Producing Eggs from a Single Worker Honey Bee (Hymenoptera: Apidae)

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ABSTRACT Because a solitary worker honey bee (*Apis mellifera* L.) will not lay eggs, eggs were produced from small populations of caged workers. First, worker bees from four colonies were evaluated for their rate of worker ovary development. Groups of 25 workers were kept in incubator cages at 34 ± 1°C, and each cage was given an empty section of drone comb and unlimited supplies of honey, water, and pollen. Workers from colonies A and B began laying eggs rapidly (about 9 d), workers from colony D were intermediate (about 12 d), and workers from colony C required about 25 d. Five workers from colony A were mixed with 25 workers from the same colony, from colony B, or from colony C and maintained for 10 d. The five workers developed their ovaries most rapidly with their own sisters or with workers from colony C and slowest with unrelated, fast-developing workers from colony B ($P < 0.05$). When a single worker from colony A was caged with 25 workers from colony C, all eggs were laid by the worker from colony A. Overall, 50% (19/38) of the workers from colony A became egg layers when they were caged alone with workers from colony C. Although workers from colony A developed their ovaries sooner than workers from colony C, the two groups had the same number of ovarioles. Yet, among sisters, ovaries with more ovarioles were significantly more developed than ovaries with fewer ovarioles.

KEY WORDS Insecta, laying workers, breeding

ALTHOUGH THE QUEEN honey bee (*Apis mellifera* L.) normally lays all the eggs in a colony, worker bees can and do lay eggs. Worker bees begin to develop their ovaries when the queen and all brood are removed from a colony (Jay 1968, 1970, 1972). Many factors are known to affect the rate of ovary development of queenless groups of bees including worker age (Delaplane & Harbo 1987), worker population size (Pain 1960), nutrition (Pain 1961, Jay 1968, Kropacova et al. 1968), and the degree of ovary development of other worker bees (Velthuis et al. 1965, Jay & Nelson 1973).

Racial differences in rates of ovary development for worker honey bees have also been described. Ruttner & Hesse (1981) ranked various honey bee races with regard to rapidity of ovary development, from fastest to slowest: *A. m. capensis* (Escholtz) > *A. m. intermissa* (von Buttel-Reepen) > *A. m. scutellata* (Lepeletier) > *A. m. ligustica* (Spinola) > *A. m. carnica* (Pollmann) > *A. m. mellifera* L. Generally, African races require 5–12 d of queenlessness before workers begin to lay eggs, and European races require 14–35 d.

Such a racial difference has enabled researchers to selectively obtain eggs from single workers of the Cape bee (*A. m. capensis*) (Velthuis 1976, Velthuis & van der Kerk 1988, Hillesheim et al. 1989). However, the Cape bee is unique. Workers of the Cape bee develop their ovaries (15–20%) in the presence of the queen (Anderson 1963), and they produce parthenogenic female progeny (diploid) rather than the male (haploid) progeny produced by workers of other races (Onions 1912, 1914; Anderson 1963; Verma & Ruttner 1983).

Our objective is to breed directly from worker bees. Although nearly all of the economically important traits in honey bees are characteristics of worker bees, the traditional approach in bee breeding is to evaluate a number of colonies and then breed from queens in selected colonies. Some important qualities of honey bees are group traits, such as honey production and colony defense, and these need to be measured at the colony level. However, many important traits such as disease resistance, longevity, and development time are measurable on single worker bees. Breeding from workers would not displace breeding from queens, but it would give a bee breeder more options, and it may provide the precision needed to detect and propagate genetic combinations that occur at very low frequencies (such as resistance to the ectoparasitic mite *Varroa jacobsoni* Oudemans).

The objective in this study was to obtain eggs from a single, preselected worker bee. Because a solitary worker will not lay eggs, the problem is to get a specific worker to lay and then to determine which eggs are hers. We surveyed colonies for differences in rate of ovary development and then put one or more workers from fast ovary developing colonies into cages with twenty five workers that had slower developing ovaries.

Materials and Methods

Four unrelated colonies of bees were used in this study. Each colony was headed by a queen that

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was instrumentally inseminated with a single drone. The drones used in the inseminations were unrelated to each other and to the queens.

