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AN ANATOMICAL STUDY OF THE MATING PROCESS IN THE HONEYBEE

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In recent years our knowledge of the mating biology of the honeybee has increased considerably, and research workers in many countries have contributed to this knowledge. At the XVI International Beekeeping Congress in Vienna in 1956, many discussions centred round the problems in this field which had not yet been cleared up. In particular they were concerned with the anatomical structures which make it possible for the queen to mate with several drones on the same flight, in the short space of about 15 minutes. We wanted to know exactly how copulation takes place, how the pair separates, how the mating sign is formed, and how it is later removed from the sting chamber of the queen.

The decision to write the present paper was made at this Congress; originally it was intended that V. V. Tryasko should be a third collaborator in the work of collecting together what has recently been discovered about the mating process in the honeybee. This proved to be impossible, and the two authors therefore decided to limit themselves to their own material. Particular attention is given to the exact process of the eversion of the endophallus, and to its position during ejaculation, to the detachment of the mating sign from the queen, and to the position of the queen and drone during copulation. The large amount of earlier work on mating in the honeybee has been reviewed by Fyg (1952), Ruttner (1956*a*) and Snodgrass (1956).

I. EXPERIMENTAL EVERSION OF THE ENDOPHALLUS

Both initial and final stages of the eversion of the copulatory organs of the drone have been studied by other workers, but the intermediate stages have not previously been described. In our opinion a clear description of these stages is necessary for an understanding of the processes of mating and the formation of the mating sign ; we have therefore recorded them carefully in Fig.1–Fig. 7. These drawings were made from preparations obtained by inducing artificial eversion in drones (caught as they were flying out of the hive) by pressure on the thorax or abdomen. Staining with Congo red (0·0025%) was used to show fissures or detached layers in the chitinous wall.

The eversion of the endophallus results from the pressure of the haemolymph which is itself pushed backwards by the contraction of the strong abdominal muscles. First the penis valves and the laminae paramerales (claspers) open, then comes the eversion of the abdominal wall with the basal part of the endophallus which carries the hairy triangular plate, and finally the two bursal cornua appear. The eversion and function

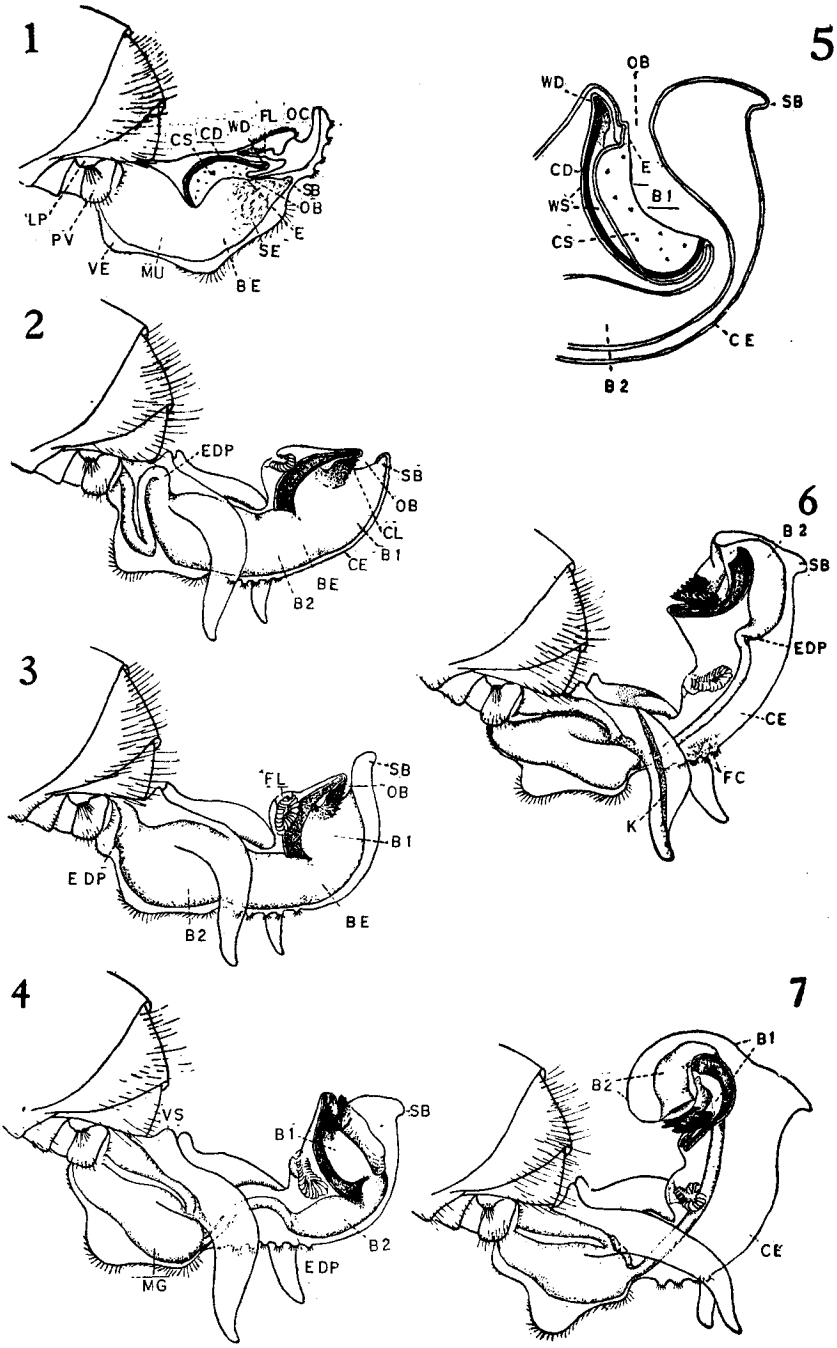

Fig. 1 – Fig. 7. Stages in the eversion of the endophallus ; Fig. 1 and Fig. 5 are in section.
For key to abbreviations see page 17.

of the bursal cornua have been already described (Woyke, 1955). Inside the everted base (vestibulum) of the endophallus, the bulb with its chitinized plates has emerged (Fyg, 1952, Fig. 3). This is the phase usually obtained by chloroforming drones.

