

REPRODUCTIVE STUDIES OF A NEOTROPICAL BUMBLEBEE, *Bombus atratus* (HYMENOPTERA, APIDAE)

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ABSTRACT

The paper presents some reproductive studies of a neotropical bumblebee, *Bombus atratus*. All the males and queens utilized were collected from nests maintained in the laboratory. Most males left the nest when they were four days old. The number of spermatozoa produced by males increased with body size. Most matings occurred in the morning and the behavior patterns during mating are described. The amount of sperm received by a queen after one copulation was proportional to the amount of semen found in the male with which she copulated. The males were able to copulate repeatedly and discharged less spermatozoa as the matings occurred. The duration of mating, in general, increased with the number of matings. The average quantity of sperm in the spermathecae of queens increased with the number of matings. However, in most cases the number of matings cannot be deduced from the number of sperms since the individual values obtained for queens copulating twice or three times were similar to those for queens copulating only once.

INTRODUCTION

There is a large number of papers dealing with the general biology of bumblebees, as can be seen in the books by Sladen (1912), Plath (1934), Free and Butler (1959), Alford (1975) and Heinrich (1979) and recent reviews by Sakagami (1976), Morse (1982) and Plowright and Lavery (1984). In spite of this, there is little information (Frison, 1927; Cumber, 1953; Postner, 1953 (quoted by Zucchi *et al.*, 1969); Pouvreau, 1963; Katayama, 1964; Hobbs, 1967; Roseler, 1973;

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Garofalo, 1980a; Jonghe, 1982) concerning the mating behavior, duration and frequency as related to time of day, amount of sperm produced by males and received by queens during the matings and on the occurrence of single or multiple matings for both sexes, although the mating of bumblebee queens can be readily induced by merely confining young queens and males together within a small container (Free and Butler, 1959). The aim of this paper is to present some observations on the reproduction of *Bombus atratus*, one of the six bumblebee species found in Brazil (Moure and Sakagami, 1962).

MATERIAL AND METHODS

All the males and queens utilized in this work were collected from nests maintained in the laboratory; the nests were placed in unheated glass-covered wooden boxes, 28 x 28 x 8 cm; a plastic tube going through the laboratory wall gave free access to the outside. The data were analyzed statistically by the Student test, Newman-Keuls test (Snedecor and Cochran, 1967) and Spearman rank correlation test (Siegel, 1956).

Determination of age of departure from nest by males

Fifty newly-emerged males were caught, marked with differently colored paint and reintroduced into the nests; the age of departure from nests was determined by daily observation of the activities of the males.

Matings

Newly-emerged males and queens were collected from the nests and maintained in separate boxes; 50% sugar syrup and pollen from *Bombus* nests were given to them daily. To permit mating, males and queens at least three days old were placed daily within a glass-covered wooden box, 100 x 80 x 80 cm. The experiments started at 7 AM and ended at 5 PM. After a mating ended, the male and queen were transferred to individual boxes and maintained there until the number of spermatozoa was counted.

To obtain multiple matings, several males and queens were marked with differently colored paint after the first mating and returned daily to the wooden box with other males and queens; the experiment ended 10 days after the first mating. The behavioral patterns shown by male and queen during the mating were recorded by direct observation.

Male body size and amount of sperm found in males and queens

The body size of the 70 males was determined by measuring the length of the forewing from the proximal portion of the 1st M cell to the distal end of the radial cell. The amount of sperm found in unmated males was determined using a sample of 70 males caught when they tried to leave the nest. The spermatozoa in the male testes and seminal vesicle, as well as in the queen spermathecae, were counted by the methods of Jaycox (1956) and Garofalo (1974).

RESULTS**Age of departure from nest by the males**

Before leaving the nest the males exhibited the nectar dehydration behavior and once they had left their nests they were never seen to return to them again. Table I shows that the males began to depart from the nests when they were 3 days old and the highest frequencies occurred when they were 4 or 5 days old.

Table I - Age of departure from nests by males of *Bombus atratus*.

Age (in days)	Frequency of departure
1	0
2	0
3	4
4	24
5	12
6	4
7	3
8	2

Male body size and amount of sperm

Male body size varied from 5.4 mm to 7.8 mm with an average of 6.84 ± 0.56 mm and the amount of sperm varied from 512,000 to 3,030,000 with an average of $1,945,871.43 \pm 591,973.10$ spermatozoa. A positive correlation ($r = 0.611$, significant at $P < 0.01$) was found between male body size and amount of sperm (Figure 1).

