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BREEDING THE HONEYBEE UNDER CONTROLLED CONDITIONS

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INTRODUCTION

Although the honeybee has been kept by man since long before the Christian era, yet in the course of the centuries little or no change has been wrought in it by breeding if the development of the so-called "golden" strain of Italians in the United States and of the "veredelte" strains of the German brown race in central Europe is excepted. The fact that in nature the honeybee mates only on the wing is commonly given as the principal reason for this situation.

Even though bee breeders have relied on natural matings in their work with the honeybee during the centuries, they would have made progress more comparable with that accomplished in the breeding of man's other useful animals had it not been for two factors. In the first place, even less than 100 years ago the bee breeder did not have adequate knowledge of reproduction in the honeybee since he did not know how drones develop. In the second place, until recently he had devised no means of distinguishing definitely between races of the honeybees and their various strains. Even Mendel (Iltis, *19, p. 142–157*)[1], the father of modern genetics, lacked such means when he endeavored to work out problems of bee genetics in addition to those on peas which brought him fame.

[1] Italic numbers in parentheses refer to Literature Cited, p. 47.

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It is unfortunate that Mendel's detailed records have never been brought to light. His studies were not confined to one race of the species, since it is reported (2) that he worked on the heather or Dutch bee, the Italian, the Carniolan, the Egyptian, and the Cyprian, in addition to the native bee of his region. He used colonies kept under natural apiary conditions in his cloister garden at Brno. (Fig. 1.)

Modern scientific information on reproduction in the honeybee had its beginning in 1669, when Swammerdam (*36, pt. 1, p. 159*) described clearly the egg-laying function of the queen. The question of the place of mating remained unanswered until over a

Figure 1.—Mendel's apiary in his cloister garden in Brno, Czechoslovakia. (Illustration published with permission of Julius Springer, Berlin.)

hundred years later, when Huber (*18, p. [illegible]*) established the fact that the queen bee mates outside when in flight. This problem had baffled even so keen an observer as Réaumur, whose interest in it (*[illegible], p. [illegible]*) led him to attempt to have a queen and drone mate in a glass dish on a table. Huber also tried the same experiment before reaching his final solution. Another important advance had taken place shortly before Huber's discovery, when Schirach, according to Christ (*7, p. [illegible]*), showed definitely that a queen can be reared from any worker larva if it is given proper food and care early enough in its larval life. Not until 1845, however, was Dzierzon's amazing discovery made public (*12, p. [illegible]*) that the drone is produced parthenogenetically. Little progress could be expected of the bee breeder without at least knowledge of these four fundamental truths.

It is only since 1900 that a way has been opened to the bee breeder for distinguishing accurately between various races and strains of the honeybee, as a result of studies undertaken to determine, especially from the standpoint of biometrics, characteristics marking them. The pioneer in this type of bee research was Koshevnikov, of Russia. Among others who have made contributions in this field are Alpatov and Mikhailoff, also of Russia; von Buttel-Reepen, Zander, and Götze, of Germany; Rytíř, of Czechoslovakia; Tokuda, of Japan; and Phillips and Kellogg, of the United States.

Within the past decade another tool has been placed in the hands of the bee breeder with the establishment of the fact that queen bees can be inseminated artificially. This makes possible full control with regard to the drones used in breeding. It is true that artificial insemination of the honeybee is, as yet, only an instrument of the scientific investigator and that the commercial breeder must continue, for a time at least, to rely on natural inseminations for his work. The way has been opened, nevertheless, for the investigator in any locality to keep pure stock of various races and strains as well as to develop special strains of the honeybee. The commercial breeder can thus, in turn, be provided with a continuous reserve from which to replenish his stock if natural matings fail him.

Many problems await the bee breeder. He might develop strains which would be gentle, disease-resistant, capable of carrying larger honey loads, capable of flying longer distances, or which would have tongues of the proper length to secure nectar from floral sources not now available to the honeybee. For some regions it would seem desirable to develop strains that fly at lower temperatures than do bees now commonly found in the United States. This is of particular importance to those interested in bees as pollenizing agents.

In general, breeding of the honeybee in modern beekeeping practice falls under two main headings: (1) Breeding under natural or uncontrolled conditions, and (2) breeding under artificial or controlled conditions. By natural or uncontrolled conditions are meant those obtaining in nature or ordinarily in the apiary, where virgin queens and drones fly freely and mating takes place only on the wing. By artificial or controlled conditions are meant both those which, while permitting natural insemination to occur, obtain through some restriction by the beekeeper as to the particular drone or drones with which the queen may mate, the flight range of the queen, or the flight range of both queen and drones, and those which obtain when the beekeeper causes the transfer of spermatozoa from drone to queen to be made otherwise than as in nature.

The following outline, in connection with the text which immediately follows, may prove helpful in keeping clear the distinction between the various methods which are in use or have been tried for breeding the honeybee:

BREEDING OF THE HONEYBEE

1. Uncontrolled conditions (little or no restrictions on queen or drones; mating on the wing).
2. Controlled conditions.
    - A. Natural insemination.
        1. Available drones restricted (mating stations).
        2. Flight range of queen restricted (tethering, etc.).
        3. Flight ranges of both queen and drones restricted (tents, greenhouses).

Controlled conditions—Continued.
- B. Artificial insemination.
  1. By placing drone organs in position in queen.
     - *a.* Before separation from drone.
     - *b.* After separation from drone.
  2. By removing sperm from drone organs first.
     - *a.* External application (brush, squeezing out sperm, etc.).
     - *b.* Internal application (injecting within genital opening).

It is to be noted that this outline does not include such methods as daubing the eggs with sperm, since this is an attempt to accomplish fertilization of the egg itself and not to simulate mating of the queen bee.

STATUS OF WORK ON BREEDING UNDER CONTROLLED CONDITIONS

WITH NATURAL INSEMINATION

The mating stations of Europe furnish a practical example of controlled conditions which restrict the drones with which the queen may mate, but which still permit a natural insemination to take place. These stations are in isolated localities in which the beekeeper has endeavored to have only drones of his own choice present. The virgin queen is taken there and is free to fly from her nucleus and to mate on the wing with these drones. The breeding experiments conducted a number of years ago in Texas by Newell (*29*), as well as those in progress on an island in Lake Ontario by Sladen, of Canada, at the time of his death in 1921, also come under this category.

Many good results are reported from the breeding work carried on at these stations in Europe, especially in Switzerland. The work in Switzerland is linked with the name of Kramer (Goldi, *14*), who started the movement in his country to maintain in purity and to improve the native Swiss bee by having selected stock mate in isolated localities. (Fig. 2.) The results of this work from 1915 to 1928, according to a survey of certain apiaries in Switzerland which contained colonies both of the improved (veredelte) and of unimproved stock (*21, p. 30*), show that the honey crop of the improved stock averaged higher per colony each year than that of the unimproved stock, ranging from 20 per cent in 1923 to 100 per cent more in 1924. These percentages, however, were based on the relation of the average per-colony yield for improved and unimproved stocks, respectively, to the sum total of the two averages, and the number of improved colonies used in the survey was larger each year than the number of unimproved colonies, being in 1928 about two and one-half times and in 1924 more than seven times as large.

In the United States isolated locations free from wild swarms or colonies of neighboring beekeepers are not easily accessible to most queen breeders. Consequently the mating station has played little or no part in beekeeping here, although certain commercial queen breeders in this country have attempted to duplicate, in a degree, conditions found in an ideal mating station by endeavoring to rid the territory in the immediate vicinity of their mating yards of undesirable colonies of bees. Their methods have consisted of buying up colonies found in the neighborhood, requeening near-by colonies with desired stock free of charge, paying rewards for wild swarms, and similar measures. The possibility that the queen may mate with a stray drone even in a locality thought to be well isolated from local bees reduces somewhat the value of mating stations in a scientific breeding program.

Attempts to mate queens tethered at the end of a pole are examples of a partial restriction of the flight range of the queen bee, while attempts to have a queen and chosen drones mate inside a tent or other inclosure are examples illustrating both a restriction of the drones available for mating and a partial restriction of the flight range of queen and drones. In all these cases, however, there is an endeavor to have mating take place on the wing—in other words, to effect a natural insemination. Mendel was one of many who

FIGURE 2.—Mating station at Bruggen bei St. Gallen, Switzerland. (Kramer) (Published by permission of Verein Deutsch-Schweizerischer Bienenfreunde)

have unsuccessfully attempted to obtain matings of queen bees within inclosures (*19, p. 147*). This phase of controlled mating was also investigated in the Bureau of Entomology in 1907 (*17*). Since no satisfactorily verified reports of success are available for matings attempted under the conditions given in this paragraph, no further attention will be paid this category at the present time.

WITH ARTIFICIAL INSEMINATION

When conditions are so controlled that both queen and drone are deprived of flight, the insemination must be accomplished artificially. This may be effected either by placing the drone organs in proper position in the queen to accomplish insemination or by removing the sperm from the drone organs and transferring it to the queen.

The methods proposed for bringing the drone's organs into proper position within the queen can be divided into two classes: (1) Causing these organs to evert in the proper position in relation to the queen and then severing them from the drone, and (2) separating them from the drone and then placing them in proper position in the queen. The methods for first removing sperm from the drone and then transferring it to the queen can also be divided into two classes: (1) Dropping or brushing the sperm on or near the exterior genital opening of the queen, and (2) introducing the sperm within the opening.

One of the early attempts at the artificial insemination of queen bees, and one which is often quoted, was that by Huber, who followed the suggestion of Bonnet in 1789 that he endeavor to inseminate a queen bee by introducing within her vagina, through the use of a brush, sperm from a drone. Huber (*18, v. 1, p. 60*) failed in such an attempt, as have doubtless most of his successors, regardless of the method employed.

According to his statements before the Wanderversammlung der Imker deutscher Zunge, held in Leitmeritz in 1927, Wankler, of Germany (*48*), was one of the earliest modern queen breeders to attempt to make use of instruments in the insemination of queen bees. He affirmed that as early as 1885 he had resorted with some success to mechanical means for inseminating queen bees. He also referred to success attained by himself, even in the eighties, in fertilizing drone eggs from drone cells by daubing them with sperm.

Wankler (*48*) mentioned, among his instruments, a pipette (Spritze) to which could be attached a model (künstliche Glied) of the drone organs, a means for moving the pipette, and a block on which to hold the queen. He stated that the semen is forced into the pipette and then the model is attached and introduced into the vagina of the queen, which is bound to the block. The sperm is next forced out of the pipette into the model through pressure on a screw knob. The pipette, having served its function, is detached, but the model remains attached to the queen at least long enough for the semen to be taken up by the spermatheca. The queen is put back on a frame of brood under a cage of wire screening (Pfeifendeckel) fine enough to catch the model when it finally becomes detached from the queen and drops off.

It is to be noted that the talk by Wankler was given after Watson's announcement of his discovery and Wankler referred to it. Armbruster, however, in a preface to Wankler's book (*47, p. 5*) written in 1924, mentioned that Wankler had presented him with one of the pipettes made of silver and with a model of a drone organ fashioned in metal.

McLain (*22*) claimed that he had achieved success in 1886 in artificially inseminating queen bees with an instrument consisting of a fine nozzle fitted to a hypodermic syringe in place of the regular injecting needle. After the nozzle had been filled with sperm from the drone, a small tube was slipped over it. The queen was held in a clamp made of the two halves of a block of wood 2 inches square and 4 inches long in which a hole had been hollowed out to hold her body with the exception of the tip of the abdomen. McLain also reported success in having queen bees mate in a greenhouse, and even in squeezing sperm from a drone directly into the vestibule of a queen bee.

No other nineteenth-century attempts to inseminate queen bees artificially need be discussed here, since in no instance were such attempts repeated successfully by others to the conviction of the beekeeping world.

The first announcement of importance in this field in the twentieth century was that of Jager and Howard (*20*) in 1914 regarding success in artificially inseminating one out of eight queen bees worked with that summer. These investigators reported that, of the first 3,000 eggs laid by this queen, all but 4 proved to be worker eggs. She was wintered successfully (*16*), but in the spring she laid drone eggs as well as worker eggs and soon was laying drone eggs exclusively. After three weeks of drone laying she was dissected, but her spermatheca was found packed with living spermatozoa. In this work (*16*) the sperm from the drone was diluted with salt solution before being taken up in a fine pipette. The queen was then held in the left hand, while the pipette was introduced into the genital opening with the right hand and the sperm was forced out of the pipette by blowing with the mouth.

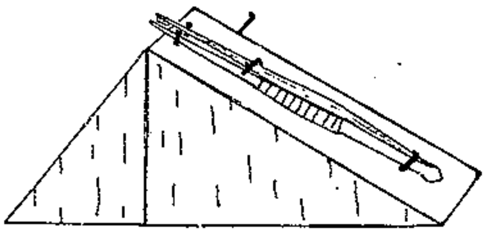

FIGURE 3.—Forceps and block used by Shafer in mating tests (Shafer)

During the next two years Howard and France (*16*) endeavored to carry this method further, but were successful with only 3 out of 55 queens. For one of the 3 success was gauged by the finding of sperm in the spermatheca upon dissecting the queen 26 days after the attempted insemination. One of the others laid only 5 per cent of worker eggs. In this work the sperm was not diluted with salt solution.

The success reported by Jager and Howard may have led Shafer (*45*) to his investigations in the same field. Shafer's main work was directed "through squeezing the drone—to evaginate the male organ into the vagina of the queen in the normal position." To keep the abdominal tips of the queen apart and to hold the sting out of the way in the process, a pair of forceps was mounted on one of the inclined surfaces of a triangular block in such a way as to allow the forceps to open only a quarter of an inch. (Fig. 3.) A small, thin plate was attached to one point of the forceps to aid in holding the sting back after the forceps had been introduced between the abdominal tips. A binocular microscope was used during the attempt at insemination.

Shafer later contrived a device which consisted of a No. 5 insect pin bent at right angles one-sixteenth inch from the head end and inserted firmly in a block. The pinhead could be inserted in the "sting notch" and as the queen's abdominal tips separated she could be moved so that the pin pressed against the sting and held it dorsalward. Shafer did not report any successful insemination, however.

In 1923 Quinn (*43*) announced success in artificially inseminating queens by a method which involves causing the drone to evert its genital organs into proper position in the queen for the transfer of sperm to take place. No details of the method were published, but the actual operation appears to have been performed by his grandson, Harry Laidlaw, instead of by Quinn himself. In demonstrations witnessed by the writer in 1928 (*1*) and in the spring of 1930 (*8*), Laidlaw supported the queen with his left hand while making use of a pair of protractors to hold the abdominal tips apart, somewhat as did Shafer (*45*). The drone was held in the right hand while being caused to evert its organs in the proper position in the queen. No microscope was used.

