

## Standard methods for *Apis mellifera* anatomy and dissection

Norman L Carreck, Michael Andree, Colin S Brent, Diana Cox-Foster, Harry A Dade, James D Ellis, Fani Hatjina & Dennis van Engelsdorp

To cite this article: Norman L Carreck, Michael Andree, Colin S Brent, Diana Cox-Foster, Harry A Dade, James D Ellis, Fani Hatjina & Dennis van Engelsdorp (2013) Standard methods for *Apis mellifera* anatomy and dissection, Journal of Apicultural Research, 52:4, 1-40, DOI: [10.3896/IBRA.1.52.4.03](https://doi.org/10.3896/IBRA.1.52.4.03)

To link to this article: <https://doi.org/10.3896/IBRA.1.52.4.03>



Published online: 02 Apr 2015.



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REVIEW ARTICLE



# Standard methods for *Apis mellifera* anatomy and dissection

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Received 3 July 2013, accepted subject to revision 15 July 2013, accepted for publication 1 August 2013

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## Summary

An understanding of the anatomy and functions of internal and external structures is fundamental to many studies on the honey bee *Apis mellifera*. Similarly, proficiency in dissection techniques is vital for many more complex procedures. In this paper, which is a prelude to the other papers of the COLOSS BEEBOOK, we outline basic honey bee anatomy and basic dissection techniques.

## Métodos estandar para la disección y anatomía de *Apis mellifera*

### Resumen

El conocimiento de la anatomía y las funciones de las estructuras internas y externas es fundamental para muchos estudios sobre la abeja de la miel *Apis mellifera*. Del mismo modo, el dominio de técnicas de disección es vital para muchos procedimientos más complejos. En este trabajo, que es un prelude de los demás documentos del BEEBOOK COLOSS, describimos la anatomía básica de abejas y las técnicas básicas de disección.

## 西方蜜蜂解剖学和解剖的标准方法

### 摘要

在西方蜜蜂的很多研究中都很有必要了解蜜蜂解剖学和内外结构的功能。同样地，熟练的解剖技术对于许多复杂的研究也很重要。作为COLOSS BEEBOOK的开篇，本文概述了基本的蜜蜂解剖学和基本解剖技术。

**Keywords:** COLOSS, BEEBOOK, honey bee, *Apis mellifera*, anatomy, dissection, autopsy

**Footnote:** Please cite this paper as: CARRECK, N L; ANDREE, M; BRENT, C S; COX-FOSTER, D; DADE, H A; ELLIS, J D; HATJINA, F; VANENGELSDORP, D (2013) Standard methods for *Apis mellifera* anatomy and dissection. In V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS BEEBOOK, Volume I: standard methods for *Apis mellifera* research. Journal of Apicultural Research 52(4): <http://dx.doi.org/10.3896/IBRA.1.52.4.03>

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## 1. Introduction

This paper is placed first in the COLOSS *BEEBOOK*, because an understanding of honey bee anatomy is essential for much of the work described in the other papers. Similarly, basic dissection techniques are also fundamental to many facets of the study of honey bees. Man has kept honey bees for many thousands of years, and they have long held a fascination for those keen on understanding natural history. The development of our understanding of the anatomy of honey bees has been outlined by Crane (1999). In the modern era, two textbooks have become standard, those by Snodgrass (1956; 2004), and Dade (1962; 2009), and these are still readily available. For the purposes of this paper, we have therefore tried to give essential information only, and we suggest that the reader seeking further information consults these. Much of the section on dissection here is taken from Dade's (1962) work, and for reference we have reproduced his fine plates, and retained the same numbering for these and the figures. The plates themselves are also available separately from the International Bee Research Association in enlarged and laminated form for use at the laboratory bench: [http://ibrastore.org.uk/index.php?main\\_page=product\\_info&cPath=4&products\\_id=176](http://ibrastore.org.uk/index.php?main_page=product_info&cPath=4&products_id=176)

Those seeking further information about the functions of the structures shown here are suggested to consult two other books, those by Goodman (2003), and Stell (2012). Specific techniques of dissection for the diagnosis of nosema infection and tracheal mite infestation are given in the relevant *BEEBOOK* papers (Fries *et al.*, 2013; Sammataro *et al.*, 2013).

## 2. Anatomy

Table 1 summarises the external and internal anatomy of the honey bee. The list of structures is not exhaustive, but is presented based on the structures discussed in the text and shown in the figures and plates of Dade (1962), which are reproduced here for reference using his original numbering. Only notable muscles, tergites, sternites, ganglia, and other "minor" or "repeated" structures labelled in the figures or plates are included in the table. Most information regarding structure definition or function is from Dade (1962) but supplemented with information from Goodman (2003), the social insect anatomy glossaries at antbase.org (2008) and the Hymenoptera Anatomy Ontology Portal (2013). If column 2 shows "not labelled", the structure is not labelled in any plate or figure, though we felt it necessary to define. Though unlabelled, the plate or figure where the structure is shown is included in column 2. If "not shown", then the structure is not shown in any plate or figure, but is worth mentioning nonetheless. We list in column 3 the caste(s) in Dade (1962) figures or plates for which the structures are shown. "Generic" means that the image is

presented stylized for two or more castes. For example, the abdomen is shown for the drone, worker and queen in different figures or plates. However it is shown generically (no specific caste) in other figures or plates. Columns 4 and 5 list the life stage (egg, embryo, larva, prepupa, pupa, adult) and structure location (head, thorax and/or abdomen and internal/external) shown in the figures or plates. That does not mean the structures are unique to that life stage or figure or plate, only that they were presented as such in Dade (1962).

## 3. Dissection

### 3.1 Apparatus and materials

#### 3.1.1. The dissecting microscope

The type of instrument required for dissection is a prismatic dissecting microscope and suitable lighting source. Instruments of this kind embody prisms which erect the image, so that we can see our tools moving in the correct directions. A compound microscope is not suitable as it inverts the image. Binocular instruments give stereoscopic vision, enabling us to perceive depth in the object as well as to use both eyes. The most useful magnification is about x 20, and higher powers are neither useful nor desirable.

#### 3.1.2. Dissecting instruments

Only a few instruments are needed (Fig. 36), but it is important that three of them should be of exactly the right kind.

- The scissors should be 'cuticle' scissors, not less than 90 mm in length, and not much longer, with very fine points which cut cleanly right up to their tips. Looked at sideways, they should be very slim.
- Forceps need to have very fine points and grip very firmly at their extreme tips.
- A very sharp and finely-pointed knife is the third important tool. The Swann-Morton scalpel No. 3 with replaceable Swann-Morton No. 11 blades of the correct shape is widely used.
- A pair of needles, mounted in metal handles.
- Pasteur pipettes.
- Coarse forceps.
- A stout wire, bent into an L-shape, its long limb being about 150 mm long and the short one 20 mm long. The best material is brass rod, 5 mm thick. This brass wire has to be heated (Section 3.1.5.; Plate 1A).
- Two or three dissecting dishes need to be made from flat round metal tins about 75 mm in diameter. These are to be filled with melted beeswax to within 6 mm of the top of the rim; the wax must then be allowed to solidify. The surface of the wax has to be re-melted frequently, and this is done most conveniently by turning a Bunsen burner flame downwards over the dish.



**Table 1.** External and internal anatomy of the honey bee. The figure and plate numbers apply both to this paper and to Dade (1962).

Structure: singular(plural)	Figures (F) or Plates (P) where labelled	Caste presented in Figures (F) or Plates (P)	Life stage shown in Figures (F) or Plates (P)	Structure location	Definition/function
abdomen(s)	F20, 22, P4, 5, 8-10, 14-17	drone (P14, 15), worker (P4, 8, 9, 10), queen (P16, 17) generic (F20, 22, P5)	adult	3 <sup>rd</sup> main body section	Third tagma of the body. It is located after the thorax.
abdominal muscle(s)	P10	worker	adult	abdomen, internal	Muscles responsible for general abdominal movement and movement of internal abdominal organs.
acid gland(s) of sting	P10, 11	worker (P10), generic (P11)	adult	abdomen, internal	A long, forked tubule with slightly expanded trips. The single, proximal tube widens for form the venom sac, in which the gland secretion is stored. Also known as the "venom gland".
acinus(-ni)	not labelled				One of the small, sac-like dilations composing a compound gland. For example, they are present in the hypopharyngeal glands and look like a string of onions.
adult(s)	not labelled				The mature stage of honey bee.
alkaline gland(s) of sting	P10, 11	worker (P10), generic (P11)	adult	abdomen, internal	White, strap-shaped organ that empties its secretion into the sting chamber.
antenna(-ae)	F5, 28, P2, 18B, C	drone (F28), all (P2), generic (F5, P18B, C)	embryo (F5), larva (P18B, C), adult (F28, P2)	head, external	Important, paired sensory organs on bees. They aid in tactile, olfactory, and gustatory sensory perception.
antenna(-ae) cleaner	P6	generic	adult	foreleg, thorax, external	A notch with comb structure on the basitarsus of the foreleg of the honey bee. When the leg is bent, the fibula and notch form a structure through which the bee pulls its antennae to clean it.
antennal lobe(s)	F24, P9, 10, 13	worker (P9, 10, 13), generic (F24)	adult	head, internal	Areas in the deutocerebrum composed of bundles of nerve fibres that connect with the sense organs of the antennae.
antennal vesicle(s)	F20	generic	adult	head, internal	Small pulsating area under the base of the antennae. Small vessels run from the vesicles into the antennae to supply them with haemolymph.
anus(-ni)	P5, 16C, 19	generic (P5, 19), queen (P16C)	adult (5P, 16C), larva (P19)	abdomen, external	An orifice in the proctiger through which wastes are expelled.
aorta(-ae)	F20, P12	generic	adult	head, thorax, internal	Continuous with the heart, it is a blood vessel that runs through the thorax to the head, where its end opens below the brain, thus supplying haemolymph to the brain/head.
apodeme(s)	P12	worker	adult	abdomen, internal	Peg-like, ingrowth extensions of thickened plate edges of the exoskeleton. They serve as points of attachment for muscles and may support internal organs.
arcus(-ci)	P6	generic	adult	legs, thorax, external	An arc on the ventral surface of the "foot" or pretarsus to which the dorsally-located manubrium is attached.
arolium(-ia)	P6	generic	adult	legs, thorax, external	A pad on the pretarsus that is normally folded and raised between the claws. Bees unfold the arolium for adhesion when walking on a smooth surface.
auricle(s)	P7	worker	adult	hind legs, thorax, external	A sloping shelf on the end of the basitarsus (i.e. in the pollen press). It has a textured surface and a fringe of hairs to facilitate pollen processing.
basalare(s) and basalare muscle(s)	F9	generic	adult	thorax, external (basalare) and internal (basalare muscle)	A plate on the thorax at the base of the wing that is hinged to a pleurite and attached to a muscle. Contraction of the muscle (basalar muscle) causes the basalare to swing inward on its hinge, thus pulling the leading edge of the wing down during flight.
basement membrane(s)	not shown			all, internal	Supportive layer for epidermal cells composed of basal lamina and collagen fibres. It separates the haemocoel from the exoskeleton.
basitarsus(-rsi)	P6, 7	worker (P7), generic (P6)	adult	legs, thorax, external	The first, and largest, tarsomere (or subsection of the tarsus).
bulb(s) of endophallus(-lli)	P14, 15	drone	adult	abdomen, internal	An ovoid body on the distal end of the endophallus, being crescent-shaped and having roughly triangular sclerotized plates in its walls and trough-like internal projections. Dorsal and lateral plates of bulb are seen in P15.
bulb(s) of sting	F12, P11	generic	adult	abdomen, internal	Inflated organ between the ramus and shaft of sting. It is continuous with the stylet and full of venom.

**Table 1. cont'd**

<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
bursa(-ae)	F32, 33	queen	adult	abdomen, internal	A wide, membranous pouch at the anterior end of the sting chamber.
brain(s)	F20, 23, 24, P13	worker (P13), generic (F20, 23, 24)	larva (F23), adult (P13, F20, 24)	head, internal	The centre of sensory perception in the bee. It principally receives stimuli from the eye and antennae and transmits the nervous impulses to the motor centres (ganglia) of the ventral nerve cord.
cardo(-dines)	F3, 4A, P2D, 3	worker (P2D), generic (F3, 4A, P3)	adult	head, external	The basal hinge of the maxilla, located on the proximal end of the proboscis. The cardines are instrumental in extending the proboscis.
cervix(-ices)	not labelled but shown in P15	drone	adult	abdomen, internal	The part of the shaft of the endophallus in drone honey bees between the horns and bulb.
chitin(s)	not shown			all, external	Tough, protective polysaccharide that forms much of the insect exoskeleton.
cibarium(-ia)	F15, P13	worker (P13), generic (F15)	adult	head, internal	Food chamber in the bee mouth. Muscles attached to it cause it to act as a sucking pump, thus raising fluid through the proboscis.
clasper(s)	P15	drone	adult	abdomen, external	External part of the reproductive organs of drones. They are reduced to small sclerites attached to the sternite of A9.
claw(s)	P6	generic	adult	legs, thorax, external	Pair of strong, recurved structures present on the pretarsus (foot). They provide secure footing on rough surfaces. Also known as unguis.
clypeus(-pei)	P2C	worker	adult	head, external	A plate of the exoskeleton on the "face" of the bee.
compound eye(s)	P2C, 9, 13	worker	adult	head, external	The two, large eyes on the head of the bee. The external surface of both eyes is an elongated oval, strongly convex and consists of the lenses of thousands of ommatidia.
corbicula(-ae)	P7	worker	adult	hind leg, thorax, external	The "pollen basket" or pollen carrying apparatus on the hind leg of the worker bee.
cornua(-nua)	See "horn"				Synonymous with "horn".
corpus (-pora) allatum (-ta)	P13	worker	adult	head, internal	Paired endocrine glands behind the brain. They produce juvenile hormone.
corpus (-pora) cardiacum (-iaca)	not shown			head, internal	Two small knots of tissue just in front of the corpora allata, on either side of the aorta. They pour the hormones they produce into the haemolymph, stimulating larval prothoracic endocrine glands to produce ecdysone.
corpus (-pora) pedunculatum (-ta)	not shown			head, internal	Also called "mushroom bodies". Buried in protocerebrum, under the ocelli, they contain small groups of nerve cells. They coordinate the actions of the insect according to information received from the sense organs.
coxa(-ae)	P5, 7	worker (P7), generic (P5)	adult	legs, thorax, external	Segment of the leg, closest to the thorax.
crop(s)	P8B, 9	worker	adult	abdomen, internal	Also known as the "honey stomach", the crop is a transparent bag in which foraging bees store nectar after it has been collected from flowers and while it is being transported to the hive. The crop is the last section of the foregut.
crystalline cone(s)	F26	generic	adult	head, internal	A transparent area behind the ommatidium lens that is surrounded by pigment cells.
cuticle(s)	not shown			all, external	The main layer of the exoskeleton. It is secreted by epidermal cells which lie beneath it. It has two layers: exocuticle and endocuticle. It is covered by the epicuticle.
deutocerebrum(s)	not shown			head, internal	Part of the brain. It is composed of bundles of nerve fibres connected with the sense organs of the antennae.
dorsal diaphragm(s)	F20, P8C	worker (P8C), generic (F20)	adult	abdomen, internal	A thin, transparent membrane spread over the roof of the abdomen and attached to the apodemes of the tergites and sternites. Responsible (with the ventral diaphragm) for setting up circulation inside the abdomen and for drawing blood from the thorax into the abdomen.
duct(s) of hypopharyngeal gland(s)	F15	generic	adult	head, internal	Ducts leading from the hypopharyngeal gland to the back of the hypopharynx. The duct carries the secretions of the hypopharyngeal gland to the mouth of the bee.

**Table 1. cont'd**

<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
duct(s) of salivary gland(s)	F15	generic	adult	head, internal	Ducts leading from the salivary glands of the thorax to the salivarium in the bee mouth, at the base of the glossa.
duct(s) of spermatheca(-ae)	F32, 33	queen	adult	abdomen, internal	Duct connecting the spermatheca to the vagina. At mating, the drones deposit spermatozoa into the oviducts. The spermatozoa then migrate up the spermathecal duct into the spermatheca.
duct(s) of venom gland(s)	F12	generic	adult	abdomen, internal	A tube that runs from the venom gland to the bulb of the sting.
egg(s)	P20	generic	egg		Also known as "ovum", the egg contains the embryo, the youngest juvenile form of the honey bee. They are about 1.5 mm long and 1/3 mm in diameter at the apex.
egg cell(s)	F31	queen	adult	abdomen, internal	The cells present in the ovarioles that later become the mature eggs.
ejaculatory duct(s)	P14	drone	adult	abdomen, internal	The duct in the endophallus through which spermatozoa pass at the moment of copulation.
embryo(s)	P20	generic	egg	egg, internal	The youngest juvenile form of the honey bee. It is present in the egg.
endocuticle(s)	not shown			all, external	The innermost layer of cuticle. It is a soft layer, composed mostly of chitin.
endophallus(-lli)	P15	drone	adult	abdomen, internal	The part of the drone reproductive organ that is inserted into the queen at copulation. It is stored, inverted, in the abdomen.
epicuticle(s)	not shown			all, external	The thin, waxy/waterproof layer covering the exoskeleton. It has the following layers (from outside to inside): cement, wax, polyphenol, and cuticulin. It reduces water loss and blocks invasion of foreign matter.
epidermis(es)	not shown			all, internal	Single layer of epithelial cells that secrete the rest of the layers of the exoskeleton. They are located below the endocuticle and are attached to the basement membrane.
epipharynx(-nges)	F4A, 15, P13	worker (P13), generic (F4A, 15)	adult	head, internal	A soft pad on the inner surface of the labrum that is shaped to fit closely (i.e. form an airtight seal) against the proboscis when the proboscis is in use.
exocuticle(s)	not shown			all, external	The outermost layer of cuticle. It is hard and composed mainly of sclerotin and chitin. It hardens in a process called sclerotization, thus forming the sclerites.
exoskeleton(s)	not shown			all, external	The protective outer shell of the bee. From outside to inside, it is composed of the following layers: epicuticle, cuticle (exocuticle and endocuticle), epidermis, and basement membrane. It protects the internal organs and serves as locations for muscle attachment.
fat body(ies)	P10	worker	adult	abdomen, internal	A layer of conspicuous, creamy cells concentrated principally on the floor and roof of the abdomen. The fat body contains fat cells, which are mainly fat but also may contain protein and glycogen.
femur(-mora)	P6, 7	worker (P7), generic (P6)	adult	legs, thorax, external	From the thorax, the 3 <sup>rd</sup> segment of each bee leg.
fibula(-ae)	P6	generic	adult	foreleg, thorax, external	A jointed spur, present on the foreleg tibia, that is part of the antenna cleaner complex.
flabellum(-lla)	P3	generic	adult	head, external	The small, rounded scoop at the end of the glossa. Also known as the labellum.
flagellum(-lla)	F28A	drone	adult	head, external	The largest part of the bee antenna. It contains 11 segments in queens and workers and 12 segments in drones.
fold(s), forewing(s)	P6	generic	adult	wing, external	Folds on the trailing edge of the forewing to which the hamuli on the leading edge of the hind wings connect.
follicle cell(s)	F31	queen	adult	abdomen, internal	Cells that cluster around the egg cells in the ovariole to form a sheath around each egg cell. They disappear once the egg is mature, thus leaving the network of markings on the exterior of the egg.