Even in this early phase of eversion, the lumen of the endophallus is connected with the outer surface (between OB and OC, Fig. 1). The connection consists only of a very small slit, and since its inner opening does not lie at the point of the lumen, there is generally no penetration of the sperm or mucus from inside to outside, but—as an artifact of *artificially induced* eversion—this is ejaculated *into* the lumen of the penis bulb.

The most difficult part of the eversion, which requires the greatest force, is that of the middle portion of the organ—often referred to as the cervix. The sac of the bulb of the endophallus (Fig. 1, SB) and the ends of the chitinized plates enter the everting cervix, dilating it and, in consequence, rupturing the thin outer layer of its wall, producing one or more long fissures through the hairy areas on each side of it (Fig. 6, FC). At the same time the folds of the endophallus which come from the lateral chitinized plates* are everted. These everted folds now form the terminal part of the everted endophallus (Fig. 2, CE). After this part of the endophallus has passed through the narrow part of the cervix, the whole bulb pushes very quickly backwards inside the everting endophallus. As a result the pointed ends of the chitinized plates quickly reach the end of the everted endophallus. The ends of the lateral chitinized plates now protrude freely, externally, but the pointed ends of the dorsal chitinized plates are wrapped in two folds of the dorsal wall (Fig. 5, WD). The enveloping folds do not allow the plates to push themselves out of the endophallus, but give them a fulcrum about which they will turn, later on in the eversion.

After complete eversion of the middle portion, and the freeing of the pointed ends of the lateral plates (Fig. 2, CL), the dorsal wall of the sac of the bulb everts. This causes a considerable widening of the slit-like aperture of the endophallus (Fig. 2, OB); as a result, sperm begins to flow out from inside the bulb, being pushed out by the mucus. If eversion stops at this instant, it may happen that the mucus, being more viscous, remains inside ; this is a desirable condition for collecting pure semen for artificial insemination. The bulb fills almost the whole lumen of the everted part of the endophallus (Fig. 2, BE). The thin-walled part of the bulb is everted more quickly and easily than the dorsal wall, with its chitinized plates, which meet considerable resistance. So the sac of the bulb everts (Fig. 3, SB), and the chitinized plates are almost vertical within the endophallus (Fig. 3). The semen and mucus in the secretory ducts, which are under pressure from the muscles and haemolymph, meet with no resistance and can easily spurt out.

After ejaculation the membranous part of the bulb, which has so far been much dilated (Fig. 3, B2), collapses (Fig. 4, B2). It frequently happens that the two mucous glands and seminal vesicles are now pushed into the empty space within the base of the endophallus (Fig. 4, MG, VS). Meanwhile, under the pressure of the haemolymph, the eversion of the endophallus continues. The chitinized plates of the bulb incline more and

*For the sake of consistency the terms used by Snodgrass (1956) are retained here ; we should however prefer *long, broad plates* for *dorsal, lateral plates*, since the ' dorsal ' plate becomes the ventral one when the endophallus is in the queen.

more forwards, until finally their tips point towards the drone's head, and their broad bases are at the end of the everted endophallus (Fig. 6). Because of this, the thick-walled part of the bulb is also everted, and the lateral plates and the membrane of what was the ventral side of the bulb, the bow of the bulb, now stand free (Fig. 6).

After the eversion of the chitinized plates, the full eversion of the endophallus is completed quickly and comparatively easily. Because the ventral wall of the bulb everts more powerfully and more quickly, the membranous end of the bulb curves upwards and forwards, until the opening at the end of the endophallus is turned into a position within the everted plates ; here the remaining contents of the bulb flow out (Fig. 7). In the final phase of eversion, however, the end of the endophallus (with the opening of the ejaculatory duct) is free and points forwards, towards the drone's head. This phase is well illustrated in Fyg's Fig. 2 (1952), Woyke's Fig. 6 (1955) and Snodgrass's Fig. 107A (1956). The fimbriate lobe is everted only at the finish. The pressure of the haemolymph and air in the everted endophallus can be so great that the thin wall at the end of the bulb bursts, and the haemolymph flows out (Woyke 1955, Fig. 7).

2. THE MATING SIGN

The mating sign can easily be seen in the sting chamber of the queen when she returns from her mating flight. There has not yet however been agreement as to what the mating sign consists of. According to Zander (1951) and Snodgrass (1956), it is the bulb with its chitinized plates (separated from the endophallus) filled with mucus; according to Fyg (1952, Fig. 5) it is no more than a plug of mucus. The present authors have each independently examined a great number of queens on their return from the mating flight, and have found that either view can be correct. In most queens the bulb, with the chitinized plates, was found in the sting chamber, the plates, or at any rate the attached mucus, being always visible from outside (Fig. 19; Ruttner 1956, Fig. 10). In some of the queens, however, there was merely a plug of coagulated mucus (Woyke, 1956, Figs. 2, 4, 6, 8); this could be so small that the terminal sclerites of the queen had closed again, and no mating sign was visible from outside to show that the queen had successfully mated. Alber, Jordan, F. & H. Ruttner (1955) and Woyke (1955, 1956) recorded about 30% of matings ' with no mating sign ' and Taber (1954) 10%. In one series of observations on 184 queens returning from a mating flight (Woyke, 1956), a portion of the endophallus was found in the mating sign of 131 queens, and only a small plug of mucus in a further 53 queens.

