Breeding Honey Bees (Hymenoptera: Apidae) for More Rapid Development of Larvae and Pupae

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ABSTRACT A shorter development time for the honey bee (*Apis mellifera* L.) would allow the parasitic mite *Varroa jacobsoni* Oudemans less time to reproduce and may provide the bee colony with some resistance to the mite. I developed an accurate way to measure development time, measured variance and heritability of development time of honey bees in Baton Rouge, and determined if colonies with rapidly developing workers produced more rapidly developing queens. Newly hatched larvae were obtained by placing combs that contained eggs into an incubator with no adult workers present. After waiting 1–3 h, newly hatched larvae were identifiable because they had no brood food in their cells. These unfed larvae from different colonies were transferred to a single comb and reared in a nurse colony. Workers (n = 180) from 26 different colonies averaged (mean ± SD) 114.5 ± 4.3 h for the uncapped larval period and 285.4 ± 5.1 h for the capped period. Heritability ± SEM was 0.41 ± 0.15 for the uncapped and 0.61 ± 0.19 for the capped period. Stocks with rapidly developing workers did not always produce rapidly developing queens (queen–worker regression slope not > 0), so workers must be evaluated rather than queens. These data predict that selective breeding from 10% of the population should reduce the mean capped period of workers by 5 h in a single generation.

KEY WORDS *Apis mellifera, Varroa jacobsoni,* selection

WHEN DISCUSSING the development of immature, worker honey bees (*Apis mellifera* L.), a beekeeper describes an egg stage (≈3 d), a period of uncapped brood (≈5 d), and a period of capped brood (≈12 d). These times are quite uniform because bees keep their brood area at a constant temperature (≈34.5C).

Despite this uniformity, development time is known to be genetically variable. In an incubator test, Harbo et al. (1981) showed that eggs of Africanized bees hatched ≈4 h sooner than eggs of European bees. When comparing other stages of development, Bolten (1986) found that larval and pupal development times were also based on genotype rather than on cell size or on the genotype of the nurse bees. Moritz (1985) found that the mean duration of the capped period of the South African cape bee (*A. mellifera capensis* [Escholtz]) was ≈2 d shorter than that of a European subspecies (*A. mellifera carnica* Pollmann), and that this character was highly heritable (h^2 = 0.8).

The capped period is of special interest because reproduction of the parasitic mite *Varroa jacobsoni* Oudemans occurs only within the capped cell. The uncapped period consists of the egg stage and most of the larval stage, but the capped period includes the last 2 d of the larval stage, a 2-d prepupal stage, all of the pupal stage, and the first half day of the adult stage (Bertholf 1925). The cap of the cell refers to a thin layer of wax that adult workers place over the brood cells. After a cell is capped, the bee larva consumes the remainder of the food in the cell, spins a cocoon, and pupates. After shedding its pupal skin, the teneral adult ends the capped period by chewing through its cocoon and wax cap and emerging from the cell.

A shorter capped period for the bee would provide less time for *V. jacobsoni* to reproduce and may provide the bee colony with some resistance to the mite. A female mite enters a cell shortly before it is capped and cannot leave until the cell is uncapped. A mite requires ≈240 h in a capped cell to produce one mature female and additional females are produced at 30 h intervals (Rehm & Ritter 1989). Males and immature female mites die when the cell is uncapped by the adult bee that emerges from the cell. Therefore, a capped period of 300 h (12.5 d) could produce three new female mites from a cell that was initially infested with only one; a capped period <240 h (10 d) would produce none. Thus resistance to this parasite might be acquired by selecting bees for a shorter capped period. Büchler & Drescher (1990) found that a 1-h reduction in capping period corresponded with an 8.7% reduction in the mite population.

The three objectives of this study were (1) to develop a method for accurately measuring the capped and uncapped periods of brood development, (2) to determine if this measuring technique can be used to select successfully for rapid development time within the Baton Rouge population of honey bees, and (3) to determine if one can select for rapidly developing workers by selecting rapidly developing queens.

