Number of Ovarioles in Workers Descendent from Crossings Between Africanized and Italian Honeybees (*Apis mellifera* L.): Comparing Stock, Inbred and F1 Colonies

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Número de Ovaríolos em Descendentes de Cruzamentos entre Abelhas Africanizadas e Italianas (*Apis mellifera* L.): Comparação entre as Gerações Parental, Endocruzada e F1

RESUMO - Realizou-se uma análise comparativa do número de ovaríolos em operárias de duas colônias parentais (Italiana e Africanizada), duas colônias endocruzadas e onze colônias híbridas da geração F1 da abelha *Apis mellifera* L. Não houve diferença no número de ovaríolos dos ovários direito e esquerdo das operárias das populações estudadas. A colônia parental italiana apresentou uma amplitude de variação maior (6-26) que a da africanizada (2-16), no que se refere ao número total de ovaríolos. É provável que a variabilidade observada para esse caráter, nos híbridos, seja pelo menos parcialmente devida a diferentes constituições genotípicas das abelhas de cada população.

Palavras-Chave: Insecta, Hymenoptera, Apidae, ovário, abelhas híbridas.

ABSTRACT - A comparative analysis of ovarioles number in workers of two stock colonies (Italian and Africanized), two inbred colonies and eleven hybrid colonies (F1) of the honeybee *Apis mellifera* L. was carried out. No difference between the number of ovarioles in right and left ovaries of workers from the colonies studied was detected. The range observed for the number of ovarioles/worker was greater (6-26) in the italian stock colony than in the africanized one (2-16). The great variability observed for this morphological trait in the hybrid colonies may be at least partially due to different genotypes of the bees from each population.

Key Words: Insecta, Hymenoptera, Apidae, ovary, hybrid bees.

During the first two days of life, queens and workers of *Apis mellifera* L. have ovaries of similar size. From the 3rd day on, the ovaries of queens grow much more rapidly than those of workers (Meier 1916). During larval development, queens and workers have approximately the same number of ovarioles up to the 5th day, after which the ovaries of workers progressively degenerate with a drastic reduction in ovariole number, a fact that does not occur in queens. Therefore, newly emerged workers and queens commonly have 2 to 20 and 160 to 180 ovarioles, respectively (Velthuis 1970). The difference is partially

due to the deficient diet of workers after 60 hs of larval life, compared with the complete diet of royal jelly received by queen larvae (Weaver 1966, 1974, Tsao & Shuel 1973). In *Apis dorsata* F. the workers have 11 to 53 ovarioles/ovary, and queens 124 to 134 (Velthuis *et al.* 1971). *Apis cerana* F. workers commonly have 1 to 12 (Kapil 1962), although individuals may have 30 ovarioles (Sakagami & Akahira 1958).

Weaver (1956) reported that environmental factors have little effect on ovarian development, though such factors may affect nurse bees by altering the amount of food they offer to larvae. The author concluded that, if variation occurs and is not caused by environmental factors, it must be regulated by genetic factors, as also suggested by Chaud-Netto & Bueno (1979). S.A. Talamoni (unpublished) did not detect significant differences in mean number of ovarioles between right and left ovary of Africanized and Caucasian bees. Using reciprocal crosses, she found that workers from hybrid colonies resulting from the two groups were closer to the parent that supplied the drone.

Diniz-Filho *et al.* (1993) estimated narrow and broad sense heritabilities (h^2) of the number of ovarioles in *A. mellifera* workers. For this purpose they used data sets based on groups of half sisters (queens inseminated by several drones) and super-sisters (single drone insemination). The values obtained were in the usual range for economically important characters, as honey for instance (between 0,25 and 0,38).

The objective of this study was to determine the number of ovarioles in Africanized and Italian honeybee workers and also to investigate how this trait is expressed among descendants of crossings between drones and queens of these two races.

Material and Methods

Colonies used in the study belonged to the Department of Biology, of São Paulo State University, Campus of Rio Claro. The first phase of the study was carried out with two bee hives, an Africanized stock colony (nº 221), containing one Africanized queen mated with several drones and an Italian stock colony (nº 224), containing one Italian queen, also mated with several drones. Two inbred parental colonies (aunt x nephew matings), an Africanized (nº 228) and an Italian (nº 59), were produced from each stock colony. Eleven hybrid colonies were then obtained by crossing drones from colony nº 5 with queens from colony nº 59. All queens were obtained by simple larval transference, each one of them mated to a single drone by instrumental insemination, except queens of both stock colonies. The crossing scheme utilized (Fig. 1) was a modification of the method proposed by Rothenbühler (1960) and Cale & Rothenbühler (1979), and has been used by Gonçalves (1970) and Stort (1971) to study morphological traits and defensive behavior on Africanized honeybees. Fifty worker pupae were collected from each colony, fixed in Bouin's solution and preserved in 70% alcohol. Bees were dissected and the ovaries excised to establish the normal variation in the number of ovarioles in the two races.

