



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2020; 8(2): 1448-1452

© 2020 JEZS

Received: 25-01-2020

Accepted: 27-02-2020

Miriam RosenboimKibbutzim College of Education,
Tel Aviv, Israel

Mating behavior of the black carpenter bee *Xylocopa sulcatipes* (Hymenoptera: Apidae)

Miriam Rosenboim**Abstract**

The courtship and mating behavior of *Xylocopa sulcatipes* is reexamined and described here. The study sought to determine whether a courted female may already be mated or is still virgin, and whether females are selective or not, with emphasis on precopulatory behavior and possible mate selection. All the observed copulations in the study were preceded by grasping behavior, in which the male holds the female by the legs. Eighty-five percent of females caught during the grasping behavior were virgin, as indicated by their empty spermatheca. The duration of grasping behavior by smaller males was short, suggesting their immediate rejection by the female; whereas a prolonged grasping behavior in larger males tended to end in copulation. The new behavior described here – grasping behavior – is interpreted as providing a mechanism for inspection of the male by the female prior to mating. It is suggested that without an 'I am ready' signal from the female the sequence of grasping that leads to copulation will not proceed, implying that females can prevent the sequence and thereby manipulate inter-sexual selection.

Keywords: *Xylocopa*, female choice, mate selection, mating behavior, inter-sexual selection

1. Introduction

Inter-sexual selection occurs when one sex is in the position of choosing an individual of the other sex as a mate. Usually, females do the choosing and males compete among themselves to be chosen. Females generally have more to lose than males when choosing a poor-quality mate^[1]. In several *Xylocopa* species in which males defend resources in order to gain access to females, males will immediately try to mount and copulate with a female upon encountering her^[2, 3]. In some *Xylocopa* species it is clear that female choice is strong, with males seemingly having no chance of forcing copulation^[4].

The mating systems of *Xylocopa* carpenter bees have been studied for many years^[5-7]. Mating behavior of the black carpenter bee, *X. sulcatipes*, generally conforms to the pattern in which males defend territories at either nesting or resource sites^[7, 8], occasionally marking the area with their mandibular gland secretion^[9]. A nectar-collecting female that lands on flowers and is assumed by a male to be unmated, is immediately antennated by the male. Following antennation, the male mounts the female once she becomes airborne^[8]. It has also been suggested by Velthuis and Gerling^[8] that if a female rejects a male, it is because she is already mated and therefore non-receptive. Stark^[10] described the copulation as taking place on the wing and lasting for 60-70 seconds. Many questions, however, have yet to receive answers, including that of whether the courted females are already mated or unmated?

Alcock^[3] in his study of *Xylocopa californica arizonensis*, asked: "Why do males defend nest sites if the females with which they interact are not receptive?" It is impossible to answer this question without knowledge of these females' sexual history. Therefore, we suggest that the first question should be: "Are the females that reject the males receptive?" If the answer is negative, we can then proceed with Alcock's question. If the answer is positive, we can ask "Why do receptive females reject males", or "Do receptive females really reject males or could this behavior be something else?" – e.g. an initial inspection to assess quality/size? These are the questions we asked here. The general objective was to investigate the effects of inter-sexual selection on reproduction success. The findings from this study, based on 138 days of observations, contribute further information on the courtship and mating behavior of *X. sulcatipes*.

Corresponding Author:**Miriam Rosenboim**Kibbutzim College of Education,
Tel Aviv, Israel

2. Materials and Methods

2.1. Bees

Male and female *X. sulcatipes* were individually marked with dots of enamel paint on the thorax. Bee size was assessed by measuring width of the head (between the eyes) and thorax (between the tegulae) with a calliper. Nest development was followed using X-ray radiography, as described in Gerling *et al.* [11].

Some of the bees were dissected to assess their mating and reproduction status through examination of the spermatheca and measuring the length and width of the ovarioles.

Virgin female *X. sulcatipes* for this study were obtained by removing female pupae from the nests and allowing them to emerge inside small lidded boxes. They were supplied with a mixture of bee bread and diluted honey for 1-10 days before being tethered by the petiole and released either into a male territory or into a wire-mesh cage.

2.2. Study sites

Semi-field studies were conducted in the bees' natural habitat in the Arava valley, at the Hatzeva Field School, about 50 km south of the Dead Sea [7-9]. Fifty nests of *X. sulcatipes* were known to be active at the research site annually. Observations were conducted between June-October of 1988-1990, for a total of 48 days (on average 6 hrs daily).

