Journal of Apicultural Research 33(1): 15–20 (1994)

Copulation of *Bombus terrestris* L. (Hymenoptera: Apidae) in captivity

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(Received 10 March 1993, accepted subject to revision 29 September 1993, accepted for publication 12 October 1993)

SUMMARY

The mating behaviour of *Bombus terrestris* was studied using wooden observation cages (50 × 50 × 70 cm) in the laboratory. Queens and males from reared colonies were isolated from the odour of the opposite sex before the experiments. Mating pairs (one per box) consisted of one 10-day-old virgin male and one 5-day-old virgin queen. The behaviour of each of the 80 pairs used was observed for 15 min, during which time 47 of the pairs copulated. Male behaviour could be divided into approach, inspection and attempt to copulate. Queen reactions to the male were immobility (13.8%), threat (53.9%) or flight (32.3%). Queens could also display inspection behaviour. The mating success of a behavioural sequence was positively correlated with the frequency of inspections by both sexes and negatively correlated with queen immobility. Flow diagrams showing sequences of types of behaviour are presented. The role of sexual pheromones in mating is discussed.

Keywords: bumble bees, *Bombus terrestris*, mating behaviour, sexual behaviour, copulation

INTRODUCTION

Bumble bees are well-known important pollinators in their natural habitats. Recently, their use to pollinate various glasshouse crops (e.g. tomatoes) has been developed and, accordingly, their industrial rearing (Ravestijn *et al.*, 1991). Mass production needs to master the reproductive behaviour in order to obtain large quantities of founding queens.

In the field, the nuptial flight of bumble bee males has been described by Newman (1851); Darwin (1886), Sladen (1912), Frank (1941), Haas (1949, 1967), Kullenberg (1956), Awram (1970), Krüger (1951), Schremmer (1972), Bringer (1973), Svensson (1980) and Williams (1991).

The nuptial flight of *Bombus terrestris* L. males has been described by Krüger (1951) and by Haas (1949, 1967) and consists, like that of many bumble bees, of patrolling behaviour during which secretions from the labial gland are deposited on marking spots. It is supposed, although not proved, that females are attracted to these places and thereby mating is facilitated. Free flying queens have been seen to visit the sites established by males along flight routes but observations associating queens with flight routes are, unfortunately, not common, probably because the system is so efficient that the queens are quickly mated (Free, 1987). The composition and action of the male pheromone have been studied by Stein (1963), Bergstöm & Svensson (1973), Bergström *et al.* (1967, 1973), Calam (1969), Kullenberg (1973a, 1973b), Ågren (1979), Svensson (1979), Cedeberg *et al.* (1984), Descoins *et al.* (1984) and Williams (1985). The active component has been identified as farnesol.

Young queens emit sex pheromones as well but these are released from the mandibular glands as shown by Honk *et al.* (1978, 1980). These authors have also described the mating behaviour of captive *B. terrestris*.

The aim of our research was to establish an ethogram of the precopulatory behaviours in captive *B. terrestris* as well as making a frequency analysis of the different behaviours.

MATERIALS AND METHODS

Bumble bees used for the experiments were taken from reared colonies. Newly emerged queens were collected from the nests and maintained in a cage for five days until the copulation experiments took place. This isolated the queen from the male's odour and from the copulations which may occur in the nest.

The males were taken directly from two different nests just before the experiment and introduced into the cage. Like the queen, they were isolated from the odour of their future partner (queen) by removing the queens from the male's nest.

For the mating experiments, one ten-day-old virgin male and one five-day-old virgin queen were introduced into a wooden observation cage, 50 × 50 × 70 cm, covered with wire mesh. The experiments started at 08:00 h and ended at 16:00 h.

The temperature in the copulation room was maintained at 20°C, 70% RH and light intensity at 2 000 lx (halogen lamp). The behaviour of the bees was observed for 15 min (80 pairs observed). If a copulation occurred during this period, observation continued for five minutes after the end of the time limit. Each male and queen were used only once in this study.

A sequence was considered successful if it ended in a copulation.

