

# Background Reading

## HONEY BEE BIOLOGY

## Introduction

These notes are personal to the author and contain inaccuracies, typos and grammatical errors for which I apologise.

I have tried to credit all sources and in particular artwork, if I have missed any credits please let me know and I will sort.

Where possible I have used my own drawings which are based upon those in the reference documents below:

Anatomy and Dissection of the Honeybee, H. A. Dade  
Understanding Bee Anatomy: a full colour guide, Ian Stell  
The Honey Bee Inside Out, Celia F Davis  
The Biology of the Honey Bee, Mark L. Winston  
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The Wisdom of the Hive, Thomas d. Seeley  
The Honey Bee Illustrated, Margaret Cowley  
The Anatomy of the Honey Bee, 1910, R. E. Snodgrass  
Form and Function in the Honey Bee, Lesely Goodman  
The Insects: structure and function, R. F. Chapman  
Pheromones of Social Bees, J. B. Free

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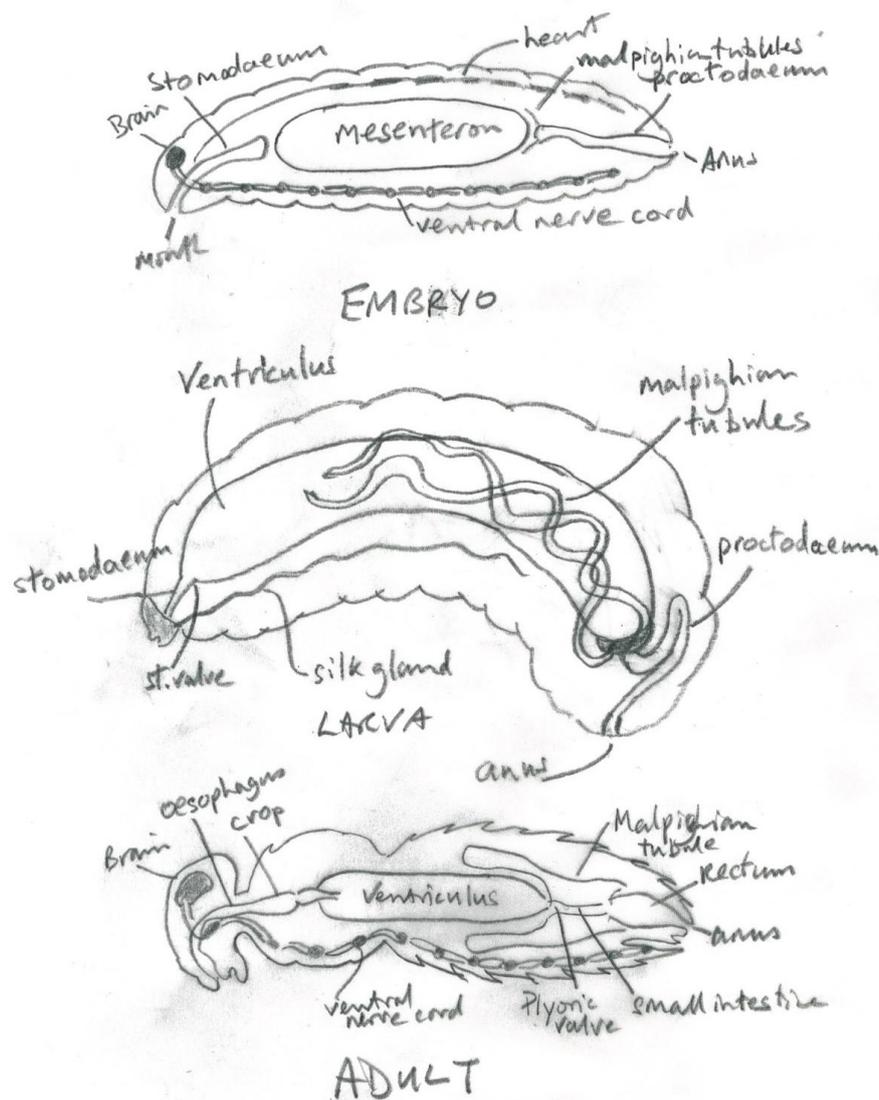
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## 1.1. the function of enzymes.

**An enzyme is a protein molecule that is a biological catalyst** with three main characteristics. First, they increase the rate of a natural chemical reaction. Secondly, they typically only react with one specific substrate or reactant, and thirdly, enzyme activity is regulated and controlled within the cell through several different means, including regulation by inhibitors and activators. It is possible to group enzymes into different categories, including oxidases, transferases, hydrolases, lyases, isomerases and ligases. In naming enzymes, the "-ase" suffix is often appended to the name of the substrate molecule upon which the enzyme reacts. For example, the enzyme sucrase catalyzes the transformation of the sugar sucrose into glucose and fructose. In this case, the "sucr-" suffix represents the molecule upon which the sucrase enzyme reacts. Not all enzymes are named according to this convention.

## 1.2. the structure and function of the alimentary canal.



The alimentary system has three distinct phases as it evolves to the final system in the adult bee.

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In the embryo the basic elements form but are not initially connected. The stomodaeum (fore gut) and proctodaeum (hind gut) form through invaginations of the ectoderm. The mesenteron (mid gut or ventriculus) forms from the endoderm that initially encompasses the yolk of the egg.

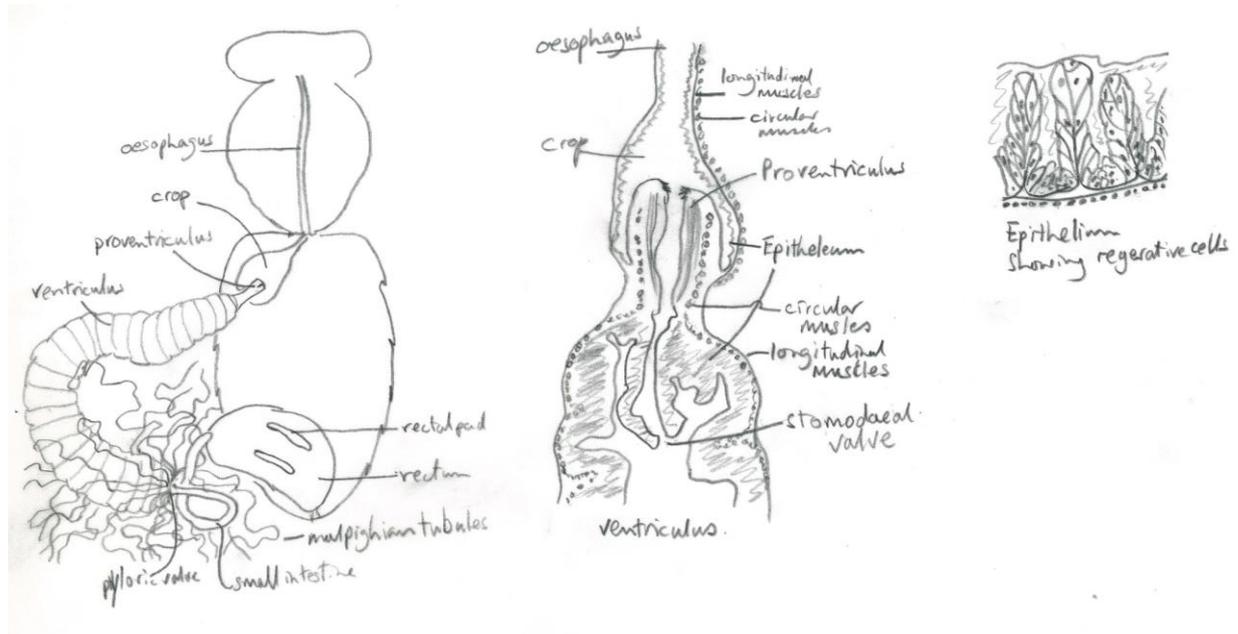
In the larva the stomodaeum is joined to the ventriculus via a basic valve, the proctodaeum is not connected to the mid gut and the 4 malpighian tubules terminate at a membranous thickening between the ventriculus and the proctodaeum.

During the pupa stage the alimentary system is completely remodelled:

- The stomodaeum becomes the pharynx, oesophagus, crop and proventriculus
- The ventriculus is remodelled to include muscles, folds and new lining
- The hind gut (joined to ventriculus at 5<sup>th</sup> moult) develops small intestine, over 100 malpighian tubules all joined at the pyloric valve, and rectum

The function of the alimentary system is to store and filter nectar, remove pollen, digest the pollen and nectar where appropriate. Collect waste from the haemolymph and debris from digestion, recover salt and water before passing on to the rectum.

### 1.3. the histology of the alimentary canal and the production of the enzymes of digestion.



#### Foregut

The cells of the foregut are flattened and undifferentiated as they are not involved in absorption or digestion. The cuticular lining is unsclerotized and consists only of endocuticle and epicuticle. The foregut comprises 4 regions:

- The pharynx
- Oesophagus
- Crop
- Proventriculus

The oesophagus is a narrow tube passing through the petiole. It is surrounded by an inner layer of longitudinal muscles and an outer layer of circular muscles, the antagonistic action of these muscles causes the contents to be moved towards the crop, this movement is called peristalsis. The inner

wall of the oesophagus is lined with a thick cuticular intima which allows for the expansion caused by peristalsis.

The crop is the extensible part of the foregut.

The proventriculus extends into the crop at its anterior end and into the Ventriculus at its posterior end, stomodeal valve. The proventriculus has 4 lips with slender recurved spines that work independently to filter out pollen from the contents of the crop. The wall of the crop is continually writhes and pulsates vigorously keeping the contents well stirred. The pollen is collected into a bolus before it is passed into the Ventriculus. The proventriculus prevents nectar being passed to the midgut unless required for digestion.

### **Ventriculus**

The Ventriculus is considered the true stomach of the honeybee. It forms a U shape within the abdomen, it has numerous transverse constrictions that form deep internal folds. When dissecting a bee the colour of the Ventriculus can be brown, this is due to the colour of the contents, usually it is pearly white when empty.

The epithelial wall of the Ventriculus is continuous throughout the midgut, it is surrounded by muscles circular surrounded by longitudinal (opposite to oesophagus). The epithelium cells are many and are continually increasing through mitotic division. Cells are cast off and degenerate releasing digestive enzymes and absorption nutrients. Other cells prevalent are enteroendocrine, which secrete hormones which regulate the function of the midgut and intestinal stem cells, from which differentiated cells are derived.

The peritrophic membrane separates the volume of the midgut from the epithelial wall, it comprises proteoglycan gel on a framework of chitin microfibrils. It is produced by the epithelial cells throughout the midgut. It allows passage of digestive enzymes to the ingested food and digested food out to pass through the epithelial to the haemolymph.

The enzymes produced by the epithelial include sucrose (breaks down sugars), amylase (breaks down starch), proteases (breaks down protein in pollen to amino acids), lipases (digest lipids to produce fatty acids and glycerol).

Absorption into the haemolymph is across enterocytes in the epithelial.

### **Hindgut**

The proctodaeum or hindgut is differentiated into two principle regions, the small intestine at the anterior end and the rectum at the posterior. The small intestine is a slender tube, the epithelial has 6 longitudinal folds, the outside is a thick layer of circular muscle (no longitudinal muscles), it joins the ventriculus at the pyloric valve.

The Malpighian tubules open into the pyloric lumen after the ventriculus and before the pyloric valve.

The small intestine opens into the rectum, there is no valve but a constriction of the internal folds reduces the aperture. The rectum is a large thin walled sac, the epithelial is highly folded, surrounded by circular muscles and smaller widely spaced longitudinal muscles. The rectum opens into a small tube to the anus. The structure of the rectum allows it to balloon and hold a volume of faeces.

Thickenings in the rectum wall (6 in total) are called rectal pads, their role is not clearly understood.

#### 1.4. ingestion, digestion, assimilation, and excretion in the honey bee.

**Ingestion** is the process by which food is taken into the alimentary canal. It includes the processes that take place while the food is in the mouth, the lubrication and chemical effects from the salivary and other glands. The swallowing of the food, which sends it onwards in the case of the honeybee to the Ventriculus.

The honeybee adds enzymes to breakdown the sugars within the nectar it collects within the mouth. The enzymes are produced in the salivary gland and hypopharyngeal glands and include sucrase, glucose oxidase and amylase.

The nectar is swallowed through a process known as peristalsis and mixed/stored in the crop of the honey bee. The food is filtered in the crop by the proventriculus and pollen removed from the liquid. The pollen as well as other impurities is passed into the Ventriculus in the form of a bolus.

**Digestion** is the process by which ingested material is broken down in the Ventriculus into a form that can be absorbed and assimilated into the tissue of the honeybee.

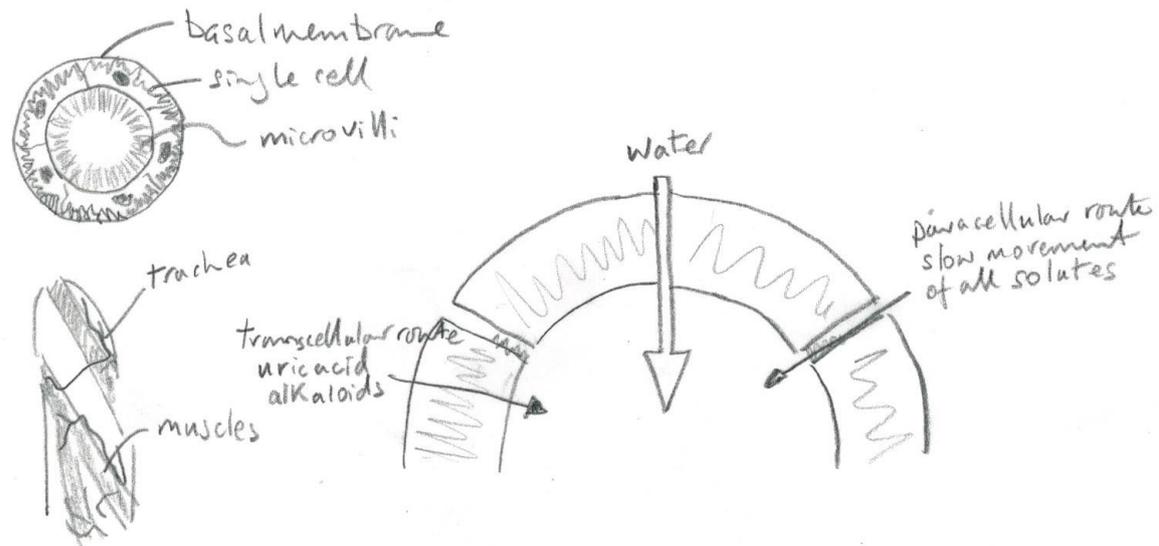
This is primarily performed through enzymes. The process starts in the mouth with the addition of enzymes from the head glands. Most of the chemical digestion processes occur within the Ventriculus where digestive enzymes are generated.

The ingested food bolus is surrounded by a peritrophic membrane which on the one hand protects the fragile epithelium and on the other hand permits the digestive enzymes to access the food. The enzymes enter the pollen through their pores and apertures and split the proteins within into their amino acids. The digested food passes through the peritrophic membrane to the epithelium to be absorbed into the haemolymph.

Assimilation is the absorption of digested food into the haemolymph for transport to the point of need within the body. The digested food include monosacarides, amino acids and lipids. Passive absorption across the epithelium via concentration gradients across the epithelium enterocytes cells.

Some absorption of foods minerals and water is also carried out in the small intestine.

**Excretion** of metabolic waste products is carried out via the rectum. First the products are collected from the haemolymph by the Malpighian tubules which wave in the haemolymph extending throughout the abdomen. The tubule has a single cell wall with an inner basal plasma membrane deeply infolded. The lumen of the cell is includes close packed microvilli. The tube is surrounded by tracheoles and muscles.



The tubule works through both passive filtration and active secretion from the haemolymph. The filtration is through the paracellular route (gaps between cells) and transcellular route (through cells), it is reliant up gradients across the epithelium. The issue with passive filtration is that good substances such as water and salts can be absorbed, these are reabsorbed into the haemolymph towards the base of the tubule.

The open end of the tubule connects between the Ventriculus and the pyloric valve where the uric acid from the tubule joins other waste such as pollen husks from the Ventriculus and passes into the small intestine.

Some further absorption of water and minerals occurs in the small intestine. The excreta is passed on to the rectum where it is stored. The excreta is evacuated as faeces by the honeybee outside of the hive.

### 1.5. the structure, function and histology of the respiratory system.

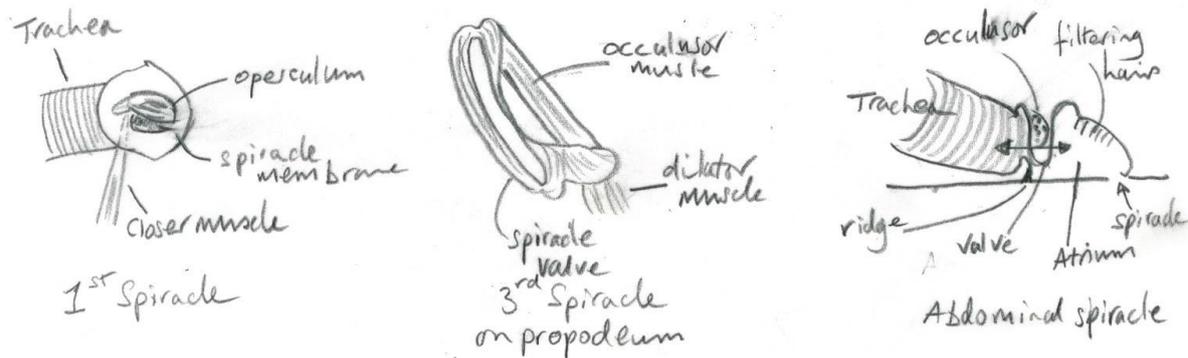
The elements of the respiratory system within a honeybee are:

- Spiracles
- Trachea
- Air Sacs
- Tracheole

Both the larva and the adult bee have 10 pairs of **spiracles**, from T2 – A8. The spiracle is to the anterior end of each plate, all bar T3 have valves that that open and close. T3 is the smallest and A1 on the prodeum is the largest. The spiracle on T1 does not fully close. Spiracle A8 is within the sting chamber.

The opening and closing of the spiracles is controlled by muscles. When is seen to land the contraction and relaxation of the abdomen can be clearly seen, this is the bee drawing in and expelling oxygen and carbon dioxide.

The three types of spiracle are shown in the diagram:



The **first spiracle** on T2 has the trachea connecting directly to the spiracle membrane. The spiracle is hidden under a spiracle lobe which is lined with fine hairs. The spiracle has an external closing mechanism, the operculum, a sclerotized plate that does not fully cover the opening. The operculum is controlled by an occlusor (closing) muscle which connects to a small arm beneath the spiracle membrane. When the muscle contracts the operculum closes over the opening and when it relaxes the operculum opens.

The **second spiracle** is small and permanently open so not discussed here.

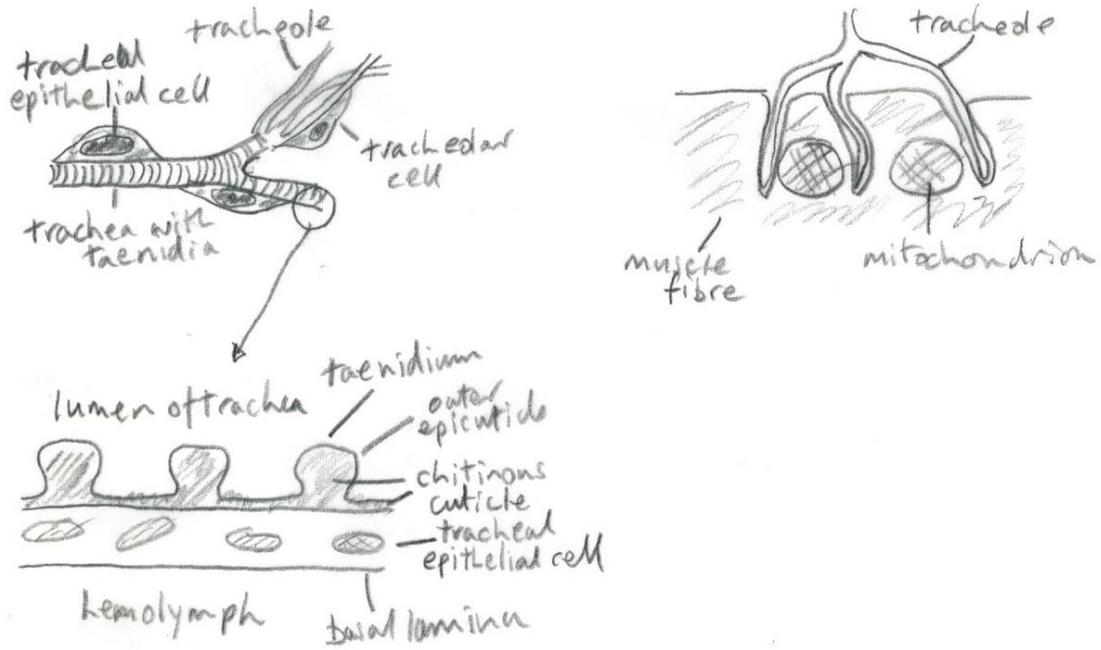
The **3<sup>rd</sup> spiracle** on A1 (propodeum) has a long aperture of 0.23mm by 0.06mm when open. It is surrounded by an elevated rim creating an external atrium. Within the entrance to the opening is controlled by two muscles connected to a valve made up of a soft integumental fold with a strongly sclerotized margin. The contraction of the occlusor (closing) muscle causes the valve to move forward closing the aperture, the relaxing of the occlusor and contraction of the dilator muscle causes the valve to release and reveal the aperture.

