Mating in Bees: How Males Hug their Mates

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ABSTRACT

In several taxa of bees males have independently evolved modes of a mating behaviour by which sex-pheromones are applied directly on the antennae of the female.

1. In *Anthophora plumipes* the males' middle legs are elongate and bear long brush like hairs but no odour glands. Befront mounting the female the male brushes secretions from glands in the abdomen onto his hind legs, then transfers the odour to the middle legs and finally brushes the secretions during mating onto the female's antennae.
2. In megachilid and *Coelioxys* bees of the New and Old World males have modified front legs which bear the odour glands in the tibia and/or in the basitarsus. To apply the contact sex-pheromone, a megachilid male mounts the female, hold the female's antennae with special structures of his mandibles, covers her compound eyes with blind shields on his frontlegs and secretes the paste like pheromone from pores in his front legs.
3. In various genera of New- and Old-World Xylocopa, males have similar structures of the front legs and exhibit a similar behaviour as described for megachilid bees. However, in some Xylocopa species it is the middle-legs which are modified to hold the female's antennae and secrete the odour.
4. In several species of the cuckoo bees *Nomada* males bear pores of pheromone glands in various segments of the flagella. During mating the male winds its flagella around the female's antennae and by slowly pulling them upwards applies paste like secretions onto the female's antennae. The significance of this chemical communication is discussed.

INTRODUCTION

Several case studies show a vast range of structures and functions of modified legs and other body parts which are involved in mating behaviour. There are several types of holding structures like in the males of the dwarf honey bee *Apis florea* which bear such structures on their hind legs with which they grasp the hind legs of the female during copulation (Ruttner 1975). A similar structure is described in *Monia nevadensis* (O´Neil and Bjostad 1987). Males of *Xylocopa torrida* bear a hook protruding from the femur of their middle leg with which they fixate the wings of the females during copula, while males of *Xylocopa tenuiscapa* have two spines protruding from the coxa of their front legs with which they cling

to the scutellum of the female (Osten 1989). Furthermore, the slightly prolonged front leg in *X. tenuiscapa* and the prolonged middle leg of *X. perforator* bear fans of long hair in a concave oval structure, exactly matching the shape of the compound eyes of the female. During copula, which in both species is initiated in flight, the males press these blindfolds on the eyes of the female (Anzenberger 1977, Osten 1989). This not only hinders the female to decide on flight direction but also gives, through transparent parts of the dilated tarsi, a species specific pattern of visual stimuli to certain sets of ommatidia (Low and Wcislo 1992, Wittmann and Blochtein 1995). Modified legs are also used for brushing odours on substrate in order to mark mating territories (Velthuis and Camargo 1975a, 1975b) or to spread odour from glands in e.g. the tergites or in the legs over the body of territorial males (Schlumpberger and Wittmann 2000). In several species of megachilid bees the basitarsi of the front legs are widely dilated, bear blindfolds like those described for *Xylocopa* and have peculiar lateral excavations (Wittmann and Blochtein 1995). *In Anthophora plumipes* which is widely distributed in Europe, we also found a conspicuous sexual dimorphism: The middle legs of the males are prolonged and bear long fringes of hair on the tarsal segments. Friese (1898, 1923) describes these legs as secondary sexual tools and attributes them the function "to grasp and embrace the female" during mating. Later observations by Batra (1994), Stone et al. (1995) and Jacobi (1997) revealed that, while in copula position, the males move their prolonged middle legs over the eyes and over the antennae of the female. What is the functional significance of those modified legs and other structures during mating? Do they exert mechanical stimuli, are visual signals given or is chemical communication involved in this behaviour? If so, where are the odour glands located?