Workers from these four colonies, hereafter called A, B, C, and D, were evaluated in small groups in cages kept in an incubator (34 ± 1°C and ≈50% RH). The worker bees emerged in the incubator from brood combs removed from their respective colonies, and the young workers (<24-h-old) were then placed into cages for testing. The cages were those described by Kulinčević & Rothenbuhler (1973), measuring 7.5 by 11 by 12.5 cm. Each cage had a two-sided section of empty drone comb (5 by 8 cm) suspended in the center of the cage and a constant supply of honey, water, and corbiculated pollen.

Experiment 1. The four colonies were compared for the time required for workers to lay eggs and for the numbers of eggs laid. On 6 June 1989, 25 newly emerged (<24-h-old) workers from each colony were put into each of five cages. The cages were maintained in an incubator for 14 d or until they produced eggs. The time to lay eggs and the number of eggs produced were compared using a single factor analysis of variance (ANOVA). A least significant difference multiple comparison was used for mean separations analysis after significant differences were indicated by ANOVA (PROC GLM; SAS Institute 1985).

Experiment 2. A second evaluation used only workers from colony A (with more rapidly developing ovaries) and colony C (the slowest ovaries to develop in Experiment 1). Workers from colony A were wild-type in body color, and workers from colony C were homozygous cordovan. The experiment began on 21 June 1989. Thirty newly emerged worker bees (<24-h-old) were put into each cage, with 10 replicates for each of the two colonies. The 20 cages were maintained in an incubator for 14 d; mortality and egg production were recorded at 24-h intervals. At the end of the experiment, all ovaries were examined in a sample of 10 bees from each cage.

The two sets of caged workers were compared for ovary development after 14 d. Ovaries were evaluated using a grading system described by Velthuis (1970). Class 1 ovaries were undeveloped; class 2 ovaries were slightly developed with rounded to bean-shaped eggs; and class 3 ovaries were fully developed with sausage-shaped eggs. Individual bees were assigned the value of their highest developed ovary (1, 2, or 3), and the number of workers in the various classes of development were compared between colonies by calculating a χ^2 value for a 2 × 3 contingency table, source colony by level of ovary development (PROC FREQ, SAS Institute 1985).

The two sets of workers were also compared based on the number of ovarioles per bee (hierarchial analysis of variance, PROC GLM, SAS Institute 1985). The cage factor was nested within treatment for this analysis. Because the sampling error was not significantly larger than the residual error ($P \geq 0.25$), a pooled mean square error term (residual error + sampling error) was used to test main colony effects.

Within workers from colony A, we measured the correlation between ovariole number and ovary development. Ovaries with 1–3 ovarioles were compared with ovaries having ≥4 ovarioles. The Chi-square contingency table compared the three levels of development and two classes of ovary size (PROC FREQ, SAS Institute 1985).

Experiment 3. To test effects that mixing different workers might have on the ovary development of worker bees, five workers from colony A were marked with paint and placed into each of 40 incubator cages. Each cage was given an additional 25 unmarked workers from colony A (10 cages), from colony B (15 cages), or from colony C (15 cages). All bees were less than 24-h-old at the start of the experiment (2 July 1989). The cages were held in an incubator for 10 d. Egg production and mortality were recorded at 24 h intervals. At the end of the experiment, all marked workers were removed from their cages and frozen until their ovaries could be examined. Any eggs were also removed. The unmarked workers were kept in their cages for an additional 24 h to detect egg production from them.

The three treatments were compared as to the number of eggs produced. Comparisons were made for the total number of eggs produced before and after removal of the marked workers. The Kruskal–Wallis nonparametric analysis of variance for ranks (Kruskal & Wallis 1952) was used to make these comparisons. Cages that produced equal numbers of eggs were given equal ranks, the average of ranks that individual cages would have been assigned. The Kruskal–Wallis H test was then corrected for tied ranks (H_c). A nonparametric Tukey-type multiple comparison of mean ranks (Dunn 1964, Wilcoxon & Wilcox 1964) was used for mean separation analysis after differences were indicated by the Kruskal–Wallis H_c test.

The three treatments were also compared for the ovary development of the marked workers. The frequencies of marked worker bees in the three categories of ovary development for the three treatments were tabulated and tested by χ^2 analysis (PROC FREQ, SAS Institute 1985). Separate 2 × 2 contingency tables compared ovary development between marked workers caged with 25 workers from colony A and those caged with workers from colony B. A second 2 × 2 contingency table compared marked workers caged with workers from colonies A and C. These tables were formed by considering the source colonies as rows and the conditions developed (class 2 and class 3) or undeveloped (class 1) ovaries as columns.