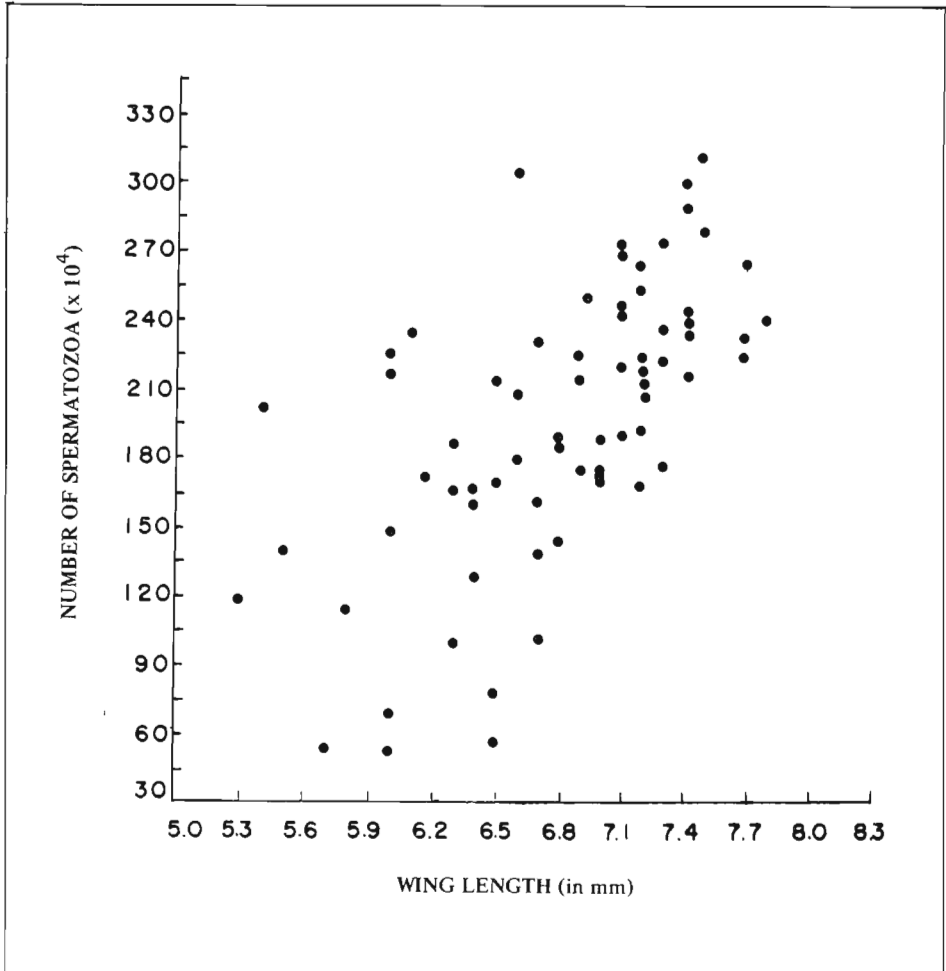


Figure 1 - Relationship between body size (wing length) and number of spermatozoa produced by males of *Bombus atratus*.

Matings

Several observations carried out in the field showed that the mating behavior begins when the male grips a flying queen. The couple then flies down and the mating may occur on the ground, on a branch or other substrate. The male places its middle legs over the queen's wings and prevents her from flying, but during this pre-mating phase the couple may take wing and shift to a different place. Observations carried out in the laboratory showed that after gripping the queen by her metasoma the male

begins periodical tentative genital insertions and simultaneously touches the queen's thorax with his antennae. The mating begins after the queen opens the abdominal tip, and exposes the vagina by releasing the sting, thus permitting penis insertion. The male then falls back and when the mating ends the queen forces the male off with the spurs of her hind legs. Figure 2 shows the behavior patterns involved in mating of *B. atratus*.

