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INFLUENCE OF COLONY SIZE AND SEASON ON THE HONEY BEE (*APIS MELLIFERA* L.) WORKER HYPOPHARYNGEAL GLAND DEVELOPMENT AND LIFETIME

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ABSTRACT

The influence of season and type of colony on the development of the hypopharyngeal glands of *Apis mellifera* workers was investigated in the present study. Three types of colony were observed: strong (with a population of about 50.000 bees), median (about 20.000 bees) and weak (about 3.000 bees). The results showed that the average of worker lifetime is longer in the more populous colonies and in times of higher temperatures, nevertheless the weak colonies were more susceptible to the weather conditions than the strong ones. Although the colony size affects the worker lifetime and its glandular development, this not changes the pattern and sequence of tasks in the three types of colonies.

KEYWORDS: *Apis mellifera,* hypopharyngeal glands, colony size, season, lifetime.

INTRODUCTION

Since Rosch (1925, 1927, 1930), Lindauer (1953), Cruz-Landim and Silva de Moraes (2000) up to Deseyn and Billen (2005) the labor division among *Apis mellifera* workers has been correlated to the particular stage of some exocrine gland development, mainly the hypopharyngeal, wax and mandibular glands. The ability of workers to nurse the brood and feed the queen depends on the degree of functioning of the hypopharyngeal glands, as well as the ability to build new combs depends upon the wax production by the wax glands. Therefore the sequence and duration of the tasks performed by the workers in their function of colony maintenance depends on the sequence in which these glands mature and the time span during they are active. The division of labor in advanced eusocial bees is known as a polyethism etary in the sense that the sequence of tasks is connected to the worker age, although being consensus nowadays that is more related to the worker physiological maturity than the chronological age (Lindauer 1952, 1953, 1961; Free 1965; Ribbands 1952, 1953; Sakagami 1953; Gracioli *et al.* 1999; Deseyn and Billen 2005). Nevertheless is equally well known that the sequence of tasks performance is not rigid (Free 1965) and may be influenced by environmental factors from inside or

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outside the colony as nutritional factors or colony specific needs (Kratky 1931; Ribbands 1952; Sakagami 1953; Gracioli *et al,* 1999; Gracioli and Silva de Moraes 2002; Huang *et al.* 1989; Huang and Otis 1989). For instance if foragers are being necessary for attending the colony need in food collection, some workers may jump over anterior functions and become foragers, independently of later return to the functions that they passed over (Free 1965).

Particularly about the hypopharyngeal gland, factor as nutrition, juvenile hormone titers in the hemolymph of workers (Rutz *et al.* 1976; Fluri *et al.* 1982; Liu 1989; Gracioli *et al.* 1999; Gracioli and Silva de Moraes 2002) and colonial conditions, as absence or presence of open brood (Browers 1982, 1983; Gracioli *et al.* 1999; Gracioli and Silva de Moraes 2002), are known as determinant factors of its development and lasting of its functioning. Huang *et al.* (1989) used colonies divided into brood-right and broodless portions with single or double screens, what resulted that the signal from the honey bee brood which activates the protein synthesis in the hypopharyngeal glands of nurse honey bee could only be obtained by workers that had direct access to the brood. The results showed that the brood had a significant effect on the hypopharyngeal glands activity, since only nurse workers from the brood-right sides of hives had elevated rates of protein synthesis in the hypopharyngeal glands.

This study reports the influence of the colony size and season on the development of the hypopharyngeal glands in workers of the honey bee.

MATERIAL AND METHODS

Workers with controlled age were obtained from colonies with different population sizes, located in the apiary of the UNESP, Rio Claro, SP, Brazil. The studied workers were all daughters of the same queen in order to avoid general genetically differences. Combs containing eggs from the chosen queen were transferred to the folling types of queen right colonies:

1. Strong colony or brood-chamber having an estimated population of 50 to 70 thousands of bees (C3);
2. Median colony or nest having an estimated population of 20 to 25 thousands of bees (C2);
3. Weak colony or nuclei having an estimated population of 3 to 5 thousands of bees (C1).

