

# Does the diapause experience of bumblebee queens *Bombus terrestris* affect colony characteristics?

M. BEEKMAN and P. VAN STRATUM Institute for Systematics and Population Biology, University of Amsterdam, The Netherlands

**Abstract.** 1. Bumblebee colonies show much variation in the number of workers, drones, and queens produced. Because this variation prevails even when colonies are kept under identical conditions, it does not seem to be caused by extrinsic factors but rather by differences between founding queens.

2. The most likely factor that could cause differences between queens is diapause. Although colonies are raised under standardised conditions, the queens often experience diapause of different length. If there are costs associated with diapause that influence post-diapause reproduction, the diapause history of the queens could affect colony characteristics.

3. Here, several colony characteristics are compared: number of first and second brood workers; total number of workers, drones, and queens; energy spent on sexuals; sex ratio; rate of worker production; time to emergence of first reproductive; and colony lifetime. Colonies were used where the queens experienced a diapause treatment of 0 (*nondiapause* queens), 2, and 4 months.

4. Although no proof was found for the existence of costs associated with diapause, the colony characteristics of nondiapause queens were significantly different from those of diapause queens. Colonies of nondiapause queens produced the lowest number of workers but the highest number of young queens.

5. It is argued that these nondiapause colonies are more time-constrained than diapause colonies because nondiapause colonies produce two generations within the same season and should therefore be more efficient in producing sexual offspring.

6. Moreover, nondiapause colonies should rear a more female-biased sex ratio because they can be certain of the presence of males produced by other (diapause) colonies.

**Key words.** *Bombus terrestris*, bumblebees, colony characteristics, costs of diapause, diapause, nondiapause, trade-off.

## Introduction

Bumblebees are social insects that are found mainly in temperate regions of the world. Only queens are able to survive winter, in a state of diapause, and they hibernate in small cavities (hibernacula) that are often situated on north-facing banks or slopes (Alford, 1969). In early spring, queens that have mated the year before leave their hibernation sites to found new colonies. The queen initiates the colony by producing a batch of diploid eggs that will develop into the

first workers. These workers assist the queen in raising subsequent broods. These subsequent broods first develop into more workers, while sexual individuals (young queens and males) emerge later in the colony cycle. After mating, the young queens go into diapause while the males and the maternal colony with the old queen and workers die in late summer.

One of the most striking phenomena of bumblebee biology is the fact that colonies are so variable (Müller & Schmid-Hempel, 1992a,b). The observation that this variation in, for instance, number of workers and sexuals produced prevails even if colonies are kept under identical (laboratory) conditions with ample food (M. Beekman and P. van Stratum, pers. obs.) suggests that it is not caused directly by extrinsic factors.

Correspondence: Madeleine Beekman, Department of Animal and Plant Sciences, Sheffield University, Sheffield S10 2TN, U.K. E-mail: M.Beekman@Sheffield.ac.uk

There could however be factors that affect colony characteristics indirectly by influencing the queen's ability to produce (sexual) offspring.

Diapause is a probable cause for variation among queens that might lead to differences in the ability to produce offspring. Bumblebee queens often experience diapause of different length. Because diapause is generally believed to entail costs that become visible after diapause completion as decreased survival, development, or reproduction, differences in the length of diapause experienced by bumblebee queens could result in variance in post-diapause reproduction.

A trade-off in the allocation of metabolic reserves between diapause maintenance and post-diapause reproduction has been suggested (Danks, 1987) and has been shown in several insect and mite species. For instance, in the predatory mite *Amblyseius potentillae* (van Houten *et al.*, 1991), preliminary experiments revealed a negative relationship between the duration of diapause and lifetime reproductive success of females. A trade-off between the length of diapause and post-diapause reproduction has also been suggested for the spider mite *Tetranychus urticae* (Kroon & Veenendaal, 1998). In the flesh fly *Sarcophaga crassipalpis* (Denlinger, 1981) and the bruchid *Kytorhinus sharpianus* (Ishihara & Shimada, 1995), a negative effect on female reproductive success was found with increasing diapause length. As a final example, the multi-voltine pear leaf miner *Bucculatrix pyrivorella* showed an increase in the number of eggs in the ovaries with successive generations, the number of eggs being lowest in the overwintered generation (Fujiie, 1980). These examples show that diapause often entails costs that have an effect on other life-history traits.