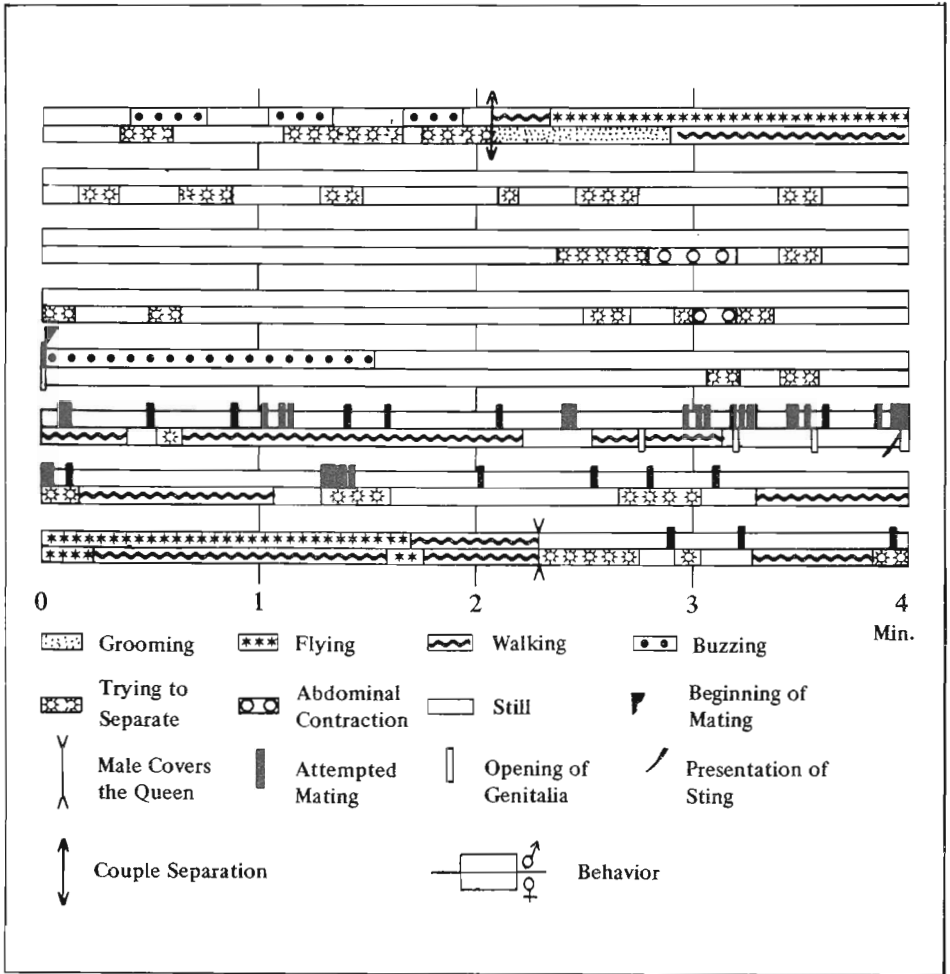


Figure 2 - Behavior patterns during the mating of *Bombus atratus*.

The maximum number of matings performed by a queen was 3, while for the males it was 4. The matings observed (N = 115) occurred between 8 AM and 4 PM but few (N = 11) were in the afternoon and most of them (N = 76) between 9 and 11 AM

(Figure 3). The duration of mating ($N = 95$) varied widely from 5 to 60 minutes with an average of 25.3 ± 14 minutes; the highest frequencies were between 10-20 and 20-30 minutes (Figure 4).