Frequency analysis

The frequency of the behaviours (number of observed behaviours/sequence duration) was calculated over a 15-min period for unsuccessful sequences, and for a variable period lasting from the beginning of observation to the beginning of copulation for successful sequences. This time interval varied from 4 s to 821 s.

The median frequency of homologous behaviours in successful and unsuccessful sequences was compared (median test).

Flow diagrams

The transition probabilities were calculated by dividing the number of transitions from one behaviour to the other by the total number of transitions observed in the whole sequence.

All the statistical tests are from Siegel (1956).

RESULTS

In 80 experiments, 47 ended with copulation within the time limit (15 min). The ethograms were identical irrespective of the success of the encounter.

Ethogram: male behaviour

The behaviours observed were similar to those described by Honk *et al.* (1978):

1. Approach (A): flight of the male towards the female once she has landed. This behaviour lasts no more than 1 s; it can be considered as an event.

2. Inspection (IF): hovering flight with antennal inspection of the queen's abdomen and head. Like

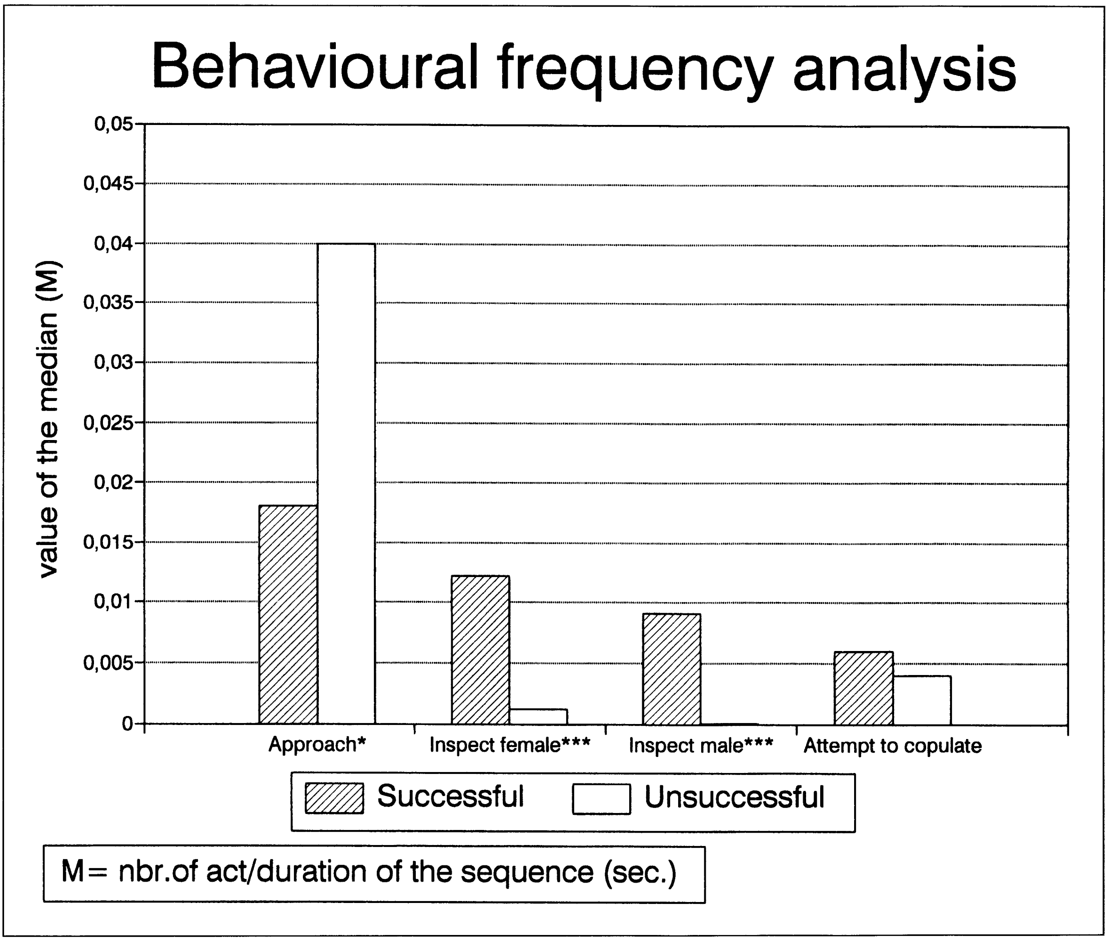

FIG. 1. Behavioural frequency analysis. Median frequency of the different behaviours (number of observed/duration of the sequence (s)) for the successful sequences (culminating in copulation) and for the unsuccessful sequences (median test, * = significant, * = very highly significant).**

the approach, inspection lasts only few seconds thus we also consider it as an event.