**Abdominal spiracle** has an internal atrium lined with filtering hairs. A domed shaped valve lies between the atrium and the trachea. Closing muscles move the valve towards the ridge and opening muscles pull the valve away from the ridge thus exposing the trachea.

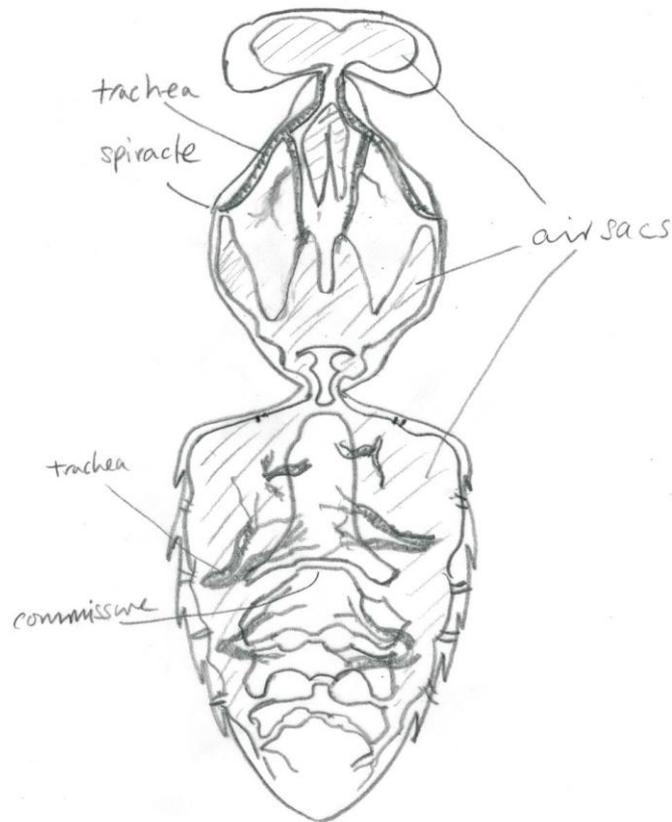
**Trachea** are formed from the invaginations of the ectoderm and thus have similar characteristics to the external cuticle of the bee. The trachea consists of an outer epicuticle with sclerotized protein/chitin beneath it, a spiral thickening of the intima runs along the trachea called taenidia. The taenidia contains protein/chitin that is sclerotized giving rise to strengthening microfibrils.

Along the trachea fine tubes called **tracheoles** arise. Tracheoles are blind-ended, air filled extensions of terminal tracheal cells and are the primary sites of gas exchange within the bee. The tracheoles are usually less than 2µm tapering to 0.3µm. some tracheoles retain their cuticle after moulting unlike trachea. Tracheoles are formed from tracheolar cells, they retain taenidia ridges but they do not contain the protein/chitin sclerotized matrix found in trachea.

There is a debate whether the tracheoles enter the cells of the receiving material or go between cells. The single cell lining of the tracheoles is very thin 16-20nm which gives a high surface to volume ratio which enables their high diffusion capacity. The diagram shows the tracheoles within invaginations of the flight muscle.



Throughout the abdomen, thorax and head there are **air sacs** which are trachea without or limited taenidia. This gives them the ability to expand with air and collapse when empty.



The air sacs provide a ready supply of oxygen to the brain and the muscles throughout the body. The air sacs in the thorax are supplied by the spiracles 1-3, the air sacs connect to air sacs in the head

and abdomen. From the air sacs are short trachea that deliver oxygen to tracheoles close to the point of need.

During flight and high activity air is mainly drawn in through spiracle T2 and exhaled from T3, during periods of inactivity has in drawn in and exhaled via T2. The abdominal spiracles are involved in intake of air during flight but by how much unknown.

Temperature affects the "breathing" activity of the bee. At 12°C there are no abdominal ventilatory movements. 12-15°C the abdomen has intermittent abdominal movements and above 28°C the movement cycles can be 100 per minute.

The purpose of the respiratory system is to deliver oxygen to the points of need and remove CO<sub>2</sub> this is carried out by nerves connecting to the ganglia recognising levels of oxygen and CO<sub>2</sub>. When CO<sub>2</sub> is high the ganglia cause the abdominal muscles to contract thus expelling the gas through the spiracles and relaxing the muscles to allow oxygen in.

### 1.6. the diffusion of oxygen through the trachea and its supply to tissues.

The ends of the tracheoles are filled with fluid. This end enters into the tissue. The ends of the tracheoles are also devoid of cuticle and therefore the respiratory surface is very thin making the diffusion of oxygen into the cells easy. As respiration occurs in the cell, the products of respiration accumulate in the cell and this forces the fluid in the tracheoles to enter the tissue. The exit of fluid creates low pressure in the tubes and draws in more oxygen to the tissues where it is needed. ???

### 1.7. the basis of cell respiration, glycolysis and energy transfer.

Definitions:

**ATP (adenosine triphosphate)** is the energy-carrying molecule used in cells because it can release energy very quickly. ATP can transfer energy and phosphorylate (add a phosphate) to other molecules in cellular processes such as DNA replication, active transport, synthetic pathways and muscle contraction.

**Mitochondria** are structures in the cytoplasm of all cells where respiration takes place (singular is mitochondrion).

The metabolic pathway involved in respiration can be split into three main parts:

**Glycolysis** - occurs in the cytoplasm.

**Citric acid cycle** - occurs in the matrix of the mitochondria.

**Electron transport chain** - occurs in the inner membrane of the mitochondria.

#### **Glycolysis**

Glycolysis is the breakdown of glucose into two pyruvate molecules. This process does not require oxygen (it is anaerobic). The glucose undergoes a series of chemical transformations.

#### **Citric acid cycle**

The citric acid cycle occurs after glycolysis only if oxygen is present (it is an aerobic process). The pyruvate enters the matrix of the mitochondria and carbon dioxide is removed.

### **Electron transport chain**

The electron transport chain is the last stage of the respiration pathway and is the stage that produces the most ATP molecules. The electron transport chain is a collection of proteins found on the inner membrane of mitochondria.

The electrons transfer their energy to the proteins in the membrane providing the energy for hydrogen ions to be pumped across the membrane. The flow of the ions across the membrane synthesises ATP by a protein called ATP synthase.

## **1.8. the production of carbon dioxide and its excretion from the body.**

During cellular respiration, a glucose molecule is gradually broken down into carbon dioxide and water.

The oxygen removed from the tracheal air space is not completely replaced by carbon dioxide, primarily because a large fraction of the carbon dioxide produced dissolves in the tissues and haemolymph (carbon dioxide, in contrast to oxygen, is highly soluble in biological fluids).

The tracheole is partially filled with liquid, energy production causes the fluid to be drawn into the muscle which enables oxygen within the tracheole to pass through the oxygen is replaced by CO<sub>2</sub>.

When internal oxygen tensions reach a low threshold, the spiracles begin to open slightly at high frequency (flutter phase), and tracheal pressures rise to near-atmospheric levels. During the flutter phase, high-frequency, but minutely sub-atmospheric, air pressures allow the animal to convectively take in oxygen with minimal emission of carbon dioxide. The spiracles remain sufficiently closed so that internal oxygen levels remain low. Carbon dioxide accumulates throughout the closed and fluttering phases, eventually triggering the spiracular open phase, when tracheal oxygen levels are restored to near-atmospheric partial pressures.

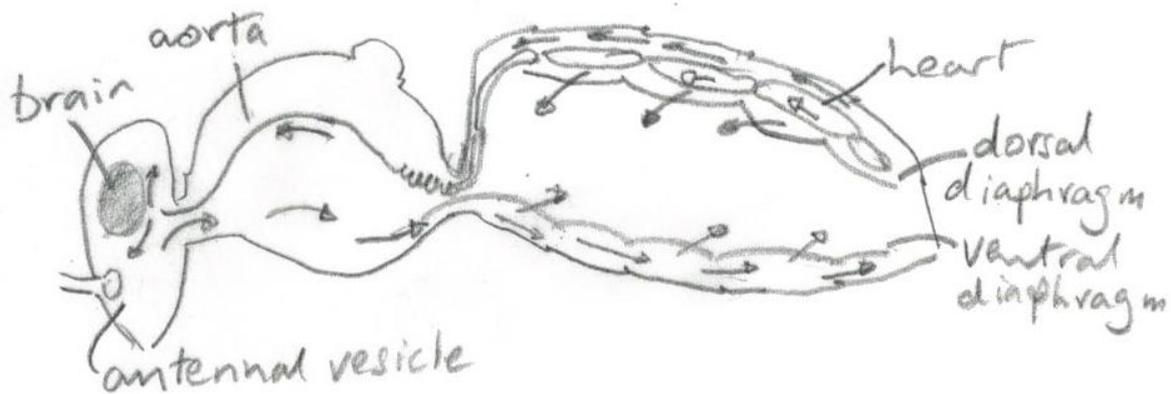
## **1.9. the structure, function, and histology of the circulatory system.**

The main functions of the circulatory system are to transport food from the Ventriculus to the body cells and transport waste products to the excretory system. This is achieved through a combination of muscular organs and diaphragms

The circulatory system of the honeybee includes:

- heart
- dorsal and ventral diaphragms
- aorta
- antennal vesicle

The circulatory system is an open system, with haemolymph circulated around the body via the movements of the diaphragms. The ventral diaphragm moves the haemolymph from the thorax.



The dorsal diaphragm moves the haemolymph forward within the abdomen. The heart closed at the posterior pumps haemolymph towards the aorta and onwards to the brain. The heart has 5 pairs of ostia which draw in the haemolymph, the valves close when the muscles forming the walls of the heart contract. The contraction drives the haemolymph forward.

Within the head there is the antennal vesicle which circulates haemolymph to the antennae, the vesicle is not muscular, the vesicle is connected by tissue to the muscles of the pharynx. Similar structures exist at the bases of the wings and legs to assist in pumping the haemolymph to the extremities.

Muscles in the mouth are able to increase blood pressure on the glossa, thus inflating it.

The dorsal diaphragm connects at points from terga segments A3 through A6. It is transparent, contains muscle fibres and supports the heart. Above the diaphragm is the dorsal sinus, movements in the diaphragm move the haemolymph forward in the sinus creating the current. A similar structure exists on the ventral side of the abdomen, except the ventral diaphragm starts in the thorax where it is attached to the endosternum of the mesothorax and metathorax and ends as two long prongs connected to the spiracle plates of segment A8.

The aorta is a narrowing of the heart that passes through the petiole, it is coiled as it enters the thorax (coiling is utilised for heat exchange between free flowing haemolymph and that within the aorta) before straightening out and opening near the brain.

### 1.10. the histology, composition and function of the blood of the honey bee.

The constituents of haemolymph include inorganic materials water, salts (sodium, chlorine, potassium, magnesium and calcium) and organic materials such as carbohydrates, proteins, amino acids and lipids.

Proteins and amino acids levels vary with the stage in the development of the bee. Proteins are specific to their function include chroma proteins, protease inhibitors, storage, lipid transport, enzymes and vitellogenins.

Hemocytes are cells that float within the haemolymph that play a role in the immune system of the honeybee.

Other organic material the haemolymph includes products of metabolism; ammonia, allantoin, uric acid and urea. Hormones are present in the haemolymph such as juvenile hormone.

The function of the haemolymph is to provide **transport** of food materials to the cells of the body and the transport of waste products to the excretory system. **Mechanical support** primarily in the larva as it does not have an exoskeleton, the support through contractions of muscles include the glossa and the endophallus of the drone. During moulting the haemolymph provides support whilst plates sclerotise. The balanced pressure of the haemolymph gives rigidity to the body parts, particularly the thorax. **Control of water content**, as the cells are bathed in haemolymph and the concentration of the materials within the haemolymph are maintained within strict bounds the cells always have sufficient water for their processes. Haemolymph does not transport **oxygen and CO<sub>2</sub>**, rather it facilitates the dilution of the gases to assist transfer. The haemolymph plays a role in the **defence against pathogen**, some proteins in the haemolymph (manufactured in fat bodies) will attack pathogens.

Two roles that haemocytes fulfil are:

- Destroying certain bacteria and parasites, this is done by a sub category of haemocytes called **phagocytes** and the process of attacking invaders is phagocytosis.
- Some phagocytes remove damaged tissues and some other haemocytes can temporarily plug damaged parts of the epidermis, this is effectively **wound healing**.

### 1.11. the exocrine glands of the honey bee, including their structure, histology and function.

The exocrine glands are defined as ones which release substance(s) to the outside world, the table below shows their presence in the two different castes and male honeybees.

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Gland	Located	Function	Composition	Caste
Hypopharyngeal	Front of head	Produces element of brood food in younger worker and enzymes in older worker  Gorging on pollen can cause gland to revert to food production	Young worker protein in form of clear liquid for making brood food  Sucrase and glucose oxidase in older/foraging worker	W
Mandibular	Above mandibles	Young worker, production of brood food and royal jelly  Mature worker, alarm pheromone issued by guard bees to ward off robbers and initiate stinging response from other bees  Queen, produces pheromones used in mating or part of queen substance	In young worker 10-HDO (10-hydroxydec-2-enoic acid) which is principle fatty acid in brood food and acts as preservative  Older worker 2-heptanone which is the alarm pheromone  Queen produces :  9-oxodec-2-enoic acid (drone attractant when mating)  9-hydroxydec-2-enoic acid (holds swarm together)	Q W D
Tergite (renner-Baumann)	Edges of abdominal tergites A3-5	Queen recognition, contributes to Queen Substance, emitted through Queen grooming and retinue palpating her abdomen with their antennae	Composition unknown	Q
Nasonov	Tergite A7	Location scent used when flying in swarm to attract other bees, marking the entrance to hive, marking source	Terpenic alcohols      Geraniol, Nerol and (E,E)-Farnesol  Terpenic aldehydes      (E)-Citral and (Z)-Citral	W

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		of water	Terpenic acids	Geranic and Nerolic acid	
Sting scent gland	Quadratate plates	Alarm pheromone, (Z).... attracts bees to sting site and stabilises Isopentyl which along with 2-heptanone elicits stinging response	(Z)-11-Eicosen-1-ol Isopentyl acetate		W
Sting acid (poison or venom gland)	Abdomen	Production of venom to be used in sting	Major ones: Mellitin (50% of dry weight), Phospholipase A, Hyaluronidase, Acid phosphatase, Allergen C		Q W
Sting Alkaline (Dufour)	Sting Chamber	Generally unknown but assumed to: lubricate sting mechanism, neutralise remaining acid and in queen protective coating to eggs or egg adhesive for cell floor	Unknown, white in colour and alkaline in nature		Q W
post cerebral	Behind brain	Salivary, no reservoir, secrete directly into outlet ducts	Water of Alkaline nature		Q W D
		Only vestigial in drone, equal development in Queen and Worker			
Thoracic	In the thorax	Salivary, developed from silk gland in larvae, have reservoir	Water of Alkaline nature		Q W D
Arnhart (tarsal glands)	5 <sup>th</sup> tarsome of each leg	Location (footprint odour) in worker bee  Constituent part of Queen Substance, queen emits factor x13 of rate of worker	Unknown except different in the two castes		Q W

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Wax glands      Sternites A4-7      Wax production

Downing (1961)

16% hydrocarbons

31% straight-chain monohydric alcohols

3% diols

31% acids

13% hydroxyl acids

6% other substances

MBBKA 2009

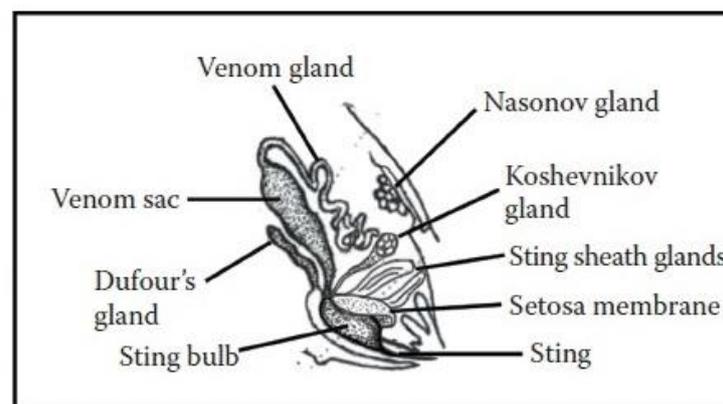
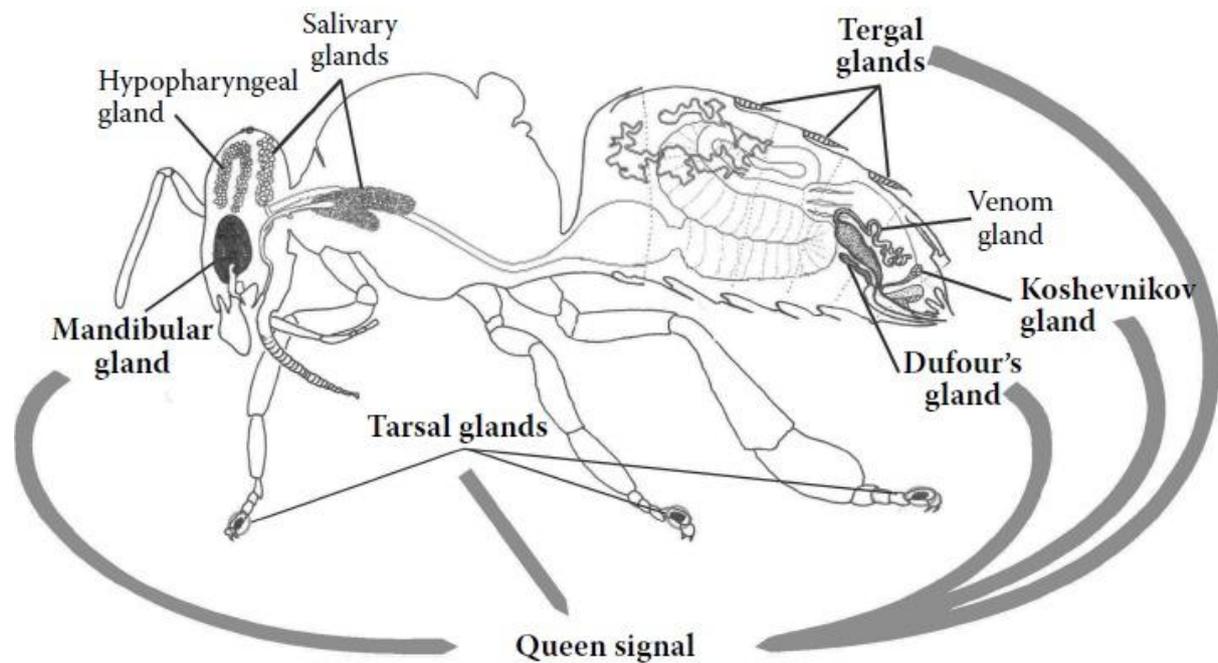
esters 70%

alcohols 1%

acids 10%

hydrocarbons 13%

W



### 1.12. the substances produced by the exocrine glands including a basic account of their chemical composition.

#### Post- cerebral and thoracic glands

Know collectively as the salivary gland. The post cerebral is located behind the brain where as the thoracic is located in the thorax. They produce a liquid passing from the glands into tubes that joins in the head passing under the pharynx and opening in the salivarium from where the secretions are used via the proboscis or in the pharynx to moisten solid food.

Secretion is mainly H<sub>2</sub>O but slightly alkaline. The thoracic glands are developed from the silk glands of the larva and have a reservoir preceding the outlet duct. They are present in Q.W.D. The post cerebral glands have no reservoir. They are equally developed in the Q and W but not in the D.

Post cerebral acini are more translucent than the creamy bodies of the hypopharyngeal gland and have a different characteristic shape. The acini are arranged in small bunches on a branching system of tubes which run into a median duct under the pharynx.

The thoracic glands are rather compact bunches of short cylindrical bodies arranged on branches of the main ducts and their secretions is stored in two small sacs from which main ducts pass to the median duct in the heads.

### **The mandibular glands**

The glands are in all three castes, the largest in the Q and the smallest in the D. Located one gland each side of the head above the mandibles.

The lumen of the gland acts as a reservoir under pressure and the outlet can be opened and closed allowing the secretion to be released as required.

The secretion runs down a groove in the mandible.

Function in the worker produces a white secretion containing 2- heptanone which acts as an alarm pheromone and 10 hydroxyl- 2 deconic acid (10 HDA) which is the principal fatty acid in brood food and royal jelly and acts as a preservative. The function of the gland changes with the age of the bee and the duties it is undertaking. 2- heptanone is not found in the secretion of very young bees. Only as they start foraging does the pheromone appear i.e. after nurse duties.