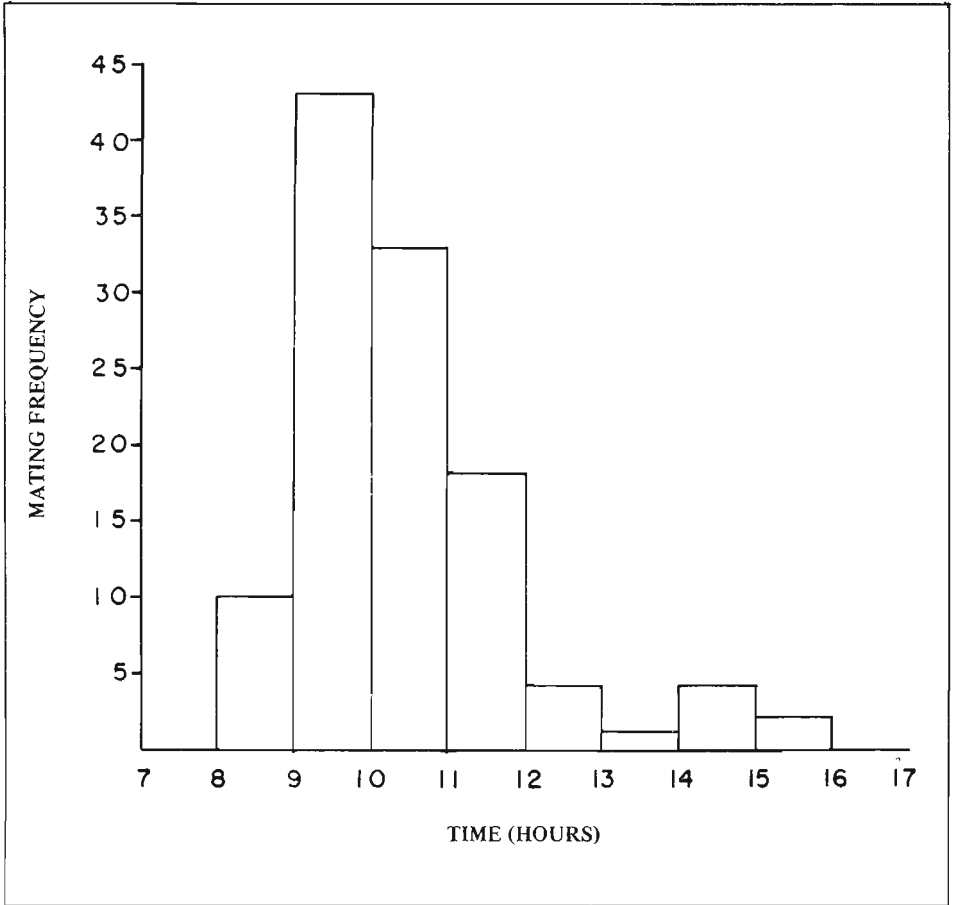


Figure 3 - Mating frequency of *Bombus atratus* according to time of day.

Amount of sperm in the queen spermathecae

The amount of sperm in the queen spermathecae ($N = 30$) after a single mating was highly variable, from 22,000 to 128,000, with an average of $68,533.33 \pm 27,379.50$ spermatozoa. A positive correlation was observed ($r = 0.632$, significant at $P < 0.01$) between the amount of sperm received by a queen and the amount in the male with which she copulated ($N = 30$) (Figure 5). After 2 matings ($N = 7$), queens

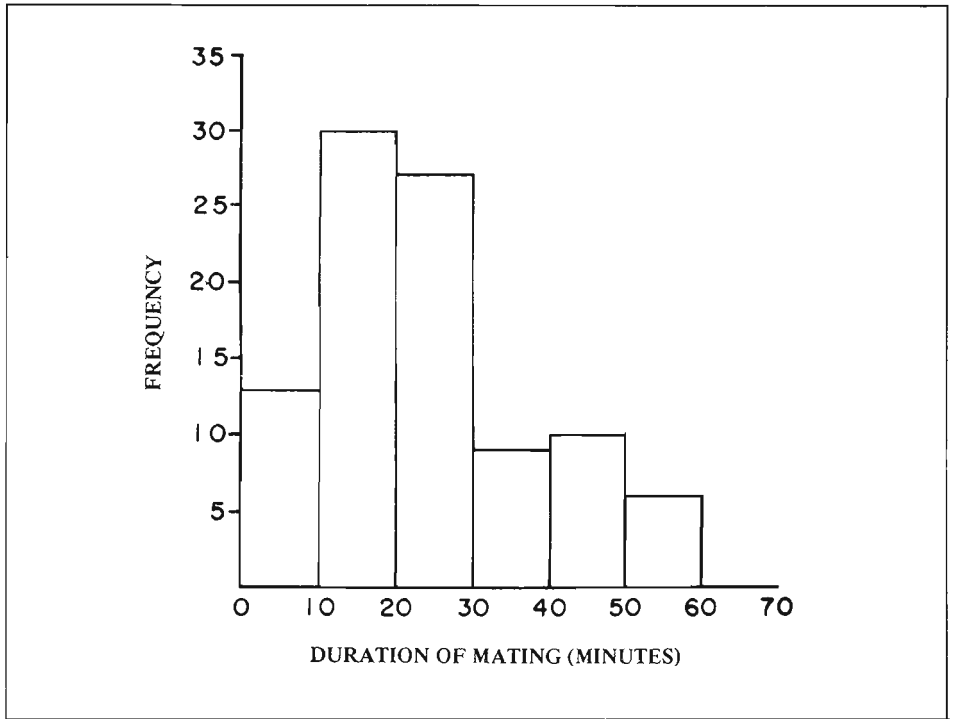


Figure 4 - Durations of matings of *Bombus atratus*.

showed an average of $92,000 \pm 31,937.44$ spermatozoa, with values ranging from 48,000 to 124,000 spermatozoa. In the case of queens copulating 3 times ($N = 3$) the number of sperms varied from 80,000 to 152,000 with an average of $124,666.67 \pm 39,004.27$ spermatozoa.

