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(BVSc)

THEME

Exploration of honey bee reproduction and the evolution of instrumental insemination in the same species (*Apis mellifera*)

Presented by:

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IN FRONT OF A JURY:

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Acknowledgement

We students are not different from sheep that we study. At times we find ourselves in an estranged place having lost our bearings. However like the good shepherds our elders always find a way to rein us in and make sure by nightfall we are back home safely.

On that note I would very much want to acknowledge the hard work put in by all the lectures and doctors who have shaped me into the person I am today. Many thanks go to the L.B.R.A laboratory in accommodating me during the whole year.

My thanks also go towards Dr. BESSAD who was gracious enough to make time for me at the end of the year and helped me finish the project; my sincerest gratitude to you and I have to say I'm grateful to have met him and honoured he is my co-promoter.

I should not forget the duty that befalls you as the jury of my end of studies project. It makes me happy to know that two of the people who went out of their way to make sure I learned are part of the jury. I only have good things to say when I'm asked about the members of the jury Dr. Boumahdi and Dr. Sellali. I appreciate the time and the effort you have taken to work through my project and it must be noted that I am grateful.

Last but definitely not least my most profound of thanks goes to one Pr. KAIDI R, my promoter for this project. I would like to thank him for the patience he has shown to me and the wise words that he has shared with me that I will definitely take with me as I continue beyond today on to a new path.

DEDICATIONS

A SPECIAL THANKS TO MY FAMILY THAT HAS ALWAYS STOOD BY ME THROUGH THE GOOD AND BAD TIMES. I LOVE YOU

VENI, VIDI,VICI…

..Julius Cesar ,47 BC

ABSTRACT

The importance of bee breeding has for long been underestimated. As studies have shown this field is still being neglected .With the contribution that honey bees give to the maintenance of what we term biodiversity, it’s only fair and fitting that we give the honeybees the attention they richly deserve.
Exploring the reproduction aspect of honey bees takes through a wonderful journey that is rich in awe and interesting aspects. The evolution of instrumental insemination is fascinating journey through the many significant developments that have led to where we are today. The early apparent failures of controlled breeding also played a great deal in this evolution, after all humans have shown a trend of learning from their mistakes

RESUME

L'importance de l'élevage des abeilles a longtemps été sous-estimée. Comme les études ont montré que ce domaine est toujours négligé, avec la contribution des abeilles à la préservation de ce que nous appelons la biodiversité, il est juste et approprié de donner aux abeilles l'attention qu'elles méritent.
Explorer l'aspect reproduction des abeilles mellifères passe par un voyage merveilleux, riche en admiration et en aspects intéressants. L'évolution de l'insémination instrumentale est un voyage fascinant à travers les nombreux développements importants qui ont conduit à ce que nous sommes aujourd'hui. Les échecs précoces de l'élevage contrôlé ont également joué un grand rôle dans cette évolution, après que tous les humains ont montré une tendance à apprendre de leurs erreurs.

الملخص:

إن أهمية تربية النحل قد تم التقليل من أهميتها منذ فترة طويلة. من السهل القيام به أعتقد إذا كنت تبحث من الخارج إلى الداخل.
من خلال جميع دراساتي، تمكنت من تقدير كامل الجانب التكاثر لجميع الأنواع التي درسناها ، الأبقار ، الأغنام ، الماعز و الأحصنة.
مع المساهمة التي يقدمها نحل العسل للحفاظ على ما نسميها التنوع البيولوجي، فإنه من العدل و الملائمة أن نعطي النحل الإهتمام الذي يستحقه.
استكشاف الجانب التكاثري للنحل من خلال رحلة رائعة غنية بالرعب و الجوانب المثيرة الإهتمام. إن تطور التلقيح الفعال هو رحلة رائعة عبر الكثير من التطورات الهامة التي تؤدي إلى ما نحن عليه اليوم.
أول فشل واضح للتربية المراقبة ايضا يلعب دورا هاما في هذا التطور، بعد كل شيء نحن بشر، و نتعلم من اخطاءنا

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INTRODUCTION

INTRODUCTION

The world as viewed today is one that has been massively influenced by the human species tenacity to evolve, along with the drive to see a tomorrow that is much better than yesterday. This instinct or unquenchable thirst that drives humans well, it drives to control, the need to know, be knowledgeable and when it suits us be actionable, well what I am rambling on about.

Today we look at a very important species to the human race; the main focus is on the honey bee, (*Apis melifera*). This species plays a very large role in biodiversity through pollination, it guarantees fruit season is never a dull moment, not to mention the vast contribution through its production of honey, propolis, beeswax, etc. but today the focus is on the reproductive side of things, after all I'm a veterinary more than I can think of being a bee keeper. Primary objective is to understand the make-up of the reproductive system on an anatomical and physiological side. Understand its natural reproductive patterns; dig deep into the need for controlled systems of reproduction that in the end leading to instrumental insemination, how it has evolved over time and how a big role it has played in human influence on the honey bee species

Chapter 1: General knowledge of honey bees(Apis mellifera)

1.1. INTRODUCTION TO THE HONEY BEE

Everything about bees has a fascinating pull to it, ranging from their distribution all around the world, different climates different modes of adaptations, to their place in the natural ecosystems especially their massive role in pollination of plants, a very important factor in plant survival, genetic diversity and in the end the bearing of fruit by said plant. Time and history has given the opportunity to study the bees in greater detail and tap into its production side for the benefit of humans, exploitation of bees for the products such as honey, beeswax, propolis, royal jelly etc.

Through research and study we know that bees exist in two sexes i.e. male and female. The female bees are represented by two groups or classes: the workers, (les ouvriers in French), make up the majority of the populous in a hive, providing the workforce that keeps the hive together; they look for food, they shelter the hive and feed the eggs laid by the queen. The latter (reine in French), which in a hive (natural or artificial) is the only one; all bees in a hive live to serve her, as she is dependent on her subjects. The hive is also dependant on the queen for survival. Her ability to give orders to the drones and workers ensures that, the hive is a properly function machine. She as well, ensures the survival and growth of the hive by continuously laying eggs.

The last class is the class of males, called: drone (faux bourdons in French) which makes up around 10% of the population in the hive. For long the sole reason for the existence of the drone has been that it has to mate with the queen, sadly as time has evolved, little to no discoveries have been made to prove that drones have other roles to play in a hive.

So with all this in mind, it is important to understand the biology of the bees.

The importance of breeding techniques and how science can help contribute to the betterment of the species and at the same time the economic benefits that such practice can bring to one. The aim of our work is to show our understanding of bees, appreciate its very diverse biology and behavior, and to show our understanding of the reproduction as influenced by science for the benefit of the species and to increase performance standards for our benefit

1.2. ECOSYSTEM ROLES

Honeybees are very important pollinators for many plants. Without honeybees, these plants have greatly reduced fertility. In North America and Australia, where there are no native bees species with large colonies, honeybees can have especially strong effects on native flowers, and on other pollinators such as solitary bee species. Honeybees' ability to recruit fellow workers by "dancing" allows them to be more efficient than other pollinators at exploiting patches of flowers. This can create strong impacts on their competitors, especially solitary bees.

Like all social insects, honeybees are hosts to a variety of parasites, commensal organisms, and pathogenic microbes. Some of these can be serious problems for apiculture, and have been studied intensively. At least 18 types of viruses have been found to cause disease in bees, including Sac brood disease. Several of them (but not *sacbroodvirus*) are associated with parasitic mites. Bacteria infect bees, notably Bacillus larvae, agent of American Foulbrood disease, and *Melissococcus pluton*, agent of European Foulbrood. Fungi grow in bee hives, and *Ascosphaeraapis* can cause Chalkbrood disease. One of the most common diseases in domesticated hives is Nosema disease, caused by a protozoan, *Nosemaapis*. An amoeba, *Malphigamoebamellificae*, also causes disease in honeybees.

In recent decades, two mite species have spread through domesticated and feral honeybee populations around the world. *Acarapiswoodi* is a small mite species that lives in the tracheae of adult bees and feeds on bee hemolymph. It was first discovered in Europe, but its origin is unknown. Infestations of these mites weaken bees, and in cold climates, whole colonies may fail when the bees are confined in the hive during the winter. A much worse threat is *Varroa destructor*. This might evolve on an Asian honeybee, *Apis cerana*, but switched on to *Apis mellifera* colonies that were set up in East Asia. It has since spread all around the world, except Australia. Juvenile mites feed on bee larvae and pupae, and adult female mites feed and disperse on adult workers. This mite is known to spread several viruses as well. Infestations of *V. destructor* often wipe out colonies. Nearly all the feral, untended honeybee colonies in North American are believed to have been wiped out by mite infestations, along with a large proportion of domesticated colonies. Other mite species are known from honeybee colonies, but they are not considered harmful.

Another commensal or parasitic species is *Braulacoeca*, the bee louse. Despite the common name, this is actually a wingless fly, which apparently feeds by intercepting food being transferred from one bee to another.

Beetles in the genera *Hylostoma* and *Aethina* are found in African honeybee nests, where they seem to do little harm. However, the "small hive beetle", *Aethinatumida*, has become a significant problem in European and North American hives. The larvae eat all the contents of comb: honey, pollen, and bee eggs and larvae. **(Adjare, 1990; Roubik, 1989; Sammataro and Avitabile, 1998)**

1.3. ECONOMIC IMPORTANCE FOR HUMANS: POSITIVE

Honey bees pollinate billions of US dollars worth of commercial agricultural crops around the world every year. They are important pollinators for economically important wild plant populations as well.

Honeybee hives provide honey and wax, and pollen, propolis, and royal jelly that are sold for medicines and cosmetics.

Honeybees are important study organisms for research in the connections between nervous system structure and behavior.

Some research suggests that honeybee venom may have medically useful applications in the treatment of auto-immune disease or inflammation. **(Adjare, 1990; Kang, et al., 2002; Sammataro and Avitabile, 1998)**

1.4. ECONOMIC IMPORTANCE FOR HUMANS: NEGATIVE

Honey bee workers will sting humans and domesticated animals in defense of themselves or their hive. A single sting is painful but not dangerous unless the target is allergic to the venom, in which case it can be life threatening. Otherwise, it takes about 20 stings per kilogram of body weight to be life threatening.

Each subspecies of *Apis mellifera* has different behavioral patterns in regards to intruders near or around the hive. The African subspecies are particularly aggressive. One of them, *Apis mellifera scutellata*, was accidentally released in South America, and has spread north to the southern United States. This is the "killer bee." It is notable for having a much higher aggressive response to disturbance; more workers attack than in other subspecies, and they pursue targets much longer than European bees do. The spread of these bees made beekeeping much more expensive and complicated, and the aggressive bees caused many deaths. **(Adjare, 1990; Sammataro and Avitabile, 1998)**

1.5. CONSERVATION STATUS

While the species as a whole is still very numerous, there is concern in Europe that widespread commercialization of beekeeping is endangering locally-adapted populations and subspecies. This, combined with higher mortality of colonies due to *Varroa* mite and tracheal mite infestations, and the recent phenomenon of Colony Collapse Disorder in North America, has cause significant concern for the health of the population. Colony Collapse Disorder (CCD) is a condition of commercial beehives, where there are sudden massive waves of mortality among the workers. Beekeepers discover their hives simply empty of workers, with so few surviving that they cannot tend the queen and brood. This condition has occurred mainly in North America, and mainly in large commercial apiaries. No single cause has been identified yet. **(Adjare, 1990; Sammataro and Avitabile, 1998)**

1.6. GEOGRAPHICAL DISTRIBUTION

Apis mellifera is native to Europe, western Asia, and Africa. Human introduction of *Apis mellifera* to other continents started in the 17th century, and now they are found all around the world, including East Asia, Australia and North and South America. **(Sammataro and Avitabile, 1998; Winston, et al., 1981)**

A number of subspecies of the Western honeybee come to mind when looking at the African continent, starting off with a subspecies *A.m.scutellata* **(lepeletier, 1836)** commonly referred to as the African honeybee or (killer bees), this subspecies is native to central and southern Africa ,and from South Africa ,the Cape honey bee as it often referred *A.m.capensis* **(escholtz, 1821).**

And here in Algeria two subspecies are prominent, first of we have *A.m.intermissa* **(Buttel-Reepen, 1906)** so often referred to as abeille itallienne,this subspecies is highly adapted to dry climates ad also found east Libya and west of Morocco , it is has a black-brown and orange striated abdomen, or can be recognized by black-brown thorax with orange fur.

Then the second subspecies native to Algeria is *A.m.sahariensis* **(Baldensperger, 1924)** which is closely related to the abeille itallienne . This subspecies is native to the desert oasis habitats, and also adapted to the date palm **(***phoenix dactylifera***)** and other Sahara flora.

1.7. GENERAL BIOLOGY OF *APIS MELLIFERA*

1.7.1. BEE

Found globally, bees are winged insects of the order *Hymenoptera*, super-family *Apoidea* , closely related to wasps and ants ,monophyletic in nature(descedants of a common ancestor).

There are nearly 20,000 known species of bees in seven recognized biological families. They are found on every continent except Antarctica, in every habitat on the planet that contains insect-pollinated flowering plants.

Megachilepluto, the largest of these creatures, is reported to be 3.9 cm long, while *Perdita minima*, the most diminutive of bees, are shorter than 2 mm long. Bees can be black or brown with red, yellow or lustrous blue stripes.

1.7.2TAXONOMY

Kingdom :*Animalia*

Phylum: *Arthropoda*

Class: *Insecta*

Order: *Hymenoptera*

Sub-order: *Apocrita*

Superfamily: *Apoidea*

Subfamily: *Apinae*

Genus: *Apis Linnaeus 1758*

Species: *Apis mellifera Linnaeus (*honeybee; abeiile domestique)

Subspecies: *Apis mellifera intermissa,*

Apis mellifera sahariens,

Apis mellifera scutellata

1.7.3. ANATOMY

The anatomy of the bee has a stunning efficiency. With honeybee anatomy, every element has a clear, well-defined purpose, to the point of being fine-tuned for the differences in roles between the worker, drone and queen bees.

As a member of the insect class (Insecta), honey bees share with other insects the following characteristics.

Honey bees are segmented in nearly all their body parts: three segments of thorax, six visible segments of abdomen (the other three are modified into the sting, legs and antenna is also segmented. Honey bees have an exoskeleton, which is rigid and covered with layers of wax, but have no internal bones like vertebrates do.

The main component of exoskeleton is chitin which is a polymer of glucose and can support a lot of weight with very little material. The wax layers protect bees from desiccation (losing water). The advantage of chitin-containing exoskeleton also prevents bees from growing continually; instead, they must shed their skins periodically during larval stages, and stay the same size during the adult stage.

Bees also have an open circulatory system, meaning that they do not have veins or arteries, but rather all their internal organ are bathed in a liquid called 'hemolymph' (a mix of blood and lymphatic fluid).

Bees breathe through a complex structure of network of tracheas and air sacs. Oxygen is vacuumed into the body through openings on each segment (spiracles) by the expansion of the air sacs, then the spiracles are closed and air sacs are compressed to force the air into smaller tracheas, which become smaller and smaller until individual tubules reach individual cells. In the following I will discuss the important structures on and inside the honey bee body.

