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## La fecundación en la abeja.

### II. El uso de los fluidos sexuales en los órganos de la hembra.

El proceso copulatorio de la abeja tiene lugar al aire libre y su naturaleza debe inferirse, por consiguiente, del exámen de los insectos antes y después del apareamiento. La configuración del tracto vaginal en la reina es tal que el pene del zángano solo puede penetrar ligeramente en el orificio, quedando el bulbo del pene, de gran tamaño, en el vestibulo génito-anal, en posición caudal a la de la vagina propiamente dicha. Las "pneumopófisis" del órgano del zángano sirven aparentemente no como organos de retención, sino para abrir el orificio vaginal para la inserción del pene, inflando los divertículos de la bolsa a cadal ado de la vagina. Los espermatozoides entran primero en los órganos y llenan los oviductos pares, penetrando después el mucus, mas viscoso, para formar un núcleo central, llenando la vagina caudalmente donde se endurece para formar un tapón vaginal cerca del orificio. El órgano del zángano puede desprenderse de la vagina de la reina al cabo de unas dos horas. Los espermatozoides y el mucus se mezclan solo parcialmente, tendiendo a separarse los primeros hacia las paredes de los oviductos y a pasar caudalmente, aparentemente por quimiotaxis, al conducto de la espermateca que se abre en la vagina. El mucus se absorbe por los oviductos mas lentamente. La mayor parte de los espermatozoides han entrado en la espermateca al cabo de unas seis horas después de la cópula, mientras que parte del mucus puede permanecer en los oviductos durante dieciocho horas. Los espermatozoides y el mucus se disponen de este modo separadamente en los órganos de la reina, de un modo ya anticipado por el examen de la estructura y funcionamiento de los órganos del zángano (descritos en un trabajo anterior).

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# FERTILIZATION IN THE HONEY-BEE

## II. DISPOSAL OF THE SEXUAL FLUIDS IN THE ORGANS OF THE FEMALE

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TWO TEXT FIGURES

CONTENTS



## INTRODUCTORY

In the paper preceding this (Fertilization in the Honey-bee, I) has been described the sexual mechanism of the drone bee and its physiological functioning in copulation. As a check to this work, a series of dissections and microscopic examinations has been made of queen bees which had flown from the hive and been mated under entirely normal conditions. Eight virgin females were watched and their time of mating recorded, and were subsequently examined to determine the behavior and the disposal of the sexual secretions of the drone in and after copulation.

These queens were killed for examination at specific intervals of time after their mating, and examined either fresh or after preparation for histological study, as conditions appeared to render desirable. The time data were obtained in the following manner. The virgins were introduced into strong nuclei of young bees, and these nuclei were inspected every half-hour during the warm bright part of the day, beginning in each case when the virgins were four days old. The bees showed little effects of handling, and the fact that queens were mated from

such nuclei repeatedly, promptly at the normal age, and at the usual time of day for mating flights (11 A.M. to 2 or 3 P.M.) indicates that for the purposes of the experiment such frequent handling did not interfere materially with the results. At each inspection the queen was actually located by observation or else searched for at fifteen-minute intervals until found or until satisfactorily demonstrated to be lost. In this way a queen found with the drone's organ attached was known to have returned from a mating flight within the past half-hour if she had been observed at the last regular inspection, or within forty-five minutes if she had been missing then, etc. The small number of queens available makes too precise an interpretation of the resultant data inadvisable, but the general agreement of the results from the respective queens allows of certain reliable and definite conclusions.

The queens were treated as follows:

Two queens, A and B, killed immediately on return to hive; one was dissected fresh and one preserved for histological study.

Two queens, C and D, killed one to one and a half hours after mating, one dissected fresh and one preserved for histological examination.

One queen, E, killed two to two and a half hours after mating. Dissected.

One queen, F, killed four to four and a half hours after mating. Preserved for histological examination.

One queen, G, killed six to six and a half hours after mating. Dissected at once.

One queen, H, killed eighteen hours after mating. Preserved for histological examination.

## HISTORICAL

There has been little reported in the literature on actual results of fertilization of the queen bee. The work of Bresslau,[1] checked by Zander,[2] appears satisfactory as an anatomical

[1] Bresslau. Die Samenblasengang der Bienenkönigin. Zool. Anzeiger, Bd. 29, 1905.

[2] Zander. Die Ausbildung des Geschlechts bei der Honigbiene. Zeit. der angewandte Entomologie, Bd. 3, 1916.

picture of the mechanism by which the queen fertilizes the egg. The fact has been noted elsewhere, however,[3] that this anatomical study of the queen's organs does not elucidate by what manner the sperms get into the spermatheca of the queen after fertilization. There is no explanation recorded of the reception of the spermatic fluid into the female organs, except a remark by Shafer,[4] that the queen's vagina, and 'in one queen' (of four available for study), the oviducts were distended with fluid after copulation. No other statement bearing directly on this subject has been found.