The averages obtained for queens copulating once, twice or three times were statistically different ($P < 0.05$); thus, on average, the amount of sperm in the spermathecae increased with the number of matings.

Duration of matings and amount of sperm transferred to queens by males that copulated repeatedly

The amount of sperm transferred to queens during the first mating ($N = 6$), varied from 29,000 to 72,000 with an average of $49,000 \pm 15,130$ spermatozoa; in the second mating ($N = 6$) the average was $30,500 \pm 10,930$, with values ranging from 15,000 to 45,000 spermatozoa. These averages were statistically different ($P < 0.01$), indicating that during the second mating the males discharged fewer spermatozoa than during the first. In the third mating ($N = 3$) the average obtained was $22,666.67 \pm$

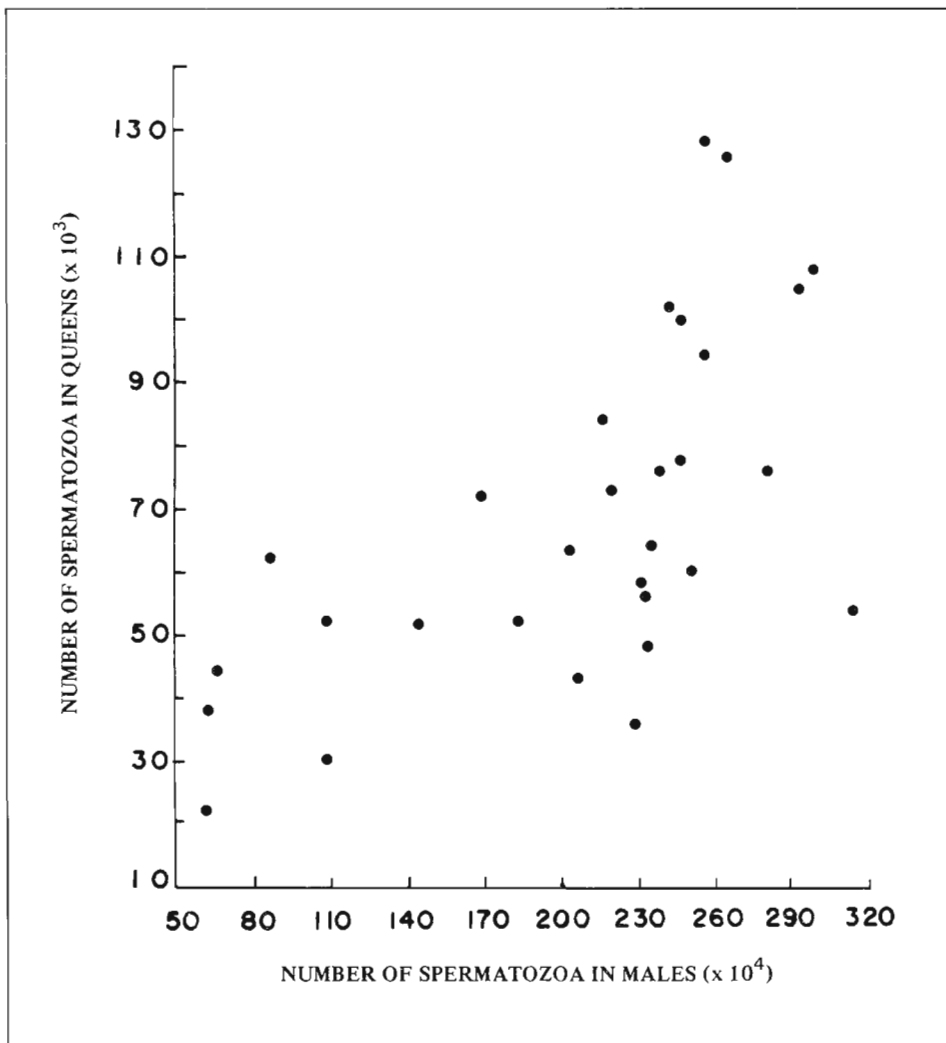


Figure 5 - Relationship between the number of spermatozoa produced by males and found in the queen spermathecae of *Bombus atratus*.