Controlled experiments were performed in a wire-mesh cage (hereafter 'cage') (4 m x 4 m x 4 m) built near Tel-Aviv, and into which bees from the Arava region were introduced. Plants of *Portulaca olearacea* and *Ocimum basilicum* served as pollen and nectar sources, respectively. In addition, bees were supplied with diluted honey ad libitum. The bees behaved normally in the cage, with males defending territories and females engaging in nesting activities. At night we closed the nests. Observations were conducted daily during July-August 1991 and 1992, with a total of 90 days of observations.

2.3. Classification of male-female interactions:

Inspection – a male approaches a female and inspects her closely, sometimes by antennating her abdomen.

Following – the male follows the female very closely, sometimes antennating her abdomen during flight.

Head to head – the female ignores the male during the "following" behavior; he flies in front of her "head-to-head" attempting to block her flight path.

Grasping – an attempt by the male to mount the female that ends in mutual leg clasping for several seconds (fig.1). During this behavior the male is usually on top, but sometimes they rotate while flying together until either separating in the air or coming into contact with a hard surface and separating.

2.4. Mating preference experiments

These experiments were performed in the cage. In the first set of experiments (season of 1991) we exposed 10 unmated females simultaneously to two males, one large and one small (thorax width 7.54 mm and 6.16 mm, respectively). In the second set of experiments (1992), we exposed 10 unmated females simultaneously to 6-10 males that were categorized as either large or small (thorax width above or below 7 mm, respectively). Males of each group size were marked with a distinct color on the thorax. Both males and females were confined to their nests and released to fly freely only during the experiment.

In the third set of experiments the female's flight path and

speed were controlled by suspending her from a moving-rail, 3.5 m-long, attached to the roof of the cage (fig.2). Live females were tethered individually at the end of a 15 cm long nylon thread attached to the motor-driven moving rail that enabled us to control both duration and speed of the females' flight. In these experiments we used four different female groups: mated females, virgin females, virgin females with excised hind legs, and virgin females that were immobilized by freezing for a few minutes.

The bioassays to determine whether females emit a sex attractant pheromone and its possible source were conducted in Hatzeva under semi-field conditions. To investigate the role of female pheromones in male attraction, we used males to which we applied a pentane extract taken from either a female's head, thorax, ventral abdomen, dorsal abdomen, or Dufour's gland, in addition to a "total female extract" that combined all the above extracts. Ten microliters of either one of the extracts or of a combination of the five were applied to the thorax of each male. The scented males were tethered and introduced into male territories.

2.5 Statistical analysis

Data were subjected to descriptive statistics, student's t-test for the comparison of means of the two samples, using SPSS 5: T-test.

3. Results

3.1. Mating behavior of *X. sulcatipes*

Mating behavior could be divided into two consecutive stages: *grasping behavior* and *copulation*.

3.1.1. Grasping behavior

Grasping behavior started with a male closely following a female, sometimes antennating her abdomen. If the female turned towards the male they flew in a mutual leg-clasping position for several seconds, in what we have termed "grasping behavior" (fig.1). If the female ignored the male, he flew in front of her while blocking her flight path (head-to-head behavior), after which they flew together in grasping behavior. Grasping behavior lasted 1-16 seconds (3.64 ± 3.08 n=37). Usually the male and female were positioned head-to-head during grasping behavior, but occasionally head-to-abdomen. The dissection of females caught during grasping behavior at Hatzeva (n=20) revealed that 85% of them were virgin, according to their empty spermatheca.

To verify that grasping behavior indeed preceded copulation, we introduced tethered virgin females (n=11) into male territories in Hatzeva. As soon as the male and the introduced female were engaged in grasping behavior, the female was retrieved and dissected. All the retrieved females had an empty spermatheca, indicating that the grasping behavior in itself did not involve copulation or sperm ejection by the males.

Males caught during grasping behavior at Hatzeva (n=29) were not significantly larger than the average male size in the population ($p > 0.07$; t test).

3.1.2. Copulation: Copulations at the field study sites were rarely seen. During 48 days of observations at Hatzeva (on average 6 hrs daily) we observed only 13 copulations, in 46% of which there was an additional male in the vicinity of the copulating couple. In eight cases in which we had observed the copulation from the start, it was preceded by grasping behavior. In these cases grasping was prolonged compared to

grasping behavior without copulation, and the pair flew up into the air, rotating until the male was finally on female's the dorsum side, clasping her body, and their abdomens connected. The duration of copulation observed in the field and in the cage was between 50-90 sec. (n=8), and during this time the pair lost height until finally flying slowly together at the same height. Males caught *in copula* at Hatzeva (n=6) were significantly larger than the average male size in the population ($P < 0.04$; t test).