New ovipositions in the transferred combs, by the resident queen, were avoided using exclusion screen. The larvae ecloded in the colonies and were treated by the resident workers. When the adults emerged, they were marked with atoxic paint and returned to the colony. The study of the developmental stage of the hypopharyngeal gland was performed in 0, 3, 7, 10, 15, 20, 25, 30, 40 and 45 days old workers. Three workers of each age had their glands dissected and stained with 1% acetic orcein. Five acini of each gland were then measured and the average calculed.

These procedures were repeated in the period of May – June (E1) when the temperatures were lower and the humidity higher and September – November when the temperatures were higher and the humidity lower.
In order to verify if the colony size or climate conditions influence the bees lifetime, 300 workers were marked at the emergency and returned to the colonies. From time to time (3 days interval) the marked bees were counted until their complete disappearance.

RESULTS

The hypopharyngeal glands of *A. mellifera* consist of acini formed by several glandular cells, linked to an axial excretory duct by a bunch of conductor canals. The aspect and size to the acini change with the gland functional state. In the normal cycle of development, the glands have small and flaccid acini in newly emerged workers, well developed and turgid acini in the nurse workers and, again, small and flaccid acini in the forager workers (Fig. 1A, B, C).

In the present experiment, the acini of the hypopharyngeal glands of the workers from the three colonies showed large at the age between 7 and 15 days, but in the weak colony, the higher developmental state of the acini last more time (Fig. 2). In this colony, the size of the acini remained unchanged in workers between 7 and 15 days old, decreasing slowly than in the other two colonies.

The weather also affected the glandular development. The size achieved by the acini was higher in the hot season (Fig. 3). The maximum size of the acini was reached in 7 days old workers, in the period of higher temperatures, while in the period of lower temperature it was observed only at workers with 10 days old.

Some workers emerged from the strong colony, with etary ranging from 10 to 20 days old, presented underdeveloped glands for the age, the acini being very small, similar those of newly-emerged workers. This event was detected in the strong colony, rarely in the median colony and never in the weak colony.

The worker longevity was affected by the colony size and weather conditions (Tables I, II). The average lifetime of the workers was 45, 42 and 40 days respectively, for the strong, median and weak colonies, in the hot season (Table II), while in the cold season this average was 40, 35 an 33 days for the strong, median and weak colonies (Table I).

Table I – Longevity of the workers in the tree types of colony in low temperature. Humidity = 79.240; minimum average temperature = 57.2 F°; maximum average temperature = 68 F°.

Colony State	Maximum Longevity (days)	Months of observations
Weak	33	May/June
Median	35	May/June
Strong	40	May/June

Table II - Longevity of the workers in the tree types of the colony in high temperature. Humidity = 63.50; minimum average temperature = 78 F°; maximum average temperature = 82.4 F°.

Colony State	Maximum Longevity (days)	Months of observations
Weak	40	September/November
Median	42	September/November
Strong	45	September/November