1.7.3.1. HEAD SECTION OF THE HONEY BEE

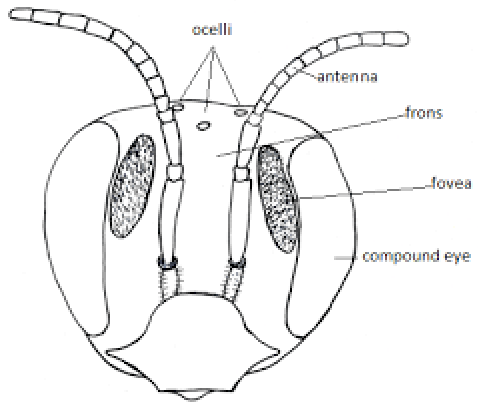

Figure 1: HEAD SECTION OF A HONEY BEE AS ILLUSTRATED IN HUTCHINGS BEE SERVICE WEBSITE

The head is the center of information gathering. It is here that the visual, gustatory and olfactory inputs are received and processed. Of course, food is also input from here.(Figure 1)

Important organs on or inside the head:

1. Antennae,

2. Eyes,

3. Mouth parts,

4. Internal structures.

1.7.3.1.1. ANTENNAE

The antennae on the head of the honey bee form a sensory power house, providing a function for a bee's sense of touch, smell, taste and even a unique form of hearing.

On the knowledge that honey bees live inside tree cavities (natural) or hives (man-made), both of which have little light away from the entrance. Smell and touch therefore are much important for them than visual when inside the colony.

Curiously in males have 13 segments making up each antenna, while females have 12. In both cases, there is an elbow-like "joint" along the antenna house thousands of sensory organs, some are specialized for touch (mechanoreceptors), some for smell (odor receptors), and others for taste (gustatory receptors).

It used to be thought that honey bees couldn't hear any airborne sound because they do not have pressure-sensitive hearing organs (like our ear-drums or similar structures on the legs of katydids).

Let's keep in mind that what we consider sound is merely vibration at frequencies we happen to detect with our ears. There is no rule of nature dictating the "vibration detection apparatus» that we happen to call ears, have to be attached to the side of the head! If a creature has a way to detect vibrations, through any mechanism, it can "hear". The mechanoreceptors on the bee's antennae respond to the movement of air particles, at frequencies associated with sound. So, through a different principle to our own ears - and because bees would look somewhat silly with ears - bees are, in fact, able to detect sound! This discovery helped the construction of robot bees that can be directed to dance (by a computer) inside a hive and guide workers to a specific location.

. Antennae also feature odor receptors, though they are not the only part of a bee's anatomy that can detect odors. In the antennae alone, bees pack a whopping 170 odor receptors, giving them an extremely well-evolved sense of smell. Bees also use their antennae to communicate with other bees, through touch. Interestingly, honeybees rely primarily on the right antennae to communicate. The favoritism shown toward the right antennae is a mystery to scientists. Studies have been conducted that prove honey bees do not function as well when forced to use only their left antennae. The proclivity to use their right antennae is akin to a human being right- or left-handed.

1.7.3.1.2. EYES

There are two types: a) compound eyes b) simple eyes

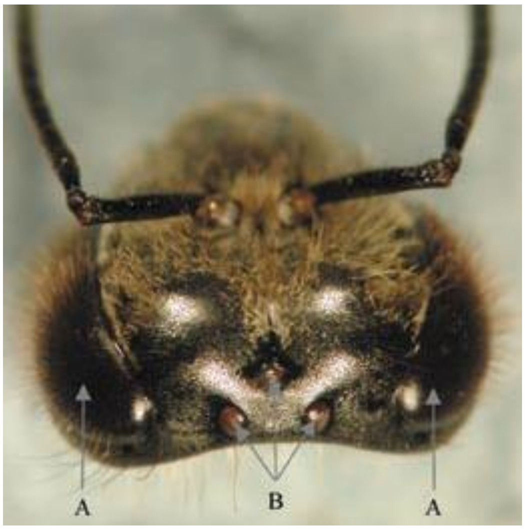

Figure 2: (A) COMPOUND EYES(B) SIMPLE EYES AS SHOWN IN ZACHARY HUANG DEPT. OF ENTOMOLOGY

a) Compound eyes

-Honey bees have two compound eyes that make a large part of the head surface. Each compound eye is composed of individual cells (ommatidium, plural ommatidia). Each ommatidium is composed of many cells, usually including light focusing elements (lens and cones), and light sensing cells (retinal cells). Workers have about 4,000-6,000 ommatidia but drones have more 7,000-8,600, presumably because drones need better visual ability during mating.(Figure 2A)

b) Simple eyes

- Bees also have three simple eyes that are called ocelli (singular: ocellus), near the top of their head (Figure 2B). Ocelli are simple eyes that do not focus but provide information about light intensity.

In conjunction with their compound eyes, the bee's UV polarized vision is the perfect tool for location of food sources.

1.7.3.1.3. MOUTHPARTS

Honey bees have a combined mouth parts than can both chew and suck (whereas grasshoppers can chew and moth can suck, but not both). This is accomplished by having both mandibles and a proboscis.

a) **Proboscis**

The proboscis is mainly used for sucking in liquids such as nectar, water and honey inside the hive, for exchanging food with other bees (trophallaxis), and also for removing water from nectar. The proboscis is another name for the tongue of a bee. It is like the human tongue, in that it is soft and can be extended. Relative to the size of the average honey bee, the proboscis is long, a result of evolution helping the bee to reach the center of a flower to collect nectar. The proboscis is also used to clean their hairs or to groom one another, especially the queen.

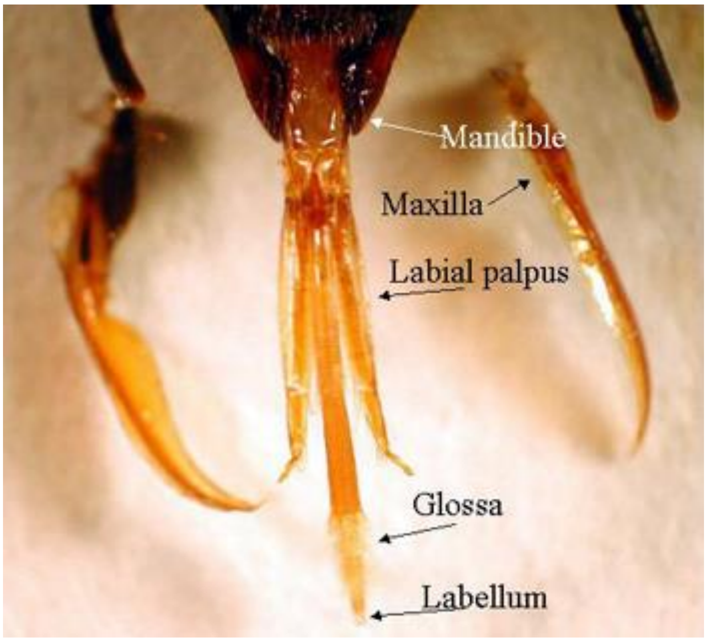

Figure 3: CLOSE CAPTION OF HONEY BEE MOUTH PARTS AS SHOWN IN ZACHARY HUANG DEPT. OF ENTOMOLOGY

b) **Mandibles**

The mandibles are the paired "teeth" that can be open and closed to chew wood, manipulate wax, cleaning other bees, and biting other workers or pests (mites).

1.2.3.1.4. INTERNAL ORGANS

The main internal organs in the head are the brain and sub esophageal ganglion, the main component of the nervous system, in addition to the ventral nerve cord that runs all the way through the thorax to the abdomen (Figure 4). Yes, the bee does have a brain, a pretty sophisticated one too.

The brain has a large area for receiving inputs from the two compound eyes, called optic lobes. The next largest inputs are from the antenna (antenna lobes). One important region in the middle of the brain is called the "mushroom body" because the cross section resembles two mushrooms. This area is known to be involved in olfactory learning and short term memory formation, and recently shown to be also important in long term memory formation in insects.

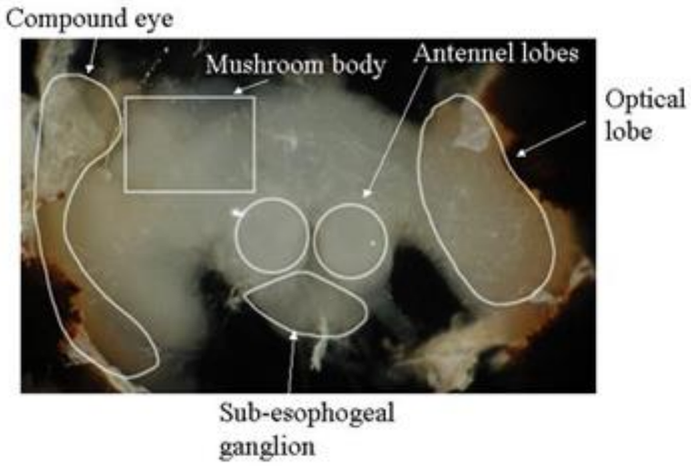

Figure 4: INTERNAL ORGANS AS SHOWN IN ZACHARY HUANG DEPT. OF ENTOMOLOGY

There are also endocrine organs attached to the nerve cords, very close to the esophagus (the food canal). One is called the corpora allata (CA, in Latin it means the body beside the food canal), which is the only source of one important hormone, juvenile hormone, which is involved in both the queen-worker differentiation, and also division of labor in workers.

The other one is called corpora cardiaca (CC, the body near the heart), which is a neurohemal organ and stores and releases another hormone (PTTH, prothoracicotropic hormone). PTTH can stimulate the production of ecdysteroids, by a gland located in the thorax, the prothoracic gland.

Lastly there are exocrine glands inside the head also, most notably the mandibular glands, the hypopharyngeal glands and the salivary glands. Mandibular gland is a simple sac-like structure attached to each of the mandibles. In the queen this is the source of the powerful queen pheromone. In young workers the gland produces a lipid-rich white substance that is mixed with the secretion of hypopharyngeal glands to make royal jelly or worker jelly and fed to the queen or other workers. In old workers (foragers) the gland also produces heptanone, a component of the alarm pheromone.

Similarly hypo pharyngeal glands produce protein-rich secretions when young (nurses), but produce invertase (an enzyme to break down sucrose into fructose and glucose) in foragers. The gland is consisted of a central duct (which is coiled between the front cuticle and the brain) with thousands of tiny grape-like spheres (acini, singular: acinus). The secretion flows to the mouth through the long duct. The glands are large in size (hypertrophied) in nurse bees but become generated in foragers.

There is also a pair of head salivary glands inside the head. The glands produce saliva which is mixed with wax scales to change the physical property of wax.

1.7.3.2. THORAX

The thorax is the center for locomotion and has three segments, each with a pair of spiracles for letting in air. Bees have 2 pairs of wings and three pairs of legs. The legs are very versatile, with claws on the last tarsomere, allowing bees to have good grip on rough surfaces (tree trunks etc), but also with a soft pad (arolium) to allow bees to walk on smooth surfaces (leaves or even glass!). There are also special structures on legs to help bee get more pollen.

Figure 5: THORAX REGION OF THE HONEY BEE AS SHOWN IN ZACHARY HUANG DEPT. OF ENTOMOLOGY

1.7.3.2.1. POLLEN BASKET

One is called the pollen basket (corbicula), located on the tibia of the hind leg, which is used for carrying pollen or propolis for foragers. It is a concave surface with hairs on the edges and a central long bristle that goes through the pollen pellet or propolis so the load would stay while bees are flying.

1.7.3.2.2. LEGS

On the front leg, there is a special structure used for cleaning antenna (when too many pollen grains stuck there), properly called the "antenna cleaner".

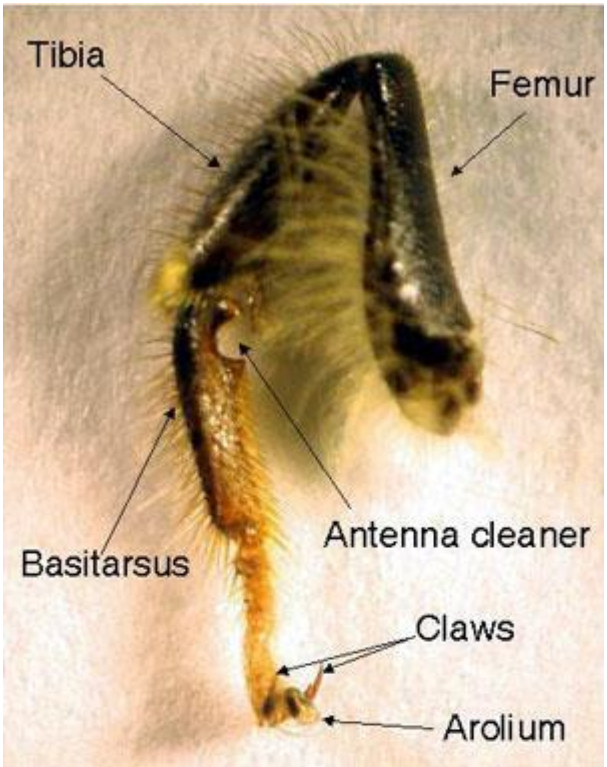

Figure 6: FRONT LEG OF THE HONEY BEE AS SHOWN IN ZACHARY HUANG DEPT. OF ENTOMOLOGY

1.2.3.2.3. WINGS

The front wings are larger than the hind wings and the two are synchronized in flight with a row of wing hooks (humuli, singular: humulus) on the hind wing that would hitch into a fold on the rear edge of the front wing (Figure 6).

The wings are powered by two sets of muscles inside the thorax, the longitudinal and verticle muscles. During flight, when the longitudinal mu scles contract, the thorax raises its height, so the wings are lowered because of fulcrum like structure (pleural plates) near the wing base. Conversely, when the vertical muscles contract, it shortens the height of thorax, raising the wings. The honey bee flight muscles can contract several times with one single nerve impulse, allowing it to at a faster rate.

1.7.3.3. ABDOMEN

Figure 7: DRONE, QUEEN AND WORKER BEE DISTINGUISHED BY SIZE AS SHOWN IN AGRITECH.TNAU

On the surface the abdomen has no special outside structures, but is the center for digestion and reproduction (for drones and queens). It also houses the sting, a powerful defense against us humans.

1) Wax scales
2) Digestive tract
3) Reproductive system
4) Sting

1.7.3.3.1. WAX SCALES

Workers around 6-12 days old can produce wax scales in their four pairs of wax glands (Figure 8). For worker bees, four pairs of wax-producing scales exist on the underside of the abdomen. The glands are concealed between the inter-segmental membranes, but the wax scales produced can be seen, usually even with naked eyes. The scales are thin and quite clear. After workers chew them up and add saliva, it becomes more whitish. Workers can create around 8 scales in a 12-hour period. Around 1,000 such scales must be created within the colony to make a single gram of wax.

Figure 8: WAX SCALE ON THE ABDOMEN OF HONEY BEES, RESPONSIBLE FOR PRODUCING WAX AS SHOWN ON HONEYBEEYSUITE.COM

1.7.3.3.2. DIGESTIVE TRACT

The digestive tract is rather typical for an insect/bird. The esophagus starts near the mouth, goes through an opening in the brain, through the thorax, and enlarges near the end to form the honey crop, which can expand to quite a large volume (nearly half of the abdomen in a successful forager). There is a special structure called proventriculus near the end of the crop. The proventriculus has sceleritized teeth-like structure, and also muscles and valves (Figure 9). These structures allow the workers to remove pollen grains in the nectar, and also stopping the backflow of food being digested into the crop, ensuring that the nectar is never contaminated. The contents of the crop can be spit back into cells, or feed to other workers, as is the case of nectar collected by foragers.

The ventriculus (midgut) is the functional stomach of bees and is the largest part of the intestine. Malpighian tubules are small strands of tubes attached near the end of ventriculus and functions as the kidney, it removes the nitrigen waste (in the form of uric acid, not as urea as in humans) from the hemolymph and the uric acid forms crystals and is mixed with other solid wastes.

The rectum is also quite expandable (just like the crop, and both are extoderma structures, and both are lined with a chitin layer, enabling workers to refrain of defecation for up tomonths (for example bees might have their last defecation flights in November, and then again in March the next year).

