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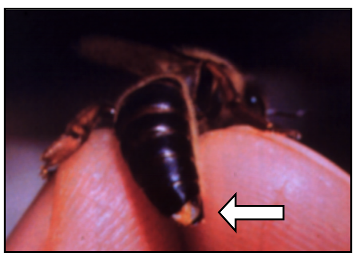
APICULTURE PROGRAM
DEPARTMENT OF ENTOMOLOGY
NORTH CAROLINA STATE UNIVERSITY

Dedicated to the dissemination of information and understanding of honey bees and bee management

EXTENSION RESEARCH INSTRUCTION

THE DEVELOPMENT OF HONEY BEE INSTRUMENTAL INSEMINATION
A TRIBUTE TO DR. HARRY H. LAIDLAW, JR.

One of the most powerful tools used in apiculture is the technique of instrumental insemination, where scientists can introduce specific drone semen into an unmated queen. By controlling the genetics of honey bees through breeding programs, researchers have had numerous successes at resolving some very difficult problems in the industry. For example, the creation of the varroa mite-resistant SMR strain, developed by the US Department of Agriculture Baton Rouge lab, would have been impossible if not for instrumental insemination. And we owe it in large part to the late Dr. Harry Laidlaw.



The mating sign of a honey bee queen. Picture by W. Mangum.

Natural insemination of honey bee queens

In order to fully understand how the instrumental insemination process works, one must first understand how queens are naturally inseminated. François Huber, a blind Swiss beekeeper working in the eighteenth century, tested whether queens mate inside the hive by restricting the entrance so that drones and queens could not leave (using an early type of queen excluder). He found that they did not lay for several weeks and produced only drone brood, indicating that queens mate outside of the hive. Later, Anton Jansha, who was the Royal Teacher of Bee Culture in Austria in the late 1700's, observed that virgin queens fly from their hives and return with a "mating sign", the lodged genitalia of the queen's mate (see Figure). It wasn't until the mid 1900's when Oertel (1940) and Roberts (1994) observed that queens often take repeated mating flights, suggesting that they mate more than once. Today, with the advent of powerful genetic analyses, we know that honey bee queens have the highest mating numbers in social insects, mating with an average of 12 males. Gregor Mendel, the father of modern genetics, endeavored to breed bees but failed because he was unaware of their complicated mating system. It is fortunate for all of us that he switched to pea plants, as his work in that system laid the foundation for modern genetics.

Our understanding of honey bee mating biology was considerably enhanced by the anatomical descriptions of the reproductive organs by Bresslau and Snodgrass

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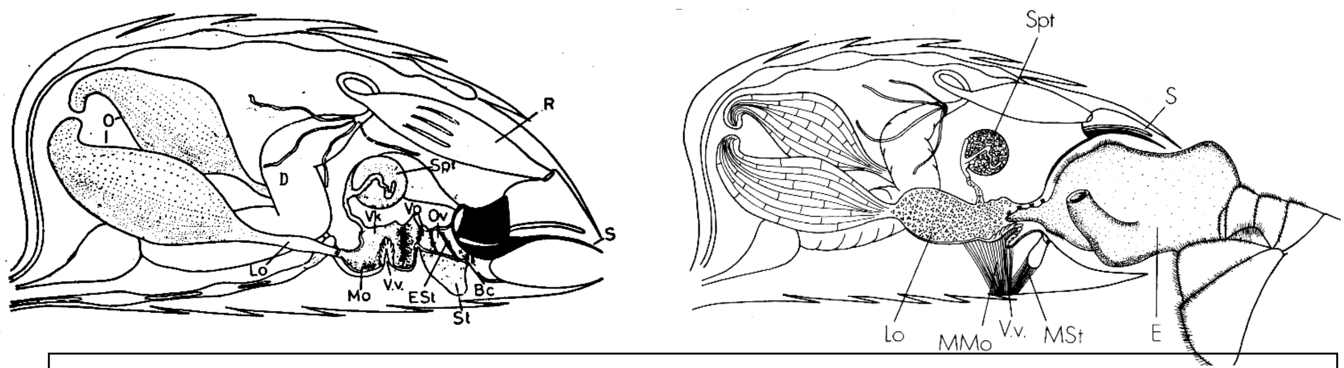