Function in the Q: produces queen substance, the main components being

1. 9-oxo-2-deconic acid (attracts drone for mating)
2. 9-hydroxyl-2-deconic acid (hols swarms together)

Inside the hive Q. substance inhibits the building of Q. cell inhibits the development of the workers ovaries and the workers are attracted to the queen.

### **The Hypopharyngeal glands**

The glands one on each side, of the workers head, each having it own duct and connected to the hypopharyngeal plate. The two connections are on the underside of the plate and the secretions runs down the upper side and collects in the labial base.

The secretion is led to the larvae the bees using its mandibles to direct the brood food into the cell. Each gland consists of 100's of pear shaped acinis each with a short duct connecting to the main axial duct. They are no apparent opening or closing valves on these glands but it is being suggested that the movement of the hypopharyngeal plate could kink the duct and stop the flow. The size of the gland depends on the duties and it is at its largest where the bee is a few days old working as a nurse bee. The glands produce a clear liquid (protein) which combines with secretions from the mandibular glands and is known as brood food or royal jelly.

Later these glands shrink and are the source of invertase in the foraging bee.

It is possible for a foraging bee to reactivate its food gland by consuming large quantities of pollen. Nosema tends to atrophy the glands prematurely.

Viruses causing ABPV and Sacbrood are both to be found in the hypopharyngeal glands. Sacbrood virus is probably fed to the larvae by the infected nurse bees. Brood food is made up of proteins, several vitamins of the B group, vitamins C and D but not E, 10HDA from the mandibular.

### **The Renner Baumann glands**

Located on the free edges of the abdominal tergites A3, A4 and A5 of the queen.

The court bees pay particular attention to the queen's abdominal.

The Q. grooms herself 3-4 times every hour spreading a combination of her pheromones over her exocuticle.

The pheromones from the tergite glands and the mandibular inhibit ovary developments in worked bees, inhibit the building of Q cells and stabilise the court of bees.

### **The Nasonov gland**

Discovered by Nasonov (Russia) 1883. Known as the "come and join us" scent

Easily observed when a worker bee flexes the top of its abdomen downwards. The Nasonov gland is then exposed on the dorsal surface of the 7th abdominal segment. It consists of glandular cells which secrete pheromone through c. 600 ducts into a groove between the 6th and 7th tergite.

Honeybees release pheromone from this gland when:

- Flying in a swarm to attract other bees
- Marking the entrance to the hive
- Marking a source of H<sub>2</sub>O or when they are foraging for water or syrup (odourless)

Components in the pheromone are terpenic alcohols, aldehydes and acids. A synthetically produced Nasonov pheromone can be used to attract a honey bee swarm to an unoccupied hive or a swarm-catching box also to attract bees to crops for pollination. Synthetically produced Nasonov consists of citral and geraniol in a 2:1 ratio.

### **The sting scent gland**

The glands are believed to be located on the inner surface of the quadrate plates. The pheromone is a powerful alarm pheromone the main chemical being isophentyl acetate which inhibits foraging and scenting.

This alarm pheromone elicits a stinging response in other bees and recruits other workers to act as guard bees. It should be noted that bee venom does not elicit a stinging response, only the alarm pheromones do this i.e. isophentyl acetate and 2- heptanone.

### **Arnhart gland**

Found on the 5th tarsomere of each leg of Q and W. Produce foot print odour. Essential for suppressing q cell production (cells above q excluder).

Foragers leave a foot print odour on flowers they visit. Lasting for 4 hours at 25°C and 4 days at 50°C

### **The sting glands (acid and alkaline)**

The main glands associated with the sting normally the acid gland (venom) and the alkaline gland (Dufour). The acid gland lies in the posterior part of the abdomen at the end of each long tubule. At the end of each tubule a glandular enlargement secretes venom which passes into the tubules. Before entering the poison sac these tubules combine into a common duct. This duct joins the anterior end of the poison sac, where the venom is stored until required. The poison sac tapering in

shape at posterior end discharges directly into the bulb of the sting shaft. There are no valves or closing mechanisms. Venom is expelled by the action of the butterfly valves associated with the bulb.

The alkaline gland is a short thick convoluted tube which opens ventrally at the base of the sting. White in colour and the secretion is alkaline in nature. Acts as a lubricant for the sting mechanism. Neutralising any remaining acid after the sting has been used, in the Q as a protective coating for the eggs or as an adhesive to stick the eggs to the cell floor.

The venom gland starts to produce venom just prior to emergence and reaches a maximum in about 12 days. It ceases secreting at about 20 days just before it becomes a forager. In total about 300mg of venom is produced and old bees cannot replenish it. Winter bees retain their ability to produce venom until the following spring. About 50-100mg is used in a sting.

The main constituents are as follows

Phospholipose A	Mellitin
Hyaluronidase	Mellitin-F
Acid phosphatase	Apamin
Allergen C	Most cell degranulators (peptide, secopin and tertins)

### Wax glands

Wax is secreted from four pairs of wax glands located inside the exoskeleton on sternites A4-A7 inclusive. These are the last visible segments on the ventral side of the bee's abdomen.

Internally, lying over each gland is a large cellular mass composed of oenocytes and fat cells. There are two glands on each sternite making 4 pairs in all.

The glands secrete a liquid which passes through the mirrors and oxidises as a flake of wax in the wax pockets. The glands, mirrors and pockets are known collectively as the "waistcoat pockets"

Wax is secreted at relatively high temp 33-36oC after consumption of large amounts of honey 1lb honey to 5-8 lbs wax.

Wax glands are best developed in worker bees 12-18 days old. When building combs bees hang in festoons near the building site after gorging themselves with honey, waiting for the wax to form.

The wax glands inside the exoskeleton are covered with far bodies.

The main constituents of wax are

Monohydric alcohols	31%	S.G-0.95
Fatty acids	31%	Melting at-64oC
Hydrocarbons	16%	Solidify-63oC
Hydroxyl acids	13%	Colour- white translucent
Other substances	6%	Insoluble in H2O
Diols	3%	slightly soluble in cold alcohol
		Soluble in Chloroforms ethers or benzene

Wax is normally white in colour but can be tinted with a yellow hue caused by pigments that originate in pollen (dandelion new comb is coloured yellow)

In new emerged workers the wax gland consists of flat epithelial cells

By day 14 these cells have enlarged longitudinally and are richly supplied with trachea from which the tracholoes branch out to penetrate into the cells and into the intercellular spaces.

Young and old bees cannot produce wax.

Queenlessness inhibits wax production

12-18 day best aged for wax production

### **1.13. the structure and position of the main endocrine glands, namely the corpora allata and the prothoracic gland of the larva.**

The endocrine glands of the larva comprise corpora cardiac, corpora allata and prothoracic glands.

The corpora cardiac appear to be little developed, difficult to impossible to see in a dissection. They appear as small masses of cells closely attached to the aorta. The aorta is enlarged and open above the oesophagus. Each cardiacum is connected via a single nerve fibre to the brain. The cells of the cardiacum resemble excretory cells of the brain. The nerve fibre continues from the cardiacum to the associated corpora allatum.

The corpora allata sit either side of the oesophagus, they are relatively large globular bodies. Composed of large polyhedral, strongly eosinophile cells enveloped in a thin membrane.

The prothoracic glands are located at the sides of the Ventriculus close to the 1<sup>st</sup> spiracle. They are connected to the sub-oesophageal ganglia. Their shape are elongated tubes.

The corpora allata and prothoracic glands work antagonistically and are key during the process of moulting in the larval stage.

The prothoracic gland begins to degenerate during the pre-pupal stage of development.

Based upon signals from the brain the corpora allata secretes juvenile hormone which suppresses the initiation of the moulting process. The moulting process is initiated in response to ecdysone secreted by the prothoracic gland. Both are hormones and are secreted by their respective gland into the haemolymph.

The brain responding to nerve sensors relating to cuticle stress reduces the production of the juvenile hormone, the ecdysone dominates causing the larva to enter a moulting stage.

### **1.14. the source, production, and effects of juvenile hormone.**

The source of the juvenile hormone is the corpora allata.

Production is initiated in response to nerve signals received from the brain via the corpora cardiac.

The effect is to maintain the larval status of the larva, i.e. no moulting.

### 1.15. the source, production, and effects of the moulting hormone ecdysone.

The source of ecdysone is the prothoracic glands.

It is produced in response to a hormone released by the corpora cardiaca in response to neurosecretory cell activity in the brain.

The hormone released by the corpora cardiaca is received by the prothoracic gland via the haemolymph.

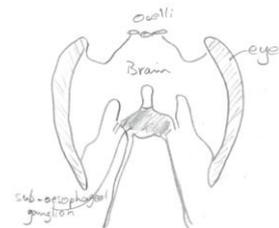
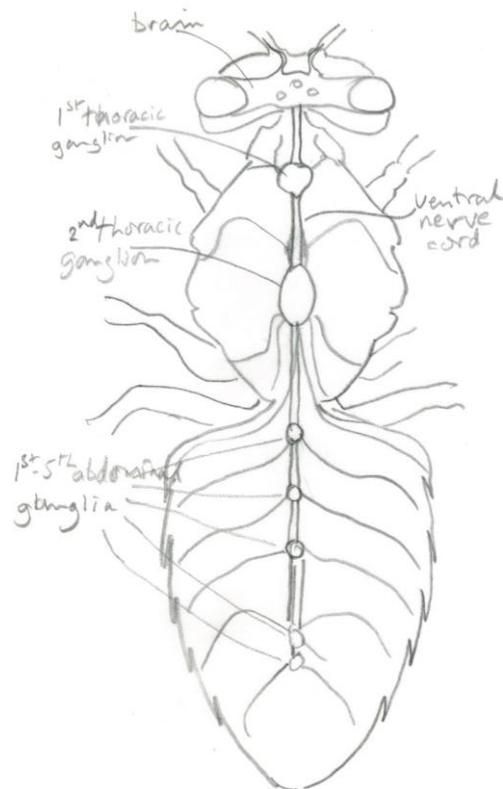
The ecdysone causes the production of enzymes that initiate moulting.

### 1.16. the structure of the nervous system of the honey bee.

The nervous system of the honeybee comprises three key elements:

- the central nervous system
- nerves
- sensed organs

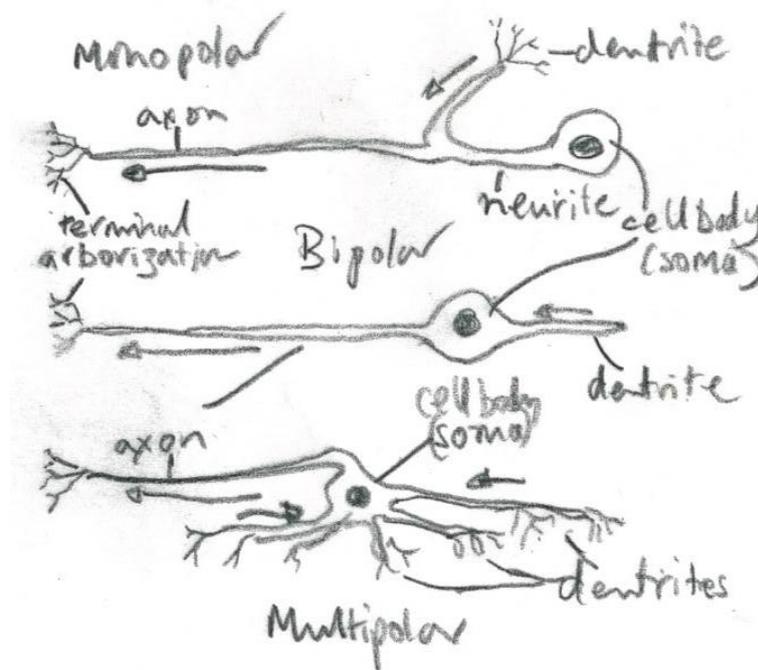
The Central Nervous System is made up of the brain and sub-oesophageal ganglion, the segmental ganglia and the ventral nerve cord.



The head of a honeybee is derived from the fusion of 6 segments each of which would have had an associated ganglia, the brain is formed from three of the ganglia and the sub-oesophageal ganglion from the remaining three. The brain and sub-oesophageal ganglion are joined by thick nerve cords and surround the oesophagus.

There are 7 pairs of segmental ganglia, 2 in the thorax and 5 in the abdomen. The ganglia are situated on the ventral side of the honeybee. The ganglia are interconnected by the ventral nerve cord which is in fact two cords of nerve fibres.

### 1.17. the basic function of nerve cells.

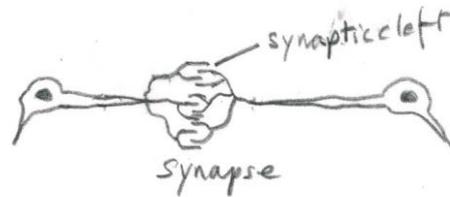


The nerve cell or neuron is the basic element in the nervous system, it consists of a cell body (soma) which has one or more cytoplasmic projections which make contact with other neurons or with organs of the body usually muscles. The cell body or soma, has a nucleus surrounded by cytoplasm.

The purpose of the neuron is to pass information from one cell to another.

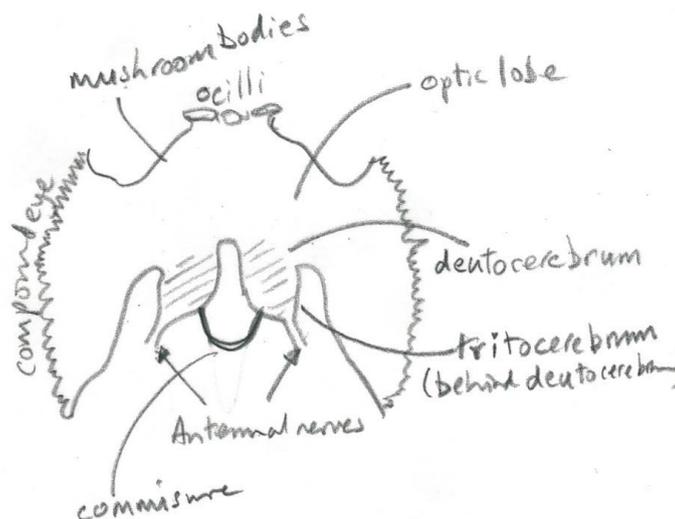
Dendrites specialise in the reception of information and axons in the transmitting of information. Generally they terminate in a branches, shown above as terminal arborisation.

The majority of cells in an insect are monopolar, with one neurite projection that branches to form axonal and dendritic regions. Sense cells are bipolar with short and usually unbranched dendrite(s). Multipolar neurons are associated with ganglia and stretch receptors.



The point at which neurons pass on or receive information via chemical messengers is known as the synapse. The plasma membranes of the two cells lie parallel to each other separated by a gap of 20-25 $\mu\text{m}$  which is known as the synaptic cleft.

### 1.18. the brain, its structure, function and histology.



The brain comprises the Protocerebrum, Deutocerebrum and Tritocerebrum.

The largest element is the **protocerebrum** which in the adult bee consists of the optic lobes, mushroom bodies and central complex:

**Mushroom bodies** or corpora pedunculata are involved in olfactory, learning and visual learning of the bee, there is some evidence that they have a role in decision making. The mushroom bodies in forager bees are more evident than in nurse bees.

The **central complex** spans the midline of the protocerebrum. The region contains neurons known to respond to mechanosensory, visual and chemical stimuli. The role of the region is not fully understood but is known to be involved in the monitoring and organising motor patterns used for

instance in walking. The region also has significant connectivity with the dorsal rim of the compound eye which is sensitive to polarised light which is used as a compass and an aid to navigation.

The **optic lobes** are lateral extensions of the protocerebrum to the compound eyes.

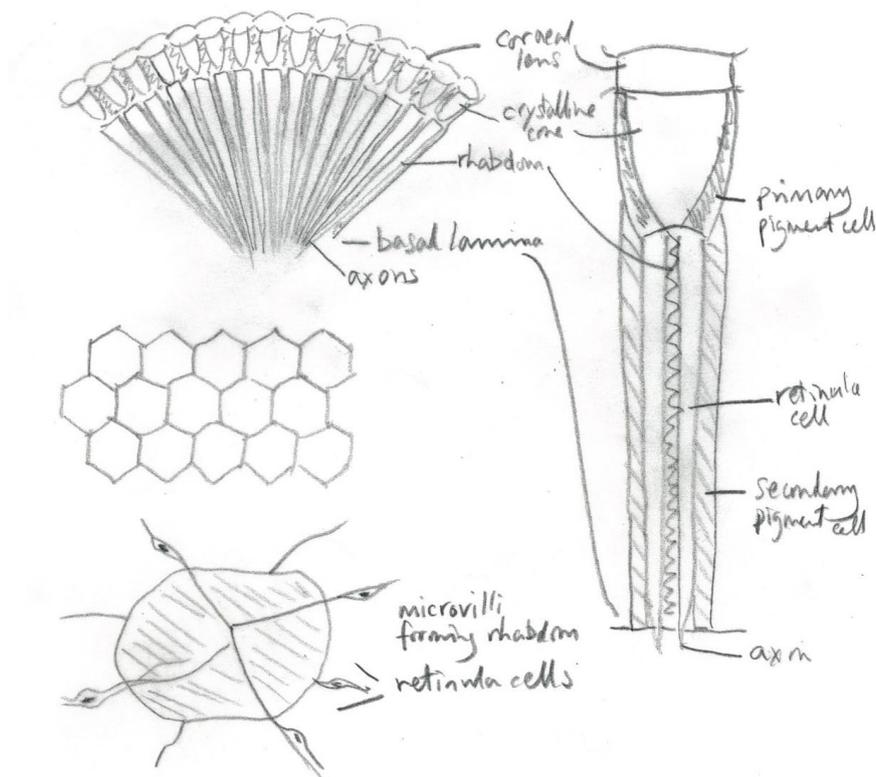
The **deutocerebrum** contains the antennal lobes and the antennal mechanosensory and motor centre. The antennal nerves terminate in the deutocerebrum.

The **tritocerebrum** is the small part of the brain consisting of two lobes sitting beneath the deutocerebrum. The circumoesophageal connectives to the sub-oesophageal ganglion come from the tritocerebrum. The two tritocerebrum lobes are connected by a commissure that passes behind the oesophagus. Nerves containing sensory and motor elements ultimately connect to the labium and the first thoracic ganglion.

### 1.19. the eyes of the honey bee, including development, structure and histology.

The honey 2 compound eyes and 3 ocellus (simple eyes).

Each compound eye is made up of 5-6,000 individual lens called ommatidium which are connected to the brain via neurons.



The development of the ommatidium arises from a thickening of the ectoderm over the surface of the optic lobe. In a newly hatched larva the ommatidium is not apparent, as the larva develops the cells join to form spindle shaped cells whose ends send out processes into the optic lobe. These cells form the retinulae, the retinulae cells move inwards away from the cuticle with the space being filled with other cells that form into groups of 4 above the retinula. The cytoplasm of the 4 cells converts

into a vitreous substance, forming the corneal lens. 2 further cells surround the lens forming the primary pigment cell.

The secondary pigment cells enclose the retinula. The retinula is formed initially of 8 cells and ultimately of 9 cells that are twisted around a core. The cells join on the axis, around the axis microvilli from each cell are orientated forming the rhabdom.

The cells that form the retinula have differing sensitivities to light wavelengths, 2 green, 2 blue, 2 ultra violet light and the 9<sup>th</sup> short cell polarised light.

The microvilli within each cell contain a photopigment called rhodopsin, this reacts with light causing a signal to be passed to the optic lobe.

The compound eye is fully formed by the pupal stage.

Summary of components:

Coneal lens, cuticle thickening

Crystalline cone, 4 cells with vitreous centre

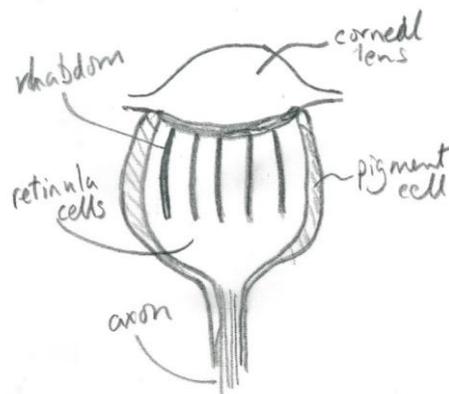
Primary pigment, 2 cells containing light in ommatidium

Retinula, 9 twisted cells 8 long one short except at eye edge 9 long

Rhabdom, microvilli of retinula cells reactive to differing wavelengths

Rhodopsin, pigment in microvilli creates energy for generation of information passed to optic lobe

Hairs protrude on surface of compound eye, no sensory purpose, disappear in older foragers



The ocelli are three simple eyes (ocellus) on the forehead of the bee. They do not focus light like the ommatidium, rather are more concerned with the recognition of the presence of light and UV light.

Like the ommatidium the ocellus has a corneal lens formed from thickened transparent cuticle. Beneath the lens are transparent cells are approximately 800 light sensitive retinal cells in a layer.

The grouping of 2-3 retinal cells which is called retinula again on an axis forming a rhabdom.

The retinula have axons that connect to the brain.

The ocellus form early in a young pupa as a thickening of the epidermis, even at an early stage some cells differentiate light and connect to the brain the optic nerve.