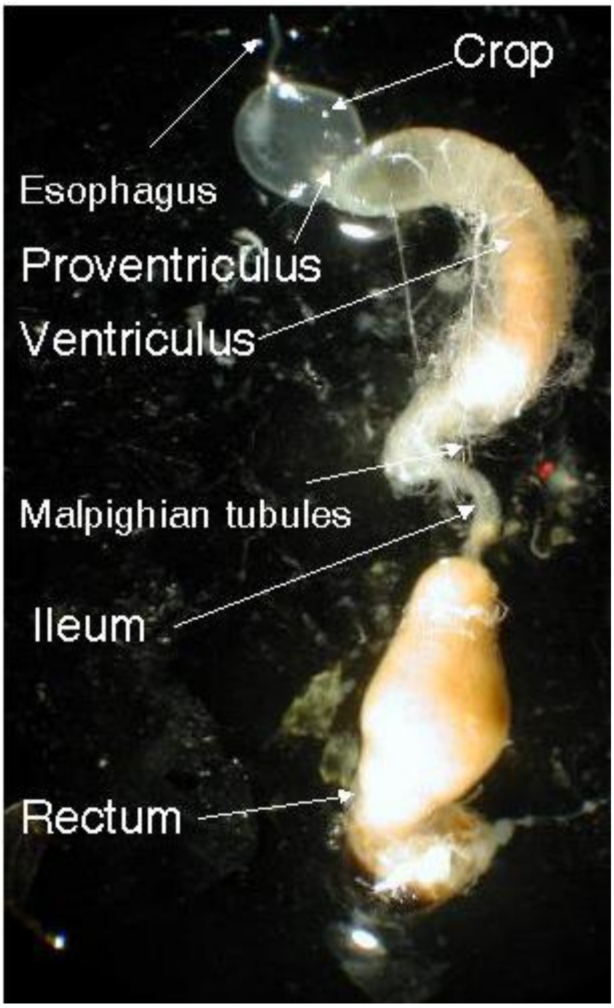

Figure 9: THE DIGESTIVE TRACT OF THE HONEY BEE AS SHOWN ON PINTEREST IMAGES

1.7.3.3.3. REPRODUCTIVE SYSTEM

Important structures in the honey bee reproductive system include the ovary and spermatheca. In the ovary of a laying queen, there are ovarioles, with mature eggs appearing yellowish. Egg cells move down the tube of ovarioles as they become larger and more mature, eventually reaching the oviduct and being laid out by the queen.

The spermatheca contains the sperm from the queen’s single mating flight during a one week window around the age of 6-16 days. She will use this sperm to fertilize all the eggs produced in her lifetime. The sperm inside can live up to 4 years.

The spermatheca is covered with a rich network of trachea. Once removed, the spermatheca is a shiny, perfectly spherical organ. In un-mated queens, the spermatheca will be clear. Queen breeders learning to use artificial insemination will sometimes check the spermetheca color in a sample of inseminated queens to see if their technique is working.

For the drone, his sexual organ is a "use once" device! After the drone mates his sexual organs are ripped from him, causing his death.Another curiosity is that his ejaculation is so explosive that it can be heard by the human ear.

Figure 10: FULLY EVERTED ENDOPHALLUS OF THE DRONE AS SEEN ON WIIKIPEDIA PAGE ON DRONE ANATOMY

1.7.3.3.4. STING

Of all components of the anatomy of a bee, the stinger is the one that the layman considers first! The stinger is the honey bees only true line of defense. Honey bees will sting only as a last resort when threatened because once they have used their stinger they typically die.

The stinger differs across worker, queen and drone as follows:

-Worker: The stinger is barbed, and once inserted into human skin it will be torn away as the bee struggles to free itself. This usually results in the death of the worker. The sting has barbs preventing the sting to be pulled out. The sting apparatus breaks off and is left behind. The sting, venom gland, and muscles controlling the gland, will work autonomously to pump venom into the victim. Alarm pheromone is also released to "mark" the victim. This sends a signal to other bees to sting you again.

-Queen: A queen's stinger has no barb and she can therefore sting repeatedly without losing it. Note, however, that stings by queen bees are quite rare.

-Drone: Nothing to worry about with drones - they have no stinger!

1.7.4. LIFE CYCLE

The life cycle of a honey bee is perennial. Each colony contains three adult castes: egg-laying queens, sperm-producing male drones and non reproductive female workers.

Figure 11: DIFFERENT STAGES OF A HONEY BEE LIFE CYCLE AS ILLUSTRATED BY JENNIFER SARTELL

1.7.4.1. EGG

A quick reminder, the queen spends her life, after her mating flights, laying eggs. She lays around 2,000 per day. The deciding factor as to whether an egg becomes a male or female is based on whether she fertilizes the egg. If she does so, she has laid an egg destined to be a worker or a queen i.e. a female. Otherwise the egg is destined to be a male bee - a drone. The life cycle of honey bees begins when an egg hatches.

1.7.4.2. LARVAE

During the first stage of its development, the offspring form a digestive system, nervous system and outer covering. The distinction between the two female castes - worker or queen - is made about three days after the transition to larvae i.e. about 6 days after the egg is laid (Figure 11). All castes are fed royal jelly in their first three days as larvae. That diet is then stopped for both workers and drones.

For queens, however, the royal (jelly) treatment is continued. Interestingly, the effect of this is not to "promote" queen-like characteristics as we might imagine, but rather to inhibit the development of worker characteristics. Before the transition to pupa, worker bees will use wax to cap the cell containing the larvae, where it will remain until it emerges as an adult bee.

*1.*7.4.3. PUPA

As the pupa stage is reached and progresses, the bee within the capped cell starts taking a more recognizable bee form. Features such as eyes, legs and wings all develop and small hairs cover the bees body.

1.7.4.4. ADULT

Finally, at the appropriate time, the bee will chew its way out from the capped cell. As soon as s/he is free and moving away from the cell, worker bees will clean up the cell and prepare it for the next egg.

1.7.4.5. DIFFERENCIES BETWEEN CASTES

Despite these commonalities, there are distinct differences between the drone, worker and queen bees as they move towards adulthood. Let's look at these in detail.

With the notable exception of whether the egg is fertilized, an egg is an egg is an egg! All three castes start life as a newly laid egg in a cell and this initial state lasts for three days. Then differences become evident.

Note: The numbers below are typical but can vary based on many conditions and factors.(different authors)

After the egg has hatched, each caste spends a different amount of time as larvae.

- **Queens** are in the larva state up **for 5 1/2 days** (till day 8 after being laid as an egg)
- **Workers** are larvae up to **for 6 days** (till day 9 from being laid)
- **Drones** spends the longest time as larvae, **for 6 1/2 days** (till 9 1/2 days from being laid)

The duration of the pupa stage is as follows:

- **Queens** are in the pupa stage **for 8 days**
- **Workers** are in the pupa stage **for 12 days**
- **Drones** are in the pupa stage **for 14 1/2 days**

Add these numbers up and the time from the egg being laid to emergence from the cell as an adult bee is as follows (these are averages and variations do occur):

- **Queen:** 16 days
- **Worker:** 21 days

- **Drones:** 24 days

Curiously, the time when cells are capped by worker bees differs between castes.

- **Queen** cells are capped about a day or so **before** the transition to pupa, about 7 1/2 days after the egg was laid

- **Worker** cells are capped around **the same time** as the transition occurs, at about 9 days

- **Drones** cells are capped a little **after** the transition to pupa has occurred, at about 10 days

TABLE 1: SHOWING *NUMER OF DAYS AT EACH STAGE OF DEVEOPMENT FOR EACH CASTE*

	QUEEN	WORKER	DRONE
EGG	3	3	3
LARVA	8	6	10
NYMPHE	4	12	11
TOTAL	**16**	**21**	**24**

1.7.4.6. LIFE EXPECTANCY

The overall life expectancy of each caste also differs considerably. Indeed, for workers alone there may be wide differences in life expectancy depending on the time of year.

1.7.4.6.1. QUEEN

One of the most important factors effecting the queen's survival is the viability of the colony through the winter months. The winter cluster of worker bees will protect the queen and regulate her temperature, even in the most extreme of conditions outside the hive. However, their success in surviving till the spring depends on a wide range of factors, not least of which is availability of resources (honey) within the hive.

1.7.4.6.2. WORKER

The expected lifetime of the worker bee also has some complications. In the summertime, workers bees will effectively work themselves to death, carrying out a large number of roles. If born any time from spring to late fall, workers will typically live around 6 weeks.

However, if a worker is born towards the end of fall or early winter, her role is quite different. These are the so-called winter bees and they are charged with helping the queen survive the cold months.

Over this period the worker bee doesn't have the strenuous obligation - or even the option - to forage. For this reason, a worker bee can live up to 4-5 months through the winter.

1.7.4.6.3. DRONE

Drones get the short straw! As something of a simplification, as earlier stated drones main purpose of existence is for reproduction purposes. They live for the chance to mate with a queen. The very act of mating is their last act and they will die after pulling away from the queen. If they don't mate, they will generally live around 5-7 weeks.

Aside from the important and essential benefit of genetic diversity, drones really add little value to the colony. They don't forage or help with the production of honey in any way. But they do consume resources.

Workers are accepting of this during the summer, when such resources are abundant. But the idea of a drone being part of the winter cluster is offensive to workers - drones haven't contributed, so why allow them to stick around through the winter?

Thus at the end of swarming season, autumn before the hive winters, most drones are evicted by worker bees to wither and die.

1.7.5 BEHAVIOUR

Honeybees are eusocial insects. They live in colonies that contain one reproductive female (the queen) and her offspring. Sterile female offspring of the queen (the workers) perform all the work of the colony and are by far the most numerous castes in the hive.

Males and queens spend all their effort on reproduction; research and experimentation has not yet managed to identify other behavioral characteristics f the male.

A. mellifera workers show what is called "age polyethism." Their behaviors change as they get older. Newly emerged worker bees’ clean cells, preparing them for a new egg or for food storage.

After a few days they shift to other hive maintenance work, removing waste and debris, fanning to maintain air circulation and temperature, processing nectar brought by foragers, and feeding the queen and larvae from glands in their head and body. In their second week of adult life workers' wax glands become active and they help build and repair the comb, while continuing to tend the queen and feed workers. Apis mellifera workers build a "comb", a sheet of hexagonal cells made of waxes they secrete. Each cell can house one larval bee, and cells are also used as protected storage space for honey (processed nectar) and pollen.

Between 12 and 25 days, workers take a turn guarding the hive, inspecting any bees that try to enter the hive - driving off strangers and attacking any other creatures that try to enter. After about three weeks, the workers food and wax glands atrophy, and they shift to foraging duty.

Foraging only occurs during daylight, but bees are active in the hive continuously.

In temperate climates, colonies store honey and pollen to feed on during the winter. During cold temperatures the workers and queen form a tight ball or cluster, working their flight muscles to generate heat and keep themselves warm. In warmer tropical regions, honeybees maintain smaller stores of food.

If a colony's nest conditions become too poor, the entire colony may move to a new site. This is particularly common in tropical honeybees that move in response to seasonal drought. Beekeepers call this "absconding", and work to prevent it in domesticated colonies.

Swarming is a behavior in a nest where a new queen is born that takes the place of the older one in that hive. The departing queen normally takes some of the workers with her. Swarming bees send out worker scouts to look for a suitable home to take the place of the one they left. The swarm of bees is just temporary. They normally swarm over a twig or branch of a tree or anywhere that can be used temporarily as an intermediate nest. **(Adjare, 1990; Sammataro and Avitabile, 1998)**

Honeybees forage as close to the hive as possible, usually within a 3 kilometer radius around the hive (i.e. an area of about about 2800 hectares). If necessary, they can fly as far as 8-13 km to reach food or water. **(Percival, 1947; Sammataro and Avitabile, 1998)**

1.7.6 COMMUNICATION AND PERCEPTION

Apis mellifera communication is based on chemical signals, and most of their communication and perception behaviors are centered on scent and taste. The members of the hive colony are bound chemically to each other. Each hive has a unique chemical signature that hive mates use to recognize each other and detect bees from other colonies.

Within the hive, bees are in constant chemical communication with each other. Workers feed and groom each other, as well as larvae, drones, and the queen. In the process they pass on pheromones, chemical signals that indicate information about the health of the queen and the state of the colony.

Chemicals not only help with detecting the right signature of hives but also with foraging. Honeybees use scent to locate flowers from a distance. When a successful forager returns to the hive, it passes the scent of the flowers to its nest mates, to help them find the same patch of flowers.

Bees also use chemicals to signal outside the hive. When a worker stings something, her stinger releases an alarm pheromone that causes other bees to become agitated, and helps them locate the enemy.

Thought it's always dark in the hive, vision is important to honeybees outside. They can see other animals, and recognize flowers. The eyes of Apis species can detect ultraviolet light wavelengths that are beyond the visible spectrum. This allows them to locate the sun on cloudy days, and see markings on flowers that are only visible in ultraviolet light. One portion of honeybee's eyes is sensitive to polarized light, and they use this to navigate.

Workers and queens can hear vibrations. New queens call to each other and workers when they first emerge. Workers hear the vibrations of the waggle dances made by returning foragers.

Apis species have a particularly notable form of communication called "dancing." Foragers that have located an abundant supply of food do a dance to communicate the location of the patch to other foragers. A "round dance" indicates food within about 300 meters of the hive, and only communicates the presence of the flowers, not the direction, though workers will also get the scent from the food the forager has brought back. The more complicated "waggle dance" indicates the direction and distance of food further away, using the location of the sun and the bee's memory of the distance it flew to return to the hive. Symbolic communication is quite unusual among invertebrates, and these honeybee "dances" have been intensively studied. **(Breed, et al., 1985; Milne and Milne, 2000; Reinhard, et al., 2004; Roat and Landim, 2008; Sammataro and Avitabile, 1998; Sandoz, et al., 2002; Sherman and Visscher, 2002)**

CHAPTER 2.REPRODUCTION: A QUALITATIVE & QUANTIATIVE OVERVIEW

2.1 REPRODUCTION IN HONEY BEES

The importance of the role of bees as pollinators should never be taken for granted, never more so than right now when the human race continues to strain the environment with our inherent demands. Therefore it is imperative that honey bees reproduce on a grand scale.

2.1.1 REPRODUCTIVE STYEMS

<u>Worker bee</u>

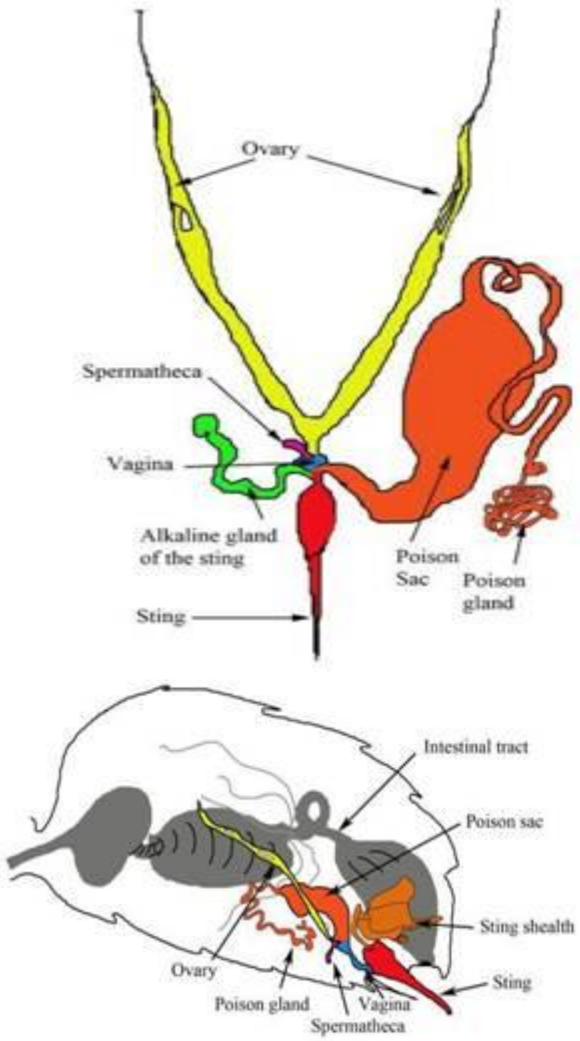

Figure 12: REPRODUCTIVE TRACT OF THE WORKER BEE, AS SHOWN IN CARRSCOUNSTILNG.COM IMAGES

Each worker develops a sting as well as a sting gland. Note the stinger has two components, the poison gland (filled with colorless liquid when fresh) and the alkaline gland (which may appear yellow) together with the stinger. Each sting contains 150 mg of venom.