Zander (1951) described the mating sign sticking into the sting chamber like a shoe horn, the chitinized plates having their convex sides turned to the ventral surface of the queen, and pointing forwards. In the following description we orient the endophallus as it lies in the queen; the wall of the ' sign ' which has the chitinized plates is ' below ', the direction of the points of the plates is ' forward '. One of us (Woyke) made a close anatomical and histological study of 60 mating signs which contained parts of the bulb of the endophallus. The findings may be summarized as follows. The mating sign is approximately cylindrical. Whilst its curved sides are covered with tissue, at either end its contents — mucus from the drone's mucous glands — form a free surface. The appearance of the anterior part of the mating sign varies according to the amount of mucus which we

Fig. 8, Fig. 9. Mating sign

Fig. 10. Chitinized plates with the bow of the bulb, prepared from the endophallus

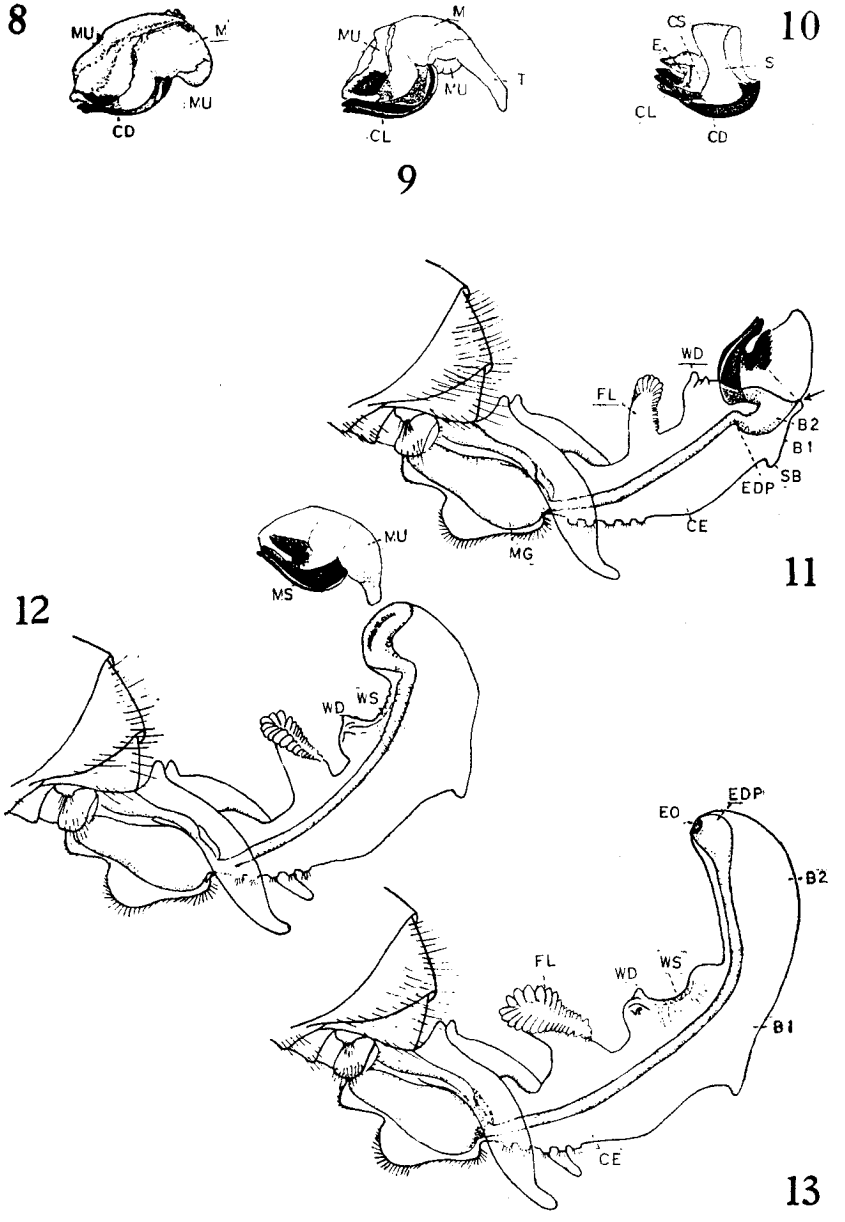

Fig. 11, Fig. 12. Separation of the mating sign from the endophallus

Fig. 13. Endophallus after the separation of the mating sign

For key to abbreviations see page 17.

remove with the bulb; a mating sign prepared from a queen treated with fixative is, so to speak, a casting of the part of the queen from which it is taken. The posterior end of the mating sign either finishes off bluntly (Fig. 8) or with a short or long thread (Fig. 9, T); this thread consists exclusively of coagulated mucus and never contains the ejaculatory duct ; we have in fact never found this in any mating sign. The structure which covers the sides of the mating sign is formed below by the dark-brown chitinized plates ; on their concave inner side is an amorphous, putty-like mass of connective substance (Fig. 5, CS), which holds the plates together and which behaves quite differently from the mucus on staining. According to Snodgrass (1956) this must be regarded as a thickening of the cuticular intima. In young drones the cuticular intima is missing, and after eversion the plates therefore fall apart.

The other part of the covering of the endophallus is formed by the bow of the bulb. This strong bow-shaped membrane stretches across between the edge of the chitinized plates, spanning their concave side like a bow (Fig. 10, S). In an intact uneverted endophallus, the bow occurs as an inner lining of the hollow interior of the bulb. If in the last phase of eversion the chitinized plates tilt with their tips forwards, and the bulb is everted, then the bow of the bulb comes to lie on its convex side. This makes it possible to determine, without exception, whether the chitinized plates are everted or not (Fig. 14).