## 1.20. the sight of the honey bee and of its perception of colour, polarised light and form.

Honeybee sight is shifted towards the ultra violet end of the spectrum, red is seen as black (devoid of colour) and the bee is able to perceive ultra violet sensitive patterns on plants.

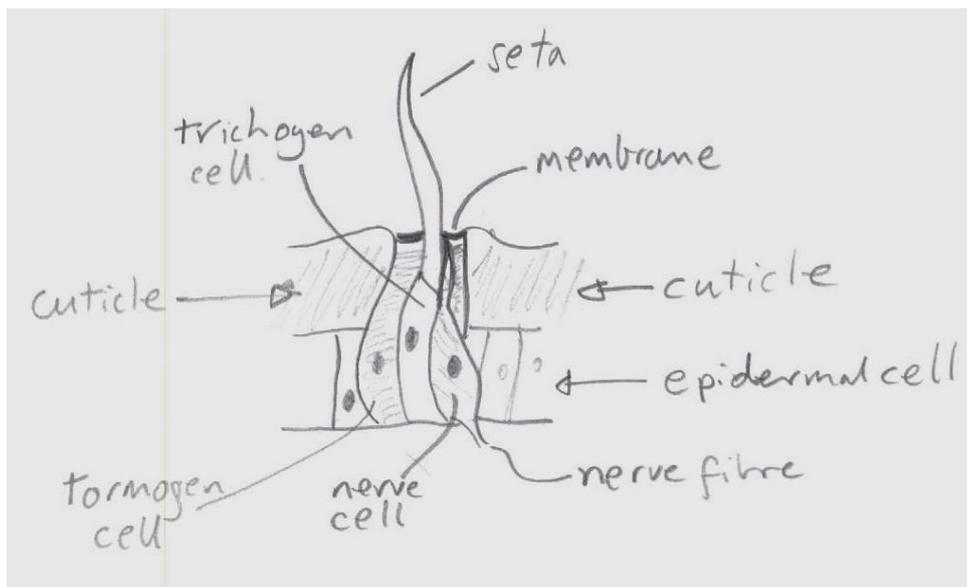
Each ommatidium focuses an inverted image at the tip of the rhabdom, because the rhabdom are fused rhabdomeres (microvilli of single cell) the image is blurred. Collectively the rhabdoms across the ommatidia transmit an erect mosaic image made up of the adjacent contributions from all ommatidia.

Colour is determined by the sensitivity of the rhodopsin as described above.

Polarised light through ommatidia on the periphery of the eye, the 9<sup>th</sup> retinula cell extends the full length of the sensory part of the ommatidium.

## 1.21. the structure of the sensory receptor organs of the honey bee.

Sensilla is the generic name given to the sense organs, below is the structure of a sensillum trichodeum which employs a hair as the principle structure. All sensilla have a similar structure with principle structure changing with the particular role.



The trichoid sensillum is principally a seta or hair which when it moves causes a pulse to be generated within the nerve cell and onwardly transmitted to the "brain".

The seta protrudes above the cuticle and is produced by the trichogen cell. Butting up to the seta is a thin membrane which is produced by the tormogen cell, the membrane allows movement of the cuticle.

The nerve cell thins and runs along the base of the seta terminating in the scolopale which picks up the movement in the hair in the dendrites within the scolopale. Movement causes an impulse to travel along the dendrite and onto the central nervous system. Some impulses are dealt with by the

local ganglion rather than the Brain, this depends on which sensillum is activated and what response is required.

## 1.22. the main senses of the honey bee namely smell, taste, touch, hearing, gravity perception, heat perception and time sense.

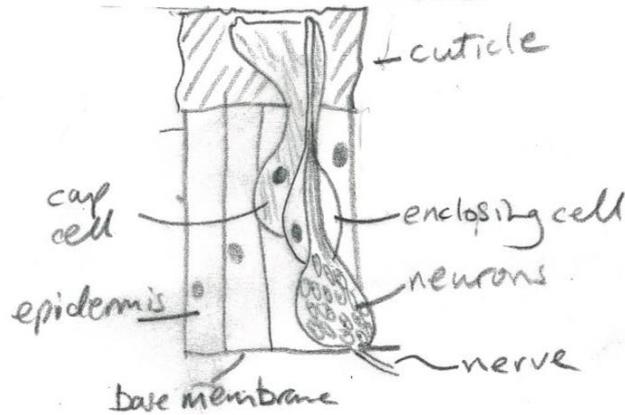
Summary of senses and mechanism employed:

<b>Sense</b>	<b>Device/Organ</b>	<b>English descriptor</b>
Smell	Sensilla placodea	Plate organ
Taste	Sensilla basiconica	Peg organs
Touch	Sensilla trichodea	Hair
Hearing	Sensilla scolopophora	Subgenual organs
Gravity perception	Proprioceptors	Hair plates of mechanoreceptors
Heat perception	Sensilla coeloconica	Pit-peg organs
Time sense	X and Y type receptors	POL area of compound eye . Narrow band of ommatidia on dorsal rim rim 2-3 deep

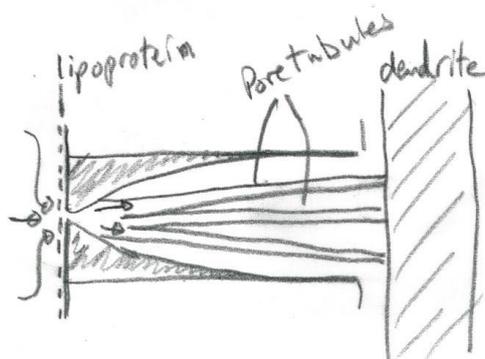
### Smell

The majority of sense plates (sensilla placodea) on the antenna of a bee varies by the sex and caste of the bee. On a single drone antenna there are approximately 500,000 sensilla, of which 30,000 are sense plates. The queen has 2-3,000 and the worker 5-6,000 sense plates.

The sensillum placodeum is shown below.



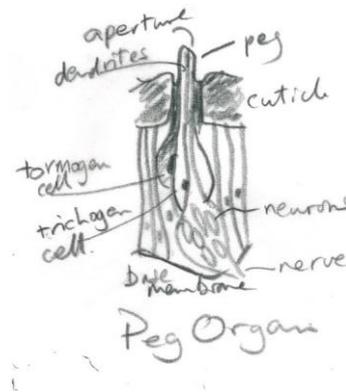
There can be up to 50 nerve cells within a single sense plate. The dendrites within the enclosing cell extend around the edge of the cavity formed by the cap cell. The cuticle over the cap cells is porous with some 2,400-3,000 pores. The image below shows the structure of a pore.



Lipoprotein traps the molecules over the surface of the of the plate, the molecules pass through the pore tubules to the surface of the dendrite. The same structure operates for seta sensilla.

**Taste**

Sensilla basiconica or peg organs are generally associated with taste, they exist at the terminal 8 sub sections of the flagellum and in the mouth of the bee. There are 3-5 nerve cells within the organ and the dendrites extend to apertures at the end of the peg. The structure is very similar to the trichodea with the peg being a very short hair.

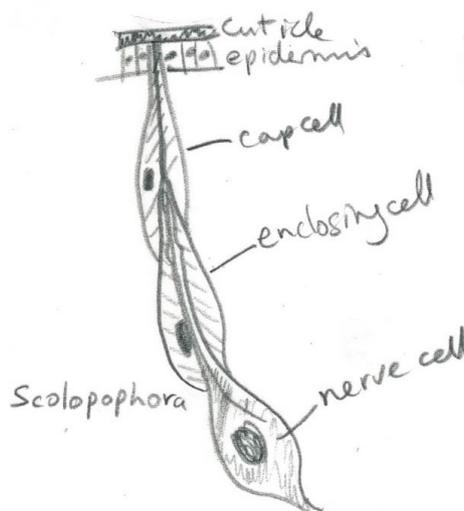


### Touch

This sensilla is shown above and is found all over the body. There are 5 different types of trichoid sensillae ranging in form from short, slender, flexible to long, stiff, thick walled sensillae.

### Hearing

The Sensilla scolopophora is associated with hearing, the organs differ from others in that they are elongated and the dendrites are embedded in two moveable parts that make them sensitive to vibration or movement. The subgenual organs are present on the legs of the bee in groups. The Organ of Johnston on the distal end of the pedicel is a sensilla scolopophora.



### Sense of Gravity

The bee has "hair plates" at critical junctures of the body:

- in the neck region
- at the petiole
- where the legs meet the thorax

The plates are groupings of mechanoreceptor sensilla, they act as proprioceptors which detect changes in the body. For instance the head of the bee hangs differently if it is facing up or down a comb. The hair plate therefore can detect the change and relate it to gravity.

### Heat perception

Sensilla coeloconica are peg like sensilla but the peg is sunk into a cavity, they are believed to be responsible for detecting carbon dioxide levels, temperature and relative humidity.

There are more than one nerve cell per organ, the organs appear in groups and are present at the distal edge of the flagellum.

### **Sense of time**

The sense of time is not the result of the activity of an organ, rather the perception of UV and polarised light and the learning of the trajectory of the sun.

The honeybee cannot see the sun, rather it is aware of a space in the ultra violet and polarised light that it can detect. There is a thin band of ommatidia on the dorsal edge of the compound eye that have 9 cells in their retinula the full length of the sensory part. The ommatidia are also raised with grooves in the lens. This gives the ommatidia a wider vision and enables them to perceive light rather than focus on it.

The forager bee over the first few days outside the hive learns the trajectory of the sun, along with the change from night to day it is able to “predict” the time of day based upon where the sun is in the sky.

## **1.23. the structure, function, and histology of the fat body of the honey bee.**

Fat body consists of thin sheets or ribbons, usually one or two cells thick, or of small nodules suspended in the hemocoel by connective tissues and trachea. The fat body is generally found in the abdomen.

Within the honeybee there are three key fat bodies:

- trophocytes
- oenocytes
- urate cells

Urate cells exit in the larva and pupa, they store nitrogenous waste in the form of uric acid. Once the adult Malpighian tubules form the role of the urate cells disappears and so do the cells.

The principle fat body cells are trophocytes.

The fat bodies of the larva die off and are completely replaced with new fat bodies in the adult. During the larval life the fat bodies build up, they contain oil globules, glycogen and protein. During the pupa stage the fat bodies disintegrate releasing their content into the haemolymph.

In the adult bee the fat cells contain fat and protein, the mix differs by age and season:

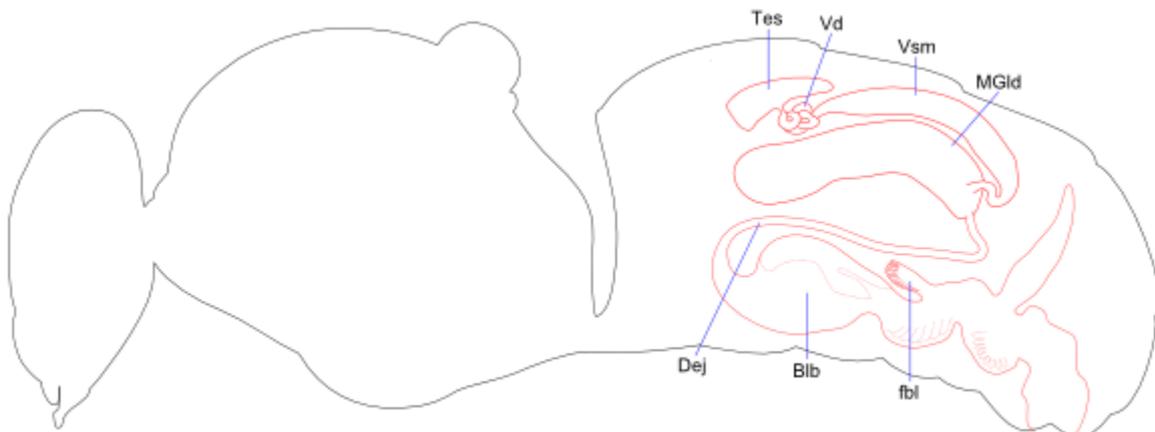
- young worker mainly fat globules
- older bees protein levels increase due to not feeding young
- mainly protein in fat body

Oenocytes are generally responsible for lipid processing and detoxification. In the worker honeybee they are found in great quantity above the wax glands and in the queen is part of the yolk production for eggs.

### 1.24. the changes which occur in the fat body of the worker honey bee both during its life and at different times of the year.

Stage	Cells Present	State of cells
Hatched larva	Trophocyte, Oenocyte, Urate	Oil globules, very few
Growing larva	As above	Cells increase in number and size
Larva 4 <sup>th</sup> moult	As above	White colour of larva fat body Body cavity full of fat body Contain oil globules and glycogen
Prepupa	As above	Fat body in haemolymph Protein increases in cells
Pupa	Disappear	Cells disintegrate releasing carbohydrate and proteins into the haemolymph
Adult	Trophocytes, oenocytes	New fat bodies built, see above for content

### 1.25. the reproductive system of the drone honey bee including structure, development and histology.



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Reproductive system of honey bee drone.

- Blb - bulb of penis
- Dej - ductus ejaculatorius
- fbl - fimbriated lobe of penis
- MGld - mucus gland
- Tes - testis

02/04/2025

Vd - vas deferens

Vsm - vesicula seminalis

The testes, vasa deferentia, seminal vesicles and mucus glands are formed in the embryo. The ejaculatory duct is a tubular ingrowth of the ectoderm that eventually opens into the mucus glands. The primitively external opening of the ejaculatory duct is the male gonopore, the penis is formed as a second ingrowth of the ectoderm that carries the gonopore inward.

In the larva the testes are a pair of elongated bodies lying on either side of the heart. Each testis opens to a rudiment of the vas deferens. The testis continues grow and with greatest development in pupa stage. After emergence as an adult bee the testis begin to shrink. When the drone reaches sexual maturity 12 days after emergence the testes have shrunk to a third of their size.

Male germ cells known as the primary spermatogonia divide into secondary spermatogonia which become encased in a thin walled cyst where it develops into spermatocytes. The spermatocytes convert into spermatids which appear as ordinary cells, the spermatids develop into spermatozoa. Spermatozoa have the form of long threads with a swelling or head. This final development is achieved 4 days before emergence.

The spermatozoa goes down the vasa deferentia into the seminal vesicles three days after emergence.

The vasa deferentia is a narrow coiled tube leaving the testis, leading to the enlarged sausage shaped vesicular seminalis. The spermatozoa embed their heads in the epithelium and take their final form.

The vesiculae seminales join the mucus glands, the glands are filled with a slightly alkaline liquid that quickly coagulates on contact with water or air. The mucus glands increase in size during the 1<sup>st</sup> 9 days after emergence.

The ejaculatory duct is a long slender tube from the anterior end of the inverted penis to the base of the mucus glands junction. Until eversion there is no link between the duct, mucus gland and the vesiculae seminales.

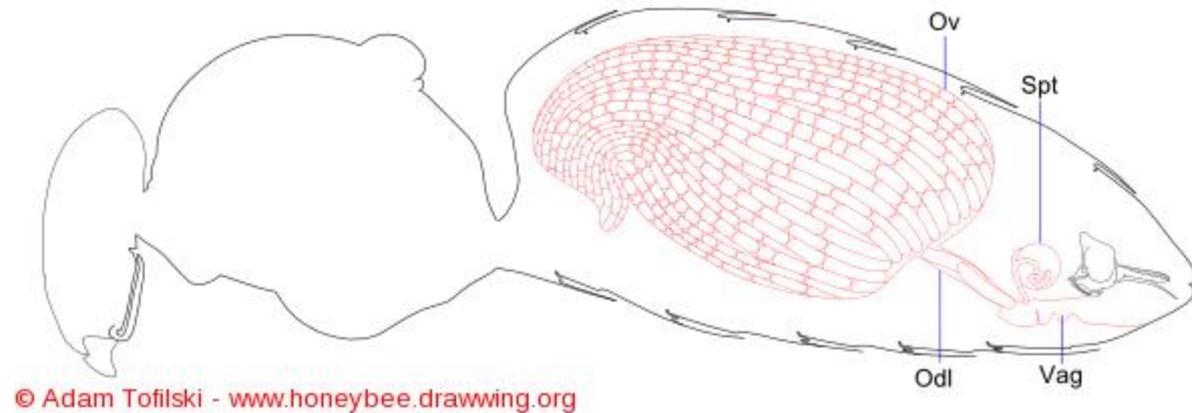
The endophallus connects to the ejaculatory duct at a swollen structure known as the bulb. The elements of the endophallus include:

- the cervix
- the vestibule
- the phallotreme

The cervix has sclerites on the surface, the phallotreme is the opening to the outside. On the phallotreme there are a pair of horns known as cornu.

The act of mating causes the endophallus to evert, the phallotreme becoming the base, the ejaculatory duct form a central tube, the muscles on the vesiculae seminales forcing the spermatozoa out followed by the mucus.

## 1.26. the reproductive system of the queen and worker honey bee, including structure, development and histology.



The reproductive system of the queen bee comprises:

- a pair of ovaries which each contain 150-180 ovarioles
- lateral and median oviducts
- vagina
- bursa copulatrix
- spermathecal

The ovaries occupy a large part of the abdomen and the ovarioles are capable of producing millions of eggs over the lifetime of the queen.

The unfertilised eggs travel via the lateral oviducts and median oviduct to the vagina. Within the vagina there is a fold which the queen employs in the process of egg fertilisation. The egg passes through the bursa copulatrix as it laid in a suitable cell.

Above the valve fold in the virgina lies the spermatheca, which connects to the vagina via the spermathecal duct. Within the duct there is a valve and pump that controls the release of sperm to the vagina.

On the surface of the spermathecal there are two spermathecal glands which provide nourishment to the sperm within the speratheca. The spermathecal can store and nourish about 6 million sperm following mating and for the lifetime of the queen.

## 1.27. spermatogenesis in the drone honey bee.

**Spermatogenesis** is the process in which spermatozoa are produced from spermatogonial stem cells by way of mitosis and meiosis. The initial cells in this pathway are called spermatogonia, which yield primary spermatocytes by mitosis. The primary spermatocyte divides meiotically (Meiosis I) into two secondary spermatocytes; each secondary spermatocyte divides into two spermatids by Meiosis II. These develop into mature spermatozoa, also known as sperm cells. Thus, the primary spermatocyte gives rise to two cells, the secondary spermatocytes, and the two secondary spermatocytes by their subdivision produce four spermatozoa.

## 1.28. oogenesis in the queen and worker honey bee.

The bee has telotrophic ovarioles which at their tips trophic, oogonia and prefollicular material exists.

Through division germinal cells are produced which divide to form the egg. There are three types of cell:

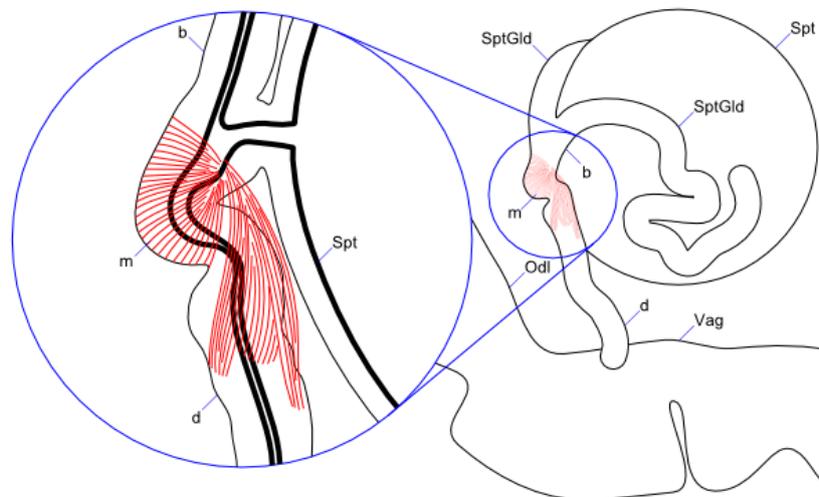
- oocyte which will become the egg
- 3 x trophocytes which through mitosis divide a further 4 times to become 48 in total these cells will nourish the oocytes
- Follicle cells, these enclose the oocyte with a small gap to the trophocytes. The follicle cells will eventually develop into the chorion

As the oocytes travel down the ovarioles they increase in size, food is absorbed through the thin wall of the ovarioles and the egg nourishes on the trophocytes which diminish and eventually disappear. After the follicle cells disappear (leaving a network of markings on the chorion) there remains a small area known as the micropyle where the trophocytes and oocyte joined, this is covered with a fine membrane.

The oocyte towards the end of the ovarioles will contain up to 90% yolk which is produced outside of the oocyte and is rich in lipids and proteins.

The oocyte undergoes a meiotic division before leaving the ovarioles.

## 1.29. the fertilisation of the egg of the honey bee.



Within the spermathecal duct there is effectively an S bend with three attached muscles, the queen is able to control the aperture of the duct. Thus she is able to release small doses of sperm on demand.

Within the vagina there is a valve fold which the queen is able press the unfertilised egg up towards the entrance to the spermathecal duct.

The released sperm will enter the egg via the micropyle.