Note bees, unlike wasps, die after using the stinger as the organ is left in the victim.

The queen bee

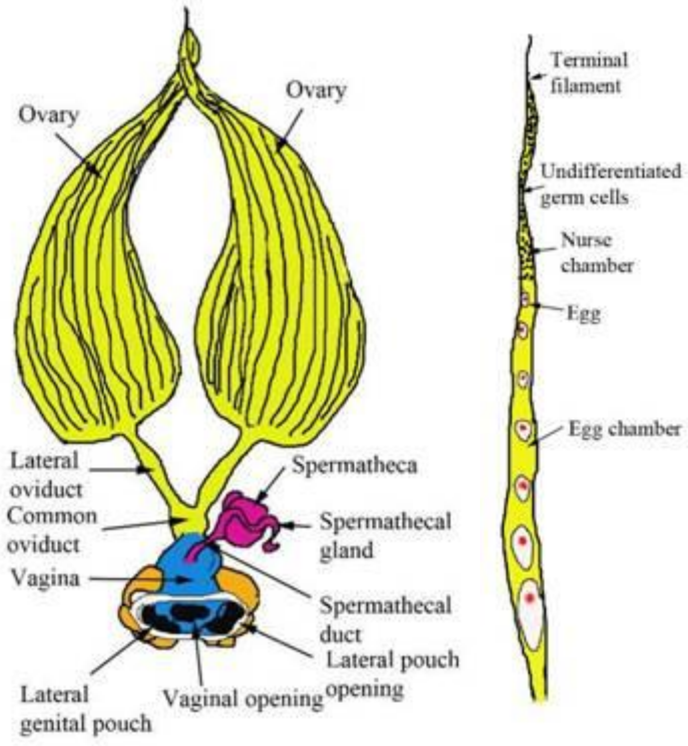

Figure 13(A) REPRODUCTIVE TRACT OF THE QUEEN, (B) OVIDUCTS AS SHOWN IN CARRSCOUNSTILNG.COM IMAGES

During the mating flight the queen the drone's penis bulb is discharged by eversion of the penis into the queen's vaginal pouch.

After mating the queen separates from the drone and the male penis bulb remains in the female. The organ is torn from the male at the penis neck. The queen may mate with several drones during the flight. The spermatozoa are discharged in the distended lateral oviducts (Figure 13B).

Once back at the hive, the workers remove the penis bulb from the queen. The spermatozoa are then moved into the vagina and then the spermatheca gland where they remain for the productive life of the queen – up to 5 years.

If the queen "runs out of semen" she will only lay unfertilized drone eggs.

The drone bee

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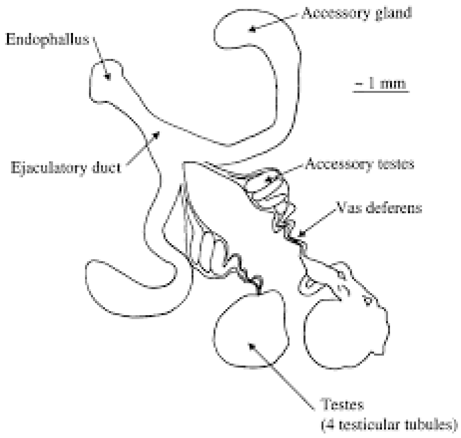

Figure 14: REPRODUCTIVE TRACT OF THE DRONE AS SHOWN IN CARRSCOUNSTILNG.COM IMAGES

During mating, sperm mass stored in the bulb of penis is discharged by eversion into the queen's vagina. After mating the queen separates from the male with the bulb of penis remaining in her genital tract. The male reproductive organs tear at the penis neck. The drone subsequently bleeds to death.

2.1.2 REPRODUCTION OF BEES ACROSS THE SPECIES

Before we look at honey bee reproduction, it is worthwhile considering that other varieties of bees reproduce entirely differently.

***Bumble Bees.** The male bumble bee is aggressive in his mating practices. **He knocks the queen to the ground to climb on her thorax, after which mating occurs.**

***Carpenter Bees**. The carpenter bee mating process usually begins with a dance. **12 males will join a group of 3-4 female carpenter bees and begin doing a 'bobbing' dance.** The females will take flight because carpenter bees must be in the air to mate. A male will then try to climb on her back, with the goal of getting his abdomen underneath hers for mating to occur.

***Sweat Bees**. Sweat bee mating is like a honey bee mating, but with a few differences. Male sweat bees are not particular as to whether a female sweat bee has previously mated. Additionally, **there is no mating flight**. Female sweat bees mate and lay eggs when needed to ensure the survival of the colony.

***Africanized Honey Bees**. The mating ritual of the Africanized honey bees is another very interesting phenomenon. Instead of taking a mating flight, Africanized bees synchronize their flight. **At a certain time of the year, the bees will all take off to find new homes**. When they do this, they mix with other hives flying, break apart and find their new homes. This mixing period is when mating occurs, and this also helps to reduce inbreeding within the hive.

2.1.3. THE MECHANICS OF MATING

By the time that adult drones emerge from pupation spermatogenesis and spermiogenesis are complete **(Bishop, 1920; Hoage&Kessel, 1968)**. During the first week of adult life, the sperm migrate from the testes to the seminal vesicles **(Snodgrass, 1963)** where they undergo the final stages of maturation.**(Figure 15)** shows the dramatic difference in size of the testes of immature **(Figure 15a)** and mature **(Figure 15b)** drones. Also note that the seminal vesicles and the mucus glands become filled in the mature drone. The epithelial cells lining the seminal vesicles secrete a small amount of seminal fluid during the maturation, making up about half of the total semen volume **(Verma&Shuel, 1973)**. At the same time mucus is being produced and stored in the mucus glands.

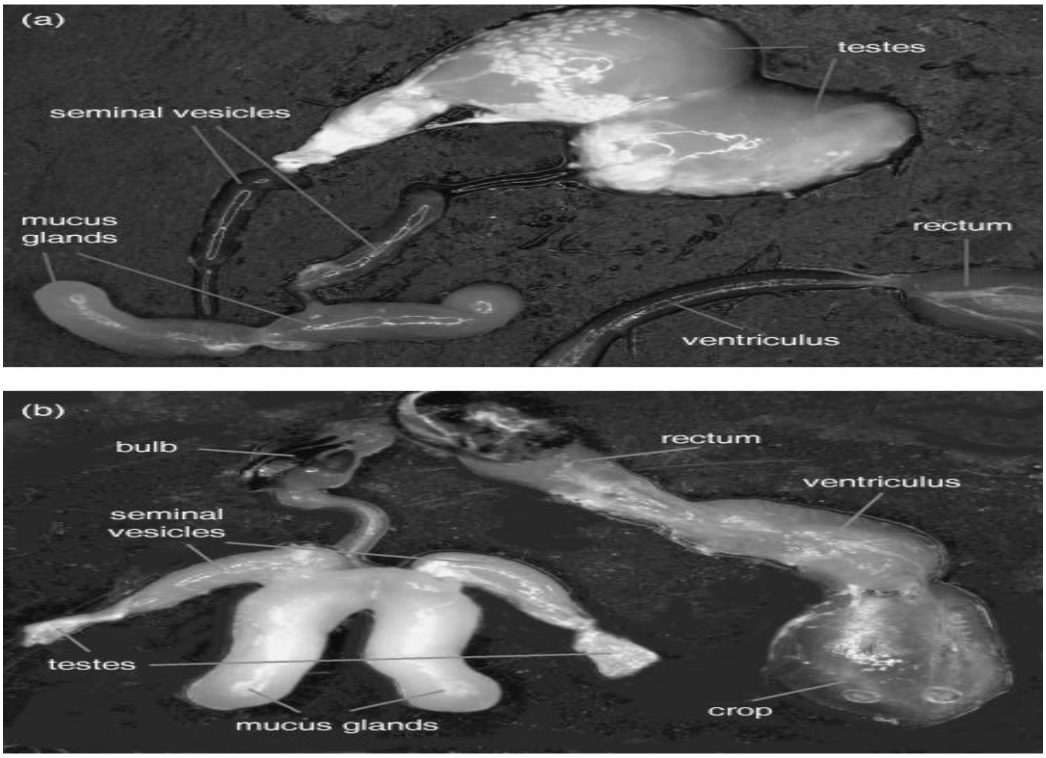

Figure 15: DIFFERENCE IN THE REPRODUCTIVE TRACT BETWEEN A SEXUALLY IMMATURE DRONE AND A MATURE ONE AS SHOWN IN IMAGES OF THE OPEN-i

At the time of mating, muscles in the abdomen of the drone contract creating pressure that everts the genitalia into the queen, turning the reproductive system inside out **(Koeniger, 1986).** The muscular contractions of the seminal vesicles aid in the ejaculation of the semen into the queen's vaginal passage and on to the median and lateral oviducts. The products of the male mucus gland **(Colonello&Hartfelder, 2003)** follow the semen and harden on contact to air **(Bishop, 1920; AC and VW, personal observation)**. Immediately after eversion, the male is paralyzed and falls away from the queen. The mucus plus the endophallus and cornua portions of the male genitalia, which are left behind, become the mating sign, visible outside of the queen's abdomen. From comparisons of mating behavior in several species of *Apis*(**Koeniger, 1990**; **Koeniger&Koeniger, 2000)** it appears that the mucus and genital structures hold the drone in place during sperm transfer and the mating sign becomes a signal to other males following the queen. **Koeniger (1986)** proposed that the mucus may also serve to hold the queen's sting out of the way of the next mate.

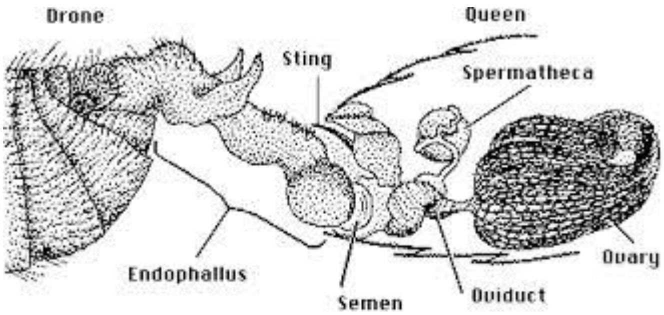

Figure 16: ILLUSTRATION OF EVENTS DURING A MATING FLIGHT BETWEEN A QUEEN AND A DRONE AS SHOWN IN PINTEREST IMAGES

The honey bee queen makes one to four flights **(Roberts, 1944)** away from the colony when she is about a week old, mating with from seven to 44 males in rapid succession **(Taber, 1954; Moritz et al., 1996; Neumann et al., 1999; Tarpy& Page, 2001).** Over the next 24–48 h, the sperm migrate to the spermatheca**(Ruttner et al., 1971)**. This movement is enabled by contractions in the queen's abdomen **(Koeniger, 1986)**, by sperm motility **(Collins, 2000a)** and the presence of spermathecal fluid **(Gessner&Ruttner, 1977)**. Only about 3–5% of the sperm are actually retained in the spermatheca**(Koeniger&Koeniger, 2000)**, the rest are lost from the queen.

Genetic studies using phenotypic markers have shown that sperm from all of the mated drones become randomly distributed within the spermatheca**(Page et al., 1984)** and relatively constant levels of all paternal types are represented in the worker offspring. Therefore there is little or no sperm competition, although different drones may contribute variable numbers of viable sperm **(Woyke&Jasinski, 1978; Collins, 2004).** The creation of colonies of multiple paternal lines of workers, or subfamilies, is evolutionarily desirable, as these colonies have the capability of responding readily to wide changes in the environment **(Jones et al., 2004)** and extreme polyandry increases the fitness of the queen by reducing the colony-level impact of her laying non-viable, diploid drone eggs **(Tarpy& Page, 2001)**.The queen uses only a few of these sperm at a time in order to fertilize eggs throughout her life. If a queen runs out of sperm in her lifetime, new generations of queens will mate and produce their own colonies.

2.1.4. DRONE CONGREGATION AREAS

Drones do not usually mate with a queen from their own hive. They fly to areas known as Drone Congregational Areas (DCAs) to seek other queens.

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Before take-off a drone cleans its antennae and eyes. This often occurs at the nest entrance. After take-off drones produce characteristic sound which is different from sound produced by flying workers. Drones perform flights not only from the nest but also from swarm cluster.

Scientists are still struggling to locate hard evidence why drones pick particular areas to congregate. It is possible that drones choose these areas based on magnetic force. When drones are older than 6 days they have a sudden increase in magnetite within their abdomen. This could literally pull them to certain locations.

Drones return to these congregational spots year-after-year. Often, they will visit multiple congregational spots in one day, each of which can hold a few hundred to a few thousand drones at a time. It is obvious when a place has been chosen as a drone congregational area because they create a buzzing sound almost like a swarm.

These congregational areas could have range as small as 30m or could be as large as 200m in diameter and 15- 40m above the ground. Drones are so focused on these areas that if a queen flies by outside of these boundaries they will completely ignore her. The drones give off a certain pheromone to draw queens to them.

2.1.4. DETERMINING THE SEXES

Honey bee queens control the sex of their offspring: as eggs pass through the ovary into the oviduct, a queen can determine whether a particular egg is fertilized or not. Unfertilized eggs become drone honey bees, while fertilized eggs develop into female workers and queens. Female workers do not mate, but they can lay infertile eggs, which in turn become male honey bees.

Queens lay their eggs in structural oval-shaped cells, which stick to the nest ceiling. Worker honey bees fill these cells with royal jelly to prevent larvae from falling. Soon-to-be workers are fed royal jelly during the first two days, while future queens are given royal jelly throughout the entire larval period. The development of each member of a colony differs depending on caste: male honey bees need 24 days for proper growth from eggs to adult, while workers need 21 days and queens require only 16.

> Worker bees are female and can lay eggs as well. But because they do not take a mating flight their eggs are unfertilized and they will only produce drones. The queen is the only bee who can lay both male and female bees.