**Table 1. cont'd**

<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
food canal(s)	F4B	generic	adult	head, internal	A tube formed when the galeae and labial palps are brought close together. It surrounds the glossa and is used to suck nectar, honey, water or other liquids.
foramen(-mina)	P2D	worker	adult	head, external	A hole below the occiput on the posterior portion of the head through which the organs inside the head are connected to the thorax.
foregut(s)	not labelled			head, thorax, abdomen, internal	The mouth cavity, oesophagus, and crop of the honey bee. It is lined with cuticle. Also called stomodaeum in embryonic and larval bees.
foreleg(s)	F5, P6	generic	embryo (F5), adult (P6)	thorax, external	The front pair of the three pairs of legs of the honey bee. These are located on the prothorax and contain the antenna cleaner. Also called proleg.
forewing(s)	not labelled but seen in P6			thorax, external	The larger of the paired wings on both sides of the body. The forewing is located on the mesothorax.
fossa(-ae)	P2D, 3	worker (P2D), generic (P3)	adult	head, external	A U-shaped hollow on the posterior portion of the head.
frons(-ntes)	P2C	worker	adult	head, external	An area on the anterior portion of the head (face). It is the segment best seen below the ocelli but above and beside the antennae. Also called the "brow".
furca(-ae)	P9, 10, 12	worker (P9, 10), generic (P12)	adult	thorax, internal	Hardened processes of the thoracic sternites that reach inside the thorax. They may protect internal organs or have other functions.
furcula(-ae) of the sting(s)	P11	generic	adult	abdomen, internal	Small, recurved process rising from the base of the bulb as two branches which unite after curving over it dorsally. It provides sites to which muscles attach in the sting.
galea(-ae)	F4B, P2D, E, 3	worker (P2D, E), generic (F4B, P3)	adult	head, external	Paired parts of the proboscis that, with the labial palps, form the food canal.
ganglion(-ia)	F23, P10, 11, 12	worker (P10, 11), generic (F23, P12)	larva (F23), adult (P10-12)	thorax, abdomen, internal	Nerve centres located in the thorax and abdomen. They are responsible, primarily, for movement and organ control.
gena(-ae)	P2C, 13	worker	adult	head, external	The "cheek" region or lateral plates on the bee head.
glossa(-ae)	F4B, 15, P2D, E, 3	worker (P2D, E), generic (F4B, 15, P3)	adult	head, external	The bee "tongue", the distal tip of which contains the flabellum. It is a hollow tube of thin, tough membrane and is flattened and curled at its sides. It is covered with small hairs.
glossal rod(s)	F4B	generic	adult	head, internal	A slender rod that stiffens the glossa and which can be drawn backwards by muscles in the prementum.
hamulus(-li)	P6	generic	adult	wing, external	Hooks on the leading edge of the hind wing that latch onto a fold on the trailing edge of the forewing, thus joining the fore- and hind wings.
head(s)	P2	drone (P2B), queen (P2A), worker (PC-F)	adult	1 <sup>st</sup> main body section	First tagma of the bee body. It is located before the thorax.
heart(s)	F20, P8C	worker (P8C), generic (F20)	adult	abdomen, internal	Elongated organ lying just under the roof of the abdomen and attached to the dorsal diaphragm. It has muscular walls and small holes (ostia) which allow haemolymph into the heart. The heart pumps the haemolymph forward through the abdomen and into the thoracic aorta.
haemocoel(s)	not shown			all, internal	The internal body cavity of the bee.
haemolymph	not shown			all, internal	Bee blood.
hind gut(s)	not labelled			abdomen, internal	The Malpighian tubules, small intestines, rectum and anus of the bee. Also called proctodeum in embryonic and larval bees.
hind leg(s)	F5, P7	worker (P7), generic (F5)	embryo (F5), adult (P7)	thorax, external	The last pair of the three pairs of legs of the honey bee. These are located on the metathorax and contain the pollen press, pollen brush, and corbicula (pollen basket).
hind wing(s)	not labelled but seen in P6			thorax, external	The smaller of the paired wings on both sides of the body. The hind wing is located on the metathorax.

**Table 1. cont'd**

<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
horn(s) of endophallus, also cornea(-nua) or pneumophysis(-ses)	P15	drone	adult	abdomen, internal	Conspicuous projections on either side of the drone vestibule.
hypopharynx(-nges)	F15	generic	adult	head, internal	A plate on the floor of the cibarium. It is hardened and the front lobe bends downward.
hypopharyngeal gland(s)	P9, 13	worker	adult	head, internal	Paired glands in the head with ducts opening at the base of the hypopharynx. They produce components of brood food.
ileum(-lei)	not labelled but seen in F17, P8B, 9	worker (P8B, 9), generic (F17)	adult	abdomen, internal	Part of the hindgut. Also called the "small intestine". It is a narrow tube, surrounded by circumferential muscle fibres. It is pleated into 6 longitudinal folds.
imago(-gines)	not labelled				The adult or sexually developed insect.
instar(s)	P20	generic	larva and pupa		The developmental stage of the bee between each moult, until sexual maturity (adulthood) is reached.
Johnston organ(s)	not shown			head, internal	A sense organ located in the antennae pedicel. The cells are arranged around the nerve trunk and are believed to be speed-of-flight indicators and also sensitive to gravity and electromagnetic fields.
labellum(-lla)	not labelled as labellum but shown in P3				The small, rounded scoop at the end of the glossa. Also known as the flabellum.
labial palp(s)	F4B, P2D, E, 3	worker (P2D, E), generic (F4B, P3)	adult	head, external	Paired parts of the proboscis that, with the galea, form the food canal.
labium(-ia)	F5, P18B, C	generic	embryo (F5), larva (P18B, C)	head, external	The "lower lip" of the bee from which the inner members of the proboscis are all derived. These include the postmentum, prementum, labial palps, glossa, two paraglossae and the labellum.
labrum(-ra)	F5, P2C, E, 13, 18B, C	worker (P2C, E, 13), generic (F5, P18B, C)	embryo (F5), larva (P18B, C), adult (P2C, E, 13)	head, external	A sclerotized flap hinged to the clypeus on the anterior side of the head.
lacinia(-ae)	F4A, P3	generic	adult	head, external	Part of the maxillae. They press against the epipharynx when the proboscis is in use. This forms an airtight joint to facilitate sucking through the proboscis.
lancet(s)	F12, P11, 18D	generic	adult (F12, P11), prepupa (18D)	abdomen, external	Paired, hardened shafts that are part of the sting apparatus. They are pointed and barbed on the distal end. When the sting is used, muscles cause the lancets to dig into the victim at the sting site.
lancet track(s)	P5, 11	generic	adult	abdomen, internal	A semicircular path on which the ramus of the lancet runs in the process of deploying the sting.
larva(-ae)	P20	generic	larva		The immature stage of the honey bee that emerges from the egg. Larvae spend all their time feeding and growing. This stage immediately precedes the prepupal stage of bee development.
lateral pouch(es) of bursa(-ae)	F32	queen	adult	abdomen, internal	Bulbous sacs located on both sides of the bursa (1 sac per side).
lens(es)	F26	generic	adult	head, external	The outer layer of the eye, both on the ocelli and the ommatidium.
ligula(-ae)	not shown				The region where the base of the glossa and the small pair of paraglossal lobes join the prementum.
lip(s), proventriculus(-li)	F16A-D	generic	adult	abdomen, internal	4 triangular flaps on the apex of the proventriculus can be closed/opened. They are fringed with hairs. The hairs filter pollen and other particles from nectar.
longitudinal commissure(s)	P10	worker	adult	abdomen, internal	Twin nerve trunks that connect ganglia.
longitudinal muscle(s), thorax(-aces)	P8A, 12	worker (P8A), generic (P12)	adult	thorax, internal	Two bundles of muscles that run side by side from the mesothorax tergite and 1 <sup>st</sup> phragma to the 2 <sup>nd</sup> phragma. Contraction of this muscle squeezes the anterior and posterior ends of the thorax together, raising the roof of the thorax and forcing the wings down.
lorum(-ra)	P3	generic	adult	head, external	V-shaped submentum that join the stipites together. It is located between the cardines.

**Table 1. cont'd**

<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
lumen(-mina) of the proventriculus(-li)	F16B	generic	adult	abdomen, internal	The inside space of the proventriculus.
Malpighian tubule(s)	F17, P8A, B, 9, 19	worker (P8A, B, 9), generic (F17, P19)	adult (F17, P8A, B, 9), larva (P19)	abdomen, internal	Function as the "kidneys" in the bee. They filter nitrogenous wastes from the haemolymph and pass it through the small intestines to the rectum.
mandible(s)	F5, P2C, E, 3, 13, 18B, C	worker (P2C, E, P13), all (P3), generic (F5, P18B, C)	embryo (F5), larva (P18B, C), adult (P2C, E, 3, 13)	head, external	The "jaws" which are hinged to the genae. They are strong, spoon-shaped organs in the worker, concave and ridged on the inner side. The queen's mandibles are toothed, and, along with the drone's, are unspecialized. Mandibles have many functions in the worker bee.
mandible groove(s)	P3	generic	adult	head, external	A straight depression on the mandible down which secretions from the mandibular gland flow.
mandible orifice(s) of gland	P3	generic	adult	head, external	An opening on the mandible that leads to the mandibular gland.
mandibular gland(s)	P13	worker	adult	head, internal	A pair of glands above the mandibles. They are single, somewhat lobate sacs lying under the genae. They are developed in the worker, rudimentary in the drone, and very large in the queen. The secretion of the mandibular glands in queens is known as "queen substance", which has multiple, important functions in the colony.
manubrium(-ria)	P6	generic	adult	legs, thorax, external	A dorsal plate on the pretarsus. It has 5 or 6 long bristles and is attached to the arcus of the arolium.
maxilla(-ae)	F5, P2D, E, 18B, C	worker (P2D, E), generic (F5, P18B, C)	embryo (F5), larva (P18B, C), adult (P2D, E)	head, external	Part of the proboscis composed of the stipites, galeae, laciniae and the maxillary palps.
maxillary palp(s)	P3	generic	adult	head, external	Small appendages that are part of the maxilla of the bee. They are vestiges, with no clear function.
median oviduct(s)	F32, 33, P16C	queen	adult	abdomen, internal	The duct formed where the two lateral oviducts join. Eggs pass from the ovarioles into the lateral oviducts, and then into the median oviduct. The median oviduct opens into the vagina.
mesenteron(s)	labelled as ventriculus in P19	generic	larva	abdomen, internal	The midgut of the immature bee. It becomes the ventriculus in the adult bee.
mesothorax(-aces), T2	P4	generic	adult	thorax, external	The second thoracic segment. The middle legs and forewing are attached to this segment.
metathorax(-aces), T3	P4	generic	adult	thorax, external	The third thoracic segment. The hind legs and hind wing are attached to this segment.
micropyle(s)	not shown			egg, external	A location at the apex of the egg that is not covered by follicle cells but rather by a thin membrane. It is this area through which spermatozoa penetrate when the egg is fertilized.
middle leg(s)	F5	generic	embryo	thorax, external	The middle pair of legs on the thorax. It has a characteristic spine on the distal end of the tibia. Also called mesoleg.
midgut(s)	labelled as ventriculus and/or mesenteron P9			abdomen, internal	Also called ventriculus or mesenteron, the latter in embryonic and larval bees.
mucus gland(s)	P14, 15	drone	adult	abdomen, internal	Club-shaped sacs in the drone, associated with the endophallus. It produces mucus and increases in size as the drone matures. The mucus may be deposited in the mated queen to prevent the escape of spermatozoa.
Nasanov gland(s)	not labelled				Synonymous with scent gland.
nerve(s)	P10	worker	adult	head, thorax, abdomen, legs, antennae, internal	Cells responsible for sensory transmission and processing to/in the ganglia and brain and for muscle movement.
nurse cell(s)	F31	queen	adult	abdomen, internal	Clusters of cells that follow each egg cell in the ovarioles. The nurse cells provide nutrients to the growing eggs and are absorbed as the egg approaches full size.
oblong plate(s)	F12, P5, 10, 18D	generic	adult (F12, P5, 10), prepupa (P18D)	abdomen, external	One of three pairs of plates in the sting which are moved by muscles and cause the sting to deploy.
occiput(s)	P2D	worker	adult	head, external	The upper posterior region of the head.

**Table 1.** cont'd

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ocellus(-li)	F24, P2C, 9, 13	worker (P2C, 9, 13), generic (F24)	adult	head, external	The three simple eyes on the dorsal surface of the head, between the two compound eyes. They consist of a lens above a layer of simple, elongated retinal cells. Used to measure light intensity.
oenocyte(s)	not shown				Type of cell in the fat body. Function possibly linked to wax production.
oesophagus(-gi)	P9, 12, 13	worker (P9, 13), generic (P12)	adult	head, thorax, internal	The tube connecting the mouth to the crop. It is part of the bee foregut.
ommatidium(-ia)	F26, P13	worker (P13), generic (F26)	adult	head, internal	Elongated bodies of the compound eyes, tapering towards their inner ends. It consists of a lens, crystalline cone (surrounded by pigment cells), a bundle of eight retinula cells (also surrounded by pigment cells).
optic lobe(s)	F24, P9, 13, 18B	worker (P9, 13), generic (F24, P18B)	adult (F24, P9, 13), larva (P18B)	head, internal	Bundles of crossing nerve fibres (chiasmata) connected with the thousands of units in the compound eyes. The lobes are part of the protocerebrum, which is one of the brain's three component parts.
ostium(-ia)	P8C	worker	adult	abdomen, internal	One-way valves in the heart that allow haemolymph to enter the heart when it is dilated, but confined it and force it forward when the heart contracts.
ovariole(s)	F31	queen	adult	abdomen, internal	A long tubule located in the ovary. It contains the egg cells, nurse cells, and follicle cells. Groups of ovarioles (150 or more) coalesce to form the ovary.
ovary(-ies)	P10, 16A-C	worker (P10), queen (P16A-C)	adult	abdomen, internal	Paired organs in the queen, each consisting of a bundle of about 150 ovarioles. They are greatly reduced in the worker.
oviduct(s), lateral	F32, 33, P16B, C	queen	adult	abdomen, internal	A broad tube that is attached to both ovaries. The two lateral oviducts connect directly to ovarioles on their posterior side and to one another to form the median oviduct on their anterior side.
ovum(-va)	not labelled				Another term for "egg".
paraglossa(-ae)	P3	generic	adult	head, external	Part of the proboscis, they are a pair of small appendages of the ligula.
pecten(-tines)	not labelled				Another term for "rastellum".
pedicel(s)	F28A	drone	adult	head, external	The second segment of the bee antenna.
peduncle(s)	not labelled				Another term for "petiole".
petiole(s)	not labelled but shown in P4, 5			thorax, abdomen external	The restricted (narrowed) area linking the thorax to the abdomen. Often called the "waist" or "peduncle".
phallotreme(s)	not shown			abdomen, internal	Genital opening of endophallus.
pharynx(-nges)	F3, 15	generic	adult	head, internal	The gullet-cavity behind the cibarium.
phragma(ae)	P12	generic	adult	thorax, internal	Fence-like ridges that are ingrowths of the exoskeleton. They serve as strong places of attachment for muscles, and struts to stiffen the shell in places where added strength is needed.
pigment cell(s)	F26	generic	adult	head, internal	Cells in the ommatidium that are located around the crystalline cone and the retinula cells. They appear to exclude the light which enters neighbouring ommatidia, thus ensuring stimulation is applied only by the light entering a single ommatidium.
pit(s), anterior head	P2C	worker	adult	head, external	Two depressions at the edges of the clypeus that mark the position of the upper ends of the tentoria.
pit(s), posterior head	P2D	worker	adult	head, external	Two depressions adjoining the foramen that indicate the position of the ends of the tentoria.
planta(-ae)	P6	generic	adult	legs, thorax, external	A sclerite of the pretarsus between the unguitractor and arolium.
pleurite(s)	P4, 5	worker (P4), generic (P5)	adult	thorax, external	In honey bees, this mainly refers to lateral plates (sclerites) of the thoracic exoskeleton. The legs on the pro-, meso-, and metathorax are articulated to the pleurites located on the respective thoracic segments. Pleurites are absent in abdomen.
pneumophysis(-ses)	labelled as horn in P15				Synonymous with "horn".
pollen brush(es)	P7	worker	adult	hind legs thorax, external	On the hind leg, it is the inner side of the flat, broad basitarsi. It is covered with rows of closely set, stiff hairs. It is used for brushing pollen from the abdomen after visits to flowers.

**Table 1. cont'd**

<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
pollen press(es)	P7	worker	adult	hind leg, thorax, external	The tibio-tarsal joint on the hind leg of the worker bee, modified for use as a pollen manipulator.
postcerebral gland(s)	P9, 13	worker	adult	head, internal	Glands that lie behind the brain. Produces enzymes that are a component of bee saliva.
postmentum(-ta)	P3	generic	adult	head, external	Part of the proboscis. It is articulated to the middle of the lorum and between the cardines.
pouch(es), proventriculus(-li)	F16B-D	generic	adult	abdomen, internal	Pockets behind the proventricular lips in which pollen is collected after being filtered from nectar. Pollen masses pass from here into the ventriculus when full.
prementum(-ta)	F15, P2D, E, 3	worker (P2D, E), generic (F15, P3)	adult	head, external	A sclerite of the proboscis to which is joined the labial palps, glossa, and two paraglossae.
prepupa(ae)	P20	generic	prepupa		The immature stage of bee development between the larval and pupal stages. The prepupal stage occurs in capped cells.
pretarsus(-si)	P6	generic	adult	legs, thorax, external	The bee "foot".
proboscis(es)	P2C, 3	worker (P2), generic (P3)	adult	head, external	An anatomical cluster composed of the labium and maxillae. It has many component parts. It is used by the bee to suck in liquids (nectar, water, honey, etc.), for exchanging food with other bees, and for removing water from nectar.
proctiger(-ra)	P5, 11	generic	adult	abdomen, external	The remains of the 10 <sup>th</sup> abdominal segment. It carries the anus and is fixed to the sting.
proctodeum(-ea)	P19	generic	larva	abdomen, internal	The hindgut of the embryo and larva. It does not connect with the midgut (mesenteron) until the full grown larva has taken its last meal and is ready to pupate. It becomes the Malpighian tubules, small intestine, rectum, and anus in the adult bee.
propodeum(-ea), A1	P4	worker	adult	thorax, external	The first abdominal segment located immediately behind the metathorax. Its tergite (A1, tg) nearly encircles the rear part of the thorax and narrows to fit around the petiole. Its sternite (A1, st) is a small strap underneath the petiole. The precursor for this is shown for larvae in P18A and for prepupae in P18 E (in both, labelled A1).
prothorax(-aces), T1	P4	worker	adult	thorax, external	The first thoracic segment. The two forelegs are attached to the pleurites of this segment. Its tergite encircles the bee neck like a collar. A lobe on either side projects backwards to cover the first spiracle.
protocerebrum(-ra)	F24	generic	adult	head, internal	Part of the brain. It is primarily composed of the optic lobes.
proventriculus(-li)	F16, P9	worker (P9), generic (F16)	adult	abdomen, internal	Valve between the crop and ventriculus that prevents the collected nectar from running into the stomach. It also comprises a filtering apparatus for extracting pollen.
pupa(-ae)	P20	generic	pupa		The immature stage of bee development that occurs after the prepupal stage and before the adult stage. This stage occurs in capped cells and is when the grub-like body of the prepupa begins to develop into that of an adult.
pyloric valve(s)	F17	generic	adult	abdomen, internal	Valve that regulates the passage of material from the ventriculus into the intestine.
quadrate plate(s)	F12, P11	generic	adult	abdomen, external	One of three pairs of plates in the sting which are moved by muscles and cause the sting to deploy.
ramus(-mi)	F12	generic	adult	worker	Proximal end of the lancet. It is flexible and runs on a semicircular track. Pressure by the triangular plate pushes the ramus round its track, thus forcing the lancet in the same direction.
rastellum(-lla)	P7	worker	adult	hind legs, thorax, external	A row of wide and pointed spines on the distal end of the tibia. It is used to rake pollen out of pollen brushes and prevent the pollen mass from escaping the pollen press. Also known as "pecten".
rectal pad(s)	P8A, 9	worker	adult	abdomen, internal	Six partly chitinized pads arranged around the rectum. They reabsorb ions and water (used to collect wastes by the Malpighian tubules) from the rectum.