### 1.30. the storage of sperm in the spermatheca of the queen honey bee.

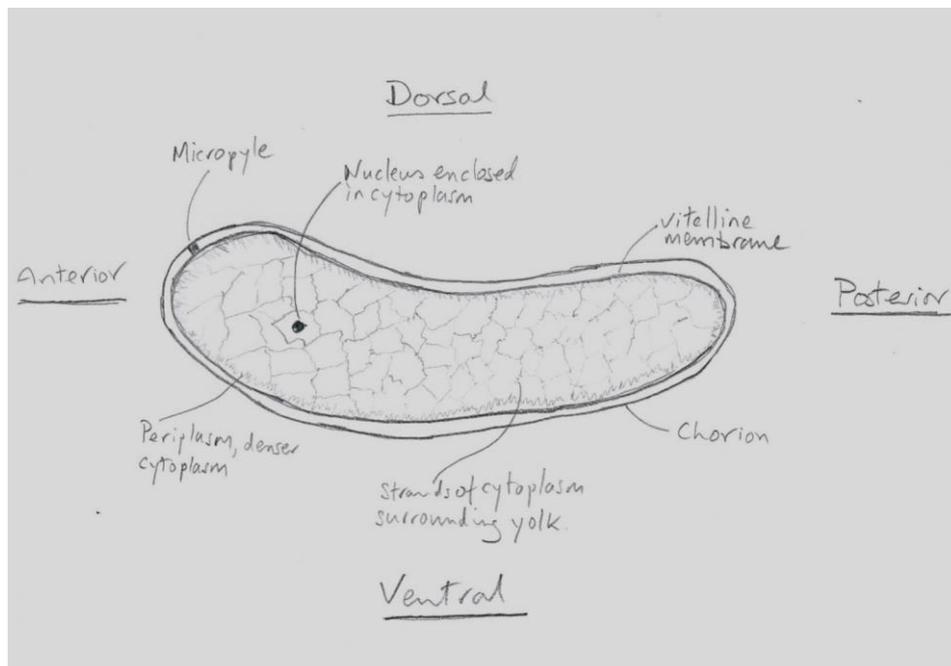
After mating the queen stores spermatozoa in her spermatheca, the spermatozoa are the result several encounters with different drones. One mating would produce sufficient sperm for the queen but sperm from several drones mixed in the spermatheca improves genetic diversity.

The spermatozoa are bathed in a spermathecal fluid high in proteins produced by the spermathecal glands.

### 1.31. the structure of the egg of the honey bee.

The honey bee egg is attached to the bottom of the cell at the posterior end.

The egg has an outer pearly white Chorion shell which is marked on the outside. The vitelline membrane, which is the true cell wall, resides just inside the chorion layer.



The nucleus of the cell is surrounded by cytoplasm and resides closer to the anterior end. The anterior end of the egg includes a micropyle through which the male gamete entered the egg. The cytoplasm within the cell is comprised of a mesh of strands that enclose globules of yolk. The outer area just within the vitelline membrane is a denser area of cytoplasm forming the

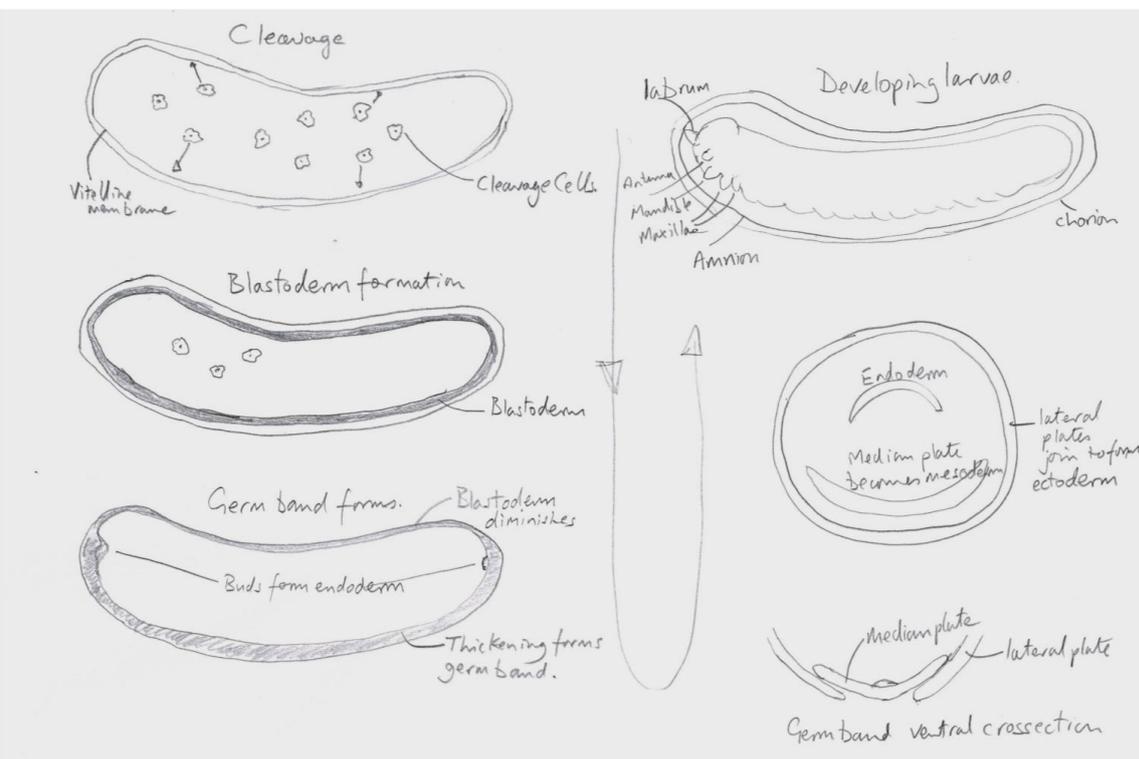
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periplasm.

## 1.32. the development of the embryo within the egg of the honey bee and the hatching of the larva.

The nucleus undergoes a series of mitotic divisions to form an increasing number of nuclei, each nucleus is surrounded by cytoplasm. This process is known as syncytial cleavage, the resulting cleavage cells surrounded by cytoplasm are known as energids. The initial mitotic divisions are synchronised across all energids, the energids are scattered throughout the yolk. After a few cycles the cleavage cells migrate towards the vitelline membrane forming a continuous cell layer known as the blastoderm.

The mitotic divisions at this stage are no longer synchronised, with divisions taking longer in different parts of the blastoderm. The blastoderm on the ventral side thicken whilst those on the dorsal thin. The thickening becomes the germ band which is the beginning of the embryo.



The germ band becomes divided due to lengthwise grooves, forming a median plate and a pair of lateral plates. The lateral plates expand and eventually join becoming the ectoderm. The median plate moves into the yolk and becomes the mesoderm. Anterior and posterior rudiments form the endoderm and in time these invaginations will form the fore and hind gut.

From the ectoderm the nervous system, tracheal system form as well as the legs, wings, mouthparts and sting. The nervous system comes from thickenings running the length of the ectoderm, the tracheal system from invaginations of buds and the external parts from further buds.

Segmentation occurs early in the process.

The mesoderm develops into the internal structures of the honeybee including the heart, aorta, diaphragms and reproductive system. The whole embryo is enclosed in a thin cellular membrane called the amnion.

The larva bursts the amnion and the chorion disappears, possibly digested by larval enzymes.

### 1.33. the external and internal anatomy of the honey bee larva.

The larva comprises 6 segments of the head, 3 thoracic segments and 10 abdominal segments.

The head segments are on the whole fused together and there are still disputes as to the total number.

The head comprises a rudimentary mouth with labrum (upper lip) and labium (lower lip) which along with maxilla and mandibles form an opening used by the larva to suck in liquid food. Other external parts of the larva are discernable as buds i.e. wings, legs, optics, antennae.

There are 10 pairs of spiracles from the second thoracic through to 8<sup>th</sup> abdominal segment.

The larva internal structure primarily comprises:

- Ventriculus which occupies the majority of the internal space
- Small foregut
- Hind gut that is not connected to mid gut (Ventriculus)
- 4 Malpighian tubules that retain waste materials until guts are joined on 5<sup>th</sup> moult
- Silk glands, used to spin cocoon

The digestive system of the larva revolves around the Ventriculus as this is key to the growth of the larva. The foregut is not required to be complex as the larva is provided with liquid feed processed by the nurse bees containing all the required nutrients.

The excretory system is basically the Malpighian tubules which store the waste, the hind gut is not connected at this point.

The brain and sub-oesophageal ganglion are formed from the segments making up the head. Each segment has its own ganglia pair apart from abdominal segments 8-10 which are fused to form one pair.

The cavity of the larva is mainly filled with fat body and the heart exists with 11 chambers and 10 Ostia. **Need more description!**

### 1.34. the metamorphosis of the honey bee larva, including the effects of juvenile hormone.

Metamorphosis of Honey Bees

Development of honey bees is similar to the metamorphosis in butterflies. The stages of development and the duration of each stage for a worker bee are given by the following sequence:

egg (3 days) --> larva & prepupa (8 days) --> pupa (9 days) --> adult

total development time = 19.5 - 20 days

The egg and early larval stages live in uncapped brood cells. The last two days of the larval stage, the pupal stage, and the first half-day of the adult stage occur beneath capped brood cells. The following description of honey-bee metamorphosis contains references of time in days. The reference point is the moment the egg was laid by the queen bee.

Table 1. Moults of the Honey Bee

Time	Workers		Queens		Drones	
Day	Stages	Moults	Stages	Moults	Stages	Moults
1						
2	egg	egg	egg	egg	egg	egg
3	1st Larval	1st Moults	1st Larval	1st Moults	1st Larval	1st Moults
4	2nd Larval	2nd Moults	2nd Larval	2nd Moults	2nd Larval	2nd Moults
5	3rd Larval	3rd Moults	3rd Larval	3rd Moults	3rd Larval	3rd Moults
6	4th Larval	4th Moults	4th Larval	4th Moults(sealed)	4th Larval	4th Moults
7						
8	gorging	sealing	gorging		gorging	
9						
10						
11	prepupa	5th Moults	prepupa	5th Moults		sealing
12					prepupa	
13			pupa			
14						5th Moults
15		pupa				
16			imago	6th Moults (Emergence)		
17						
18						
19					pupa	
20						
21	imago	6th Moults (emergence)				
22						
23					imago	6th Moults
24						Emergence

During the first 4 larval moults the larva continues to take the form described in section 1.1.3 after the shedding of each skin.

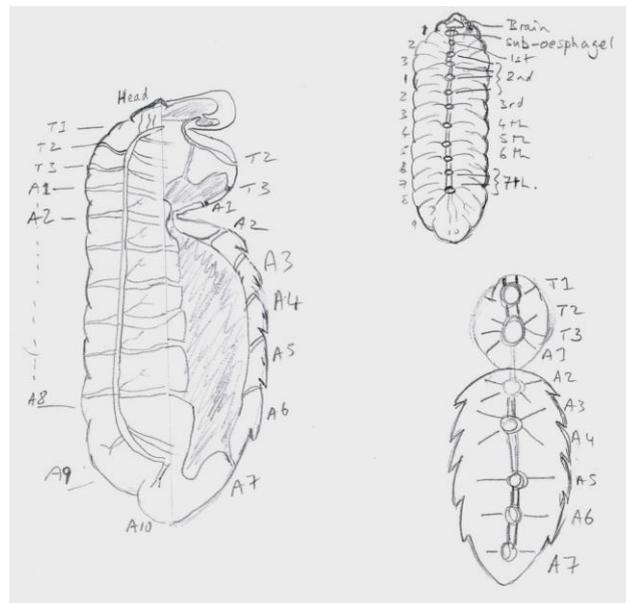
It is not until after the 5<sup>th</sup> moult that the larva begins to take on the shape of a bee. In fact the shape is formed before it finally sheds its skin on the 5<sup>th</sup> moult, this is called prepupa (sometimes propupa).

On capping of the cell the larva turns and stretches in the cell with its head towards the cap. During this process the mid and the hind gut are joined and the larva excretes into the cell. The larva begins to spin a cocoon using the debris within the cell along with silk spun from its spinneret. The prepupa retains the larval skin until the 5<sup>th</sup> moult completes between 3-5 days after capping.

On shedding the larval skin the external characteristics of a honeybee are evident; the head, eyes, antennae, mouthparts, thorax, legs and abdomen. The wings at this stage are small and undeveloped.

During the pupae stage which occurs before the final 6<sup>th</sup> moult the musculature and the alimentary canal are restructured, the 4 malpighian tubules of the larva are replaced with 100 new ones. The reproductive organs begin to develop. The brain increases in size, through fusion of some ganglia there remain 7 segmental ganglia. The adult cuticle forms within the pupa cuticle, darkening with time. The wings complete their eversion at a very late stage of the pupa.

The imago emerges from the last moult as a hairy insect covered in sensilla. The imago remains in the sealed cell for up to a day to allow its cuticle to harden and the wings/hairs to dry out before eating its way out of the cell as an adult bee. Images redrawn from Dade and Davis.



The shedding of the larval skin is in part controlled by the interplay of two hormones ecdysone and juvenile hormone. Ecdysone secreted by the prothoracic gland promotes the development of a new exoskeleton. Juvenile hormone suppresses the activity of ecdysone therefore delaying the shedding of the larval skin. Juvenile hormone is secreted by the Corpora Allata. Juvenile hormone is also key in caste determination, age roles of adult bee and the production of germ cells.

Queen bee lays the egg.

Only one queen lives within a colony of honey bees. She lays up to 2,500 eggs per day. The worker bees care for the queen and the young brood.

A single egg is laid in a brood cell.

The life of a worker honey bee begins when the queen lays a fertilized egg onto the base of a worker-sized brood cell.

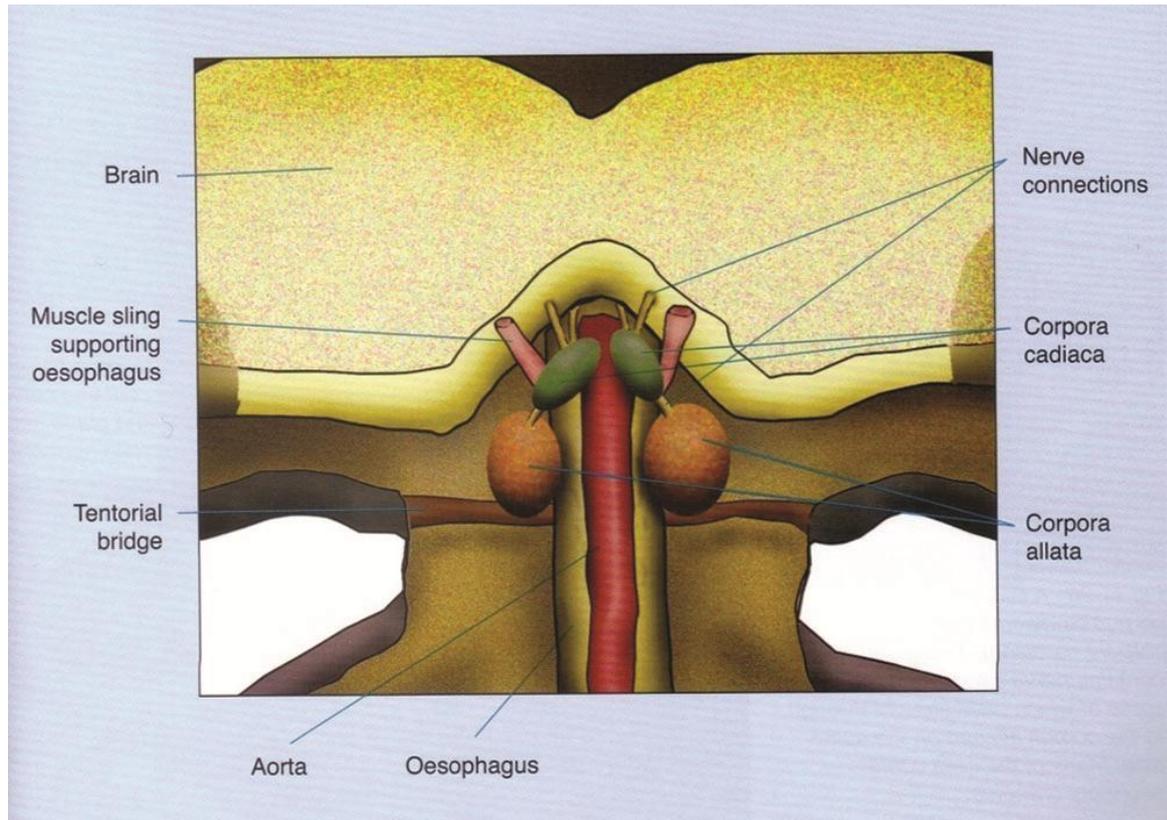
Step 2: A larva hatches from egg after three days.

Nurse bees feed brood food to the larva within minutes of hatching. Glands in the head of nurse bees secrete the liquid diet. The nurse bees continue to feed the larva until the cell is capped.

### 1.35. ecdysis (moulting) and its control by hormones.

The definition (Snodgrass) of moulting is the separation of the outer layer of the cuticle by a dissolving of the inner layer (endocuticle). It is only when the epidermis is thus free of the old cuticle that it can form a new cuticle and begin a new phase of growth. The final emergence of the insect from the larval skin is its ecdysis(coming out).

The process of moulting is controlled by the endocrine system. The medium for sending control signals are hormones.



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Brain neuro-secretory cells release brain hormone in response to both internal and external stimuli. The brain hormone is passed down the nervous connections to the corpus cardiaca where it is released into the haemolymph. The brain hormone causes the prothoracic gland to produce ecdysone (moulting hormone) which initiates changes in the epidermis.

Firstly the cells of the epidermis divide becoming closely packed. The procuticle becomes detached from the epidermis through moulting fluid secreted by the epidermis filling the space between the two layers. The moulting fluid is inactive at this point. The epidermis secretes a new epicuticle to protect it when the moulting fluid becomes active and digests the old endocuticle through enzymes within the fluid (proteinases and chitinase).

The products of digestion are absorbed by the epidermis and used in the secretion of the new procuticle. Once the endocuticle has been fully digested the old exocuticle and epicuticle are split by action of the larva swallowing air and causing it to rupture the exocuticle.

Juvenile hormone is secreted by the corpora allata, it suppresses the expression of adult characteristics. Juvenile hormone and ecdysone work together to manage the process of growth and moulting. Juvenile hormone production ceases prior to the prepupa final moult, it resumes again when the bee reaches the adult stage.

Juvenile hormone levels are at their highest between moults 3 and 4.

Ecdysis is the final emergence of the bee from its larval skin, i.e. the final stage of the moult.

### 1.36. the sealing of the cell, larval orientation in the cell, defecation and cocoon spinning.

From Snodgrass:

Some time before the last moult (6 days after hatching) the cell is sealed by the worker bee with a wax cap mixed with pollen to give air holes. The larva gorges itself on the remaining food and spins a cocoon of silk from the silk gland via the spinneret.

The larva stretches out causing the mid and hind gut to join so evacuates the contents of the alimentary system. The head is towards the cap. The cocoon is mixed with the faeces.

### 1.37. the anatomy and metamorphosis of the prepupa and pupa.

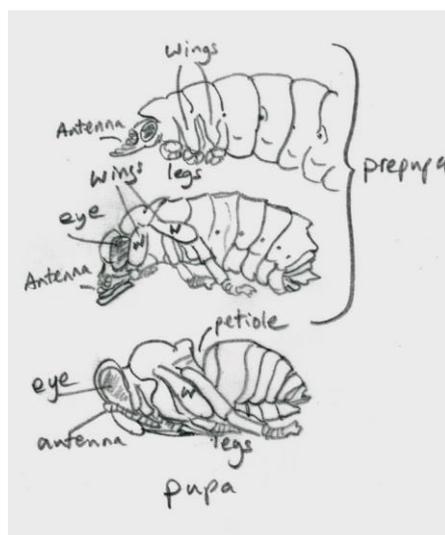
In the early stages of the prepupa it begins to take on the characteristics of the adult in the head and thorax. The abdomen remains larva like and there is no distinction from the thorax, i.e. no constriction that develops into the petiole.

The head includes compound eyes, basic mouth parts structure and antennae.

Small legs are present and are jointed. The wings are flaps that hang limply.

The sting has developed since last larval stage.

When 5<sup>th</sup> moult completes the pupa has the characteristics of an adult except the wings.



The bee larva becomes a prepupa.

The bee larva changes into a prepupa within hours of finishing the cocoon. The prepupa lies motionless in the brood cell as it prepares to shed the last larval skin. This stage lasts for nearly two

days spanning the 10-11th days of bee development.

The prepupa becomes a pupa.

The head of the prepupa enlarges by the end of the 11th day, marking the beginning of the pupal stage of bee development. The tissues of the pupa will continue changing to form the adult insect.

The most noticeable changes are an increase in pigmentation of the eyes and body as the pupa ages.

The eyes of the pupa begin pigmentation.

Around day 13, the eyes become pigmented before other parts of the bee. There is no movement of legs, antennae or mouthparts during the early pupal stages.

Eye pigments darken.

The compound eyes and the ocelli (the three small eyes at the center of the head) appear pink on the 14th day. The pupa still does not move its appendages.

Eyes are purple on the 15th day.

The body is still white or slightly yellow, but some brown pigment appears in the antennae and mouthparts at this stage.

The bee's body darkens.