MATERIAL AND METHODS

Newly emerged males and females of *Anthophora plumipes, Megachile rotundata, M. willoughbiella, Nomada fucata and N. lathburiana,* parasites of *Andrena spp.* were collected, cooled on ice and transferred to the laboratory. They were either kept in a flight cages (4 x 2 x 2 m) or were transferred in petri-dishes for observation and video recording. Their mating behaviour was recorded on video film and on photographs. To observe the movements of the legs in males of *Anthophora plumipes* during courtship we fixed a male with a wire on his thorax in front of the video camera. When a virgin female was brought in his visual field, he started to show courtship behaviour which we recorded. Video recordings were analysed frame by frame giving special attention to sequences which showed males approaching females as well as courtship and copulation behaviour.

For histological studies males of *A. plumipes, Xylocopa perforator, M. rotundata, M. willughbiella N. lathburiana and N. fucata w*ere fixated in Bouin and then transferred to 70% ethanol. The legs, sternites, tergites and antennae were dissected, kept for 10 min in 70-90% ethanol, and 20 min in propylenoxide and finally embedded in EMBEG 812. Cross-sections were stained with toluidin-blue and fuchsin and analysed under a photo-microscope. For SEM studies of the outer and inner surface of the mentioned body parts were macerated in 5% KOH solution for 24 h, were kept for 10 min each in 70-90% ethanol and 100% isopropanol, were dried at 60 °C for 10 h and sputtered with gold.

RESULTS

A. plumipes

Middle legs of *A. plumipes* are 16.5 mm long (n=7, sd=0.25) and are therefore about one third longer then the front legs. Middle leg femur and tibia have anterior and dorsal 0.1-0.3 mm long hair; on the posterior and ventral side plumose hairs reach a length of 1.0-1.8 mm (Fig. 1a). The metatarsus is apical slightly dilated and bears anterior a conspicuously dense brush of 0.4-0.6 mm long black hairs. Posterior we find comparatively long black hairs (2.0-3.0 mm) which are pointing downwards by about 45 °. The prolonged tarsal segments II-IV also bear posterior long hairs. Tarsal segment V has anterior and posterior long hairs which give this segment the shape of a broad brush. SEM as well as histological studies showed no indication of odour glands in the segments of the front, middle and hind legs. However, in those areas of the tergites which are covered by the anterior tergite we found fine hairs of 0.01-0.3 mm length and pores with a diameter of 1.8-2.0 µm. On the inner side of the tergites we found chitinous ducts inserting in these pores and terminating in an end apparatus, typical for ducts of odour gland cells.

In the flight cage *Anthophora plumipes* males approached females resting at the gaze and hovered in front of them, or followed females in flight for several minutes. During hovering flights males frequently brushed with their hind legs over the sides of the abdomen and then rubbed them to each other. Analysis of video recordings of a male fixed at the thorax in front of the camera revealed the following behavioural sequence(Fig 1b).: The male extended and bent his abdomen downwards thereby exposing the areas on the tergites where pores of glands are located. Simultaneously the male brushed with the ventral sides of the hind tibiae and metatarsi alongside the exposed pores. While moving the abdomen upwards he rubbed the hind legs to each other. Then he pressed one of the prolonged middle legs between the extended hind legs and rubbed it against them. These movements were repeated 3-4 times within 5 sec. Then he conducted the same movements with the other middle leg

Males who tried to mount a female were rejected in 25% of the attempts. In all other cases the male successfully mounted the female by clinging to the bases of the wings with his front legs and by holding the abdomen with his hind legs. During courtship the male stretched one of his prolonged middle legs over the head of the female and vibrated the tarsal segments 3-4 times up and down alongside the female's flagellum which was stretched upwards. While doing so he held the other middle leg stretched out laterally and upwards. Then he conducted these movements with the other middle legs. While shaking his middle legs, the male pulled the female's abdomen upwards with his hind legs and bent his abdomen ventrally to get in copula position. To avoid copulation, females bent their abdomen downwards and push the male from her abdomen with their hind legs. Females successfully rejected the males during 66% of the initially successful mountings. In 34% of the mountings the male was accepted and the couple stayed in contact without any movement of the legs or wings.

