

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY**Number of Ovarioles in Workers Descendent from Crossings Between Africanized and Italian Honeybees, *Apis mellifera* L.: Comparison among Backcrosses and Ancestors Colonies**REGINA H.C. THULLER¹, OSMAR MALASPINA¹, ODAIR C. BUENO¹ E JOSÉ CHAUD-NETTO¹¹Departamento de Biologia, UNESP, Caixa postal 199, 13506-900, Rio Claro, SP.

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Número de Ovariólos em Descendentes de Cruzamentos entre Abelhas Africanizadas e Italianas, *Apis mellifera* L.: Comparação entre os Retrocruzamentos e as Colônias Ancestrais

RESUMO - Comparou-se o número de ovariólos de operárias de abelhas *Apis mellifera* L. de 36 colônias de retrocruzamentos (Africanizadas e Italianas) e operárias de colônias ancestrais parentais, endocruzadas e híbridas (F1). Não houve diferença no número de ovariólos dos ovários direito e esquerdo das operárias. As abelhas híbridas da geração F1 apresentaram de 2 a 31 ovariólos. O número de ovariólos nas operárias dos retrocruzamentos africanizados variou de 2 a 56 e nos retrocruzamentos italianos de 2 a 117. A grande variação no número de ovariólos das abelhas dos retrocruzamentos deveu-se a variabilidade observada na geração parental (abelhas africanizadas: 2 - 16; italianas: 6 - 26) e no F1 (2 - 31).

PALAVRAS-CHAVE: Insecta, Hymenoptera, Apidae, ovário, abelhas híbridas.

ABSTRACT - A comparative study of ovarioles number in workers of bees, *Apis mellifera* L. of 36 backcrosses colonies (Africanized and Italian), and stock, inbred and F1 colonies was carried out. No difference on the number of ovarioles in right and left ovaries of workers from the colonies used was detected. The hybrid bees from F1 generation presented from 2 to 31 ovarioles. The number of ovarioles observed in backcrossed Africanized workers varied from 2 to 56 and from 2 to 117 in the Italian ones. The range observed in these bees may be partially explained by the variability registered in stock colonies (Africanized workers: 2 - 16; Italian workers: 6 - 26) and in F1 colonies (2 - 31).

KEY WORDS: Insecta, Hymenoptera, Apidae, ovary, hybrid bees.

Environmental factors that may affect nectar and pollen flow into the hive, such as climate, amount of flowers or number of bees in the colony have little effect on ovarian development, though such factors may influence

nurse bees by altering the amount of food they offer to larvae (Levin & Haydak 1951, Weaver 1956).

During the development of *Apis mellifera* L. workers, a significant increase in number

of ovarioles is noted at the various larval stages, peaks at the end of the larval period, even though this value is lower than the observed in queens. As metamorphosis starts, a marked reduction in ovariole number occurs in the worker caste up to the white-eyed pupal stage, when the definitive number is established (Bueno 1981).

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 13% of cases the left and right ovaries of *A. mellifera* workers have the same number of ovarioles, but in 76% of cases the left ovary has more ovarioles than the right one (Hess 1942). Pain & Verge (1950) confirmed these results and discussed the relative importance of the left ovary in relation to the right one, a fact also supported by Chaud-Netto & Bueno (1979).

Levin & Haydak (1951) observed symmetrical ovaries in 19.9% of the bees they examined, whereas Weaver (1956) found 19%. Bueno (1981) registered the same characteristic in 15.8% of Africanized bees (*A. mellifera*) examined. Comparisons of the mean number of ovarioles for both ovaries were non significant in most cases, leading the author to repropose the hypothesis that there is no importance of one ovary over the other.

Bueno (1981), in a study of the variation in ovariole number among descendants of crosses between queens of colonies, whose workers showed a small number of ovarioles, and drones of colonies in which workers showed a large number of ovarioles, observed that these descendants are closer to the father side. He also observed that this trait is not related to bee weight at emergence from the cell. Ceccato *et al.* (1984) found no relationship between ovariole number and maximum larval weight (L5).

Diniz-Filho *et al.* (1993) estimated nar-

row 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).

Thuller *et al.* (1996) made 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 *A. mellifera*. 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 same authors concluded that the great variability observed in the hybrid colonies (2-31) was at least partially due to different genotypes of the bees from each population.