## ANATOMY OF THE FEMALE SEXUAL APPARATUS

Before passing to the consideration of these fertilized queens, the configuration of the female genital tract merits consideration.

In figure 1, A, is shown diagrammatically the dorsal aspect of the sexual organs of the virgin queen. This is the view which shows most of the gross anatomy and the one conventionally presented. This aspect, however, demonstrates most inadequately several structural details and relationships which are significant in fertilization. Figure 1, B, is a diagram of the sexual apparatus in profile, showing particularly the configuration of the lumen of the tract. This is partially figured (in the region of the 'sperm pump' leading to the spermatheca) in Bresslau's work on the seminal receptacle and sperm pump[1] but the significance of the more general relationships is not there brought out.

The queen and drone, in copulation, meet face to face while in rapid flight. The drone's copulatory organ (fig. 2, A), by an explosive contraction of the abdominal muscles, is everted from the body of the drone (fig. 2, B or C) into the copulatory bursa of the queen, and there it lodges. The queen twists off the drone's organ and returns to the hive with the end of it attached in the vagina.

The complex morphology of the penis is usually interpreted as facilitating this penetration of the drone's organ as it everts

[3] Snodgrass. Anatomy of the honey-bee. U. S. Dept. of Agr. Bur. Entomol. Tech., Series 18.

[4] Shafer. A study of the factors which govern mating in the honey-bee. Mich. Agr. Col. Exp. Sta. Tech., Bul. 34, 1917.

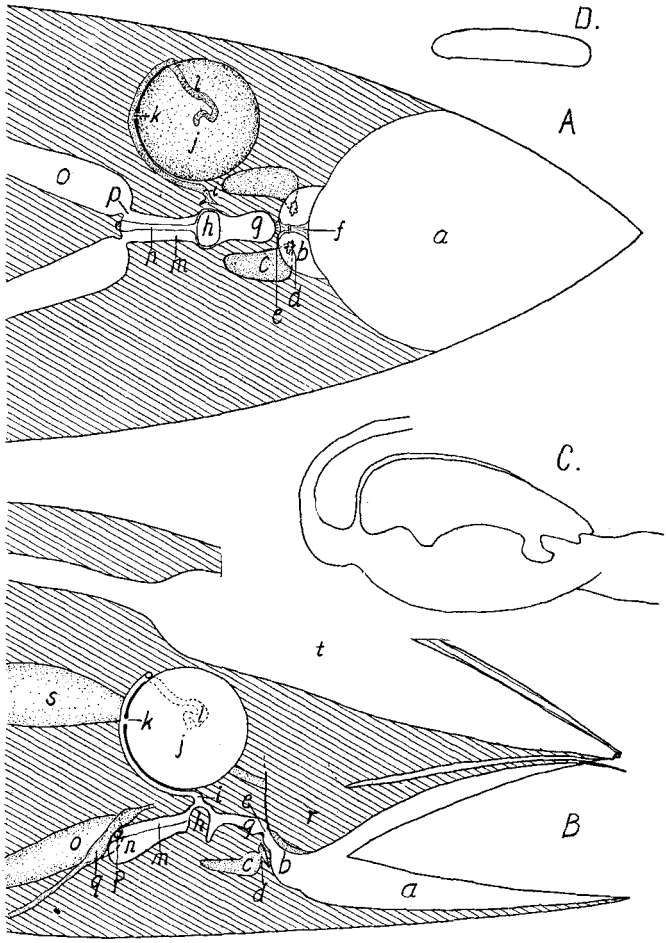


Fig. 1 Dorsal (A) and lateral (B) views of female fenital tract of bee, with part of male organ (C) drawn to the same scale. *a*, ventral floor of genito-anal vestibule; *b*, bursa copulatrix; *c*, bursal pouch; *d*, aperture of pouch; *e*, vaginal orifice; *f*, medial ridge of ventral floor of bursa; *g*, posterior region of vagina; *h*, tongue-like organ situated in medial region of vagina; *i*, duct to spermatheca, or 'sperm pump;' *j*, spermatheca; *k*, its opening from the sperm duct; *l*, glandular continuation of sperm duct; *m*, posterior region of vagina; *n*, fossa in floor of same; *o*, oviduct; *p*, aperture from oviduct to vagina; *q*, ganglion straddling end of vagina; *r*, base of sting; *s*, poison sac of sting; *t*, rectum. All drawings free-hand, but drawn proportional to measurements of camera-lucida projections of cross-sections, longitudinal sections, and whole mounts of the organs represented.