The body of the bee has a yellowish tan appearance on the 16-17th day of development. The pigmentation of the antennae, mouthparts and legs increases. Some slight movements of the legs can be seen at this time.

### **1.38. the moult from pupa to imago.**

The pupa-to-adult molt occurs about 12-20 hours before the adult bee emerges from the cell. The young bee expands her wings and finishes hardening her exoskeleton during this period of time. Her body movements are frequent and strong.

The pupa does not grow or change shape, the cuticle hardens. The final moult to the imago exposes the furry covering and exposes the completed appendages.

The wings are fully formed in the pupa stage.

The imago remains within the sealed cell for a day before emerging, this is for the wings and hairs to dry out.

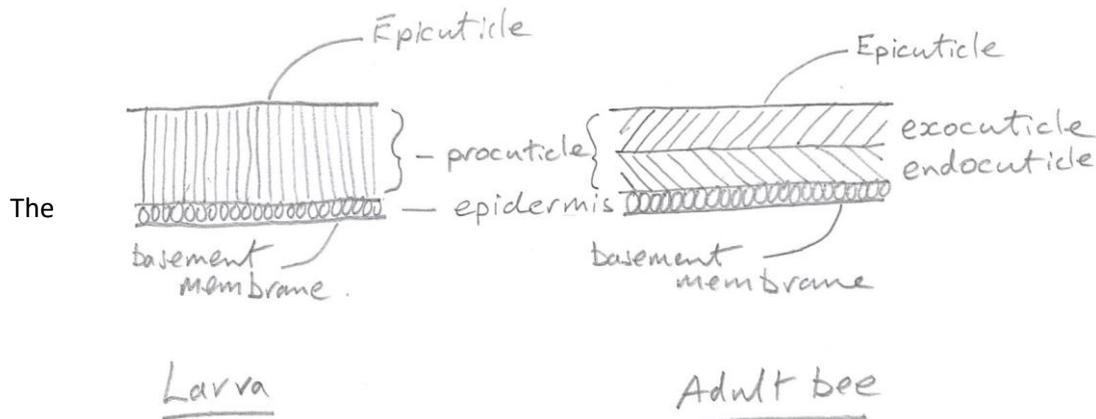
### **1.39. the emergence of the imago from the cell.**

The adult bee chews away cell cap.

Adult bee exits the cell.

The young adult bee leaves her brood cell about 20-21 days after the egg is laid.

### **1.40. the structure and main constituents of the cuticle of both larval and adult honey bees, including internal cuticular structures.**



cuticle comprises several layers residing above the living cellular layer known as the Epidermis.

The Epidermis is attached to the basement membrane which forms the boundary between the inside and outside of the body.

The layers forming the cuticle comprise the procuticle and the epicuticle. In the adult honeybee the procuticle is further divided into the endocuticle and exocuticle.

The endocuticle primarily contains chitin which is derived from chitin, this layer is relatively flexible. The exocuticle also contains chitin but also has sclerotin in large amounts. The sclerotin is very hard and dark in colour.

The epicuticle is made up of layers of sclerotin but has no chitin, the outer layer is waxy forming a waterproofing layer.

The plates that make up the exoskeleton of the honeybee are called sclerites and are formed through the bonding of the exocuticle and epicuticle, the flexible membranous connections between the plates is the endocuticle.

Some plates have inward growths (apodemes) of sclerites, these are places where the muscles attach or form strengthening to the body. The outer surface of the sclerites reflect the internal parts, forming grooves (sulcus) where the internal structure is a ridge and pits where the internal structure is a peg.

#### 1.41. the effect of feeding on caste determination in females of the honey bee.

The type of feeding of a larva is determined by the type of cell in which a larva resides (worker/drone or queen cell), worker and drone cells will be fed brood food and queen cells copious amounts of royal jelly. Sticking to the differences in feeding and food between worker and queen,

there are three elements within brood food and it is the mix of elements as well as the feeding programme that determine the caste.

Larval food comprises:

- White, produced by the mandibular gland
- Clear, produced by the hypopharyngeal gland
- Yellow, from pollen

Composition of feed:

- Queen larva (Royal Jelly)
  - o First three days mostly white
  - o Last two days ratio white to clear 1:1
- Worker (Brood Food)
  - o white:clear:yellow in ratio 2:9:3 average

Points to note:

- queen is fed 10 times volume of worker, queen “swims” in royal jelly, worker fed as needed
- larva up to 3 days old transferred between cell types and hence feed change will mature to appropriate caste i.e. queen cell produces queen, worker produces worker.
- Larva transferred 3-4 days old will mature to appropriate caste but may exhibit traits of other caste e.g. pollen baskets on queen.
- Sugar composition in royal jelly is 34% and in brood food 12% for first three days. Brood food rises to 47% after 3 days with addition of honey to mixture. Sugar stimulates to consume more.
- Higher levels of Juvenile Hormone in larva triggers queen development, royal jelly stimulates JH production by the early development of the endocrine system

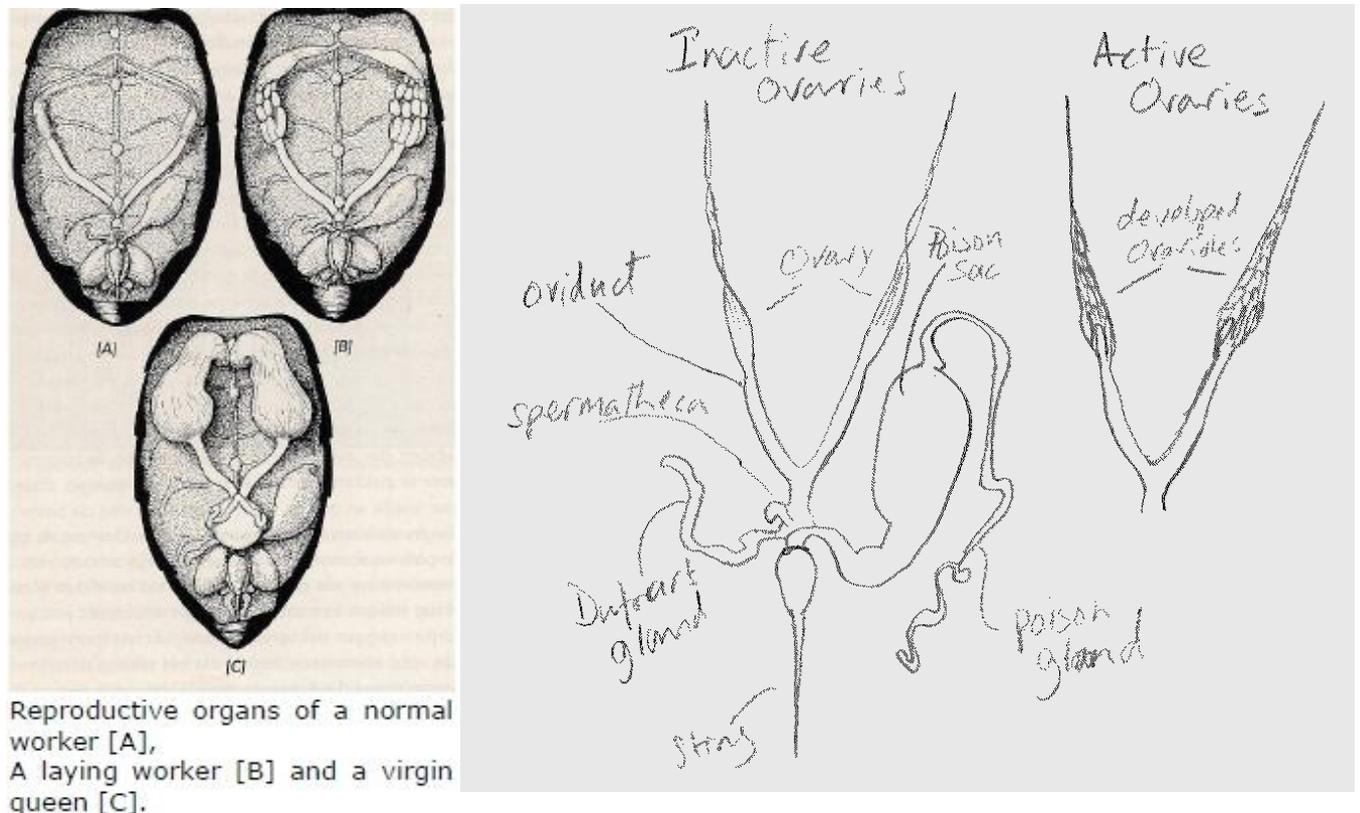
#### **1.42. the composition of royal jelly and of brood food.**

Royal jelly is composed of 67% water, 12.5% crude protein, including small amounts of many different amino acids, and 11% simple sugars (monosaccharides), also including a relatively high amount (5%) of fatty acids. The main acid is the 10-hydroxy-2-decenoic acid (10-HDA) (about 2 - 3%). It also contains many trace minerals, some enzymes, antibacterial and antibiotic components, pantothenic acid (vitamin B5), pyridoxine (vitamin B6) and trace amounts of vitamin C,<sup>[2]</sup> but none of the fat-soluble vitamins, A, D, E, and K.

Brood food dry weight contains 15-25% protein, 30-60% sugars, lipids decline in volume with age of larva, unprocessed pollen also added.

### 1.43 the physiological and structural differences between laying workers and normal workers and the role of pheromones in bringing about these differences;

The image to the left is the classic Dade drawing, to the right is taken from Seeley Honeybee ecology.



Reproductive organs of a normal worker [A], A laying worker [B] and a virgin queen [C].

Development of laying worker bees

In a honeybee colony, under normal conditions female worker bees' ovaries are inactive as their development is prevented by brood pheromone (a 10-component mixture of methyl and ethyl fatty esters) and Queen Mandibular Pheromone (9-ODA). However, when a colony loses its queen and there are no fertile eggs or worker larvae of an appropriate age to raise a new queen from, one or more worker bees will partially activate their ovaries and commence to lay eggs due to the absence of queen and brood pheromones. The process of developing a laying worker usually takes weeks (3-4 weeks) after the loss of the queen, usually by the time all the brood has emerged.

Seeley writes that 15 days after the loss of the queen 5% of workers possess ovaries with mature eggs rising to 50% of workers 30 days after the loss of the queen. The extent of the ovary activation depends mainly upon the season and level of colony nutrition. A colony which loses its queen in the Spring with a high level of pollen stores is more likely to produce laying workers.

A few of the workers with developed ovaries will become false queens with the development of the mandibular and Dufour's glands producing queen substance. A retinue will form around these workers and drones may attempt to couple with them. False queens will have slightly swollen abdomens. The production of pheromones by the false/pseudo queens suppresses the development of ovaries in other workers.

The ovaries of the normal worker are narrow with a few short ovarioles. They lead via oviducts to a very small vagina with a vestigial spermatheca projecting from the top.

A worker with activated ovarioles will commonly have 2-3 per ovary and sometimes up to 6.

The eggs will be laid in worker cells with several in the same cell and produce eventually undersized drones. Drones produced from laying workers are sexually viable.

Below is a table summarising the differences between workers with activated and inactive ovaries.

	Laying Worker	Normal worker
Ovarioles	10 – 12	Nil or vestigial
Laying rate	50 eggs per day	Nil
Spermatheca	Nil	Nil
Hypopharyngeal gland	Enlarged	Atrophied in older bees
Fat bodies	Increased	Low in summer
Age	Extended	Circa. 6 weeks
Pheromones	Mimic queen substance	Nil
Colony	Disorganised	Orderly
Behaviour	May attract court	Maul laying workers

#### 1.44 the differences between summer and winter worker honeybees

A key difference between summer and winter bees is their lifespan, the summer bee will survive around 6 weeks whereas the winter bee will survive several months which enables the colony to survive the winter.

- Winter bees contain large amounts of stored glycogen and fat in the fat bodies
- The hypopharyngeal gland is plump and full of brood food in the winter bee
- Metabolic rate of winter worker is lower than summer worker
- Winter bees do less work i.e. no foraging
- The life span of winter worker is months compared with summer worker of circa 6 weeks.

A key protein for the age task determination in bees as well as survival of winter bees is vitellogenin. Vitellogenin is employed by most animals as part of egg production, in sterile worker bees the protein has been adapted to perform other roles. Bees have made it much more important in their physiology and behaviour, using it additionally as a food storage reservoir in their bodies, to synthesize royal jelly, as an immune system component, as a “fountain of youth” to prolong queen and forager lifespan, as well as functioning as a hormone that affects future foraging behaviour.

Honey bees deposit vitellogenin molecules in fat bodies in their abdomen and heads. The fat bodies act as a food storage reservoir. The glycolipoprotein, meaning that it has properties of sugar (glyco, 2%), fat (lipo, 7%), and protein (91%), vitellogenin has additional functionality as it acts as an antioxidant to prolong Queen bee and forager lifespan as well as a hormone that affects future foraging behavior. The health of a honey bee colony is dependent upon the vitellogenin reserves of the nurse bees – the foragers having low levels of vitellogenin. As expendable laborers, the foragers are fed just enough protein to keep them working their risky task of collecting nectar and pollen. Vitellogenin levels are important during the nest stage and thus influence honey bee worker division of labour.

A nurse bee's vitellogenin titer that developed in the first four days after emergence, affects its subsequent age to begin foraging and whether it preferentially forages for nectar or pollen. If young workers are short on food their first days of life, they tend to begin foraging early and preferentially for nectar. If they are moderately fed, they forage at normal age preferentially for nectar. If they are

abundantly fed, immediately after emergence, their vitellogenin titer is high and they begin foraging later in life, preferentially collecting pollen. Pollen is the only available protein source for honey bees.

### **Juvenile hormone feedback loop**

For the majority of insect species it has been documented that juvenile hormone stimulates the transcription of the vitellogenin genes and the consequent control of vitellogenin production.

The vitellogenin expression is part of a regulatory feedback loop that enables vitellogenin and juvenile hormone to mutually suppress each other. Vitellogenin and juvenile hormone likely work antagonistically in the honey bee to regulate its development and behaviour. Suppression of one leads to high titers of the other.

It is likely that the balance between vitellogenin and juvenile hormone levels is also involved in swarming behaviour.

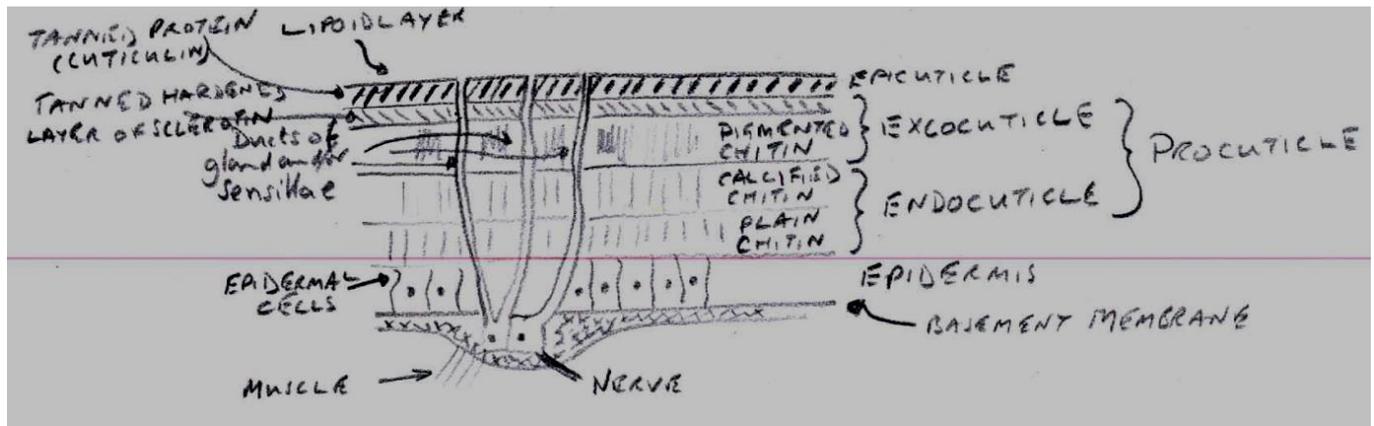
Juvenile hormone levels drop in honey bee colonies pre-swarming and it is expected that vitellogenin levels would therefore rise. One may surmise, that swarming bees would want to pack along as much vitellogenin as possible to extend their lifespan and to be able to quickly build a new nest.

## **1.45 the structure and main constituents of the cuticle with an outline account of its invagination within the body to form linings of the gut and tracheae**

In arthropods, the integument, the external "skin", or "shell", is the product of a single layer of ectodermal epithelium. That layer is attached to the external or distal surface of the deepest layer, the non-cellular internal membrane of the integument. That non-cellular membrane is called the **basement membrane**. The layer of epithelium on the basement membrane produces the cuticle, which begins as a tough, flexible layer of chitin. Such thin, flexible chitin is the major structural part of the integument where flexibility is necessary, such as in bodily parts that must stretch to contain accumulated liquids, or that form joints between rigid parts of the exoskeleton. In other parts of the cuticle the function of the integument demands more rigid materials, such as armored regions or the mandibles, or where the exoskeleton forms the limbs of the honey bee. To achieve such rigidity the outer chitin layer of the cuticle is impregnated, thickened, and reinforced with harder, more brittle materials such as sclerotinised proteins. This main chitinous layer of the cuticle is called the **procuticle**.

The procuticle in most land insects is covered externally with a thin, waxy, water-resistant outer layer containing no chitin. That outer layer is the **epicuticle**, and it is much thinner than the procuticle. The chitinous procuticle consists of two major layers, the outer **exocuticle** and the inner **endocuticle**. The tough and flexible endocuticle is a laminated structure of layers of interwoven fibrous chitin and protein molecules, while the exocuticle is the layer in which any major thickening and armoring occurs. The exocuticle is greatly reduced in many soft-bodied larvae of the honey bee.

Hardened plates in the exoskeleton are called sclerites. Sclerites may be simple protective armor, but also may form mechanical components of the exoskeleton, such as in the legs, joints, fins or wings. In the body segment of an insect there are four principal regions. The dorsal region is the **tergum**; if the tergum bears any sclerites, those are called **tergites**. The ventral region is called the **sternum**, which commonly bears **sternites**. The two lateral regions on the thorax are called the **pleura (singular pleurum)** and any sclerites they bear are called **pleurites**.



The cuticle sits on the epidermis, a layer of living cells that secrete substances including:

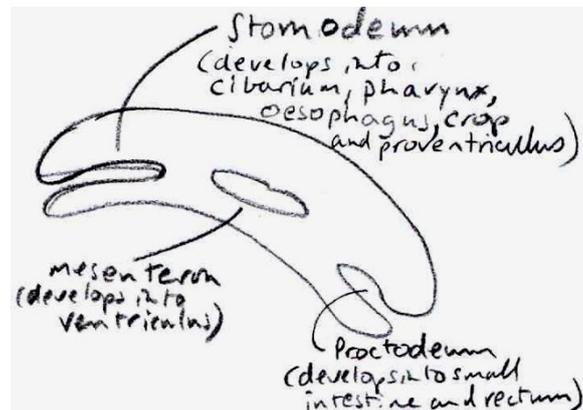
- Chitin
  - o Chemically, chitin is a long-chain polymer of a N-acetylglucosamine, which is a derivative of glucose
- Arthropodin
  - o protein injected between the chitin threads
- Polyphenols, for tanning the arthropodin and eventually forming sclerotin
- Cuticulin, tanning protein
- Cuticular lipids, paraffin hydrocarbons used for waterproofing the epicuticle

The thickness of the cuticle is circa 0.2µm.