Megachile, Coelioxys and Xylocopa

In leafcutter and in Coelioxys males of various genera the sexual dimorphism includes

modifications of the mandibles and of the head capsules. Mandibles may have pronounced teeth and the head capsule may have deep and elongate excavations beneath the mandibles or, namely in *Coelioxys* males, behind the compound eyes. Furthermore their front legs may bear spines at the coxa, while the basitarsi are dilated and prolonged. In the strongly modified megachilid males the basitarsi bear marginal u-shaped excavations and fringes of long hairs (Fig.2a). In *Xylocopa perforator* the front legs also bear such excavations and fringes on the front or middle legs. In all of the mentioned males we found pores of odour glands in the modified legs. On the inner surface of these legs cuticular ducts insert at these pores, leading to odour glands.

Males of *M willughbiella* have also odour glands at the sternites V and VI. These males mark rendezvous sites with odours from the sternal glands. Females frequently fly over such odour marks and land on some of them more frequently than on others. During courtship and mating the male mounts the female in a position in which his head is in front of the female's head. In this position the spines of the coxa are inserted behind the head capsule of the female. He then grasps the female's scapus with his modified mandibles and catches the flagella with his front legs. Finally the flagella are inserted into the u-shaped lateral excavation of the basitarsus. At the same time the hairy fans at the basitarsi are held like a blind shield over the female's compound eyes (Fig. 2b). With his middle legs the male press down the wings of the female and lifts her abdomen with his hind legs. Having achieved these positions with his body parts the male may still be rejected by the female who might fling him away by rapidly moving up her abdomen. Out of 34 couples of *M. rotundata* observed only 44% of the males were accepted for copulation.

Nomada fucata

Males of *Nomada fucata and N. lathburiana* show no conspicuous modifications of mandibles and legs. However, SEM studies revealed modifications of several segments of the flagellum. In *N. fucata* they bear elevations on which we detected pores of 0,6-1 µm diameter (Fig.3a). Histological studies corroborated that within the segments odour glands are associated to these pores. In various specimens of *N. fucata* paste like secretions protruded from the pores. Field and laboratory observations and video films revealed that males of both species immediately after mounting the female clinch with their front legs to the thorax of the female. With their middle legs they fixate the female's wings. Then males start to wind both flagella simultaneously around the flagella of the female and slowly pull their enrolled flagella upwards over the female's antennae (Fig.3b).

DISCUSSION

A common feature of all the males studied here is that during courtship and mating they transfer secretions from odour glands – no matter in which body part they are located – to the antennae of the female. This trait has evolved independently not only in megachilid bees, carpenter bees, anthophorine bees, as well in the parasitic Nomada bees, but also in sphecid wasps (Wittmann and Blochtein, 1995). The key to the understanding of the courtship and copulation behaviour associated with these morphological, physiological and behavioural traits

might be the fact that in all cases the male has first of all to achieve a very close contact to the female so that he can apply his odours directly on the female's flagella. This indicates a rigid control and selection system by the female as the complex behavioural sequence during courtship offers several instances in which female choice can operate before the male is finally accepted for copulation. Such a series of check-ups might be involved in what we can observe e.g. in megachilid bees: The males mark a rendezvous site with individual odour. The area marked and the odour concentration at the site might be a first cue for a female to land there. As these rendezvous sites are also frequented by neighbouring or satellite males, females must make sure to meet the right mate. Females might achieve this by comparing the odour marks at the selected site with the odour applied directly on their antennae while they are in physical contact with the sender. Similarly in *Anthophora plumipes* the females have to differentiate between several males who are hovering in their surrounding and emitting odour and the male who is finally mounting her. Subsequently, the male who was accepted for mounting can be checked for finely tuned motor coordination while he uses his front- or middle leg or his antennae to apply odour to the female's antennae. Finally the quality and quantity of glandular secretions applied on the antennae may indicate further physical properties of the male. In Megachilid, Xylocopa and Anthophora bees visual and tactile stimuli exerted by blindfolds might also play a role in female choice. Due to the low abundance of *Nomada fucata* we have so far not sufficient observations to make any statement when and how females may reject mates who try to hug them.

