The Development of Instrumental Insemination

First of a Series

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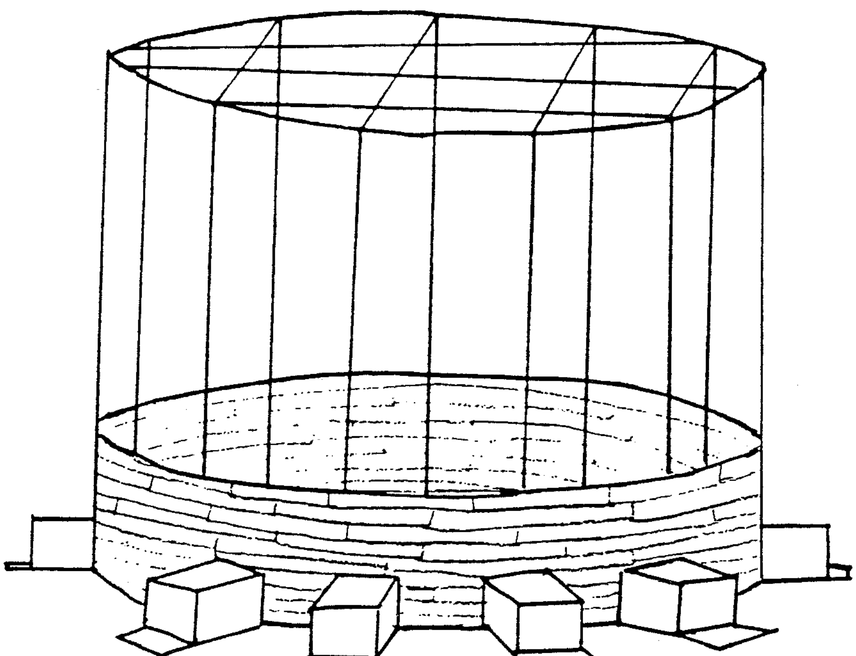
THE development of instrumental insemination is a fascinating history which makes one appreciate the capability we now have today. The attempts to control honey bee mating were numerous, frustrating, and full of sporadic claims that could not be verified or repeated. Beekeepers were not able to make the progress comparable to that accomplished by other plant and animal breeders, though queens and drones were easily reared from any stock. The fact that bees mate in flight and, until recent history, our lack of knowledge of the mating process created a large stumbling block.

The first recorded attempts to control mating date back more than 200 years. At that time, the honey bee mating process was full of mystery and misconception. Queens and drones were thought to mate inside the hive at frequent intervals. Others thought the eggs, after being laid by the queen, were fertilized by drones inside the hive.

Many tried to develop methods of natural mating control. Reaumur, as early as 1740, attempted to observe the mating by placing a queen and drone in a small glass dish, without success (Laidlaw, 1949). Several attempts of fertilization were made by introducing semen onto the queen at various stages. Swiss naturalist, Francis Huber (1814), painted semen on the queen's sting chamber, also without success. Fluid from the drone larvae was squeezed onto the queen larvae by Kohler (1868). McLain (1885) tried to fertilize the queen pupae and adults with a drop of semen, and claimed partial success with adults. Fresh semen was painted on drone eggs which were reported to have developed into queens by Barrat (1919).



Davitte's tent for confined mating, built in 1901. He suggested 500 queens a day could be mated in a tent such as this.

The exciting discovery that queens mate outside the hive was made by Anton Janscha in 1771 (Roberts, 1944). Huber (1814) confirmed this fact in 1787 by placing queen guards at the hive entrance and observing virgins flying and returning with evidence of mating.

The discovery that queens mate in flight led to numerous attempts over the next 50 years to confine mating to various enclosures, and a variety of flight chambers. Gregor Mendal, the father of modern genetics, was among those who unsuccessfully tried to solve the problem in his endeavers to understand honey bee genetics.

Timing of the mating flight was another technique used in attempt to control mating. Colonies were screened or placed in cool dark cellars until late afternoon. The screens were removed or colonies moved outside to permit mating when normal drone flight had ceased. These methods were tried by several investigators and some success claimed; as Dathe (1868), Kohler (1868) and Kruger (1868).

Some experimenters of controlled mating attempted to restrict the flight range of queens by tethering. Shrimplin (1861) described the mating of a virgin he caught leaving the hive and tethered on a silk thread. Shuck (1882) tethered a virgin at the end of an 18 foot pole and also claimed success. Attempts by others, as Demaree (1881), were admitted failures.