A. Dorsal aspect, diagrammatical optical section of female genital tract. The spermatheca is shown rolled over to one side of its normal position immediately dorsal to the anterior portion of the vagina, and therefore presents a lateral view. Drawn as if everything dorsal to the genital tract were dissected away, and the lumen of this laid open.

B. Lateral aspect, profile of genital tract, drawn as above.

C. Uneverted bulb of penis and adjoining regions. Comparison of the size of this organ with the dimensions of the female genital tract casts some doubt upon the assumption that this organ everts into the vaginal tract, *g*. The only part comparable in size to this region is the extreme tip of the everted organ (fig. 2, C, *c'*), formed by the eversion of the ejaculatory duct beyond the penis bulb; while the bulb itself would scarcely be completely contained in the much larger bursa.

D. Egg drawn to same scale as genital tract.

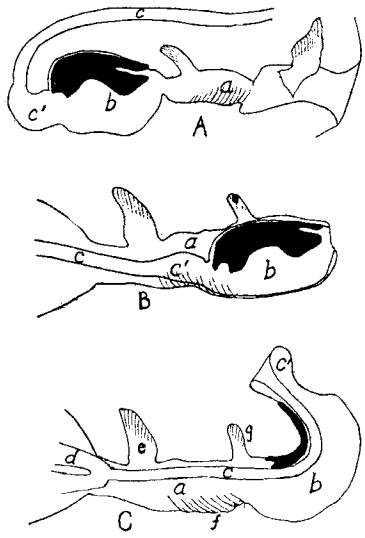


Fig. 2 Diagrams of drone's organs, showing uneverted, partially everted, and completely everted relationships of the various portions, in A, B, and C, respectively. *a*, posterior or proximal tubular portion of the penis with modifications for facilitating copulation, *e*, *f*, *g*; *b*, bulb portion of penis with lateral chitinous plates shown in black; *c'*, proximal end of ejaculatory duct *c*, expanded where it joins the bulb of the penis; *d*, the mucous glands. For further explanations see text.

into the vagina of the queen, and its retention within it when everted. Certain conformations of the penis have been described with considerable ingenuity and in great detail as 'fitting,' or formed so as to lodge in, corresponding regions of the bursa or vagina, as the eversion of the copulatory organ proceeded. The impossibility of observing copulation, which takes place high in the air, makes such inferences the best evidence available as to the manner of coitus.

While the detailed description of this correspondence between the anatomy of queen and drone is irrelevant to the object of this paper, and has been described by other writers, a brief analysis of the process of coition is necessary to the interpretation of some of the data. Two points discussed in recent literature seem to have been inadequately treated. The functioning of the pneumophyses (fig. 2, C, *e*) has not been satisfactorily analyzed, and the extent of the eversion of the copulatory organ —whether this takes place up to the bulb of the penis (fig. 2, B) or whether the bulb also everts (fig. 2, C)—is open to question; as is also the distance to which it enters the vagina of the queen.

In figure 1, C, is drawn, uneverted, the bulb of the penis of the drone to the same scale as that of the queen's organs in figure 1, B, and from the same lateral aspect. It may be observed (fig. 2) that as the penis extrudes from the male genital aperture the first portion to evert, after the short proximal tubular section, will be the penumophyses (*e*). If queen and drone are in position for coitus (tip of drone's abdomen inserted between the anal plates of the female) these pneumophyses presumably evert into the paired bursal pouches opening from the copulatory bursa by slits on either side of and below the vaginal orifice (fig. 1, A and B, *b*). The hypothesis is hardly tenable, however, though it has been presented, that their function is to hold the organ momentarily in the queen's bursa while further eversion of the organ takes place. In the first place, their eversion coincides with a violent contortion of the drone's abdomen (when protrusion of the organ is artificially induced, and presumably in the natural act), which would render any holding function for such soft and pliant organs not only relatively insignificant, but

superfluous. In the second place, their size is so great that their complete expansion into the pockets designed to receive them would force the insects apart rather than hold them together. Thirdly, the next section of the penis which shows definite adaptation for resisting withdrawal (fig. 2, C, *a*, *f*, *g*) is so far from the pneumophyses that these will be forced back from the bursal pouches before the other section everts. Upon careful inspection of the queen's anatomy, especially of the profile of the genital tract, another function for these pneumophyses becomes apparent, namely, to open the orifice of the vagina at copulation, as described hereafter.