**Endocuticle** – contains a large amount of chitin which is tough but flexible

**Exocuticle** – contains a large amount of sclerotin which is hard and dark in colour

**Epicuticle** – is made up of several thin layers of hard sclerotin and cuticulin but no chitin



The stomodeum and proctodeum are invaginations of the epidermis and are lined with cuticle (intima). They form:

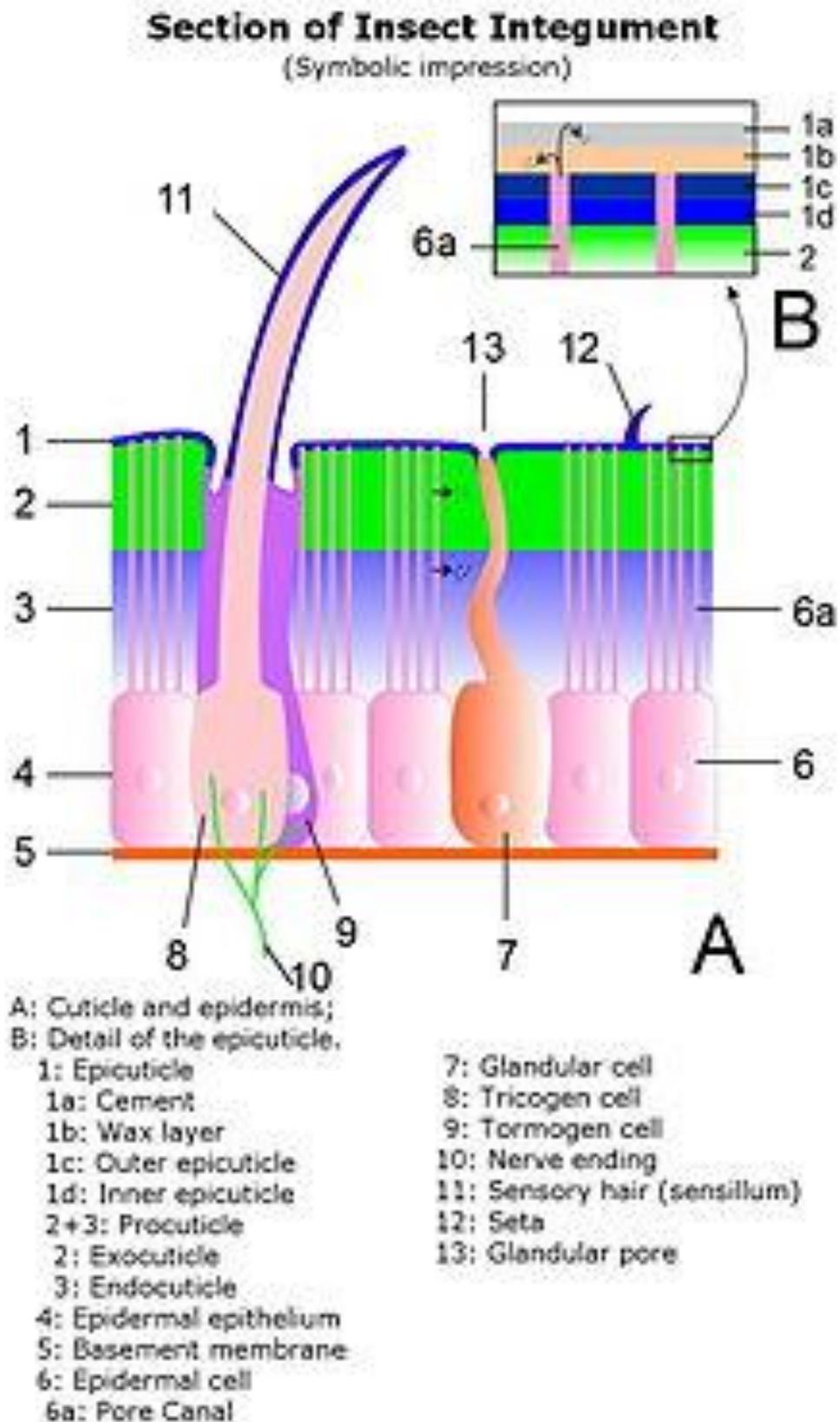
**Oesophagus** – slender tube lined with thick cuticle and surrounded with circular muscles

**Crop** – an extension of the oesophagus which is capable of stretching in order to take a heavy load

**Proventriculus** – walls are lined with dense cuticular intima

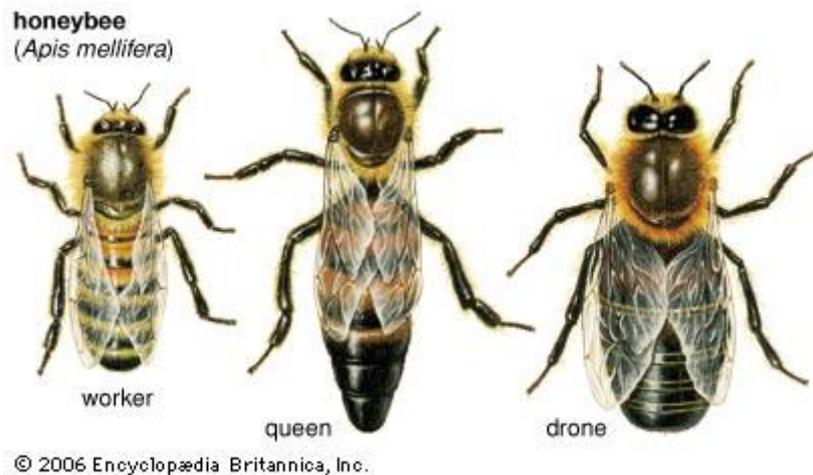
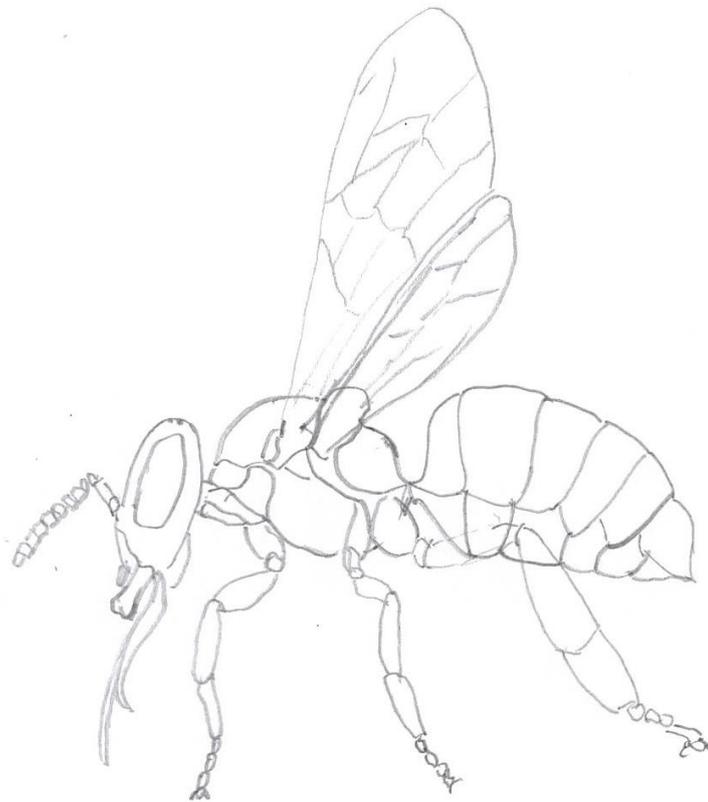
**Rectum** – has an epithelial wall lined with thin cuticular intima

The tracheal system forms from inwards growths arising from pits in the ectoderm of the embryo. The air sacs, trachea and tracheoles have a thin cuticular intima covering the epidermal layer. The tracheae have thickenings of chitin known as taenidium (plural tenidia) to ensure they remain open. Tracheoles and air sacs do not normally have tenidea.



### 1.46. the external anatomy of all castes.

The three Thoracic segments have pleuron between Tergum (dorsal) and Sternum (ventral), effectively forming as box.



There are two sexes and two female castes of the *Apis mellifera* honeybee.

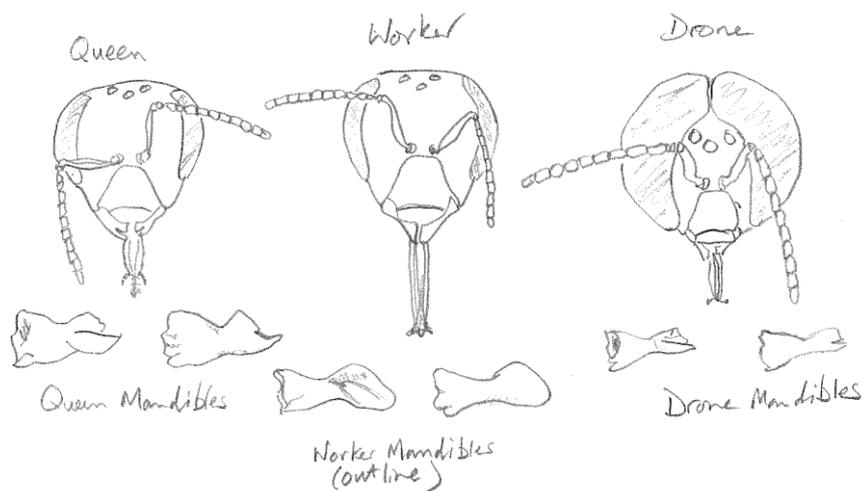
All three have the same overall structure:

- Head with 5 eyes, two antennae and mouthparts
- Thorax comprising three thoracic segments and one abdominal segment
- Petiole

- 6 legs on the thorax, each pair from a different tergite
- Two pairs of wings on the thorax from T2 and T3

There are differences on several of the features and in some cases parts are missing, these are primarily due to the differing roles of each sex and castes. The table below identifies some of the differences.

Feature	Worker	Queen	Drone
Antennae			
Flagellum	10 sub-sections	10 sub-section	11 sub-sections
Sensilla			500,000 total
- sense plates	5-6,000	2-3,000	30,000
Eyes, ommatida	6,900	<worker?	7-8,000
- compound	Side of head	Side of head	Meet in forehead
- ocelli	On top of head	On top of head	Forehead below eyes
Proboscis	Long 5.5-7mm	Short than worker	Very short
Thorax			Largest
Abdomen	Same length as wings	Longer than wings, long pointed	Shorter than wings, large, round
Legs	Corbicula	No corbicula	No corbicula
Sting	Straight barbed	Curved no barb	Not present
Wings			Broader than worker



<b>Characteristic</b>	<b>Worker</b>	<b>Queen</b>	<b>Drone</b>
<b>Sensory</b>			
No. facets of compound eye	4,000-9,000	3,000-4,000	7,000-8,600
Optic lobe of brain	Medium	Small	Large
No. antennal plate organs	3,000	1,600	30,000
Relative ratio of antennal surface	2	1	3
<b>Glandular</b>			
Hypopharyngeal	Present	Vestigial	Absent
Mandibular	Large	Very large	Small
Head salivary	Large	Large	Vestigial
Thoracic salivary	Large	Large	Small
Wax	Present	Absent	Absent
Nasonov	Present	Absent	Absent
Alkaline (dufour)	Reduced	Present	Absent
Koshevnikov	Reduced/absent	Present	Absent
<b>Reproductive and sting</b>			
Ovaries or testes	Reduced ovaries	Enlarged ovaries	Testes
No. ovarioles	2-12	150-180	None
Spermathica	Rudimentary	Large	None
Sting barbs	Strong	Minute	No sting
Sting plates	Loosely attached	Strongly attached	None
<b>Mouthparts</b>			
Mandibles	Slender	Robust	Small
Mandibular groove	Present	Absent	Absent
Proboscis	Long	Short	Short
<b>Leg and wing</b>			
Pollen press and combs	Present	Absent	Absent
Pollen basket	Present	Absent	Absent
Wing sensilla	Medium	Fewest	Most
Re-written from Winston, The biology of the Honey Bee			

### 1.47. the structure of the wings of the honey bee, their articulation and action in flight.

The wings grow from small pouches containing trachea. The membrane that forms the wings is contiguous with the gaps between the tergites and pleurites of T2 and T3. In the larva they started as pockets of epidermis below the larval cuticle.

The wings are above their respective spiracles, although they start below in the larva. The wing veins are laid out at thickenings of the wing surface. The trachea penetrate the thickenings. The wings develop during the pupa stage.

The trachea carry a little haemolymph and some fibres.

The forewing is larger than the rear wing, in flight they are linked through hamuli.



Worker wings showing veins

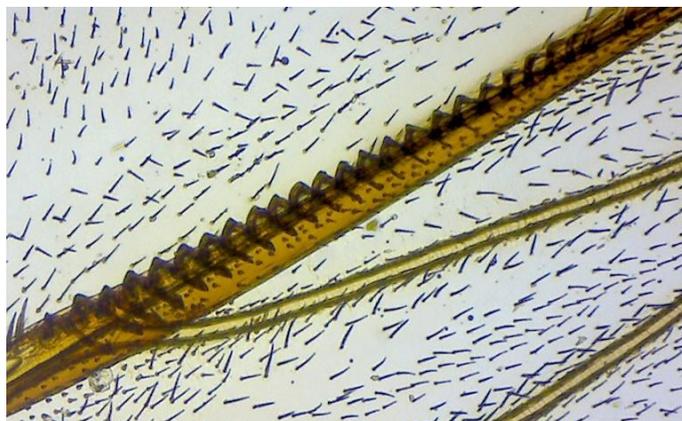
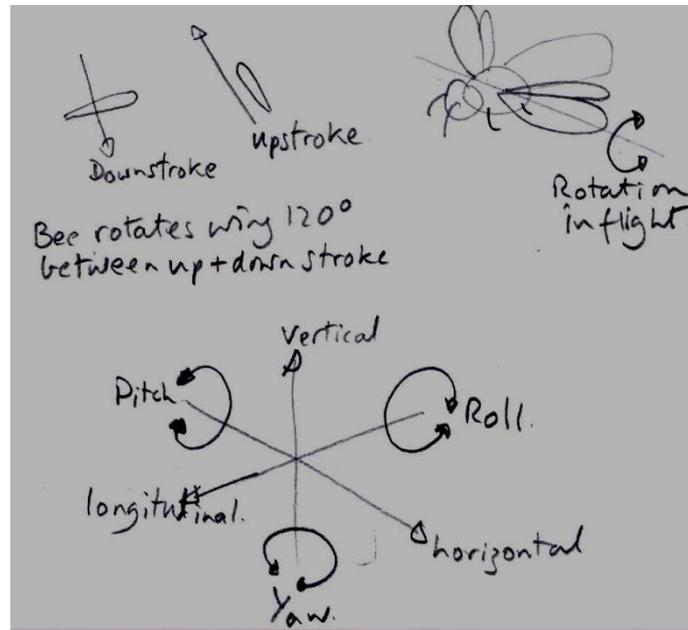


Image showing hamuli and sensilla

The root of each wing thickens into an intricate system of articular sclerites which include the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> Axillary. The 2<sup>nd</sup> Axillary rests upon the pleuron and is the fulcrum for the wing movement. AX2 connects to two small plates that are hinged to the pleurite, called basalare and subalare. Muscles are anchored to the inside of the pleurite, the action this muscle controls the wing.



The basic movement of the wing is for it to move up and down, during this motion the wing is rotated in order to give lift in both directions of movement. Three variations of wing movement creating uplift are:

- Delayed drag, where the wing is at a steeper angle in the down stroke causing additional uplift from the leading edge vortex formed
- Rotational circulation, where the wing rotates between cycles
- Wake capture, the wing rotates before the start of the upbeat causing it to intersect its own wake and again gaining extra uplift.

Slight variations in the actual angles of the wings determine whether the bee hovers, moves forwards or turns.

When the bee hovers, the wings are producing a vertical force exactly equal to the weight of the bee. Subtle change in the wing angles produces an overall force that is not vertical. This will make the bee move forwards or sideways. When the wing angles are slightly changed on one side only, different forces on each side of the bee are produced. This will cause the bee to turn in the same way that a boat will turn if one oar is producing more force than the other.

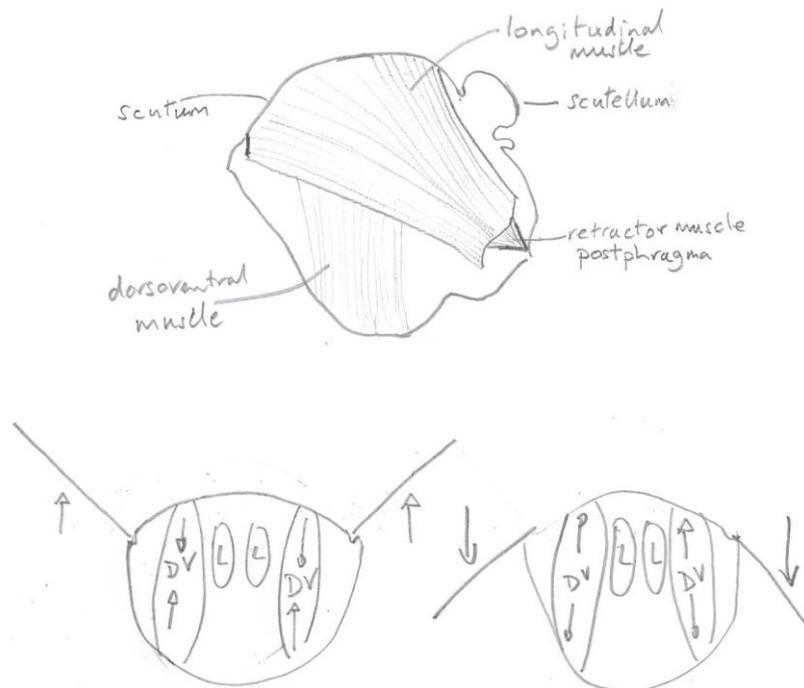
### 1.1.13. the muscles associated with the wings of the honey bee and their control.

Muscles attached to the Axillary's causes control movements on the wings:

- AX1, controls figure of 8 wing tip pattern
- AX2, enables the wings to be twisted to correct yaw, roll and pitch
- AX3, used for furling and un-furling forewing beneath hind wing
- AX4, assists AX3

The hind wing has AX1-3 and a similar muscle structure.

The force in the wings is provided by 2 pairs of indirect muscles, dorsoventral and longitudinal. The dorsoventral muscles, known as elevator muscles, are connected to the scutum and the lower sides of the mesothorax. The longitudinal muscles are connected to the mesoscutum and the rear of the mesothorax which is part of propodeum.



Contracting the elevator muscles releases the longitudinal muscles and pulls down the notum (tergum) raising the wings. Relaxing the elevator muscles the longitudinal (depressor) muscles contract notum expands and wings drop.

#### 1.48. the structure of the legs of the honey bee, their articulations, associated muscles, action when walking and special uses.

Key points:

- Hollow tubular outgrowths of the body
- Leg single tube, sclerotized parts, unsclerotized "joints"
- Act as tripods when walking
- Articulating processes in connecting membrane
- One articulation monocondylic, two dicondylic
- Leg has 6 segments
  - o Coxa, trochanter, femur, tibia, tarsus, pretarsus
  - o Tarsus has 5 segments called tarsomeres, basitarsus plus 4 small segments
- Large joint coxa to thorax allows muscle entry
- Coxa joins between pleura and sternum, one leg per thoracic segment

- Coxa articulation
  - Forelegs coxa downwards between pleural and sternal articulations so legs move forward and back
  - Middle and hind legs set obliquely to thorax and slanting posteriorly so legs turn out and in with movement
  - Hind legs directed backwards
- Trochanter has dicondylic articulation with coxa in horizontal plane, movement up and down (raises body)
- Femur connected obliquely to trochanter with articulations that allow forward and back but not vertical so femur becomes extension of trochanter in vertical movements
- Tibia connected to femur via “knee joint” has vertical articulations
- Tarsus joint with tibia is monocondylic as are the joints between tarsomeres
- 4 small tarsomeres do not have muscles, rather controlled via tendon that extends to the pretarsus
- Pretarsus has own muscles, projects from the end of the 5<sup>th</sup> tarsomere
  - Two individually articulated claws
  - Arolium, soft pad between claws

Special leg features:

- Foreleg antenna cleaner
- Worker hind leg corbicular, pollen basket, outside tibia
- Worker hind leg pollen bush, inside basitarsus
- Worker hind leg pollen press

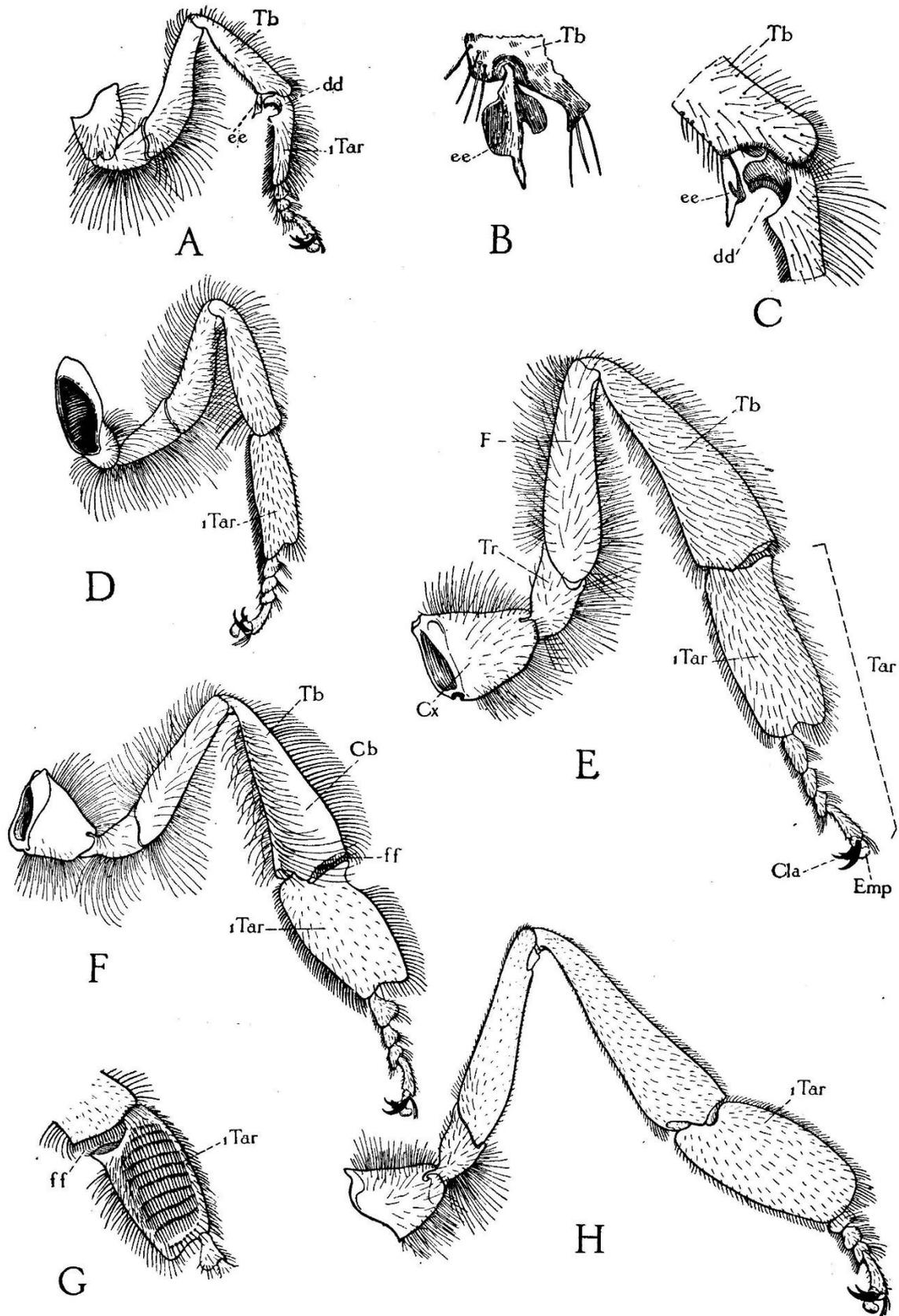
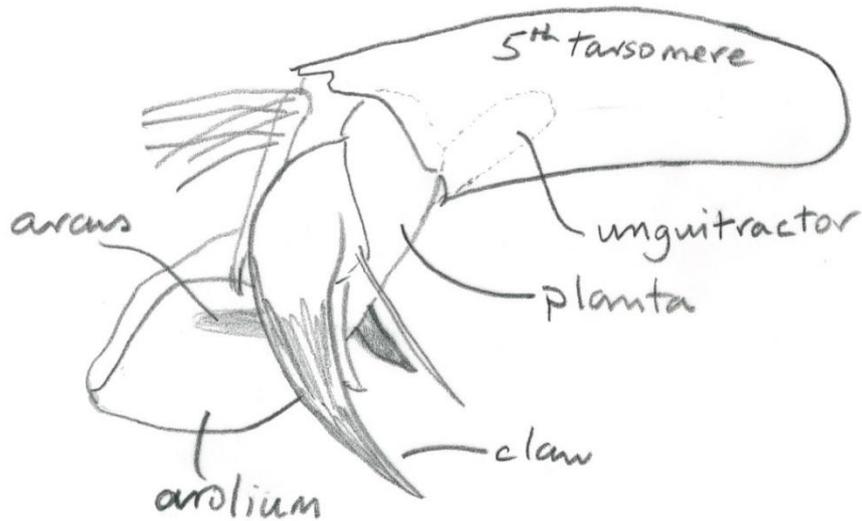