**Table 1. cont'd**

<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
rectum(-ta)	P5, 8A, B, 9, 16A	generic (P5) worker (P8A, B, 9), queen (P16A)	adult	abdomen, internal	Part of the hind gut into which the contents of the small intestines empty and out of which wastes pass through the anus. It can expand greatly to hold wastes when bees are unable to leave the colony to defecate.
retaining hair(s) or auricle(s)	P7	worker	adult	hind legs, thorax, external	Hairs on the fringe of the auricle that keep pollen from falling out of the pollen press.
retina(-ae) of ocellus(-lli)	P9, 13	worker	adult	head, internal	Part of the ocellus that detects relative intensity of flight which falls on the lens.
retinula(-ae) cell(s)	F26	generic	adult	head, internal	A bundle of 8 cells in the ommatidium. They are surrounded by pigment cells. The edges of the retinula cells, which meet in the axis of the ommatidium, combine to form a long, narrow rhabdom.
rhabdom(s)	F26	generic	adult	head, internal	A transparent rod that is formed by the hollow left when the edges of 8 retinula cells meet in the axis of the ommatidium. It is striated and likely diffuses light laterally into the retinula cells.
salivarium(-ia)	F15	generic	adult	head, internal	A pouch under the hypopharynx into which opens the common duct of the postcerebral and thoracic salivary glands.
salivary gland(s), thorax	P9	worker (P9)	adult	thorax, internal	Glands in the thorax which are partially responsible for producing enzymes in the saliva.
scape(s)	F28A	drone	adult	head, external	The first antennal segment.
scent canal(s)	P12	worker	adult	abdomen, external	Structure on the 7 <sup>th</sup> abdominal tergite onto which the pheromone of the scent gland is released.
scent gland(s)	P4	worker	adult	abdomen, internal	Lies under the front part of the 7 <sup>th</sup> abdominal tergite. Secretes a pheromone into the scent canal, which is dissipated by the bees when they fan their wings. Also called Nasanov gland.
sclerite(s)	not labelled			all, external	Hardened plates of cuticle.
sclerotin(s)	not labelled			all, external	A tanned protein present in the exocuticle.
scopa(-ae)	not labelled			abdomen, external	Pollen-carrying apparatus on bee body. Also known as "corbicula" or "pollen basket" for honey bees.
scutal fissure(s)	P5	generic	adult	thorax, external	A fissure (line that divides a sclerite) between the scutum and scutellum.
scutellum(-lla), T3, tg	P4, 5	worker (P4), generic (P5)	adult	thorax, external	The tergite of the 3 <sup>rd</sup> thoracic segment. It is visible as a prominent "roll" behind the scutum.
scutum(-ta), T2, tg	P4, 5	worker (P4), generic (P5)	adult	thorax, external	The tergite of the 2 <sup>nd</sup> thoracic segment. It is strongly domed and covers most of the thorax.
semen	P15	drone	adult	abdomen, internal	A mixture of spermatozoa and glandular secretions produced by the drone reproductive organs.
seminal vesicle(s)	P14, 15	drone	adult	abdomen, internal	Two curved, sausage-shaped organs which increase in length and girth as they receive spermatozoa from the testes. Their walls are muscular and lined with glandular tissue. When the drone is sexually mature the vesicles are packed with spermatozoa and glandular secretions.
sense hair(s)	F28C, 29B	drone (F28C), generic (F29B)	adult	head, external	Sensilla with fine bristles projecting from the cuticle on a smooth surface or from in a pit. They are tactile organs, with many located on the antenna.
sense peg(s)	not shown				Similar to sense hairs, they are sensilla with short, stout pegs rather than a bristle, projecting from the cuticle on a smooth surface or from in a pit. They are tactile organs (mechanoreceptors).
sense plate(s)	F28C, 29A	drone (F28C), generic (F29A)	adult	head, antennae, external	Sensilla that consist of a hollow in the cuticle, capped by a thin plate, level with the surrounding surface. They are chemoreceptors.
sensillum(-lla)	F29	generic	adult	head, external	A sense cell or cells with a nerve fibre connected with the central nervous system and its distal end in close connection to the cuticle. There are several types and they are involved in various types of sensory perception (principally mechano- and chemoreceptors).
seta(-ae)	not labelled			all, external	A sensillum that is multicellular, consisting of trichogen (hair shaft), tormogen (the "socket" holding the shaft), and sense cells.

**Table 1. cont'd**

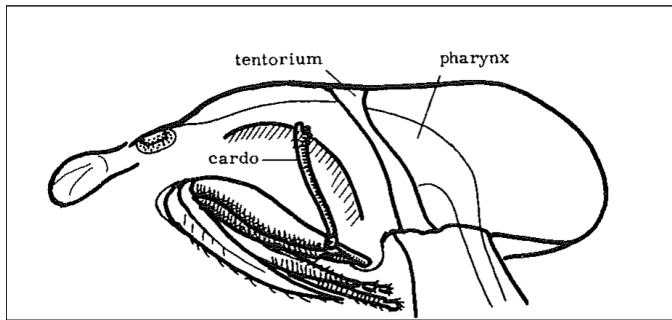
<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
silk gland(s)	F5, P19	generic	embryo (F5), larva (P19)	abdomen, internal	Long, kinked glands that extend through most of the larva's body. They unite in a common duct which opens in the spinneret on the labium. They become the thoracic salivary glands in the adult bee. The larva uses the glands to produce a silk cocoon while entering the prepupal phase.
sinus(es), dorsal and ventral	not labelled			abdomen, internal	The space between the dorsal/ventral diaphragms and the body wall. It is important for haemolymph circulation in the bee's body.
small intestine(s)	F17; P8A, 16A	worker (P8), queen (P16A), generic (F17)	adult	abdomen, internal	Derived from the proctodeum, it is a narrow tube surrounded by circumferential muscle fibres. The tube is pleated into six longitudinal folds and is coiled. It follows the ventriculus and Malpighian tubules and precedes the rectum in the alimentary tract. Also called ileum.
spermatheca(-ae)	F33, P16B, C	queen	adult	abdomen, internal	A spherical sac which holds the spermatozoa in a mated queen. The spermatozoa can be stored and kept alive here for the life of the queen.
spermathecal gland(s)	F33	queen	adult	abdomen, internal	A gland with two branches that loop over the dorsal surface of the spermatheca. The common duct of these two branches joins the spermathecal duct and the gland is believed to produce a nutrient secretion for the spermatozoa.
spermathecal valve and pump	F33	queen	adult	abdomen, internal	A fixture on the duct of the spermatheca that has muscles which draw spermatozoa out of the spermatheca and forces it into the vagina where it is available to fertilize an egg.
spermatozoon(-zoa)	F30	drone	adult	abdomen, internal	The male gametes. They are also called sperm. They are slender threads, about ¼ mm long. They have head and tail regions, using their tails to swim.
spiracle(s), sp	F5, P5	generic	embryo (F5), adult (P5)	thorax/abdomen, external	The bee's breathing holes. Spiracles are arranged on the lateral sides of the various body segments. They open to the tracheal system and facilitate air exchange into and out of the body.
spiracle plate(s) of sting, A8, tg	P5	generic	adult	abdomen, external	The remains of the 8 <sup>th</sup> abdominal tergite. It composes part of the sting.
sternite(s), st	P4, 5	worker (P4), generic (P5)	adult	thorax/abdomen, external	Ventral plates of the exoskeleton. There is one for each visible body segment of the thorax and abdomen. They are labelled 1-3 on the thorax and 1-7 on abdomen.
sting apparatus(es)	P10, 11, 16B, C	worker (P10), queen (P16B, C), generic (P11)	adult	abdomen, internal	A number of abdominal parts that collectively compose the honey bee sting. These include modified sclerites, muscles, glands, etc. The honey bee sting is used in defence.
sting chamber(s)	P5, 16C	generic (P5), queen (P16C)	adult	abdomen, external	The area covered by the 7 <sup>th</sup> abdominal segment that contains the sting apparatus.
sting shaft(s)	P4, 5, 10	worker (P4, 10), generic (P5)	adult	abdomen, external	The part of the sting that is deployed into the stung victim. It is composed of three parts, the stylet and two lancets.
sting sheath(s)	P5, 11, 18D	generic (P5, 11, 18D)	adult (P5, 10), prepupa (P18D)	abdomen, external	Two soft extensions of the oblong plates about the same length of the sting shaft. It may produce a glandular secretion that is involved in the alarm response.
stipes(-pites)	P3	generic	adult	head, external	Part of the maxillae, which, in turn, is part of the proboscis. The two stipites are joined together by the transverse lorum.
stomodaeum(-ea)	F5, P19	generic	embryo (F5), larva (P19)	head, thorax, abdomen, internal	The foregut of the immature honey bee embryo and larva. It becomes the mouth cavity, oesophagus, and crop in the adult bee.
stylet(s)	F12, P18D	generic	prepupa	abdomen, external	A slender, rigid and sharply pointed rod that, with the two lancets, compose the sting shaft. The stylet is barbed at its point. The two lancets and stylet form a canal, down which the venom flows when the sting is deployed.
subalare(s) and subalare muscle (s)	F9	generic	adult	abdomen, external (subalare) and internal (subalare muscle)	A plate on the thorax that is hinged to the notched pleurite of the mesothorax. Inside the body, this plate is attached to the subalare muscle, the other end of which is anchored to the coxa of the second leg. Contraction of the subalare muscle pulls down the subalare and with it, the trailing edge of the wing.

**Table 1. cont'd**

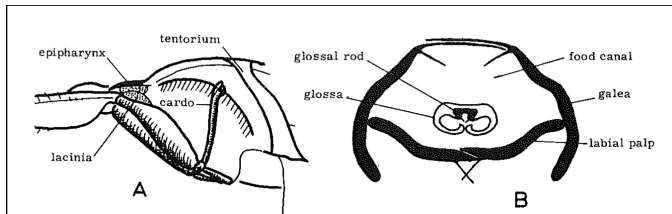
<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
suboesophageal ganglion(-ia)	F23, 24, P13	worker (P13), generic (F23, 24)	larva (F23), adult (F24, P13)	head, internal	The ganglion in the head that sends nerves to the mandibles and proboscis.
taenidium(-ia)	not shown			all, internal	Spiral thickenings of the cuticle in the walls of trachea. They keep the trachea open or dilated.
tagma(-mata)				head, thorax, abdomen	The three major body regions of the bee: the head, thorax and abdomen.
tarsomere(s)	P6	generic	adult	legs, thorax, external	The five subdivisions of the tarsus, of which the first and largest is the basitarsus.
tarsus(-si)	P7	worker	adult	legs, thorax, external	A part of the bee leg that contains 5 subdivisions. It follows the tibia.
tegula(-ae)	P4, 5	worker (P4), generic (P5)	adult	thorax, external	A large scale on the mesothorax that overlaps and protects the root of the forewing
tentorium(-ia)	F3, 4A, P3, 13	generic (F3, 4A, P3), worker (P13)	adult	head, internal	Struts which strengthen the head framework.
tergite(s), tg	P4, 5, 8C	worker (P4, 8C), generic (P5)	adult	thorax/abdomen, external	Dorsal plates of the exoskeleton. There is one for each visible body segment of the thorax and abdomen. They are labelled 1-3 on the thorax and 1-7 on abdomen.
testis(-tes)	P14, 15, 19	drone (P14, 15), generic (P19)	adult (P14, 15), larva (P19)	abdomen, internal	Two bundles of tubules in which spermatozoa are produced and matured. They occupy much of the abdomen in newly-emerged drones but they shrink to a smaller size in sexually-mature drones. This occurs when the testes empty their contents into the seminal vesicles.
thoracic collar(s), T1, tg	P4, 5	worker (P4), generic (P5)	adult	thorax, external	A notable thoracic tergite located on the prothorax. It encircles the neck like a collar. A lobe ("lobe" in P4 and "lobe covering spiracle" in P5) on each side projects backwards to cover the spiracle through which tracheal mites ( <i>Acarapis woodi</i> ) enter.
thorax(-aces), T1-3	P4, 5	worker (P4), generic (P5)	adult	2 <sup>nd</sup> main body section	The 2 <sup>nd</sup> tagma of the body. It occurs after the head and before the abdomen. It is the centre of bee locomotion, being the tagma where the wings and legs are attached.
tibia(-ae)	P6, 7	worker (P7), generic (P6)	adult	legs, thorax, external	The 4 <sup>th</sup> segment of the bee leg. It is proximal to the tarsus and distal to the femur.
tormogen(s)	not labelled			all, external	Cell that secretes the cuticle of the "socket" holding the trichogen of the seta.
trachea(-ae) or tracheal trunk (s)	F21, 22, P9	worker (P9), generic (F21, 22)	larva (F21), adult (F22, P9)	all, internal	Tubes in the body connected to spiracles through which respiratory air is delivered to organs. They are maintained in a dilated state by spiral thickenings of cuticle (taenidia) in their walls. They resemble the tracheal of terrestrial vertebrates. They expand into tracheal sacs.
tracheal sac(s)	not labelled but seen in F22			all, internal	Large air bags formed by longitudinal tracheal trunks. They serve as bellows to move air into and out of the body.
tracheoles(s)	not labelled			all, internal	The smallest of the branches that radiate from the tracheal trunks. They have no spiral thickening of the cuticle (taenidia). They are closely applied to the tissues. Here, oxygen brought to the tracheoles is dissolves in the haemolymph and carbon dioxide is removed.
triangular plate(s)	F12, P5, 11, 18D	generic	adult (F12, P5, 11), prepupa (P18D)	abdomen, external	One of three pairs of plates in the sting which are moved by muscles and cause the sting to deploy.
trichogen(s)	not labelled			all, external	The "hair shaft" of the seta.
tritocerebrum(-ra)	not labelled			head, internal	The smallest of the three component parts of the brain. It is obscured by the other parts and sends nerves to the labrum and frons (for the cibarium).
trochanter(s)	P7	worker	adult	thorax, external	The second leg segment. It is proximal to the femur and distal to the coxa.
unguis(-ues)				thorax, external	Synonymous with "claws".
unguitractor plate(s)	P6	generic	adult	thorax, external	The sclerite that corresponds to the site of insertion of the tendon which flexes the pretarsus.
valvefold(s)	F33, P16C	queen	adult	abdomen, internal	A muscular fold in the floor of the vagina that projects upwards as a flap. It may be used to press eggs against the opening of the duct as sperm are released.

**Table 1. cont'd**

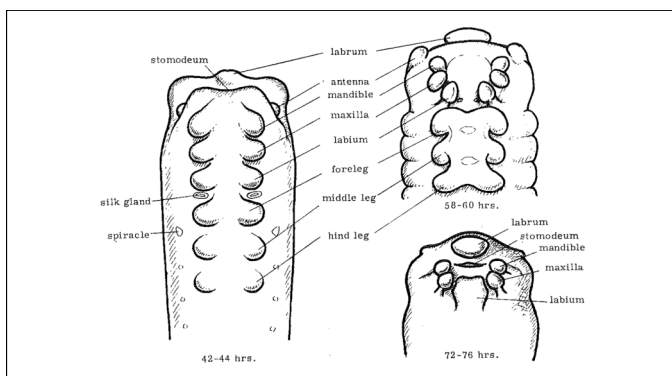
<b>Structure: singular(plural)</b>	<b>Figures (F) or Plates (P) where labelled</b>	<b>Caste presented in Figures (F) or Plates (P)</b>	<b>Life stage shown in Figures (F) or Plates (P)</b>	<b>Structure location</b>	<b>Definition/function</b>
vagina(-ae)	F32	queen	adult	abdomen, internal	The sac-like pouch that follows the median oviduct. Eggs pass through the vagina and the queen can release spermatozoa from the spermatheca, through a duct, to fertilize the egg.
vaginal orifice(s)	F32	queen	adult	abdomen, internal	Opening leading to the vagina.
vas(-sa) deferens(-ntia)	P14, 15	drone	adult	abdomen, internal	Coiled tubes leading from the testes to the seminal vesicles. Spermatozoa pass through the vas deferentia.
venom canal(s)	F12	generic	adult	abdomen, internal	The canal formed by the pair of lancets and the stylet. This is the canal through which venom passes on its way to the victim.
venom gland(s)	labelled as "acid gland"				Synonymous with "acid gland".
venom sac(s)	P10, 11	worker (P10), generic (P11)	adult	abdomen, internal	A single tube proximal to the venom glands (acid glands) that widens to form a large, club-shaped sac in which the secretion of the venom gland is stored. The sac tapers to a narrow duct which connects with and opens into the bulb of the sting.
ventral diaphragm(s)	F20	generic	adult		A thin, transparent membrane spread over the floor of the abdomen and attached to the apodemes of the sternites. Responsible (with the dorsal diaphragm) for setting up circulation inside the abdomen and for drawing blood from the thorax into the abdomen.
ventriculus(-li)	F17, P8A, B, 9, 16A, 19	worker (P8A, B, 9), queen (P16A), generic (F17, P19)	adult (F17, P8A, B, 9, 16A), larva (P19)	abdomen, internal	Also known as mid-gut or stomach. It is derived from the embryonic mesenteron. It is a long, wide tube, lying in a loop in the abdomen. It is constricted at intervals by contracted circumferential muscles. It is lined with epithelium cells which aid in digestion and nutrient uptake.
vertex(-tices)	P2C	worker	adult	head, external	The crown or top of the head.
vertical muscle(s), thorax(-aces)	P8A, 12	worker (P8A), generic (P12)	adult	thorax, internal	Two bundles of muscles that run side by side from the domed scutum of the mesothorax to the sternite of the same. When they contract, the roof of the thorax is pulled down, thus raising the wings.
vestibule(s)	not labelled but shown in P15	drone	adult	abdomen, internal	The part of the shaft of the endophallus in drone honey bees between the horns and the genital opening at A9.
wax gland(s), w	P4	worker	adult	abdomen, internal	Glands located on inner sides of abdominal sternites 4-7 that secrete wax.
wax mirror(s)	P12	worker	adult	abdomen, external	Areas on abdominal sternites 4-7 onto which wax is secreted by the wax gland.



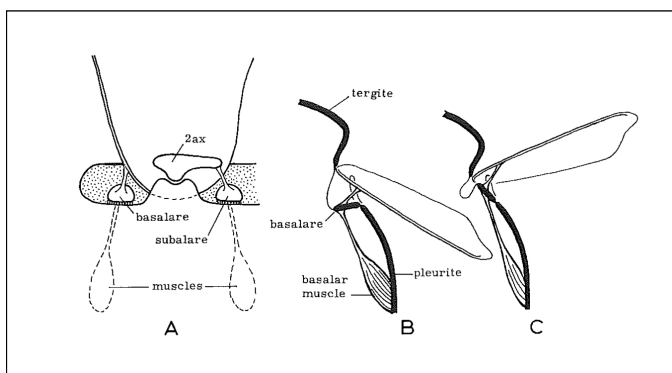
**Fig. 3.** The proboscis folded, cardines swung back.



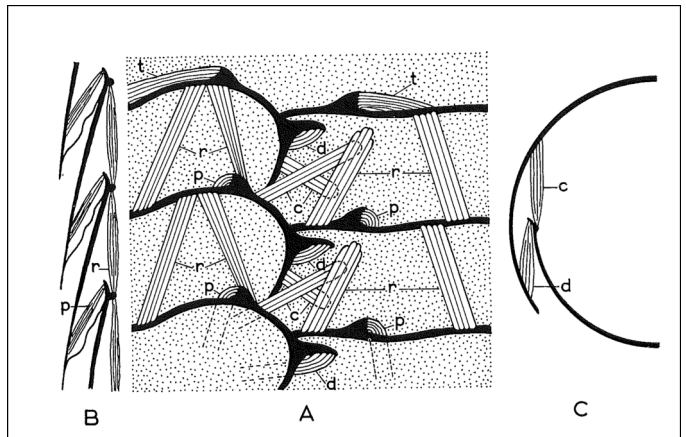
**Fig. 4. A.** The proboscis extended, cardines swung forward, laciniae pressed against the epipharynx. **B.** cross-section of proboscis.