3. Attempt to copulate (AC): the male grips the queen, places his middle legs on her abdomen and tentatively begins periodic genital insertions and simultaneously touches the queen's thorax with his antennae. This behaviour lasts from 1 s to 840 s.

Ethogram: female behaviour

As noticed by the authors, the female is far more passive than the male. When she is not reacting to an approach of the male, she is usually settled on the cage, immobile.

However, she reacts to the approach of the male in three ways:

1. Immobility (O): the queen stays immobile during and after the male approach.

2. Threat (+): the queen is settled on the cage, immobile. When the male approaches she raises towards him her middle legs and moves away. This behaviour is generally considered as a threat.

3. Flight (–): when the male approaches the queen, she flies away from him.

Moreover, the female can also display inspection (IM) of her partner. She flies towards the male and touches his abdomen and head with her antennae. This behaviour is identical with that of the male. In this behaviour the female flies behind the male with her antennae pointed towards him.

Frequency analysis

The success of the sequence was correlated with the frequency of inspections by both sexes (IF and IM) (fig.1).

Analysis of the female reactions to the approach of the male (A) (immobility: 13.8% of her reactions;

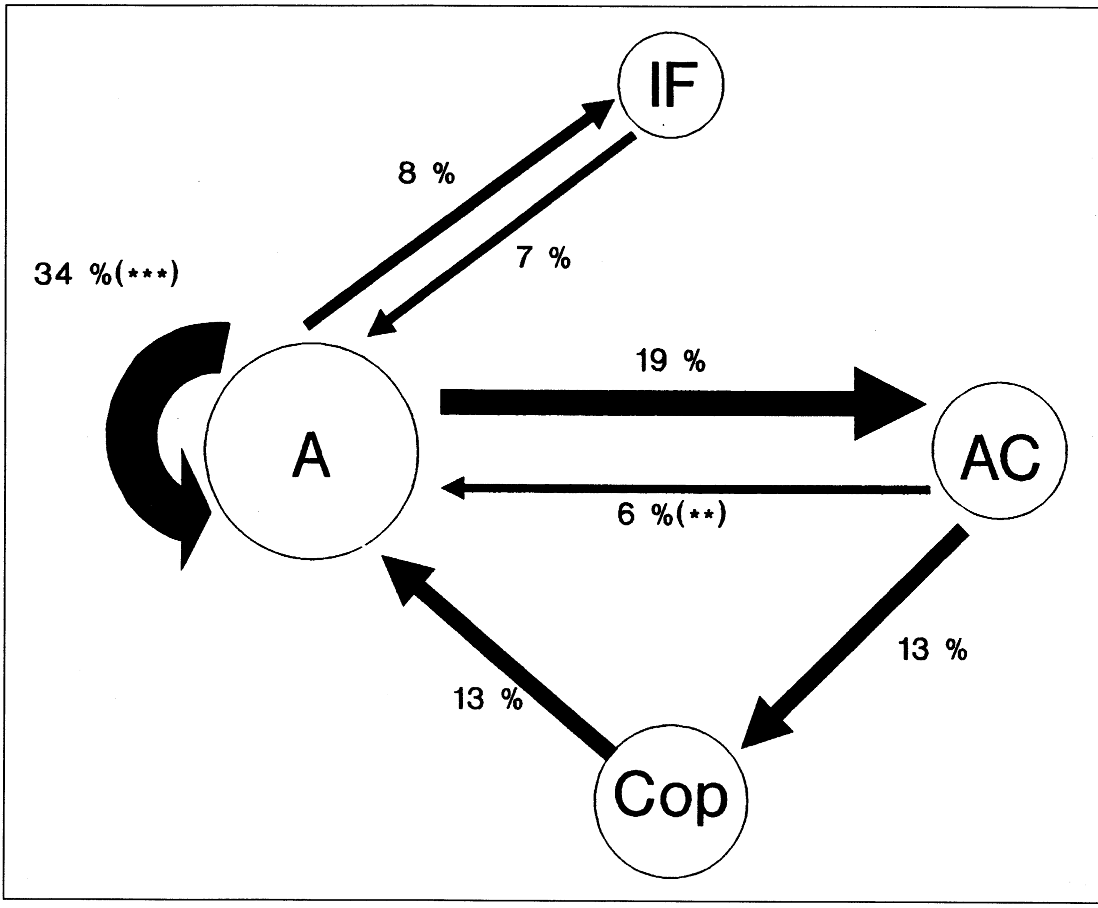

FIG. 2. Flow diagram of successful courtship (the sequence ends in copulation). The thickness of the arrows is proportional to the probability of the transition from one behaviour to another (number of a given transition/total of all observed transitions). The area of the circles is proportional to the frequency of the behaviour. A = approach, IF = inspection of the female by the male, AC = attempt to copulate and COP = copulation. (χ^2 test, * = significant, ** = highly significant, * = very highly significant). Total number of observed transitions = 352.**