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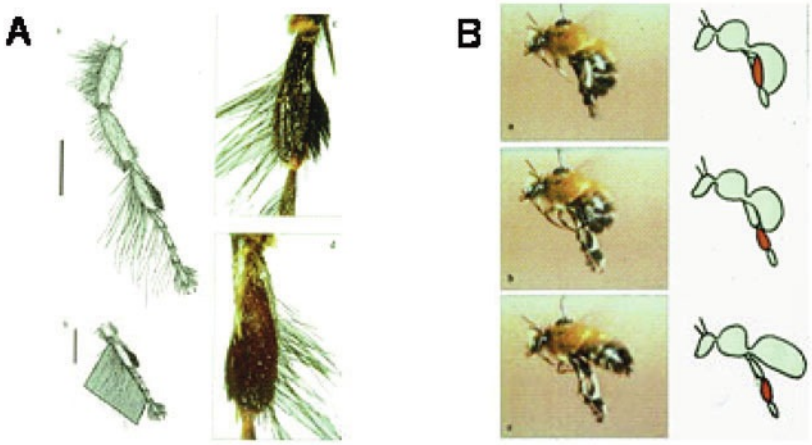

Fig. 1. A. *Anthophora plumipes*; prolonged middle leg with fringes of long hairs (ventral and dorsal view of the tibia). B. Male fixed at the thorax beats wings and takes up secretions from glands in the abdomen with his hind legs and transfers them to the middle leg.

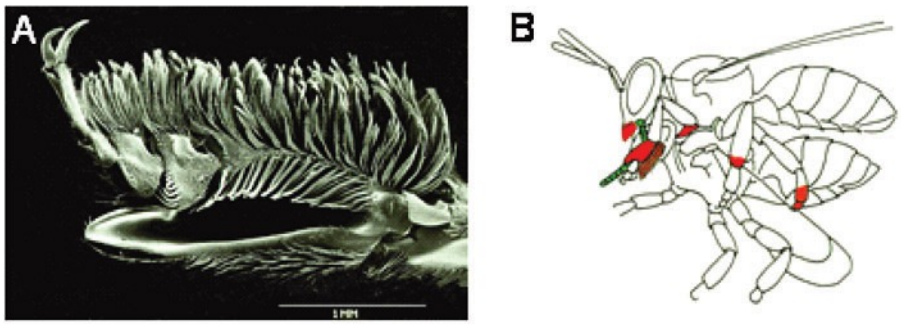

Fig. 2. Megachile spp. A. Front leg basitarsus of *Megachile curvipes* with marginal excavation and fringes of hais serving as blindfolds. B. Megacilid couple in courtship position. Details in text.

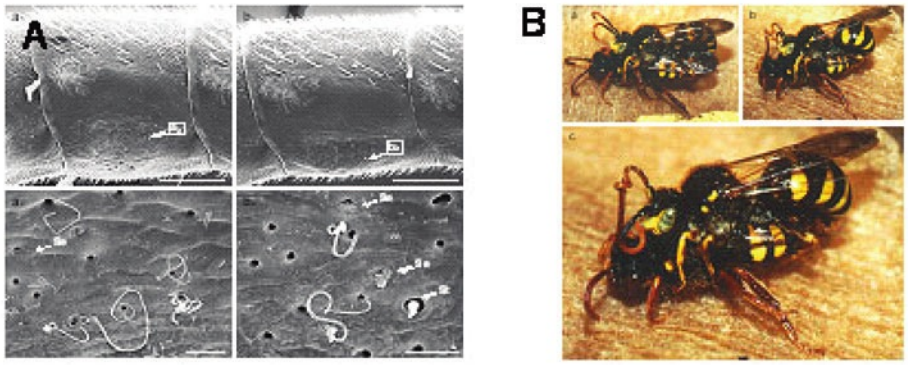

Fig. 3. *Nomada spp. A. N. fucata*; segments of the flagellum with elevations and pores of glands with protruding secretions. B. *N. lathburiana*; sequences during courtship. The male winds his flagella around the antennae of the female.