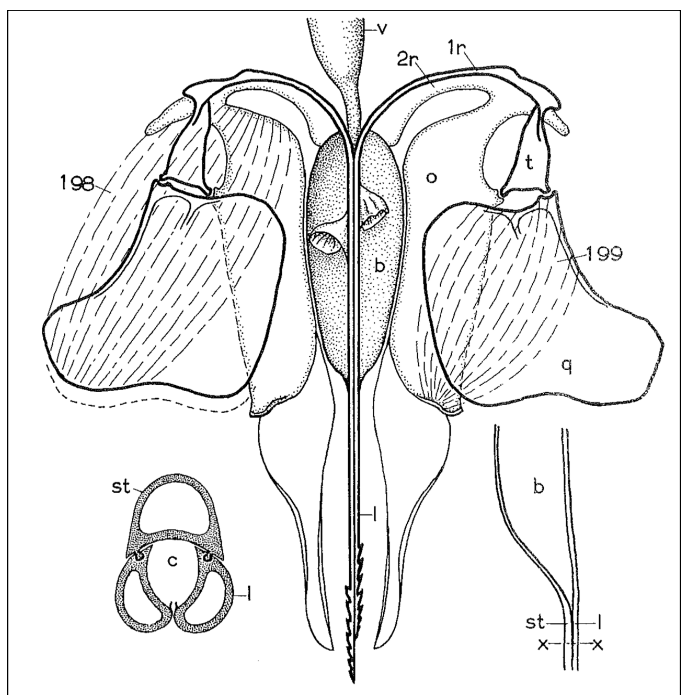
**Fig. 5.** Development of embryonic mouthparts, showing their progressive arrangement round the mouth, and the final fusion of the two parts of the labium.



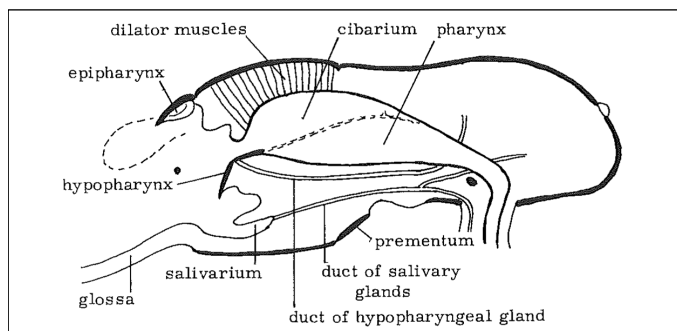
**Fig. 9.** Diagram, greatly simplified, showing the feathering action of the direct wing muscles. **A.** left forewing, seen from outside the body: the 2nd axillary sclerite (2ax) of the wing is articulated in a notch on the pleurite; the basalare and subalare are hinged to the pleurite and float on the membrane (dotted); their direct muscles are inside the thorax. **B.** cross-section of part of thorax: basalare pulled down by its muscle, drawing down with it the leading edge of the wing. **C.** basalare muscle relaxed, while the subalare muscle (hidden) pulls down the subalare and with it the trailing edge of the wing.



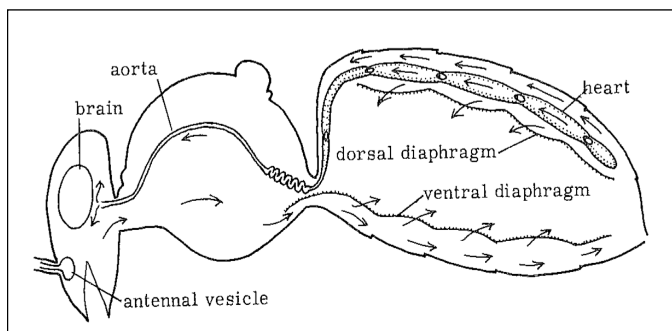
**Fig. 11.** The principal abdominal muscles. **A.** the diagram shows half of some abdominal segments, laid out; **B.** section through the sternites, showing how the protractor and retractor muscles act on the plates; **C.** half of cross-section of the abdomen, showing how the compressor and dilator muscles work. p. protractors; r. retractors; c. compressors; d. dilators; t. muscles in anterior segments which produce torsion.



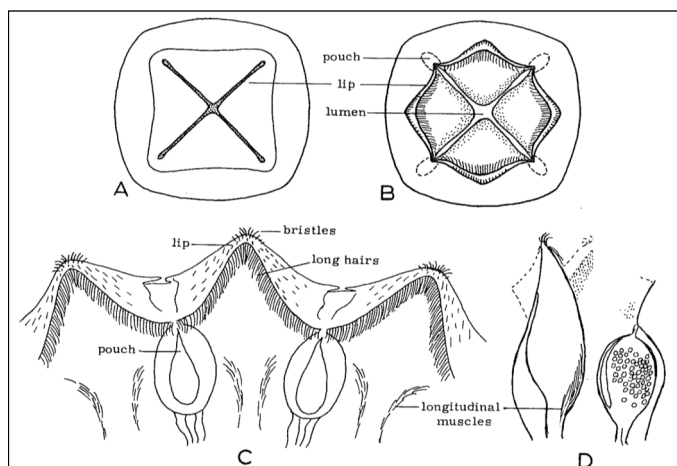
**Fig. 12.** The sting mechanism: movements of the plates and lancets. The diagram shows the sting flattened out, the moving parts in thick lines. On the left side, the protractor muscle (198) contracts, pulling the quadrate plate forward and thus causing the triangular plate to swing on its articulation with the oblong plate; the movement is transmitted to the ramus of the lancet (1r), which slides on the ramus of the oblong plate (2r) and causes the lancet to be protracted. On the right hand side the lancet is simultaneously retracted by the reverse movements produced by the contraction of the retractor muscle (199). The small drawings show: on the right, a longitudinal section through part of the bulb and shaft; on the left, a cross-section through the shaft. v, duct of venom gland; b, bulb; l, lancet; o, oblong plate; q, quadrate plate; t, triangular plate; 1r, first ramus; 2r, second ramus; st, stylet; c, venom canal.



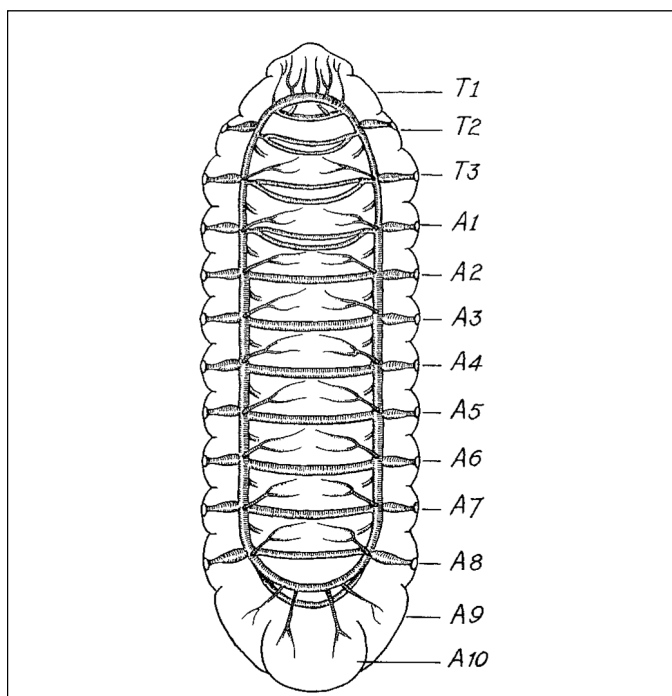
**Fig. 15.** The cavity of the mouth and the associated structures. A longitudinal section through the head, diagrammatic.



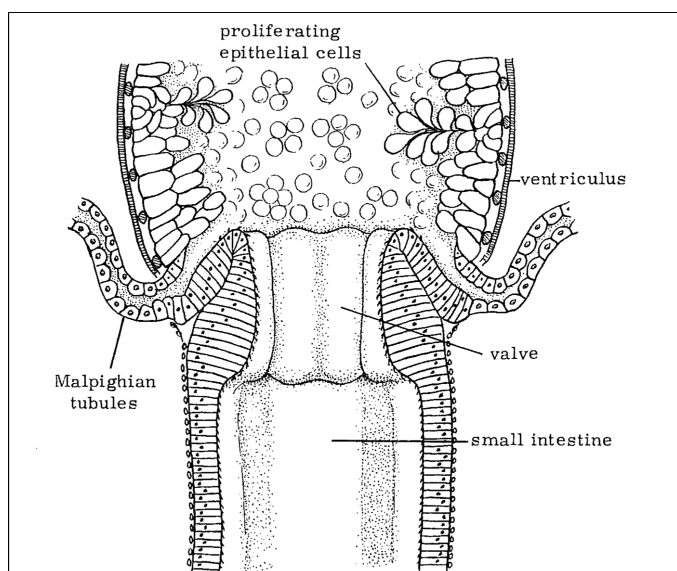
**Fig. 20.** Diagram illustrating the action of the heart and diaphragms.



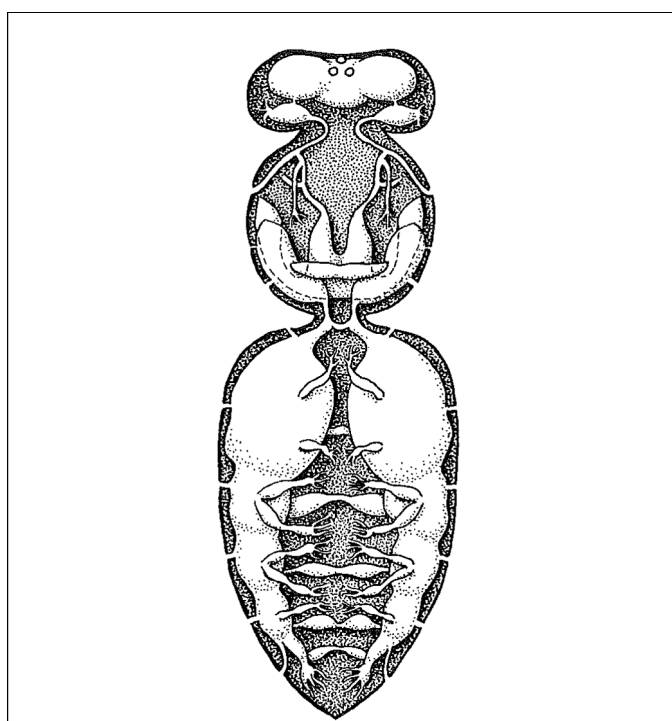
**Fig. 16.** The proventriculus. **A.** anterior aspect, lips closed; **B.** ditto, lips open to show the short spines and long hairs of the lips, and the lumen partly closed by the muscles below the lips, also the pouches which open into the lumen. **C.** part of the proventriculus laid out after slitting up on one side; three of the four lips are shown, with the pollen pouches between them. **D.** sketch of a longitudinal section, on the left through a lip, on the right through a pouch.



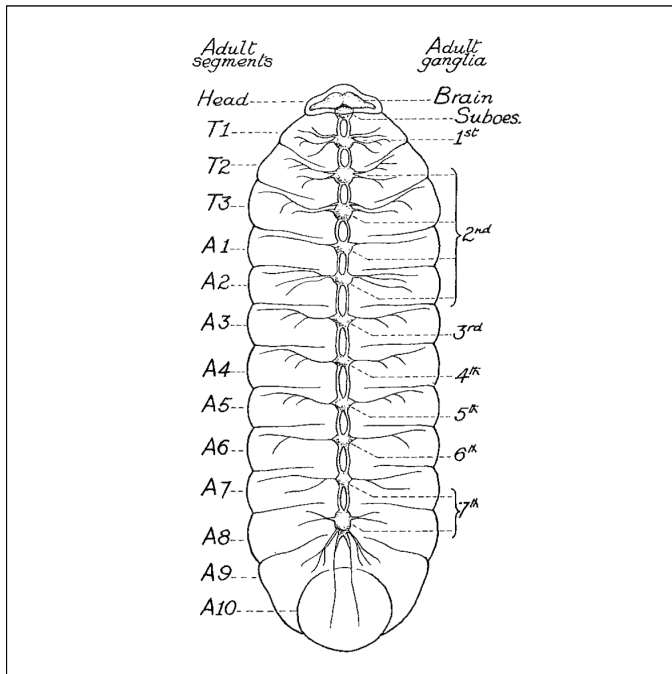
**Fig. 21.** The larval tracheal system.



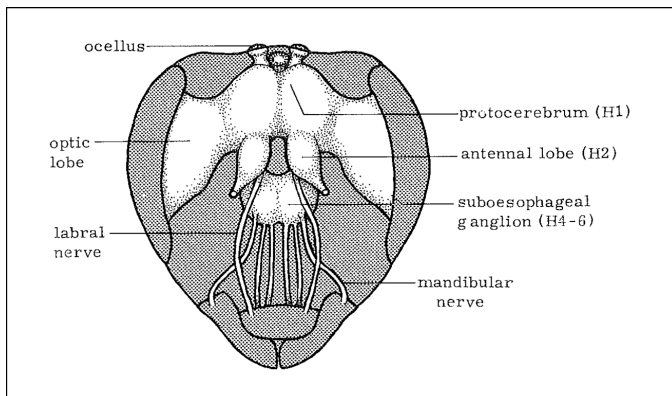
**Fig. 17.** The pyloric region. Half of the canal is cut away, showing the interior of the other half, with the junction of the ventriculus and the small intestine, and the valve, also the insertion of the Malpighian tubules. Note the small recurved setae lining the valve, also the proliferating digestive cells of the ventricular epithelium.



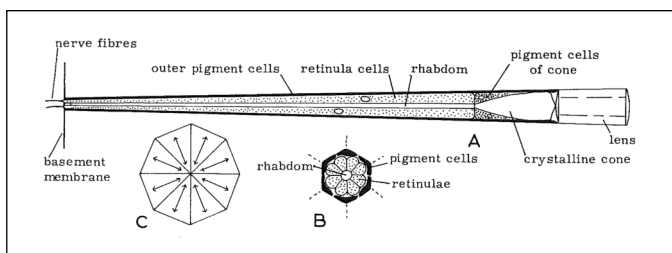
**Fig. 22.** The principal tracheal sacs and trunks of the adult bee.



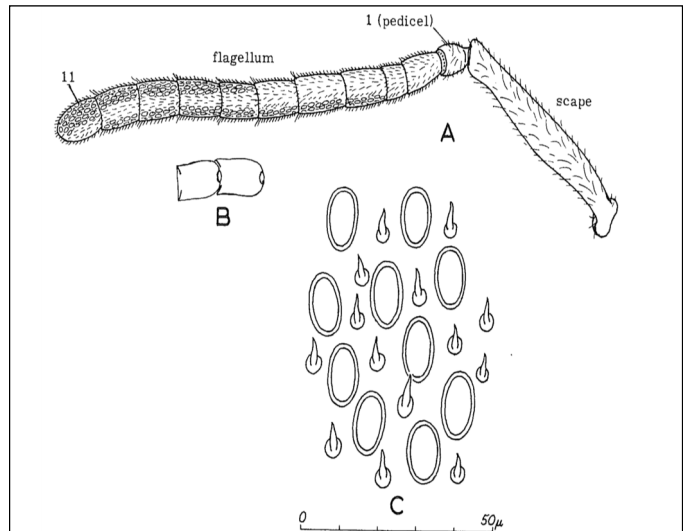
**Fig. 23.** The larval nervous system. The six ganglia of the head are already combined to form the brain and suboesophageal ganglion. Those of T2 and T3, and A1 and A2, are still discrete; that of A7 is still discrete, but those of A8 to A10 are already fused.



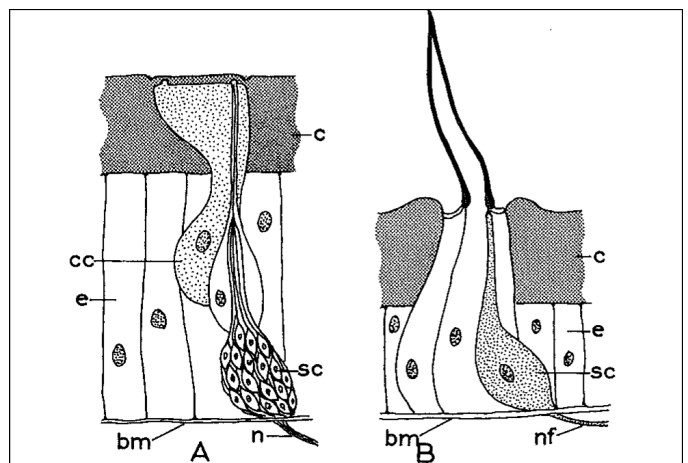
**Fig. 24.** The brain and principal nerves of the head, anterior aspect. The labral nerves come from the very small tritocerebrum (H3), concealed behind the antennal lobes. The mandibular nerve comes from H4. Two other pairs of nerves (shown but not flagged) come from H5 and H6, and go to the maxillae and labium respectively.



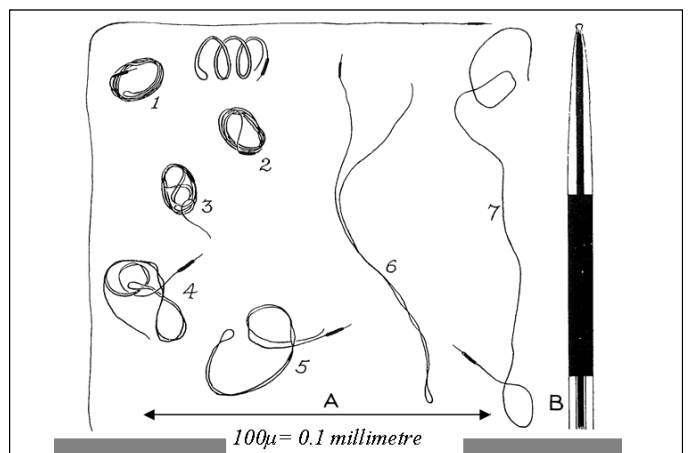
**Fig. 26.** An ommatidium. **A.** longitudinal section; **B.** transverse section through the rhabdom and retinula cells; **C.** von Frisch's polaroid model of B, consisting of 8 triangles with axes of polarization shown by double arrows.



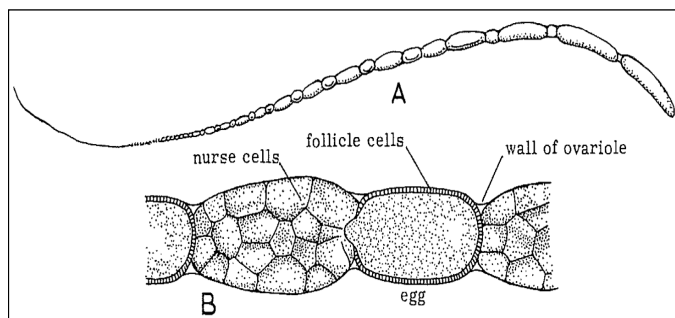
**Fig. 28.** **A.** antenna of worker; **B.** jointing of segments; **C.** sense plates and sense hairs on the antenna.



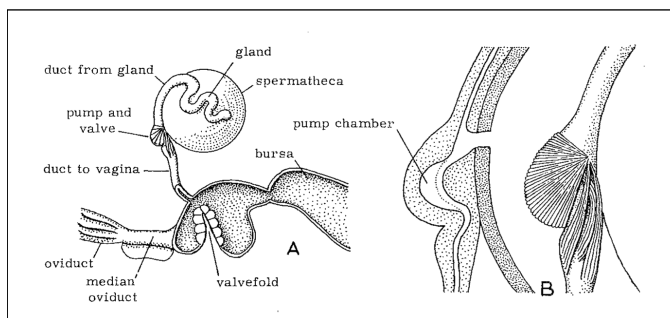
**Fig. 29.** Sensilla. **A.** a sense plate in section; **B.** a sense hair. c, cuticle; cc, cap cell; sc, sense cells; e, epidermis; bm, basement membrane; n, nerve; nf, nerve fibre.