Materials and Methods

General Design. I measured the time required for a newly hatched larva to develop into an adult worker. For each larva, I measured the time from egg hatch to capping (hereafter called the uncapped period) and the time from capping to emergence from the cell (capped period).

To maintain uniformity, newly hatched larvae were evaluated at the same time in the uniform environment of a single colony. Newly hatched larvae were identified by making use of the facts that adult workers put food into a cell within minutes after an egg hatches in that cell and that eggs will hatch in an incubator without the presence of adult bees.

After colonies from which to measure development time were chosen, combs were collected from those colonies and placed in an incubator (34.5°C; 50–60% RH). The combs contained eggs about to hatch; no adult workers were present in the incubator. After 1–3 h, suitable larvae were identified as those without brood food in their cells. These larvae had hatched since the comb had been taken from their colony, and their age could therefore be accurately determined.

A single comb and a nurse colony were chosen to receive the larvae for testing. The comb chosen to receive the larvae for testing had young worker larvae (aged 1–2 d) in the center and eggs at the periphery. This arrangement ensured that the brood being tested was surrounded by brood of about the same age. Larvae were removed from cells on this comb to create three areas, each 4 cells high and 10 cells wide. If larvae from fewer than 10 colonies were tested, then the areas were made narrower. The test areas looked like three parallelograms near the center of the comb.

After brood combs had been in the incubator for about 1 h, I began to transfer larvae to the cells in the prepared comb. Within each area (4 by 10 cells), I randomly determined the column that was assigned to each colony. The resulting sequence of columns (left to right) dictated the order of the larval transfers. Larvae of known age from one colony source were placed in a column of four cells in each of the three test areas.

The transfer process required about 1 min per larva, so transferring a group of 10 colonies (120 larvae) required ≈2 h. To find and manipulate these tiny larvae, I used a dissecting microscope fitted with a 0.5× objective to obtain a focal distance of 14 cm and a magnification of 3.5. The dry larvae were picked up by touching their rear dorsal side with a dry, flattened wire. After all larvae had been transferred, the larvae (now in one comb) were placed into a populous colony to be reared.

Development time was established for each worker by checking the comb at 2-h intervals during the capping and emergence periods. A pin was placed near each test area to mark where it began. Capping time was determined by checking the cells in the colony starting 108 h after the larval transfer. Emergence times were measured with the test comb in an incubator (34.5°C).

Experiment 1. Because the basic procedure often requires a newly hatched larva to remain unfed for 3–4 h, this experiment tested the effect of not feeding on the subsequent development time of the bee. I used a 2 X 2 factorial design. Factor 1 was time (the age of the larvae [6–8.5 h or 0–2.5 h] when they were moved from the incubator to a nurse colony); factor 2 was whether or not larvae were fed while in the incubator. The test comb differed from that described above in that it consisted of a single test area with nine columns and five rows. Each treatment was randomly assigned two columns except the unfed, 6–8.5 h treatment, which was assigned three columns. The larvae were supersisters, (i.e., daughters of a queen mated to a single drone).

This experiment was begun on 20 July 1990. At 1315 hours, I removed brood food and larvae or removed only larvae and left the food in the cells of the 5 by 9 test area. At 1330 hours, I transferred 10 larvae (aged 0–2.5 h) to the test comb into cells with brood food and 15 into dry cells; the test comb was kept in the incubator. Another comb from the same colony was put into the incubator at 1650 hours, and at 1920 hours I transferred 20 larvae (aged 0–2.5h) into cells with and without brood food. The test comb was put into a nurse colony at 1930 hours.

Experiment 2. To assess the potential for selective breeding for shorter development time of the worker caste, I measured the variability and heritability of development time. The test population consisted of honey bees in the Baton Rouge area.

For this experiment, I prepared a group of 26 colonies from bees in the Baton Rouge area. The 26 queens for these colonies were produced from 22 different sources (colonies) and each queen was inseminated with semen from a single male (the 26 males [drones] were taken from 19 different colonies). Because a drone honey bee is haploid and produces genetically identical spermatozoa, the worker progeny in each colony had a relatedness of 0.75.