The female genital tract has not been adequately described. Regions in the genital tract may be distinguished as follows (fig. 1, A and B): 1) a genito-anal vestibule (*a*), the space between dorsal and ventral genito-anal plates, enclosing the shaft of the sting and receiving the outlet of the rectum, or anus; 2) the copulatory bursa (*b*), lying ventral and anterior to the base of the sting and extending forward on either side into the bursal pouches (*c*), and which is divided from the vestibule by a slight ridge in the floor of the tract (*f*), and 3) the vagina proper, or unpaired oviduct, of which three regions may be distinguished, the posterior (*g*), flattened dorsoventrally and lying just anterior to the base of the sting, divided off from the bursa by a very definite constriction (*e*); medially, a slight enlargement of the lumen enclosing a tongue-like organ (*h*), and opening dorsally into the duct to the spermatheca (*i*), and anteriorly, a region with a T-shaped cross-section (*n*, *m*), into which open on either side the oviducts (*o*).

The configuration of this tract will be described in more detail. Reference to the figure (fig. 1, A and B) will demonstrate the relation of the copulatory bursa to the base of the sting. The floor of the genito-anal space (*a*) is formed by a heavily chitinized membrane attached at the lateral margins to the ventral terminal sclerite, the anterior margin being free from this sclerite and separated from it by a space. From this margin, which is curved convexly to the anterior, extends a thinner and less heavily chitinized membrane, to form the ventral wall of the bursa.

From the line of division of these two areas, marking the outer limits of the bursa proper, the ventral wall of the bursa, in the closed condition of the parts, envelops closely and lies in contact with the base of the sting (*r*); posterior to this line the tract opens out into a considerable cavity, enclosing the shaft of the sting and the tip of the rectum. The base of the sting fitting the bursa is slightly bilobed; that is, its two halves are separated by a flattened furrow, which runs up around the anterior surface of the sting's base, and forms the posterior and dorsal wall of the bursa. The anterior and ventral wall of the bursa (the membrane above mentioned) is correspondingly sculptured into two cup-shaped areas (*b*) symmetrically disposed on either side a median ridge (*f*), which ridge extends from the midpoint of the posterior margin of the bursa to the vaginal orifice, and fits the furrow along the sting's base. From either cup-shaped half of the bursa opens anteriorly a bursal pouch (*c*), by a slit with crenelated margins (*d*). The bursal region of the tract slants dorsally and anteriorly, and narrows laterally to the vaginal orifice (*e*).

Of this orifice, the lower margin is the most prominent, and consists of a V-shaped lip, the lower point of which is continuous with the median elevation of the ventral bursal wall (fig. 1, A). The dorsal margin of the orifice, a lesser elevation, lies in the groove of the sting's base aforementioned, and comprises but a slight distortion of the smooth dorsal wall of the tract.

The remainder of the sexual tract, the vagina proper, may be described here, since no complete description has been noted in the literature, although its elaborate sculpturing appears to be more concerned with oviposition than with copulation.

Just anterior to the vaginal opening, the first region of the tract (*g*) widens out considerably and extends horizontally forward for a short distance without significant modification. The next region (*h*) gives rise dorsally, from a cone-shaped prominence, to the duct and sperm pump (*i*) (described fully by Bresslau) leading to the spermatheca. Ventrally a tongue-shaped lobe extending from the ventral floor of this region fits, in the collapsed state of the organs, into the cone-shaped protrusion at the mouth

of the duct, and occludes the lumen in this region. To it the function has been assigned by Adam of holding the egg against the mouth of the duct when fertilization is to be accomplished. A space posterior to this tongue allows for its depression on passage of the egg.

Beyond this region and anterior to it, the vagina shows a curious modification. The main tract extends forward (*m*), slanting a little ventrally, as a passage with a deeply folded wall, broad in the transverse direction, flattened dorsoventrally. From the anterior angles of this rectangular passageway lead the oviducts (*o*), by lateral passages that open from the outer sides of the vagina into the medial aspects of the posterior ends of the oviducts (at *p*). Along the midventral line of this portion of the vagina runs a narrow slit, or fossa (*n*), deepening toward the anterior end, and extending slightly beyond the apertures of the oviducts, to terminate as a short blind pocket just under the ganglion (*q*), which straddles the end of the vagina and the crotch of the oviducts. The whole region is enveloped by a heavy muscle mass, the finer anatomy of which has not been analyzed.

In the first or ascending region of the vaginal tract (*g*) the dorsal and ventral walls of the vagina are apposed, and a cross section shows as a horizontal slit. The walls of the medial portion (*h*) are deeply folded and wrinkled, and a cross-section approaches a horizontal oval with wrinkled margins. Anteriorly to the ventral tongue the horizontal wall is apposed to a ledge on either side of the deep fossa (*n*), while the walls of this fossa are apposed laterally. The cross-section of this region therefore shows as a T.

In copulation the arrangement of the bursal structure facilitates the penetration of the drone organ. When the genital aperture is opened, by depressing the ventral anal plate, and the sting is slightly withdrawn, the bursa may be readily observed. This condition is also obtained by holding the female between the fingers or by etherizing her, when the anal plates gape open.