In the summers of 1930 and 1931 Laidlaw was given temporary employment as field assistant by the Division of Bee Culture at its station in Baton Rouge, La. His work during 1930 led to the statement in the annual report of the Chief of the Bureau of Entomology (*24, p. 71*) that "the results obtained so far indicate that the method has considerable promise, for a partial degree of insemination can be effected in all cases." In December, 1931, Laidlaw reported before the American Association for the Advancement of Science that he had improved upon his technic somewhat, having devised a small spring to fit inside the queen's abdominal tips and hold them apart and having adopted the use of a microscope.

Apparently Laidlaw has been the only successful exponent of his method thus far, although, according to a recent statement (*2*), A. Z. Abushady, of Egypt, was successful in 1930 in inseminating three queen bees artificially without instruments. Unfortunately no details were given as to Abushady's work, however.

Malyschev (*23*), of Russia, in 1923 reported success obtained the year before in the artificial insemination of one queen bee. He dissected out the organs of a drone that had been caused to ejaculate by being chloroformed and placed them in the proper position in a queen that had been subjected to ammonia. Malyschev reported that this queen laid eggs normally in a nucleus and was then transferred to a queenless colony. He did not record her further history.

In October, 1926, Watson satisfied a special committee at Cornell University by a demonstration that he had developed a method which gave results far superior to any that had previously been published (*6*). According to his method (*49*) the sperm is injected with a microsyringe or pipette into the queen, which is bound to a small wooden block, or miniature operating table, hollowed out to fit her body. (Fig. 4.) The queen is held in position on the block by a thread passed several times around her thorax and the anterior part of her abdomen, the posterior tip of her abdomen being left free. The injection is accomplished under a binocular microscope.

To facilitate insertion of the microsyringe, Watson places a fine-pointed forceps between the queen's abdominal tips in such a way as to hold the sting back out of the way and to spread the tips. The microsyringe is held in a Barber pipette holder or micromanipulator which allows movement in three directions. Sperm is drawn in and is forced out of the tube of the pipette, which is about 0.5 mm in diameter, by a fine wire plunger moved by a screw. According to Watson's technic as first published (*49*), with the bulb of the drone held on the index finger of the left hand, the end of the pipette is forced into the mucus and a small quantity is drawn in. The pipette is then forced more deeply into the bulb and the full load of sperm is taken up. In a well-developed drone the white mucus is readily discernible in one end of the bulb and the cream-colored sperm in the other end. The mucus is taken up first so that it will be forced out of the tube last and thus serve its purpose, as set forth by Bishop (*5*), of acting as a plug to the sperm owing to its property of congealing on contact with air.

In subsequent work Watson (*52*) minimizes the importance of the mucus plug, and describes a small "saculus" which is formed in the end of the bulb containing the sperm when pressure is applied at the other end. By cutting this sac and inserting the pipette there,

FIGURE 4.—L. R. Watson demonstrating his method of artificial insemination of queen bees. (Copyright by the American Bee Journal)

instead of pushing it through the mucus first, it is possible to take up in the syringe a large charge of sperm which contains practically no mucus.

As to his own success with his method, Watson (*52*) states that, of 42 queens operated upon in 1926, he found evidence, either by the microscope or by the rearing of worker brood, of some degree of insemination in about half this number. Of 96 queens operated upon in 1927, 65 per cent showed some degree of insemination, and 67 of 93 queens operated upon in 1928 showed some insemination. In a private communication to the writer, dated January 6, 1932, Watson stated that, out of 42 queens treated in 1929, 35 proved to be inseminated in some degree and that the remaining 7 were lost. In 1930 Watson, as reported by Disbrowe (*11*), operated on 40 queens. Nine of these queens were lost, but the remaining 31 showed some degree of insemination. About 12 appeared to be normally inseminated, while about 12 or 14 appeared to be only about 2 per cent inseminated. In 1931, according to the communication mentioned above, Watson treated 13 queens. Of these, 8 proved to be inseminated in some degree, 1 is recorded as a failure, and 4 were lost. Watson states that "of the queens treated in 1931 only three, or possibly four, were anything like copiously inseminated."

To determine the degree of insemination with the aid of the microscope, Watson (*50*) compares the quantity of sperm in the artificially inseminated queen with that in the normally inseminated queen. The spermatheca of the normally inseminated queen at the beginning of egg laying is so filled with sperm that it is opaque, while that of the virgin queen remains filled with a clear liquid. It is, of course, difficult to judge accurately the degree to which the spermatheca is really filled, although with practice results can be obtained that will afford interesting comparisons.

One feat performed by Watson through his method was the transfer of living spermatozoa from the spermatheca of one queen to that of another (*50*). On another occasion he was able even to rear worker bees from eggs laid by a queen that had been artificially inseminated with spermatozoa from the spermatheca of a queen that had been mated a year before (*53*). To be able to transfer or to save in this way the spermatozoa contained in the spermathecae of queens mated with drones of certain desired strains or races should prove valuable at times in breeding work in preserving and propagating those strains or races.

The summer after Watson's discovery, Prell (*41*) announced that he, too, had successfully inseminated queen bees artificially by a method which, from the description given, seems to be like that used by Malyschev. Although Prell refers to Malyschev's work, he ascribes to him a method employing a pipette. Unfortunately, Prell gives no details as to the queens or the number with which he had success. He only reports that brood rearing began normally within a few days after the queens had been released. In a report of his work for 1928 Prell states (*42*) that in that year he attempted unsuccessfully to inseminate drone-laying queens. He did not state that he had been successful otherwise with his method during the year.

Apparently the first person to verify Watson's method was the writer (*32*). Within nine months from the time Watson had given publicity to his method, worker bees which were the offspring of a queen bee that the writer had subjected to the Watson method were emerging in the apiary of the bee culture laboratory of the Bureau of Entomology at Somerset, Md. The results of this work were not made public until the December meeting of the American Association of Economic Entomologists in the following year (1928), although the work had been mentioned previously by Alpatov (*3*) and by Hambleton (*15*).

The only other published reports of success by the Watson method are those by Mikhailoff, of Russia, and by Disbrowe, of Canada. Watson has informed the writer personally, however, that G. H. Cale also has performed the operation successfully, if a microscopic examination of the spermathecae of the queens may be used as a criterion.

During his first season's work on the artificial insemination of queen bees, conducted during the summer of 1928, Mikhailoff (*25*, *27*) performed 113 operations with apparatus made by himself in following Watson's book (*49*) as a guide. In addition he performed 30 operations by Malyschev's method. These 143 operations were performed on only 105 queens, which means that some queens were treated more than once. Success was had in 18 instances, as was evidenced by the presence of sealed worker brood in 6 cases, and by spermatozoa found in the spermathecae of 12 queens. All-worker brood developed from the first eggs laid by 2 of the queens, but success in the case of 1 of these is attributed to the Malyschev method, because it was used on the queen after an apparently unsuccessful use of the Watson method 4 days earlier.

Mikhailoff (*26*) continued his experiments in 1929 with the aid of a helper, I. A. Licenko, and used a microsyringe made by Watson. During the year 114 queens were operated upon, some of them more than once, the total number of operations being 138. Twenty-four of the queens, or 21 per cent, showed some degree of normal insemination. Mikhailoff states that eight of these queens gave all-worker brood.

In 1930, according to a communication to the writer, Mikhailoff treated about 100 queens. These treatments were 27 per cent successful, as gauged from the actual rearing of worker brood. Of these successfully treated queens 10 per cent gave evidence of complete insemination.

Disbrowe's experiments date from 1929 (*9*). He states (*10*) that approximately 25 queens were operated upon during that season. Of this number more or less success was had with 5. In a later article (*11*) he mentions having continued the work in 1930, but gives no results.

BREEDING WORK AT THE BEE CULTURE LABORATORY

Work on a bee-breeding program was begun at the Bureau of Entomology's bee culture laboratory at Somerset, Md., before the announcement of Watson's discovery. A study, then under way, of queen rearing and of the biological and physical characteristics of the leading recognized races (*30*, *31*, *37*, *38*, *39*) was one step in this program. It was planned to make later a thorough trial of mating stations on the European model, but when Watson's method was made public it was considered advisable to test out methods of artificial insemination first and to endeavor to improve the technic of those found acceptable.

In any bee-breeding program, regardless of whether artificial or natural insemination is employed, there are numerous manipulations which must be carried on. Even when artificial insemination is used, it is not the greatest consumer of time in a large-scale breeding project. The manipulations belonging to the technic for breeding the honeybee under controlled conditions, whichever type of insemination is used, cover the maintenance, supply, and checking of the queens, drones, and workers used in the work. In the technic employed by the writer these manipulations include queen rearing to supply the necessary virgins, maintenance of nuclei to test the queens treated, marking queens, checking up on queens after the operation, maintenance of supply colonies both to rear queens and to provide honey and brood for nuclei as required throughout the season, making provision to obtain sufficient drones of the right stock, marking drones, and other necessary operations. As beekeepers know, the successful carrying out of all this procedure is, in part at least, dependent on weather and floral conditions.

As commonly performed by the beekeeper or queen rearer, many of the manipulations just mentioned require too many bees, or too much time and equipment, to be economically or efficiently used in an intensive study of the genetics of the honeybee. Consequently the writer has endeavored to develop a technic for breeding the honeybee under controlled conditions which will permit the use of a maximum number of queens per queen breeder with a minimum requirement per queen of time, bees, and equipment. This technic, together with new technic for use with the Watson method of artificial insemination, is described in the text that follows. A discussion of the results obtained on queens used in the work on controlled mating is given first, however.

In this bulletin, unless stated otherwise, or unless specific reference is made to the spermatheca, the term " percentage of success " in connection with the writer's work refers to the percentage of worker cells in the first two weeks' total of sealed brood. As applied to the spermatheca, the term is used to denote the relative degree to which the spermatheca appears filled with sperm, as compared with the spermatheca of a normally inseminated queen. The actual emergence of worker or of queen bees is, of course, the final criterion for the purpose of the breeder or geneticist as to whether a queen is inseminated and as to the degree of insemination, but this gauge can not always be readily applied. Thus, when a queen lays few worker eggs in relation to the number of drone eggs, the actual emergence of the resulting workers is not always observed, and in such a case the story is told by the finding of worker cappings. When a queen is killed before she has laid any eggs, an examination of her spermatheca is the only way of determining whether or not she has been inseminated.

When a queen is laying mostly drone eggs and is using worker cells for them, it is sometimes easy on casual observation to mistake the cappings over the resulting pupae for worker cappings, but a little practice on the part of the observer should eliminate error from this source. On the other hand, spermatozoa may be found in the spermatheca of an artificially inseminated queen that has been injured sufficiently in the course of the insemination to prevent normal egg laying. Such an insemination could not be called successful from its utility in a genetical study, although it would be successful as far as the introduction of living spermatozoa within the spermatheca is concerned.

RESULTS OF ARTIFICIAL INSEMINATION

ON QUEENS USED IN 1927, 1928, AND 1929

In 1927, 20 queens were subjected to the Watson method by the writer. Positive results were obtained in 5 instances, including the completely successful case mentioned previously. For 1 of the other 4 queens the degree of success was 50 per cent, for 2 it was between 5 and 10 per cent, and for 1 it was below 5 per cent.

In 1928 experiments were conducted on 16 queens. Six of these queens were subjected to Watson's method and the last 3 were inseminated successfully. In 2 cases the degree of success was over 70 per cent and in 1 it was 100 per cent. The other 10 queens were subjected to the Quinn-Laidlaw method, but without success. Since then the writer has done little with either this or the Malyschev method. Details on the work in 1927 and 1928, as well as the method of rearing the queen bees and handling them in the nuclei, are given elsewhere (*32*, *33*, *34*).

In 1929, 16 queens were operated on and placed in nuclei for observation. Of these, 1 was accidentally lost too soon to determine whether or not it had been inseminated, 4 were lost by balling or otherwise within 2 or 3 days after being put in the nuclei, 1 was lost 8 days later, and 2 were lost when their nuclei were robbed out within 3 days afterwards. This left only 8 queen bees on which to continue observations. Four of the 8 queens gave evidence of insemination, 2 of them beginning their egg-laying careers as if 100 per cent inseminated, while the other 2 appeared to be about 50 per cent inseminated.

ON QUEENS USED IN 1930

In 1930, 52 queens were operated upon by the Watson method. One other queen was operated upon by the Malyschev method, but she died from an undetermined cause too soon to ascertain the results. The data for each of the 52 queens are given in Table 1.

Sixteen queens received some degree of insemination, as was evidenced by the presence of emerging workers, worker pupae, or spermatozoa in the spermathecae of the queens. Success in the case of 10 of these queens was determined solely by observing under the microscope living spermatozoa in their spermathecae, in the case of 2 solely by observing worker pupae or emerging workers, in the case of 3 others by observing both sealed brood and sperm, and in the case of 1 by observing both emerging brood and sperm. These data alone show that insemination resulted in practically 31 per cent of the work in 1930.

Flat cells, which were undoubtedly those of worker brood, were observed in the case of 7 other queens. These cells did not occur in such abundance that workers were seen emerging, and such cells as were opened for observation did not contain brood old enough for the sex to be determined on superficial examination. A microscopic examination of 2 of these queens made, respectively, 33 and 124 days after insemination revealed no spermatozoa. The other 5 queens disappeared without such an examination having been made. If it is admitted that these 7 queens were also inseminated, the percentage of success for 1930 becomes 44.2 instead of being 31, as mentioned previously.