Table 1. Analysis of variance of development times of larvae that were fed or not fed during their time in the incubator (experiment 1)

Source	df	Uncapped period			Capped period		
		MS	F	P	MS	F	P
Food	1	406.8	29.3	0.0001	17.5	2.8	0.11
Time	1	192.1	13.9	0.0012	2.8	0.9	0.56
Food*Time	1	430.8	31.1	0.0001	10.9	1.4	0.25
Error	22	13.9			7.9		

Side-by-side comparisons of larvae from the different colonies followed the general procedure described above, but the experiment was run during four different testing periods that began on the following dates: 1 June, 8 June, 19 July, and 2 August 1990.

Larvae were transferred from 8–10 colonies during each test period. Twelve larvae were transferred from each colony, and data were used from a colony as long as at least three workers survived to adulthood. A few of the colonies were evaluated at two or more test periods, but data from these colonies were used only from one test period. Each test period provided usable data from five to eight colonies.

Experiment 2 was evaluated as a random design, with colonies nested within time, using the general linear models procedure of SAS Institute (1979). Colonies were compared for mean duration of the uncapped larval period, the capped period, and total of both periods. Heritabilities (h^2) for the three traits (uncapped, capped, and total) were estimated with the variance components from the analysis of variance (Rinderer 1977, Oldroyd & Moran 1983, Collins 1986). The VARCOMP procedure (SAS Institute 1979) identified the between-colony variance and the within-colony variance. Because workers within each colony had a relatedness of 0.75 and those in different colonies were unrelated, heritability was calculated by dividing the intraclass correlation (between-colony variance divided by the sum of the within- and between-colony variances) by 0.75. Standard errors were computed with the formulas of Oldroyd & Moran (1983). The expected response to selection (R) was calculated with the equation $R = ih^2\sigma_P$, where i equals the intensity of selection and σ_P equals the phenotypic standard deviation (Falconer 1981, Rinderer 1986).

Experiment 3. In this experiment, I used three rapidly developing and three slowly developing stocks to see if sisters of rapidly and slowly developing workers would be rapidly and slowly developing queens. I used only total development time in this experiment.

Larvae were transferred into queen cells in the same way as they were moved into worker cells. Worker and queen cells were all furnished with 4 μl of fresh royal jelly that had been diluted 1:1 with water. The queens were reared on three bars with 18 cells per bar. Three larvae from each of the six stocks were randomly placed on each bar. Four larvae from each stock were placed in each of three areas that had been prepared in a comb of worker brood (as described in the general procedure).

A disadvantage of evaluating queens is that they cannot be placed as close together as worker cells, so temperature and feeding are apt to be more variable for a group of queens than for a similar number of workers. The temperature effects are also a factor in an incubator, so special cages were built to keep the queen cells within a radius of 10 cm while in the incubator.

Four analyses were done on these data. First, I used analysis of variance to determine if colony source affected the development time of queens. Second, workers were analyzed in a similar way, even though experiment 2 had already shown a significant colony effect for workers. Third, each worker mean was paired with its respective queen mean from the same colony to plot six points of a simple linear regression. A positive slope significantly >0 ($P < 0.05$) would indicate that the development times of queens and workers follow the same trend. Finally, the six worker means were similarly paired with previous worker means from the same colony (data from experiment 2) and analyzed by linear regression. Because test period (called "time") was a significant factor in experiment 2, I added the overall mean to the residual for each observation from experiment 2 to minimize the effect of time from that data.

Results and Discussion

Experiment 1. For newly hatched larvae that were unattended by workers in the incubator, both the duration of their stay and the presence of food affected the length of their uncapped period in the nurse colony (Table 1). Mean uncapped periods were 127.5 h (not fed, 6–8.5 h [n = 3]), 110.1 h (fed, 6–8.5 h [n = 8]), 112.8 h (not fed, 0–2.5 h [n = 8]), and 113.0 h (fed, 0–2.5 h [n = 7]); mean capped periods were 283.5, 283.9, 282.8, and 286.0 h, respectively.