Whether these costs of diapause influence post-diapause reproduction in bumblebees is unknown, but it seems likely because diapausing bumblebee queens require large fat reserves (Horber, 1961; Holm, 1972). Because these fat reserves are used during diapause, the amount of metabolic reserves left after completion of diapause depends on diapause length. A previous study (Beekman *et al.*, 1998b) showed that weight prior to entering diapause has an important effect on the diapause survival of bumblebee queens: queens with a wet weight below 0.6 g were unable to survive diapause irrespective of diapause length. Although these results showed the importance of queen quality (weight) for survival, no relationship was found between weight prior to entering diapause and the ability of the queens to lay eggs.

In the study described here, characteristics of 58 colonies of the bumblebee *Bombus terrestris* were measured. The number of first and second brood workers, total number of workers, drones, and queens, energy spent on sexuals, sex ratio, rate of worker production, time to emergence of first sexual, and colony lifetime were assessed. The queens that started these colonies had experienced diapause for 2 or 4 months (*diapause* colonies) or had not entered diapause (*nondiapause* colonies). If there is an effect of diapause length experienced by the queen on post-diapause reproduction, an effect is expected on (1) the number of first and second brood workers, (2) the rate at which workers are produced in the linear growth phase of the

colony (which starts after the workers of the second brood have emerged; Duchateau & Velthuis, 1988), and (3) the queen's lifespan (which determines colony lifetime). The queen has a direct influence on the number of first brood workers produced because these workers are reared solely by the queen. As soon as the larvae have entered the pupal phase, the queen lays the eggs of the second brood on top of these pupae (Duchateau & Velthuis, 1988). Thus, the workers of the second brood are reared by the queen and the first brood workers. The rate at which workers are produced in the linear growth phase of the colony (as a measure of the egg-laying rate) is also under direct influence of the queen and could therefore be affected by the length of diapause experienced. Longer diapause is more costly because more metabolic reserves are used, so it is expected that the number of first and second brood workers, the rate of worker production, and the colony lifetime will decrease with increasing diapause length.

As soon as the workers of the second brood have emerged, workers take over all foraging activities from the queen, and feed and tend the brood. From that moment, the queen solely lays eggs. The timing of the production of sexuals is determined by the amount of food brought into the colony by the workers (Beekman *et al.*, 1998a) and thus by the efficiency of the worker force. Moreover, although the queen determines whether diploid or haploid eggs are laid, the workers decide whether they raise workers or queens from the diploid eggs and they could even kill haploid eggs or larvae selectively (Shykoff & Müller, 1995). It is therefore assumed that total number of workers, timing of the production of sexuals (measured as time to emergence of the first sexual), number of sexuals, and sex ratio are not influenced directly by the condition of the queen. Thus, it is predicted that the diapause experience of the queen does not have an effect on these colony characteristics.

## Materials and methods

Bumblebee colonies were kept in the laboratory from 1993 and provided with ample pollen and sugar water (Bee-Fit<sup>®</sup> 'Bijenhuis', Wageningen, The Netherlands). Queens were collected from several colonies and assigned randomly to different diapause conditions. Forty-eight queens were used that had been in diapause under one of the following conditions: 2 months ( $n=7$ ) or 4 months ( $n=10$ ) at  $-5^{\circ}\text{C}$ , 2 months ( $n=6$ ) or 4 months ( $n=11$ ) at  $0^{\circ}\text{C}$ , 2 months at  $5^{\circ}\text{C}$  ( $n=14$ ). Although more queens were subjected to the different diapause conditions (Beekman *et al.*, 1998b), these either did not survive or did not lay eggs. In addition, 10 laboratory-reared queens were used that had not been in diapause (*nondiapause* queens: queens that started to lay eggs without a period of cold storage or  $\text{CO}_2$  anaesthetisation; Beekman *et al.*, 1996; Beekman, 1998). In a previous study, it was shown that diapause length rather than temperature during diapause determines the survival and post-diapause performance (the ability to start a new colony) of queens (Beekman *et al.*, 1998b). Queens with

the same diapause length were therefore treated as one group irrespective of temperature. This yielded the following treatments: diapause length of 2 months ( $n=27$ ) and 4 months ( $n=21$ ), and nondiapause ( $n=10$ ). Five queens were placed together in a perforated plastic box with sugar water and stored in an incubator (in continuous darkness;  $RH \geq 60\%$ ). After 1 week, the sugar water was removed because it was assumed that by that time the queens were physiologically adapted to diapause (queens in diapause do not feed).