6,027.71, with values ranging from 17,000 to 29,000 spermatozoa. Although this average is smaller than the one obtained for the second mating the difference between them was not significant, probably on account of the small sample size. In the single fourth mating investigated, 24,000 spermatozoa were transferred.

The average duration of the first mating, 19.17 ± 12.42 minutes, was significantly shorter ($P < 0.05$) than that of the second mating, 31.83 ± 19.60 minutes, with

the values for the former ranging from 5 to 50 minutes while those for the latter ranged from 13 to 80 minutes. Durations of the third mating varied from 10 to 90 minutes, with an average of 40 ± 43.59 minutes. Although the average was higher for the second mating, the difference between them was not significant. The fourth mating showed a duration of 30 minutes.

DISCUSSION AND CONCLUSIONS

Bumblebee males usually leave their nests a few days after maturing and do not return (Free and Butler, 1959; Alford, 1975; Morse, 1982). Although Frison (1917, 1928) reported that the males of certain species sometimes return after leaving their nests (in Free and Butler, 1959) this seems to be an unusual event. In this respect, the males of *B. atratus* were similar to most other species.

Free (in Free and Butler, 1959) marked many males of several species and found that most of them departed from their nests when they were between two and four days old; Pouvreau (1963) has also obtained the same results for *B. hypnorum*, whereas Richards (1973) observed that the males of *B. polaris* and *B. hyperboreus* remained in the nest for two days. The results obtained for *B. atratus* were similar to those reported by Free and by Pouvreau (1963), although the males began leaving the nests when they were three days old.

Nectar dehydration, which is usually observed in honeybees and stingless bees, had not been previously reported for bumblebee males. However, the males of *B. atratus*, before leaving the nests, spend some time dehydrating nectar. A similar behavior has been observed by Deleurance (1952) in *Polistes gallicus* females, West-Eberhard (1969) in *Polistes fuscatus* and *P. canadensis* males and females, Zucchi (1973) in *B. atratus* queens and Garofalo (1976) in *B. morio* males and queens. According to West-Eberhard (1969), this behavior may serve to concentrate dissolved sugar by evaporation, thus increasing the energy/volume ratio of food held in the crop during a period of decreasing food availability. As *B. atratus* males have never been observed to place the dehydrated product in food pots, West-Eberhard's suggestion is probably valid although, in this case, decreasing food availability cannot completely fit since in our region the nests are active all year long.

Our results show how size polymorphism, a well known trait of bumblebees, affects the amount of sperms produced by males. A progressive size increase of workers in relation to season or colony age has been recorded for several species (Richards, 1946; Knee and Medler, 1965; Plowright and Jay, 1968; Pouvreau, 1971; Zucchi, 1973; Röseler and Röseler, 1974; Garofalo, 1980b; Honk *et al.*, 1981) and Saraceni (1976) observed that it occurs for males of *B. atratus* as well, so that they are smaller at the start of male production and gradually increase in size until the first queens appear.

A comparative analysis of the mating behavior described for some species (Free and Butler, 1959; Pouvreau, 1963; Katayama, 1964) indicates that this behavior is very similar among the species. Cumber (1953) observed one pair of *B. pratorum* that copulated for nearly 90 minutes. According to Pouvreau (1963), the sexual activity of *B. hypnorum* males was most intense between 12 and 16 hours, when the temperature reached 28 to 33°C, with matings lasting from 25 to 55 minutes. Katayama (1964) found that the duration of matings was more than 48 minutes in *B. ardens*. Hobbs (1965a,b, 1967) verified that in *B. nevadensis*, *B. rufocinctus* and *B. nearticus* the queens mated in the morning and entered hibernation in the afternoon. Postner (1953) (quoted by Zucchi et al., 1969) observed that the duration of matings in *B. lapidarius* was 180 minutes. Röseler (1973) noticed that the average duration of matings in *B. hypnorum* and *B. terrestris* was 24.7 ± 6.6 and 36.2 ± 12.1 minutes. Jonghe (1982) when trying to obtain interespecific matings among several species, observed that the copulations occurred between 9 AM and 7 PM, lasting in general from 20 to 45 minutes. The results obtained for *B. atratus* can be considered similar to those for *B. nevadensis*, *rufocinctus* and *nearticus* as the highest frequencies of matings occurred in the morning. Concerning the duration of matings, with the exception of *B. lapidarius* and *B. pratorum*, with long matings, and *B. terrestris*, with an average duration longer than that for *B. atratus*, for all other species the results were similar.