A highly significant interaction indicated that feeding affected the uncapped period of older and younger larvae in different ways (Table 1). The presence of food reduced the uncapped pe-

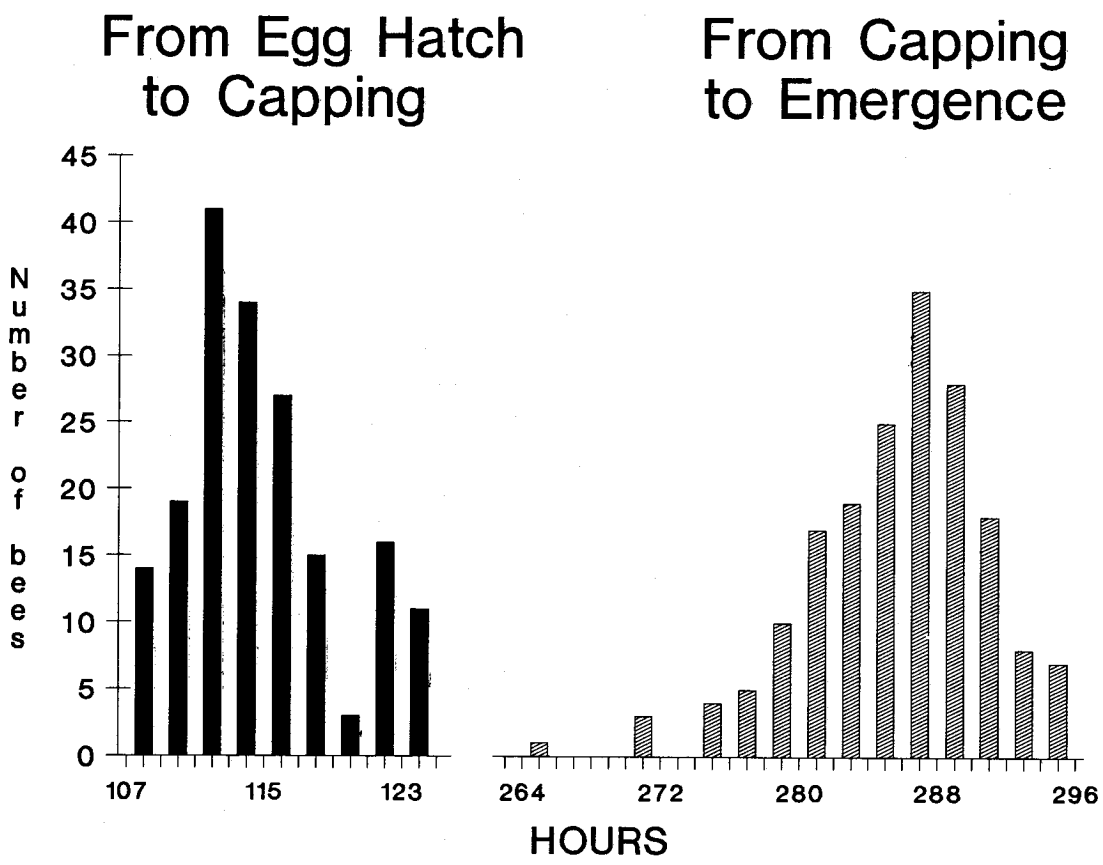

Fig. 1. Development times of 180 worker bees from 26 different colonies in Baton Rouge, LA (experiment 2).

riod for larvae kept in the incubator for 6–8.5 h, and the absence of food extended it. Food had no effect on larvae in the incubator for 0–2.5 h.