The orifice of the vagina remains tightly closed. If the membranous floor of the genito-anal vestibule is depressed further, however, the median ridge of the bursa is pulled ventrally, and the V-shaped lower lip of the vaginal opening is drawn away from the base of the sting. The inflation of the bursal pouches, by the eversion into them of the drone's pneumophyses, and especially by the withdrawal of the pneumophyses as the eversion of the rest of the organ proceeds, would pull the whole ventral wall of the bursa ventrally, and the median ridge would draw the lower vaginal margin away from the upper, thus opening the vaginal orifice to the drone's organ. The function of the pneumophyses thus appears to be performed upon their withdrawal from, as well as by their eversion into the bursal pouches. This would allow the tubular portion of the drone organ which precedes the bulb (fig. 2, *a*) to penetrate the vagina, thus allowing connection even though the bulb itself should not succeed in everting.

That this relatively massive bulb enters the vaginal orifice at all is doubtful. In the first place, its size relative to the dimensions of the vagina (fig. 1, B and C) seems to render this impossible, even though one allows for considerable elasticity in the walls of this region. But other evidence also renders this improbable. As the penis extrudes, the ventral side being longer than the dorsal, the organ bends dorsally. This is especially noticeable at the tip, the dorsal curvature of which is occasioned by the eversion of the bend by which the bulb of the penis (fig. 2, C) tapers into the ejaculatory duct (at *c'*). This recurved tip will fit neatly into the upward bending copulatory bursa and vagina. If this condition obtains in copulation, the bulb of the penis with its chitinous plates obviously does not enter the vaginal orifice at all, but merely forces the tapering end of the ejaculatory duct (*c'*) within the orifice. Further evidence that this is the true state of affairs is obtained from the appearance of queens that return from the mating flight with the drone organ attached. Often, I believe generally, the brown chitinous plates of the bulb can be seen just within or partly extruding from between the anal plates; although the bursa is so large that if they lodged, even partly, within the vagina, they must be com-

pletely hidden from the outside. Under these conditions these chitinous plates could not act, as Shafer infers,[4] as a means of holding the organ in the vagina, nor would they serve to prevent the escape of seminal fluid when the queen had freed herself from the drone. This fluid, unless otherwise prevented, could return by the route by which it entered, through the ejaculatory duct; for the same fullness of the bulb wall opposite these plates which allows them to evert (emphasized by Shafer) would allow the ejaculatory duct to extend back through the bulb without being compressed by them. In a former paper (p. 267) prevention of the escape of spermatic fluid has been ascribed to the immediate coagulation of the mucus from the accessory gland upon exposure to the air.

Assuming this explanation to be correct, the second point in question, the extent of the eversion of the drone's copulatory organ, resolves itself into the following: granting that the usual process results in complete eversion of the bulb and end of the ejaculatory duct (fig. 2, C), is it possible for a queen to be fertilized by a drone whose organ has not been extruded so far (fig. 2, B)? Of the eight queens to be described, three still carried the drone organ when killed, and of these three, in two the bulb was fully everted. In one, however, it was apparently not. One of the oviducts of this queen was normally distended with sperm and mucus, the other was slightly distended, and the bulb of the penis retained a mass of secretion sufficient to distend the lesser one as fully as the other. How this queen retained the organ securely enough to twist it off from the drone, or even how the connection was made at all, it is difficult to say; unless one assumes, as described elsewhere, that the section of the penis tube immediately preceding the bulb (fig. 2, *a*) failed to turn inside out, and held in the vagina as the end of the ejaculatory duct is assumed to do in complete eversion. This might result if the force of the sexual act were insufficient to cause the eversion of the bulb, which if it took place would cause the withdrawal of this section of the tube from the orifice of the vagina. One assumes in this case that the everting end of the penis (*a*) enters the vagina as the pneumophyses open it; and then withdraws

again normally as the bulb everts, since the bulb itself would not enter the orifice. The presence of this bulb in the bursa would operate, by depressing the ventral floor, in the same manner as the pneumophyses do; that is, the vaginal orifice would be widely opened, and the dorsally recurving end of the ejaculatory duct could enter it. More evidence is needed on this phase of the matter.

The distance to which the drone organ enters the vagina may be judged from a consideration of the relative sizes of the respective parts. In figure 1, A and B, are drawn in outline the dorsal view and the profile of the female genital tract, and in figure 1, C, the lateral view of the end of the everted drone organ. The vagina can undoubtedly be expanded to a larger dimension than its relaxed state would show, but it is hardly possible that the large bulb with its heavy chitinized plates could be made to enter the vaginal orifice, to say nothing of turning inside out as it entered. It seems certain, therefore, that the eversion of this part takes place in the outer space comprising the bursa copulatrix and the region posterior to it (the genito-anal vestibule). The curved end of the ejaculatory duct is probably normally the only part of the drone organ to remain in the vagina proper, though, as stated, the section of the penis preceding the bulb in eversion probably lodges there momentarily. The bulb would then lie between the dorsal and ventral genito-anal plates, and its size is such as would cause the pronounced gaping of the queen's last segment, and which would leave the tips of the brown chitinized plates on the dorsal side of the bulb to protrude visibly from the genital aperture, as may usually be observed in newly mated queens.