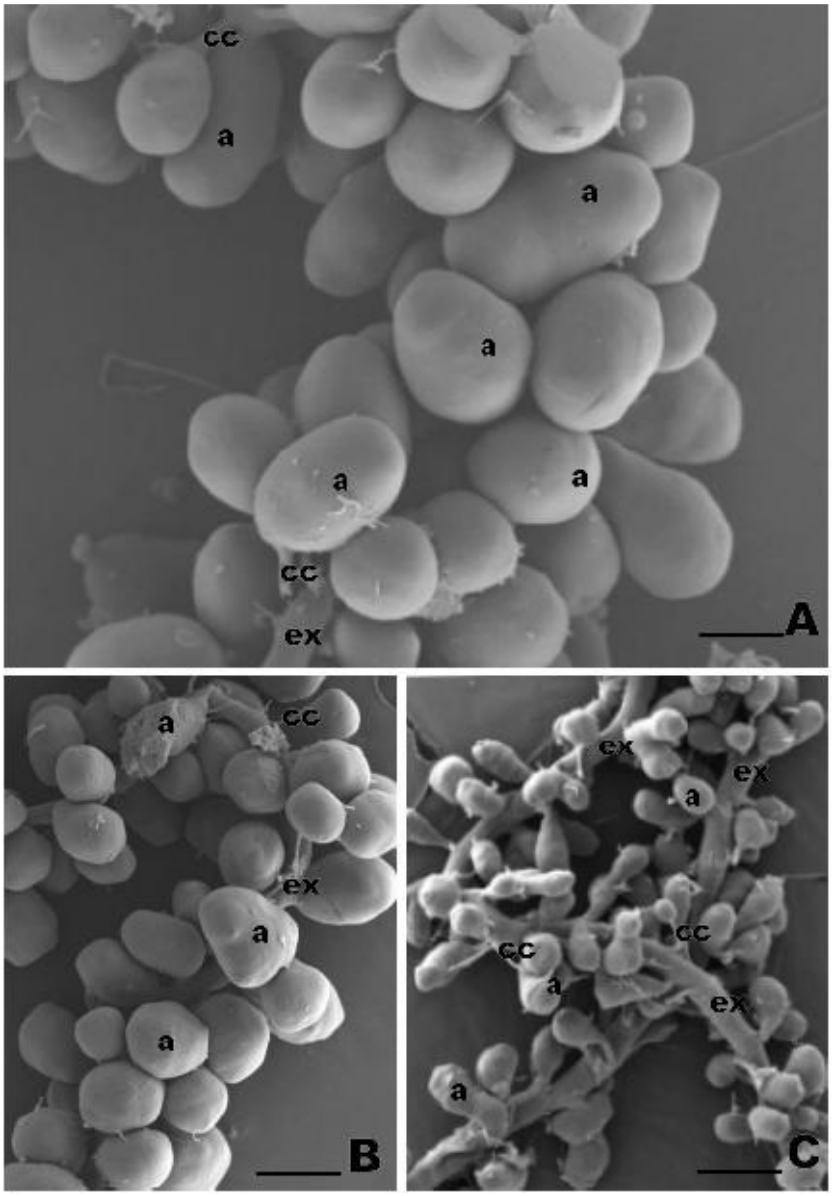

Figure 1 – Scanning electron microscopy of the hypopharyngeal glands of *Apis mellifera* workers. A. glands of workers obtained from strong colony, B. glands of workers obtained from median colony, C. glands of workers obtained from weak colony. (a= acini of hypopharyngeal glands, ex= excretory duct, cc= conducting canals, bars= 75 µm).

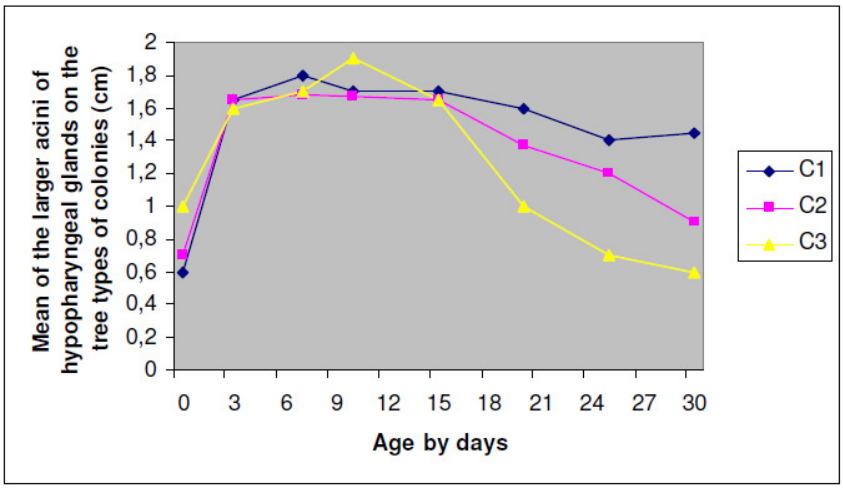

Figure 2 – Development bend of the hypopharyngeal glands in the tree colonies: weak (C1), median (C2) and strong (C3), in the long of lifespan.