The chitinized plates and bow of the bulb do not, however, form the outermost layer of the mating sign. Closer observation shows yet another transparent, yellow, layer (Fig. 8, Fig. 9, M), which lies close to the bow of the bulb and also covers (to a varying degree) the chitinized plates. The edges of this layer are mostly irregularly corrugated or indented. The front edge generally corresponds with the edge of the bow of the bulb, which lies below it; often it allows a narrow edge to protrude. At the top, opposite the chitinized plates, this covering layer is generally torn (Fig. 8). Observations on the different positions of the mating sign in the sting chamber suggest that this is due to movements of the sting apparatus.

It might be supposed that this yellow layer is the same substance which covers the pneumophyses, and which has somehow got on to the mating sign, but a closer study shows that it must have been in existence originally, and is not a substance acquired later. This is borne out by the fact that its extension is very regular, and that individual variations do not exceed certain limits. The identity of this layer only became apparent when the bulb wall was examined histologically.

Transverse sections through the uneverted bulb of the endophallus have shown the following structure of the membranous wall of the bulb (Fig. 15): the inner layer (1) is formed by the strong bow of the bulb; outside this there is a thicker layer of transparent chitin, which is itself clearly divided into an inner and an outer layer (2 and 3); the outermost layer is formed by the epithelium (4) on the basal membrane. On transverse sections through the mating sign, one finds, in the corresponding order, the bow of the bulb and a chitinized layer which is however much thinner than that in the intact bulb. The average thickness of the chitin covering the mating sign is 0·0025–0·0035 mm., but the chitinized layer of the bulb wall is about 4 times as thick—averaging 0·0115 mm. No epithelium is to be found in the mating sign.

The covering of the mating sign thus consists not of the whole bulb

wall, but only of its two inner layers : the bow of the bulb and the inner chitinized layer. This makes it clear that the mating sign is not produced by the endophallus tearing off transversely, but by the *longitudinal* separation of the wall layers : the mating sign is *pushed out* backwards from the endophallus. The chitinized layer of the bulb wall thus functions as a ' slide ' which both holds in place the part of the bulb which forms the mating sign, and also allows its separation. The experimental data cited in the next section support this hypothesis. Tryasko (1957) also recently came to the conclusion that a membranous covering from the bulb wall is missing in the mating sign, and hence that this must have been pushed out from—not torn off—the endophallus.

3. EXPERIMENTAL SEPARATION OF THE MATING SIGN FROM THE ENDOPHALLUS

We wished to ascertain whether it was possible, by dissecting off the layers of the bulb wall, to produce a preparation similar to the mating sign, that is, a preparation consisting of the chitinized plates and the bow of the bulb, covered with a thin layer of chitin. It proved possible to accomplish the following, even with drones preserved in alcohol. First

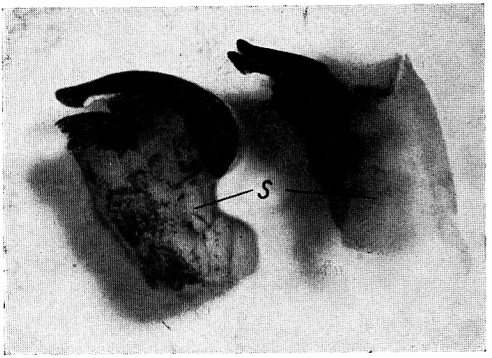

Fig. 14. Chitinized plates with the bow of the bulb
left, uneverted (mating sign)
right, everted (from preparation of fully everted endophallus)
S bow of the bulb

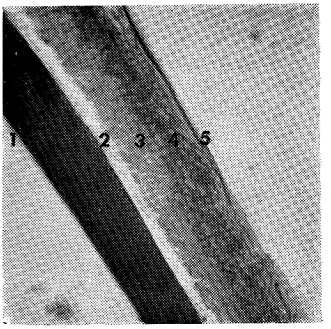

Fig. 15. Section through bulb wall (photomicrograph taken with polarizing microscope)
1 bow of the bulb
2 chitin layer next to the bow of the bulb
3 chitin layer next to the epithelium
4 epithelium
5 basal membrane

the outer layer, the epithelium, can be loosened from the covering of the bulb. Then the fairly thick transparent chitinized layer of the covering can be separated. On the outside of the chitinized plates and the bow of the bulb there still remains a thin layer of chitin; in the uneverted bulb of the endophallus this structure formed the thin inner chitinized layer (Fig. 15, 2). *What is now the outer wall of the bulb very much resembles the outer wall of the natural mating sign.* It proved possible finally to remove also the third layer of the bulb covering. Of the bulb itself there then remained only the chitinized plates (Fig. 10, CD, CL), which are united by connective tissue (CS), and the bow of the bulb (S) which arises from

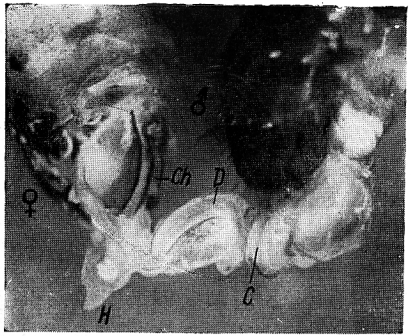

Fig. 16. Pair *in copula*; in this preparation the two end sternites of the queen are removed to show the chitinized plates (Ch) of the bulb, partly separated from the bulb wall; H is the pocket in the bulb wall