Treatment groups were compared based on the number of ovarioles per marked worker. This comparison was made using a hierarchial analysis of variance (PROC GLM, SAS Institute 1985). The

Table 1. A comparison of egg production between four different groups of worker bees (Experiment 1) ($\bar{x} \pm SD$)

Source colony	Onset of egg-laying, d[a]	No. eggs laid during the first 24 h of egg-laying[b]	No. eggs laid in the first 14 d[c]
A	8.8 ± 0.4c	9 ± 5.0	185 ± 49a
B	8.0 ± 1.0c	9 ± 5.8	131 ± 67ab
C	24.8 ± 1.5a	7 ± 4.6	0
D	12.4 ± 2.0b	9.2 ± 4.0	42 ± 76b

Each cage contained 25 newly emerged bees (<24-h old), and 5 cages for each colony were maintained in an incubator through 14 d or until they laid eggs.

[a] Onset of egg-laying was significantly different between colonies ($F = 156.9$; df = 3, 16; $P = 0.0001$).

[b] The numbers of eggs laid during the initial 24 h of oviposition were not significantly different ($F = 0.22$; df = 3, 16; $P = 0.8784$).

[c] The numbers of eggs laid during the first 14 d were significantly different ($F = 6.13$; df = 2, 12; $P = 0.015$). Only colonies A, B, and D were compared.

cage factor was nested within treatment (sampling error) as in experiment 2.

The three treatment groups were also compared for worker mortality. Analysis of variance (SAS Institute 1985) compared dead bees per cage for marked and unmarked workers.

Experiment 4. One worker from colony A and 25 workers from colony C were placed into each of 10 incubator cages on 1 August 1989. All bees were less than 24-h-old at the start of the experiment. Cages were maintained through 14 d, and egg production and mortality were recorded at 24-h intervals. All workers from colony A and a sample of 10 workers from colony C per cage were examined for extent of ovary development at the end of the experiment. From cages that produced eggs, all workers from colony C were examined for ovary development to determine if they were capable of laying eggs.

Experiment 5. This experiment was similar to experiment 4, except the workers from colony A were aged before putting 1 per cage with 25 workers from colony C. This was done to see if more workers from colony A would develop their ovaries in the presence of sisters rather than with unrelated bees. On 16 August 1989 thirty workers from colony A (24-h-old) were placed into a cage. The cage was kept in an incubator for 14 d; egg production and mortality were recorded at 24-h intervals. On the 14th day, 28 workers (two had died) were removed, and each worker was placed into its own cage with 25 cordovan workers (<24-h-old) from colony C. Eight days later (6 September), all the workers from colony A were removed and evaluated for ovary development. Any eggs were also removed. The workers from colony C remained in their cages for three more days to detect egg production from them.

Results

Experiment 1. Workers from the four colonies differed in the time until onset of egg laying (Table 1). Workers from colonies A and B became laying workers rapidly; those from colony C were slowest, and workers from colony D were intermediate. These three groups were significantly different from one another at an $\alpha = 0.05$ as indicated by the LSD multiple comparison level (MSE = 1.925, df = 16, lsd = 1.9 d).

Workers from all four colonies produced about the same number of eggs during the first 24 h of oviposition, but they differed in the numbers of eggs produced within the first 14 d of the experiment (Table 1). We compared only the three stocks that produced eggs before 14 d (MSE = 4213.4, df = 12, lsd = 89.45).

Worker mortality was not different among the four sets of bees ($F = 0.37$; df = 3,16; $P = 0.7772$). During the 14 d, cages with workers from colonies A, B, C, and D averaged 1.0 ± 1.5, 2.0 ± 0.7, 1.2 ± 1.3, and 1.6 ± 1.1 ($\bar{x} \pm SD$) dead bees, respectively. Cages with workers from colony C lost an additional 4.3 ± 2.1 bees from day 14 through day 27.

Experiment 2. Ovary development between the workers from colonies A and C was different (Table 2). Only cages containing workers from colony A produced eggs, and workers from colony A had more class 2 and class 3 ovaries than workers from colony C. There was no difference in the number of ovarioles per bee between the two stocks (Table 2).