threat: 53.9%; flight: 32.3%, $n = 207$) showed that the success of the sequence is negatively correlated with immobility (0) of the female ($P < 0.01$, χ^2 test). The other reactions of the queen are not correlated with the result of the sequence ($P > 0.05$, for the flight and for the threat, χ^2 test).

Flow diagrams

Two flow diagrams have been established according to the success of the courtship (figs 2 and 3).

The differences between the two diagrams are minor: there is an increase in the frequency of A–A and AC–A transitions when courtship is unsuccessful. This could be tautologically linked to the absence of the transition from AC to copulation in (by definition) the unsuccessful sequences, which redirect the behaviour towards the A–A and AC–A transitions. What is notable is that there is no transition between the behaviours 'inspection of the female by the male (IF)' and 'attempt to copulate (AC)'.

Some discrepancies between the transition frequencies linking two behaviours (i.e. the transitions A–IF = 8% and IF–A = 7% in fig. 2) are due to the fact that the duration of the observations is not infinite and limited to 15 min at most.

DISCUSSION

In the field, male bumble bees, *B. terrestris,* fly along special routes, linking sites which have been scented with their sex pheromone. The male marking pheromone is produced in the cephalic part of the labial glands.

When young virgin queens reach maturity, they leave their colony and are thought to fly along the routes established by the males, visiting the scent sites. The male marking pheromone seems to act as an