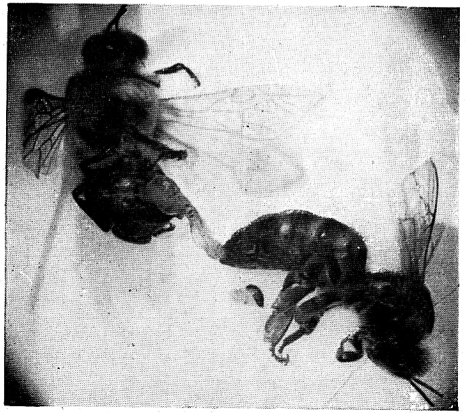

Fig. 17. Pair *in copula*; under the abdomen of the queen is a mating sign from a previous mating.
Figs. 14, 16, 17 are from *Z. vergl. Physiol.* 39 1957

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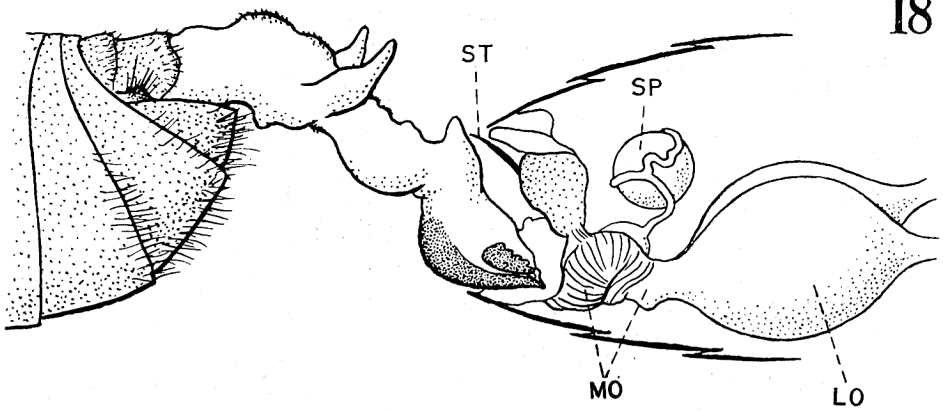

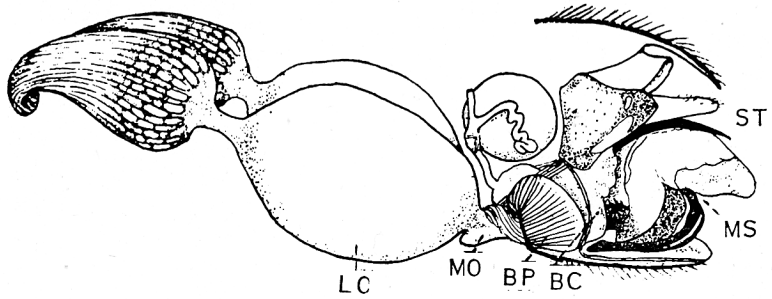

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Fig. 18. Pair *in copula*; in this preparation the great mass of sperm in the lateral oviducts of the queen has been made visible
Fig. 19. Position of the mating sign in the sting chamber of the queen

them. The bow becomes thinner and thinner the further it is from its point of attachment; it is thinnest at the summit of the bow which it forms. Thus the bow of the bulb, together with the chitinized plates and the connective tissue which unites them, form a closed ring (Fig. 10), which lines the *inside* of the bulb, and holds the contents of the mating sign together from the *outside*.

The endophallus only everts in the way described in Section 1 if nothing prevents its complete eversion. This is not so during mating, and we therefore next investigated the eversion of the endophallus in circumstances more like natural mating. Against the abdomen of a drone we held a glass tube whose diameter was the same as that of the sting chamber of the queen. Eversion took place as far as the stage shown in Fig. 2. If the diameter of the tube was smaller than the length of the dorsal plates, eversion ceased for a time. *Although the bulb was then not everted, ejaculation of the sperm from the end of the endophallus followed. Even in the glass tube there was no mixing of the semen with the mucus.* After the bulb was partly emptied, the eversion of the chitinized plates followed (in the tube) under the action of the mounting pressure. This occurred even when the diameter of the glass tube was only 1·2 mm. (with the dorsal chitinized plates 2·25 mm. long.) But the plates did not now part from the endophallus ; the conditions for eversion in the tube must therefore be different from those in the genital organs of the queen. In the tube the endophallus has unlimited opportunity to evert backwards, which is not so in the queen. To reproduce the conditions in the genital organs of the queen more exactly, the backward eversion must be made more difficult. It is sufficient to press with the fingers on the thin-walled part of the bulb at the end of the endophallus, and at the same time on the abdomen of the drone. If this pressure is maintained during all further stages of the eversion, the following procedure is observed. Because of the impossibility of further eversion, the pressure of the haemolymph within the endophallus rises; this pressure is also exerted on all folds and protrusions not yet everted. The fimbriate lobe therefore becomes everted. As the pointed ends of the dorsal chitinized plates are now not pointing straight backwards, the folds and protrusions of the covering which surrounds these ends (Fig. 5, WD) begin to draw away from them. After the folds are freed and everted (Fig. 11, WD), the pointed ends of the dorsal plates project freely, as do the lateral plates (Fig. 11). The detachment occurs, as we saw above, at the chitin layer of the bulb wall.

The subsequent detachment of the sheath from the entire surface of the plates occurs very easily ; only at their point of attachment at the broad end of the dorsal plates does the process come to a temporary stop. Because of the further eversion of the endophallus, the sheath of the artificially formed mating sign (Fig. 12, MS) is finally freed completely, and the mating sign is thrust out backwards (Fig. 12). But the endophallus does not tear after the mating sign has become detached ; instead it everts still further. The individual folds of the fimbriate lobe now become everted, so that the whole lobe bends forward (Fig. 12, Fig. 13). The thin-walled portion of the bulb also everts (Fig. 13, B2). In place of the endophallus, which earlier carried the chitinized plates, there is now a longish indentation with two small protuberances (Fig. 13, WS, WD); these correspond to the folds which enveloped the pointed ends of the dorsal plates before eversion.