The objective of this study was to determine the number of ovarioles in honeybee workers from Africanized and Italian backcrosses, comparing the data with those registered for stock, inbred and F1 ancestors colonies.

Material and Methods

In the 1st phase, the following material was used: an Africanized stock colony (n° 221), containing an Africanized queen natural mated with several drones; an Italian stock colony (n° 224), with Italian queen, also mated with many drones; an Africanized inbred colony (n° 228) with queen used as source of drones to make the F1 crosses; an Italian inbred colony (n° 59) with queen used as source of queens, and 11 colonies of F1 worker bees from the mating of Italian queens with Africanized drones. In the 2nd phase backcrosses gave origin to 36 colonies: 16 with bees from backcrosses to Africanized ancestors and 20 with bees from backcrosses to Italian ones.

All colonies belonged to the Department of Biology, São Paulo State University - Campus Rio Claro. The 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). Fifty worker pupae

detect any differences among backcrosses colonies.

Results and Discussion

The number of ovarioles in the hybrid colonies (F1) ranged from 2 to 31 ovarioles/

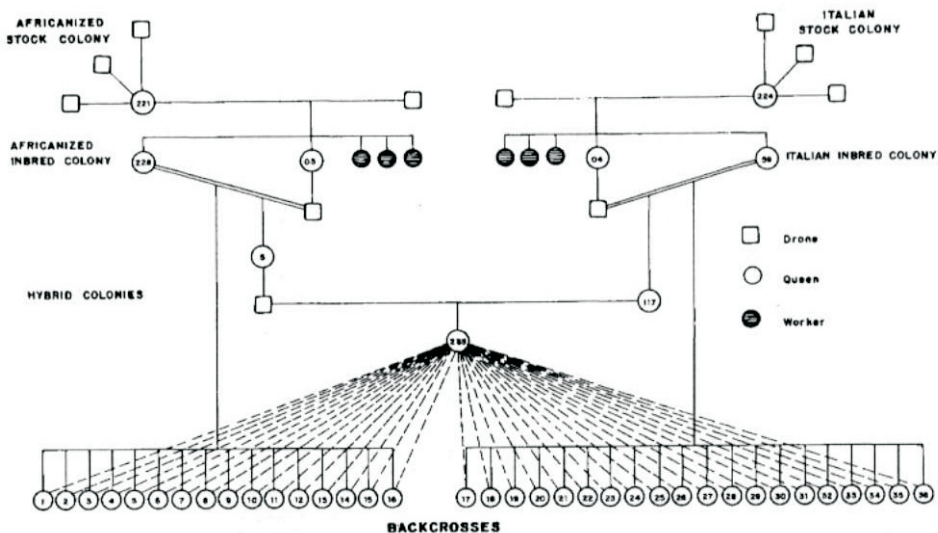


Figure 1. Scheme used of crossings between Africanized and Italian honeybees, *Apis mellifera*, comparing stock, inbred, F1 and backcrossed colonies to determine the number of ovarioles in descendent workers.

were collected from each colony, fixed in Bouin's solution and preserved in 70% alcohol. The bees were dissected and the ovaries excised to establish the normal variation in the number of ovarioles in both types of backcrosses.

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 1979, Sokal & Rohlf 1981). The mean numbers of ovarioles in right and left ovaries of each worker were compared using *t* test. Wilcoxon test was also applied to the data in order to

worker. 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). In this research we registered from 2 to 56 ovarioles/worker in the Africanized backcrosses and from 2 to 117 ovarioles/worker in the Italian ones (Table 1). In a previous research Thuller *et al.* (1996) found from 2 to 16 ovarioles in Africanized stock workers, and from 6 to 26 in Italian ones. The same authors observed a reduction on the average number of ovarioles for both Africanized (3 - 13) and Italian (3 - 17) inbred colonies. In these cases the range was reduced probably because each queen was instrumentally mated to a single drone. The great variation registered considering both

Table 1. Number of ovarioles in honeybee *Apis mellifera* workers (n = 50) from Africanized and Italian backcrossed colonies.