The normality of the data was verified by using test of normality with an alternative of asymmetry (Levy 1974). The homogeneity of variances was verified by the Bartlett test and since the variances were found not to be homogeneous, the Mann Whitney U test or the Kruskal-Wallis test was used (Siegel 1975, Sokal & Rohlf 1981). The mean numbers of ovarioles in right and left ovaries in workers of each race were compared using *t* test. The Wilcoxon test was applied to the data to detect any differences among colonies.

Results and Discussion

The number of ovarioles/worker varied from 2 to 16 in Africanized honeybees and from 6 to 26 in *A. m. ligustica* (stock colonies) but, the variation observed in the inbred colonies was not so great, i. e., from 3 to 13 ovarioles in the Africanized bees, and from 3 to 17 ovarioles in the Italian (Table 1). The highest variation was observed in the

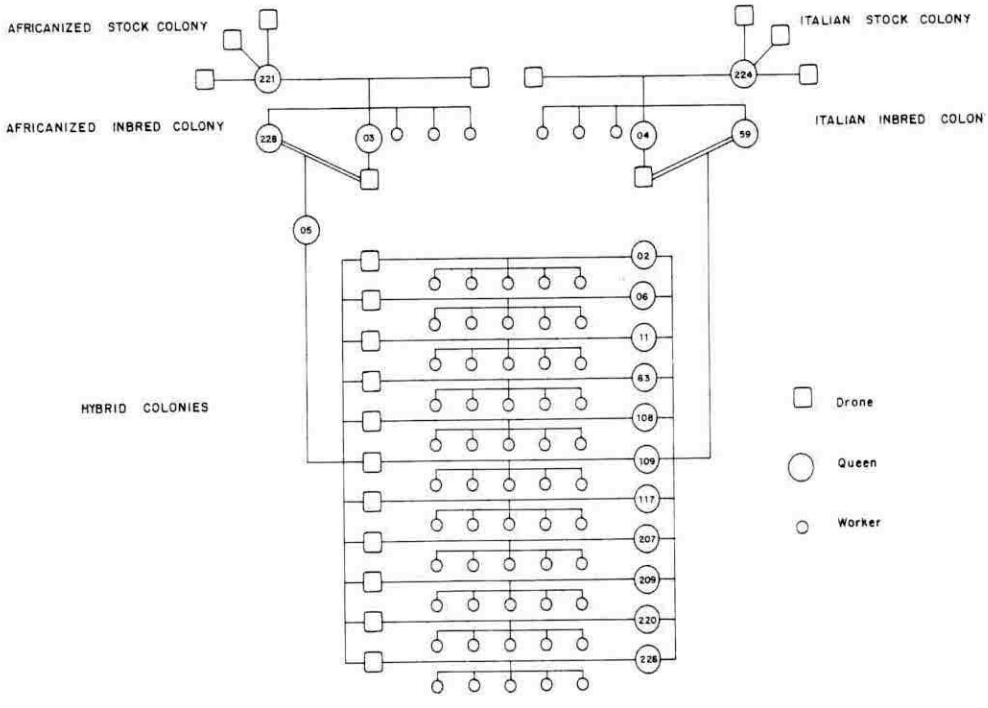

Figure 1. Scheme used of crossings between Africanized and Italian honeybees, *Apis mellifera* comparing stock, inbred and F1 colonies to determine the number of ovarioles in descendent workers.

hybrid colonies, i. e., from 2 to 31 ovarioles per worker (colony nº 207). The range of variation usually observed for the number of ovarioles in *A. mellifera* workers is from 2 to 20 (Velthuis 1970, Chaud-Netto & Bueno 1979). Since the experimental hives were maintained under the same environmental conditions, and had approximately the same quantity of food (honey and polen), and the same number of bees, we believe that the differences observed on the number of ovarioles may be partially determined by a genetic component, as suggested by Chaud-Netto & Bueno (1979) and later confirmed by Diniz-Filho *et al.* (1993). However, this genetic component may be associated with differences in environmental conditions of the colonies during the larval period, resulting in a variation on the quantity of royal jelly furnished to the worker larvae. This important association of factors may contribute to the expression of the differences detected among the colonies used in this research.