As for the males of *B. atratus*, the number of spermatozoa found in the queens was highly variable. This variation is due to the fact that the amount of semen received by a queen from one copulation is proportional to the amount of semen in the male with which she copulated. Thus, the variation in size of males not only influences the amount of semen produced by them but also the amount received by the queens. These aspects have not been studied in other bumblebee species.

Concerning the number of spermatozoa found in spermathecae of queens, Röseler (1973) reported the following figures: *B. variabilis* = 12,200; *B. pratorum* = 13,200; *B. hortorum* = 14,700; *B. agrorum* = 17,300; *B. silvarum* = 19,000; *B. hypnorum* = 23,300; *B. lucorum* = 33,500; *B. lapidarius* = 41,600; *B. terrestris* = 52,800. The average obtained for *B. atratus* was higher than those for the nine species mentioned. Röseler (1973) showed that at the end of colony development the quantity of spermatozoa in the spermathecae of old queens did not differ significantly from the quantity measured when the colony was started; thus, according to this investigator, the quantity of sperm cannot be a limiting factor in colony strength. On the other hand, Sakagami (1976) mentions that the number of sperms carried by each post-hibernating queen is roughly comparable to the colony size attained. Thus, although Röseler's affirmation is correct, the values obtained here for *B. atratus* support Sakagami's comparison because the colonies of this species become larger than those of the species previously mentioned.

As observed by Röseler (1973) for *B. hypnorum* and *B. terrestris*, in *B. atratus*

the average quantity of sperm in the spermathecae of queens increased with the number of matings. However, in most cases the number of matings cannot be deduced from the number of sperms in a queen since the individual values obtained for queens copulating twice or three times (with a single exception) were similar to those for queens copulating only once. This occurs because the amount of semen received by queens is related to the amount of semen produced by males with which they copulated. The results obtained about the amount of sperm transferred to queens by males that copulated repeatedly were different from those reported by Röseler (1973) for *B. hypnorum* and *B. terrestris*. Thus, the males of *B. atratus* discharged less spermatozoa as the matings occurred. On the other hand, the durations of matings increased with the number of copulations, as observed by Röseler (1973) for the above species.

Queens are generally believed to mate only once; however, multiple matings are common in a few species, as observed by Pouvreau (1963) for *B. hypnorum*, Hobbs (1967) for *B. huntii* and Röseler (1973) for *B. hypnorum* and *B. terrestris*. Although a queen of *B. atratus* was observed to copulate three times, we believe that the queens ordinarily mate only once. Unlike the queens, the males of *B. atratus* can copulate several times and this fact seems to be true for all bumblebee species.

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RESUMO

Nesse trabalho são apresentadas algumas observações sobre aspectos reprodutivos de *Bombus atratus* (Hymenoptera, Apidae). Todos os machos e rainhas utilizados foram obtidos a partir de ninhos mantidos no laboratório. A maioria dos machos abandona o ninho com a idade de quatro dias. O número de espermatozóides produzido pelos machos aumentou com o tamanho do corpo. A maioria dos acasalamentos ocorreu no período da manhã e os padrões de comportamento do macho e rainha durante o acasalamento são apresentados. A quantidade de sêmen recebida por uma rainha, após um acasalamento, foi proporcional à quantidade de sêmen encontrada no macho com o qual ela copulou. Os machos copularam várias vezes e transferiram às rainhas quantidades de sêmen cada vez menores conforme os acasalamentos ocorriam. A duração dos acasalamentos, em geral, aumentou com o número de acasalamentos. A quantidade média de sêmen encontrada na espermateca das rainhas aumentou com o número de cópulas. Contudo, na maioria dos casos, o número de acasalamentos não pode ser extrapolado a partir da quantidade de sêmen pois os valores individuais obtidos para rainhas que copularam duas ou três vezes foram similares àqueles encontrados em rainhas que copularam uma única vez.

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