

## Multiple Mating of the Honeybee Queen (*Apis mellifica* L.) in One Nuptial Flight

by

J. WOYKE

*Presented by S. A. PIENIAZEK on February 26, 1955*

The natural mating process in bees takes place in mid-air, and, consequently, man has no influence on the selection of parent couples. Controlled fertilisation in honeybees is possible solely by way of artificial insemination.

First the structure and functioning of the reproductive system of the queen and drone honeybees, and then the natural mating process itself have to be learned in order to provide a firm basis for artificial insemination.

### Methods

To investigate the structure of the drone's reproductive system and its content, these organs were removed from the body of a live drone. Preliminary experiments have demonstrated that all other methods, calling for prior killing of the drone or for injecting a fixative into its body, elicited changes in the reproductive organs.

To observe the order in which the various parts of the copulatory organ are everted, slight pressure was exerted on the thorax of examined drones. To establish the influence of the drones' flight on the eversion of the copulatory organ, returning and leaving drones were caught directly at the beehive entrance and their thorax was immediately pressed. The amount of sperm ejaculated during eversion of the organ was determined by collecting the sperm during ejaculation in a 1 - 3 mm. diameter glass tube placed against the abdomen end.

For purposes of examination, queens returning from a mating flight were caught with the aid of special traps at the beehive entrance before they managed to get inside.

### Results

The investigations have demonstrated that a sexually mature drone everts the copulatory organ upon pressure on the thorax. First the bursa of endophallus up to the neck, and the bursal cornua are everted. The

cornua turn upwards (Fig. 1) and sideways (Fig. 3). Then the cornua bend downwards (Fig. 2) and their apices converge (Fig. 4). The cornua have no muscles, yet they are made to bend by the increased pressure inside the organ and as a result of the bursting of the external viscous layer covering the cornua. The layer bursts in a longitudinal fissure on the central wall of the cornua (Figs. 1 and 3); subsequently the fissure grows larger and the layer slides off outwards (Figs. 2 and 4) making the above described motion possible.

Arnhart [2] has drawn and erroneously described the verge of the broken layer as a sort of reinforcing lath, and according to Fyg's [5] drawing and description the cornua become flabby and then bend downwards, which is also at variance with actual facts.

The fimbriate lobe and the bulb of endophallus together with the chitinized plates slide into the thus everted bursa. Normally in all drones, up to the moment of eversion, there is no sperm or mucus in the bulb of endophallus. Statements to the contrary have resulted from the application of erroneous methods of investigation. We term the copulatory organ in the above described stage as a partly everted endophallus (Fig. 2).

Further eversion of the organ occurs in older, sexually mature drones but after a brief interval. First the bulb of endophallus twists by 90° inside the bursa, so that the chitinized plates take a dorsal position, then the fimbriate lobe and the bulb of endophallus squeeze through the neck of endophallus. The fimbriate lobe does not evert yet. When the copulatory organ is turned inside out to such an extent that the chitinized plates of the bulb are at the end of it, without becoming everted however, then the sperm comes out of the end opening of the organ (Fig. 5). This sperm does not pass from the seminal vesicles to the bulb but at the moment of the endophallus eversion. Now the chitinized plates and the rest of the bulb of endophallus evert rapidly, and only then does the fimbriate lobe also evert (Fig. 6).

The mucus follows after the sperm which is expelled in the meantime on to the abdomen of the drone. The mucus is pushed out of the ejaculatory duct by the epithelium which, almost as a whole, is torn off the mucus glands. Nearly the whole ejaculatory duct is filled with this epithelium. We have established that the mucus glands and the seminal vesicles, and even the guts slide into the bursa of endophallus. At this stage of eversion we call the copulatory organ fully everted (Fig. 6). A further rise of the internal pressure makes the organ burst; its end section shrinks (Fig. 7) up to the place where, starting from the chitinized plates, an elastic layer extends and clamps the organ. This layer, contrary to existing descriptions, embraces the whole surface of the organ.

Drones less than 9 days old everted the organ only partly; in older ones complete eversion was not a rare occurrence. According to our counts, the ratio of drones which everted their copulatory organ at all (partly or