Of the remaining 29 queens, 5 (Nos. 4, 5, 9, 14, and 24) were found dead and 3 (Nos. 17, 22, and 27) had disappeared within 2 days of their attempted insemination. One queen (No. 1) died within 2 days of a second insemination attempted 14 days after the first one. Nine more queens (Nos. 10, 23, 25, 26, 28, 29, 33, 41, and 51) were found dead or had disappeared within 3 to 9 days of the attempted insemination. Queen No. 10 was found balled to death. Three others of the foregoing were given a second operation. Owing to other work, queens Nos. 1, 4, 5, 9, 10, and 14 were not examined under the microscope. One queen (No. 47) was killed accidentally on the seventh

TABLE 1.—*Record of queens operated on in 1930 by the Watson method* [1]

Queen No.	Cross [2]	Date of first insemination	Date of second insemination	Ether used	Type of plunger [3]	Drones used for first insemination	Drones used for second insemination	Age of queen at first insemination	Period between first and second inseminations	Period between last insemination and egg laying	Period lived after egg laying	Period lived after last insemination	Total length of life	Flatly sealed brood seen	Workers or virgins reared	Sperm seen in spermatheca
						Number	*Number*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*			
1	I×I	Apr. 26	May 10	X	G	1	?	7	14			2	23			
2	I×I	Apr. 30	Apr. 30	X	G	1	1	11	0	3	156	[4] (159)	170	X		
4 [5]	I×I	May 3	May 3	X	G	1	1	10	0			2	12			
5	I×I	May 5		X	G	2		?				1	?			
6	I×I	do		X	G	1		?		10	2	12	?	X		
7	I×I	May 6		X	G	1		?		10	143	153	?	X	X	
8	I×I	May 7		X	G	1		?				19	?			
9	I×C	May 22		X	G	1		1				1	2			
10	I×C	May 28		X	G	1		5				8	13			
11	C×C	June 4	June 19	[6] X	G	1	1	7	15	3	34	(37)	[7] 59			X
12	C×C	do		O	G	1		7				36	43			
13	C×C	June 5		O	G	1		8				47	55			
14	C×C	do	June 5	X	W	1	1	9	0			2	11			
15	C×C	June 6		X	W	1		?				[7] 45	?			O
16	C×C	do		X	W	1		10		7	117	(124)	[7] 134	X		O
17	C×C	June 10		X	G	1		?				2	?			
18	C×C	June 13		X	G	1		15				38	53			
19	I×C	do		X	W	1		21				1	22			X
20	C×C	June 18		O	G	1		21		10	23	(33)	[7] 54	X		O
21	C×C	do		O	G	1		22				33	55			
22	I×C	June 19		X	W	1		?				2	?			
23	I×I	June 20		X	W	1		4				5	9			O
24	I×C	June 23		X	W	1		7				1	8			O
25	C×C	June 30	June 30	X	G	1	1	8	0			3	11			O
26	C×C	July 1	July 1	X	G	1	1	8	0			7	15			O
27	C×I	July 2	July 2	X	G	1	1	9	0			1	10			
28	C×I	July 3	July 3	X	W	1	1	10	0			7	17			O
29	C×I	July 7		X	W	1		13				3	16			O
30	C×I	July 8	[8] July 8	O	W	1	1	14	0			1	15			X
31	C×I	July 9		O	W	1		15				2	17			X
32	C×I	do		O	G	1		13				12	25			
33	C×I	July 10		O	W	1		14				5	19			
34	C×I	do		O	W	1		13				11	24			X
35	C×I	do		O	W	1		15				17	32			O
36	C×I	July 11		O	W	1		16				10	26			
37	C×W	July 14		O	W	1		18		24	62	(86)	[7] 104	X		X
38	C×W	July 15		O	W	1		18		23	68	(91)	[7] 109	X		X
39	C×W	do		O	W	1		19		28	23	(51)	70	X		

40	7-1×W	do		O	W	1		8				17	25			
41	7-1×W	July 17		O	W	1		22				4	26			
42	W×W	July 18		O	W	2		10		6		?	?	X		
43	7-1×W	do		O	W	1		10				6	16			X
44	7-1×C	July 23	July 25	O	W	1	1	15	2	12	98	(110)	[7] 127	X	X	X
45	7-1×I	do		O	W	1		15		14	109	(123)	138	X	X	
46	7-1×I	July 24		O	W	2		17				4	[9] 21			X
47	7-1×I	July 25		O	W	1		9				7	[9] 16			
48	7-1×I	July 28		O	W	2		20		30	9	(39)	[7] 59	X		X
49	I×I	July 31		O	W	3		31		12		18	61	X		
50	I×I	do		O	W	2		31				9	[7] 40			X
51	I×?	Aug. 1		O	W	?		32				9	41			
52	I×I	Aug. 4		O	W	5		22				1	[9] 23			X
53 [10]	I×I	Aug. 5		O	W	3		22				1	[7] 23			X

1 X indicates positive, O, negative results, except in column headed "Ether used," in which X indicates that ether was used, and O that ether was not used.
2 The first symbol used refers to stock of queen; second symbol to stock of drone. I=Italian, C=Caucasian, W=white-eyed Italian, 7-1=F_1 generation of queen No. 7.
3 G=glass; W=wire.
4 Numbers in parentheses not included in total length of life.
5 Queen No. 3 was operated on by Malyschev method.
6 No ether was used in first insemination.
7 Killed for dissection.
8 Two additional operations on this queen were made on this date.
9 Killed accidentally.
10 Actual total is 52.

day after the operation, but when found her spermatheca had dried out too much for proper examination. Queen No. 35 survived 15 days after the attempted insemination and was 1 of 7 of the foregoing for which negative results were obtained under the microscope.

The remaining 9 queens lived from 10 to 47 days after their operations without showing any evidence of insemination. Four of these 9 queens (Nos. 12, 13, 21, and 40) disappeared; 1 (No. 36), when found, was too dry for examination, while the spermathecae of 3 (Nos. 8, 18, and 32) were not examined microscopically. No sperm was seen in the spermatheca of queen No. 15.

In only 1 (No. 45) of the 3 queens from which workers or queens were reared in 1930 did all-worker brood develop. Queen No. 52, however, which was killed accidentally on the day after the operation, proved to contain such a large quantity of living spermatozoa in her spermatheca as to warrant the assumption that all or practically all her brood would have been workers, at least in the early stages of her egg laying.

In the case of most of the other 13 queens in whose spermathecae living spermatozoa were found, the degree of insemination appeared relatively small. Five of these queens were in hives 4 days or less after being inseminated, while one was in a hive only 9 days. As this period was scarcely long enough for these queens to begin egg-laying, there was no opportunity to determine the degree of insemination from the quantity of worker brood reared. Worker bees had been reared from one (No. 44) of these queens, however. The examination of the spermatheca of this queen was made more than 3 months after the insemination. Although flat-sealed cells were found on brood reared from queens Nos. 37 and 38, no worker pupae or emerging workers were observed. On examination 80 days after insemination the spermatheca of No. 37 had a light, milky appearance and living spermatozoa were found, while 91 days after insemination the spermatheca of No. 38 showed that the sperm had collected into a small spherical mass floating in the clear spermathecal liquid.

Another interesting case was that of a queen (No. 48) born on July 8 and inseminated on July 28. On September 5 only 5 flatly sealed cells were found out of a total of 150 sealed. The queen was killed, and on examination of her spermatheca a spherical mass of sperm much larger than in the case of No. 38 was seen floating in the clear liquid which ordinarily characterizes the spermatheca of an uninseminated queen bee. Further examination showed living spermatozoa to be present. Such cases are quite comparable to that of the queen bee operated upon by Jager and Howard, which, as mentioned earlier, contained spermatozoa in her spermatheca even after she had been laying only drone eggs.

Another queen (No. 11) was born on May 28 and inseminated on June 4. No eggs being seen in the meantime, she was reinseminated on June 19. Eggs were seen on June 22 but, although larvae were seen later, none of these were found to develop into adults. The queen was killed on July 26 and, although her spermatheca showed only a slight degree of cloudiness, living spermatozoa were discovered.

In queen No. 19 the sperm was found massed within the spermatheca in a shape like a smoke ring which revolved rapidly. This queen and queens Nos. 52 and 53 were the only ones whose spermathecae appeared under the microscope to be more than 10 per cent filled with spermatozoa. Of these three, the spermatheca of No. 52, as mentioned earlier, appeared to be well filled, or between 75 and 100 per cent. That of No. 19 appeared to be at least 20 per cent filled, while that of No. 53 appeared to be 10 to 20 per cent filled. The spermatheca of the queen which produced all-worker brood (No. 45) was not examined microscopically.

ON QUEENS USED IN 1931 AND 1932

In 1931 a total of 103 queen bees were treated, 97 by the Watson method and 6 by the Malyschev method. (Table 2.) Of the latter, 5 disappeared within 1 to 29 days after the performance of the experiment, while the spermatheca of the sixth (No. 11) showed negative results under the microscope.

Of the 97 queens on which the Watson method was used in 1931, both queens and workers were reared from 8, or 8¼ per cent. Workers but no queens were reared from 3 others, thus making 11 queens, or 11⅓ per cent of those treated, from which the F_1 generation was reared. As indicated by the proportion of sealed drone to sealed worker cells which were found, some of these queens (Nos. 5, 12, 33, 34, 37, and 51) laid no drone eggs; others (Nos. 47, 79, and 84) laid only a very small proportion in relation to worker eggs—no more, however, than certain normally inseminated queens in neighboring colonies; while about half the eggs laid by 2 (Nos. 78 and 82) proved to be drone eggs. Of the 11 queens, 5 (Nos. 51, 78, 79, 82, and 84) were alive and laying at the end of the brood-rearing season and entered the winter of 1931–32.

In addition to the 11 queens successfully inseminated as evidenced by the production of F_1 queens or workers, the spermathecae of 25 other queens lost by balling, by accident, or otherwise were found to contain living spermatozoa. This makes a total of 36 queens inseminated, or slightly more than 37 per cent of indisputable insemination for the season. If to this total are added 2 queens (Nos. 30 and 48) whose only indication of being inseminated was the presence of some flatly sealed cells among their brood, the grand total for 1931 becomes 38, or slightly more than 39 per cent of successful insemination. These last 2 queens, however, laid relatively few eggs, although sufficient to have rendered possible a continuation of their particular strain under favorable circumstances.

Of the 25 queens whose degree of insemination was determined from their spermathecae, the spermathecae of one-fifth (Nos. 4, 29, 53, 86, and 89) were estimated to be 50 per cent or more filled with sperm, those of about one-sixth (Nos. 45B, 52, 59, and 93) to be 25 per cent or more filled, those of more than one-fourth (Nos. 3, 21, 40, 42, 71, 80, and 81) to be 10 per cent or more filled, while those of slightly more than the remaining third (Nos. 8, 10, 20, 22, 24, 44, 46, 88, and 99) were estimated to be less than 10 per cent filled. Flatly sealed brood was found in 2 of these cases (Nos. 10 and 20), although no emerging adults were seen.

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TABLE 2.—*Record of queens used in 1931* [1]

Queen No.	Cross [2]	Date of first insemination	Date of second insemination	Date of third insemination	Type of plunger or method used [3]	Drones used for first insemination	Drones used for second insemination	Drones used for third insemination	Age of queen at first insemination	Period between first and second inseminations	Period between second and third inseminations	Period between last insemination and egg laying	Period lived after egg laying	Period lived after last insemination	Total length of life	Flatly sealed brood seen	Workers or virgins reared	Sperm seen in spermatheca
						Number	*Number*	*Number*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*			
1	C×I	May 3			W	2			13					5	18			O
2	C×I	May 5	June 4	June 12	W	2	1	2	4	30	8			10	52			
3	C×C	May 6			W	2			16					9	25			X
3A	C×C	May 15			W	3			14					1	15			O
4	C×C	May 18			W	2			6					2	8			X
5	C×C	May 18			W	2			6			14	5	[4] (19)	25	X	X	
6	C×C	May 19			M	1			6					3	9			
7	C×C	May 19	June 4		W	1	1		6	16		7		11	40	O	O	O
8	C×C	May 19	May 20		W	1	1		6	1				6	13			X
9	C×C	May 20			W	1			7					7	14			
10	C×C	May 20			W	1			8			13	163		184	X		X
11	C×C	May 20			M	1			8					9	17			O
12	C×C	May 21	June 5		W	1	1		6	15		3	98	(101)	122	X	X	X
13	C×C	May 22			W	2			7					10	17			
14	C×C	May 22	May 23		M	1	1		7	1				1	9			
15	C×C	May 25			W	2			10					8	18			
16	C×C	May 28	June 5		M	1	2		11	8		10	4	(14)	33			
17	C×C	May 28			M	1			12					7	19			
18	G×I	June 3			W	2			7					23	30			
19	C×C	June 3			M	1			21					29	50			
20	C×C	June 5	June 29		W	2	1		1	24		18	110	(128)	153	X		X
21	C×C	June 5			W	2			1					5	6			X
22	C×C	June 8			W	1			3					1	4			X
23	C×C	June 8			W	1			3					4	7			
24	C×C	June 10			W	1			5					3	8			X
25	C×C	June 11			W	1			6					2	8			O
26	C×C	June 11			W	2			6					7	13			
27	C×C	June 11			W	2			6					5	11			
28	C×C	June 12			W	2			7					14	21			
29	C×C	June 12			W	1			7					1	8			X
30	C×C	June 12	June 12		W	3	1		7	0		29	96	(125)	132	X		O
31	C×C	June 15			W	1			10					28	38			O
32	G×I	June 18			W	2			14					11	25			O
33	C×I	June 19			W	2			14			6	34	(40)	54	X	X	?
34	G×I	June 22			W	2			[5] ?			12	9	(21)	?	X	X	X
35	C×C	June 23	June 23		W	1	1		7	0				10	17			O