Table 2. A comparison of the ovary development between workers from colonies A and C

Source colony[a]	Onset of egg-laying, d[b]	Total no. eggs in 14 d, $\bar{x} \pm SD$	No. workers with ovaries in each class[c]			No. ovarioles, $\bar{x} \pm SD$[d]
			1	2	3	
A	9.0 ± 2.0	61 ± 39	15	33	52	7.4 ± 2.0
C	—	0 ± 0	96	4	0	7.6 ± 2.2

Thirty newly emerged workers (<24-h old) were placed in each cage for 14 d (10 cages per colony) (see Experiment 2).

[a] Each replicate represents a sample of 10 bees (20 ovaries).

[b] Stock C did not produce eggs.

[c] The frequencies of bees having ovaries in the three classes of ovary development (see text) were significantly different ($\chi^2 = 133.8$, df = 2, $P < 0.001$).

[d] The number of ovarioles per bee were not significantly different ($F = 0.93$; df = 1, 18; $P > 0.05$).

Table 3. Effect of ovariole number on extent of ovary development

No. ovarioles	No. ovaries in each class[a]		
	1	2	3
1–3 ovarioles	19	56	35
≥4 ovarioles	13	21	56

Ovaries from workers from colony A (Experiment 2) were classified according to size.

[a] See text for class descriptions. Ovary development between the two size classes was significantly different ($\chi^2 = 20.1$, df = 2, $P < 0.001$).

Within workers from colony A, ovaries with ≥4 ovarioles had greater development than ovaries having 1–3 ovarioles (Table 3).

Mortality was not different between the two stocks; both lost six bees.

Experiment 3. The three combinations of mixed workers differed in time of initial egg laying (Table 4). All cages with 30 workers from only colony A (10 cages) or with five workers from colony A and 25 workers from colony B (15 cages) produced eggs within 10 d, and there was no difference in time for onset of egg-laying between the two groups. The third combination was different. Of the 15 cages with five workers from colony A and 25 workers from colony C, only 10 had eggs. Moreover, the bees began laying eggs significantly later than the other two treatments (MSE = 0.1125, df = 32, lsd = 0.3).

The three treatment groups differed in the number of eggs produced during the first 10 d (Table 4). Cages with 25 workers from colonies A and B produced similar numbers of eggs, but cages with 25 workers from colony C produced significantly fewer [$SE_1 = 4.77$; $SE_2 = 4.26$; Q ($\alpha = 0.05$, df = 3) = 2.39].

After marked workers from stock A were removed from the cages, the three sets of workers differed in numbers of eggs produced (Table 4). Workers from colonies A and B produced similar numbers of eggs, but workers from colony C produced no eggs [$SE_1 = 4.77$; $SE_2 = 4.26$; Q ($\alpha = 0.05$, df = 3) = 2.39]. Because workers from colony C produced no eggs after the five workers from colony A had been removed, all eggs were probably laid by the workers from colony A.

Among the three treatments, marked workers differed in the extent of ovary development (Table 4). Marked workers placed with workers from colonies A and C did not differ in their development ($\chi^2 = 2.552$; df = 1; $P = 0.110$). Marked workers caged with workers from their own colony (A) had more developed ovaries than did those that were caged with workers from colony B ($\chi^2 = 13.5$, df = 1, $P < 0.001$). Thus, for marked workers, the order of highest ovarian development (percent workers having class 2 or class 3 ovaries) was when they were caged with workers from colony A (68%) ≈ colony C (53%) > colony B (34%). The treatments did not differ in regard to the number of ovarioles per marked worker ($F = 1.33$; df = 2,190; $0.10 > P > 0.001$). Marked workers with workers from colonies A, B, and C had 7 ± 2, 8 ± 2, and 8 ± 2 ovarioles, respectively ($\bar{x}$ ± SD).

The treatment groups did not differ in the number of marked workers that died during the experiment. One marked worker died from the colony A treatment and one died in each of three cages from both the colony B and colony C treatments. The treatments also did not differ in the number of unmarked workers that died ($F = 3.09$; df = 2,11; $P = 0.0862$); colony A lost eight, B lost four, and C lost nine.