Africanized Colonies	Ovarioles/number			C.V.	Mode	Italian Colonies	Ovarioles/number			C.V.	Mode
	X	SD	Range				X	SD	Range		
01	8.0 ± 2.94	2 - 14	0.36	8	17	44.8 ± 24.58	9 - 117	0.54	34		
02	16.0 ± 9.87	5 - 56	0.61	14	18	9.0 ± 4.92	2 - 32	0.54	7		
03	6.0 ± 6.20	2 - 46	1.30	5	19	5.3 ± 2.19	2 - 10	0.41	6		
04	5.2 ± 1.63	2 - 8	0.31	5	20	6.5 ± 1.88	3 - 11	0.29	7		
05	12.9 ± 3.65	6 - 26	0.28	12	21	4.5 ± 1.78	2 - 11	0.39	3		
06	10.6 ± 4.68	5 - 29	0.44	8	22	35.8 ± 19.36	12 - 86	0.54	16		
07	8.3 ± 3.05	3 - 16	0.36	6	23	5.9 ± 1.98	2 - 10	0.33	6		
08	10.3 ± 4.97	3 - 33	0.48	9	24	8.7 ± 5.19	2 - 28	0.59	6		
09	5.5 ± 2.49	2 - 12	0.45	5	25	13.0 ± 5.93	3 - 32	0.45	13		
10	7.1 ± 2.60	2 - 14	0.36	6	26	9.6 ± 3.64	3 - 19	0.38	8		
11	8.4 ± 2.57	4 - 16	0.31	7	27	5.6 ± 2.20	3 - 11	0.39	4		
12	7.2 ± 2.87	2 - 14	0.39	7	28	10.5 ± 3.98	4 - 24	0.37	8		
13	13.6 ± 3.89	4 - 22	0.28	14	29	9.1 ± 3.13	2 - 17	0.34	9		
14	6.4 ± 1.73	3 - 10	0.27	5	30	10.3 ± 4.79	3 - 27	0.46	9		
15	11.0 ± 3.67	2 - 20	0.33	12	31	5.2 ± 1.87	2 - 11	0.36	4		
16	6.6 ± 2.16	3 - 11	0.32	8	32	7.2 ± 2.34	2 - 13	0.32	9		
					33	9.7 ± 8.89	2 - 38	0.92	5		
					34	11.7 ± 5.54	4 - 38	0.47	8		
					35	13.7 ± 4.72	5 - 32	0.34	13		
					36	11.2 ± 3.43	4 - 19	0.30	14		

stock colonies (from 2 to 26 ovarioles/worker) may explain why a part of the F1 colonies presented a mean number of ovarioles closer

to the value obtained for the Italian stock colony, and the other near to the Africanized one (Fig. 2). The same reasoning can be used

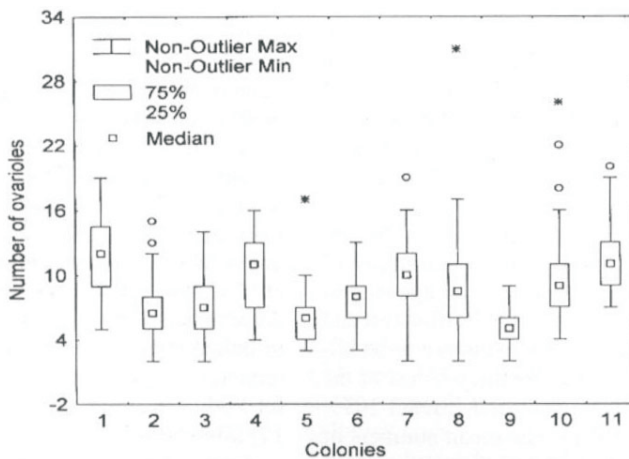


Figure 2. Number of ovarioles in hybrid honeybee workers, *Apis mellifera*, from F1 colonies obtained by crossing queens and drones through instrumental insemination.

to explain the large range observed in both types of backcrosses (from 2 to 117 ovarioles/worker), considering the results registered for F1 colonies (Figs. 3A, B). From the 1800 backcrosses worker honeybees dissected

19.5% had the same number of ovarioles in the left and right ovaries, 39.4% had a larger number of ovarioles in the right ovary and 41.1% had more ovarioles in the left. The means for right and left ovaries were similar