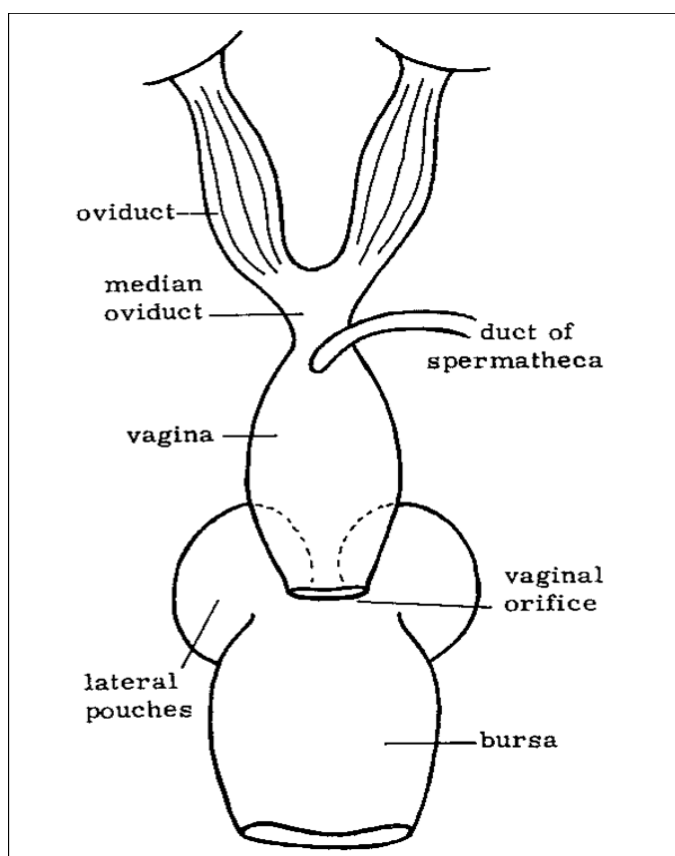
**Fig. 30.** **A.** spermatozoa, as they appear in a stained smear: 1, 2, coiled, inactive; 3 to 7, stages in uncoiling. The total length of a spermatozoon is about 0.25 mm; the head is about 10 μm long and 0.5 μm in diameter. **B.** structure of the head and part of the tail. (A, drawn from smear; B, simplified after Rothschild.). Bar represents 100 μm.



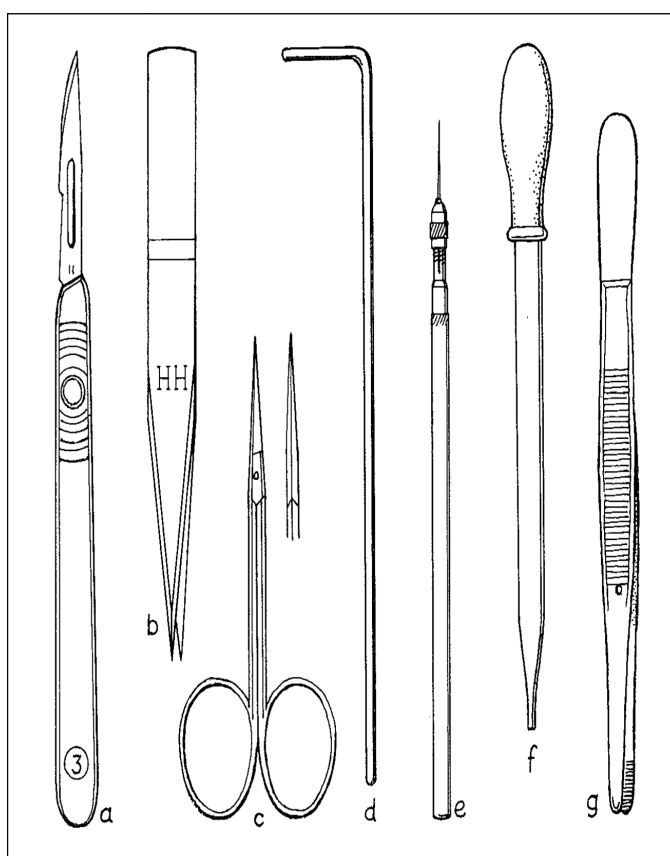
**Fig. 31.** **A.** an ovariole with eggs and nurse cells, in all stages of development. **B.** an egg with its nurse cells, drawn from a microtome section; a plug of the egg's cell plasma is in direct contact with the nurse cells through an opening in the layer of follicle cells.



**Fig. 33.** **A.** the spermatheca and vagina, with adjoining organs, of a queen. **B.** the spermathecal valve and pump, in section, and external view, showing muscles.

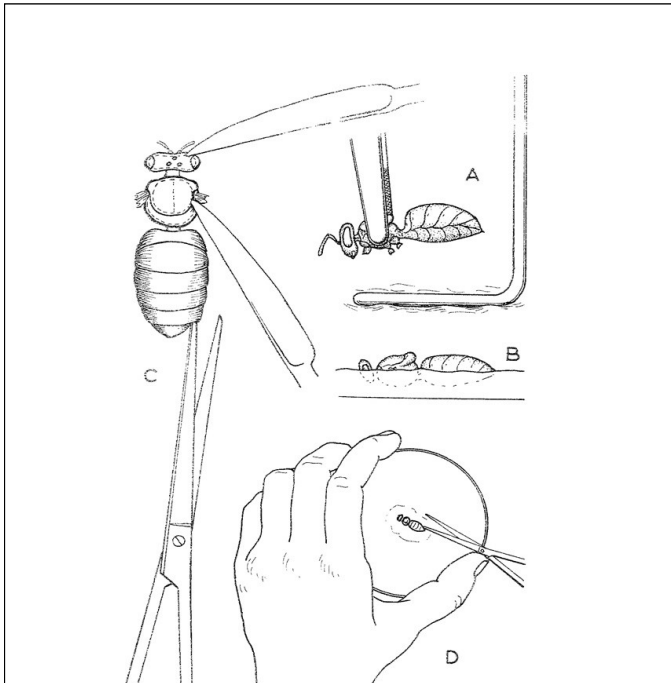


**Fig. 32.** The reproductive tract of a queen, dorsal aspect. Diagrammatic.

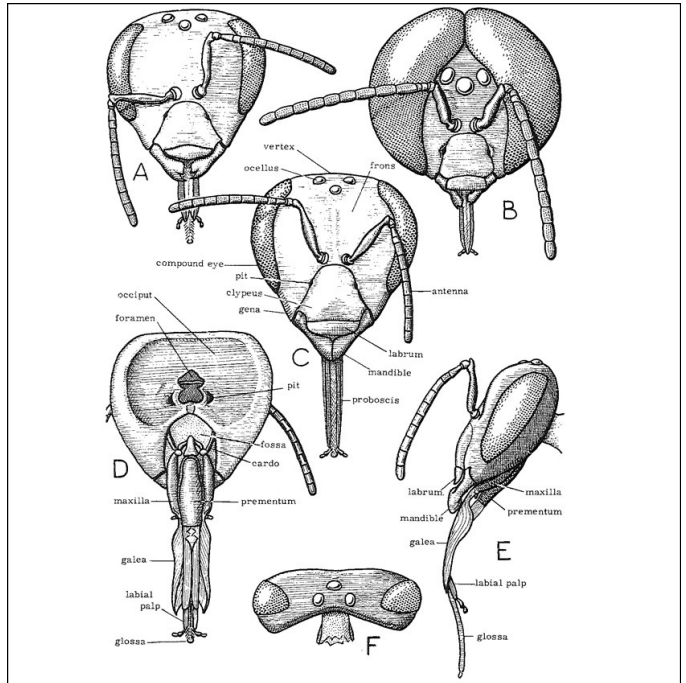


**Fig. 36.** Dissecting instruments. **a.** scalpel with No. 11 blade; **b.** watchmaker's forceps, HH pattern; **c.** cuticle scissors; **d.** bent wire; **e.** needle in chuck-top holder; **f.** pipette with teat; **g.** coarse forceps.

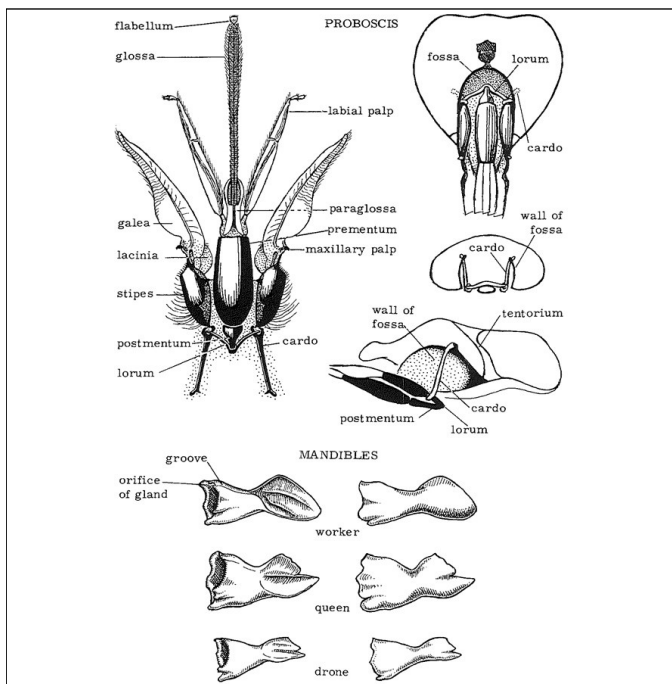




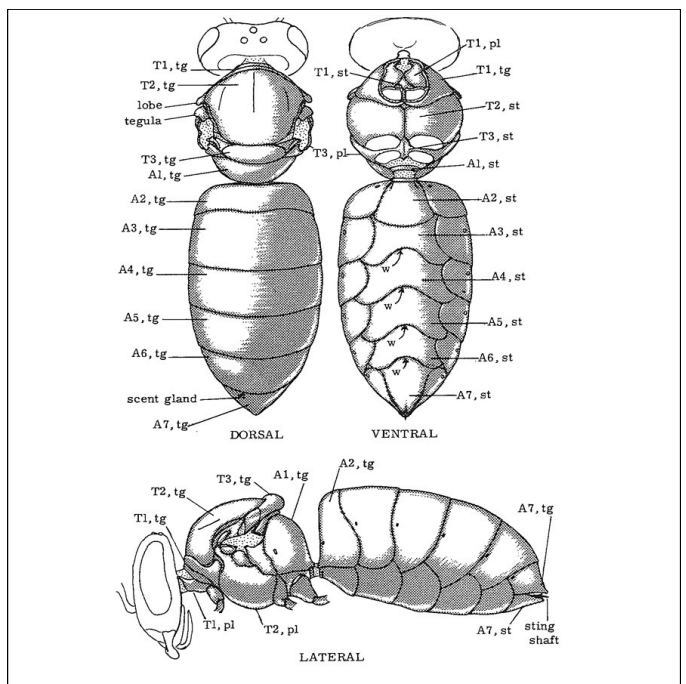
**Plate 1.** Preparation for dissection and use of instruments.  
**A**, anchoring bee in pool of melted wax. **B**, bee fixed in wax.  
**C**, methods of using scissors and scalpel. **D**, holding dissecting dish and steadying scissors against thumb.



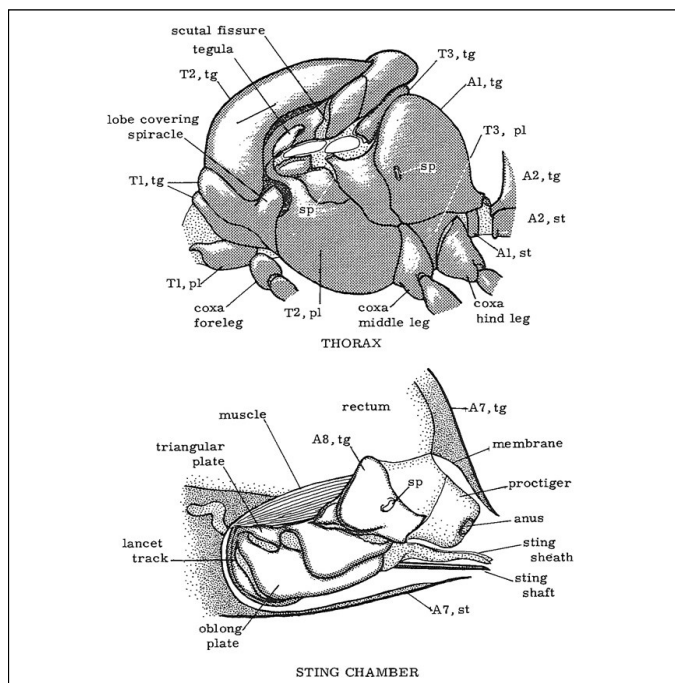
**Plate 2.** External anatomy of the head. Faces of queen (A), drone (B), worker (C).  
 Other aspects of worker's head: posterior (D), lateral (E), dorsal (F).



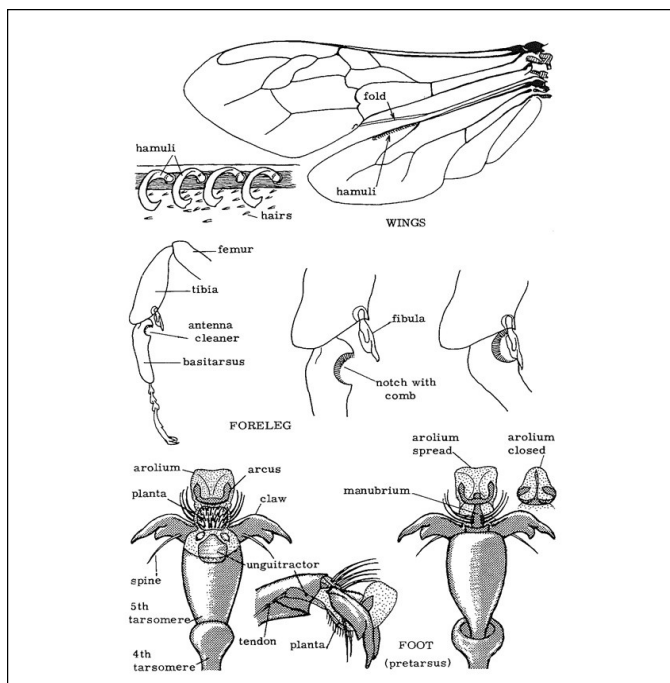
**Plate 3.** Mouthparts: proboscis, parts and suspension; mandibles, all castes.



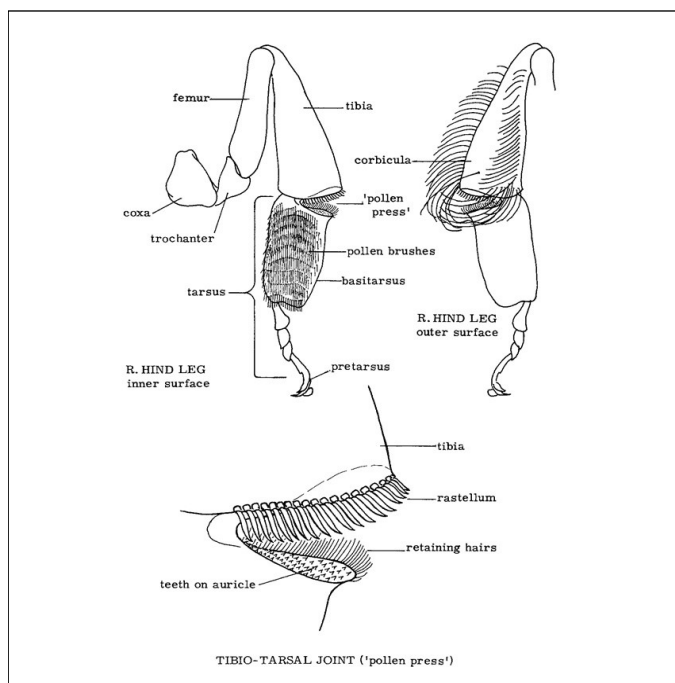
**Plate 4.** Thorax and abdomen of worker, external features from all aspects.  
 (For details of lateral aspect of thorax, see Plate 5.)



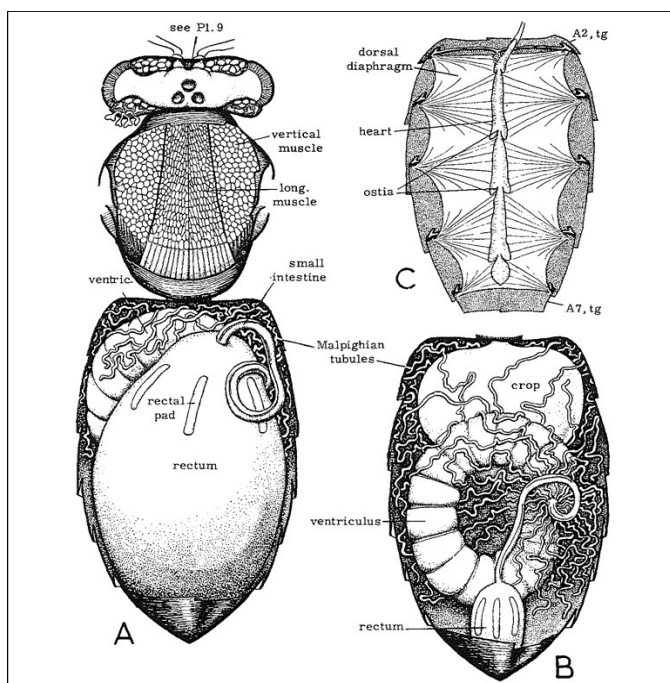
**Plate 5.** Above: lateral aspect of thorax. Below: lateral dissection of sting chamber, from the left side.



**Plate 6.** Above: the wings, with details of the hamuli. Centre: the foreleg with the antenna cleaner; the antenna cleaner open and closed. Below: the foot (pretarsus); left to right, ventral, lateral, dorsal aspects.