Experiment 4. Single workers produced eggs in four of the 10 cages. In the four cages with eggs, none of the bees from colony C had developed ovaries (95 bees examined). The same was true for the 60 bees from colony C that were sampled from the remaining six cages. Because the workers from colony C had undeveloped ovaries, we concluded that all eggs in the four cages were laid by the four workers from colony A. The four workers from colony A began laying after 13 ± 0.8 d ($\bar{x}$ ± SD) and averaged 2.75 ± 1.7 eggs through day 14, and they all had class 3 ovaries. From the remaining six cages, workers from colony A had class 2 ovaries in three of the cages and undeveloped ovaries in the other three. The workers from colony A av-

Table 4. Effect of mixing workers from different colonies on worker ovary development (Experiment 3)

Colony used for the 25 bees	Onset of egg-laying, d[a], $\bar{x}$ ± SD	No. eggs ($\bar{x}$ ± SD) within 10 d[b]	No. eggs ($\bar{x}$ ± SD) from day 10–11[c]	No. marked bees in each class[d]		
				1	2	3
A	8.2 ± 0.4b	32 ± 24a	12 ± 8a	16	19	14
B	8.0 ± 0.4b	28 ± 17a	11 ± 12a	48	15	9
C	10.0 ± 0.3a	6 ± 6b	0b	34	11	27

Five marked workers from colony A were placed into cages with 25 workers from colony A (10 cages), colony B (15 cages), or colony C (15 cages). Marked workers were removed on the 10th day, and the other 25 workers were left in the cages for an additional 24 h.

[a] Mixtures began laying at significantly different times ($F = 118$; df = 3, 32; $P = 0.0001$).

[b] Significantly different ($\alpha = 0.05$) by Kruskal–Wallis nonparametric comparison ($H_c = 19.7$, df = 2, $P < 0.001$).

[c] Significantly different ($\alpha = 0.05$) by Kruskal–Wallis nonparametric comparison ($H_c = 23.62$, df = 2, $P = 0.001$).

[d] See text for class descriptions. The numbers of bees in each class were significantly different between the treatment groups ($\chi^2 = 23.0$, df = 4, $P < 0.001$).

eraged 6.9 ± 0.9 ovarioles per bee (n = 10 bees). The bees from colony C averaged 7.3 ± 2.1 ovarioles per worker (n = 155 bees).

Experiment 5. Bees in the cage that initially contained 30 worker bees from colony A began laying eggs during the 10th day (7 eggs). On the 14th day, just before the bees were separated, the total number of eggs present in the cage was 63. The workers in the cage produced 14 eggs between day 13 and day 14.

On the first day after the bees from colony A were put alone with young bees from colony C, three cages produced a total of 5 eggs. By the 8th day, 15 of the 28 cages had produced an average of 11 ± 12 ($\bar{x}$ ± SD) eggs. The workers from colony C were monitored for 3 d after removal of the workers from colony A, and no eggs were found. In cages that produced eggs, all workers from colony A had class 3 ovaries. Of the remaining 13 workers from colony A, nine had class 2 and four had class 1 ovaries.

Discussion

This study suggests that a worker from colony A was as likely to become a laying worker if she was alone with 25 bees from colony C, in a group of 5 with 25 bees from colony C, or with 29 of her sisters. When workers from colony A were caged in groups of 30 bees for 10 or 14 d (Experiments 3 and 2), 44% (66/149) of the workers had class 3 ovaries. When groups of five were caged with workers from colony C (Experiment 3) for 10 d, 38% (27/72) had class 3 ovaries. When only one was caged with 25 from colony C for 14 d, 50% (19/38) of the workers had class 3 ovaries. These three frequencies were not significantly different (χ^2 = 1.742, df = 2, P = 0.419).

Are some workers unable to become laying workers? This consideration is important when selecting and breeding from workers. Under our conditions, about half the workers from colony A had class 3 ovaries, and another 34% (64/187) had class 2 ovaries. These ratios were fairly constant, whether the bees were in groups of 30, five workers with 25 unrelated bees, or 1 with 25 unrelated bees. If we count class 2 ovaries as those that would produce eggs in a short time, then 84% of the workers from this colony would probably produce eggs using this technique. Because ≈15% of the bees from colony A did not develop their ovaries regardless of how they were treated (especially when put alone with slow developing workers), we suspect that they may be incapable of laying eggs.