## DESCRIPTION OF FERTILIZED QUEENS

Of the eight queens the fertilization of which was successful and the time of mating recorded, four were fixed and sectioned for histological study and four were dissected in the fresh condition. The following data describe the gross appearance of the oviducts, vagina, spermatheca, and, when present, the penis of the drone, with which the queen habitually returns to the hive,

and in those sectioned, the histological appearance and the disposal in the female organs of the male sexual products.

A (not over one-half hour mated). In this queen, on opening the abdomen, both oviducts and the anterior end of the vagina were widely distended with fluid, which appeared through the thin walls of the parts in this fresh specimen to be of the yellow color characteristic of sperm rather than white like mucus. On opening the oviducts under the microscope, clear active sperm flowed from the anterior end of each, followed by a mixture of sperm and mucus, which was not coagulated until it came into contact with the air. The vagina contained chiefly mucus. There were no sperms in the spermatheca. The drone's penis attached in the bursa copulatrix had been fully everted, so that the end of the ejaculatory duct had been carried through the everted bulb and formed the end of the organ in its extruded condition (fig. 2, C). There was a little mucus in the end of the ejaculatory duct lying within the bulb of the penis.

B (not over one-half hour mated). One oviduct of this queen was widely distended, the other slightly. Sections showed mucus and sperm both present. The sperms were scattering in the mucus, but by far the greater number of them were found to be in clumps or masses surrounded by mucus, and especially outside the mucous mass altogether and next the oviduct wall. Here they clumped in masses so dense that a 10 $\mu$ section was almost impervious to light, because of the dark-staining heads. The filaments lay side by side in wavy bands perpendicular to the oviduct wall, in much the same way as the sperm are arranged in the seminal vesicle of the drone. In the anterior portion of the vagina where the oviducts enter it, and whence the duct leads off to the spermatheca, the sperms were very densely collected, especially on the dorsal side of the vagina. Masses of pure sperm without mucus occurred in the folds of the vaginal wall in the vicinity of the duct opening. The duct itself in cross-section showed sperm masses, but no mucus that could be distinguished. The spermatheca showed a faint sprinkling of sperms in a clear transparent non-staining medium—a medium which gave exactly the same appearance as the content of the spermatheca of the virgin queen.

The penis attached to this queen was distended with white mucus to a point slightly beyond the tips of the queen's last segments. It was fixed with the queen; then dissected out, and an attempt made to section it. But either the fixative used (Gilson's) or else exposure to the air had so hardened the mucous content as to nick the microtome knife and destroy most of the significant sections. Several points could be made out: first, that the mass contained mucus and few, if any, sperm; second the bulb of the penis was not everted (fig. 2, B) (though the organ was firmly attached to the queen), and, third, the mucus was contained in the bulb of the penis and in the enlarged end of the ejaculatory duct adjoining it. The sheath of the penis surrounding the uneverted bulb was torn from the bulb in removing the organ, exposing the bulb and ejaculatory duct. In this case, therefore, these parts served analogously to a spermatophore, except that the contained fluid was mucus without sperm, all the sperms having apparently been forced on into the queen's oviducts.

C (mated not over one and one-half hours). This queen was inspected at once. Both oviducts were distended with sperm and mucus, still uncoagulated (until exposure), and the spermatheca contained many sperms in a clear medium. The penis attached in the vagina was shrunken considerably and had apparently been extruded completely with eversion of the bulb, as in extrusions produced experimentally (previous paper, p. 250). It contained but a small amount of mucus, no sperms, and it was torn off close behind the bulb.

D (same time as C). This queen had already lost the drone organ. Histological sections showed in general the same conditions as obtained in B, but both oviducts were distended, and yellow in the fresh condition as before. Sperms had scattered somewhat through the mucous mass, but were mostly still unmixed with the mucus, and most of them were gathered along the oviduct walls, especially dorsally. The mucus appeared less densely staining and showed evidence of solution along the edges of the mass, except that in the posterior region of the vagina the mucus stained intensely, as mucus does which has been

hardened or coagulated by exposure. Near the duct leading to the spermatheca, which was dorsal to this densely staining region, were clusters of clear sperm as in B, and the spermatheca itself was quite densely populated with sperms in a clear plasma They were perhaps twice as abundant as in the case of B.