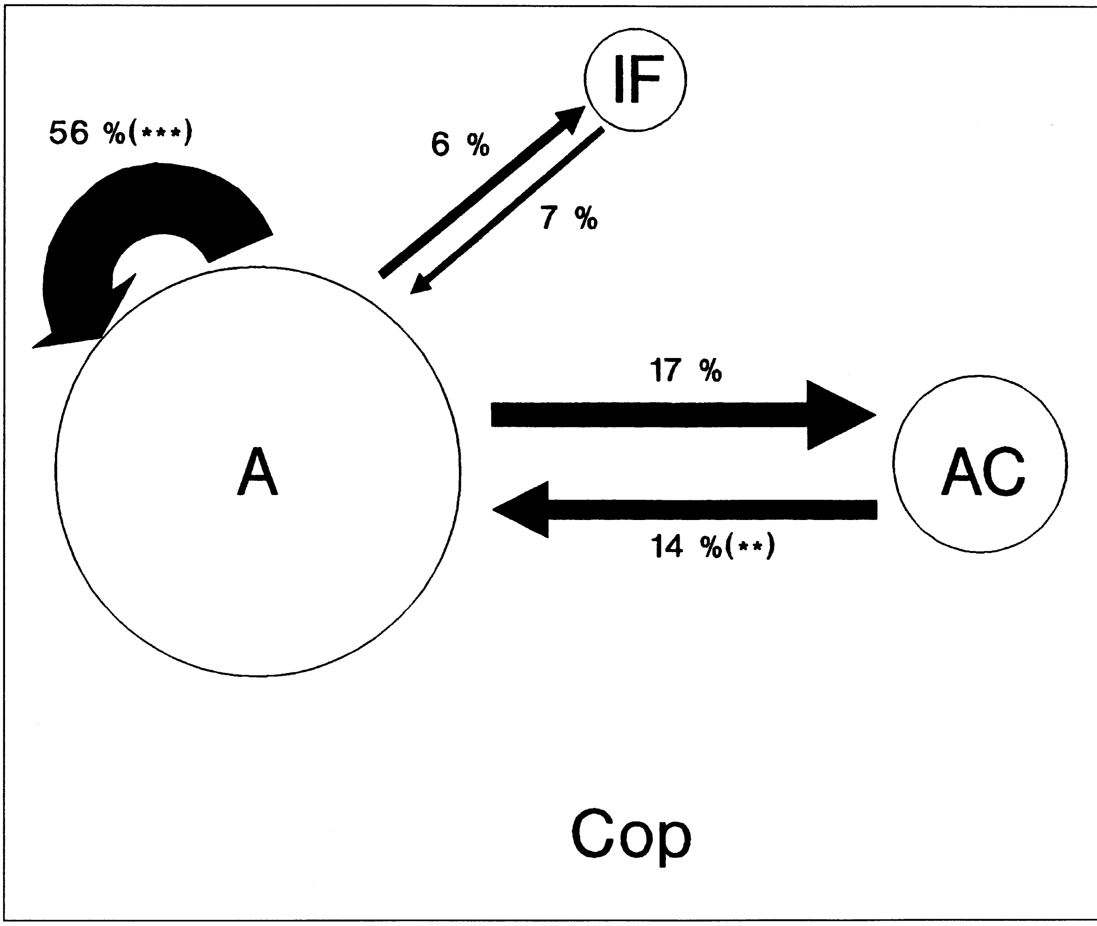

FIG. 3. Flow diagram of unsuccessful courtship (the sequence does not end in copulation). The thickness of the arrows is proportional to the probability of the transition from one behaviour to another. The area of the circles is proportional to the frequency of the behaviour. A = approach, IF = inspection of the female by the male, AC = attempt to copulate and COP = copulation. (χ^2 test, * = significant, ** = highly significant, * = very highly significant). Total number of observed transitions = 1 029.**

attractant and arrestant for the queen. When queens are inspecting scent sites along the flight routes, males are thought to be attracted to them and may attempt to copulate. It seems that the odour of the queen is important in eliciting contact and an attempt to copulate.

As is the case in other insects with low visual acuity, mistakes made during visual approach can be rectified by scent stimuli at close quarters (Free, 1970, 1987). The odour of the queen comes from a pheromone produced by the mandibular glands.

In our experiments, unlike the situation in the field, males did not establish a nuptial route; the space was too restricted. In other respects, we observed the same male behaviours as in the field (approach, inspection and attempt to copulate). Since the male did not mark scent sites in our experimental cage, we noticed that he was directly inspected by the queen. This is the first time this queen behaviour has been observed.

Previous authors have stressed the importance of male and female sex pheromones in the courtship behaviour of bumble bees (Sladen, 1912; Frison, 1927; Frank, 1941; Haas, 1949, 1967; Krüger, 1951; Cumber, 1954; Kullenberg, 1956; Stein, 1963; Awram, 1970; Free, 1970, 1987; Schremmer, 1972, Bringer, 1973; Honk *et al.*, 1978; Svensson, 1980).

This study conforms with these results since the antennal inspections seem to be a key factor in the success of the sequence. However, the analysis of the flow diagrams suggests that if the female pheromone elicits copulation it does not do so immediately: antennal inspection of the female by the male is never directly followed by an attempt to copulate. So the sex pheromones, in addition to their attractive and species-recognition role, seem to have a contextual effect and act like aphrodisiac substances. We must keep in mind that these results have been obtained in the laboratory, far from field conditions. Nevertheless, such studies are most useful in the context of industrial applications.

ACKNOWLEDGEMENTS

We sincerely thank Duclos Agro-Biotech (Marseille) for sending us colonies of *Bombus terrestris* necessary to our study.

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