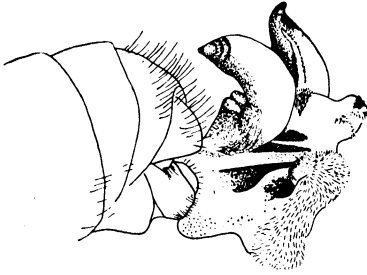


Fig. 1

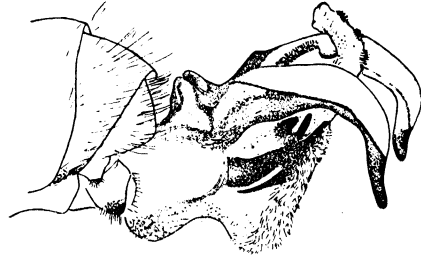


Fig. 2

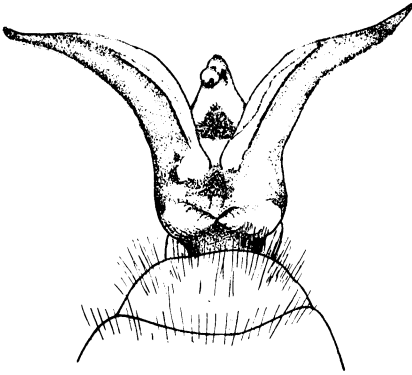


Fig. 3

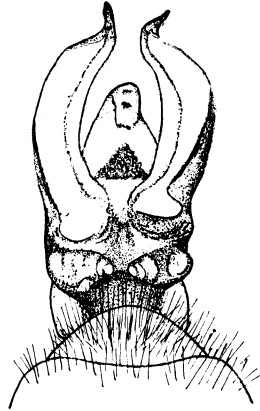


Fig. 4

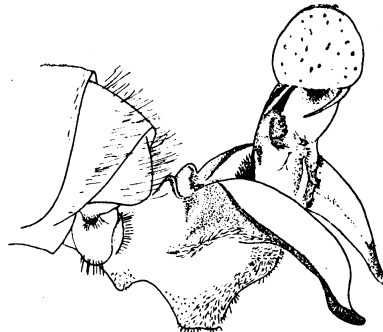


Fig. 5

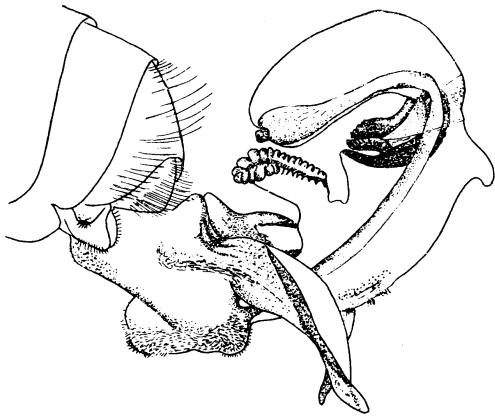


Fig. 6



Fig. 7

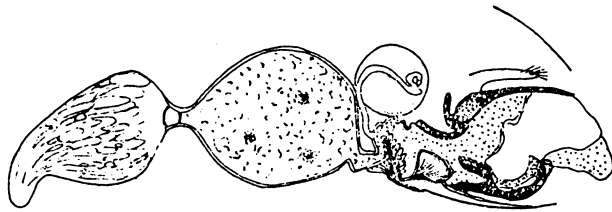


Fig. 8

fully taken together) was about the same in drones departing and returning to the hive. In returning drones, however, the ratio of partial eversion was twice that found in departing drones, while the ratio of complete eversion was, in departing drones, four times that found in returning ones.

While everting the copulatory organ, the drones eject 1.5 - 3.0 cu. mm., on the average 2.2 cu. mm., of sperm. When the ejaculation was directed into a glass tube, it could be clearly seen that the sperm and mucus were ejected separately in that order, without the two mixing in the tube either.

When the diameter of the tube was 1.2 mm. or less, complete eversion of the organ failed to occur. In such instances the eversion attained the stage reproduced in Fig. 5. Despite the failure of the chitinized plates to evert, the sperm was ejaculated.

Observing the mating flights of the queens and examining the latter immediately upon their return to the hive, we noticed that queens less than 5 days old, put out in a Zander type nucleus, were fertilised later and in a lower percentage than older queens put out at age of more than 5 days.

Upon returning from the nuptial flight the queen looks as follows (Fig. 8). The paired oviducts are considerably dilated and their interior is filled with sperm, undiluted with mucus only occasionally small clumps of mucus can be seen in the oviducts, but they do not mix with the sperm. There are 6-20 cu. mm. of sperm in the oviducts alone. The common oviduct is compressed by strong muscles. We have observed queens in whom the spermatheca was depressed. As yet, there are very few spermatozoa in the spermatheca. The sperm is in the forepart of the vagina and the mucus fills the remaining section mostly from the end to the rugose appendage. The sting cavity of the queen holds the drone's torn off bulb of endophallus. This part is not everted. As can be seen from the illustration, only the chitinized plates and a small section of the wall are inside the body of the queen. The bulb of endophallus is completely filled with mucus and we never found any sperm in its front part. Upon returning to the hive, the queen immediately begins to remove the bulb of endophallus from the sting chamber, and the movements of the sting cavity are not intended to transfer the sperm from the bulb into the vagina and oviducts. We have also established that leaving for another mating flight the queen carries spermatozoa in her spermatheca.

#### Conclusions

Since the drones ejaculate 1.5 - 3.0 cu. mm. of sperm during eversion of the copulatory organ, while the fertilised queen's oviducts alone contain 6.0 - 20.0 cu. mm. of sperm undiluted with mucus, it follows that the queen mates with several drones in one flight.