After the diapause treatment, queens were put singly into small cages in a climate room ( $29^\circ\text{C}$ ,  $62\%$  RH, continuous darkness) and provided with ample pollen and sugar water. To stimulate egg laying, two to four callow honeybee workers were added to each queen. Nondiapausing queens were treated in the same way, apart from the diapause treatment. Queens, both diapause ( $n=48$ ) and nondiapause ( $n=10$ ), that started laying eggs within 6 weeks after honeybee workers were added were selected and used for the experiment.

As soon as the first workers emerged, the colony was transferred to a larger box. If necessary (depending on the size of the colony), a second box was later connected to the first. Brood development was monitored three times a week. One of the aims of the study was to determine the rate at which workers are produced in the linear growth phase of the colony (Duchateau & Velthuis, 1988). The number of first and second brood workers was therefore adjusted to 40 workers to exclude the possible effect of different numbers of first and second brood workers. This was done by removing surplus workers randomly or by adding workers of about the same age from colonies with a surplus of first and second brood workers.

After this adjustment, the number of new workers was counted three times a week and the rate of worker production was calculated. Time to emergence of the first sexuals and the total number of sexuals was also determined. Colony lifetime was defined as ending with the emergence of the last sexual. Because the average developmental time of a male is 26 days and the average developmental time of a gyne is 30 days (Duchateau & Velthuis, 1988), colonies were not discarded until 30 days after the death of the queen to assure that all gynes produced by the queen had emerged. Because workers are able to produce males and will do so after the queen has died, colonies were discarded 26 days after death of the queen if no more gyne pupae were present (these can be distinguished easily from male pupae by their size).

Energy spent on sexuals (in kJ) was calculated by multiplying the number of queens by 7.83 and adding the number of males multiplied by 2.35 (7.83 and 2.35 kJ are needed to produce one queen and one male, respectively; Beekman & van Stratum, 1998).

Because the different colony characteristics were determined for each colony and were thus not independent, MANOVA was used to test for differences among treatments. In addition to comparing the colony characteristics assumed to be under the control of the queen (i.e. number of first and second brood workers, rate of worker production, and

colony lifetime), the whole set of colony characteristics measured was also compared among the treatments.

## Results

The characteristics of colonies headed by queens that had experienced different diapause treatments are shown in Table 1.

Is there any effect of diapause duration on the characteristics that are assumed to be under queen control: number of first and second brood workers, rate of worker production, and colony lifetime? Comparing the three treatments of different diapause length (0, 2, and 4 months) did not yield a significant effect on these characteristics (MANOVA,  $P > 0.5$ ). Nor were the differences significant if the 2- and 4-month (diapause) treatments were combined and compared with nondiapause ( $P > 0.5$ ) or among 2- and 4-month ( $P > 0.5$ ) treatments. If all characteristics measured were compared, however, there was a significant effect of diapause length ( $P < 0.05$ ). This effect became larger if diapause colonies were compared with nondiapause colonies ( $P < 0.001$ ). No significant difference was found when the 2-month diapause treatment was compared with the 4-month treatment ( $P > 0.05$ ) or when temperature during diapause treatment was included in the analysis ( $P > 0.05$  for any colony characteristic and for the characteristics under queen control), confirming that temperature was relatively unimportant.

Because there were no significant differences in the colony characteristics assumed to be under queen control, all colony characteristics measured are henceforth considered.

Although the number of first and second brood workers (before adjusting the total number to 40 workers) did not differ among the treatments (one-way ANOVA,  $P > 0.05$ ), colonies headed by nondiapause queens produced the smallest number of workers and the largest number of young queens. As a result of the high number of queens produced, the total amount of energy spent on sexuals was also largest because the amount of energy needed to raise one queen is much larger than the amount needed to raise one male.