36	C×C	June 23	June 23		W	1	1		7	0				6	13			O
37	C×C	[5] ?	[7] June 23		W	?	1		[6] ?	?		15	12	(27)	65	X	X	X
38	C×C	June 24			W	1			7			17	6	(23)	30	O	O	O
39	C×I	June 24			W	1			2					1	3			O
40	C×I	June 24	June 24		W	1	1		2	0				2	4			X
41	C×C	June 25			W	1			3					7	10			O
42	C×I	June 25	June 25		W	1	2		3	0				2	5			X
43	C×I	June 26			W	1			4					7	11			O
44	C×I	June 26			W	2			4					4	8			X
45	C×C	June 26			W	2			7					12	19			O
45A	C×C	[5] ?			W	?			[6] ?					?	15			O
45B	C×C	[5] ?			W	?			[6] ?					?	15			X
46	12-1×C	July 1	July 2		W	1	1		6	1				7	14			X
47	C×C	July 3			W	1			20			8	90	(98)	127	X	X	X
48	C×C	May 4	July 3		W	1	1		14	60		12	30	(42)	116	X		
49	C×C	[5] ?	[6] July 6		W	?	1		[6] ?	?				9	40			
50	C×C	[5] ?	[5] July 6		W	?	1		?	?				14	45			
51	C×C	[5] July 9	(9)		WG	?	1		?	?		12			(10)	X	X	
52	C×C	[5] ?	[7] July 13		W	?	1		[6] ?	?				7	45			X
53	C×C	June 18	July 9	July 13	W	1	1	1	13	21	4			11	40			X
54	I×C	July 17			W	1			3					7	10			
55	I×Ch	July 15	July 21		G	1	1		2	6				3	11			O
56	I×C	July 16	July 16	July 21	G	2	1	1	3	0	5			10	18			
57	Cp×C	July 30	Aug. 5	Aug. 6	W	2	1	1	7	6	1			7	21			
58	12-1×I	Aug. 4			W	1			6					8	14			
59	47-1×C	Aug. 6			W	1			6					21	27			X
60	47-1×C	Aug. 6			W	1			6					6	12			O
61	47-1×C	Aug. 6			W	1			6					6	12			
62	I×C	Aug. 7			W	1			9					4	13			O
63	G×I	Aug. 7			W	1			9					8	17			
64	51-1×C	Aug. 10			W	1			5					5	10			
65	51-1×I	Aug. 10			W	1			5					3	8			O
66	G×I	Aug. 10			W	1			5					23	28			O
67	51-1×C	Aug. 13			W	2			8					14	22			O
68	51-1×C	Aug. 13			W	1			7					14	21			
69	51-1×I	Aug. 14	Aug. 14		W	1	1		8	0				1	9			O
70	51-1×I	Aug. 14			G	1			8					20	28			
71	G×I	Aug. 14			W	1			16					16	32			X
72	G×I	Aug. 14			W	1			8					13	21			O
73	51-1×I	Aug. 18			W	1			8					12	20			
74	47-1×C	Aug. 19			W	1			8					8	16			
75	51-1×I	Aug. 24	Aug. 24		W	1	1		13	0				11	24			
76	47-1×C	Aug. 24	Aug. 24		W	1	1		14	0				11	25			
77	47-1×C	Aug. 24	Sept. 21		W	1	2		13	28					(11)			
78	47-1×I	Aug. 24			W	1			13						(11)	X	X	
79	I×I	Aug. 26			W	1			21			9			(11)	X	X	
80	I×C	Aug. 27			W	1			19					3	22			X
81	I×I	Aug. 28	Sept. 23		W	1	3		10	26				0	45			X
82	I×I	Aug. 28			W	1			21			6			(11)	X	X	
83	47-1×C	Aug. 28	Aug. 28		W	1	1		13	0				10	23			

See footnotes at end of table.

TABLE 2.—*Record of queens used in 1931* [1]—Continued

Queen No.	Cross [2]	Date of first insemination	Date of second insemination	Date of third insemination	Type of plunger or method used [3]	Drones used for first insemination	Drones used for second insemination	Drones used for third insemination	Age of queen at first insemination	Period between first and second inseminations	Period between second and third inseminations	Period between last insemination and egg laying	Period lived after egg laying	Period lived after last insemination	Total length of life	Flatly sealed brood seen	Workers or virgins reared	Sperm seen in spermatheca
						Number	*Number*	*Number*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*			
84	I×C	Aug. 20			W	1			19			18			(11)	X	X	
85	51-1×I	Aug. 31			W	1			22					1	23			O
86	G×C	[5] ?	Aug. 31	Sept. 1	W	1	1		?	26	1			1	27			X
87	G×C	Sept. 1			W	1			20					16	36			
88	37-1×I	Aug. 17	Aug. 25	Sept. 1	W	?	?	1	14	8	7			7	36			X
89	47-1×I	Sept. 4			W	1			11					3	14			X
90	51-1×I	Sept. 8	Sept. 8	Sept. 9	W	1	1	1	14	0	1			11	26			
91	51-1×C	Sept. 9			W	1			15					1	16			O
92	51-1×C	Sept. 10	Sept. 10		W	1	1		16	0					(10)			
93	47-1×C	Sept. 16	Sept. 16		W	2	1		17	0				6	23			X
94	47-1×C	Sept. 16	Sept. 16	Sept. 16	G	1	1	1	8	0	0			36	44			
95	82-1×I	Sept. 29			W	1			6					5	11			O
96	82-1×I	Sept. 30			[6] W	2			7					36	43			
97	82-1×C	Oct. 1			W	2			8					20	28			O
98	82-1×C	Oct. 6			W	1			13					4	17			
99	47-1×C	Oct. 15			W	2			17					8	25			X
100 [12]	79-1×I	Oct. 15			W	1			15					21	38			

[1] X indicates positive, O negative results.
[2] First symbol refers to stock of queen; second symbol to stock of drone. C=Caucasian; Ch=Chinese; Cp=Cyprian; G=German brown; I=Italian; 47-1, etc.=F_1 generation of queen No. 47, or of queen represented by first number.
[3] G=glass (Watson); W=wire (Watson); M=Malyschev method.
[4] Numbers in parentheses not included in total length of life.
[5] Put in a nursery cage after operation and no record taken.
[6] Virgin used came from other than queen-rearing colony.
[7] 38 days old at second insemination.
[8] 31 days old at second insemination.
[9] 34 days old at second insemination.
[10] Still alive on Nov. 1, 1931, but missing on Feb. 10, 1932.
[11] Still alive on Feb. 10, 1932.
[12] Actual total is 103.

Of the 59 queen bees operated upon by the Watson method in 1931 and for which no positive results are recorded, the spermathecae of 26 proved to be clear on examination. Two of the remaining 33 queens were inseminated late in the year, and in the slackening of brood rearing, which occurred soon after all were successfully introduced, the results of the attempted inseminations were not determined. These queens, however, were still in their nuclei at the beginning of winter. The spermathecae of the other 31 queens were not examined microscopically.

Although the season is not yet over, it seems in order to state that 353 queens were subjected to the Watson method in 1932 before September. With two exceptions only one operation was given each. In the two exceptions two operations were given but neither proved successful. Of the 353 queens, 115, or nearly one-third, were successfully inseminated. From 43 of the 115, adult workers or queens, or both, were reared. The majority of the 43 laid only worker eggs. The egg-laying performance of some of them could not be differentiated from that of naturally inseminated queens. Certain of these queens were given 10 frames. As about 50 queens yet remain to be checked, the total number of successes for the season should prove somewhat higher than that just given.

NEW INSTRUMENTS AND METHODS FOR ARTIFICIAL INSEMINATION

In 1927 the technic originally described by Watson was carried out in the main, with some minor exceptions. For instance, no attempt was made to feed the queen while she was bound to the operating block, as the writer has repeatedly observed that the queen bee can go without food with no apparent injury during the time required for the operation. Illumination was obtained by using a standard microscope lamp and at times even an ordinary desk lamp without any special reflector or filter. A microscope lamp is still being used and is brought as near the queen as necessary for sufficient illumination during the operation. No ill effects from heat have been detected. In private discussion with the writer Watson stated in 1931 that he now uses a lamp that is attached to his microscope.

GLASS HOLDER

In 1928 the first major modifications in the technic were made. One of these, as described elsewhere (*32*, *33*), does away with the necessity of binding the queen down with thread to the operating table. A small glass tube, devised by Jas. I. Hambleton, is used instead. The queen is backed into this tube until her abdomen projects just far enough from the narrowed opening at the other end for the operation to be performed. A stopper formed of another piece of glass tubing prevents her escape once she is in position.

As originally described, the glass stopper was held in the outer tube by a small wooden wedge. The writer later adopted the plan of dipping the end of the stopper in melted paraffin so that a coating is formed which will serve to retain the stopper in the outer tube when it is shoved in the desired distance. In hot weather it may be necessary to recoat the stopper frequently if paraffin with too low a melting point is used, and, at times, the wooden wedge can be used in addition. It has been found most convenient of all, however, to fasten the stopper in place by a small piece of DeKhotinsky cement. The stopper is thus held firmly in place during the operation and is easily loosened by turning it enough to crack the cement.

The writer has found that the queen is easily made to back into the tube by letting her first run into another similar tube. When her head touches the constricted end she usually begins to back of her own accord, and if the two tubes have been brought together in the meantime, she will continue backing until she is in proper position in the holder.

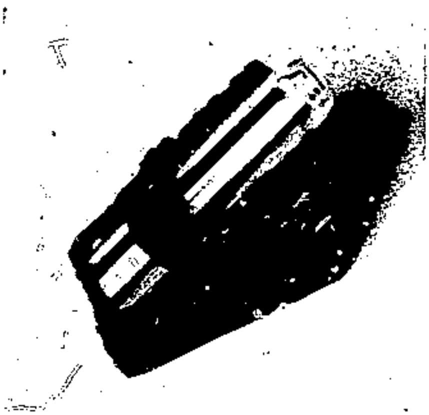

FIGURE 5.—Glass holder supported on wooden block

MOUNT FOR GLASS HOLDER

At first the glass holder was mounted on a triangular wooden block (fig. 5), which permitted an elevation at three different angles. The tube could be turned to any desired angle in the plane of the face of the block on which it rested, because it was bound to the block only by rubber bands. Such a block serves very well.

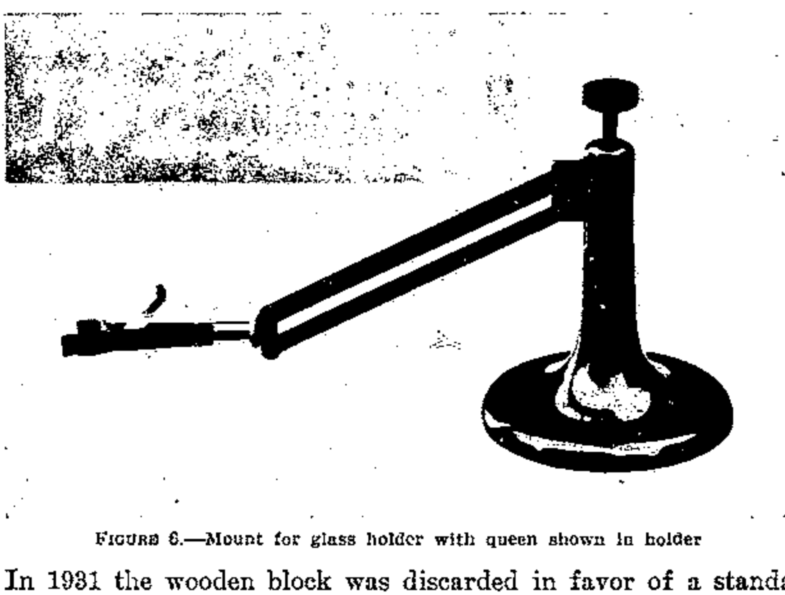

FIGURE 6.—Mount for glass holder with queen shown in holder

In 1931 the wooden block was discarded in favor of a standard dissecting lens stand. (Fig. 6.) This apparatus, entirely of metal, consists of a long arm fitted to an upright rod by a rack and pinion, the upright itself being supported by a heavy base. At the end of the arm is a revolvable clamp which holds the glass tube and permits it to be turned about the arm as an axis. The tube can also be readily set at any desired angle in relation to the arm, since it is held only by pressure from the two sides of the clamp.

In 1932 a different type of mount was devised which is now used exclusively. Included in its construction (fig. 11) is a narrow strip of wood with a hole in each end through which passes an upright metal rod. The strip can be readily pushed up and down on these rods, but yet fits tightly enough on them to stay at any desired height. Through the center of this strip is a narrow slot about 2 inches long for the insertion of a thumbscrew which also passes through a smaller wooden block. The thumbscrew is provided with a wing nut for fastening the two blocks firmly to each other at any vertical angle. The slot permits a certain horizontal adjustment of the smaller block in the lengthwise direction of the long block. The glass holder fits into a hole through the smaller block, and can be rotated in it. To hold the tube in the desired position in this hole, a thumbscrew is provided. Since this mount allows the holder to be

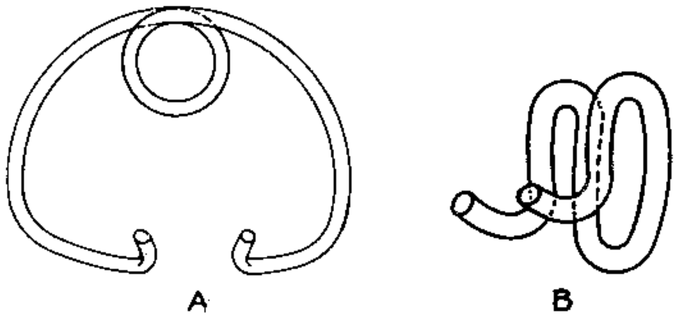

FIGURE 7.—Clips: A, Old type; B, new type. Both enlarged

adjusted in position for height, to be rotated on its own longitudinal axis, to be revolved in a vertical plane, and to be moved for a limited distance horizontally in the lengthwise direction of the longer block, ample provision is made for bringing the glass holder into proper position in relation to the pipette.

SPREADING AND HOLDING ABDOMINAL TIPS

CLIP

Another innovation dating from 1928 was the use of a U-shaped clip of fine wire with each end bent at a right angle to form a small prong. These prongs were so inserted as to hold the abdominal tips of the queen bee apart while the pipette was manipulated into position. The sides of the U were bowed outward to give some tension and, when the prongs were in position between the abdominal tips, projected at right angles from the queen. Instead of making a simple U-bend in the clip, the writer later found it advisable to secure more tension by making a small loop at what corresponds to the bottom of the U. (Fig. 7, A.)

Such a clip was first suggested by L. C. Spencer, of Louisiana, for use with the Quinn-Laidlaw method, but, as described earlier (*32*, *33*), its use with the Watson method has proved more convenient than using a pair of forceps for the same purpose. This type of clip, however, possibly owing to the wire used, proved a trifle too heavy, since movements of the queen often caused it to swing downward with the points of its prongs serving as pivots and then to drop out at inconvenient moments.

In 1931 the writer devised an entirely new type of wire clip which stays when put in position. It is U-shaped with the two sides of the U straight and bent back on themselves nearly as far as the closed end. At this point the rebent sides are bent upward at right angles and are left just long enough to be grasped conveniently with the points of a fine dissecting forceps. The width of this clip approximates the lateral width of the vestibule of the queen, while the length is approximately the height of the vestibule when the queen's abdominal tips are spread apart. (Fig. 7, B.)

The clip is easily introduced between the queen's abdominal tips. During this procedure it is held in a pair of dissecting forceps. With the point of a transferring needle the abdominal tips are first spread apart just enough to permit the insertion of the closed part of the U between the sting and the dorsal abdominal tip. Then, by pressing against this tip with the closed end of the clip and by pressing at the same time against the ventral abdominal tip with the transferring needle, the tips are spread far enough apart so that the sides of the clip can be dropped down within the sides of the vestibule. (Fig. 8.)