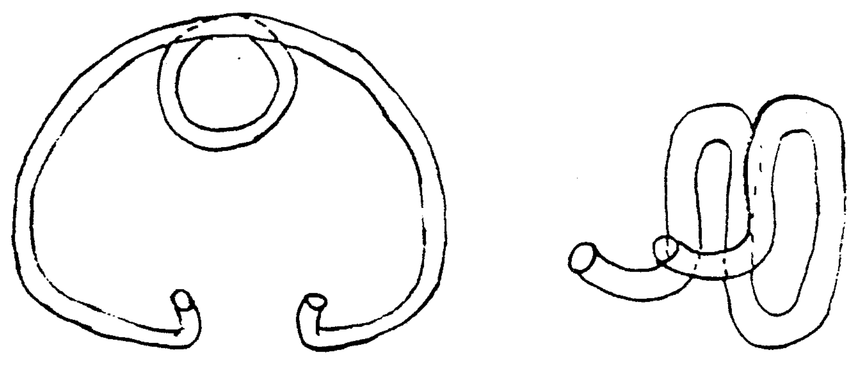
The trails and methods of confined mating were numerous and diverse. Boxes of various sizes and shapes, a glass quart jar and sugar barrels were all used to confine the bees. Flight chambers were built of muslin, mosquito netting and wire cloth. Some had roofs made of glass, others shaded and one was reported to have an adjustable ceiling.

Hasbrouck (1878) claimed successful mating in a 3 inch cubical box with a glass top placed in the sunlight. Several others tried this method and reported failure. Cramer (1881), who claimed a glass top would not work, reported success with a 2 foot high fertilizing box made of muslin attached to the top of a hive. A mating tent of brown cloth was built by King (1872), who claimed the secret was to exclude the workers and use drones which had never flown. Success by this method was also claimed by Hohenshell (1887). A large tent of mosquito netting, 30 feet in diameter and 30 feet high, was reported to have been the mating site of 100 queens by Davitte (1901). He suggested 500 queens a day could be mated in such a structure. Several others tried Davitte's technique and reported no matings. Church (1906) substituted wire cloth which he reported caused torn wings and blamed as the reason for failure. Large buildings were used in attempts to confine matings without success. This included the largest greenhouse in America measuring 600 feet long, 60 feet wide and 30 feet high. Extensive experimentation by Root et al (1917) reported flight, but no matings.

The methods of the many investigators who claimed successful controlled matings were not repeatable. The leading beekeepers of the time; A. J. Cook, C. C. Miller, J. Heddon, C. P. Dadant, J. E. Pond, Jr., G. M. Doolittle, G. L. Tinker and G. W. Damaree (1885), unanimously agreed that mating in confinement was not possible. W. H. Furman challenged the claims of confined mating, calling it an "unmitigated humbug." He offered $500 to anyone who could mate 50 queens in confinement at his apiary. Doolittle (1888), frustrated by his own failed attempts, also offered $500 for a plan by which his queens could be mated with selected drones. Today we have yet to develop a technique for confined mating.

This led many researchers to explore the mechanical transfer of semen. Francis Huber was the first to attempt artificial insemination by means of a tool. It wasn't until 100 years later that this approach was taken seriously by others.

The U.S.D.A. hired their first apiculturist, Nelson McLain, in 1885. His job included the development of a method of artificial insemination. McLain used a hypodermic syringe with a modified needle to collect semen and inject it into the queen held in a clamp (Nolan, 1932). Others attempted to collect semen from the drone and transfer it into the queen by means of a capillary tube or pipette. Jager and Howard (1914) reported success by this method in one of eight queens with semen that was diluted with saline solution.



Clips of fine wire used to hold the abdominal tips of the queen apart during artificial insemination in the late 1920's and early 1930's.

There were many attempts of hand mating. Shafer (1917) and Bishop (1920) tried to obtain fertilization by everting a drone into the queen's genital chamber. A similar technique was being tried in Russia about the same time.

Partial success of manual manipulation was reported by Charles Quinn and his grandson, Harry Laidlaw. Laidlaw (1932) improved this technique by devising a small spring to hold the queen open, and he used a microscope. Using this procedure, the queens were known to lay fertile eggs but soon became drone layers.