In contrast, neither feeding nor time in the incubator affected the duration of the capped period (Table 1). Therefore, if one is interested only in the capped period, keeping newly hatched larvae in the incubator as long as 8.5 h should not alter the results. However, of the larvae kept in the incubator for 6–8.5 h, those without food had a significantly lower survival rate (3/15) than those with food (8/10) ($\chi^2 = 6.5$; df = 1; $P < 0.05$).

Experiment 2. Development times (mean ± SD) for 180 workers from 26 different colonies were 114.5 ± 4.3 h for the uncapped larval period and 285.4 ± 5.1 h for the capped period (Fig. 1). Combined times for the uncapped and capped stages ranged from 379 to 417 h, so by adding 71 h for the egg stage (Harbo & Bolten 1981), total development times ranged from 450 to 488 h (18.8–20.3 d) with a mean of 19.6 days. None of these worker bees had a development time as long as 21 d, the generally accepted period.

The statistical model required that the four groups of randomly chosen colonies have equal means. There were differences in the four time periods (based on mean squares in Table 2; $F = 21.5$ and 1.6 for the uncapped and capped periods (df = 3, 22; $P < 0.001$ and ns, respectively), but these were factored out of the analysis. Two colonies served as a base line by being evaluated in all four test periods. The development times of bees from these colonies showed the same changes in their means as did the group means. I therefore concluded that group means were equal and that differences in time periods were caused by the environment of the nurse colony.

Estimates of heritability indicate that a selection program is likely to be successful. By selecting within the Baton Rouge population, it should be possible to use standard breeding methods and this measuring technique to produce bees with a shorter development period. Heritability (h^2) was 0.41 for the uncapped larval period, 0.61 for the capped period, and 0.52 for the total development time after egg hatch (Table 2).

Table 2. Sibling analysis of 180 worker bees from 26 different colonies to estimate the heritability of development time of the worker caste (Experiment 2)

Variable	Source	df	MS	Composition of mean square[a]	Components[b]
Uncapped period	Time	3	568.1	$= \sigma_W^2 + k_2\sigma_Q^2 + k_3\sigma_T^2$	
	Colony (time)	22	26.4	$= \sigma_W^2 + k_1\sigma_Q^2$	$\sigma_Q^2 = 2.9$
	Progenies	154	6.6	$= \sigma_W^2$	$\sigma_W^2 = 6.6$
$h^2 = 0.41 \pm 0.15$ (± SEM)					
Capped period	Time	3	147.2	$= \sigma_W^2 + k_2\sigma_Q^2 + k_3\sigma_T^2$	
	Colony (time)	22	93.0	$= \sigma_W^2 + k_1\sigma_Q^2$	$\sigma_Q^2 = 11.6$
	Progenies	154	13.8	$= \sigma_W^2$	$\sigma_W^2 = 13.8$
$h^2 = 0.61 \pm 0.19$					
Uncapped and capped period	Time	3	3357.8	$= \sigma_W^2 + k_2\sigma_Q^2 + k_3\sigma_T^2$	
	Colony (time)	22	136.6	$= \sigma_W^2 + k_1\sigma_Q^2$	$\sigma_Q^2 = 16.2$
	Progenies	154	25.6	$= \sigma_W^2$	$\sigma_W^2 = 25.6$
$h^2 = 0.52 \pm 0.17$					

[a] The ks are constants based on estimates of average numbers of workers sampled; $k_1 = 6.85$; $k_2 = 7.15$; $k_3 = 43.5$.
[b] σ_W^2 is the within-colony variance; σ_Q^2 is the betweeen-colony variance. $h^2 = t/r = \sigma_Q^2/(\sigma_Q^2 + \sigma_W^2)/0.75$.

The lower heritability for the uncapped larval period probably reflects environmental variability. During the uncapped period, larvae interact directly with adult workers, and this interaction (especially with food and feeding) is a likely cause of variability. In contrast, the capped stage has no direct contact with the adult population and can develop normally in an incubator without adult bees.