3.2. Are females that display grasping behavior receptive?

3.2.1. Duration of interactions between males and unmated females

Observations on the unmated females released into the cage together with territorial males are summarized in Table 1. Small males demonstrated more interactions with females (n=359) than large males (n=175), but the durations of interactions involving large males were longer. The average female-following time of small males did not significantly differ from that of large males ($P > 0.5$; t test) (Table 1). However, the average grasping time of the large males was significantly longer than that of the small males ($P < 0.001$; t test) (Table 1).

Comparing mean male-grasping time observed in Hatzeva with mean grasping time of the two males in the cage, we found mean grasping time of small males in the cage to be significantly shorter than that in the field at Hatzeva ($P < 0.001$; t test). There was no difference between the mean grasping time of large males in the cage and that in the field at Hatzeva ($P < 0.092$; t test). None of the females released into the cage copulated directly after emergence (n=20).

3.2.2. Size of the ovarioles

There was no significant difference between the mean length of the terminal oocyte of females caught in Hatzeva during grasping behavior and that of females caught *in copula* in Hatzeva ($T=0.335$; t test), indicating that the developmental stage of the ovarioles is not a criterion for this behavior.

3.2.3. The ability of unmated female to oviposit

During the 13 days of observations in the cage described above, five females initiated nesting behavior. They provisioned the nest and then oviposited. Males later emerged from the cells. It is possible that the fact that these specific females had oviposited even though they were unmated, indicates that oviposition too cannot be considered as a criterion for the receptivity of females: they can oviposit and also still be receptive.

3.2.4. Female sex pheromone

Generally, territorial males inspect any bee entering their territory and either chase it away if it is a male, or initiate courtship if the bee is a virgin female. The test subjects here were therefore tethered males applied with the scent of either one of five female extracts or with a combination of all five (Table 2), and introduced into another male's territory. Of the extracts tested, only the total female complex and the extract derived from of the ventral part of the abdomen were effective in mimicking virgin females. The territorial males followed these scented males and engaged in grasping behavior, the typical precopulatory behavior. The other three extracts were not effective individually in attracting males, and the scented male was generally evicted from the territory by the resident male.

3.3. What are the roles of the male and the female in the process of grasping behavior?

Females were tethered to the flight-instrument (fig. 2 & Materials & Methods), and the response of the males in the cage towards them was documented. Mated females (n=15) were inspected by the males and then abandoned. Unmated females were in every case (n=20) inspected, followed, and engaged in grasping behavior by the males. As soon as the female began to move her legs, the male clasped them. The unmated females devoid of hind legs (n=10) were also inspected in every case, then followed and engaged in grasping behavior by the males. The unmated immobilized females too were inspected in every case and then followed by the males, but these did not engage in grasping behavior.

4. Discussion

We separated the courtship behavior of *X. sulcatipes* into two consecutive stages – grasping behavior and copulation – based on cases in which we had observed the copulation from the start (n=8), and which were also preceded by grasping behavior. Courtship behavior of *X. sulcatipes* has been described in general terms in previous studies^[8, 11]. It has been assumed that pollen-collecting females are mated and non-pollen-collecting females are unmated^[8, 12]. Based on our current observations in the cage however, and as also noted by Stark^[10], pollen-collecting females can be either. There is no way to predict whether the female is mated or not: neither from the female's behavior nor from the male's behavior. Gerling *et al.*^[7] assumed that carpenter bee females start laying eggs only following insemination: and, therefore, they suggested that mating may be the physiological trigger for oogenesis and for nesting behavior. Stark^[13], in contrast, described unmated females that had demonstrated nesting behavior and laid eggs, and his observations are confirmed by the present study. *X. sulcatipes* can indeed demonstrate nesting behavior without being mated (Table 1). Consequently we suggest that for *X. sulcatipes* grasping behavior is a precopulatory stage during courtship, prior to accomplishing copulation.

The duration of copulation observed in both the field and the cage was between 50-90 sec. (n=8). Although the smaller males spent more time than the larger ones in following females and in grasping with them, the males that finally achieved copulation were significantly larger than the general male population. These findings suggest that there is a link between duration of grasping behavior and copulation success.

4.1. Are females caught in grasping behavior receptive?

According to the empty spermatheca of females caught during grasping behavior at Hatzeva, we found that 85% of them were virgin. We can therefore assume that in most of the cases in which grasping behavior takes place it is with receptive females.

4.2. Is grasping behavior necessary for males?

As revealed by the experiment with the extract of unmated females applied to tethered males, the territorial males in the field perceived these "scented" males as females, and all three usually male-female interactions were observed: inspecting, following, and grasping. Since we can be certain that the tethered male did not consider itself as a female, and normally no such interaction takes place between two males, we suggest that territorial males necessarily initiate and perform grasping behavior before copulation.