When in position the rebent ends of the sides of the clip rest against the queen's ventral abdominal tip, while the closed end of the clip rests against her dorsal abdominal tip. The base of the clip rests on the membranous covering of the posterior end of the body cavity. The pressure of the tips plus that of the sides of the vestibule holds the clip in this position regardless of respiratory movements by the queen. The swinging movement, which was an objectionable feature of the older clip, is absent. The sting and sting palpi are free, but, possibly owing to stretching of sides or ends of the vestibule, remain out of way and at the same time are kept from injury to themselves.

HOLDING HOOK

In connection with the clip, the writer in 1930 introduced the use of a teasing needle with its point bent at a right angle to form a hook. By means of this needle, or holding hook, the queen's ventral abdominal tip is pulled to the left the desired distance when the pipette is brought within the vestibule. The clip may be removed at this time since the pipette itself then serves to hold the queen's dorsal abdominal tip to the right. The sting remains out of way between this tip and the pipette. In 1931, as a means for keeping it in the proper position without holding it by hand, the hook was fastened to an adjustable clamp mounted on a supporting rod. When thus held it is easily brought into place by moving the base to which the rod is attached.

In 1932 a method was devised for spreading and holding the queen's abdominal tips apart by the use of two holding hooks alone. In connection with the mount devised that year for the glass holder, a small wooden block to support a holding hook (fig. 11) was attached to each of the two metal rods. These blocks can be shoved up and down or be turned on the rods, but fit tightly enough to stay in the desired position. The holding hooks resemble teasing needles, having wooden handles and points of fine spring wire bent in proper shape to hold the abdominal tips of the queen without injuring her. The handle passes through a hole in the block large enough for freedom of movement in inserting the hook between the queen's abdominal tips, while a thumbscrew is provided to fasten it securely in position. The ventral abdominal tip is held by one hook and the dor-

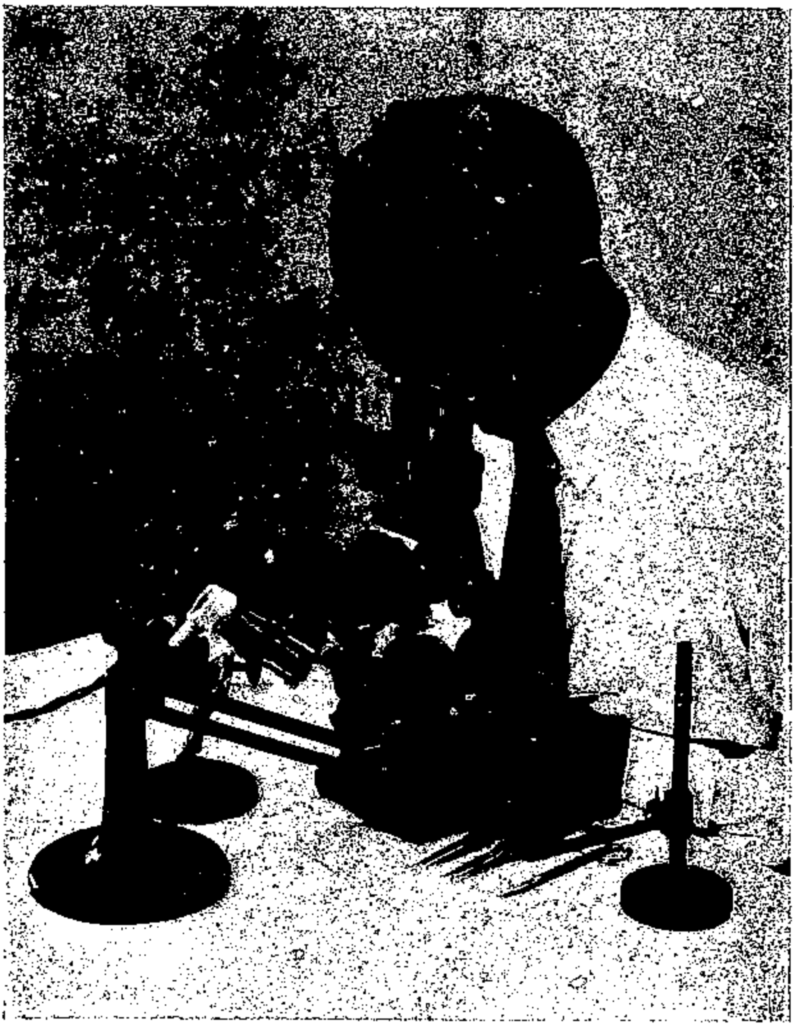

FIGURE 8.—Inserting the clip

sal abdominal tip by the other. Thus, merely by means of these two holding hooks the tips are easily spread and held apart the proper distance and the clip is eliminated altogether. When the pipette is in position, the hook holding the dorsal tip can be withdrawn.

In the procedure just described the combined use of two hooks, or of a hook and a clip, does away with the need of a forceps during the insertion of the pipette, and the queen's genital opening is

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more readily located than when her abdominal tips are kept apart by a pair of forceps held by hand. Consequently, this procedure speeds up the bringing of the pipette into proper position in relation to the genital opening, and permits the operator to leave the microscope during the operation at any time after the insertion of the

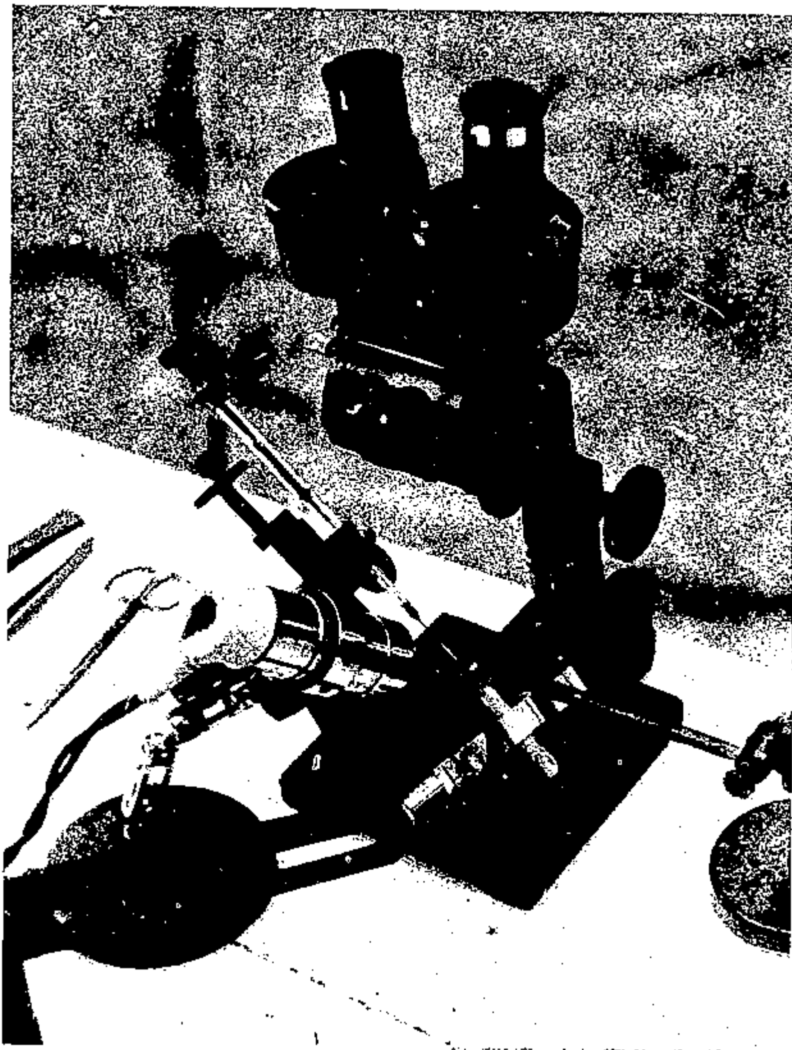

FIGURE 9.—View of apparatus during insemination of queen with technic as modified in 1931

pipette and, on returning, to find relatively the same view still under the microscope, provided the queen has not had too great freedom of movement. The view presented in the field of the microscope is the following: At the right, the pipette; in the center, the end of the queen's abdomen protruding from the glass holder with her dorsal abdominal tip at the right and her ventral abdominal tip at the left; and, at the extreme left, the hook. (Fig. 9.)

MULTIPLE SET-UP

The time required for the part of the insemination accomplished under the microscope varies considerably, depending upon the quantity of sperm in the pipette, how properly the pipette has been inserted in the queen, how rapidly the sperm is forced out of the pipette, and similar factors. Sometimes the insemination has been completed in half an hour, but often it has taken longer. This does not include time spent in getting the queen ready for the operation, filling the pipette, and other preliminary manipulations.

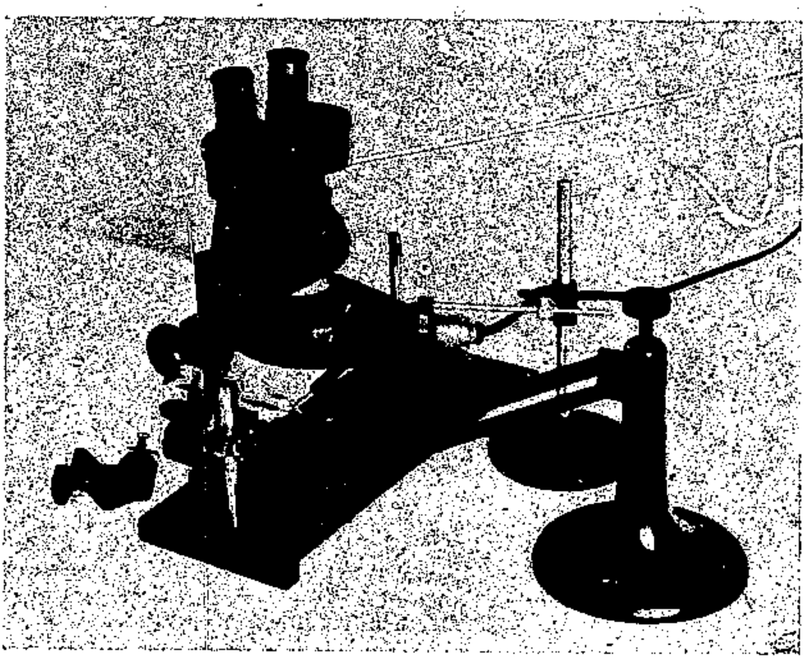

FIGURE 10.—Unit of multiple set-up at first devised in 1932. There is shown the microscope, false stage, new pipette manipulator, rod support for manipulator, micropipette, glass holder for queen but without queen, mount for glass holder, holding hook with its support, and new-style lamp

From experience thus far, the writer prefers that the operation should not be hurried. During the actual operation the screw of the pipette is turned slowly for the purpose of forcing the sperm out just fast enough for it to enter the queen without leaking back out. If it does begin to leak, the operation is stopped to allow the sperm and mucus exposed to the air to harden, as described later under the heading "Mucus Plug." For this and other reasons, the operator must often wait during the operation before proceeding further.

In order to utilize any extra time on hand during an operation, the use of a multiple set-up for the work in 1932 was adopted so that several queens might be operated upon more or less simultaneously. This multiple set-up contains several individual units (fig. 10), each of which consists of the apparatus used in the artificial insemination of a queen. To reduce the expense of each unit, however, and to provide greater convenience in the work, the writer devised a pipette manipulator and also a false stage which is not attached to the microscope but yet serves as the base for the support of the manipulator. The stage and manipulator are described under separate headings. The method of holding each individual queen is that already described which permits the operator to leave the microscope once the pipette is properly inserted in the queen. The false stage is so constructed that, even if the Barber micromanipulator is used, only one miscroscope and one lamp are needed for the entire set-up regardless of the number of units. The writer's equipment, however, includes a lamp for each unit because it is much more convenient and saves time.

By the use of a multiple set-up any tendency to hurry the operation should be reduced because the operator has more than one queen with which to occupy his time. The number of queens to be provided for in one set-up will depend upon the skill and convenience of the operator. The set-up used by the writer in 1932 consisted of six units.

FALSE STAGE

In the writer's equipment for work with the Watson method in 1932, instead of the false stage pictured in Figures 9 and 12, which slips tightly over the base of the microscope stand and holds the Barber micromanipulator, there is used a stage (figs. 10 and 11) made of a sheet of metal supported at each end by wooden blocks of a height sufficient for the base of the microscope stand to be slipped in and out readily beneath the stage without touching it. Instead of using wooden blocks for supports, the sheet of metal may be bent at each end for this purpose. If the Barber micromanipulator is used, provision is made for attaching a removable support for it, while for the pipette manipulator described in the next section provision is made for attaching to the stage the rod which supports the manipulator. This stage is made long enough to attach to it the rod for the holding hook at the left of the operator, or to serve as a base for the support of the holding hook in case a movable support is used. It is also long enough not to tip up if the Barber micromanipulator is being employed with it, but is not wide enough to hold the lamp or the base of that type of mount for the glass holder shown in Figure 6. If desired, it can be bolted or clamped to the table or other support on which it rests. When the stage is equipped with a rod at the right for the new pipette manipulator (fig. 11), and one at the left for the holding hook, these two rods also serve to hold the new mount devised for the glass holder in 1932. In the multiple set-up these stages are placed in a row, or otherwise, for conveniently transferring the microscope from one to the other, as well as to provide for shifting the miscroscope lamp if only one is used.

PIPETTE MANIPULATOR

The new pipette manipulator devised by the writer in 1932 consists of two small oblong wooden blocks joined together by a thumbscrew and wing nut which permits fastening the two pieces at various angles in relation to each other. (Fig. 11.) One of the blocks is made into a clamp which is fitted to an upright rod attached to the false stage. By means of another bolt and wing nut this clamp can be fastened firmly at any height on the rod and at any horizontal angle. Through the free end of the other block is a hole just large enough to permit the pipette to be shoved through freely, a small screw being provided to fasten the pipette at the proper position. An additional clamp just beneath that holding the manipulator on the rod serves to hold the manipulator at the proper height when rotating it in a horizontal plane.