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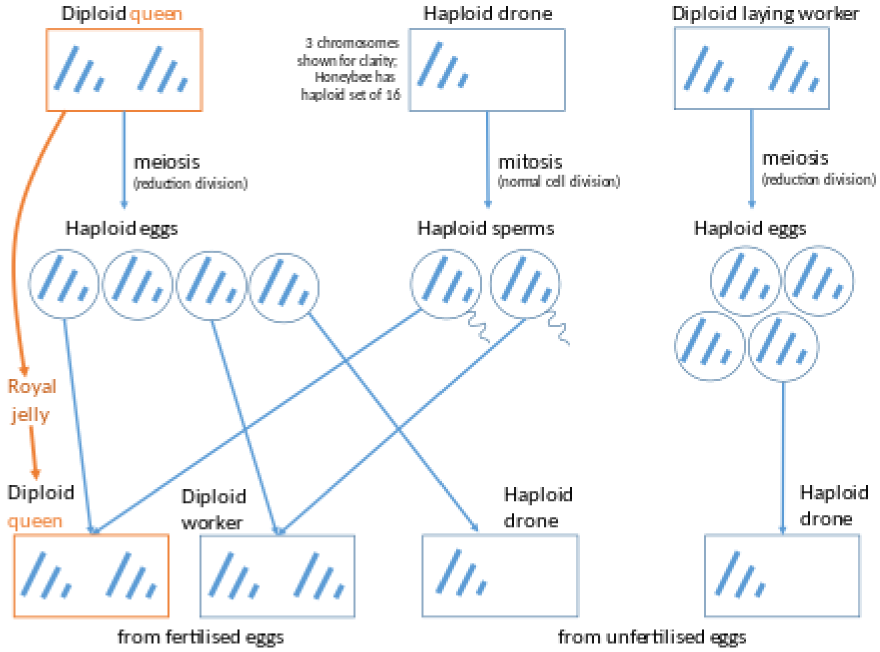

Figure 17: DETERMINATION OF SEXES IN HONEY BEES SHOWN WIKIPEDIA PAGE ON DRONES

So, drones have no real purpose beyond mating, essential though that is. Drones do mate with the queen, but they offer something so much more important than simply sperm.

They offer genetic diversity.

Female bees have 32 chromosomes. This means they get 16 chromosomes from their mother and 16 from their father. Drones only have 16 chromosomes. Therefore, eggs only have the option of holding half of the queen’s genetics. This is significant because genetics determine how efficiently and effectively a hive runs, as well as being a factor in resistance to disease.

When the queen mates with drones on her mating flight, she benefits from the genetic diversity they offer. Each egg that hatches will be slightly different, based on its genetics. Because of this the colony has a greater potential for success.

Drones have one role: to mate!! What happens if they don’t mate?

Drones only have a 1-in-1,000 chance of mating with a queen; so many drones will not mate and die from the process. Some drones actually survive the mating process.

So, what happens to the drones that don't die from mating or the ones that never get the chance? They still have a pretty grim future.

Drones that are not able to pass on their genetics - or in a freak turn of events, survive the mating process - are allowed to remain at their hive during the warm seasons. However, when winter approaches and bees must live only on what they have stored, drones become just one more mouth to feed.

At this point, worker bees will round them up and kick them out of the hive. Remember, drones do not have stingers which mean they have no defense system and are incapable of collecting food for them. This means they die from cold or starvation.

Though these tiny creatures have such a large impact on the reproduction of bees, their outlook is certainly on the bleak side when it comes to individual survival.

2.2. INFLUNCE OF BEE KEEPING ON REPRODUCTION

2.2.1. SWARMING

Healthy, well-fed honeybee colonies reproduce by "swarming." The workers in the colony begin by producing numerous queen larvae. Shortly before the new queens emerge, the resident, egg-laying queen leaves the hive, taking up to half the workers with her. This "swarm" forms a temporary group in a tree nearby, while workers scout for a suitable location for a new hive. Once they find one, the swarm moves into the space, and begins building comb and starting the process of food collection and reproduction again.

Meanwhile at the old hive, the new queens emerge from their cells. If the population of workers is large enough, and there are few queens emerging, then the first one or two may leave with "afterswarms" of workers. After the swarming is completed, any remaining new queens try to sting and kill each other, continuing to fight until all but one is dead. After her competition is removed, the surviving queen begins to lay eggs.

Normally the pheromones secreted by a healthy queen prevent workers from reproducing, but if a colony remains queen less for long, some workers will begin laying eggs. These eggs are unfertilized, and so develop as males. **(Adjare, 1990; Milne and Milne, 2000; Sammataro and Avitabile, 1998; Tarpy and Page Jr., 2000)**

2.2.2. QUEEN GRAFT

If the beekeeper does not want to delay the newly created colony and give him the maximum chance of success, he can quite possibly combine artificial swarming with the introduction of a virgin queen bought on the market. Thus, it frees itself from the period of breeding of the queens as well as from the lapse of time between hatching and nuptial flight, ie at least 3 weeks **(Tarpy et al, 2000)**

The introduction of queen can be carried out apart from the creation of an artificial swarm, to replace an old queen or a queen having generated an aggressive colony for example, it is anyway more prudent to introduce the queen in an excluder cage where the entrance is clogged with food to allow workers to get used to it for about 3 days, which is the time needed to allow free entrance **(Moore et al, 2015).**

2.3. EVALUATION OF REPRODUCTION AND OF DRONE SPERME

A newspaper headline: (**NO OFFENSE AMERICAN HONEYBEES YOUR SPERM IS NOT GOOD ENOUGH**) reads the article in one Washington newspaper in the usa, july 2007. So why the harsh words.

Well, According to the WSU research team, the root cause of the U.S. honeybees' vulnerability to varroa is dwindling gene pools that has left them short on genetic traits that help honeybees resist varroa elsewhere in the world. This is certainly not a problem only in the usa but the whole world, which is why it is necessary to collect sperm and evaluate it before in the end having to artificially inseminate.

2.3.1. COLLECTION OF SPERM

Sperm can be collected by one of two methods; directly from the seminal vesicles by form of dissection of the male abdominal region, **(Mackensen, 1955)**. Sperm collection directly from the seminal vesicles results in more viable sperm but it is not as convenient for artificial insemination, because it takes longer to perform, as well as significantly low quantity of sperm that is collected,**(Collins, 2004).**

The drone is dissected carefully with a pair of dissection scissors and two watchmaker forceps under a microscope. Intact seminal vesicles are taken out with the forceps and then washed with a drop of Kiev buffer to prevent semen from contamination by hemolymph. Seminal vesicles are placed in a sterile watch glass with Kiev buffer **(Moritz 1984)**.

Semen in each seminal vesicle is released by grasping its one end with one of the forceps while compressing it gently through the other end with one of the forceps in the Kiev buffer. Emptied seminal vesicles are discarded.

Semen collection can also be accomplished by manual eversion of the sexual organs as described by **(Woyke 2008).** This method has been used in a lot of studies assessing semen properties of drone semen, **(Collins and Donoghue 1999) (Nur et al,**

2012) (Rhode et al 2010). Also a commonly used method in instrumental insemination of honey queens, **(Mackensen and Tucker1970), (Harbo ,1985).**

An initial vertical pressure on the head of the drone with the thumb and index finger produces a partial eversion of the endophallus. Subsequent horizontal pressure from anterior to the posterior of the abdomen results in full eversion of the genitals.

In mature drones, semen is cream coloured and is found on the tip of the genitalia on a bed of white mucus. Semen is collected using Harbo large capacity syringe.

2.3.2. CONSERVATION OF SPERM

A queen bee can store sperm in its spermatheca for a period of up to five years , more or less, after its initial mating flight. It is a phenomenon which is highly revered in the science world.

Natural sperm storage mechanisms in honey bees (*Apis mellifera*) have an additional applied importance, because the exploitation of these mechanisms could help breeding processes and efforts to store and maintain genetically diverse lines **(Collins, 2000).**Despite these strong motivations, genetic mechanisms behind sperm storage in honey bees and other social insects remain unexplored.

Antioxidative enzymes can increase sperm longevity by reducing levels of damaging reactive oxygen species (ROS). ROS, including hydroxyl and hydroperoxyl radicals, as well as hydrogen peroxide, are produced by enzymatic and non-enzymatic reactions during biological processes **(Pardini, 1995)**, and are known to impact survivorship of sperm from diverse organisms **(Ball et al 2000; de Lamirande& Gagnon, 1992)**.

Because honey bee sperm are stored in an aerobic environment and retain some metabolic activity during storage **(Blum & Taber, 1965; Koeniger, 1986)**, they most likely face some level of oxidative stress throughout storage.

Antioxidative enzymes superoxide dismutase (SOD), catalase and glutathione S - transferase (GST) have been proposed to reduce oxidative risk during sperm storage in bees **(Weirich et al, 2002)** and catalase as an antioxidant **(Collins et Evans)**

To preserve the honeybee species from a possible extinction natural breeding is being supplemented by the rise in artificial insemination practices on honeybees. Valuable and varid genetic traits can now be preserved and passed on to a targeted colony or area for better resistance to diseases, better breed of worker bees, subsequently new stronger queens and colonies.

That leaves us with our main characters of the preservation process being; the amount of TIME, we can store the sperm, be it for a short period of time, for 24hr-48hr periods , to a few weeks or preservation for a long period of time , a year or perhaps more.

2.3.3. DILUTION AND DILUENTS

Sperm can survive for very long time in the spermatheca of a mated queen. Survival of sperm in the spermatheca is of great interest to us, we try to replicate the conditions that allow for this phenomena so we can artificially store sperm be it for a short period of time or longer periods. Diluents are our srtificial replication of the spermathecafluid .many diluents have been proposed or are in use each with its set of pros and cons.

Investigations into diluents suitable for honeybee sperm storage by **Verma (1978)** found sperm survived longer in Tris buffer diluent at pH 7.19 than at pH 8.4. Balance of nutrients and chemical components make up a diluent. J**ohn Harbo in 198X**used a dilution of (60% semen, 10% DMSO, 30% saline) .another diluent that is often used(40% semen , 60% Kiev diluent). Each diluent has the added character of either allowing for storage either at above freezing temperatures or cryogenic freezing.

2.3.4. TEMPERATURE

Categorically honeybee sperm can be stored at above freezing temperature or be cryo-frozen.The development of practical techniques for the storage of honey bee, ApismelliferaL., semen would significantly improve our ability to breed for desirable genotypes and maintain genetic diversity in populations.

Artificial insemination of queens has been possible for some time, but the semen used is usually freshly collected, or held for < 1 week at room temperature. Examining the limitations of spermatozoal survival at nonfrozen temperatures. Pooled, diluted semen was stored in sealed capillary tubes at room temperature (25 degrees C) or in a refrigerator set to 12 degrees C, for periods up to 1 yr.

Survival of spermatozoa was assayed by a dual fluorescent staining technique using SYBR-14 and propidium iodide stains, which readily distinguishes live and dead cells. No significant loss of viable spermatozoa occurred within the first 6 weeks. Between weeks 6 and 9, the percent live spermatozoa fell from 80 to 58%, and remained at that level until after 39 wk. By week 52, samples at room temperature, but not at 12 degrees C, fell to 18.9% live spermatozoa. Nonfrozen storage of honey bee semen has potential for short-term preservation of germplasm, however several factors need to be studied further to optimize survival rates**(Coliins 2000)**

2.4. QUANTITATIVE ANALYSIS

2.4.1. VOLUME OF SPEMEN AND NUMBER OF SPERM PER DRONE

Honeybee sperm are 230 µm long cells consisting of an 8 µm head with an acrosome on its end and a long tail. The tail contains an axoneme and two different sized accompanying mitochondrial derivatives. Spermatogenesis takes place in the testes of the haploid drone during its larval and pupal life **(Hoage and Kessel, 1968, quoted in Pabst and Pfeiler, 1994).**

Rhodes (2002) obtained the numbers of sperm present in the spermathecae of 260 Apismellifera queen bees examined between 14 and 35 days of age over three years with 56.6% containing < 3 million, 24.4% containing 3-4.5 million, and 19.0% containing > 4.5 million sperm/queen, range 0.25-5.21 million sperm/queen. **Mackensen and Roberts (1948)**, (quoted in **Ruttner, 1956**) stated that the full spermatheca of a naturally mated queen holds, on average, 5.73 million spermatozoa. **Taber** (quoted in **Jay and Dixon, 1984)** stated that queen bees with sperm counts of less than 3 million are unable to head commercial honey production colonies for one season.

The two factors from **Rhodes (2002)** of overall low sperm counts per queen and a wide range of sperm counts between queens suggest a problem during queen bee mating. The problem could be insufficient numbers of mature age drones, inadequate quality of drones, or a combination of both factors for drones present in the mating area when the queens were mating.

Mackensen and Roberts (1948) described their methodology for “Estimating the number of sperms present” either from a queen bee’s spermatheca or from a drone’s seminal vesicles by diluting each sample in either 5cc or 10cc of tap water and counting the number of sperm present in the known volume, 0.8 mm3 , contained in a counting chamber (haemocytometer). They conceded that there is “considerable chance of error in making counts from such a small sample” and that “greater accuracy can be obtained by counting a larger sample”.

Mackensen (1955), when experimenting with Artificial Insemination techniques of queen bees collected sperm directly from seminal vesicles removed from drones.**Mackensen**reported drones 7-8 days of age produced an average of 9.89 (range 8.36-10.63) million sperm per drone, n = 6. Köhler**(1955, quoted in Ruttner, 1956)**, found the average count of spermatozoa in a drone to be 4.5 million sperm, **Jaycox (1961)** considered this number to be abnormally low, however the method of collecting the sperm sample was not presented. **Woyke (1962)** found drones yielded an average of 1.7 mm3 (range 1.5-1.75 mm3) of semen with each drone producing, on average, 11.0 million spermatozoa, n = 78.

Woyke refers to the “ejaculate” which suggests the samples were obtained from manual eversion of drones. Of the above authors, **Woyke (1962)** did not state the methodology used for calculating sperm numbers per drone. **Mackensen (1955)** and **Ruttner (1956)** cited **Mackensen** and **Roberts (1948)** in their References although they did not state the methodology used for counting sperm numbers.**Collins and**

Pettis (2001) stated that a healthy drone will produce up to 1.25 µl of semen with close to 10 million sperm with the majority of sperm present being alive.

2.5. QUALITATIVE ANALYSIS

2.5.1. SPERM VIABILITY

Evidence showing that sperm competition selects for higher sperm quality in insects was provided by **García-González and Simmons (2005)** who found that paternity success was determined by the proportion of live sperm in a male's ejaculate and were able to predict the paternity patterns observed on the basis of the male's relative representation of viable sperm in the female's sperm-storage organ.

Hunter and Birkhead (2002) examined the importance of sperm quality in determining which male's sperm had the advantage when sperm from two or more males were competing to fertilise a female's ova, finding that, all else being equal, males vary in their ability to fertilise ova on the basis of sperm viability alone suggesting that sperm viability is one of a suite of male adaptations to sperm competition in insects.

Kraus et al. (2003) investigated male fitness of drone honeybees finding that selection through the male side appears to be an extremely important factor for colony fitness as the number of mating drones and the individual siring success of each drone is determined by the colony and/or the genotype of the mother queen.

Attention to drone semen quality was raised by **Vesely (1970**, quoted in **Locke and Peng, 1993)** who studied the retention of semen in the lateral oviducts of artificially inseminated queen bees which he suggested was due to factors affecting semen quality and which often resulted in infertility and death of the queen bee. **Woyke and Jasinski (1978)** examined the effects of drone age on semen quality finding that as the age of drones increased, mostly, a lower number of spermatozoa entered the spermatheca of queens, and the percentage of queens with semen residue in their oviducts increased.

Effects of drone age on semen quality were examined by **Locke and Peng (1993)**. They found that sperm viability, identified by supravital staining, was adversely affected by drone age. They suggested the decline in viability may be an indicator of a natural ageing process with sperm in the seminal vesicles reaching some age at which sperm senescence begins, with the final stage being sperm membrane disruption and death.

Viability assessment of honeybee sperm was investigated and improved by **Collins and Donoghue (1999)** who developed and validated the use of the living:dead fluorescent stains SYBR-14 and propidium iodide (PI) with honeybee sperm. In an experiment examining the relationship between semen quality, i.e. percent viable sperm, and queen performance using instrumentally inseminated queen bees inseminated with known ratios of live:dead sperm, **Collins (2000a)** determined that sperm survival levels of 50% were sufficient for the queen to produce only fertilised eggs, at least early in their lives.