Colonies are divided according to the sex of the first-emerged sexual in Table 2. Colonies that produce queens first produce a more female-biased sex allocation (difference in total number of queens is statistically significant: Mann–Whitney  $U$ -test,  $P < 0.001$ ) and start producing sexuals later (Mann–Whitney  $U$ -test,  $P = 0.001$ ; see also Beekman & van Stratum, 1998). Because the share of queen-first colonies is highest in the nondiapause colonies, emergence of the first sexual is delayed compared to the diapause colonies (Table 2). Even though the nondiapause colonies produce the highest number of sexuals both in number and in energy (due to the female-biased sex allocation), this does not result in a longer colony lifetime because this is shortest in the nondiapause colonies. Nondiapausing colonies seem to be able to rear more queens with fewer workers in a shorter time.

**Table 1.** Characteristics of colonies headed by queens that experienced different diapause durations. Given are average values  $\pm$  SD. Time is measured after emergence of the second brood. Differences in colony characteristics that are assumed to be under control of the queen (number of first and second brood workers, rate of worker production, and colony lifetime) do not differ among the three treatments ( $P > 0.05$ ) or between nondiapaused and diapause (2 and 4 months) together, ( $P > 0.05$ ). Neither do these characteristics differ between the diapause treatment (2 and 4 months,  $P > 0.05$ ). Differences in all characteristics measured among the three treatments ( $P < 0.05$ ) and between nondiapaused and diapause ( $P < 0.001$ ) are significant. Differences in all characteristics between diapause treatments (2 and 4 months) are not significant ( $P > 0.05$ ). Significances are based on MANOVA.

Diapause duration (months)	Number of workers in		Total number of		Energy in sexuals (kJ)	Numerical sex ratio (M/M+F)*	Rate of worker production (workers/day)†	Emergence of first sexual (days)	Colony lifetime (days)†
	First and second brood	Total colony	Queens	Males					
0 ( $n = 10$ )	46 $\pm$ 10.5	118 $\pm$ 20.3	108 $\pm$ 79.0	364 $\pm$ 138.0	1696.7 $\pm$ 580.3	0.77 $\pm$ 0.17	5.52 $\pm$ 1.40	18.7 $\pm$ 4.88	60.8 $\pm$ 25.8
2 ( $n = 27$ )	43 $\pm$ 11.2	128 $\pm$ 55.5	70 $\pm$ 60.1	266 $\pm$ 160.1	1170.3 $\pm$ 680.4	0.77 $\pm$ 0.20	5.21 $\pm$ 2.07	15.2 $\pm$ 5.50	64.6 $\pm$ 21.6
4 ( $n = 21$ )	42 $\pm$ 10.5	138 $\pm$ 49.2	59 $\pm$ 55.7	383 $\pm$ 228.9	1362.7 $\pm$ 741.1	0.84 $\pm$ 0.17	5.65 $\pm$ 1.85	14.2 $\pm$ 5.81	68.2 $\pm$ 23.9

\*Sex ratio is not tested in the MANOVA.

†See Materials and methods for details.

## Discussion

Does the length of diapause experienced by the founding queen affect colony characteristics? It is argued here that, although differences in characteristics between colonies headed by queens that differ in diapause history were found, these are unlikely to be caused by costs associated with diapause that influence post-diapause reproduction.

If nondiapaused colonies are compared with diapause colonies, there are differences in colony characteristics, whereas no difference is found between colonies of the two diapause treatments (2 and 4 months). Some trends are apparent if the three treatments are compared (Table 1). Total number of workers and colony lifetime increases when the length of diapause increases from 0 to 4 months, whereas the number of young queens decreases. Time until the first sexual emerges increases with decreasing diapause length. The increase in time to emergence of the first sexual with a decrease in sex ratio (fraction males) is explained by the change in the share of colonies that produce queens first (Table 2). Colonies in which queens emerge before males have a more female-biased sex ratio (Beekman & van Stratum, 1998; Table 2).

If the observed differences in colony characteristics between diapause and nondiapaused colonies are caused by costs associated with diapause, an effect would be expected on traits that are under direct influence of the queen because it is the queen that goes into diapause. Yet, no effect was found of treatment on number of first and second brood workers and rate of worker production (Table 1). There was an effect on colony lifetime, but this was in the opposite direction. If diapause entails costs, it would be expected that colonies where the queens had experienced the longest diapause would have the shortest colony lifetime, however colony lifetime increased with diapause duration. Differences were also found between diapause and nondiapaused colonies in total number of workers, timing of the production of sexuals, and number of sexuals. Because these traits are more likely to be under the control of the workers (see Introduction), it is concluded that these differences are not caused by costs associated with diapause. The fact that nondiapaused colonies produce by far the most queens but the lowest number of workers seems counter-intuitive and needs to be explained. Moreover, these queens are reared in a shorter time because the nondiapaused colonies have a shorter colony lifetime than the diapause colonies (Table 1).