The rod supporting these clamps is threaded at one end. This end is passed through a hole in the false stage and a nut fitted to it permits fastening the rod rigidly in place. If this rod and another for a holding hook are fastened to separate bases like those used for

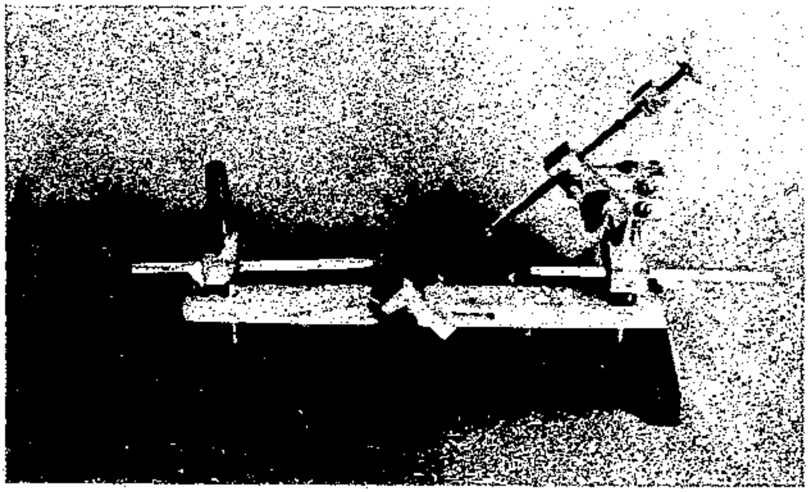

FIGURE 11.—Equipment devised and used by writer in 1932, showing false stage, mount for glass holder, two holding hooks and their mounts, and pipette manipulator with extra clamp just beneath it on its supporting rod. The pipette is slightly modified from the standard Watson type as regards the style of activating screw and the fact that it passes through a wooden instead of a metal cap fitted over the outer glass sheath

the holding hook in 1931 (fig. 9), these two rods can be used as a support for the new type of mount for the glass holder (fig. 11) devised in 1932 and the stage may be dispensed with. However, the writer has found it more convenient to use the false stage, since it is more rigid and lessens the strain on the long strip of the mount. It is possible to operate the new manipulator when its supporting rod is mounted on a separate base alone in conjunction with the mount for the glass holder used in 1931 (fig. 6) and a separate base for a holding hook, but the lack of rigidity plus the risk of hitting either base with the microscope or body makes this arrangement much less desirable than the use of the false stage.

This type of pipette manipulator evidently does not give so fine an adjustment as does the Barber micromanipulator, but its cost is negligible, and, if properly made, it will serve the purpose if used by a person with a steady hand. It has the further advantage of rotation in a vertical plane. It was the only manipulator used by the writer in 1932.

PIPETTE

At one stage of the work it seemed desirable to use in the microsyringe a glass tube of smaller diameter than the one furnished by Watson. A smaller pipette meant using a correspondingly smaller plunger. After some experimenting in 1929, glass tubes of less than half the diameter of those furnished by Watson were made and fitted with glass plungers (*34*). All the writer's work in 1929 was carried out with such pipettes. In 1930, 20 queens (Table 1) were operated on with them, and 5 in 1931. (Table 2.) Some success was had.

Watson had experimented with glass plungers but gave them up because he considered them too fragile (*49*). He attempted to wind his glass plungers with thread to secure better suction, whereas the writer has had success in using them unwrapped.

For use with the small tubes glass plungers present certain desirable features, among these being their rigidity when in the tube and the ease with which they can be drawn out from a piece of glass rod to fit any such tubes. On the other hand, the tubes can be drawn to fit the plungers. One end of the plunger is carefully smoothed off in a flame and the other end is fastened by DeKhotinsky cement directly onto a glass stem drawn out to the proper shape to permit the spring to act on it. Otherwise, construction of these small pipettes is the same as that in the standard Watson pipette except that an outer piece of glass tubing is fitted over the plunger tube to give it greater strength.

Although such pipettes appear to offer certain advantages, it is evident that if the tubes are kept short enough to permit convenient manipulation and to reduce danger of breakage they will not hold so much sperm as does the original Watson pipette. The smaller the diameter of the pipette, the more this criticism applies. Consideration of the quantity of sperm available in the pipette at the time of the operation in relation to success of the insemination was one reason for the exclusive use of the regular Watson pipette throughout the latter part of 1930 and for most of the work in 1931.

However, since the results in 1929, although on a small number of queens, indicate that, within the limits of the sperm available, satisfactory results can be obtained by the use of the smaller pipettes if employed under proper conditions, the question of size of pipette merits further study. For the work in 1932, consequently, pipettes were prepared which are smaller than the standard Watson pipette but larger than the pipettes used in 1929. Plungers for these new pipettes were made of nichrome wire.

USE OF AN ANESTHETIC

Although at times in previous work it had seemed that greater success might be attained through anesthetizing the queen, this was first attempted by the writer in 1930. Ether was the only agent used. A swab of cotton batting that had been dipped in ether was shoved into the glass tube holding the queen and was left there until her movements had practically ceased except for the rapid protrusion and retraction of her sting.

Twenty-four queens were operated upon with the aid of ether, 15 with the small pipette and 9 with the regular Watson pipette. (Table 1.) Of these queens 6 proved to be inseminated, 4 of these successes being with the small pipette and 2 with the standard Watson pipette. Twelve of the other 18, or 50 per cent of the total, succumbed through balling or otherwise within 5 days after the final operation. Eight of the 12 were operated upon only once, 3 were operated upon twice on the same day, while 1 had two successive operations 14 days apart.

Of 28 queens operated upon without ether in 1930, only 4 (Nos. 30, 31, 33, and 41), about 18 per cent, were lost through natural causes within 5 days after the operation. Two queens (Nos. 46 and 47) were accidentally killed within 7 days of the insemination, while 2 others (Nos. 52 and 53) were killed for dissection purposes on the day following the insemination.

Owing to the high mortality so soon after the operation, possibly due in part to the after effects of the ether, the work for the remainder of 1930 was conducted without the use of ether. It may be that the anesthetic was not properly applied. The use of some other anesthetizing agent, such as carbon dioxide as used by Hambleton for several years in various types of work on honeybees, might prove advantageous, but in the satisfactory results reported by Watson, Mikhailoff, and Disbrowe no mention is made of the use of an anesthetic and the work of the writer in 1931 was all accomplished without it.

FILLING THE PIPETTE

Watson mentions holding the bulb of the drone on his index finger while filling the pipette. The writer finds it more convenient to retain the bulb by the forceps after pulling it loose from the drone and to fill the pipette while the bulb is being thus held. (Fig. 12.) An incision is first made in the sperm sac with fine scissors. If the incision is made at the end of the bulb containing mucus, care is taken to cut through the mucus at a point where the sac is at least as large in diameter as the pipette so that the latter will slip in easily. Sometimes there is too much mucus in a drone in relation to the sperm, or it congeals so rapidly that the pipette can not readily be forced through it to the sperm. In such cases another drone has to be obtained.

On several occasions in 1930, and more frequently in 1931, the writer cut an opening for the pipette through the "saculus" of the sperm sac described by Watson (*52*). Although in many instances it was found possible, on inserting the pipette into the "saculus," to take up large loads of sperm apparently free from mucus, this was not true in every case. The writer is therefore not yet in a position to say whether or not this method gives more success than does the method by which the pipette is inserted into the sperm from the other end of the sac without any special attempt to take up some mucus first. Success has been achieved with both methods.

MUCUS PLUG

In a previous publication (*34*) the writer mentioned that he did not consider the use of mucus essential to success in artificial insemination by the pipette method. This viewpoint, as stated earlier in this bulletin, is that most recently expressed by Watson (*52*). This does not mean that the mucus plug is held unimportant. In the writer's opinion it does play, in nature, the important rôle ascribed to it by Bishop (*5*). In the Watson method of artificial insemination it is difficult to secure this plug, however, and a certain degree of success has been attained without it. If its use were easier, perhaps successful insemination by the pipette method might be more frequent and the degree of insemination uniformly higher. In this re-

FIGURE 12. Filling the pipette

spect the Quinn-Laidlaw and the Malyschev methods appear to have an advantage over the Watson method.

In the writer's work with the Watson method, particularly in 1930 and 1931 when a large number of queens were treated, no special effort was made to get a mucus plug. If during the operation sperm begins to leak back around the pipette, the exposure to the air soon causes it to harden on the surface sufficiently to keep the remainder from running out of the genital opening. Possibly this hardening is due to the presence of some mucus that has mingled with the sperm in the process of filling the pipette. In any event it seems best to provide a superabundance of sperm to take care of any wasted by leakage.

It is now the writer's usual practice to delay the withdrawal of the pipette for a few minutes after the completion of the operation and also whenever sperm begins to ooze out around the pipette. This allows the sperm to harden around the end of the pipette and thus form a sort of collar through which the pipette can be pulled out gradually without bringing the entire mass of sperm along with it. The pipette is removed by raising it slightly and loosening it from surrounding mucus with a teasing needle with bent point if this can not be done readily with the sharp end of a transferring needle.

In disregarding the mucus plug it seems essential to apply the end of the pipette squarely to the genital opening and then so to manipulate the plunger that the sperm enters without being forced back out around the end of the pipette in too large a quantity. With the improved apparatus described in this paper, this procedure is rendered much easier to achieve than under the older technic, especially since the genital opening is more readily found.

MARKING INDIVIDUALS

In any breeding experiment it is important to know as exactly as possible the genetic history of the individuals being bred from. This implies being able to distinguish between various individuals. There are other reasons why one must be able to pick out with certainty this or that bee for use in breeding work. In the first place, according to Bishop (*5*) and others, the drones must be of a certain age before they are sexually mature. Even queens are held to have optimum age limits for mating. In the second place, because of drifting, in an apiary of bees of the same race one can not be sure that a drone found in a given hive belongs to that hive. The writer has found marked drones from one hive in as many as three other hives 12 hours after marking, and further search might have shown more cases of such drifting. Other drones were found to stay in one hive over a period of weeks. Workers often make themselves at home in hives other than their own. Even when queens are confined to their hives by queen excluders, they sometimes drop to the ground and may be lost when their hives are being manipulated.

Such considerations as the foregoing render it imperative to have some means of distinguishing definitely given individuals within a hive or individuals of one hive from those of another. In the case of a queen bee this can be done easily, since her wings can be clipped to prevent flight away from the hive and a distinctive mark can be painted on her. Drones and workers present more of a problem in this respect, since there are so many more of them than queens.

One way to make sure of a drone's origin is to rear and keep it behind a queen excluder. This was proved practical in 1929 in at least one instance, and in two instances in 1930. However, the need of flight on the part of the drone is an objection to this method.

A better method, and one which allows the drone free flight throughout its life, is to give it a distinguishing mark when it emerges. Mikhailoff mentions having done this in 1929 (*25*). The writer did not begin any extensive use of such a method until 1930. As used in 1931 it consisted in marking the thorax of the drone with the number of its mother and placing on the abdomen a number to mark the week of emergence. Although this method gives the age of the drones only by weeks, and somewhat roughly at that, it can be of service in determining the relative range of age in weeks during which drones are best for use. Those desiring to know the exact age of any drone in days can give it a separate number on emergence. Queens and workers were also marked by numbering.

The method used for numbering drones, queens, and desired workers is an adaptation of that given by Von Frisch (*13, p. 42*). Ten pigments are chosen, one to represent each of the 10 digits. By combining these in dots on the thorax, any desired number can be represented. Units are represented by a dot of the proper color on the left rear of the thorax, tens by a dot on the right rear, hundreds by a dot on the left front, and thousands by a dot on the right front of the thorax. For instance, if the numeral "one" is represented by a blue pigment, bee No. 1 would carry a blue dot on the left rear of the thorax and bee No. 11 would carry a blue dot on the right rear and also one on the left rear of its thorax, bee No. 111 would be marked as described for No. 11 plus a blue dot on the left front of its thorax, and No. 1111 would carry three blue dots as for No. 111 plus one blue dot on the right front of its thorax. A similar system can be used for marking the abdomen. (Fig. 13.)

FIGURE 13.—Marked drones

DRONES

OBTAINING DRONES

It is not always easy to secure drones in sufficient quantity during the active season, especially at its beginning and end. In an endeavor to get an early supply in 1930, drones were purchased from the South about three or four weeks before their usual appearance in the apiary of the bee culture laboratory at Somerset. They were successfully united to strong colonies and consequently the season for artificial insemination opened earlier than would have been the case under natural conditions. Since, in purchasing drones commercially, it is not always possible to get the desired stock, this procedure is practical only for certain purposes.

Late in 1931 limited trials were made of stimulative feeding to induce drone rearing during a normal slackening of brood rearing.

A certain measure of success seemed to attend these efforts, since drones were present in these colonies until late in the fall. The exceptionally warm weather may have been a contributing factor.

When the first queens reared in early spring in 1932 were ready for treatment, drones were on hand from an overwintered queen which had been successfully treated the previous fall but which proved a drone layer in spring. This points to one way of securing an early supply of drones.

Prior to 1931 most of the drones used from the Somerset apiary were caught by hand at the hive entrance as they were returning from a flight, either on the wing or just as they alighted. Such drones proved more satisfactory than those taken from combs. This method was slow at times, especially when drones were scarce or when their flight was not active; so in 1931 queen-and-drone traps were put on the hives at the entrances just before the hours of greatest flight, which have been found to occur at Somerset shortly after noon. Usually more than sufficient drones were easily collected in this way, and by keeping the excess caged overnight in proper containers it was possible to have available the next morning a supply of drones suitable for the work on artificial insemination. Candy was supplied as food for the drones kept overnight and, when it seemed necessary, some workers were caged with them as attendants. Usually sufficient workers for this purpose were collected in the traps. An electric incubator provided proper temperature for the drones on cool nights. On several rainy days in 1931 the work was continued by the use of such drones when otherwise it would have been necessary to suspend operations pending better weather.

DETERMINING SEXUAL MATURITY OF INDIVIDUAL DRONES

Since in picking drones at random from a hive it is not always possible to get only those that are sexually mature even if their calendar age is marked, it is desirable to have some simple criterion as to their sexual maturity—in other words, some indication as to whether or not a drone, regardless of age in days, will furnish a supply of active spermatozoa. In experiments conducted thus far there has been found less correlation between calendar age and physiological age in drones, as far as sexual maturity is concerned, than might be anticipated. For example, some drones 2 or 3 weeks old were found without sperm or with less than was possessed by younger drones.