Lodesani et al. (2004) when examining the effect of time on the viability of sperm in a queen bee's spermatheca found a relatively low percentage of dead sperm in the spermatheca (20.5%) of two month old inseminated queen bees suggesting to them that sperm in the spermatheca undergo an initial selection for quality. However, after inseminating queen bees with only dead sperm, **Collins (2000b)** found dead sperm in the vaginal area of inseminated queen bees but none in the spermatheca suggesting that activity of the sperm themselves is critical in their migration to the spermatheca.

Dead sperm were found in the spermathecae of queen bees inseminated with a mixture of live and dead sperm allowing **Collins (2000b)** to suggest that live sperm drag dead sperm along with them when moving towards the spermatheca. **Bresslau (1905**, quoted in **Collins, 2000b)**, identified a muscular sperm pump contained in the sperm duct which was considered to support migration of sperm into the spermatheca as well as the release of spermathecal contents for fertilisation.

2.5.2. SPERM MOTILITY

Sperm, generally, are motile cells with sperm motility being critical at the time of fertilization which allows sperm motility to be used as a measure of sperm quality. **Tourmente et al. (2007)** considered semen quality analysis a powerful tool for evaluation of fertility potential of males and stated that sperm motility, evaluated as the sperm velocity and the percentage of motile spermatozoa, is positively correlated with fertilization success in several species.

Kaftanoglu and Peng (1984) found honeybee sperm motility rates were higher at pH 6.35-8.40 in hypertonic than in hypotonic solutions and no vigorous sperm motility was observed in saline and trisbuffer diluents, both of which were hypotonic to seminal plasma.

Locke and Peng (1993) did not find significant differences between sperm motility from drones 14, 28 and 42 days of age. Should an age effect be present, then an expected result would have been the 14 day old drones with the highest motility and the 35 day old drones with the lowest motility.

CHAPTER 2 : REPRODUCTION, A QUALITATIVE & QUANTITATIVE OVERVIEW

Results **(Rhodes 2008)** suggest that drone sperm motility is a selectable trait able to be improved by a breeding program, and a significant seasonal effect suggests that a higher quality sperm, based on motility rating, may be produced by drones during certain periods each year

CHAPTER 3: INSTRUMENTAL INSEMINATION

3.1 COMMONLY ASKED QUESTIONS:

a. The difference between Instrumental and artificial insemination

The terms, artificial insemination and instrumental insemination, are used interchangeably. The term “instrumental insemination” was coined by Dr. Lloyd Watson, the first to successfully demonstrate a technique of instrumental insemination in 1926, He disliked the term artificial. The term artificial insemination is more commonly used and recognized by other industries such as; cattle, poultry, sheep, swine, equine etc

b. The choice to use instrumental insemination

Instrumental insemination is a simple tool to control breeding. It also provides a means to create novel crosses for research purposes. Honey bee mating behaviour is highly random and difficult to control. Queens mate in flight with numerous drones, averaging 15 to 20. Virgin queens fly to drone congregating areas, consisting of 10,000 to 30,000 drones from diverse genetic sources. The queen, who only mates during the first week or two of her adult life, stores the sperm collected in her spermatheca for use over her lifetime. The queen stores only a small percentage of sperm from each drone she mated with and this is mixed in her spermatheca. All the drones the queen mated with are represented in the many subfamilies of her colony, although the ratio of these may change over time.

c. The need for instrumental insemination

I.I. is simply a tool for the bee breeder and researcher requiring specific crosses. It provides a method to control honey bee mating. This technique enables the control of who the queen mates with, the number of drones she mates with and the semen dosage given and stored in her spermatheca. Just because a queen is instrumentally inseminated does not mean she will be superior. The goal of producing Top Tier and “Rock star” breeder queens is dependent upon the selection of stock and the breeding program employed. In this process, many queens must be culled.

d. The equipment needed

The insemination instruments are specialized and vary widely in quality and price. The technique requires precision and accuracy in fine adjustments and this will determine the ease and repeatability of the procedure. There is no standardization in equipment and some parts are not interchangeable between instruments. Therefore, research the options. The basic instrument consists of a stand, a set of hooks or forceps, queen holder assembly, syringe and syringe tips. A microscope with a magnification of 10X to 20X, cold light and compatible stand with sufficient depth of field and instrument clearance are required. A cold light prevents heating and drying of tissues, a gooseneck L.E.D. light works well. A source of carbon dioxide, with a flow regulator and flexible tubing to the instrument, is used to anesthetize the queen during the procedure. Modern instruments offer micro-manipulators that provide fine precision in movements. Large capacity syringes provide efficiency in semen collection, storage and shipment of semen. Various designs of sting manipulation tools offer personal choice in techniques. The Schley Instrument is currently the most widely used instrument, valued for its fine precision and wide range of flexibility in adjustments. The Harbo large capacity syringe, designed for ease of semen handling and storage, is also popular and compatible with most instruments.

e. The most common problems

Good sanitation and proper techniques are critical to success. Injury and infection are the most common problems for beginners. Drones tend to defecate during the eversion process for semen collection, and can be very messy. Care must be given to maintain highly sanitary conditions and avoid faeces contamination during the procedure. The insemination procedure is very delicate and injury to the queen will produce poor results. Manipulations; opening and positioning the queen, bypassing the valve fold and insertion of semen, must be precise and brief. Queens vary physically, especially between the subspecies, and these nuances of differences must be learned and recognized. Care of queens, their pre and post insemination treatment and introduction method, require careful attention. The queen signals her many changes of reproductive status to the workers, from virgin to mated and laying. These changes vary more among IIQs and therefore introductions need attentive treatment. These factors are discussed in the reference listed: Comparison of instrumental inseminated and naturally mated honey bee queens and factors affecting their performance, **(Cobey 2007)**

f. The most overlooked aspect

Rearing a plentiful supply of select drones to maturity can be a major limiting factor. Queen production methods have been perfected and are routine. Most beekeepers are not accustomed to giving the same detailed attention to drone production. Drones appear plentiful during peak season, yet are seasonally produced and the most vulnerable to stressors; such as parasites and pathogens, malnutrition, miticide and pesticide residues, poor weather conditions, etc. The colony will regulate the seasonal drone population based on many factors. Strong healthy, well fed colonies are required for drone production. A colony can rear about 2000 drones during peak season, of which about half will be immature. Drones are mature at 2 weeks post emergence and peak at 3 weeks. Stressed colonies will eliminate mature drones and often continue to rear a new batch of young drones for the future. Colonies headed by older queens tend to rear and care for more drones. In extreme cases when seasonal conditions are unfavourable, queen less colonies will hold and care for drones, although this method requires intensive management.

g. Time for procedure to be completed

The timing required for the insemination procedure is dependent upon the skill of the inseminator, the quality of the equipment used and the quality and quantity of the live material produced. It is always best to overproduce queens and drones to ensure an adequate supply and allow for culling. In mastering this skill, it takes time to perfect techniques and gain proficiency. The beginner must learn proper procedures. Beekeeping skills essential to rear and care for virgin queens and drones are also essential. The actual insemination procedure is very quick. Once the semen has been collected, it is a matter of seconds to insert the semen and inseminate the queen. The semen collection process is more time consuming and tedious. Timing is generally determined by the quality and maturity of drones. In general, about half the drones will yield usable semen. Many semen loads must be discarded due to contamination during the explosive force of the eversion. Some drones may not be mature and some simply will not yield semen. A skilled inseminator can collect semen and inseminate 50 queens in 5 hours. The standard semen dosage per queen is 8 to 10 microliters. The procedure of semen collection and insemination can be separated. Collection of a 100 microliter tube of semen takes about 40 minutes. Each drone yields about one microliter of semen. Once the semen is collected, 40 - 50 queens can be inseminated in one hour. It is helpful to have a “runner” assist in supplying virgins to the table and caring for the inseminated queens after the procedure, including record keeping and marking queens.

h. The success rate

Success depends upon two major factors; the skill of the inseminator and the beekeeping skills to provide proper care of queens and drones. Assuming this expertise has been mastered, instrumentally Inseminated queens (IIQs) have the capability to perform as well or better than naturally mated queens, (NMQs). Better performance of IIQs is based upon the ability to do selection and control the semen dosage given. The longevity and performance of the queen is largely based upon the genetic diversity of drones she mates with and how much semen she stores in her spermatheca. The pre- and post-insemination care given to IIQs affects sperm migration and queen performance. The claim that IIQs do not perform as well as NMQs is unfounded. Tiring of this perception, I wrote a review of supporting studies.

This discussion, listed in the references, **(Cobey, 2007)** clearly demonstrates how the differences in quality of care affect queen performance, over the actual procedure of insemination

3.2 INSTRUMENTAL INSEMINATION OF A QUEEN

The successful insemination of a queen artificially, rests on the now most established of aspects of the procedure, that in their own way pose a risk to the success of this procedure just as much as they contribute hugely to is success. 1- Semen collection; 2-anaesthesia; 3- apparatus; 4- breeding practices

a) semen collection

The amount and consistency of semen obtained varies, generally each drone will yield about 1 µl. The standard dose per queen is of 8 to 10 µl. Initially, collect an air space to separate semen from the saline column in the syringe. Collect a drop of saline in the tip to make contact with the semen on the endophallus. For collection of each semen load, expel a drop of semen from the syringe to make contact. Avoid collecting air bubbles and avoid the underlying mucus layer. Draw the semen into the syringe, skimming this off the mucus layer.

Figure 18: COLLECTING SEMEN INTO A SYRINGE AS SHOWN IN SUSAN COBEY JOURNAL ON INSTRUMENTAL INSEMINATION

b) Anaesthesia

since the early recoded use of ether as anaesthesia in the early 1900s , there has only been one other used, carbon dioxide, its added effects make it the perfect fit, its ability to stimulate juvenile hormone in queens and oviposition , getting the queens ready to lay eggs as earliest as possible, solving the problem of delayed egg laying (**Mackensen& Roberts 1947)**

c) Apparatus

The Schley instrument designed by Dr Peter Schley is one of more refined mackensen's apparatus that is available, increased level of precision and freedom of manipulation.

d) Breeding practices

The availability of numerous healthy virgin queens; a sufficient supply of sexually mature drones because sexually immature drones don’t produce any sperm during manual eversion of the endophallus

3.3 EVOLUTION OF INSTRUMENTAL INSEMINATION

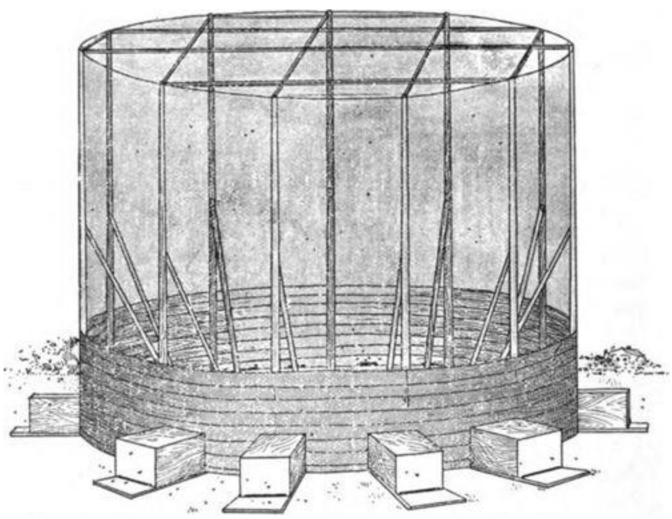

Figure 19: MOSQUITO NETTING IN THE 1900s USED TO TRY AND CONTROL HONEY BEE MATINGAS SEEN IN LAIDLAW DIARY ON ORIGINS OF INSTRUMENTAL INSEMINATION

Bee enhancement can only be achieved if control is exercised on couplings; either by developing methods of control of the coupling natural, by developing methods of artificial insemination. These have evolved according to two conceptions:

1) Direct introduction of the organ of copulation of the male in the genital tract of the Queen. This is the method of manual insemination.

2). Introduction of semen into the genital tract of the queen by means of instruments. This is so-called instrumental insemination.

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 endeavour to inseminate a queen bee by introducing within her vagina, through the use of a brush, sperm from a drone. Huber (1788-91) was the first to attempt instrumental insemination utilizing a fine brush to try to introduce sperm into the queen's vagina. Other authors tried to introduce sperm by means of a syringe; ANKLBR in 1883 and, McLain in 1886 also practiced this method, but without recording any successful outcome.

McLain 1885, after having managed to stop drone and queen flight by clipping the wings, the next step was finding a way to transfer sperm to the queen. The methods proposed to bringing the drone's organs into proper position within the queen were then divided into two :(1) causing these two 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 which now can be classified as efforts at manual insemination.

After failed attempts at manual insemination, McLain also proposed two methods of first removing sperm from the drone and then transferring it to the queen :(1) Dropping or brushing the sperm on or near the exterior genital opening of the queen, and (2) introducing the sperm within the opening.

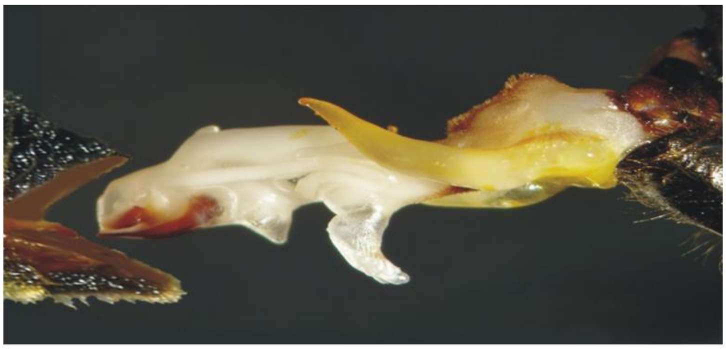

Figure 20: ILLUSTRATION OF AN ATTEMT AT MANUAL INSEMINATION BY EVERTING DRONE SPERM DIRECTLY INTO A QUEEN AS SHOWN ON WIKIPEDIA IMAGES OF ARTIFICIAL INSEMINATION

McLain 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 It queen bee.

The first announcement of importance in this field in the twentieth century was that of Jager and Howard in 1914 regarding success in artificially inseminating (3 out of8 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, 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 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.

During the next two years Howard and France devoted 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 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, drawing inspiration from the work done by Huber 1891. To keep the abdominal tips of the queen apart and to hold the sting out or 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 . 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.

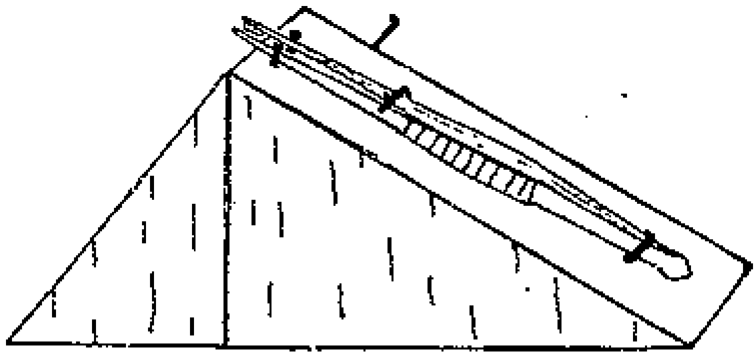

Figure 21: INSTRUMENTS USED BY SCAFER DURING MANUAL INSEMINATION USING THE BLOCK TO KEEP THE QUEEN STEADY AS SEEN IN LAIDLAW DIARY ON ORIGINS OF INSTRUMENTAL INSEMINATION

Shafer later contrived a device which consisted of an 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 dorsal-ward. Shafer did not report any successful insemination, however in 1923 Quinn 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 and in the spring of 1930 , 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 Schafer . 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 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 statement 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.