E (mated not over two and one-half hours). This queen's organs were dissected out in a fresh condition, and showed essentially the same condition as case C, as far as inspection without sectioning could show. Both oviducts contained sperm and mucus, the sperm exuding first when the oviducts were opened. The penis had been detached. The spermatheca contained numerous sperms in a clear fluid.

F (mated not over four and one-half hours). Sectioned for histological study. Both oviducts were considerably distended, the penis had been detached. The vagina contained only a little mucus at the posterior end, which stained very densely, and a few sperms were visible at the edges of this mass even posterior to the aperture of the spermathecal duct. One oviduct had received mostly sperms with little mucus, the other showed the characteristic picture of a central core of mucus surrounded by a dense layer of free sperms, not so numerous, however, as in specimens killed earlier. There seemed to be not many more sperms scattered through the mucus than in earlier cases, but there were fewer areas of clear sperms within the mucous mass, as if in this case the sperms had had time to work out toward the oviduct walls. The mucus appeared to be less densely granular, stained less densely than other cases described (except in the vagina as noted), and was probably beginning to dissolve.

The spermatheca was densely crowded with sperms, for the first time in these cases presenting the characteristic picture of the laying or fertilized queen's spermatheca in the numbers present, and exhibiting the characteristic tendency to gather into wavy masses or whorls with heads together and filaments parallel.

G (not over six and one-half hours). Queen was dissected fresh. The oviducts were equally but not greatly distended, and on opening them, what fluid was present consisted of few sperms and considerable mucus with sperms scattered homogene-

ously but sparsely through it. This mucus was distinctly more fluid than in earlier queens or than that obtained from drones. The spermatheca when punctured on a slide poured forth seething masses of sperms, with no sign of mucus in the fluid that contained them. The penis of the drone was not present.

H (not over eighteen hours). The oviducts of this queen were but slightly distended. Sections showed a light-staining and but slightly granular mucous mass in the oviducts, not more than one-fourth the volume of that in newly mated queens, with sperms so scattering that they were found only with considerable difficulty. The vagina was empty, except for a few sperms in the folds of the dorsal wall near the aperture of the duct to the spermatheca. The spermatheca burst in fixation (either through osmosis or handling), and most of the content escaped, but what remained showed dense clumps of sperms. At this rate, it is estimated that the oviducts would have become empty in about twenty-four hours from the time of mating.

## SUMMARY AND CONCLUSIONS

These observations may be summarized in the following account:

The result of copulation in the bee is not immediate filling of the final sperm reservoir, the seminal receptacle of the queen, but the sexual secretions of the drone are received temporarily in the vagina and oviducts. The two components, spermatic fluid from the seminal vesicles and mucus from the accessory glands of the drone, are not intimately mixed in copulation, nor do they later mix homogeneously in the queen's organs. The white viscous mucus is found as a white central core in (typically) each oviduct (in one case most of the mucus was in one oviduct, most of the sperm in the other) surrounded by the less viscous spermatic fluid; as if the oviduct, partially distended with the spermatic fluid which had first entered, then received the more viscous mucus as a rod-shaped column through the center of its lumen. The vagina is filled with mucus alone, indicating that pure mucus was the last to be injected at copulation. The end of the copulatory organ brought back by the queen after

mating also contains pure mucus. In the oviducts the sperms tend to separate out from the mucus, to gather along the walls of the oviducts, and in the folds of the chitinous wall in the region of the aperture of the 'sperm pump' leading to the spermatheca; and they alone, without the mucus, enter the spermatheca.[5] The mucus disappears gradually from the oviducts. It is probably absorbed.

The bulk of the sperms have entered the spermatheca within four and one-half hours after copulation; practically all of them may enter within six and one-half hours. Their manner of getting there has not been determined, though their progress is not altogether passive, and is possibly guided by a chemotaxis. The mucus is absorbed more slowly, being still present in small amounts (in one case cited) at eighteen hours after mating. One of its functions is evidently mechanical, to follow the spermatic fluid through the organs of drone and queen, forcing all of this fluid well up into the oviducts, where it will be retained even though some loss of content of the vagina might follow the tearing of the penis from the drone. Another function is apparently to seal off, by almost instantaneous coagulation on contact with air, the torn end of the copulatory organ, and thus prevent backflow of secretion upon rupture of this part (as coagulation of blood prevents loss from a wound in higher animals). Concerning its physiological function in the queen, if it has any, these data give no information, though the obvious inference is that it may be a stimulus to the ovaries.[6]

[5] The cavity of the spermatheca is far too small to contain the mucus or any considerable part of it (fig. 1, B and C) that is injected into the queen's oviducts at the time of fertilization; nor is any trace of it found there, nor in the duct leading to it, nor in the vagina immediately adjacent to the duct. The sperms alone are so densely packed into the spermatheca as to preclude the presence of any great amount of material of any kind as a medium for them; and in the virgin queen the spermatheca is well distended with a fluid of its own into which the sperms pass after the queen's mating.