The mating sign (Fig. 12, MS) obtained experimentally by this method resembles that found in the queen. The only difference seems to be that the artificially produced sign has a rather smaller yellow chitin layer, This is probably due to the difference in the conditions of separation, for instance the length of time involved, and the physiological state of the drone. But the main features are the same. In the experimental mating signs, the chitinized plates, together with the bow of the bulb, also form a closed ring which surrounds the mucus inside the mating sign ; in fact it is comparable with the natural mating sign before this becomes damaged by the sting apparatus of the queen.

If this experimentally produced process corresponds to the process of natural mating, then the drone after mating must possess a fully everted endophallus, but without its chitinized plates and the bow of the bulb. So far only Tryasko (1955) has reported upon drones which have been found after complete mating. These drones were in the same condition as those from which the mating sign had been experimentally produced (Fig. 13).

These experiments and their results lead us to conclude that, during natural mating, the mating sign becomes detached from the endophallus in the manner described.

4. OBSERVATION OF THE MATING ITSELF

The most conclusive verification of the above interpretation would be one obtained by the observation and investigation of a queen and drone *in copula*, but so far no exact description of such a pair has been available.

It is extremely difficult to obtain observations of the actual course of mating, and all efforts to obtain mating in an enclosed space have so far failed. An attempt was therefore made to obtain the required conditions, not by limiting the queen's flying space, but by limiting her ability to fly. In the summer of 1956 the front wings of a number of queens were clipped by such an amount that the queens were only just able to fly. A similar reduction of the queen's ability to fly can be obtained by sticking a small metal plate (about 50 mg.) on her thorax. Queens with clipped wings fly more slowly than others ; also the note emitted is higher and harsher, and therefore more easily distinguished from those of flying workers and drones. If the weather is not very favourable for mating, clipped queens usually circle in the immediate neighbourhood of the mating apiary. In very warm sunny weather, however, they too fly so high that it is easy to lose sight of them. Even so, a considerable part of the mating flight can be followed, and interesting details of the mating display observed (Ruttner, 1957).

The final phase of one mating was observed, and the pair *in copula* could be investigated (Fig. 17). The queen went for her first flight on July 26th. After making some loops close to her hive, she circled higher and higher until she could not be seen. Ten minutes later she reappeared about 12 metres above the ground, at the centre of a swarm of about ten drones ; details could not be ascertained because of the height, and the bees' rapid movements. Suddenly the whole swarm fell vertically to the ground. The accompanying drones gradually flew up and away, but the pair was found *in copula* among the grass, the drone lifeless on his back, and the queen whirring her wings vigorously (Fig. 17). Close by the queen, the mating sign of another drone was sticking to a blade of grass.

The queen was killed in a cyanide bottle about one minute after falling, and the following observations were made on the subsequent preparation.

1. The endophallus was everted up to the bulb.
2. The chitinized plates of the bulb were not everted but, together with the bow of the bulb, were pushed out of the membranous endophallus.
3. The convex side of the chitinized plates was separated from the endophallic membrane. The mating sign was attached to the endophallus only by the broad end of the dorsal plates and by the posterior edge of the bow of the bulb (Fig. 16); this condition corresponds nearly to that in Fig. 11.
4. The everted endophallus was undamaged, except in one place near the point of attachment of the mating sign, where the sting apparatus of the queen had pierced the membrane. The endophallus had thereby lost its turgidity. The fimbriate lobe had everted, but had collapsed again.
5. The chitinized plates were in the same condition, and in the same position in the sting chamber, as in every queen studied on returning from a mating flight ; it was therefore concluded that this example represented a normal mating.
6. The queen had been mated several times during the unobserved ten minutes, for the mass of sperm in the lateral oviducts was estimated to correspond to the quantity produced by 6 drones (Fig. 18). In at least one of these earlier matings, a mating sign had been formed which was probably only removed from the sting chamber during the mating with the final drone, and which subsequently became detached from the body of the queen or drone. The spermatheca contained only a small amount of sperm.

The mating sign in this naturally mated pair was therefore formed in the same way as in the experiments described on page 11. These also explain the delay in the pair becoming separated; because the damage to the membrane of the endophallus destroys its turgidity, the mating sign does not peel off from the outer covering so easily and quickly as from an intact organ with a tightly stretched wall.

5. THE POSITION OF THE MATING SIGN IN THE GENITAL ORGANS OF THE QUEEN

Fig. 19 shows the position in which the mating sign is generally found —in so far as the mating sign contains a portion of the bulb of the endophallus at all. In the queen the chitinized plates of the mating sign reach as far as the bursa copulatrix ; indeed the points of the lateral plates project into it, and the dorsal plates press in under the folds of its lower edge ; the broad ends of the dorsal plates lie just above the tip of the final sternite of the queen. The sting apparatus is pushed upwards and backwards by the mating sign, so that the sting, usually hidden, projects freely (Ruttner 1956*a*, Fig. 10).

Occasionally one finds deviations from this position. The mating sign may not lie symmetrically, but be turned somewhat on its longitudinal axis in the sting chamber (Ruttner 1956*a*, Fig. 10; Woyke 1956, Fig. 1, Fig. 3). Or the two chitinized plates may reach further into the bursa copulatrix ; they are then invisible from outside. In rare cases they may even be found within the vagina (Bishop, 1920), or one of the two bursal pouches (Ruttner 1956*b*).