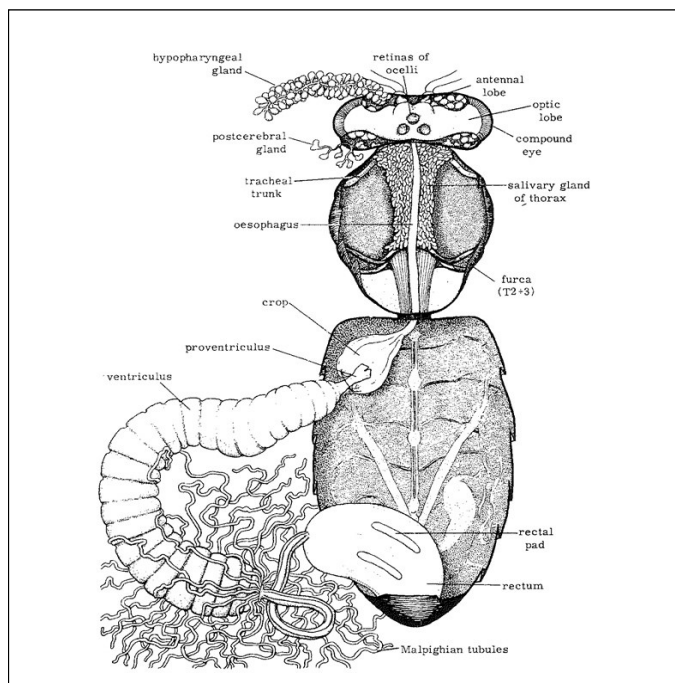


**Plate 7.** The hind leg, inner and outer surfaces, and details of the tibio-tarsal joint ('pollen press').

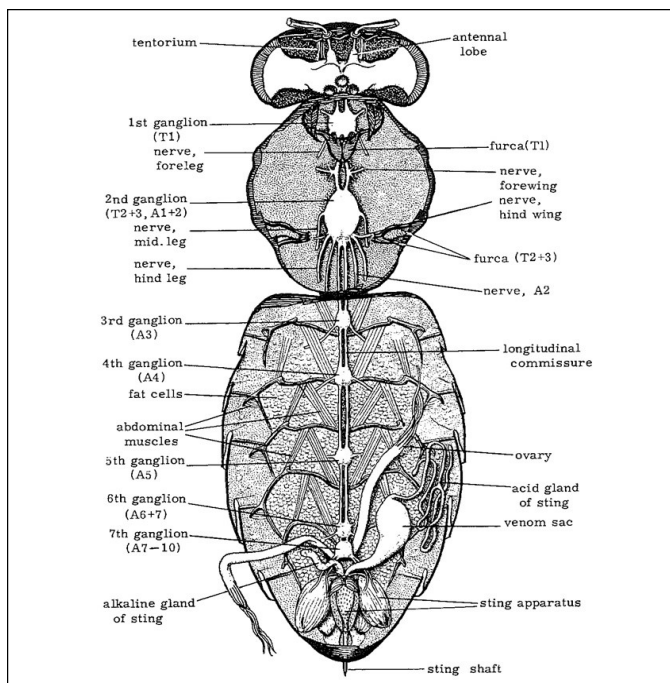


**Plate 8.** Dissection of the worker from the dorsal aspect, Stage 1.

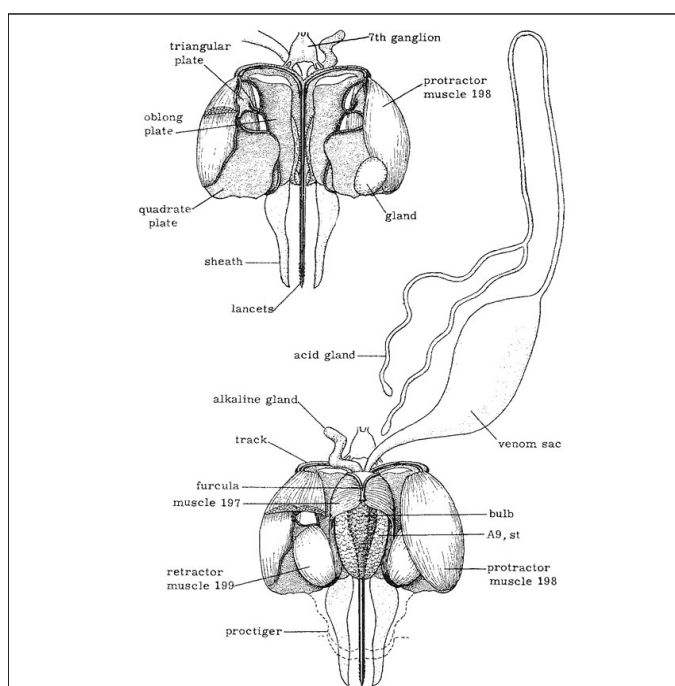
**A**, rooves of the head, thorax, and abdomen removed, and underlying organs undisturbed; abdomen shows condition of bee confined to hive with full rectum; for details of head, see Plate 9. **B**, abdomen shows condition of bee returning to the hive, rectum empty, crop full of nectar. **C**, roof of abdomen inverted, showing heart and dorsal diaphragm attached to tergites.



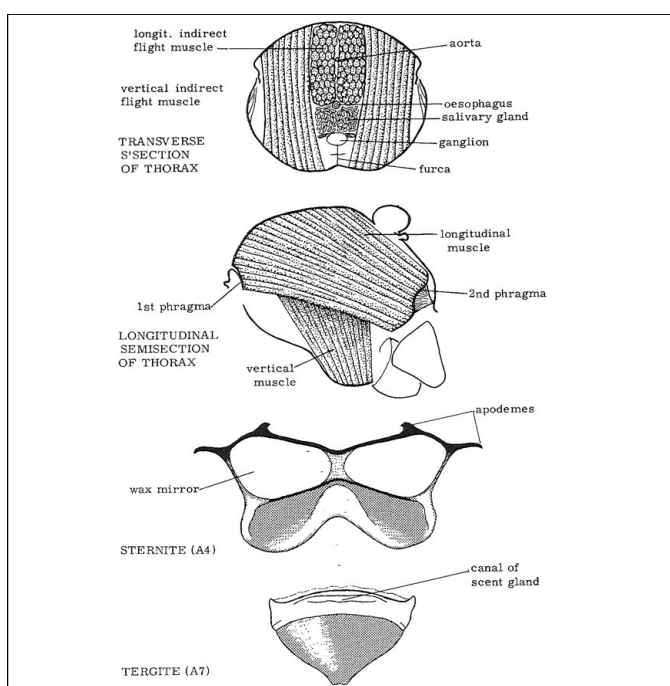
**Plate 9.** Dissection of worker, Stage 2. Glands of head lifted out, indirect flight muscles removed from thorax to expose underlying organs, and alimentary canal displayed.



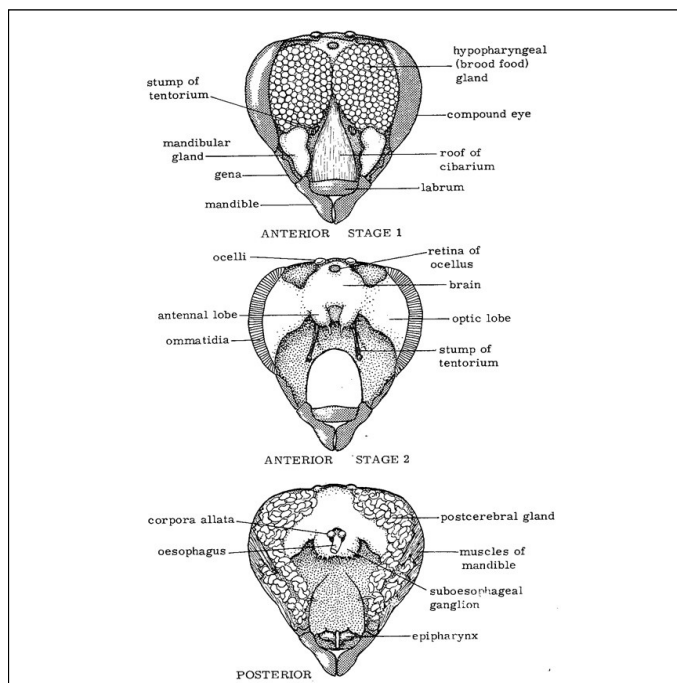
**Plate 10.** Dissection of worker, Stage 3. Head canted back to show some parts more clearly, glands removed; alimentary canal removed to expose nervous system, sting apparatus, and floor of abdomen.



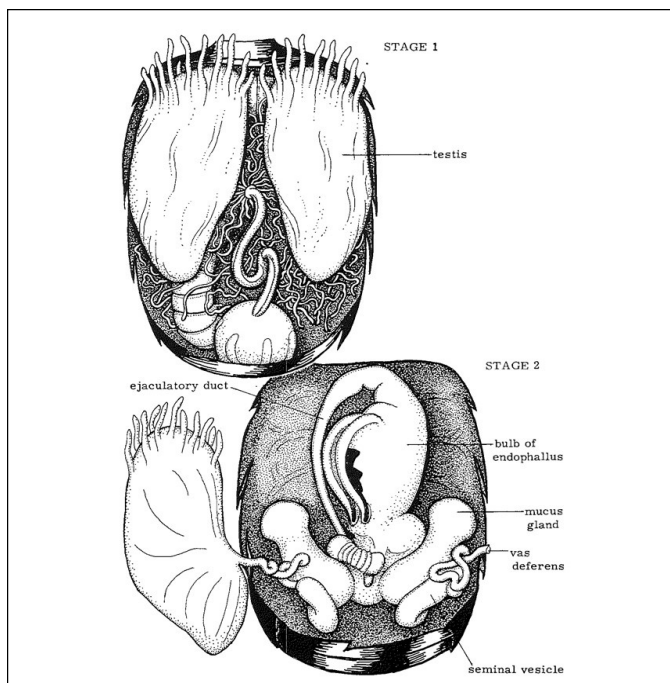
**Plate 11.** The sting apparatus. Below: dorsal aspect, as in Plate 10. Above: ventral aspect, revealed when apparatus is lifted out and turned over.



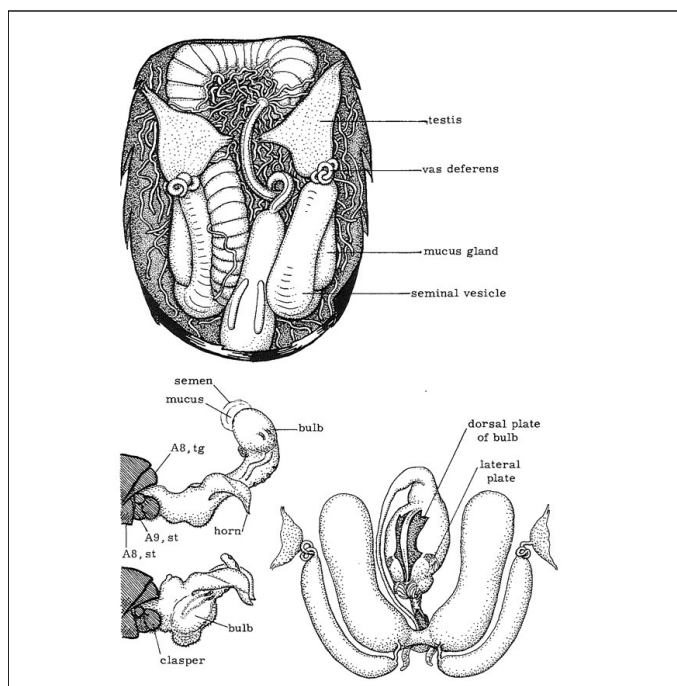
**Plate 12.** Above: transverse and longitudinal 'semisections' of the thorax, to show the indirect flight muscles, etc. Below: the fifth abdominal sternite with 'wax mirrors', and the seventh abdominal tergite, with the canal of the scent gland.



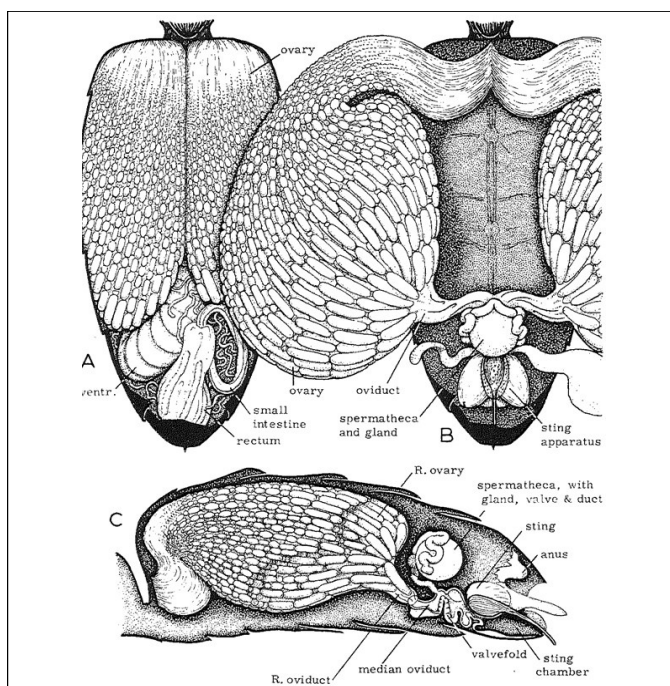
**Plate 13.** Dissections of head of worker. Above: from the anterior aspect, Stages 1 and 2. Below: from the posterior aspect.



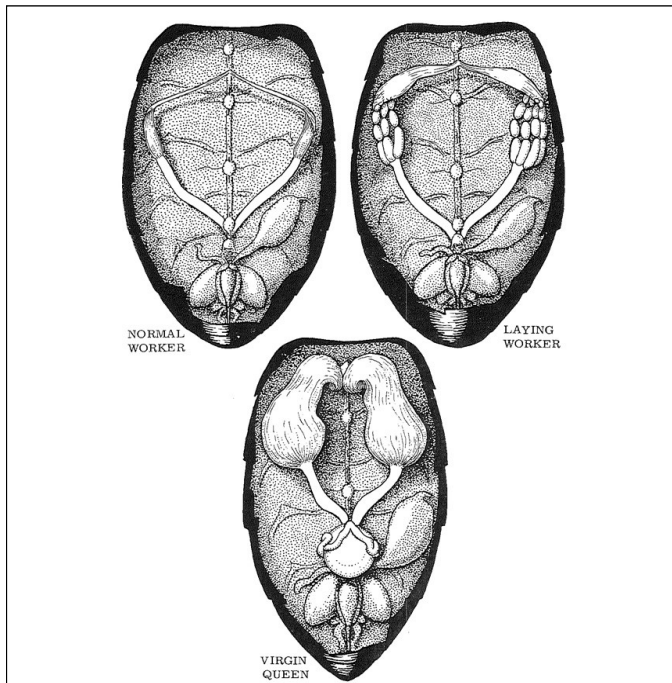
**Plate 14.** Dissection of immature (newly emerged) drone. Stage 1, viscera undisturbed. Stage 2, testes laid out and alimentary canal removed to expose the complete reproductive apparatus.



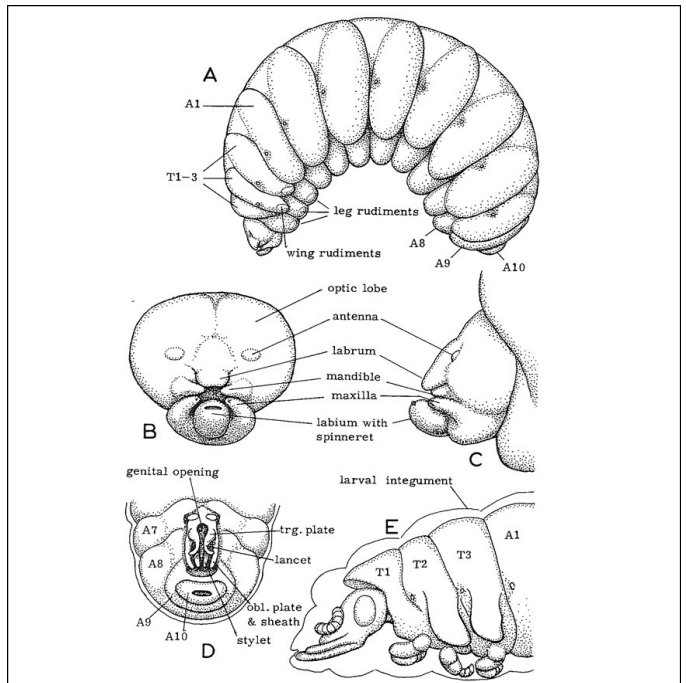
**Plate 15.** Dissection of maturing drone, viscera undisturbed. Below, right: the reproductive apparatus removed and laid out. Below, left: two stages in induced eversion of the endophallus.



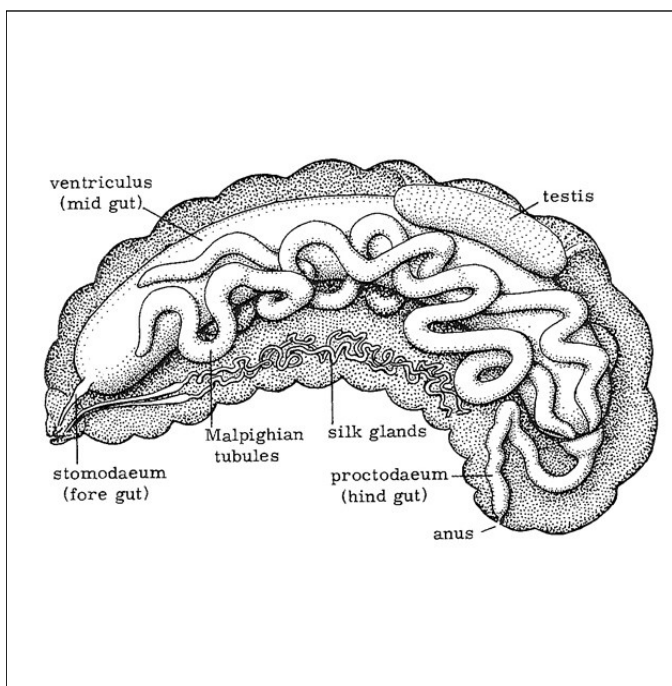
**Plate 16.** Dissection of the fertile queen. **A**, Stage 1, viscera undisturbed. **B**, ovaries laid out and alimentary canal removed. **C**, longitudinal 'semisection' right side, viewed from the left.



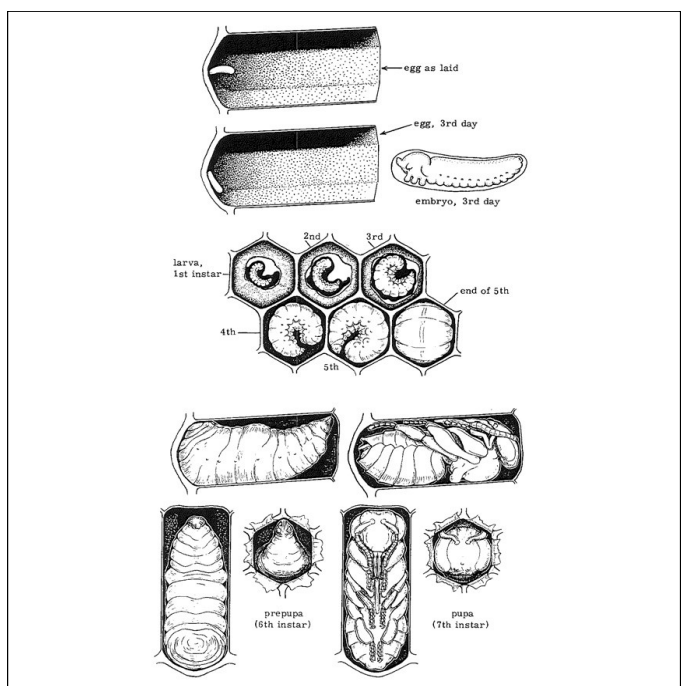
**Plate 17.** Reproductive organs of normal worker, laying worker, and virgin queen. Compare with Plate 16.



**Plate 18.** External anatomy of larva and prepupa. **A**, larva, lateral aspect. **B**, face of larva. **C**, head, from left side. **D**, posterior segments of prepupa, ventral aspect, showing sting initials. **E**, head and thorax of prepupa, from left side, showing the rapid reorganization of thoracic segments and head.



**Plate 19.** Dissection of larva from lateral aspect.



**Plate 20.** Stages in development from egg to pupa.

If a Bunsen is not available, a hand gas tool may be used or a butane blow-lamp; otherwise the whole of the wax will have to be melted. When dissections are done to prepare glands (e.g. mandibular glands) for chemical analyses, wax contamination can be a problem. In this instance a clean glass Petri dish can be used. There the bee cannot be fixed, but with some training, this is not a problem.

### 3.1.3. Reagents

- Alcohol has a variety of uses. The stock should be 95% Industrial Denatured Alcohol (IDA). The best dissecting fluid is 30 or 50%, made by diluting the stock with water (30 parts spirit to 65 parts water by volume, or 50 to 45, respectively). Water cannot be used by itself because it will not wet the hairy bodies of bees.
- Formalin (38-40% solution of formaldehyde) will be needed as preserving fluid. Caution: toxic compound.
- Bees can be killed with many volatile substances such as ethyl acetate or chloroform, but freezing is also a good method of killing bees (see Human *et al.*, 2013).
- Glacial acetic acid will be required if Carl's solution is preferred for preservation; it has many advantages (see section 3.1.4.).
- Glycerol, diluted with water, is an excellent preserving fluid for finished dissections.
- Insect saline solution is a good preservative for dissections: 7.5 g NaCl, 2.38 g Na<sub>2</sub>HPO<sub>4</sub>, 2.72 g KH<sub>2</sub>PO<sub>4</sub> in 1 l of distilled water. Insect saline is preferable to alcohol as it preserves the tissues for the time of dissection, but it does not harden the tissues as alcohol does.
- Either saline solution or Grace's insect tissue culture medium should be used when observing organs *in vivo*. These solutions are isotonic relative to the organisms body fluid. Sterile bee saline is appropriate for honey bees and can be easily made. Sterile bee saline is composed of 130 mM NaCl, 6 mM KCl, 4 mM MgCl<sub>2</sub>, 5 mM CaCl<sub>2</sub>, 160 mM sucrose, 25 mM glucose, 10 mM 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid in distilled water (pH 6.7, 500 mOsmol) (Richard *et al.*, 2008). Alternatively, Grace's Insect Tissue culture medium can be purchased from various biological supply companies or made according to Grace (1962).