Although the results were with just one colony, similar percentages have been found with other colonies. When a selected worker was replaced with newly emerged bees (<24-h-old) at 5-d intervals, 7 out of 8 workers had class 2 or 3 ovaries after 10 d) (Harris & Harbo 1988). When 85 newly emerged bees (<24-h-old) were taken from 17 colonies (five bees per colony, each colony with a naturally mated queen, all queens were sisters) and placed into two cages (40 bees in one cage, 45 in the second), we found that 54% (46/85) of the bees had class 3 ovaries, 32% (27/85) had class 2 ovaries, and 14% (12/85) had class 1 ovaries. Thus, 86% of the workers had developed ovaries after 14 d. Hess (1942) also found 80–90% of workers to be developed after a few weeks of queenlessness. Again, these results indicate that 10–15% of the workers may not be capable of developing.

Certainly one can expect a worker from colony A to lay eggs >50% of the time. This is probably also true for workers from other colonies with rapid ovary development. More time would be needed to produce eggs from workers from colony C or from workers with similarly slow developing ovaries, and the success rate is likely to be lower. Nevertheless, all cages with workers from colony C (Experiment 1) produced eggs, and numbers of eggs produced during the initial 24 h of egg-laying were not significantly lower than those from the other three colonies examined.

Based on our results, the following procedure would be used to produce eggs from chosen workers:

- Evaluate rates of ovary development for various colonies to find slow-developing workers that could be mixed with other workers as nonlaying workers.
- If possible, use nonlaying workers that are phenotypically mutant as a safeguard to determine that the progeny were produced by the chosen workers. For example, our nonlaying workers from colony C were homozygous cordovan and our chosen workers from colony A were wild-type.
- Use one chosen worker with 25 nonlaying workers in a cage to produce eggs from the chosen worker. The time needed to produce eggs will vary with the source colony used for chosen workers. If many workers expressing the trait of interest can be easily obtained, five chosen workers could be mixed with 25 nonlaying workers to increase the chances of producing eggs from chosen workers within a single cage.
- Feed pollen. Bees need protein for ovary development.

When five workers from colony A were caged with 25 other workers for 10 d, they had more developed ovaries when caged with workers from colonies A and C and least developed ovaries when caged with workers from colony B. If kin recognition between workers from colony A and their cagemates had affected ovary development, the five workers mixed with 25 of their own sisters should have produced significantly more developed workers. Because ovary development of workers from colony A was equally high with sisters or with completely unrelated workers from colony C, we suspect that kin recognition played only a minor role.

The differences in ovary development for workers from colony A in these different mixed populations may have involved dominance hierarchies. Various researchers have noted that in queenless groups of bees, dominant bees that are fed by subordinate bees become laying workers while the subordinate bees remain undeveloped (Korst & Velthuis 1982, Velthuis 1985). In the Cape bee, these hierarchies are well established (Moritz & Hillesheim 1985) and have been shown to have a genetic basis (Hillesheim et al. 1989). Thus, unmarked workers from colonies A and C may have been better subordinate workers in our mixtures than more dominant workers from colony B.

As an alternative, dominance hierarchies may not have been established within our mixtures, and differences in ovary development may represent competitive differences in rates of ovary development by workers from different colonies. When workers from colony A were mixed with slow developing workers from colony C, workers from colony A had an innate advantage over workers from colony C due to their rapid development. When workers from colony A were caged with their own sisters, all workers had equal rates of development and probably had equal chances of becoming egg layers. When workers from colony A were caged with fast developing workers from colony B, the workers from colony B may have had a slightly faster rate of development. Although the time required for workers from colonies A and B to lay eggs were not significantly different, workers from colony B were slightly faster (Experiment 1). Because workers with more developed ovaries have been shown to inhibit ovary development in nestmates (Velthuis et al. 1965, Velthuis 1970), the faster developing bees in workers from colony B may have inhibited ovary development of workers from colony A.

Ovaries with ≥ 4 ovarioles were more developed than ovaries having 1–3 ovarioles. Within groups of sister bees in other experiments (unpublished), we also found that ovaries with more ovarioles developed more rapidly. Velthuis (1970) reported the opposite, and Allsopp (1988) reported no correlation.

Among sister workers, ovaries with more ovarioles developed faster than those with fewer ovarioles, but potential ovary development was not limited by ovariole number. Large differences in ovary development between workers from our fast and slow developing colonies were independent of ovariole number, because both groups had equal numbers of ovarioles per bee. We also found single bees in which an ovary with only one ovariole became fully developed while the other ovary with 3–5 ovarioles remained undeveloped.

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