[6] In one case of artificial fertilization, reported by Jaeger and Howard (Artificial fertilization of queen bees. Science, N.S., vol. 40, p. 720, 1915) to have resulted in a short period of fertile egg production, injection of the drone's secretions with a pipette is understood to have been responsible for what degree of insemination was obtained. As to whether this was due to the injection of

The results of these observations, furthermore, bear out in certain details the conclusions of a former paper on the functioning of the sexual apparatus of the drone. They corroborate the thesis that the two secretions of the drone which make up the sexual fluid—the spermatic fluid of the vesicles and the mucus of the glands—have a separate function and are not mixed prior to copulation, and that the mucus acts as a plug to prevent backflow of fluids, by coagulating in the torn end of the penis. The mucus does not form an essential part of the spermatic fluid in the sense in which the mucous secretions of some other animals do. It forms neither a vehicle for their transfer from male to female, nor a nutrient fluid, nor, probably, does it provide an important stimulus to their activation (since they separate from it, and pass toward the sperm duct evidently under some other stimulus).

Finally, from the two series of observations (that of the former paper and this) may be derived a more complete account of the mechanics of fertilization in the bee than has been proposed heretofore. The stages of this process may be summarized as follows:

*1. Coition.* The insects meet and clasp face to face; the female on being grasped allows the tip of the male's abdomen to enter the genito-anal vestibule; the drone, by explosive contraction of the abdominal walls everts the organ into the female genital tract. The pneumophyses first dart into the bursa pouches, and by their expansion depress the ventral floor of the bursa, and thus pull open the vaginal orifice. As the next region of the penis, the median tubular portion with its modifications, proceeds to unfold, the pneumophyses are forced back and withdrawn, opening the vagina as they retract to the widest possible extent, and probably allowing this tubular section to enter the

pure spermatic fluid, however, or to the injection of both sperms and mucus, and how long after copulation egg-laying commenced, their preliminary note did not state. Virgin queens will often lay drone eggs if for some reason (defective wings, etc.) they are unable to mate, but only after a prolonged period. Queens usually lay within two or three days after fertilization, conditions being favorable, and sometimes after as short a period as thirty-six hours.

orifice temporarily. If the contraction of the drone's abdomen is violent enough to cause the eversion of the bulb, the medial tubular section must be withdrawn, for the bulb itself is too large to be able to enter the vagina. Its size, however, serves to keep this orifice open until the final portion, the tapering end of the ejaculatory duct adjoining the bulb, everts through the bulb and enters the vagina.

*2. Ejaculation.* With the insects in connection, the drone's seminal vesicles and accessory glands contract by spasmodic alternate twitchings of the circular and longitudinal muscles enveloping them (see former paper). In the gland the musculature is so arranged about its base that the contraction of either set of muscles or of both of them serves to occlude the gland's lumen, and prevent outflow of the mucous content through the ejaculatory duct. At the same time this contraction forces the mouth of the vas deferens from the seminal vesicle close against the chitinous membrane which covers the blind end of the ejaculatory duct penetrating the gland's base. This is burst through, and the sperms from the vesicle are the first to be ejaculated. Upon relaxation of the first spasmodic contraction of the musculature of the gland's base, the lumen of the gland is again allowed to open, and the mucus in the distal reservoir region of the gland flows through the basal region and into the ejaculatory duct, following the sperm. In this manner all the sperms are forced over into the queen's organs.

*3. Separation.* If eversion of the drone's organ has been only partial (fig. 2, B), the uneverted bulb may be distended with mucus, but the sperm will have been forced on into the vagina. If eversion was complete (fig. 2, C), the bulb will still be distended by reason of its stiff chitinous structure. In either case it remains attached to the queen, lying in the genito-anal vestibule and bursa between the sting (dorsal) and the ventral floor of the passage. The queen breaks this organ off from the drone, usually close behind the bulb, by crawling or flying in a circle around him, and returns with it to the hive. The character of the mucous content is such that it coagulates immediately on contact with the air, thus preventing backflow of the sexual fluids through

the torn end of the penis. The organ gradually dries and shrinks when it becomes dislodged or is pulled away by the bees in the hive within a few hours.

*4. Disposal in organs of queen.* The secretions distend the oviducts and vagina, the former with an outer layer of almost pure sperm, and an inner core of almost pure mucus, the latter with mucus alone. The sperms appear to separate out of the mucus and pass by way of the sperm duct to the spermatheca by their own activity, leaving the mucus to be absorbed from the oviducts.