4.3. Is the female's cooperation necessary to accomplish grasping behavior?

The results of the third set of experiments, described in Materials and Methods, showed that when we used immobilized, unmated females in the cage to attract males, they detected them as unmated females, inspected and followed them, but grasping behavior did not occur, possibly as a result of the restricted situation of the female. When unmated females without their hind legs were the test subject, inspection, following, and grasping were all observed. Consequently we suggest that the females must cooperate in order to accomplish grasping behavior. It is possible, as Leys [4] suggested, that males of *X. aeratus* wait for an 'I am ready' signal. Accordingly, we suggest that male *X. sulcatipes* too wait for an 'I am ready' signal. This might be the reason why grasping behavior did not occur with an immobilized female – she was not able to give a signal; but did occur with the females devoid of their hind legs, but which still had their middle legs by which to transfer the signal.

We can assume that when a male receives a "freezing" signal from the female this functions as a rejection and the male does not proceed to grasping. Such behavior by the female would seem to indicate a case of female choice. Alcock & Johnson [14] found that for species in the subgenera *Neoxylocopa* and *Kaptortosoma*, in which males exclusively use non-resource defense strategies (i.e *X. varipuncta*), there is a high level of female choice. Leys [4] also found a high level of female choice in the territorial species *X. aureus*.

4.4. Why do females need to inspect the males?

Females usually do the choosing and males compete among themselves to be chosen; and females generally have more to lose than males by choosing a poor-quality mate [1]. In many species of *Xylocopa* it is the female that chooses the male, whether the males employ lek polygyny or female-defense polygyny. A possible explanation for this lies in the greater maternal care invested by these species. Stark [15] found that the genus *Xylocopa* displays a very high value of egg index, indicating the great investment by the female. This egg index value is the highest among the Hymenoptera [16], and may be one of the factors influencing courtship behavior. For females investing such a high degree of maternal care, it is important to have the opportunity to compare male quality before choosing a mate. Every female undergoes some/much grasping behavior with several males before copulation take place. It is possible that during grasping behavior she is inspecting the male. Let us imagine that a female enters a territory to which she has been attracted by its odor or quality, while that territory's potentially best male is busy elsewhere chasing away another male. In such a case, a satellite male, which is not the one to which she was attracted, will have the chance to copulate with her. It is therefore necessary for the

female to be able to ensure the quality of her mate. Here, we suggest that grasping behavior can give her that answer. Goodenough *et al.* [1] described the adaptations of non-dominant males to secure copulation: mimic the female or be a satellite male. It is possible that in the case of *X. sulcatipes*, participating in grasping behavior is the evolutionary response that enables the female to avoid confusion/error and select the appropriate mate.



Fig 1: Grasping behavior during courtship behavior in *X. sulcatipes*

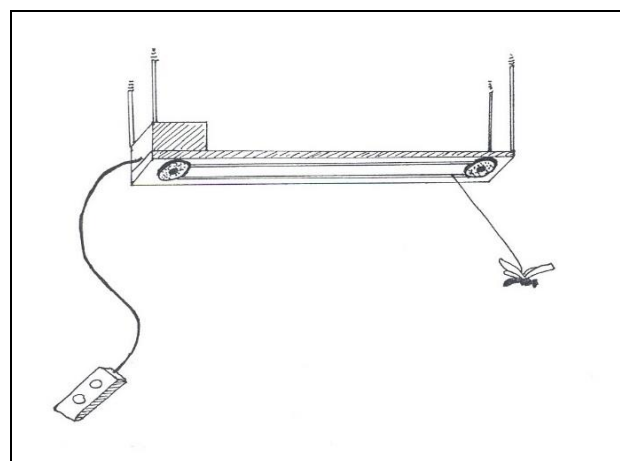


Fig 2: Scheme of the device attached to the ceiling of the cage and controlling the bee's flight

Table 1: Time (sec) spent by males in following and grasping females in the cage during 13 days of observations (1991). Total number of interactions with large males (n=175), and with small males (n=359).

Female type	Following time (sec)			Grasping time (sec)		
	Large male Thorax width=7.54 mm	Small male Thorax width=6.16 mm	t-test	Large male Thorax width=7.54 mm	Small male Thorax width=6.16 mm	t-test
Unmated female	27.2 ±35.99 n=34	34.79±61.07 n=73	NS	3.66 ±3.33 n=44	2.24 ±1.24 n=83	P<0.001
Unmated female collecting pollen	29.4 ±46.88 n=52	19.93±25.43 n=112	P < 0.096	2.71 ±1.83 n=45	1.93 ±1.08 n=90	P<0.002
Unmated female (both groups)	28.53 ±42.44 n=86	25.79 ±43.5 n=185	NS	3.18 ±2.69 n=89	2.08 ±1.67 n=174	P<0.01

Table 2: Tethered males applied with the scent of either one of five female extracts or with a combination of all five, and introduced into male territory.