Cross sections of a queen's abdomen showing her reproductive organs. Left: typical view showing her ovaries (O), lateral and median oviducts (Lo and Mo), the valve fold (V.v.), and spermatheca (Spt). Right: view during natural mating, showing the male's inserted genitalia. Semen is stored in the lateral oviducts, past the valve fold, and the sperm migrate into the spermatheca. Figures from Moritz (1989).

in the early 1900's. Their dissections and drawings illustrate the basic structures that are involved during the insemination process. A queen's lateral and median oviducts, the tubes that enable eggs to be carried from the ovaries out through the vaginal orifice, become filled with semen as the queen mates with numerous drones. Posterior to the median oviduct is the *valve fold*, a flap of tissue that projects dorsally and blocks direct passage into the lateral oviducts. During copulation, a drone is able to bypass this structure, enabling him to inseminate the queen. Sperm from a queen's mates are stored in the spermatheca for her to use over the course of her egg-laying lifetime.

A brief history of instrumental insemination

François Huber is considered to be the first person to attempt to artificially inseminate queen bees (despite his blindness!). He did this by trying to introduce drone semen into the vaginal orifice of virgin queens on the tip of a fine paint brush. Needless to say, these attempts failed.

H. Wankler was a German beekeeper and clockmaker in the late 1800's. Using his skills with fine tools, he constructed what he called an "artificial penis" out of silver to deliver semen into the queen's reproductive tract. This attempt also failed.

Also in the late 1800's, Nelson McLain, a USDA honey bee researcher, began a series of experiments to inseminate queens. First, he placed drops of semen on the queens vaginal opening while holding open her sting chamber with forceps. He also tried to constrain queens with wooden clamps and inject the semen into her with a hypodermic syringe. Unfortunately, both of these techniques were also unsuccessful.

It wasn't until the 1920's that Lloyd Watson was able to demonstrate to a now-skeptical beekeeping community that instrumental insemination was possible. Watson used a stereomicroscope, a light source, and hand-held forceps to open a queen's sting chamber. He was then able to inseminate her with capillary syringe filled with drone semen. Although not always reliable, his refined technique had some success, which was a vast improvement over previous attempts.

Learning from Watson, W. J. Nolan at the USDA developed an instrument and syringe of his own. The main differences from Watson's device were that the queen holder was fixed in place and that two laterally-positioned hooks there were used to open the queen's sting chamber. This was an improvement, but the technique was still not 100% reliable.

Finally, in 1944, Harry Laidlaw at the USDA Baton Rouge lab succeeded where others had failed. Laidlaw discovered that the valve fold hindered injection of semen into the lateral oviducts, and bypassing it enabled him to inseminated semen directly into the oviducts. Circumventing the valve fold was the key to successful inseminations, enabling subsequent attempts to be routine.

There have been many devices constructed over the years, but the model that is most commonly used was developed by Otto Mackensen. It consists of a fixed tube in which the queen is held in place and anesthetized with CO_2, a ventral hook to pull the lower sting chamber open, a sting hook to pull the sting apparatus dorsally, and a syringe with which to inject drone semen. Syringes can be made with a plunger, tapered tip, and a barrel with a threaded rod that pushes a rubber diaphram to collect and eject semen from a plastic tip. The most popular syringe today was developed by John Harbo at the USDA Baton Rouge lab. It consists of a glass capillary tube, a drawn glass tip, fine rubber tubing, and a Gilmont micrometer syringe.

Dr. Harry H. Laidlaw, Jr.

Dr. Harry Laidlaw passed away in September, 2003 at the age of 96. His contributions to honey bee genetics and bee breeding are among the most significant of all apiculturists, and he will be greatly missed.

Readings

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