By selecting two or three breeders from the population of 26 colonies (10% of the population selected from one tail of the distribution), $i = 1.6$ and the predicted response to selection (R) = 4.9 h for the capped period. This result means that the mean duration of the capped period is expected to change by 4.9 h with one generation of selection. If selecting for duration of the uncapped or total development times, $R = 2.0$ h and 5.4 h, respectively.

This estimate of response to selection was very close to the actual response of the F_1 generation. After propagating from two colonies with the shortest period of capped worker brood and using the same evaluation techniques as described for experiment 2, worker bees in the F_1 generation had a capped period of 281.8 h (mean of 95 workers from 15 colonies). Those similarly selected for long capped period averaged 291.4 h (mean of 69 workers from 13 colonies).

Based on the results above, I conclude that the estimate of heritability for duration of capped period seems to be accurate. The design of my experiment allows dominance effects to increase the estimate of heritabilty falsely. However, because the actual response to selection was so close to the predicted response, genetic dominance seemed to have had little effect.

Experiment 3. The development time of queens was affected by colony source, but the effect was not as strong as that shown by workers from those colonies (Table 3). Because difference in worker development time was the basis for choosing the six colonies for this experiment, one cannot conclude that the development times of workers show more intracolony variation than that of queens. Instead, both workers and queens showed significant intracolony variation.

Stocks with rapidly developing workers did not always produce rapidly developing queens (Fig. 2). The regression equation for the total development times of workers (x) and queens (y) from the same colonies was $y = 210 + 0.20x$, and the slope was not significantly different from zero ($F = 0.93$, df = 4, $P = 0.39$, $R^2 = 0.19$). When the development times of workers (x) was compared with previous measurements of workers (y) from the same six colonies, the slope was significantly different from zero ($y = -19.6 + 1.06x$; $F = 36.0$, df = 4, $P = 0.004$, $R^2 = 0.90$) (Fig. 2).

Therefore, I conclude that selection and propagation cannot be effectively done in a single step by simply selecting rapidly developing queens during stock propagation. Nevertheless, queen selection may still have a function. The correlation between the development times of workers and queens was weak ($r = 0.4$), but it was a positive relationship. As long as queens need to be produced for propagation, the step

Table 3. Effect of bee parentage (colony) on the total development times (after egg hatch) of 24 queens and 46 workers (experiment 3)

Variable	Source	df	MS	F	P
Queen development time	Colony	5	40.1	3.6	0.019
	Error	18	11.1		
Worker development time	Colony	5	298.8	19.3	<0.001
	Error	40	15.5		

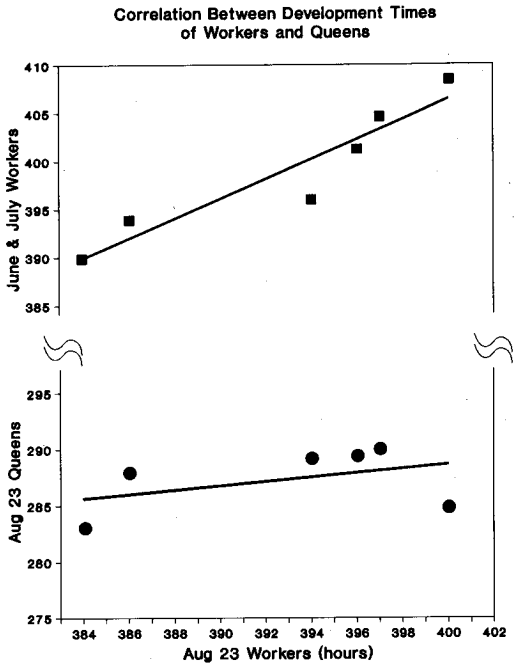

Fig 2. Each circle represents the mean development times (from egg hatch to emergence) of workers and queens from the same colony; each square represents the same worker data plotted with worker data that had been collected earlier from the same six colonies. The slope was different from zero when comparing workers to workers ($P = 0.004$) but not when comparing queens to workers ($P = 0.39$) (experiment 3).

may as well include selection. However, selection of breeder colonies should be based on data from workers rather than from queens.

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