' Double mating signs ' have been described by Tryasko (1951) and Ruttner (1956*a*, Fig. 8). It cannot be stated for certain how they come

about. Possibly one from an earlier mating becomes stuck fast to the fresh mucus of a subsequent mating sign. It is clear from the occasional irregular disposition of sperm (only) that a second mating sometimes occurs while the earlier mating sign remains in place. One of us (Woyke) was able to examine four newly mated queens in which, contrary to the usual state of affairs, there was sperm *at the back* of the mating sign. The sperm was outside on the mucus, on the chitinized plates, and on the covering of the mating sign ; the amount corresponded to that produced by one drone. One cannot assume that this sperm came from an abnormal ejaculation, in which for instance the mucus was ejaculated before the sperm, for the vagina and oviduct of these queens were filled with sperm, and the sperm from the later mating was on the outside of a normal mating sign. It seems much more likely that a drone had attempted to mate with a queen which still had a mating sign in her sting chamber, sperm being ejaculated but the mating sign remaining in place.

6. DISCUSSION

The mating of the honeybee is an unusually complicated affair, and one which is very difficult to observe. Various recent investigations have clarified the resultant condition of the newly mated queen, but there is still great dissension as to the procedure during mating (Snodgrass, 1956). The material presented in this paper enables us to answer some of the disputed questions, and attention is called to certain facts which we now regard as established.

1. The ejaculation of the sperm does not occur when the endophallus is wholly everted, but when eversion has been carried out as far as the bulb, and the points of the lateral chitinized plates (now projecting free) open up a passage out of the lumen of the uneverted bulb (Fig. 2). The sperm only enters the bulb during eversion, and from there is flung out on the sudden production of an opening in the bulb.
2. If during the process of eversion the end of the endophallus meets with some resistance, the bulb cannot evert fully. Its inner lining (the chitinized plates together with a bow-like membrane referred to as the bow of the bulb) is pushed out of the endophallus uneverted. The separation from the bulb covering occurs along a layer of chitin, whose outermost stratum remains on the endophallus. This body—the chitinized plates with the bow of the bulb, filled with mucus and covered outside with the yellowish inner chitin layer of the bulb wall—forms the normal mating sign.
3. The endophallus is undamaged when it becomes detached from the mating sign. After the end of the eversion, the pressure within can result in its wall bursting, with an audible pop. If the wall is torn earlier, its turgidity is lost, and the complete detachment of the chitinized plates is then much more difficult, and takes longer. It is therefore not necessary to assume that the endophallus is broken or twisted off. The separation of the mating sign takes place, under suitable conditions, of its own accord and very quickly.

We give below an account of the procedure of mating as we believe it to take place, limiting the account to phenomena and conditions which have been demonstrated anatomically, and then (page 17) discuss very briefly those parts of the procedure which have not yet been sufficiently

investigated, such as the beginning of the copulation, the position of the pair during copulation, and the rapid removal of the mating sign from the sting chamber for the reception of the next insemination.

At the commencement of the mating the base of the endophallus and the pneumophyses are the first organs to be everted. The points of the latter are first turned dorsally and outwards. As the internal pressure increases, the sticky, orange-yellow surface layer of the pneumophyses becomes smooth and is pushed to their lateral sides. As a result the pneumophyses bend downwards, and their points become closer, probably embracing the queen's abdomen.

The bulb, which only becomes filled with sperm and mucus during the mating process, has meanwhile entered the wide cavity of the vestibulum (Fig. 1), and is now (under considerable pressure) pushed slowly thence through the everting cervix. The partially everted penis is introduced into the actively opened sting chamber of the queen, the points of the chitinized plates — now forming the end of the endophallus — reaching into the bursa copulatrix. As eversion proceeds, the points of the lateral chitinized plates push out of the endophallus (Fig. 2), and the sac of the bulb begins to evert (Fig. 3). As a result, an opening is formed which leads to the lumen of the bulb, and out of this opening the sperm is ejected under strong pressure. The semen is guided by the lateral chitinized plates into the vagina, and from there past the depressed valvefold into the oviduct. The viscous mucus which follows can only move slowly; it fills the bursa copulatrix with its side pockets, and the sting chamber, but does not normally extend as far as the oviducts. Since ejaculation of the sperm takes place from the *uneverted* bulb of the endophallus, there is no cogent argument for assuming that the position of the queen during mating must be above the drone. The anatomical facts known are not yet sufficient to enable us to determine the relative position of the queen and drone during mating.

The further eversion of the endophallus encounters resistance in the narrow sting chamber. The endophallus pushes against the queen's abdomen, and the bulb wall is everted under increasing pressure from within. Its contents — the chitinized plates filled with mucus, and the bow of the bulb — are pushed out of the wall uneverted. The separation takes place along the surface of a preformed layer of chitin in the bulb wall. This peeling off of the wall from the chitinized plates and the bow of the bulb begins at the tip and continues to the base (Fig. 11, Fig. 12). The now fully everted endophallus separates undamaged from the mating sign (Fig. 13), and with it the separation of the queen and drone is accomplished. It is possible that the fimbriate lobe, at its eversion, helps to free the mating sign from the endophallus. When the mating is finished, the endophallus is fully everted, but it has lost its chitinized plates and the bow of the bulb. If at this instant the endophallus is under a strong pressure from within, it may burst at its tip.

During mating, the flight of the pair is largely hindered. But the pair only falls to the ground if the queen and drone have been unable to part immediately, or if they have paired at a low height. Usually the whole mating procedure takes place in the open air, during flight. The queen flies on after separation from the drone, and the dead drone falls to earth (Melzer, 1956). It seems likely that the mating act usually lasts only for a few seconds. The fact that the queen, left with the mating sign, can mate again on the same flight, was established by the finding of a mating sign

close to a copulating pair (page 12). We do not yet know how this occurs, especially how the mating sign is removed from the sting chamber during flight. On the basis of the observations set out above it is possible to make the following conjectures, which are no more than conjectures. The queen pushes the mating sign partly out of the sting chamber by movements of the abdominal segments and the sting apparatus, as she does when anaesthetized. As soon as she is again in the mating position with another drone, the mating sign becomes attached to the hairs of the triangular plate of the everting endophallus, which point backwards; one can see a similar procedure when the queen, after returning to the colony, drags the tip of her abdomen over the comb, and leaves the mating sign hanging on the edge of a cell. During flight the preceding mating sign falls to the ground, or sticks to the abdomen of the queen or the drone [see page 18].