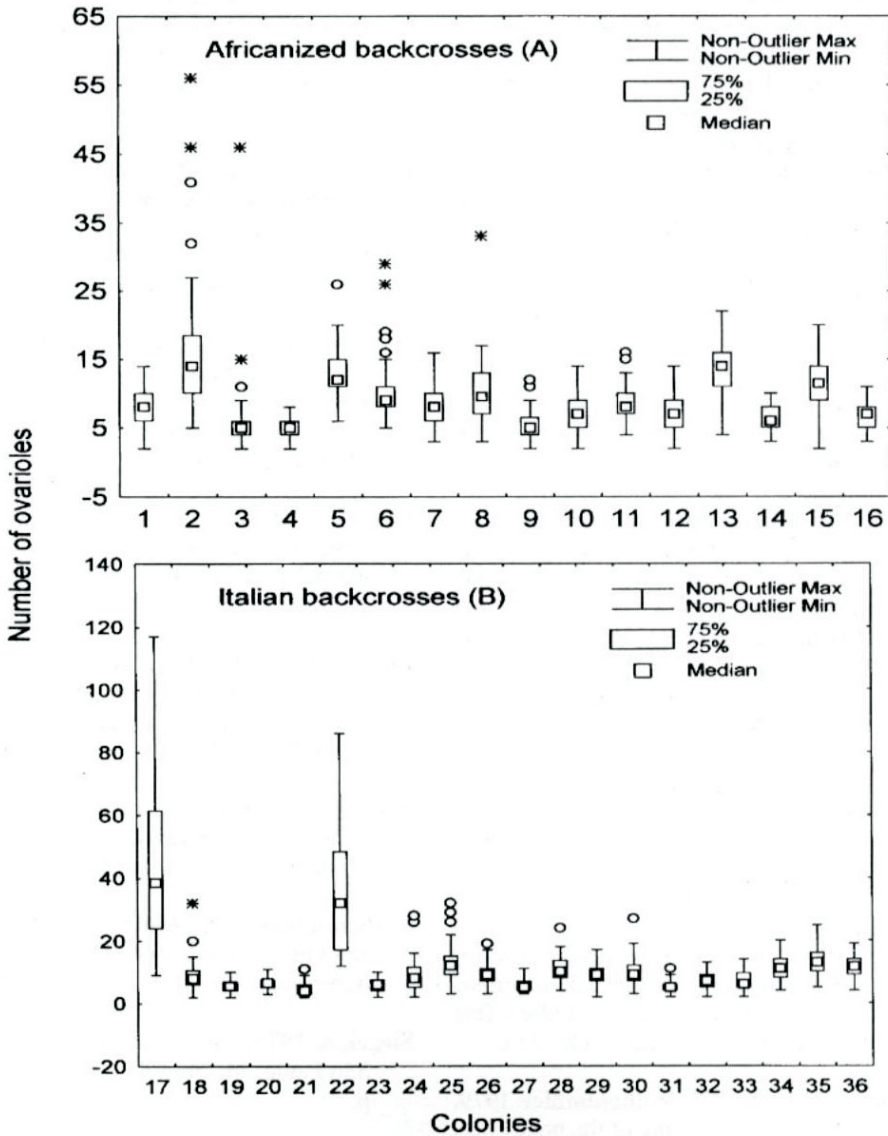


Figure 3. Number of ovarioles in hybrid honeybee workers, *Apis mellifera*, from Africanized (A) and Italian (B) backcrosses obtained by crossing queens and drones through instrumental insemination.

(*t* test, $P < 0.05$), as was observed for stock, inbred, and F1 colonies (Thuller *et al.* 1996). These data confirm the hypothesis that one ovary is not more important than the other in relation to the number of ovarioles (Bueno 1981). Significant results ($P < 0.001$) were obtained in a nonparametric analysis of variance (Kruskal-Wallis) applied to the data registered for F1 colonies ($H = 203.3$), Africanized backcrosses ($H = 365.3$) and Italian backcrosses ($H = 545.9$).

In conclusion, since the experimental hives were maintained under the same environmental conditions and had approximately the same quantity of food (honey and pollen) and the same number of bees, we believe that the differences observed on the number of ovarioles may be partially determined by genes, as was 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.

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