Statements have appeared that drones which ejaculate on decapitation are sexually mature. In 1930, of five queens (Nos. 48, 49, 50, 52, and 53) inseminated with sperm from such drones, all proved to be inseminated to a greater or lesser degree. In the operation on the other queen (No. 47) for which a record was kept, sperm from a drone not in this class was used, but the queen proved to be uninseminated. In 1931, of the 38 instances of successful insemination by the Watson method, drones which had ejaculated on decapitation were used in 25. The reaction of the drones was not recorded in 3 of these instances. For 16 of the 26 queens whose spermathecae were found clear in a post-mortem examination, such drones had also been used. The reaction of the drones used was unrecorded in only one of these instances. Since the proportion of the drones in question to the total number used in the unsuccessful cases cited for 1931 is about the same as their proportion to the total number used in the successful cases cited for the same year, these data indicate that, for 1931 at least, it was immaterial for the accomplishment of artificial insemination which type of drone was used.

QUEEN REARING AND TESTING

EXPERIMENTS IN 1930

To economize on the quantity of bees necessary in the work, the writer in 1930 adopted the use of what he terms a "nursery colony" for rearing and keeping virgin queens, as well as for testing inseminated queen bees. This colony had no laying queen of its own. It was made up in the spring in two standard Langstroth hive bodies with 10 frames full of stores in the lower story and 10 frames full of sealed brood with adhering bees in the upper one. Until summer, when the quantity of sealed brood available decreased, frames of sealed brood were added as necessary to insure a sufficient number of young bees to carry on the work given them. This colony was fed at no time.

When queen-rearing operations were begun, one of the frames in the second hive body was replaced by a frame filled with bars for cell cups with grafted larvae. Grafts were made weekly or oftener as desired. About the third day after being sealed, the accepted cells were put in Alley nursery cages in a nursery frame and left in the second hive body. Three such frames were in this hive body most of the season, room being provided by removing the original frames as necessary.

As the season advanced a third hive body was added to the nursery colony for the purpose of taking care of the inseminated queens. When first put on, this body contained brood and honey. As queens were inseminated they were placed in California introducing cages directly on a comb of sealed brood about ready to emerge but with no other bees on it. Room was made in the hive body for these cages by removing the original frames as necessary. The California cage holds one Langstroth frame. It is made of galvanized metal except for the two sides, which are of wire screening. The comb is inserted at the top of the cage, which is provided with a sliding cover made of the galvanized metal. A 10-frame standard hive body holds 6 such cages plus 1 Langstroth frame.

Up to the close of the main honey flow, whenever an appreciable quantity of the brood in a cage had emerged, the comb, with queen and adhering bees, was removed and set in another cage similar except that one of the screened sides was replaced by excluder zinc. This new cage was then put back in the space formerly occupied by the old cage, and thus worker bees from the nursery colony and those which had emerged in the cage could intermingle and pass in and out freely. The queens were left in these cages until they began to lay or until it was apparent that the attempted insemination was not successful. When a queen began to lay, she was put in a nucleus, together with her comb from the nursery cage and a frame or two of bees from the nursery colony.

As a matter of practice, the sides made of excluder zinc were faced in the same direction in any hive body which contained more than one cage with such a side. On one occasion the nursery colony contained five queens in these modified cages, and three of the queens were laying. In addition the colony contained other queens in the regular California cages in another hive body.

Satisfactory results were obtained with this system until after the close of the main honey flow, when it was found that the bees were inclined to ball some of the queens upon gaining admittance to them through the excluder cages. This may have been due in part to the presence of a comparatively large quantity of old worker bees in the hive, a condition aggravated by the fact that sealed brood became less available from the supply colonies at this period of the season. Since the nursery colony was not fed at any time, the effect of feeding in inducing acceptance of the queens when the main honey flow had passed was not determined. After the main honey flow, therefore, the use of the cage with an excluder-zinc side was discontinued and each queen was transferred directly to her own nucleus box as soon as sufficient young bees had emerged from the comb in her screened cage to keep up the activities of an independent nucleus.

APARTMENT HIVES

In 1931, as an outgrowth of the use of the equipment just described, the writer devised what may be called "apartment" hives, which are made up of one or more compartment hive bodies. A compartment hive body is a hive body separated lengthwise into two or more divisions or compartments by wire screening. (Fig. 14.) Ordinary copper window screening has served this purpose nicely. Strips of bent tin are slipped over the edges of the screening and soldered in place. The bottoms of the hive bodies and, consequently, of each compartment, are also covered with wire screening. Each compartment holds one or more full-depth frames, and thus the total number of such compartments possible in a 10-frame hive body varies from 2 to 10. The bees are provided with an entrance to each compartment by a small hole bored through the hive body. A small block at the entrance serves as an alighting board, and over the entrance is fitted a piece of queen excluder. A separate cover of thin wood is fitted to each compartment and a standard outer cover fits over the whole.

On the basis of the size of compartments found in each, the compartment hive bodies used thus far by the writer have consisted of those with five 2-frame compartments, those with two 5-frame compartments, and those with two 3-frame compartments plus one 4-frame compartment.

The compartment hive bodies are set directly over a queen-right colony from the hive of which the inner cover has been removed. Any heat rising from the colony below will pass through the screen into the compartment hive body, and heat will also pass from compartment to compartment. These hive bodies may be tiered up. (Fig. 15.) In such a case the individual covers to each compartment except those on the top hive body can be removed, so that the screening on the bottom of the hive body above fits snugly against the top of the screening forming the walls of the divisions in the hive body

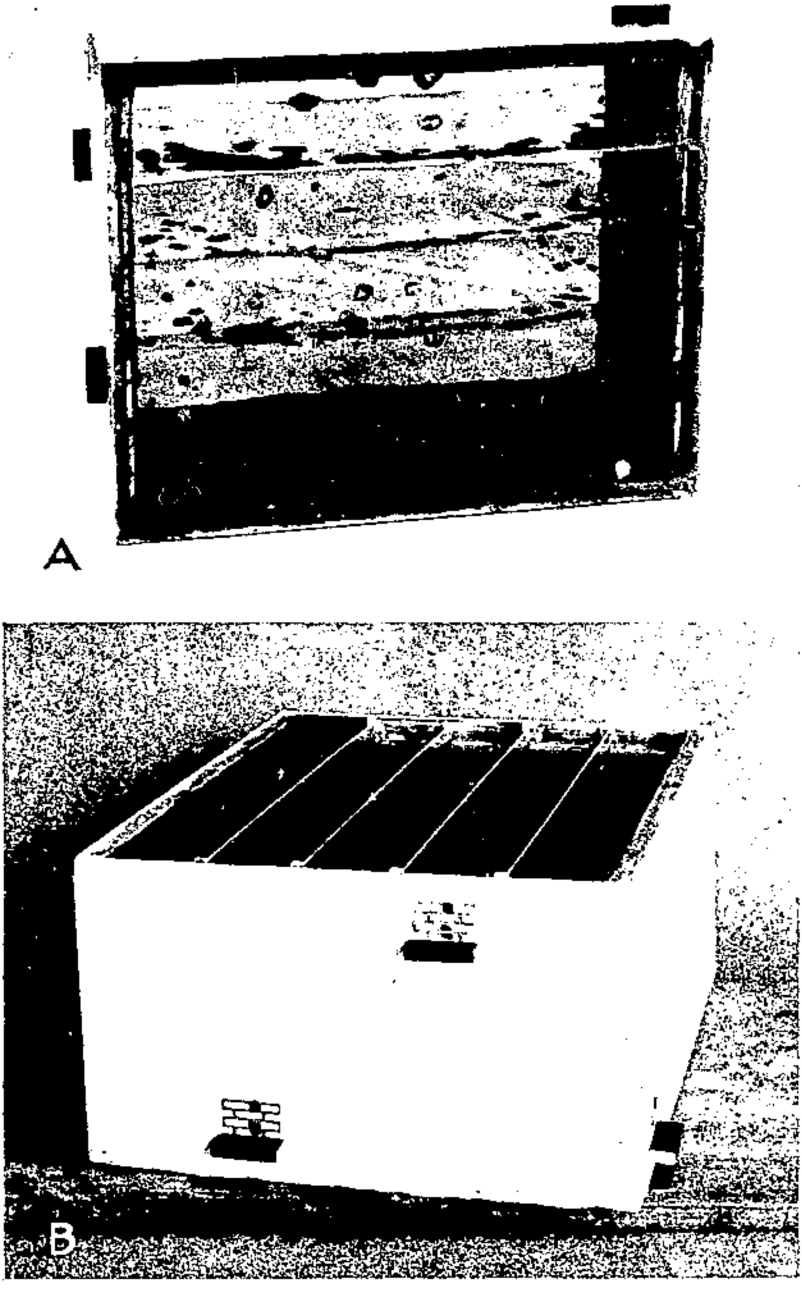

FIGURE 11.—Two views of a compartment hive body. A is without tin strips

below, preventing the passage of bees from compartment to compartment. In 1932 the largest number of hive bodies thus tiered up on one colony and manipulated by the writer was four, each containing five 2-frame nuclei. Counting the queen in the colony on the bottom, at one time there were 21 queens on this one stand, each with its own separate organization of bees. Hence the name "apartment" hive.

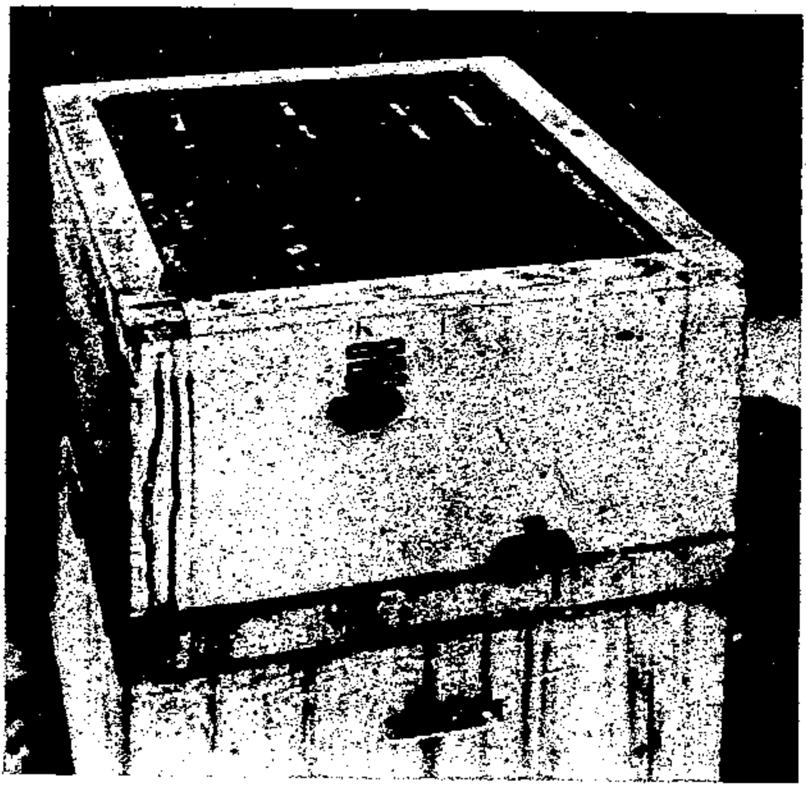

FIGURE 15.—A 5-compartment hive body tiered on a full-strength colony. The hive body is inside of the winter protective collar. The glass tube which extends through the collar as a passage for the bees shows at the entrance at which there is no excluder zinc. Aluminum tags used to keep records in the hive are visible on the frames

The apartment hives have proved to be as good as, or even better than, the ordinary type of nucleus during the robbing season, since the bees for the most part have defended each compartment well. A little care must be exercised, of course, in opening the compartments at this season. Little trouble was experienced in 1931 by bees from one compartment running into a neighboring compartment when adjoining compartments were exposed for purposes of manipulation. In 1932, in the case of tiered-up compartment hive bodies, all compartments that were not being manipulated were kept covered. It may be that all bees in the apartment hive have more or less of a common hive odor. In any event the bees in each compartment seem to remain there without any more than normal drifting elsewhere, even though the entrances were often not very far apart.

The queens are introduced into the compartments by means of a Miller introducing cage. The workers are usually denied access to the candy for three days by fastening a piece of tin across the candy hole. Frames of emerging brood are added to the compartments as necessary to insure the presence of young bees when a queen is being introduced or at other times.

The use of apartment hives is recommended for only those beekeepers who will give them the proper care and attention.

WINTERING IN APARTMENT HIVES

An experiment to winter queen bees in apartment hives was undertaken for the winter of 1931 and 1932. Two compartment hive

FIGURE 16.—Wintering in apartment hives: A, Apartment hive consisting of a 5-compartment hive body over a normal colony; B, telescope cover and tray removed to show individual inner covers of compartments

bodies, each with five 2-frame nuclei, two with two 5-frame nuclei, and one with two 3-frame nuclei plus one 4-frame nucleus were chosen. Each of the five compartment hive bodies was over a 10-frame colony in a double-wall hive, and a packed double-wall collar was fitted around it. A tray of sawdust covered the top of each hive, and over this a telescope cover was slipped. A hole drilled through the collar, into which glass tubing was fitted, connected the entrance of each compartment with the outside. A piece of queen excluder was placed over the outer opening of the tube in some cases. (Fig. 16.)

The winter was milder than is common in the region of Somerset, Md., but since there was consequently more flight activity than usual for this period of the year the weather can not be considered in itself as having been conducive to successful wintering. Nevertheless, the condition on March 1, 1932, of all those nuclei which had plenty of bees on November 1, 1931, was highly satisfactory, with the exception of one 5-frame nucleus. This nucleus, which contained queen No. 51, appeared to have sufficient bees on entering winter, but on February 10 it was queenless and only a handful of living bees remained. Of the 17 experimental nuclei, this 5-frame nucleus and a 2-frame nucleus were the only ones not strong enough in the spring to carry on brood rearing successfully. Perhaps the bees on hand in each nucleus in the fall were somewhat older than was thought. Each of the 10-frame colonies below the compartment hive bodies wintered excellently. The especially strong condition of some of the 2-frame nuclei was a pleasant surprise and showed clearly the value of this method for wintering with a minimum of bees the queens being tested, provided there has been no neglect in insuring the presence of sufficient young bees in the nuclei by the beginning of winter.

QUEEN REARING IN 1931

In line with the other efforts to reduce the number of worker bees and the labor required in the work, all queen cells upon being sealed are put in Alley nursery cages and then placed in an electric incubator and left there at brood-rearing temperature until they hatch. This incubator has glass sides, top, and door, and has sufficient room inside to hold two hive bodies. For queen-rearing purposes, however, this space is divided by a number of shelves to hold the nursery cages. The incubator is located in a building adjacent to the apiary.