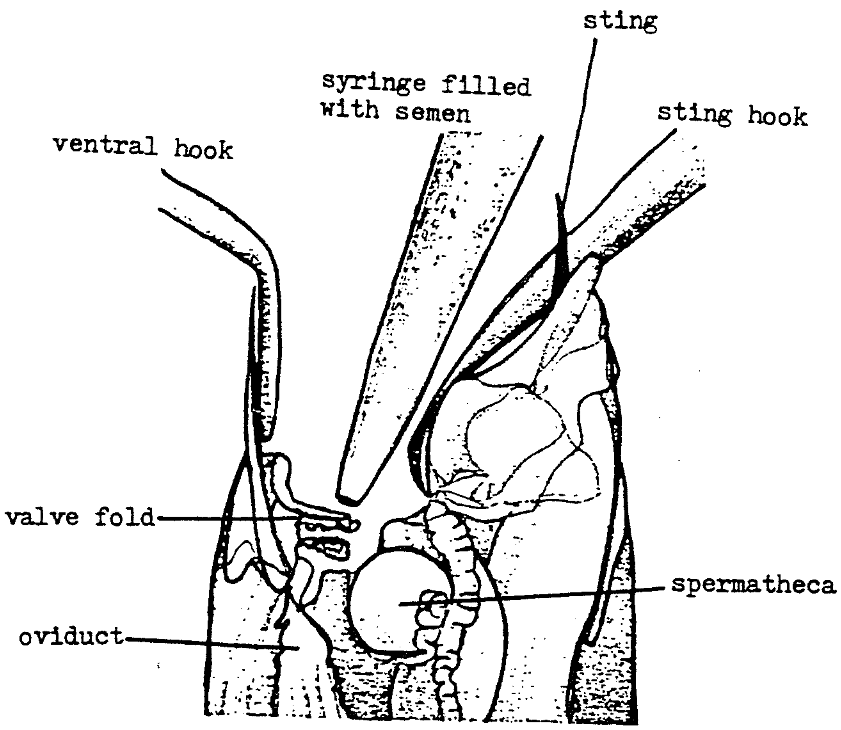
These instances of success were enough to encourage futher study by several investigators. The modern technique of artificial insemination began with Dr. Lloyd Watson, who gave the first successful demonstration at Cornell University in 1926. He was a chemist, skillful in designing and constructing the glass syringe used for insemination. The queen was stabilized on a wooden block by a silk thread, and semen was injected by a microsyringe, under the microscope (Watson, 1927). Watson's technique of "instrumental insemination," as he called it, proved superior to any previously recorded. Although at the time he did not realize that most of the semen injected was going into the pouches that lie to the side of the vagina (Laidlaw, personal communication). There were still many unanswered questions to explore and refinements in the technique to be worked out. The foundation had been laid and this work stimulated others.

Understanding the mating biology and honey bee reproductive system was recognized as essential for the development of practical artificial insemination. Success could only be measured by comparing queen performance. Early inseminations presented many problems. Queens were inseminated with semen from only one or two drones and the semen injection was very inefficient. These queens were tardy to begin egg laying and would produce only a small proportion of fertile eggs. Some queens died before laying, others became drone layers or were soon superseded. The few exceptions to this motivated futher experimentation.

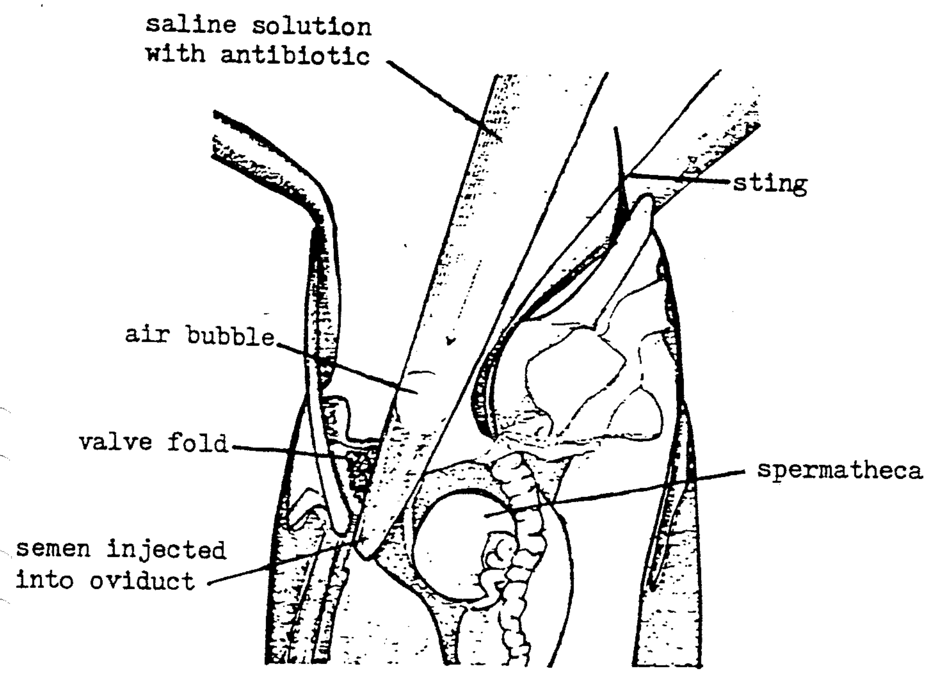
Dr. Harry Laidlaw (1934) wrote an accurate description of the honey bee reproductive system for his Master's thesis at Louisiana State University. It was here that he solved the major obstacle of getting semen into the reproductive tract of the queen. Laidlaw saw the need to lower the valve fold, an invaginated tongue-like structure blocking the oviduct and passage of semen into the spermatheca. He designed a sting hook to pull the sting over the vaginal opening and lowered the valve fold with a probe.