Malyschev , 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 the queen that had been subjected to ammonia, in this instance to immobolise the queen, ammonia was used as an anesthetic. Malyschey reported that this queen laid eggs normally in a nucleus and was then transferred to a queen-less colony. He did not record her(queen) further history.

Some successes were obtained. the work of WATSON (1927), who used a microsyringe attached to a micromanipulator. The queen was immobilized in a block of wood by silk threads. To open the sting chamber Watson made the use of a hand-held pliers.

Figure 22: DR WATSON OPERATING EQUIPMENT WHEN HE FIRST SUCCESSFULLY ATTAINED INSTRUMENTAL INSEMINATION IN 1923; SEEN HERE IN A DEMONSTRATION OF IINNSTTRUMENTAL INSEMINATION BY SUSAN COBEY 2007

In subsequent work Watson minimizes the importance of mucus plug and describes small "saculus" which is formed in the end of the bulb containing the sperm when pressure is applied at the other end. By cutting and inserting the pipette there is instead of pushing it through the mucus first; it is possible to take up in te syringe a large charge of sperm which practically contains no mucus.

As to his own success Watson states that in 1926; he operated on 42 queens, of that number either by way of microscope or breeding the worker brood, half the queens were successfully inseminated. Later own as he mastered his technic Watson in 1930, 35 of the 42 queens he had instrumentally inseminated showed show signs of a successful operation. But it should be noted that as the numbers improved in the successful column he continued to lose queens. Instrumental insemination is very invasive and bees are very prone to stress,Watsondd not have anaesthesia to calm the queens , his instruments relied on the use of silk threads used to tie down a quenn. That may have gone a long way to explain the deaths of queens soon after.

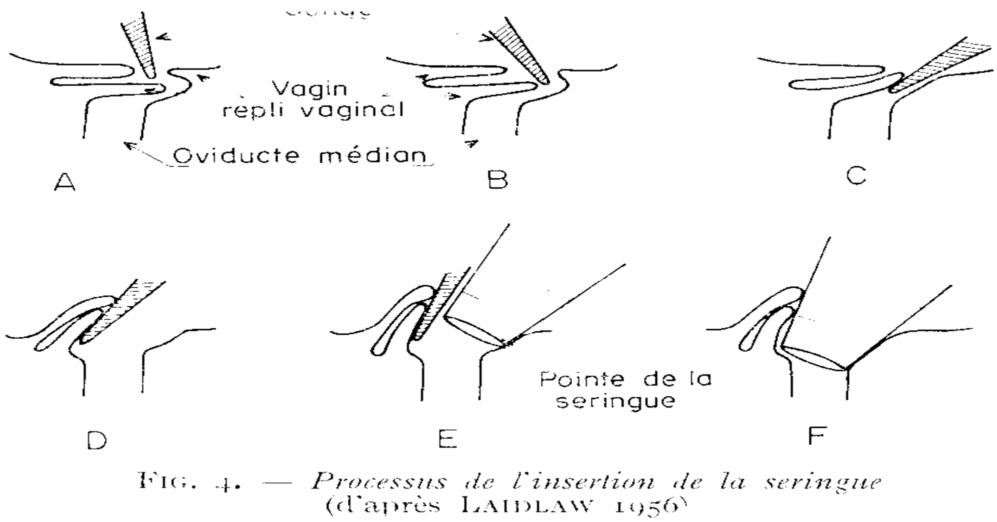

Figure 23: LAIDLAS GUIDE TO PROPERLY INSEMINATE A QUEEN AFTER HAVING DICOVERED THE EXISTENCE OF A VALVEFOLD AS SEEN IN LAIDLAW DIARY

In 1944 Laidlaw successfully dissected a queen bee to study the physiology of the reproductive system, so as to further understand the field, he discovered the valve fold and its physiological functions; blocking the oviducts; and that the queen herself was in charge of opening and closing the valve fold, probably explaining why earlier trials at instrumental or manual insemination had not been as successful; that brought him to a realization that the valve fold had to be bypassed and directly deposit the sperm into the oviduct in order to increase the chances of success in instrumental insemination.

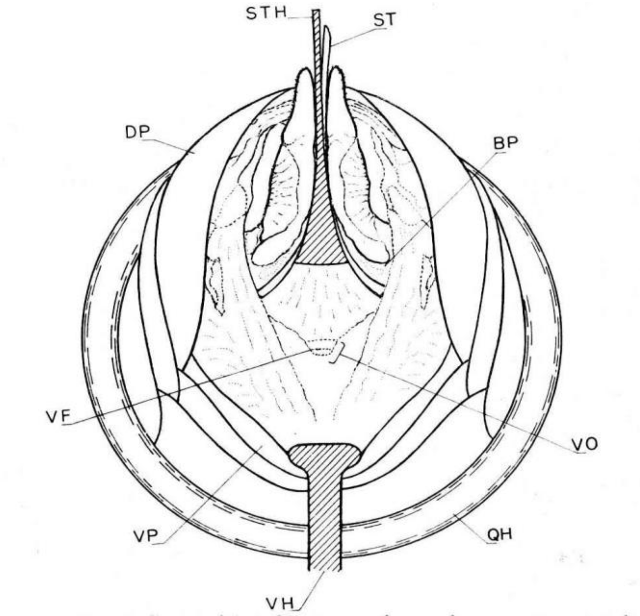

Figure 24: MACKENSEN ILLUSTRATION OF INSEMINATION USING KNOWLEDGE OF WATSON AND LAIDLAW'S WORK, MACKENSEN AND ROBERTS 1947

(STH- sting hook; ST-sting; VF-valve fold; VO-valve orifice, VH- ventral hook; QH-queen holder**)**

As the need for improved methods of artificial insemination , other adjacent fields also benefited from the research, focused his work on immobilizing the queen during insemination, he explored in different anesthesia , sticking to those that had been used in the other fields , ether was his first, it seemed to be successful, then on the day he made a significant discovery, he saw how carbon dioxide was being used in the laboratory he worked to put mosquitoes to sleep and voila , there was **mackensen's** next big idea ; the use of carbon dioxide as anaesthesia for the queen for instrumental insemination.

In using carbon dioxide as anaesthetic **Mackensen** reduces the delay before the onset of egg-laying in the vicinity of normal, the mean difference between queens naturally and artificially fertilized falls to 1.9 days. Anaesthesia of queens virgin carbon dioxide also allows to obtain easily and quickly parthenogenesis spawning queens, which lay only male eggs. They are used in selection and genetics of the bee.

So in short carbon dioxide: anaesthesia; Stimulates juvenile hormone production and oviposition , solved the problem of egg laying.

Mackensen also started using the plastic queen holder which was used open at both ends, one being the side that is pumped full of carbon dioxide to anaesthize the queen. Mackensen also designed a new operating apparatus which was more fixed, and allowed for greater accuracy , improving on the works of Laidlaw.

CHAPTER 3 : INSTRUMENTAL INSEMINATION

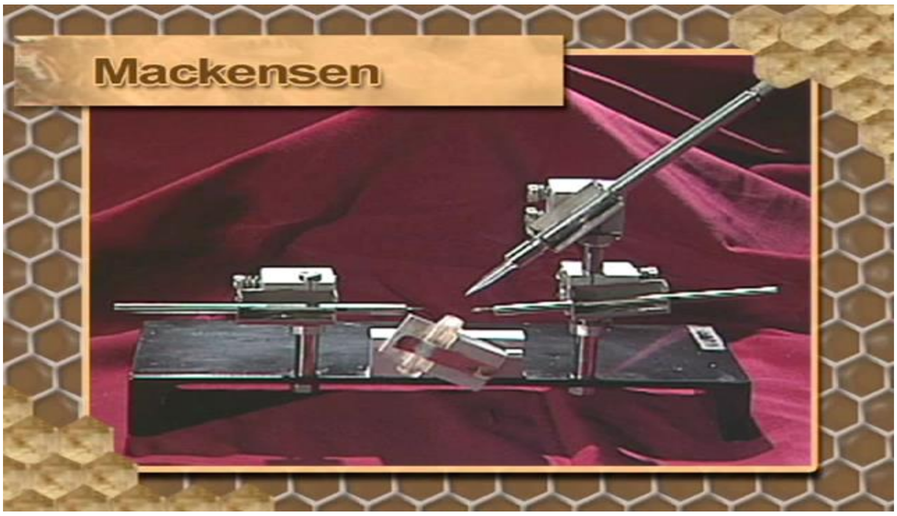

Figure 25: MACKEN SEN INSTRUMENT DESIGNED IN 1947 ; SEEN HERE IN A DEMONSTRATION OF IINNSTTRUMENTAL INSEMINATION BY SUSAN COBEY 2007

Mackensen wrote in his 1970 publication, The techniques now employed had their beginnings in the late 1920's and early 1930's. In these first approximations, semen from one or two drones was taken up into a fine syringe and then part of the semen injected into a queen's vagina.

Typical results were some partial inseminations: queens, tardy to begin laying, that produced at least some fertilized eggs. But, occasionally, inseminations were good enough to encourage further effort.

By the late 1930's it was recognized that the semen was not placed deeply enough into the queen's reproductive tract. Obstructing further penetration is a tonguelike structure, the valve fold. When it is moved out of the way, the syringe tip can be placed beyond it, and the semen discharged into the median oviduct. Placing the semen beyond the vagina and into the oviducts led to greatly improved results from instrumental insemination, even though partial inseminations were still too frequent, and queens were usually tardy to begin to lay.

Mackensen continued to write It had been established that in the 1940's, that partial inseminations could be almost eliminated by injecting a larger volume of semen. Since that time, the semen of three to 10 drones per insemination has been used with consistently good results. The problem of tardy onset of laying was solved about the same time. It was discovered that anesthesia of queens with carbon dioxide would hasten the onset of laying, not only of inseminated queens, but also of virgin queens.This discovery fits in well with insemination technique, because carbon dioxide is used to keep the queens still during instrumental insemination.

CHAPTER 3 : INSTRUMENTAL INSEMINATION

Along with the advances in technique, the equipment used for instrumental insemination was developed and improved into the two types of apparatus now used. Early means of holding the queen still by tying her with thread to a wooden support was supplanted by queen holders of the tubular or clamp type in which the queen is also anesthetized. The technique of holding the sting chamber open and the sting from over the opening to the vagina by handheld forceps was replaced by using hooks manipulated through friction mounts or by rack and pinion. For collecting and injecting semen, the screw type syringe with a fixed glass tip and a plunger extending into the tip has been modified into a disassemblable metal syringe with a plastic tip and a plunger extending not into the tip but pressing against a rubber diaphragm, which activates a column of saline solution within the tip. A probe, designed especially to move the valve fold, has been used since the importance of the valve fold was recognized.

Mackensen further studied and grew his research on the reproductive systems of both the queen and the drone, on a an anatomical level and as well as well as physiological that he declared that knowledge of this was as essential as the instruments used in instrumental insemination. In 1970 mackensen , published a basic and in detail description f the reproductive systems, quoting Laidlaw who also had taken this anatomical and physiological interest earlier in the 1930s;

The sperm cells go through development in the tubules of the testes and then pass into the vesicles where they remain until ejaculated. In the meantime the testes shrink. The seminal vesicles have muscular walls and are lined with secretory cells that provide nourishment for the sperm. At 3 to 4 days of age a few sperm are already in the seminal vesicles, and at 4 to 5 days there are about 5 million and this increases to 10 to 11 million at 8 days.

It is very important that drones receive proper care during' this period. Sperm obtainable at any age usable in artificial insemination; however, it is best to wait until all sperm have had a chance to mature. This is considered to be about 12 days of age. There seems to be no deterioration of sperm as aging continues. The mucous glands also have muscular wallswith secretory cells. As the drone maturessexually these glands become distended andwhite from the amorphous white mucus secretedinto the lumen of the gland.

Ruttner 1976, studied the effects of semen collection and its immediate storage, he stated that quoting previous work by Watson who had used saline water to dilute semen before instrumental insemination. Dilution was meant for the purpose of keeping the semen from drying up , but later on penicillin was added as addition of antibiotic was believed to clean up up sperm that may be riddled with bacteria and undesirable diseases. Many scientists at this time came up with many different solutions which they tested for the short term storage of semen, dry storage if you can call it, sperm could still be used 48hrs later and still positive insemination results were witnessed, **hyes** solution is one such example used in experiments by **Woyke,** being made up of part sodium chloride , calcium chloride, sodium hydro carbonate, penicillin, streptomycin all diluted in distilled water.

The researchers became satisfied with the level of development the apparatus of instrumental insemination had taken, their research started to broaden and encompass other areas that are directly linked to artificial insemination, such as the factors that may increase or reduce the success rate of instrumental insemination. **1985 JOHN HARBO**, after successful dry conservation tests of semen he ventured further into the cold temperature storage of semen, basically freezing of semen intended for use at a later date, he found out that just like fry conservation , freezing of sperm still needed diluents full of nutrients and antibiotics,

As further studies show Harbo realized the need of a cryoprotectant for his semen to survive the process of cryopreservation, thus he tasted the use of dimethylsulfoxide(DMSO) and NaCl as possible cryoprotectant. His research proved that sperm could be preserved at -196 degrees Celsius using DMSO as a cryoprotectant and still achieves positive results on artificial insemination.

(Susan Cobey, 1983 & 2007) worked on the history of instrumental insemination that had been done by other leading her to conclude or should I say state a more refined method of insemination, I want to refrain from using the word standard because there are more methods of achieving the same result so , according to Susan Cobey: To expose the vaginal orifice of the queen, the abdominal plates are separated using a pair of hooks or forceps. The large sting structure is lifted up and dorsally. The valvefold, a stretchy flap of tissue covering the median oviduct, is bypassed. Semen is inserted directly into the median oviduct. The syringe tip is used to bypass the valvefold. The tip is slipped beneath the valvefold and lifted ventrally. The angle of the syringe and a slight “zigzag” movement is used to manoeuvre around the valvefold.

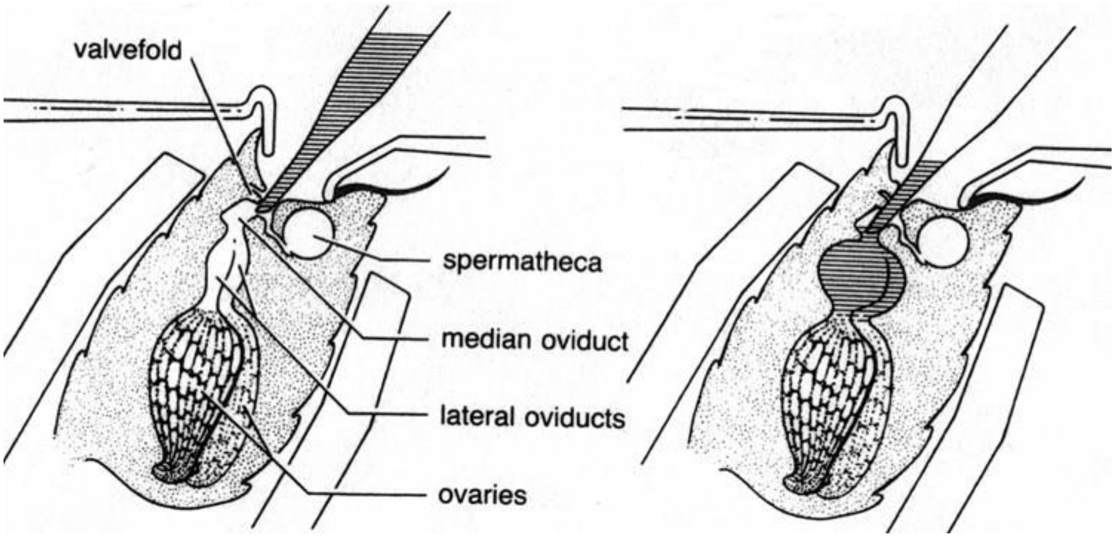

Figure 26: BYPASSING THE VALVEFOLD FOR INSEMINATION (SUSAN COBEY) ; SEEN HERE IN A DEMONSTRATION OF IINNSTTRUMENTAL INSEMINATION BY SUSAN COBEY 2007

Position the syringe tip dorsally above the "V", defining the vaginal orifice. Insert the tip about 0.5 mm, slightly forward of the apex of the "V". Positioned correctly, the tip slips easily past the valvefold without resistance. Insert the tip another 0.5 to 1.0 mm into the median oviduct. Bypassing the valvefold allows passage of the tip into the median oviduct. Test placement of the tip, preceding the insemination with a drop of saline, then insert a measured amount of semen, 8 to 10 µl is the standard dose per queen. With practice, the insertion of semen is preformed quickly and precisely, requiring only seconds per queen. There are a variety of tools to choose from. Use is based on personal preference.