### 3.1.4. Material and its preservation

For guidance on how to collect bees, see the *BEEBOOK* paper on miscellaneous methods (Human *et al.*, 2013). Freshly killed bees will be available for dissection at all times, and even in the depths of winter can be collected from the cluster. With the exception of queens and drones, preserved bees are more satisfactory for dissection. Bees outside of preservation fluid deteriorate quickly, so bees that are intended for autopsies need to be collected alive and appropriately

preserved. Black bees, or dark hybrids, are easier to dissect than pale races, the internal sclerotized parts being more deeply coloured and therefore more conspicuous. Queens and drones must be dissected immediately after killing; their reproductive organs become brittle when they are preserved by any method, and thus break up when they are disturbed.

Alcohol is commonly supposed to be a good preservative. This is a fallacy. It does not penetrate sufficiently rapidly to prevent the onset of decomposition, which ensues very rapidly after death, and it renders tissues very brittle. It is therefore necessary to add to the alcohol some substance which has great penetrating power and which tends to toughen tissues. Two such fluids are commonly used, formalin and acetic acid.

Formol alcohol is prepared by mixing the following:

- 95% alcohol 70 parts by volume
- formalin 5 parts
- water 25 parts.

The formalin penetrates quickly, and hardens soft tissues, making them leathery and easy to handle. This fluid can be used not only for preserving material, but also for fixing and hardening finished dissections of freshly killed bees, prior to mounting them in glycerol.

For preserving material, however, many prefer Carl's solution.

This is made up of:

- 95% alcohol 17 parts
- formalin 6 parts
- water 28 parts
- glacial acetic acid 2 parts.

The acetic acid should be added just before using, i.e. 2 parts of acetic acid to 51 parts of the remaining mixed constituents.

Larvae and pupae can be extracted from their cells without damage by floating them. This is quite easy if a jet of water from a fine nozzle is directed into the cells. They should be carefully handled, using a small spoon or section lifter, and at once put into formol alcohol or Carl's solution. They should be dissected two days later (see section 3.6.1.).

### 3.1.5. Mounting bees for dissection

Insects are usually dissected with their backs uppermost, though in some cases another posture may be more appropriate for some special reason.

1. Take a freshly killed or preserved worker, and cut off its wings, legs and proboscis with scissors.
2. If the bee has been preserved, dry it as well as possible by rolling it gently on blotting paper.
3. Consult Plate 1.

4. Seize the bee by the thorax, back uppermost with coarse forceps.
5. Take the bent wire with the other hand.
6. Heat its short limb in a flame.
7. Apply the hot wire to the wax in the middle of the dissecting dish, thus forming a small pool of melted wax somewhat bigger than the bee.
8. Place the bee quickly in the pool, hold it there with the cool end of the wire, and withdraw the forceps.
9. Reheat the wire and melt a little of the wax near the sides and ends of the bee
10. Push this melted wax against the body, so that it piles up slightly and makes good contact.

This will ensure that the specimen is firmly anchored and will not come adrift during dissection. The bee should be sunk nearly halfway in the wax. Plate 1A shows this operation, and B the embedded bee. All this must be done quickly and without overheating the insect. A better posture is obtained if, when lowering the bee into the pool of wax, the tip of the abdomen touches the wax first; it will adhere, and then the body can be drawn forward slightly, thus stretching the abdomen a little. Whole insects, or parts like the head, can be prepared in any posture that may be desired.

11. Pour insect saline or dissecting fluid (diluted alcohol) on at once, enough to cover the bee.

The fluid floats and supports the internal organs and of course prevents them from drying.

12. Place the dish under the microscope, it then is ready for work.
13. Focus the microscope and adjust the spot lamp.
14. Instruments should be laid out ready for use.

## 3.2. Dissection of the worker bee

### 3.2.1. The general dissection

Plates 8 to 10 illustrate the dissection of the whole body, from the dorsal aspect, in three stages. In practice it will be found convenient to begin with the abdomen, and to complete its examination before starting on the thorax and head, and the directions which follow are arranged in that order.

For the study of the alimentary canal, the heart, the tracheal sacs, and the ovaries in both normal and laying workers, bees should be dissected immediately after killing. These organs become brittle in preserved specimens. For all other purposes, preserved bees are much more satisfactory. Bees with fully distended abdomina will show the heart and nervous system to the best advantage. The specimen is prepared, fixed in the dissecting dish, back uppermost, and covered with dissecting fluid, as described in Section 3.1. and illustrated in Plate 1.

### 3.2.2. The abdomen

Turn the dish so that the head of the insect points to 10 o'clock.

During operations, remember to move the dish to suit the convenience of the right hand and scissors (see section 3.4.).

#### 3.2.2.1. Exposing the viscera (Plates 1, and 8 A and B)

To open the abdomen:

1. Steady the dish with the left hand, and the scissors by resting them against the left thumb on the edge of the dish (Plate 1).
2. Insert one point of the scissors under the overlapping edge of the tergite of A5, on the right side of the body.
3. Cut through the body wall.
4. Continue snipping through the right side, working forwards towards the thorax.

Keep the inner blade of the scissors as far as possible parallel with the side of the insect, thus avoiding thrusting it in deeply and damaging the viscera.

5. Turn the dish clockwise to suit the scissors hand when the corner at the front end of the abdomen is reached.
6. Cut across the broad front of the abdomen to the opposite corner.
7. Then again turn the dish and work down the left side.
8. Turn the dish again when the tergite of A6 is reached.
9. Cut across the tergite, taking great care not to damage the soft organs underneath, and so complete the circuit at the beginning of the first incision.
10. Lift off the roof of the abdomen gently with the point of a needle; it should be free and easily removed.

If it resists, one or more of the infolded parts of the tergites have not been severed. These uncut parts must be found and cut through, using the inner scissors point as a probe while gently lifting the roof with the needle. When it is clear that the roof is free, do not pull it off roughly, but lift it gently with two needles. There may be slight resistance from tracheae, but these will break without doing any damage. If, however, it seems that the internal organs are being pulled out or disturbed, take the roof by its edge with the fine forceps, and with the needle in the other hand break the tracheae, which will show as fine threads stretching between the roof and the organs below. Finally lift off the roof and turn it over. If the work has been done neatly, it will come off in one piece.

11. Examine the underside of the roof (Plate 8C) as it lies in the dissecting fluid.

Observe:

- 11.1. the heart, with its closed posterior chamber and its ostioles (there are five pairs, but the anterior pair may have been lost), in the mid-line of the roof;
- 11.2. the dorsal diaphragm, transparent, but clearly visible, and its attachments to the apodemes of the tergites;

- 11.3. the pericardial fat cells, large numbers of small, creamy bodies clustered against the heart;
- 11.4. the dorsal sheet of the fat body forming a pad between the heart and the body wall;
- 11.5. some of the abdominal muscles may be seen as flat, nearly transparent bands stretched across the tergites.

In preserved bees the heart and dorsal diaphragm occasionally adhere to the viscera and thus tear away from the roof. Having examined all these organs, lay aside the roof, and

12. look at the contents of the abdomen.

12.1. The appearance of the undisturbed viscera is very variable, depending on the state of the alimentary canal (Plate 8, A and B). In a bee which has been confined to the hive for some time, or a young bee which has not yet flown, the rectum is greatly distended by accumulated faeces, the bulk of which are yellow pollen husks (A). If the rectum has been damaged by instruments during the opening operations, some of the faeces will have escaped, and will litter the dissection. If the bee has just returned to the hive after a flight, the rectum will be empty and shrunk to very small proportions; if she has brought home a load of nectar or water, the crop (honey stomach) will be expanded into a large, transparent globe (B); if it is empty it will appear as a small, semi-opaque, pear-shaped body;

12.2. part at least of the ventriculus will be visible as a broad, corrugated tube;

12.3. a loop of the small intestine will be found connected to the forward end of the rectum; its other end, which joins the ventriculus, may not be visible;

12.4. the slender, tangled threads which spread all over the abdomen are the Malpighian tubules;

12.5. in a freshly killed bee, the tracheal sacs will be seen as large bags, silvery with included air (which escapes when a needle point is inserted), obscuring parts of the other organs. In preserved bees the sacs are almost invisible, filmy membranes, the air having been dissolved by the preserving fluid. When air-filled sacs obscure the view, they should be pulled out with forceps.

12.6. Tracheae in large numbers appear as silvery tubules in all parts of the body.

13. Clear away debris (faeces, fragments of tissues, etc.) which collects in the abdominal cavity from time to time during dissection.

This is done by irrigation with clear dissecting fluid, a jet of which is directed into the cavity with the pipette.

### **3.2.2.2. Displaying the alimentary canal (Plate 9)**

1. Take a needle in each hand.
2. Pass them under the rectum and ventriculus
3. Lift up the alimentary canal, gently tearing away the network of investing tracheae in which it hangs.
4. Lay it over to the left side, as shown in Plate 9.
5. Carefully tease out the tracheae and Malpighian tubules to permit the canal to lie loosely, showing all its parts.
6. Identify and examine the parts. Notice:
  - 6.1. the six rectal pads, which appear as whitish bars on the wall of the rectum;
  - 6.2. the small intestine, as a narrow coiled tube with six longitudinal pleats;
  - 6.3. at its junction with the ventriculus, about one hundred Malpighian tubules are inserted. This is the pyloric region of the canal;
  - 6.4. the ventriculus, in which food masses in the course of digestion can usually be seen, showing as dark areas where the corrugations of the ventriculus are smoothed out. If the ventriculus is torn with a needle, this food mass will exude as a brownish gelatinous substance.
  - 6.5. the proventriculus, which will be visible through the walls of the crop if it is full of nectar. In any case, tear open the wall of the crop, using needles, and turn up the proventriculus so that its four triangular lips may be clearly seen. If they are closed, the lips meet to form a cross. If they are partly open, an aperture like a four pointed star is seen;
  - 6.6. the forward end of the crop, which narrows into
  - 6.7. the oesophagus, which enters the thorax through the petiole.

### **3.2.2.3. The underlying organs (Plate 10)**

1. Grasp the alimentary canal with forceps.
2. Stretch it.
3. Cut through the oesophagus with scissors.
4. Treat the rectum in the same way, cutting through it as far back as possible.
5. Carefully cut away the remaining small triangle of the roof which was left at the tip of the abdomen.
6. Lower the side walls with scissors, giving a better view of the floor.
7. Flush out the cavity with the pipette.
8. Compare with Plate 10.
9. Identify and examine the parts. Notice:
  - 9.1. the ventral diaphragm, which may not be noticed at first, but closer inspection will reveal it as a transparent film which very slightly obscures the view of the chain of



ganglia and other underlying features. In a later dissection, the diaphragm may be studied more carefully; it is attached to the apodemes of the sternites; its anterior end extends into the thorax and is attached to the furca of T2 and T3, while its posterior end is anchored to the spiracle plate of A8.

10. Tear out the diaphragm with the fine forceps, taking care not to damage other structures in the process.
11. Observe the now more clearly visible organs lying on the floor of the abdomen:
  - 11.1. the chain of five ganglia is the most conspicuous, it is connected by twin longitudinal commissures. The last, the 7th ganglion, is attached to the sting apparatus, and comes away with the latter when it is torn out of the worker's body after stinging;
  - 11.2. the main lateral nerves which spring from the ganglia can be seen running out to right and left; those of the 7th may be seen passing to the muscles of the sting;
  - 11.3. the fat body spreads widely over the floor of the abdomen, being particularly well developed over the wax glands of the sternites of A4 to A7. Smaller clusters of fat cells occur along the sides of the abdomen. The fat body is highly developed in young bees and winter bees, where the cells are large and plump, but in old foragers they are shrunken;
  - 11.4. the abdominal muscles show clearly, some of the larger sets being very conspicuous as broad V-shaped pairs of bands stretching between the thickened forward margins of adjacent sternites;
  - 11.5. the ovaries are difficult to see, and since they encircle the alimentary canal they are torn away when it is lifted out. To prevent this, after removing the roof of the abdomen,
    - 11.5.1. lift the alimentary canal slightly from the right-hand side,
    - 11.5.2. look sideways under it, the right ovary with its oviduct will be seen as an almost transparent, narrow, flat tube running to the root of the sting,
    - 11.5.3. gently disengage the ovary from the tracheae which tie it down and attach it to the other viscera,
    - 11.5.4. repeat this operation from the other side, thus freeing the left ovary,
    - 11.5.5. go on with the dissection, removing the canal.

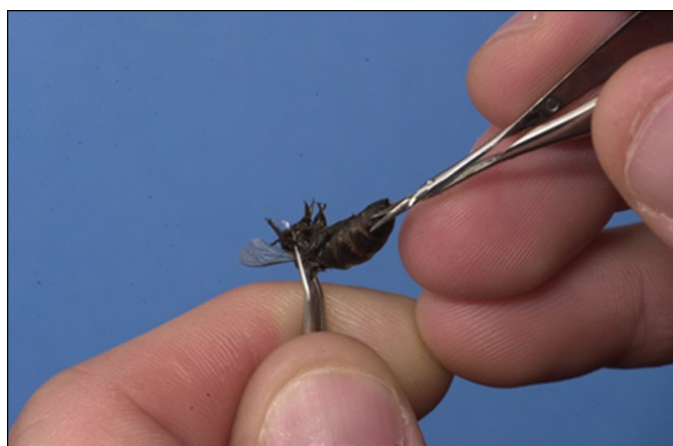
The ovaries will then be seen lying or floating in the abdominal cavity, their oviducts disappearing behind the sting, their distal ends separated. In the undisturbed abdomen, the tips of the ovaries are joined and attached to the heart (Plate 17).

- 11.6. the sting, if not wholly visible, can be examined *in situ* by removing more of the wall of the abdomen at the tip (Plate 10).

Identify the parts flagged in Plate 11, dorsal aspect. The whole apparatus can be removed intact very easily by passing needles below it and lifting it out, the small muscles which hold it giving way without offering noticeable resistance. The extracted apparatus can now be turned over, as it lies in clear fluid, and its ventral aspect (Plate 11) can be examined. Very rarely, the sting apparatus is laterally reversed, the only evidence of this being that the positions of the venom gland and the alkaline gland are reversed. The powerful muscles of the sting apparatus conceal the plates which constitute the system of levers actuating the lancets. The plates can be exposed by removing the muscles by maceration. Note that the sting apparatus is arched; it can be flattened by tearing away the proctiger, which is firmly attached to the oblong plates, and it is then easier to examine and also to mount as a microscopical preparation.

#### **3.2.2.4. Rapid removal of alimentary canal and associated glands in the abdomen**

There may be occasions when rapid removal of abdominal organs (alimentary canal, and sting glands) is needed. For instance, one may want to quantify the percentage of bees exhibiting a certain pathology such as scaring of the pyloric valve (Bailey, 1981). In these cases it may be more economical and efficient to expose the abdominal organs without mounting the bee in wax. Instead, after blotting individual bees on a paper towel, the intact bee can be held by the thorax, ventral side uppermost. Using scissors, two shallow incisions are made along the lateral sides of the sclerites (Fig. 42). The incisions should start at the posterior end of the abdomen (between tergite and sternite A6, Plate 4) and end near the petiole. It is helpful to lay the scissors flat against the sclerites while cutting between the tergites and sternites. When the incisions are complete, remove the abdomen from the thorax, and placing the abdomen in a petri dish



**Fig. 42.** An individual bee being held for rapid removal of abdominal organs.

Photo: Michael Andree.

containing the appropriate preservation solution to submerge the abdomen. With the aid of a compound microscope, remove the sclerites by grabbing the edge of the most anterior sternite (A2 st, Plate 4) with forceps and peel the sternites (st A2 - A6, Plate 4) back, exposing the gastro-intestinal tract and other abdominal organs (Fig. 43). The digestive tract and sting gland organs can be removed from the tergites by gently teasing them away from the integument beginning at the anterior end and working back toward the sting gland (Fig. 44). Examination of the desired organs can then commence.

### 3.2.3. The thorax

In a freshly killed bee it is not possible to see the structure of the indirect wing muscles, or to remove them without great difficulty, for they are extremely soft and elastic. In bees which have been preserved, however, these great muscles are hardened and tough, very easy to examine and to handle. Therefore preserved bees should always be used for work on the thorax.

#### 3.2.3.1. Exposing the flight muscles (Plates 8A and 12)

The roof of the thorax is best taken off with the knife:

1. Insert the extreme point only, as shown in Plate 1.
2. Make a short slit in the body wall by an outward and forward stroke.
3. Continue this along the dotted line in the Plate, all round the domed roof of T2.
4. Make a longitudinal slit along the mid-line.

Usually the roof is very firmly attached to the flight muscles, and must be detached, again with the point of the knife.

5. Keep the blade in a horizontal position, pass its point under the body wall, through the longitudinal slit, and separate the roof from the muscles by small forward movements, gradually working the point further under the body wall. When the first half of the roof is nearly free, steady it with forceps while completing the separation. If this is done carefully, the muscles will be undisturbed and undamaged, and will have the appearance shown in Plate 8.
6. Remove the other half of the roof.
7. Take off the remainder of the roof, along the second dotted line in Plate 1.

This is not attached to the muscles, and will come off easily.

8. If necessary, remove more of the side walls of the thorax, down to the level of the wings.

The indirect flight muscles are now exposed.

#### 3.2.3.2. Oesophagus and glands (Plate 9)

1. Remove the flight muscles: simply grip bunches of them with the forceps and pull them out (this is virtually impossible in a freshly-killed bee).



**Fig. 43.** The contents of the abdomen revealed after the sclerites have been removed.

Photo: Michael Andree.



**Fig. 44.** The alimentary canal and sting gland organs removed from a worker bee using the rapid autopsy procedure.

Photo: Michael Andree.

2. Observe:
  - 2.1. the attachment of the longitudinal muscles to the 2nd phragma, which is an extension inwards of the tergite of the second segment;
  - 2.2. the oesophagus below the longitudinal muscles, it passes from abdomen to head;
  - 2.3. the salivary glands of the thorax (derived from the silk glands of the larva);
  - 2.4. the aorta is a delicate tube which is destroyed by the removal of the indirect flight muscles; it can be found by careful lateral dissection.

#### 3.2.3.3. The nervous system in the thorax (Plate 10)

1. Remove the oesophagus and salivary glands with forceps.
2. Observe:
  - 2.1. the combined furcae of T2 and T3, which are now conspicuous.

They form a strong strut and bridge across the thorax and protect:

- 2.2. the great second ganglion, which can be seen below the furcae;
- 2.3. the first ganglion lies in front of and partly concealed by the furca of T1, at the anterior end of the thorax. To see this, it will probably be necessary to remove more of the body wall in this region. Both ganglia can be exposed by removing the overlying parts of the furcae, using the point of the knife and forceps. Sheets of semi-transparent muscles attached to the furca will also have to be removed; this must be done cautiously, for the ganglia are very easily damaged. Notice the thick twin commissures joining the ganglia;
- 2.4. commissures also run forward from the first ganglion to the suboesophageal in the head, and from the second ganglion to the abdomen, where they join the abdominal chain;
- 2.5. nerves from the second ganglion can also be seen passing to the propodeum and into the abdomen, where they serve A2.

