienic and non-hygienic lines in new and old combs, Pereira, Morais, Francoy & Gonçalves (2013) observed that, in the early hours, the cleaning activity in new combs was higher, but worked with the same efficiency in both combs at the end of 24 hours and had greater difficulty uncapping and remove dead brood from old combs.

Additionally, dark combs stimulate the activities of the workers, but the age of the combs negatively influences the morphological characteristics of workers, such as weight, wing area and length of the tibia, which may reflect the performance of the colony (Alfalah, Shaibi, Tawfiq & Mogrby, 2013). Beeswax is costly and requires a lot of energy to be produced (Nogueira-Couto & Couto, 2006); for species such as *Apis florea*, it is necessary to recover wax from the old nest, but only the one used to store pollen and nectar due to cuticular residue left by developing brood (Pirk, Crous, Duangphakdee, Radloff & Hepburn, 2011).

Considering our results, we recommend beekeepers to use better or reuse dark combs in the nest chamber, which may lower production costs and reduce the incidence of greater beeswax moth - *Galleria mellonella* (Linnaeus, 1758) or lesser beeswax moth (*Achroia grisella*, Lepidoptera: Pyralidae). Giving preference to introduce dark combs, which should be introduced according to the interest of the beekeeper, at the center they will stimulate queen laying and hence greater amount of brood, and at the sides of the hive, stimulate greater collection of food. In the case of light combs, these should be placed at the sides, because they will stimulate the collection of nectar, even indirectly.

Conclusion

Hybrid show no preference for the type of comb introduced in the hive. For storing food, nectar and pollen, honeybees prefer dark combs, except the percentage of area occupied

Considering the acceptance of dark and light combs at the center of the hive, both were used for rearing egg-larva and pupa of workers, honey and pollen (Tables 1 and 2). The absence of egg-larva and pupa of drone is justified by the fact that the foundation introduced was of worker cells, hindering the drone brood laying by the queen. So, when the interest is to produce only workers, beekeeper must introduce a frame with an entire beeswax comb or a worker beeswax foundation.

In percentage, the brood area in the central region was larger in light combs - 81.74% than in dark combs - 61.73% and the opposite was found for the food area - 18.26 and 38.26% respectively (Table 2). However, when analyzing these same values in cm^2, the area used in the dark comb was larger than in the light comb for egg-larva, pupa, honey and pollen, that is, 48.00% of area occupied with egg-larvae in dark comb corresponds to 176.70 cm^2, while 38.49% in the dark comb corresponds to 255.40 cm^2. Still at the center in the light comb, 14.48% of the area was occupied honey and 3.78% with pollen, and in the dark comb, 28.12% of the area was occupied with honey and 10.14%, with pollen.

The area used by hybrids in the central region was larger for egg larva followed by pupa, honey and pollen. With respect to the area with honey, Africanized honeybees presented the lowest percentage - 10.76% or 82.10 cm^2, while Caucasian hybrids had the largest area - 35.55% or 172.80 cm^2. Nevertheless, Africanized honeybees presented larger area occupied both at the center and sides of the hive - 762.90 and 540.80 cm^2 (Tables 1 and 2).

At the sides of the hive, the hybrids which tended to present the smallest area were the Caucasian - 74.90 and 21.00 cm^2, Italian - 98.00 and 41.50 cm^2 and Carniolan - 141.70 and 56.30 cm^2, while Africanized - 423.50 and 117.30 cm^2 maintained the largest area occupied, in light and dark combs during observations, respectively, by honey and pollen. Table 2 presents that, at the sides of the hive, the combs were not used for brood, and with this, the percentages of honey and pollen in light and dark combs were higher than those obtained at the center, 89.62 and 10 38% for light comb and 67.20 and 32.80% for dark comb, respectively.

We verified a considerable increase in the amount and percentage of pollen deposited in the dark comb compared to the clear comb in the center and sides of the hive (Tables 1 and 2). Also, the Africanized hybrid invested more in brood rearing than in food - 82.87 and 17.13%, at the center, while in the Caucasian hybrids, these values were closer - 55.17 and 44.83%.

At the sides of the hive, all hybrids presented similar results in percentage, in which the largest area was occupied mainly by honey and the rest by pollen. In this way, it can be seen that by introducing an empty honeycomb foundation at the sides of the hive, it should be used primarily to deposit honey, whereas, when introduced at the center of the hive, the comb would be mainly occupied for rearing worker egg-larva and pupa brood than by food, especially honey. Such use will be greater if the comb introduced is dark.

Several factors such as the queen laying capacity, colony population, climatic factors and the communication system influence the food collection and productivity of colonies (Ali, 2007; Kather et al., 2011) and genetic factors. It is possible to select colonies with preference for stocking more food in light comb, as described by Rinderer and Baxter (1980).

The dark wax has about three times more n-alkanes of pair-chain that the light wax, due to hydrocarbons of cuticle source from the brood that develop in the honeycomb, thus affecting the wax characteristics, making it more brittle, dark and heavier (Namdar, Neumann, Sladezki, Haddad & Weiner, 2007). Regarding the collection of honey, Rocha et al. (2010) reported increased honey acidity with advancing age of the combs, but the color of the honey was not affected by this

by honey at the lateral combs, which is higher in light combs. The use or reuse of dark combs can be an important tool to improve production and reduce costs.

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