FIG. 29.—A, left front leg of worker, anterior view, showing position of notch (*dd*) of antenna cleaner on base of first tarsal joint (*iTar*) and of closing spine (*cc*) on end of tibia (*Tb*); B, spine of antenna cleaner (*cc*) in flat view; C, details of antenna cleaner; D, left middle leg of worker, anterior view; E, left hind leg of queen, anterior or outer view; F, left hind leg of worker, anterior or outer view, showing the pollen basket (*Cb*) on outer surface of tibia (*Tb*); G, inner view of first tarsal joint of hind leg of worker showing rows of pollen-gathering hairs and the so-called "wax shears" (*ff*); H, left hind leg of drone, anterior or outer view.

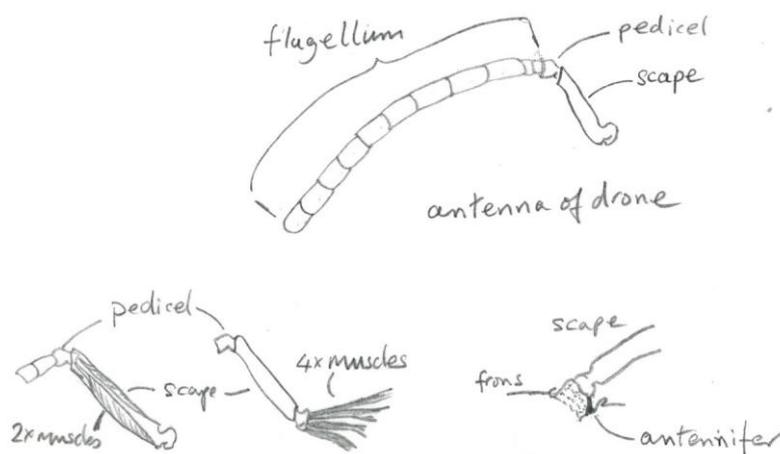


The pretarsus (foot) is similar on all the legs. The claws (ungues) are hinged to the 5<sup>th</sup> tarsomere via the plate known as the unguitactor. The claws connect to muscles in the femur and tibia via tendons which pass through the unguitactor, the pretarsus does not have its own muscles.

The normal mode of operation is for the bee to employ the claws for grip on the surface, when the claws cannot provide purchase the claws splay and the manubrium (dorsal plate with 5-6 hairs) is drawn down causing the arolium to make contact with surface.

It is known exactly how purchase is made as there is no sticky substance employed, it is assumed that suction is employed. Within the pretarsus is the Arnhart gland its secretions form part of queen substance and are employed by workers for marking locations.

#### 1.49. the antennae, their structure, associated muscles, and functions.



The antenna comprise three elements:

- scape which attaches to the frons via a basal knob into a membranous socket in the cranial wall of the bees head

- the pedicel which is hinged to the scape and can turn the final part up and down
- the flagellum, made up of 10 subsections on the worker and 11 on the drone

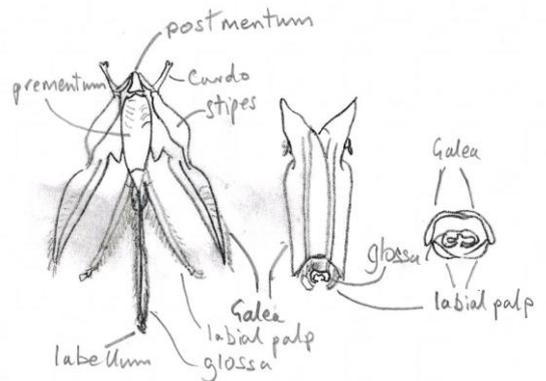
The scape contains two muscles which articulate the dicondylic hinge to the pedicel. There are no muscles within the flagellum, the subsections are joined by via a membranous neck. The scape is pivoted on an articular process called the antennifer which allows the antenna to freely move in all directions within the socket. Movement of the antenna is controlled by 4 muscles attached on the base of the scape. 2 muscles are above the antennifer and two below.

The two antennae of the honey operate independently and support thousands of sense organs.

### 1.50. the mouth parts of the honey bee, their structure, associated muscles, glandular connections, methods of use, comparative sizes and shapes for different castes and tongue length for different races.

The mouth parts are generally common to other insects:

- Labrum, the upper lip is an extension of the face
- Mandibles, the jaws in a worker are spoon shaped and smooth, their uses include
  - o Taking in of pollen, shaping and chewing wax, fighting, grooming, dragging out of debris from the nest, brood feeding, gathering and using propolis and to support other mouth parts.
- Proboscis, 5.3-7.2 mm dependent upon race, is basically a tube within a tube, the central tube called the glossa surrounded by another made up from the galeae of maxillae and labial palps (2 of each fused together). It is terminated in the labellum.



The proboscis main function is to ingest liquid (water, nectar and honey) but it also functions in trophallaxis and in the licking of pheromones and exchanging them with other workers. The proboscis can be folded in a Z shape within the mouth when the bee is resting. liquid is sucked up the glossa by the cibarial pump (the walls of cibarium expanding and contracting). The epipharynx forms an airtight seal at the top of the glossa in order to enable the suction to occur.

The glossa is very hairy and an important use is in the collection of pollen that sticks to the hairs and is removed by the fore legs.

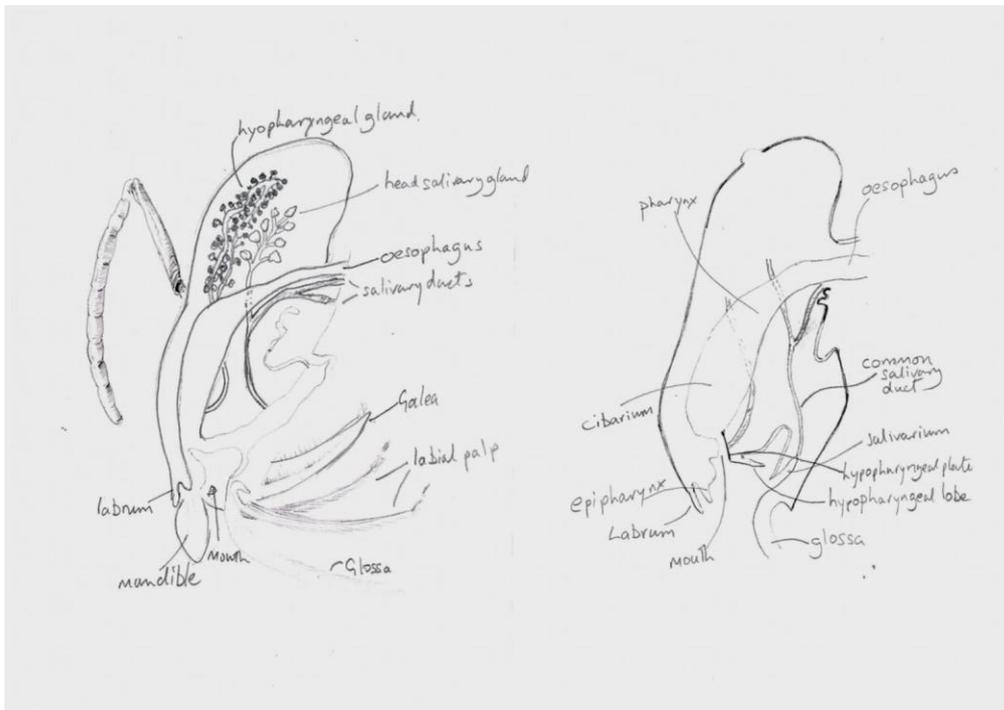
The proboscis is able to protrude and retract and used to:

- Tongue lashing, the worker regurgitates liquid (water or nectar) spreads it on comb or side of hive in a thin layer and repeatedly beats it with her tongue.
- Tongue stropping, hesitant bees when entering the wrong hive and are challenged by a guard, pull their proboscis through their front feet.
- Honey ripening, nectar is regurgitated and held in a fold of proboscis exposing it to air

To liquefy solid foods saliva travels down the glossa tube and the labellum is employed to rub it into the solid food to create a liquid to be drawn up.

Tongue length by sub species:

<i>Apis mellifera carnica</i> (carniolan)	6.3-6.5mm
<i>Apis mellifera caucasica</i> (caucasian bee)	Up to 7.2mm
<i>Apis mellifera ligustica</i> (Italian bee)	6.3-6.6mm
<i>Apis mellifera mellifera</i>	5.7-6.4mm



Parts of the honeybee head are shown in the above drawings.

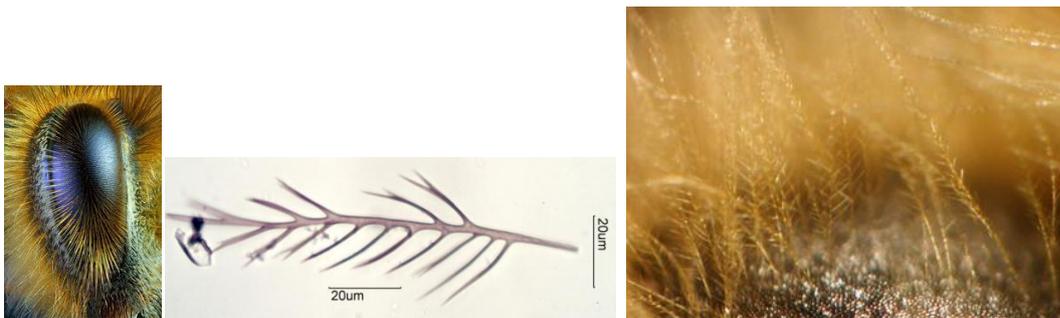
Key glands feeding the mouthparts are the salivary glands, the postcerebral within the head and the thoracic glands within the thorax. These salivary glands feed into the salivarium a cavity to the bottom and rear of the mouth. The glossa draws on the saliva for delivery in the feeding, cleaning and food liquefying roles of the bee. The saliva is mainly saline water.

In addition to the hypopharyngeal gland feed brood food (nurse bees) and enzymes in older bees. The hypopharyngeal gland opens at the hypopharyngeal plate via the hypopharyngeal duct.

The main activity of sucking food into the mouth is done so by muscles surrounding the cibarium, causing it to flex and create a pumping action through the resultant vacuum. The dilator muscles connect from the clypeus (the part above the epipharynx and between the cibarium and front of the face) to the cibarium. Transverse muscles surround the cibarium. There are two “rods that connect from the hypopharynx plate to the frons (brow of the bee). The rods hold up the hypopharynx and are used as muscle attachments.

### 1.51. the structure and function of the hairs of the honey bee.

Bees are well covered by branched (plumose) body hairs. They also have thousands of unbranched hairs covering their body which are for sensory purposes.

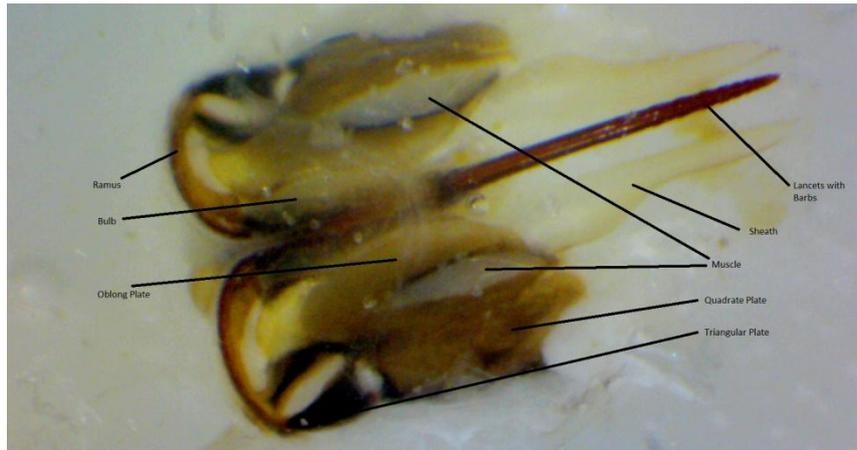


Closeup of hairs on the thorax of a worker bee. Notice that each hair is branched (plumose), enabling it to trap pollen grains more effectively. The plumose is one of the things that distinguish the honeybee from the wasp as the wasp has only straight hairs. The plumose are effective in collecting pollen and can be seen as kind of combs.

### 1.52. the sting, its structure, associated muscles, glandular connections and method of use.

The worker sting is a highly modified ovipositor which has evolved for defensive functions. Unlike most stinging insects the bee loses its sting after use, resulting in the bee's death shortly afterwards. The advantage of losing the sting is that the victim is injected with additional venom. The sting chamber is formed by the reduced and modified 8<sup>th</sup>, 9<sup>th</sup> and 10<sup>th</sup> abdominal segments attached to the bee by a thin membrane.

It is this delicate membrane that enables the sting along with the three segments, the poison glands and the terminal end of the alimentary canal to easily become detached once the bee has stung. The sting of the queen is more strongly attached and there are fewer barbs on the lancet (see later) meaning the chamber does not become detached after stinging.



The elements of the sting are:

- Three plates (per lancet) which along with muscles drive the associated lancet and valve to the bulb
- The shaft comprising the lancets and a stylet which expands into the bulb via an umbrella valve for each lancet, the valve opens when the lancet is extended and closes when it is removed from the victim
- Venom sac which holds the venom and is connected to the bulb
- Poison glands (acid glands) which secrete the venom into the sac
- Dufour gland (alkaline gland) which opens to the sting chamber to lubricate the stylet and to possibly neutralise any leaked venom

Before stinging the bee positions itself perpendicular to the victim by contracting ventral sclerites and extending dorsal sclerites.

The working of the sting mechanism comprises three main elements:

- Sting
- Plates and muscles
- Poison glands and venom sac

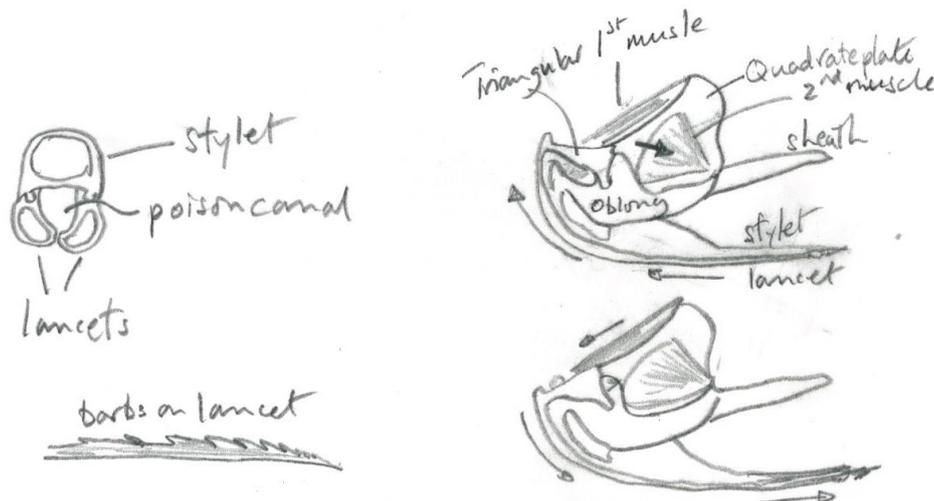
The sting is made up of two barbed lancets which slide on the tracks of the stylet or sheath. These open out into a large swelling called the bulb via umbrella valves. The bulb is full of venom from the venom sac and releases it into the poison canal formed by the linking of the lancets and sheath.

When the worker stings the first one lancet is extended by a series of plates and muscles which drive the lancet into the victim as well as opening the valve to the bulb releasing the venom. As the first lancet is withdrawn the second lancet is driven into the victim, this continues until the lancet becomes stuck and the sting is detached. The action of withdrawing the lancet causes a vacuum within the bulb drawing more venom into the bulb. On extending venom is forced into the lancets and sheath causing it to enter the victim via small pores in the lancets and sheath.

The three plates are Oblong, Triangular and Quadrate, there is one set for each lancet and each side functions alternately. The plates drive a Ramus which extends to the lancets and thus the action of thrusting and withdrawal is controlled by muscles connecting the oblong and quadrate plates which connect via joints to the triangular plate. The thrusting action opens the lancet valve within the bulb and venom is poured into the sting.

The poison glands (acid and alkaline) continually produce venom which is stored in the venom sac. The venom sac does not have a muscular wall, rather the flow of the venom is controlled by the expansion and contraction of the bulb. The pulsing of venom after the bee has detached itself is the continued action of the plates.

The Dufour gland releases alkaline solution into the sting chamber at the base of the sting, the purpose of which is not clear, it is thought to lubricate the sting and neutralise any venom that has seeped into the chamber. Snodgrass talks of both acid and alkaline poison elements making up the venom.



The action of the sting is controlled by two muscles attached to either side of the fixed quadrate plate. The 1<sup>st</sup> muscle from the rear of the quadrate attaches to the front of the oblong and the 2<sup>nd</sup> muscle from front of the quadrate to the rear of the oblong.

When the 1<sup>st</sup> muscle contracts the articulation between the oblong and triangular plates moves and hence drives the ramus which pushes the lancet into the victim. The 2<sup>nd</sup> muscle works antagonistically with the 1<sup>st</sup> muscle so when it contracts the lancet is withdrawn.

### 1.53. the composition of honey bee venom and its effect upon the insect, animal or human stung.

Venom comprises a mixture of proteins and peptides, the major component is a protein called melittin. Other components include hyaluronidase, phospholipase A, acid phosphatase and histamine.

The reason for the cocktail of components maybe due to the fact that the venom must be effective against a range of victims. For instance the histamine is highly toxic against other insects but the levels are very low for humans and have no effect.

02/04/2025

A table of components and their effect:

Compound	% in venom (dry weight)	Effects
Melittin (protein)	50	Breakdown membrane of blood and mast cells (mast cells are a form of white cell which have a role in the immune system)  Histamine and serotonin released by mast cells  Lowers blood pressure and respiration
Phospholipase A (enzyme)	12	Breaks down cell membrane  Pain  Toxicity; synergistic with melittin
Hyaluronidase (enzyme)	<3	Hydrolyzes connective tissue; opens up passages between cells for other components  Not toxic to humans
Acid phosphatase	<1	Involved in allergic reaction, possibly nausea
Histamine	<1	Itching  Pain  Not toxic, mast cells release far more histamine

In humans the reaction can be at three levels:

**Local**; localised swelling to the sting, possible swelling, itchy and tender for up to 3 days

**Systemic**; within a few minutes of the sting may involve severe rash possibly over whole body, wheezing, nausea, vomiting abdominal pains and fainting.

**Anaphylactic**; symptoms can occur within seconds, they include swelling >10cm at point of sting, difficulty in breathing, confusion, vomiting and falling blood pressure which can lead to loss of consciousness.