Considering the size and the functioning of the reproductive organs of the queen and the drone honeybees, we believe that in the first stage of mating only the end of the partly organ, the so-called neck of endophallus is introduced into the body of the queen during the mating process. The bursal cornua remain outside.

The copulatory organ of the drone is, at this stage of eversion (Fig. 4), reminiscent of the copulatory organs of other insects and can function similarly. If this is the case, the bursal cornua are organs analogous to the parameres of other insects and perform a similar function.

Since the sperm is deep in the reproductive organs of the queen returning from the nuptial flight, it must have been introduced there during the mating process.

The stage represented in Fig. 5 is the only one permitting the ejaculation of sperm without concomitant eversion of the bulb of endophallus as observed in queens returning from a nuptial flight. Our opinion is that also in the natural mating process the ejaculation of the sperm occurs at this very stage of eversion. This can be achieved likewise by placing a glass tube, not exceeding 1.2 mm. in diameter, against the end of a drone's abdomen.

If, at the above described stage of eversion, the queen compresses the drone's copulatory organ which is inside her body, then the bulb of endophallus can be torn off in such way as can be observed in queens returning from a mating flight. We take it that only during the mating with the last drone of a given flight is the queen's sting chamber contracted and the drone's bulb of endophallus severed. This view is substantiated by the fact that, even in the hive, the queen has difficulties in removing the bulb of endophallus from the sting chamber. It can thus hardly be assumed that the queen, or the next drone, does this always in flight.

It would seem that, during the mating process, the queen as a rule does not close the sting chamber thus far as to cause the severance of the bulb of endophallus except in the instance of the last drone. The ejaculation of the sperm occurs in all drones at the stage represented in Fig. 5; subsequently the bulb of endophallus and the fimbriate lobe evert. The eversion of these parts automatically induces the extrusion of the endophallus from the inside of the queen's body. This extrusion is eventually facilitated by the bursting and shrinking of the end part of the copulatory organ (Fig. 7). Mating with subsequent drones develops similarly. Eventually, when mating with the last drone, the queen contracts the sting cavity, and severs the bulb of endophallus; having thus rendered further mating impossible, she returns to the hive.

When the severed part of the male organ and excess of sperm have

been removed from the sting chamber, it often happens that the queen leaves for further nuptial flights although her spermatheca contains spermatozoa.

#### Discussion

The results of our investigations contradict the hitherto generally accepted belief that during a nuptial flight the queen mates with one drone only. But we agree with the point of view of Triasko [10] who has drawn attention to the disproportion in size between the drone's seminal vesicles and the oviducts of the queen returning from a nuptial flight, although he has not measured the amount of sperm ejaculated by the drone. While the oviducts do not contain sperm alone as is reported by Triasko [10], there is, nevertheless, too little mucus in them to permit a dilution of the sperm as is maintained by Kuriennoi [9]. The amount of mucus in the oviducts of the queen returning from a mating flight is many times smaller than the difference between the volume of oviducts and that of the sperm ejaculated by one drone.

The size and shape of the bursa of endophallus and cornua in the first stage of eversion contradict the view according to which the bursa and the cornua are introduced during the mating process into the sting chamber and lateral genital pouches of the queen respectively [4], [9]. Other facts reported in this paper contradict the belief that the endophallus everts only partly during the mating process [2], [3], [11] and that the queen herself shifts the sperm from the bulb of endophallus into the oviducts [1].

If it were true, as Bishop [4], Fyg [5], Michaelis [7], and Snodgrass [9] take it, that the copulatory organ of the drone everts in the mating process completely and that the sperm is ejected from the ejaculatory duct directly into the genital tract of the queen, the queen would have to return from the nuptial flight either with a fully everted bulb of endophallus, or without it. Actually, almost all queens return with a non-everted bulb of endophallus, which fact contradicts such a view.

The remaining drawings, microphotographs, and a detailed description of the structure and functioning of the genital organs of the drone and queen will probably be published in "Folia Biologica".

DEPARTMENT OF BEEKEEPING, SCHOOL OF AGRICULTURE, WARSAW (SKIERNIEWICE)

#### REFERENCES

- [1] L. Arnhart, Arch. Bienenk. 16 (1935), 126.
- [2] — Arch. Bienenk. 17 (1936), 63.
- [3] — Arch. Bienenk. 19 (1938), 46.
- [4] G. H. Bishop, J. exp. Zool. 31 (1920), 225, 267.

- 
- [5] W. Fyg, *Bee World* 33 (1952), No. 8.  
[6] N. M. Kuriennoi, *Ptchelovodstvo* (1953), No. 10.  
[7] G. Michaelis, *Z. Wiss. Zool.* 67 (1900), 439.  
[8] R. E. Snodgrass, *Anatomy and physiology of honeybee*, New York, London, 1925.  
[9] — *Smith. Misc. Coll.* 89 (1933), No. 8.  
[10] W. W. Triasko, *Ptchelovodstvo* (1951), No. 10.  
[11] E. Zander, *Der Bau der Biene*, Stuttgart, 1922.