From the 750 worker honeybees dissected 16.9% had the same number of ovarioles in the left and right ovaries, 44.3% had a larger number of ovarioles in the right ovary, and 38.8% had a larger number of ovarioles in the left ovary. Similar results were obtained by Bueno (1981) for Africanized bees. The means for right and left ovaries were similar (t test, $P \leq 0,05$) in worker honeybees of stock colonies, inbred, and F1 colonies, confirming the hypothesis that one ovary is not more important than the other (Bueno 1981, S. A. Talamoni, unpublished).

The range observed for the number of ovarioles in Italian workers stock (ITS - 224)

Table 1. Number of ovarioles in honeybee workers of *Apis mellifera* (A = Africanized; I = Italian) from stock, inbred and hybrid colonies (n = 50).

Bee Type	Colony Number	Ovariole/Number X ± SD	Range	C.V.	Mode
Stock	221 (A)	8.6 ± 2.56	2 - 16	0.29	9
	224 (I)	14.0 ± 5.38	6 - 26	0.38	11
Inbred	228 (A)	6.9 ± 2.68	3 - 13	0.39	4
	59 (I)	7.7 ± 2.92	3 - 17	0.38	6
Hybrid (F1)	02	12.2 ± 3.71	5 - 19	0.30	12
	06	6.8 ± 2.68	2 - 15	0.39	5
	11	7.2 ± 2.76	2 - 14	0.38	5
	63	10.5 ± 3.11	4 - 16	0.29	13
	108	6.1 ± 2.54	3 - 17	0.41	4
	109	7.9 ± 2.03	3 - 13	0.25	8
	117	10.0 ± 3.22	2 - 19	0.32	10
	207	9.0 ± 4.42	2 - 31	0.49	11
	209	5.1 ± 1.84	2 - 9	0.36	5
	220	9.5 ± 4.43	4 - 26	0.46	9
	226	11.4 ± 3.02	7 - 20	0.26	11

was greater than the obtained for Africanized ones (AFS - 221), with an overlap between both sets of data (Fig. 2). The great variation observed for both stock colonies (from 2 to 26 ovarioles) may explain why part of the F1 colonies presented a mean number of

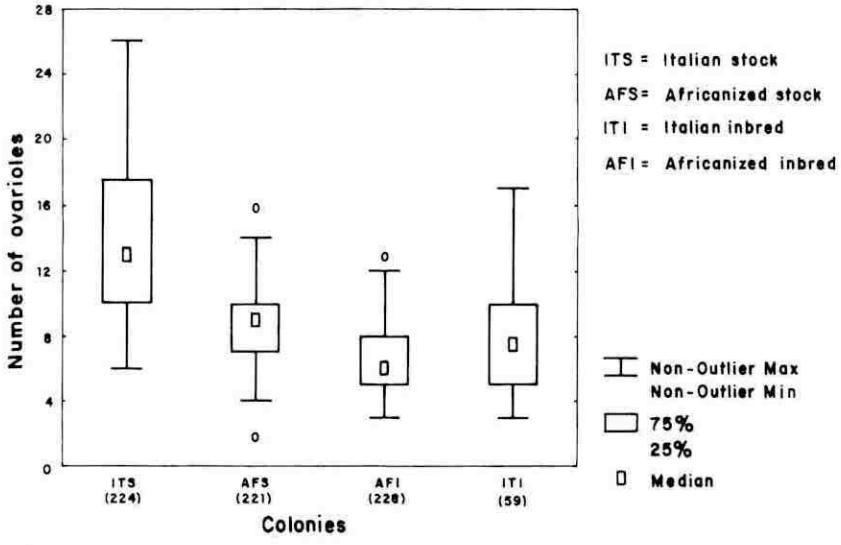

Figure 2. Number of ovarioles in Africanized and Italian honeybee workers from stock and inbred colonies of *Apis mellifera* obtained by crossing queens and drones through instrumental insemination.

ovarioles closer to the value obtained for the Italian stock colony, and the other near to the Africanized one (Table 1).

A reduction on the average number of ovarioles was observed for both Africanized and Italian inbred colonies (AFI - 228 and ITI - 59). In these cases the range was reduced probably because each queen was instrumentally mated to a single drone (Fig. 2).

Data of the Africanized and Italian stock colonies were significant ($Z = 5.34$; $P \leq 0.05$), indicating difference between races. Similar result was obtained by Chaud-Netto & Bueno (1979). A comparision of the mean number of ovarioles registered for the inbred colonies furnished a non significant value ($Z = 1.56$; $P \leq 0.05$). A nonparametric analysis of variance applied to the data (Kruskal-Wallis) registered for hybrid colonies indicated a significant result ($H = 203.3$).

In conclusion, results indicate that the variability detected in number of ovarioles was at least partially due to the different genetic constitutions of the bees from each colony.

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