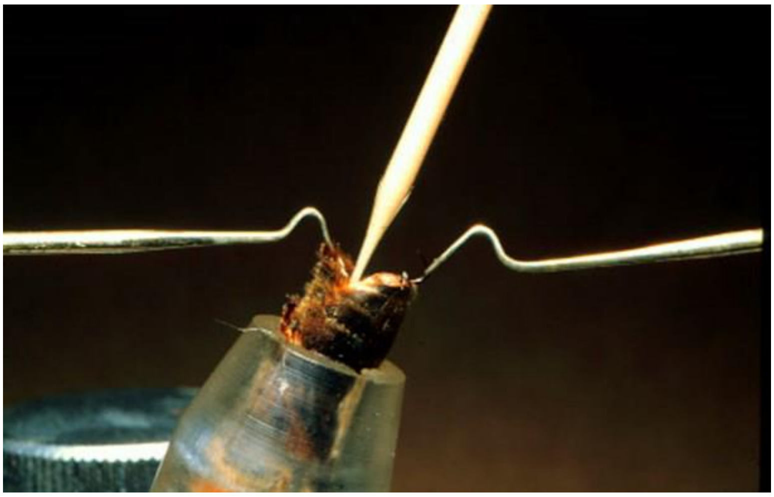

Figure 27: VIRGIN QUEEN POSITIONED IN QUENN HOLDER AND UNDER ANESTHESIA SYRINGE USED TO BYPASS VALVEFOLD AND DEPOSIT SEMEN IN THE OVIDUCTS, SEEN HERE IN A DEMONSTRATION OF IINNSTTRUMENTAL INSEMINATION BY SUSAN COBEY 2007

3.3 FACTORS AFFECTING THE RESULTS OF INSTRUMENTAL INSEMINATION

1. The optimal age for insemination of queens is 5 to 14 days post emergence.

a. Queens inseminated older than 2 weeks tend to store less sperm in their spermathecae. Queens inseminated less than 4 days old have high mortality **(Woyke and Jasinski, 1976)**.

2. The standard semen dosage given to each queen is 8 to 12 µl.

a. An insufficient semen dose can result in premature queen supersedure or premature queen failure.

3. Post-insemination care of queens influences sperm storage **(Woyke 1979)**.

a. Active movement of queen, appropriate brood nest temperatures, and attendance by worker bees promote sperm migration into the queen's spermatheca.

b. Queens confined in cages after insemination tend to store less sperm and retain semen in their oviducts

3.4 SPECIALIZED TECHNIQUES THAT HAVE COME OUT FROM THE PURSUIT OF INSTRUMENTAL INSEMINATION

Homogenizing honey bee semen

To homogenize or mix honey bee semen from numerous drones requires dilution, mechanical movement and reconstitution of semen. Current techniques using centrifugation result in a high percentage of damaged spermatozoa, although 50% viability of spermatozoa is sufficient to produce normal brood patterns (Collins, 2000). Semen is very dense, tends to clump, and the long, fragile tails of spermatozoa are subject to damage during processing and some components of the seminal fluid are removed.

Migration of sperm from the oviducts into the spermatheca is a complex process involving contraction of muscles mediated by the specialized composition of fluids in the semen and the oviduct as well as active sperm movement **(Koeniger, 1986)**. Queens are very active after natural mating which also promotes sperm migration; therefore, use a direct queen introduction release method **(Büchler et al., 2013).**

Short term semen storage at above freezing temperature

Honey bee semen can be held at room temperature for several weeks without significant loss of sperm viability. The Harbo syringe, with detachable capillary tubes, is designed for semen storage.

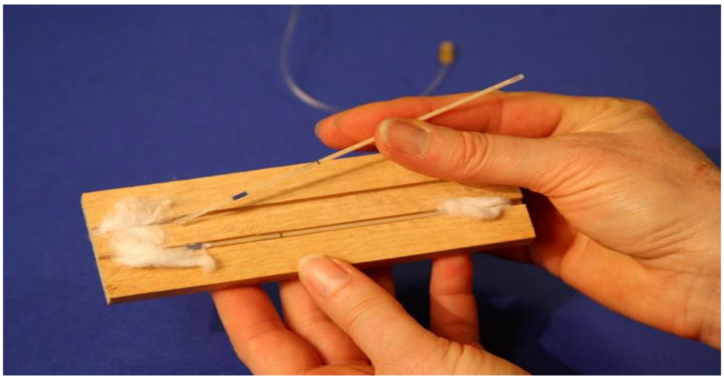

Figure 28: SEALED CAPILLARY TUBES USED TO STORAGE AND TRANSPORT OF SEMEN, SEEN HERE IN A DEMONSTRATION OF IINNSTTRUMENTAL INSEMINATION BY SUSAN COBEY 2007

Cryopreservation of semen

The maintenance of honey bee stocks currently requires costly and labour intensive annual propagation. Current threats to the biodiversity of honey bees, and the need to select lines tolerant to pests and diseases, create a need to develop techniques for the cryopreservation of honey bee germplasm. Repositories

would provide a resource for breeding purposes and the preservation and recovery of selected stocks and endangered populations.

High viability and motility of honey bee semen cryopreserved in liquid nitrogen and thawed has been demonstrated, although fertility is greatly reduced in the spermatheca of queens. Current techniques demonstrate that fertility is adequate to produce sequential generations of queens inseminated with frozen-thawed semen for breeding purposes, although they are not sufficient to head productive colonies **(Hopkins et al., 2012)**. Further research is being conducted to perfect these techniques.

Current recommendations for cryopreservation:

Cryoprotectants: dimethyl sulfoxide (DMSO) and ethylene glycol (see **Wegener &Bienfeld, 2010 and Hopkins and Herr, 2010)**

Programmable freezing rate: 3ºC / min, from 4ºC to - 40ºC, then place samples in liquid nitrogen (see Hopkins et al., 2012). Thawing rate, 40ºC for 10 seconds (see Hopkins et al., 2012)

DISCUSSION

Bees as we have analyzed, scrutinized and read all the literature we possibly could, we have been found to be still wanting, a great deal still needs to be understood, we have barely scratched the surface. Bees present us with a unique two bit anatomic characteristic, it definitely is an insect , its taxonomy is true to its word, yet further study reveals the complexities of the digestive tract, it most definitely resembles a bird, the crop, proventriculus and ventriculus system, is textbook bird digestive anatomy.

Bees live in polyethism society, by definition it is the existence of different work activities among different members of an insect community, or at different ages in the insect's life. in that community of bees we found that there are three castes of bees that exist in a hive, the ever so tireless workers make up the majority of the hive , 90% of the total population in a hive. These workers a testament to their name work tirelessly to keep the hive alive. They look for food, water; they are the premier defense for the hive using their stingers to do so. The duties of the drones also differ by age , the older the worker the less the physical task she will partake in comparison to the younger workers that still ooze with so much buzz and energy. Studies have shown that these workers have the capability to lay eggs, be as it may all the eggs will become drones.

The queen runs the hive, she gives commands through pheromones to both the drones and workers, and workers are given orders on food security, which eggs to feed. A queen's reign of power can only last as long as she is strong. She has to continue to lay eggs and not just for drones but keep repopulating the hive with more workers. The moment a queen loses the capability to her role as seen fit in the hive by her workers, an insect sized coup d'etat is at hand to remove the queen and replace her with a younger and more capable queen. And at times the queen herself may sense the need for a change, she will abandon her current hive to form a new one but having had the courtesy of ordering workers to start feeding new queens. At other times she becomes weak, can't lay eggs, her pheromones become weak in intensity, her own drones will evict her from the hive and they will raise themselves a new queen.

The eusocial nature of the hive means as the bees work tirelessly to provide for the hive. There are the drones whose sole existence is for reproduction and make up 10% of the hive. Drones wait until they are sexually mature to take that mating flight, sadly the studies that have been done and the research that has been done over the years, not much has been discovered on the other possible roles that a drone plays in a hive. Autumn brings with it a tough time for drones, the hive winters, there is simply no room in the hive for drones, workers are the more aggressive and they see to it that drones are expelled from the hive before winter, a lot to admire in that self-preservation awareness that the worker bees possess.

As we move closer to understanding the nuances of honey bee reproduction, it all becomes so fascinating. As earlier discussed bees have two female castes in a hive i.e. workers and queen. Both are capable of reproducing and laying eggs. The big difference is the haploid nature of the worker eggs because the eggs are not fertilized by sperm. Workers can only give the hive new drones.

Now the queen on the other hand has much more, she can lay the same haploid eggs that the workers do. In order to lay eggs that bring about the worker bees, the queen at an early age takes a mating flight to try and copulate with as many drones she can. Studies show that queens take one mating flight in her lifetime. So in that one mating flight she must make sure that she has enough sperm in her spermatheca that can last her a lifetime.

So talking of mating flights, we have talked about both drones and queens taking one for reproduction purposes. Drones fly out and form these drone congregation areas which vary in diameter. When the queens take their flight they are looking to mate with as many drones as possibly thus finding a drone congregation area can be very beneficial to a queen, both in terms of collecting enough sperm but also the different genetic traits that she can pick up from mating with drones from other colonies. It is describe in some texts that mating takes place in flight, and when the drone everts its endophallus to deposit sperm in the queen, the exercise can be explosive and a drone can damage its abdomen that it can no longer survive beyond that mating flight

Sperm can stay in the spermatheca for up to five years and still be able to fertilize the eggs that the queen lays and produce workers. Without sperm there cannot be workers. The physiological function of the spermatheca has so many a veterinary trying to replicate its storage conditions. In our studies we have witnessed many established researchers trying to find a way they can preserve drone semen and still maintain the high levels of sperm mobility and viability that exist naturally in the spermatheca.

We have come to understand that drones are not readily available for those that may be seeking drone semen, finding a way to store it just like the spermatheca could definitely come in handy. As early as the 1970s Tuber and later Harbo in the 1980s were already experimenting on the possibilities of preserving sperm over a long period of time. One the ideas that came to mind were cryopreservation. It became possible to preserve sperm at freezing temperatures, with the aid of liquid nitrogen and a cryoprotectant .

As suspected, sperm competition in the spermatheca was proven; the dominance of one drone’s sperm over another has a lot to do with its viability and mobility characteristics. Young and recently mature drones are suspected to be the one with sperm which presents the highest quality.

All our endeavors, through the biological understandings of the bees’ reproductive system, the nuances of the reproduction cycle , the sperm qualitative and quantitative analysis all has been leading to a grand finale that is artificial insemination or as Dr. Luke Watson called it in 1927 instrumental insemination. So many advances have been made in other breeding practices, for horses and cows. Artificial insemination is no longer a myth in those respective fields.

Apiculture still faces the same challenges it did in 1885 when McLain discussed the idea of controlled mating in bees. According FAO, a United Nations branch, bees are still not on the endangered species list. But with so many insecticides and pesticides posing a threat to the survival of the species it can be seen that this controlled mating in bees is the way forward. on that note hats off to the European Union for broadband ban on pesticides that were killing bees , (law passed in April 2018).

That said some rare but wonderful genetic traits that are exhibited by other bee species, or subspecies need to be spread around not only for the sake of the species but bee keepers who have a living to make. Resistance to diseases, quality honey making, and calm behavior are all qualities that we try to share during the instrumental insemination.

The technique of instrumentally inseminating bees has grown in strides too. Many publications make reference to earlier but not much on the statistical side of it. Little that is available showed a not so successful attempt by Jager and Howard (1914) with only one of their eight queens showing signs of a successful insemination. The exercise was taxing on the physical and psychological scale of queens, large percentage of the test subjects did not survive.

But as the instruments were introduced, microscope introduced, the practice became more successful, and the level of precision in semen collection and its delivery improved vastly over years. Dr Watson with this new strategy managed to operate on 96 queens achieving a 65% success rate.

As time went on, the use of carbon dioxide by Mackensen proved very pivotal. Carbon dioxide was applied as anesthesia but as they later discovered it improved the chances of a successful insemination. Development of new apparatus also happened at its own pace, new apparatus that was stable, and simple to manipulate and did stress out the queen are now a thing of the day.

Sadly because it’s a field that is not as common or widely regarded as artificial insemination of cows or horses, resources remain scarce hence the practice of artificial insemination will remain as it was in 1900, expensive for the simple farmer who just wants to be a beekeeper and make a reasonable profit from bee farming.

CONCLUSION AND PERSPECTIVE

As the work before us has shown bee is truly interesting, we have dug deep into our understanding of bee anatomy, physiology which is mainly the basics of what any veterinary should know. Bees should not have to be given less attention at university levels in comparison to other species that we get to study.

Just as we value the addition of cattle, small ruminants and horses as the main stakeholders of our studies we have seen that bees offer just as much of a part in our education. Our study in reproduction is very interesting when it comes to the honey bee species as well.

We managed to get to the basics of fertility of drones, factors that may influence it. We now have better understanding of the qualitative and quantitative aspect of the reproduction cycles in bee life. Be that as it may be, lot of work still needs to be done in order to further appreciate how human influence can further contribute to this wonderful species.

We have seen how long it has taken the practice of instrumental insemination, to evolve, over 200 years and yet what we have now can still not be called world standardized.

Many authors still have a few differences in what they can agree on to be standard. That still has not stopped its advancement. It has become an important tool because of the various contributions it adds to our biological aspect. Cross-breeding is one such example; the benefits of this cannot be taken lightly. A single desirable genetic trait can now be improved on we can make sure it's not lost to the species. Mixing of breeds to increase the chances of disease resistance and a bump in honey bee products is an attractive factor.

In Africa we have barely made a scratch on the research field of honey bees. That can easily be explained by the geopolitical situations in many countries on the continent. Half the countries are still struggling with civil wars and if it's not that then its famine. In all we are saying as history has taught us all the significant research that has been done has been funded by the government through agricultural ministries.

As previously stated between the fight against poverty, civil-wars, famine and natural disasters the governments in Africa have been and continue to be incapable of funding such research. The private associations of bee keepers have managed to pull together resources and do some advance work of their own, but still the work is limited because all they can do is prove what has already been stated by someone else. Some of the resources that are needed for instrumental insemination can be very pricey and learning the way to do it is also expensive. In the end a privileged few get to learn how, and have the resources to learn and improve their skills. For a continent that has lot of rural bee farmers this presents a challenge. The information is there, the expert is there nut if he is one in a province, and surely his services are way too expensive for the regular bee farmer. Knowledge not shared is knowledge lost.

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