Ten microliters of female extract applied to the thorax of tethered males	n	Territorial male's behavior		
		Chasing (%)	Following (%)	Grasping (%)
Reconstructed total	3	-	100	100
Head	5	80	40	20
Thorax	5	100	40	-
Ventral abdomen	7	14.3	100	85.7
Dorsal abdomen	4	100	25	-
Dufour's gland	4	100	25	-

5. Conclusions

In the present study it was found that male *Xylocopa sulcatipes* display a typical behavior prior to mating – grasping behavior. Female participation in this behavior would appear to be necessary prior to proceeding to copulation. We suggest that without an 'I am ready' signal from the female the grasping sequence will not proceed and the pair will separate, implying that females can prevent the sequence, and thereby manipulate inter-sexual selection.

6. Acknowledgment

I would like to thank Abraham Hefetz from the School of Zoology, Tel Aviv University, for his help in conducting the research and constructive comments on the manuscript.

7. References

- Goodenough J, McGuire B, Wallace RA. Perspectives on Animal Behavior. John Wiley & Sons, Inc. 2000, 528.
- Cruden RW. Observations on the behaviour of *X. californica* and *X. tabaniformis orpifex* (Hymenoptera: Apoidea). Pan-Pacific Entomology. 1966; 42:111-119.
- Alcock J. Mate-locating behavior of *Xylocopa californica arizonensis* Cresson. Journal of Kansas Entomological Society. 1992; 64:349-356.
- Leys R. Mate locating strategies of the green carpenter bees *Xylocopa (Lestis) aeratus* and *X. (L.) bombylans*. Journal of Zoology. 2000; 252:453-462.
- Alcock J, Barrows EM, Gordon G, Hubbard LJ, Kirkendall L, Pyle DW *et al.* The ecology and evolution of male reproductive behaviour in the bees and wasps. Zoological Journal of the Linnean Society. 1978; 64:293-326.
- Eickwort GC, Ginsberg HS. Foraging and mating behavior in Apoidea. Annual Review of Entomology. 1980; 25:421-46.
- Gerling D, Velthuis HHW, Hefetz A. Bionomics of the large carpenter bees of the genus *Xylocopa*. Annual Review of Entomology. 1989; 34:163-190.
- Velthuis HHW, Gerling D. Observations on territoriality and mating behavior of the carpenter bee *Xylocopa sulcatipes*. Entomologia Experimentalis et Applicata. 1980; 28:82-91.
- Hefetz A. Function of secretion of mandibular gland of male in territorial behavior of *Xylocopa sulcatipes* (Hymenoptera: Anthophoridae). Journal of Chemical Ecology. 1983; 9:923-931.
- Stark RE. Untersuchungen zur Bruthbiologie and zum Sozialverhalten der grobem Holzbiene *Xylocopa sulcatipes* Maa. Dissertation, Albert-Ludwigs Universitat Freiburg, 1990.
- Gerling D, Hurd PD, Hefetz A. Comparative behavioral biology of two Middle East species of carpenter bees (*Xylocopa*, Latreille) (Hymenoptera: Apoidea). Smithsonian Contributions to Zoology. 1983; 396:1-33.
- Hurd PD, Linsley EG. The principal Larrea bees of the southwestern United States (Hymenoptera: Apoidea). Smithsonian Contributions to Zoology. 1975; 193:1-74.
- Stark RE. Beobachtungen zur nstgründung und brutbiologie der holzbiene *Xylocopa sulcatipes* Maa (Apoidea: Anthophoridae). Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie. 1989; 7:252-256.
- Alcock J, Johnson MD. Female choice in the carpenter bee *Xylocopa varipuncta* (Hymenoptera: Anthophoridae). Journal of Zoology. 1990; 221:195-204.
- Stark RE. Sex Ratio and Maternal Investment in the Multivoltine Large Carpenter Bee *Xylocopa sulcatipes* (Apoidea: Anthophoridae). Ecology Entomology Journal. 1992; 17:160-166.
- Iwata K, Sakagami SF. Gigantism and dwarfism in bee eggs in relation to the modes of life, with notes on the number of ovarioles. Japanese Journal of Ecology. 1966; 16:4-16.