What causes the difference between diapause and nondiapaused colonies? An important distinction between nondiapaused and diapause colonies is that nondiapaused queens *choose* to start a second generation whereas queens of the diapause treatments were forced into a diapause regime. Nondiapaused in bumblebees can be selected for under laboratory conditions (Beekman *et al.*, 1996; Beekman, 1998). If nondiapaused is a genuine phenomenon in this bumblebee, under natural conditions bivoltinism instead of true nondiapaused would be expected because the queens of the second generation have to enter diapause in

**Table 2.** Fraction of colonies in which queens emerged first (QF colonies) and the timing of emergence of the first queen and male as well as the sex ratio in both QF and MF (in which males emerge first) colonies. Given are the average values  $\pm$  SD. Time is measured after emergence of the second brood. Differences in the total number of males, queens, emergence of first male and first queen in QF and MF colonies were tested using Mann–Whitney *U*-tests. Except for the number of males ( $P=0.05$ ), all differences were statistically significant ( $P\leq 0.001$ ).

Diapause duration (months)	In MF colonies				In QF colonies		
	Fraction QF colonies	First male (days)	First queen (days)	Sex ratio (fraction males)	First male (days)	First queen (days)	Sex ratio (fraction males)
0 ( $n=10$ )	0.60	14.2 $\pm$ 3.90	25.5 $\pm$ 2.87	0.92 $\pm$ 0.12	32.0 $\pm$ 3.16	21.7 $\pm$ 2.75	0.67 $\pm$ 0.10
2 ( $n=27$ )	0.48	13.5 $\pm$ 5.21	26.1 $\pm$ 11.8	0.92 $\pm$ 0.11	25.7 $\pm$ 7.90	17.0 $\pm$ 5.22	0.66 $\pm$ 0.14
4 ( $n=21$ )	0.29	14.0 $\pm$ 6.95	32.0 $\pm$ 14.5	0.89 $\pm$ 0.10	23.0 $\pm$ 5.45	16.0 $\pm$ 4.24	0.63 $\pm$ 0.19

order to survive winter. Because this second generation must be produced before the season ends, nondiapause colonies will be more time-constrained than diapause colonies (which produce their sexuals in summer; Alford, 1969). Natural selection would therefore favour more efficient colonies: colonies that produce more sexuals with fewer workers in a shorter time span. Although natural selection will always favour more efficient colonies, selection on efficiency will be stronger in nondiapause colonies because these colonies run the risk of having zero fitness if the seasons ends before their sexuals have emerged and the young queens have entered diapause. Moreover, colonies that produce a second generation should rear a more female-biased sex ratio because they can be certain of the presence of males produced by other (diapause) colonies (Bourke, 1997; Beekman & van Stratum, 1998).

In conclusion, the work presented here shows that diapause and nondiapause colonies differ in that nondiapause colonies produce fewer workers but more queens in a shorter time. It is argued that this difference in efficiency between diapause and nondiapause colonies is due to the fact that both colony types perform a distinct reproductive strategy, and is not caused by costs associated with diapause that affect post-diapause reproduction. Irrespective of diapause duration, colonies where the queens have experienced diapause are less time-constrained than nondiapause colonies. Because nondiapause colonies have to produce a second generation before the season ends, these colonies need to be more efficient in producing sexual offspring. By the time nondiapause colonies produce their sexuals, males will already be present, so subsequently these nondiapause colonies should specialise more on female production.

The conclusion that costs of diapause do not affect post-diapause reproduction is strengthened by the fact that efficiency in rearing sexuals is a trait that does not seem to be under direct influence of the queen. Efficiency in rearing brood is more likely to be a trait of the workers because these workers feed and tend the brood. Because it is the queen that goes into diapause, not the colony as a whole, it is unlikely that the observed differences are a result of costs of diapause that have an effect on bumblebee queens.

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