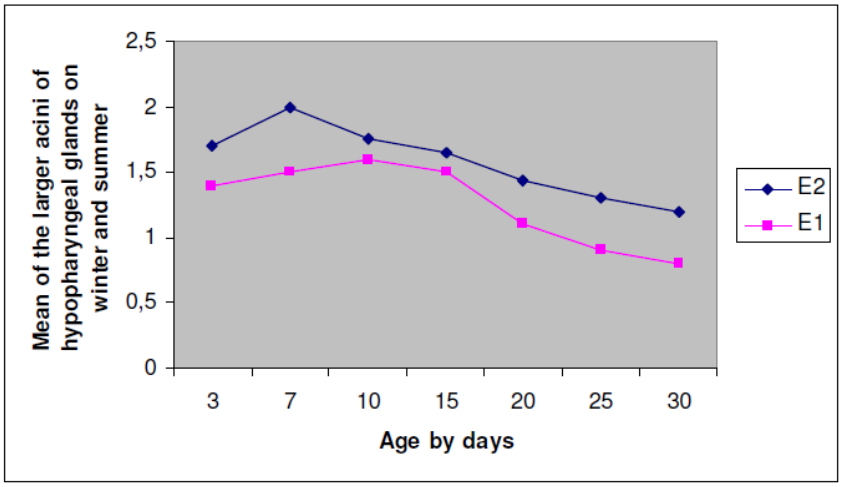

Figure 3 – Cycle of hypopharyngeal glands during the seasons: winter (E1) and summer (E2).

DISCUSSION

The results shown that in the present weather condition the average lifetime of the worker was longer in the more populous colonies and in times of higher temperatures, feature also verified by Terada *et al.* (1975) for the subtropical climate of Ribeirão Preto, SP, Brazil. Nevertheless, the weak colony seems more susceptible to the weather conditions than the strong ones (Saraiva 1985). In the temperate zones where the winter is very cold and the workers do not leave the colony they have a longer average lifespan during the winter and the workers remain in the colonies in a kind of hibernation over all the season (Sakagami and Fukuda 1968). The smaller average lifetime of the workers in the subtropical climate cold season may be due, to the depletion of the nutritional resources in the environment or to the lowering of the forager activities than due to the temperature.

Although the colony size affects the worker lifetime and glandular development, it does not change the pattern the sequence of tasks in the three types of colonies. According to Sekiguchi and Sakagami (1966) the maturity of most of the morphophysiological factors which capacitate the workers for their colony functions

are already complete after 3 - 5 days from the emergence. The present results shown that, for the hypopharyngeal gland, the whole maturation was reached only by the 7 days old workers in the weak and median colonies and delayed 10 days in the strong ones. Therefore the weak colony compensate the small number of workers and their small lifetime by beginning their work program early and lasting more time in the condition of its execution. The lasting of the glandular development in the higher stage makes possible the alternance function postulated by Lindauer (1953). Although several authors (Browers 1982, 1983; Gracioli *et al.* 1999; Gracioli and Silva de Moraes 2002) postulated the possibility of hypopharyngeal gland reactivation in response to the colony requirements, it seems that this fact is only reflex of the function alternance. Therefore, workers that have yet functional glands and are required for functions out of the colony, can return to the functions inside of the nest in answer to the colonial needs.

The possibility of the gland reply to the requirements of the environment is reinforced by the presence of workers which do not develop their glands in strong colony. This fact is in accordance with the observation of Lindauer (1961) who observed workers that remain all life in idleness in strong colony.

CONCLUSION

In conclusion, the principal determinant factors of the hypopharyngeal gland development is not the age of the workers and yes the general physiological conditions of the individual in interaction with the inner and outer environment of the colony. Therefore, studies in which the knowledge of the maturation stage of the individuals is need, it must observe the function in which it is performing than the chronological age.

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