An attempt was made in 1931, but somewhat unsuccessfully, to keep virgins in the incubator after hatching. Fresh candy was given for food, and worker bees were provided as attendants. On one occasion all of about 50 virgins so left died within three or four days after emergence. Thereafter the newly emerged virgins were given fresh candy and their cages placed in nursery-cage frames. These in turn were placed in a super directly above the brood nest of a queen-right colony, but separated from it by a queen excluder. Frames of sealed brood, taken from the brood nest below, were placed next to the nursery-cage frame. The chief disadvantage of this method is the tendency of the bees to desert the caged virgins when the weather becomes cool.

In 1932 virgins were successfully kept in early spring in 3-frame queenless nuclei in apartment hives. A special frame was used which consisted of an old brood frame with enough comb cut out of the lower portion to allow the insertion of two nursery cage bars from a standard Alley nursery frame.

In 1931 queen-rearing operations were often conducted with larvae from several queens at once. It was desired to have larvae from any one queen reared by only one colony. However, to have maintained a separate colony for rearing the larvae transferred from each of these queens, as described in the work for 1930, would have taken too much time and too many bees; hence use was made of 2-frame nuclei in compartment hive bodies. Each nucleus was kept strong in young bees. The lower portion of one comb in each compartment was cut away and a removable cell bar was inserted next to a thin strip of wood, which in turn was securely fastened next to the cut edge of the comb. Good results were obtained from these queen-rearing nuclei. In 1932, 3-frame compartments were used instead of the 2-frame compartments, since it is easier to maintain sufficient bees in the larger nuclei.

Frequently it is desirable to carry on transferring operations on cold, rainy, or dark days, or during the robbing season. In order to do this the writer devised a wooden support to hold a brood frame at a convenient angle on a stand during the process. The stand is located in the same room as the electric incubator. With a small movable electric lamp in one hand and the transferring needle in the other, transferring is accomplished under convenient conditions.

HIVE RECORDS

For keeping records within the hive concerning cell bars, queen cells, and queens, tags cut out of aluminum tape and bearing numbers or letters, or both, have proved useful. Numbers and letters can quickly be marked on such tags by a metal stamping outfit, or by scratching with a nail or other sharp instrument. The tags are easily fastened to top bar, cell cage, or queen cage by a thumb tack. The number assigned to any queen and her race or origin, as well as dates of her birth, insemination, first egg laying, and the like, may be thus recorded. In the case of cell bars, the number or origin of the queen from which the larvae are derived, the date of grafting, and the date of first sealing of cells on the bar can be placed on the tags. (Fig. 15.)

When there are many nuclei to be looked after, the use of thumb tacks on the outside of the hive will be found helpful as a means of indicating quickly those colonies needing special attention. Thus an absence of thumb tacks on a compartment may indicate that all was normal within at the last examination. One tack may mean that the queen has been successfully introduced, two tacks may have some other meaning assigned it, and so on. The position of the tacks may be given a certain meaning. The advantage of the tacks is that they are easily withdrawn but stay where placed unless forcibly removed.

VIABILITY OF SPERMATOZOA

Certain experiments performed in Europe (*35*, *36*) indicate that spermatozoa from drones may remain alive at least a number of hours in various electrolytes. Bishop (*5*) has reported that spermatozoa in a salt solution mounted on a slide were still alive after two hours' contact with ice. In order to determine how long honeybee spermatozoa would remain alive under conditions somewhat similar to those in the glass pipette, the writer mounted some on a slide with a cover glass. Under these conditions the spermatozoa at times were found motile more than three hours later at room temperature.

Of particular interest in this connection are the cases of queens Nos. 29 and 86, used in the work for 1931. One of these queens (No. 29), after an operation which seemed normal and satisfactory, was found dead in her Miller introducing cage the next morning at 8.30. A post-mortem examination under the microscope was not made until 11 a. m., at which time her spermatheca was estimated to be 50 per cent filled with sperm and an abundance of live spermatozoa were observed in highly active motion. Even more striking was the case of queen No. 86, which was found dead at 3 p. m. on September 1. She was in an Alley nursery cage in a colony at that time and was left there until the next day at 11 a. m., when a microscopic examination of her spermatheca indicated that it was 75 per cent filled with sperm. A great abundance of living spermatozoa was seen. It is to be noted that these spermatozoa had survived 20 hours in the spermatheca of a dead queen in a cage between the frames of a hive.

Certain results given by Bishop (*5, p. 247*) are in line with the findings presented for these two queens. On one occasion he subjected a "fertile" queen to a temperature 3.6° F. (2° C.) below freezing, for 15 minutes. He states that none of her eggs laid subsequently were found to be infertile.

SUCCESSIVE INSEMINATIONS

The writer has given elsewhere (*39*) a review of the numerous instances in beekeeping literature on the multiple matings of queen bees. That insemination results when the sperm from different drones is introduced into queen bees on successive occasions not on the same day has been indicated in work both by Watson (*52*) and by the writer. In 1930 the writer attempted this on three occasions and had good results in two of them. (Table 1.) For one of the successful instances the interval between operations was 2 days, while for the other one it was 15 days; for the unsuccessful case it was 14 days. In addition seven queens were given two operations in one day, but in only one case was the treatment successful. Three of the queens were found to have clear spermathecae, and three disappeared.

In 1931, 33 queens were operated upon twice by the Watson method, and 8 of these in turn were operated upon three times. (Table 2.) Sixteen of the 33, or about 48.5 per cent, proved to be inseminated. This is to be compared with 34.4 per cent of ascertained success for the 64 queens operated on only once, 22 of these having been found inseminated. In the case of 14 of the 33 queens the second operation was performed on the same day as the first, while a third operation was performed later on 3 of these, but all 3 disappeared. Of the remaining 11 queens operated on twice in the same day, 4 proved to be inseminated, 4 were lost, and 3 had clear spermathecae.

Of the other 5 queens which were given a third operation, 3 proved to be inseminated and the other 2 disappeared. In one of these successful cases (No. 86) sperm was found so massed in the queen's spermatheca that the degree of insemination was estimated to be at least 75 per cent. Queen No. 53 was found to be somewhat less inseminated, while the other case (No. 88) was classed as less than 10 per cent successful.

The results for 1930 are not comparable with those for 1931 because of the use of ether in some cases. The results for 1931, although not conclusive in themselves, seem to be in line with Watson's statement (*52*) that repeated inseminations increase the degree of success. In describing his work for 1927 (*51*), Watson mentions that he operated on some queens from 1 to 10 times.

It is possible that many of the queens that disappeared in 1931 before any check could be made as to their insemination, as well as many of those that were found dead in the cage, may have been lost in attempts to get out through the excluder to take a mating flight. That many of the queens found dead in the nuclei had tried to get out or had suffered rough treatment from their bees seems evidenced by the fact that the painted markings had been rubbed off the thorax in nearly all cases. It seems of significance in this connection that practically all those queens which laid eggs were readily accepted by the nuclei in which they were introduced.

MIXING SPERM FROM DIFFERENT DRONES

Without following the genetic composition of succeeding generations, it is hard to say in the case of insemination from the sperm of different drones on successive days whether or not the sperm from each drone was active. In the case of 8 of the queens operated upon in 1930 the sperm from more than one drone was loaded into the pipette at one filling. Seven of these operations were successful. Sperm from 2 drones was used in 5 cases, 4 of which proved successful; sperm from 3 drones was used for 2 queens, and each of these queens was found to be inseminated. In one of these instances all the first brood sealed proved to be worker. For the eighth queen sperm from 5 drones was used, and this operation was also successful.

In 1931 the sperm from more than one drone was loaded into the pipette in the operations on 29 queens. Eleven of these queens proved to be inseminated. On two occasions the sperm from 3 drones was used, and in each case a successful insemination resulted. In no instance was the sperm from more than 3 drones employed.

The foregoing examples indicate that a mixture of sperm from different drones does not destroy the vitality of all the spermatozoa at least. In each case the pipette was loaded by taking up the sperm from the drones in succession. It happened that all the drones used for any one operation came from the same hive.

In his 24 successful inseminations in 1929, Mikhailoff (*25*) used sperm from 2 or more drones for 22 of the queens. For 1 queen he reports the use of only 1 drone, while for 1 he does not give the number. In a later article (*28*) he states that he used the sperm from 2 to 4 drones for every queen.

OPTIMUM AGE OF QUEEN FOR ARTIFICIAL INSEMINATION

It is frequently stated in beekeeping books that the queen usually mates early in life and that if she delays more than three weeks she is apt to become a drone layer. Thus, Phillips (*40, p. 49*) gives the normal age of the queen for mating as 5 to 8 days. In a compilation made by Armbruster (*4*) of data from mating stations covering six years, 9 days was the age of the queen at which the greatest number of matings was found to occur. If these conditions hold in natural mating, it might be expected that more success would be attained in artificial insemination with queens only a few days old than with older queens.

Mendel (*19, p. 149; 54*), however, held that a queen remains capable of being inseminated up to the twenty-eighth to the thirtieth day after emerging, and that for some queens this time limit is even longer. Likewise, although in the data by Armbruster (*4*) on natural matings of the queen bee the earliest age at which any of the queens mated was 3 days, the oldest age is given as 32 days. The queens successfully operated upon in 1929 by Mikhailoff (*26*) ranged from 7 to 29 days in age, while Watson (*49*) reports that he has been successful in the artificial insemination of queens even 37 days old.

In 1930 a record of the data of emergence and of attempted artificial insemination was kept by the writer for 43 queens which were operated on only once or only once plus a second operation within two days of the first, and in 1931 for 82 queens which were operated on only once or, in some instances, once again within one day of the first operation, making a total of 125 queens. As a matter of fact, only 1 of the 43 queens listed for 1930 (No. 44) received a second operation, while 5 of the 82 listed for 1931 (Nos. 8, 14, 46, 86, and 90) received a second operation. Queen No. 86 received a third operation. In Table 3, which gives the results obtained from queens of various ages, these six queens have been considered as receiving their successful operation on one occasion, since the time interval between the operations is small. Of these 125 queens, 47 proved to be inseminated.

TABLE 3.—*Success attained with queens operated on in 1930 and 1931 only once or within two days of a preceding operation in age period specified*

Age of queens	Total queens inseminated	Successful operations	
	Number	*Number*	*Per cent*
1 to 8 days	59	14	23.7
9 to 16 days	41	14	34.1
17 to 24 days	20	15	75.0
25 to 32 days	5	4	80.0

From these results it is apparent that the greatest degree of success was attained by using queens at least 17 days old. The total number of queens (100) under 17 days of age which were used, however, was four times as large as the number (25) of those which were 17 days old or over, but the percentage of success with the younger queens was only 28 as compared with 76 for the older queens. It is interesting to note that No. 47, operated on in 1931, even though mated when 29 days old, proved to be one of the most prolific layers of worker eggs of the queens mated that year. In 1930 two queens were successfully mated at the age of 31 days. On the other hand, in 1931 a queen 1 day old was successfully inseminated. The data presented here, although based on a small number of cases over 17 days of age, indicate that the age limit at which queens can be inseminated artificially is higher than that commonly accepted as the limit for natural insemination.

SUCCESSION OF GENERATIONS

Regardless of any method for inseminating queen bees at will or of any method for carrying queens through either the active or the inactive season with a minimum of labor, time, and equipment, success in breeding the honeybee and other forms is dependent upon a third factor—upon having a succession of generations. The writer has been successful in the first factor mentioned since the beginning of his work in this field, while, as for the second factor, an artificially inseminated queen was wintered over in the first year of the work. However, F_1 queens from artificially inseminated mothers were not obtained until 1930. These were all reared from one mother. Six of them were successfully operated upon that year and from one of these an F_2 queen was reared. This queen was born too late for mating that year, however.

In 1931, as already stated, F_1 queens were reared from eight artificially inseminated queen bees. Unfortunately none of these was reared before July, and since August was marked by an unusual scarcity of drones, the inseminations during the latter part of the season were somewhat restricted both as to number of attempts and as to degree of success.

Of the 97 queens used in the work with Watson's method in 1931, 32, or practically one-third, were F_1 queens. Workers of the F_2 generation were obtained from one of these (No. 78) in October. It was then so late in the season that no attempt was made to rear F_2 queens from this queen, but she was still alive at the beginning of winter. Insemination in the case of 6 others of the 32 was determined by a microscopic examination of their spermathecae, making a total of 7 of the F_1 queens, or 21.9 per cent, which were found to be inseminated. Eight of the remaining 25 gave no evidence of insemination on a post-mortem examination; 15 disappeared; while the other 2 (Nos. 77 and 92), inseminated late in the fall, entered the winter season without having commenced egg laying.

In the work as conducted in 1932 there was no difficulty in obtaining a succession of generations. Of four successive generations of queens reared in that year from a certain strain, the first three had been successfully inseminated by the Watson method before September, as evidenced by the rearing of virgins and workers. Certain other strains were carried three generations, and a number were carried two. Under ordinary conditions five or six generations should be secured readily in a season such as characterizes Somerset, Md. Under optimum conditions during the active season one generation a month is not out of the question, although one every five or six weeks appears satisfactory at present.

SUMMARY

In breeding the honeybee under controlled conditions either natural or artificial insemination can be used. The only feasible method thus far developed which makes use of natural insemination is that of using mating stations. An important objection to this method is the difficulty of being absolutely sure that no wild swarms or colonies kept by beekeepers exist within a radius which would make possible crossings other than those desired.

For absolute certainty as to drone and queen, artificial insemination can be employed. Two main methods have been developed for accomplishing artificial insemination: (1) Removing the organ containing the sperm from the drone and placing it in proper position

in the queen to accomplish insemination, as done by Malyschev and by Quinn-Laidlaw; and (2) removing the sperm completely from the drone organ and then introducing it within the queen, as done by Watson. Thus far only the Watson method has been repeatedly used with success in the hands of others.

The technic originally described by Watson has been modified by the use of new instruments and methods. Methods have also been developed to use a small number of bees and to reduce the quantity of equipment required in rearing and testing queens used in breeding work.

Spermatozoa will remain alive under the cover glass or in the spermatheca of a dead queen for several hours at ordinary summer temperature.

Successive artificial inseminations of a queen, at least before she begins egg laying, can be made with good results.

Sperm from different drones can be mixed together without apparent effect on some of the spermatozoa at least.

Worker brood was reared from a queen inseminated when 29 days old. In 1930 and 1931 greater success was had with queens 17 days of age or older than with younger queens.

A succession of generations of the honeybee is possible through the use of artificial insemination. Four generations is the largest number yet obtained at Somerset, Md., in one season, although under optimum conditions it should be possible to obtain a generation a month during the active season.

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