It soon became evident that semen from several drones was necessary for complete insemination. The widely accepted belief that a queen naturally mated with a single drone was being refuted by many researchers. Numerous observations of multiple mating were reported. Queens were observed to take

ABDOMEN OF THE VIRGIN DURING INSTRUMENTAL INSEMINATION



Proper placement of hooks during I.I., notice the valve fold blocking entrance into the oviducts.



Insertion of syringe tip, bypassing the valve fold, and injection of semen into the median oviduct. (From Ruttner, 1976).

two or more mating flights (Oertel, 1940). Dr. William Roberts (1944) proved multiple mating by progeny and flight tests. Multiple matings were confirmed by other researchers, as Tryasko (1951) and Peer (1956). Queens were also observed to mate several times during each flight (Taber, 1954). These facts plus the need to increase the efficiency of sperm storage in inseminated queens led to the use of several drones and repeated inseminations.

Queens returning from natural mating flights were found to have mucus in their genital chamber. The mucus was thought to create a natural seal after mating to prevent semen leakage. The mucus plug was used in instrumental insemination in attempt to prevent the backward flow of semen. This proved a difficult and unnecessary task, and was discontinued.

The first artificial inseminations were performed on fully conscious queens. There was a need to stabilize the queen because her breathing movements made the process difficult. Ether was used but appeared to cause detrimental effects, resulting in high mortality and abnormal behavior of queens (Nolan, 1932). Laidlaw (1949), aware that carbon dioxide was used by the U.S.D.A. to anesthetize imported bees for examination of acarine mites, decided to use it during instrumental insemination. Carbon dioxide replaced the use of ether as an anesthetic in the 1930's and is routinely used today.

The use of carbon dioxide also solved the problem of delayed egg laying of instrumental inseminated queens. This discovery was made by Dr. Otto Mackensen (1947) who found that two 10 minute treatments of CO_2 stimulated egg production in instrumental inseminated (I.I.) queens as well as virgins. Before this, I.I. queens would not begin egg production for several weeks. This was an important step toward the success of instrumental insemination.

In search of a more practical method of instrumental insemination, advances were made in equipment design. Watson's technique was modified by the use of new instruments and methods. Nolan (1932) simplified the apparatus so he could inseminate several queens at once and inject semen for a longer period of time in an attempt to increase the efficiency of semen transfer. He used a glass queen holding tube, which was the idea of J. I. Hambleton, and designed a syringe from a mechanical pencil with a glass drawn tip. The queen was held open by hooks or a wire clip, instead of hand-held forceps. The holding tube, syringe, and hooks were mounted on a stage which fit under the microscope.

The early glass tips used for insemination were too large, making it difficult to inject semen past the valve fold. Mackensen drew out the tip, reducing its size to allow entry into the median oviduct. Roberts (1947) designed a syringe with a removable plastic tip which was more durable. The syringe was further improved by Mackensen, with a liquid plunger controlled by a rubber diaphram, enabling semen to be taken up and expelled. Mackensen and Roberts (1948) modified and refined Nolan's instrument with these improvements and attached a CO_2 tube to the queen holder creating an anesthetization chamber. This is the Mackensen instrument as we know it today.

Dr. Harry Laidlaw (1949) designed a different insemination device which offered greater precision of movement with racks and pinions. He used the Mackensen syringe and replaced the queen holding tube with a clamp. The queen was held between sponge pads in which the flow of CO_2 was directed over the spiracles. Laidlaw also instituted the use of an antibiotic in the saline solution used during I.I. This solved the problem of excessive queen loss from normally non-pathogenic bacteria which multiplied in contaminated semen used for insemination.

The techniques of I.I. of queens developed by Watson, Mackensen, Roberts, and Laidlaw enabled a practical method of controlled bee breeding. Based on the design of these instruments, workers in several countries have made many variations in the techniques and devices used for I.I. Among these; Ruttner, Schneider and Fresnaye designed an innovative, Mackensen type instrument with movement controlled by racks and pinions (Ruttner, 1976).

Instrumental insemination has proved a valuable tool for the study of honey bee genetics and a means to improve our commercial breeding stock. Progress certainly has been demonstrated since the days of tethered queens and attempted confined matings, and we owe much thanks to the researchers for their persistence and determination in perfecting a method of controlled breeding.

Future articles in this series will explore the developments in equipment design, care and conditions affecting I.I. queen performance, and a look at the possibility of sperm storage.

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