Since a number of queens return from their mating flights with only a lump of mucus in the sting chamber, and without any part of the bulb of the endophallus, one may assume that the chitinized plates do not become detached from the endophallus in all drones.

We have seen that a certain position and anchoring in the sting chamber is necessary for the quick detachment of this portion of the bulb; if this does not occur, the bulb sheath will not peel off the endophallus after ejaculation. The chitinized plates then also become everted, as in artificially induced eversion, and only a lump of mucus remains in the queen's sting chamber. Within the anatomical possibilities, certain variations can thus occur ; this seems to apply to the whole mating procedure, including the much disputed position of the queen and drone during mating.

A mating sign consisting entirely of mucus is mostly found in queens whose oviducts are very full of sperm, or whose ovaries are already well developed (Woyke, 1956). The termination of matings during any one flight could be due, amongst other reasons, to the fairly large volume of the ovaries and the sperm-filled oviducts, or to the impossibility of removing the former mating sign.

SUMMARY

A description is given, based on a large amount of material, of the experimental eversion of the endophallus in all its stages. It is shown that the ejaculation of sperm and mucus does not *follow full eversion*, but occurs *before the eversion of the bulb*.

In about 70% of queens examined, the mating sign contained parts of the bulb of the endophallus ; in 30% it consisted only of mucus. In the former, the mating sign consisted of the mucus-filled chitinized plates and the bow of the bulb, both covered to a certain extent by a yellowish chitin layer and not everted. An almost identical body can be experimentally detached from the endophallus if complete eversion of the endophallus is prevented. The separation of the chitinized plates and bow of the bulb from the endophallic membrane takes place along an amorphous chitin layer in the bulb wall.

In natural mating, ejaculation takes place from the uneverted bulb. The chitinized plates and the bow of the bulb, held fast in the sting chamber, are pushed uneverted out of the endophallus under the pressure of the continuing eversion ; the tube of the endophallus is therefore generally undamaged.

The anatomical condition of a queen and drone *in copula*, which were obtained by certain experimental arrangements, corresponded entirely

with the above hypothesis. In the normal course of events, mating and separation of the pair occur very rapidly, presumably during flight, and in spite of the presence of the mating sign further matings can take place during the same mating flight.

KEY TO ABBREVIATIONS USED IN FIGURES

	English	*German*
BE	bulb of endophallus ; penis bulb	Zwiebel ; Bulbus
B1	the part of the bulb which is strengthened by the chitinized plates and the bow of the bulb	Teil der Zwiebel verstärkt durch die Chitinplatten und die Bulbusschleife
B2	thin-walled part of the bulb	dünnwandiger Teil des Bulbus
BC	bursa copulatrix	Begattungstasche
BP	bursal pouch	Seitentasche der Begattungskammer
C	pneumophyses of endophallus ; bursal cornua	Hörnchen
CD	dorsal chitinized plates*	lange Chitinplatten
CE	cervix of endophallus	Mittelstück des Begattungsschlauches
CL	lateral chitinized plates*	breite Chitinplatten
CS	connective substance ; 'semi-transparent thickening of the cuticular intima' (Snodgrass 1956, p. 296)	Verbindungsmasse
D	ejaculatory duct	Ductus ejaculatorius ; Spritzkanal
E	edge of connective substance partly concealing the posterior opening of the bulb of the endophallus	Rand der Verbindungsmasse, die hintere Zwiebelöffnung teilweise verdeckend
EDP	posterior end of the ejaculatory duct	hinteres Ende des Ductus ejaculatorius
EO	opening of the ejaculatory duct ; gonopore	Öffnung des Ductus ejaculatorius ; Gonopore
FC	fissures in the outer layer of the cervix	Risse in der äusseren Schicht der Cervix
FL	fimbriate lobe	gefiederter Anhang
K	sticky surface layer pushed back from the pneumophysis	klebrige Schicht, von der Pneumophyse heruntergeschoben
LO	lateral oviduct	lateraler Eileiter
LP	lamina parameralis ; upper clasper	Deckschuppe
M	covering of the mating sign	Hülle des Begattungszeichens
MG	mucous glands	Schleimdrüsen
MO	median oviduct	medianer Eileiter
MS	mating sign	Begattungszeichen
MU	mucus	Schleim
OB	posterior opening of the bulb	hintere Öffnung der Zwiebel
OC	external opening of the cervix	äussere Öffnung des Mittelstückes
PV	penis valve; lower clasper	Deckplatte
S	bow of the bulb	Bulbusschleife
SB	sac of the bulb	Bulbussack
SE	semen	Samen
SP	spermatheca	Samenblase
ST	sting	Stachel
T	thread-like end of the mating sign (mucus)	fadenartiges Ende des Begattungszeichens (Schleim)
VE	vestibulum or base of the endophallus	Basis des Begattungsschlauches
VS	seminal vesicle ; vesicula seminalis	Samenblase
WD	doming of the bulb wall, surrounding the pointed ends of the dorsal plates	Vorwölbung der Zwiebelhülle, die spitzigen Enden der dorsalen Chitinplatten umfassend
WS	stretching of the bulb wall for the chitinized plates	Einbuchtung in der Zwiebelhülle für die Chitinplatten

* see footnote on p. 5.

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Note added in proof [*see page 16*] : Tryasco [*Pchelovodstvo* 34(12) : 29-31 (1957)] describes drones which, after mating, had a mating sign attached to the basal plate of the vestibulum.

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