### **3.2.4. The head (Plates, 8, 9, 10)**

#### **3.2.4.1. Exposing the brain and other structures.**

1. Cut along the dotted line of Plate 1C, using the point of the knife. The isolated portion of the wall will come away easily. Conspicuous on the summit of the brain are the purple retinae of the three ocelli.

2. Extend the opening to each side, cutting away part of the compound eyes (Plate 8A).

Both knife and scissors can be used for this part of the work.

3. In a freshly killed bee, clear away the large tracheal sac filled with air, which obscures the brain.
4. Observe:
  - 4.1. the protocerebrum, bearing the ocelli;
  - 4.2. the optic lobes connected with the compound eyes. The pigmented parts of the compound eyes indicate the radiating ommatidia;
  - 4.3. the hypopharyngeal glands in front of the brain. They are conspicuous in young nurse bees and winter bees, in which the acini are large and white, but in foragers they are much shrunken and may be difficult to find;
  - 4.4. another branch of the hypopharyngeals;
  - 4.5. a small part of the postcerebral glands behind the brain.

#### **3.2.4.2. Displaying the glands and antennal lobes (Plates 9, 10)**

1. Lift out the glands with the point of a needle.
2. Note:
  - 2.1. the form of the hypopharyngeals, like strings of onions;
  - 2.2. the quite different branched structure of the postcerebrals;

2.3. the mandibular glands.

Beware of rupturing them and thus losing their contents.

3. Cut away the frons down to the level of the antennae.

4. Observe:

- 4.1. the antennal lobes,
- 4.2. nerves running into the antennae.

They can be seen if the head is lying in a favourable position, as in Plate 10.

### **3.2.5. Lateral dissection of the worker**

Mount a preserved worker on its right side.

#### **3.2.5.1. The head**

The dissection is chiefly useful to demonstrate the suspension of the proboscis, but also shows different aspects of the organs which are the principal subjects of other dissections.

1. Take off the left gena.
2. Work carefully downwards, lowering the walls.
3. Note:
  - 3.1. the tentoria, two powerful struts running from the foramen to the clypeus, and joined at their feet by a small transverse bridging piece;
  - 3.2. the fossa, of which the upturned wall lies just below
  - 3.3. the gena;
  - 3.4. the cardo, its end will be found articulated to a knob on the edge of this wall;
  - 3.5. the oesophagus, it enters the head through the foramen and then expands into the pharynx;
  - 3.6. the hypopharyngeal plate, of which the long extensions embrace
  - 3.7. the pharynx;
  - 3.8. the cibarium, its dilator muscles connects it to
  - 3.9. the clypeus.

The organs revealed by this dissection will be recognized from the diagrams in Figs. 4 and 15.

#### **3.2.5.2. The thorax**

1. Remove the left side of the thorax.
2. Excavate, removing the flight muscles and lowering the walls as the work proceeds.
3. Observe:
  - 3.1. the attachment of the vertical muscles to the floor and roof of the thorax;
  - 3.2. the attachment of the longitudinal muscles to the roof and first phragma in front, and to the second phragma in the rear;
  - 3.3. the aorta, which can be found when approaching the mid-line of the thorax, between the right and left sets of longitudinal muscles, and below it
  - 3.4. the oesophagus;

- 3.5. the ganglia;
- 3.6. the commissures;
- 3.7. the anterior end of the ventral diaphragm, which enters the thorax through the petiole.
4. Remove everything else.
5. Observe:
  - 5.1. the direct wing muscles on the inner surface of the right hand wall of the thorax.

A special preparation to show the direct muscles can easily be made by slicing off one side of a thorax with a razor blade and trimming away debris.

### 3.2.5.3. The abdomen

1. Examine the sting chamber.
2. Cut a window in the side of the abdomen, starting at the rear edge of the tergite of A6.
3. Enlarge the opening towards the posterior end of the abdomen, severing the muscles which hold the isolated parts of the body wall by passing the point of the knife under them.
4. Observe:
  - 4.1. the spiracle plate of A8, it is found overlying the sting apparatus, and has something of the appearance of a dog's head (Plate 5). The spiracle plate is bound to
  - 4.2. the quadrate plate, near the dog's nose;
  - 4.3. the proctiger, attached to the sting apparatus and also to
  - 4.4. the rectum.
5. Remove the contents of the abdomen, lower the walls.
6. Survey the abdominal muscles of the right side (some of them are shown in Fig. 11).

### 3.2.6. Dissection of the laying worker (Plate 17)

See note on obtaining material in section 3.1.4. Laying workers should be dissected when freshly killed, or within two or three days after preservation, or the ovaries will become too brittle to handle.

1. Remove the roof of the abdomen.

If the crop is full and the ovaries are well developed, the anterior extremities of the ovaries may be seen crossing the crop, but this is not usual.

2. Proceed as though dissecting out the ovaries of a normal worker (see section 3.9.2.3.). The laying worker's ovaries are much more easily seen, as the ovarioles contain strings of eggs.
3. Break the tracheae binding the ovaries to the alimentary canal.
4. With care, remove the alimentary canal without tearing the ovaries apart, and they are then displayed as shown in Plate 17.

## 3.3. Dissection of the head, all castes

### 3.3.1. Preparation

Heads are removed and mounted for dissection from both anterior and posterior aspects. Beeswax alone is too soft to hold the head firmly, and a stiffer cement must be used. A wax-resin mixture is

suitable, and may be used to mount the heads on small pieces of acetate or Perspex® sheet, these in turn being temporarily anchored in the dissecting dish by melting the wax in the dish at their corners with the toe of the bent wire. The heads should be embedded deeply, with their upper surfaces almost level with the surface of the cement.

### 3.3.2. Worker's head, from the anterior aspect (Plate 13)

#### 3.3.2.1. The glands

1. Cut through the wall of the mask with the point of the knife, across the vertex, round the margins of the compound eyes, and round the edges of the mask, excluding the mandibles, labrum, and clypeus.
2. Snip off the antennae near to their insertion.
3. Lift off the mask which remains connected by tentoria.
4. Cut around the small pits in the suture surrounding the clypeus.

The mask will then lift off.

5. Hold the clypeus down firmly by the cibarial muscles (dilators of the cibarium).
6. Disengage the cibarial muscles by using the point of the knife in the same way as when taking the roof off the thorax.
7. Notice:
  - 7.1. the hypopharyngeal glands. In a young bee, five or six days old, or in a winter bee which has not yet nursed brood, they will have the appearance shown in the Plate, the acini being plump and creamy white; they will almost fill the space in front of the brain, as well as sending branches to the back of the brain. They can be lifted out to show their string-of-onions structure. In foraging bees which have completed their nursing duties, these glands are greatly shrunken, almost to the point of disappearance, leaving only thin thread-like remains;
  - 7.2. the mandibular glands, beware of rupturing them and thus losing their contents.

#### 3.3.2.2. The brain

1. Remove the hypopharyngeal glands to expose the brain.
2. Identify the structures flagged in the plate 13.
3. Cut down the compound eyes to the level of the optic lobes, and note the indications of the radiating ommatidia.
4. Remove the roof of the cibarium.
5. Examine the floor of the mouth cavity, finding:
  - 5.1. the hypopharynx, with the two pores marking the ends of
  - 5.2. the ducts of the brood-food glands.

### 3.3.3. Worker's head, from the posterior aspect (Plate 13)

#### 3.3.3.1. The glands

1. Remove the proboscis.
2. Remove the wall of the occiput and postgenae, by the same means as in the preceding dissection.

3. Lift off the back wall of the head cut down the tentoria, to give a clear view.
4. Clear the tentoria in the normal way.
5. Identify the structures flagged in the plate 13. The postcerebral glands are more translucent than the hypopharyngeals; note their branching form.

### 3.3.3.1. The brain and suboesophageal ganglion

1. Remove the glands.

The cut end of the oesophagus will be seen projecting from the space between the brain and the suboesophageal ganglion.

2. If the corpora allata are not visible, pull the oesophagus out a little way with forceps, when they should come into view.
3. Trace:
  - 3.1. the principal nerves (Fig. 24);
  - 3.2. the ducts of the glands (Fig. 15).

### 3.3.4. Isolation of the retrocerebral complex

The retrocerebral complex (RCC) is a set of neurosecretory organs that sit at the base of the brain and play a crucial role in controlling insect behaviour and physiology. The complex consists of the corpora allata (CA) and corpora cardiaca (CC), both of which are paired organs. In honey bees, the complex is located close to the suboesophageal ganglion (Fig. 45.3), and forms an incomplete ring around the aorta and oesophagus (AO-ES) (Hannan, 1955).

The CA are responsible for the production and release of juvenile hormone (Goodman and Cusson, 2012). The CC are a conduit for neurosecretory cells of the brain, responsible for storing and releasing factors such as prothoracicotrophic hormone, adipokinetic hormone and other regulators of metabolism and muscular activity (Woodring *et al.*, 1994; Lorenz *et al.*, 1999; Takeuchi *et al.*, 2003; Audsley and Weaver, 2006; Boerjan *et al.*, 2010).

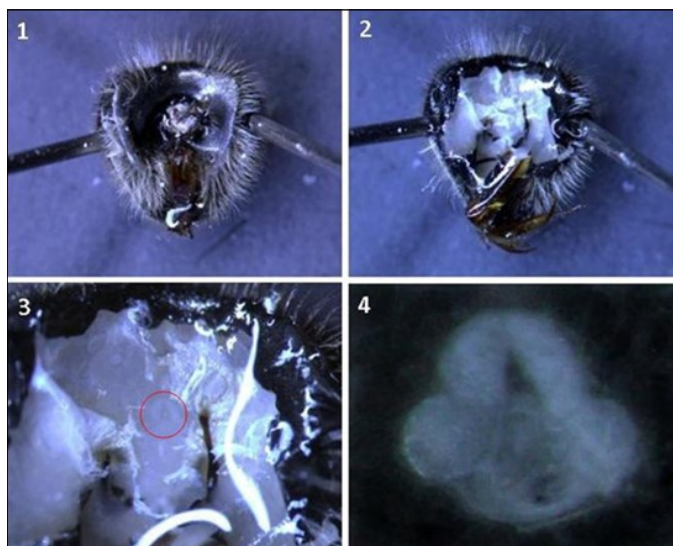
Isolation of the RCC permits quantification of these various hormonal factors, including the *in vitro* measurement of the rate of synthesis and release of juvenile hormone (Rachinsky and Hartfelder, 1990; Huang *et al.*, 1991; Hartfelder *et al.*, 2013).

#### 3.3.4.1. Dissection of the RCC from the head capsule

1. Sedate a live adult bee by chilling.
2. Decapitate the bee by gently pulling on the head to elongate the neck.
3. Sever the neck as close to the thorax as possible.
4. Secure the head, face side down, to a wax surface using a pair of pins through the eyes.

Angle the pins away from the head to maximize accessibility (Fig. 45.1).

5. Using a micro-scalpel or –scissors, cut the area (postgena) that encircles the occipital foramen.



**Fig. 45.** Dissection of the retrocerebral complex of an adult honey bee.

**1.** Head capsule viewed from behind; **2.** cuticle removed, giving a posterior view of the brain; **3.** enlarged image of Fig. 45.2, with the position of the retrocerebral complex marked by a red circle; **4.** the isolated retrocerebral complex showing the spherical corpora allata connected to the corpora cardiaca, which sits like a saddle over the foregut.

Photos: Colin Brent.

6. Widen the circle out to the inner margins of the eyes.
7. Gently tilt up the cuticle from the anterior end to gain access to any connective tissue bound to the cuticle.
8. Sever these connections.

Be sure to minimally disturb the posterior end and leave the oesophagus intact.

9. Remove the cuticle plate.
10. Add a droplet of incubation medium if dissecting to measure the rate of juvenile hormone biosynthesis by the paired corpora allata (see section 3.5 of the *BEEBOOK* paper on physiology and biochemistry (Hartfelder *et al.*, 2013)) or another appropriate physiological liquid (e.g. Ringer solution, see Table 1 of the *BEEBOOK* paper on cell cultures (Genersch *et al.*, 2013)) to ensure the interior of the head stays moist (Fig. 45.2.).
11. Examine the posterior end of the brain where the suboesophageal ganglion completes a circle around the aorta and oesophagus; the retrocerebral complex will be found in this area, also tightly associated with AO-ES.
12. If the glands are not immediately visible, gently pull on the oesophagus and the complex should come into view.

The RCC has a slightly blue colour that is distinct from the whiter nervous tissue around it (Fig. 45.3.).

### 3.3.4.2. Isolation of the RCC and CA

1. Sever the section of AO-ES just anterior to where the complex is attached.
2. Remove the whole mass from the head.
3. Grasp the AO-ES rather than the RCC to avoid damaging the CA and CC.
4. Use forceps to gently push the complex off of the AO-ES, leaving an isolated RCC.
5. Transfer the AO-ES/RCC to an incubation medium if dissecting to measure the rate of juvenile hormone biosynthesis by the paired corpora allata (see section 3.5. of the *BEEBOOK* paper on physiology and biochemistry (Hartfelder *et al.*, 2013)) or another appropriate physiological liquid (e.g. Ringer solution, see Table 1 of the *BEEBOOK* chapter on cell cultures (Genersch *et al.*, 2013)) to prevent desiccation.

For some assays, it is desirable to leave attached tracheal elements intact to enhance the buoyancy of the complex (Fig. 45.4.). Avoid grasping the glands with forceps as the endocrine tissue is readily damaged, and often the glands will adhere to the forceps and become difficult to dislodge. The CA or entire RCC can be moved between different media by suspending the glands in a droplet of liquid held by a small wire hoop.

### 3.3.4.3. Dissection of the brain from the head capsule

1. Flash freeze the bees in liquid nitrogen.
2. Disconnect the head from the body.
3. Place the head on dry ice to ensure it stays frozen.
4. Cut away the face plate using a micro-scalpel or scissors.
5. Make the incision from just above the epistomal sulcus, along the inner margin of the eyes and over the ocelli.
6. Gently lift the face away, severing any underlying connections to reveal the brain below.
7. Cut the connections between the brain and optic lobes.
8. Lift the brain away from the head capsule.

If the brain is to be further subdivided prior to analysis, ensure that it stays chilled during the dissection.

### 3.3.5. The head of the drone

Proceed as with the worker. The hypopharyngeal glands are absent, and the postcerebrals are reduced to rudiments adhering to the posterior wall of the head. The mandibular glands are also vestigial. Clusters of fat cells in some parts of the head may be mistaken for glands. The brain proper (part of the protocerebrum under the ocelli) is smaller than the worker's, though the very large optic lobes give a misleading appearance of size.

### 3.3.6. The head of the queen

Proceed as with the worker. The hypopharyngeal glands are lacking (vestiges of their ducts may sometimes be found). The postcerebrals are like the worker's. The mandibular glands (source of queen substance)

are much larger than the worker's. The brain is somewhat smaller than that of the worker.

## 3.4. Dissection of the drone

### 3.4.1. Preparation for dissection

Immature drones which have just emerged from their cells, or which can be caught actually emerging, should be killed and dissected at once. Mature drones cannot be killed without partial eversion of the endophallus ensuing. Partly matured drones, if their development has not proceeded too far, can usually be killed without inducing eversion, though ejaculation often begins. Preserved material is useless for dissection of the reproductive organs, but preserved drones may be used for the dissection of the head, of the thorax to show the powerful flight muscles, and of the abdomen to show the immensely powerful abdominal muscles.

### 3.4.2. Immature drone, dissection from the dorsal aspect (Plate 14)

#### 3.4.2.1 Exposing the viscera

1. Proceed, as in the case of the worker (see section 3.2.2.1.), to take off the roof of the abdomen.

In crossing over at the anterior end it will be difficult to avoid damage to the testes, which will then be slightly frayed out in that region.

2. Notice the resistance to the scissors offered by the greatly developed abdominal muscles.
3. Take care in all dissections of the drone to avoid damage to the mucus glands.

If these are pierced, mucus will exude, coagulate, and obscure the work.

4. Compare the undisturbed viscera with the plate 14 and identify the organs.

Note the enormous testes, composed of bundles of tubules which can be shown by teasing out one of the testes.

#### 3.4.2.2. The reproductive apparatus

1. Lay out the testes, as shown in Plate 14 (where only one testis is drawn).

If they are not very gently handled, they will break off at the vasa deferentia.

2. Grasp the ventriculus with forceps and draw it out backwards until the crop appears.
3. Cut through the oesophagus with scissors, also through the rectum.
4. Remove the alimentary canal.
5. Flush out the abdominal cavity with clean dissecting fluid from the pipette.

This exposes the rest of the reproductive apparatus.

6. Identify the parts flagged in Plate 14.

The whole of the apparatus can be removed for more detailed examination by cutting through the body wall, round the genital aperture, with the point of the knife. It can then be lifted out, very gently to avoid damage, fixed in formol-alcohol, and preserved.

### 3.4.2.3. Maturing drone (Plate 15)

Treat as in the preceding dissection. The testes are reduced in size, finally becoming thin, triangular, translucent scales. The seminal vesicles, having received spermatozoa, are increased in size, so are the mucus glands. If eversion has not occurred, there may yet be partial ejaculation, the evidence of which may be seen in the swelling of the ejaculatory duct near the bulb.

### 3.4.2.4. Mature drone

1. Catch a flying drone on the alighting board of a hive or at a DCA (see section 13.4. of the *BEEBOOK* paper on behavioural methods (Scheiner *et al.*, 2013)).
2. Kill it (see section 2.1.2. on immobilising, killing and storing adult *Apis mellifera* in the *BEEBOOK* paper on miscellaneous methods (Human *et al.*, 2013)).
3. Cut off the everted endophallus.
4. Mount the insect for dissection.
5. Remove the roof of the abdomen.
6. Look for the testes, now shrunken to mere yellowish or greenish scales.
7. In the second stage of the dissection, compare the condition of the rest of the apparatus (except the endophallus, which is now outside the body) with that of the immature and maturing drone. The ejaculatory duct will be seen passing out through the genital aperture.

### 3.4.2.5. The everted endophallus (Plate 15)

1. Kill a mature drone.
2. Examine the partially everted endophallus, and compare it with the drawing in Plate 15.
3. Take the abdomen between the thumb and forefinger and compress it laterally; eversion will continue until the bulb reaches the tip of the organ, when sperm and mucus will be liberated.

In living drones, notice how easily eversion is induced by the slightest pressure, and the 'snap' when this occurs.

## 3.5. Dissection of the queen

### 3.5.1. Preparation

Queens must be dissected immediately after killing. If they are preserved in alcohol the ovaries become extremely brittle and cannot be handled for proper examination. They can, however, be placed at -20°C alive, without alcohol, and they can be dissected even months afterwards. The remainder of the body may be preserved, however, and can then be dissected at leisure (see section 3.10.5.).

### 3.5.2. Dissection of the fertile queen (Plate 16)

#### 3.5.2.1. Exposing the viscera (Plate 16A)

1. Mount the queen for dissection, dorsal surface uppermost.

2. Open the abdomen carefully, avoiding damage to the underlying organs when taking off the roof.
3. When the roof appears to be quite free, raise the front edge of it slightly with the forceps.
4. Gently detach, with a needle held in the right hand, the soft organs which cling to the roof.

If this is not done very carefully the ovaries will be pulled out and spoiled. The undisturbed viscera will then have the appearance shown in Plate 16A. At their anterior ends, the ovarioles of the two ovaries are joined, and at this point were attached to the heart, from which they were detached before lifting the roof.

5. Identify the parts flagged in the plate, and note:
  - 5.1. the enormous ovaries that fill about two-thirds of the abdomen;
  - 5.2. the rectum that is always empty.

#### 3.5.2.2. Displaying the reproductive organs (Plate 16B)

1. Grip the ventriculus with forceps and stretch it, drawing it towards the rear end of the abdomen.
2. Cut the canal in front of the crop and through the rectum.
3. Remove the alimentary canal.

This must be done without damaging the ovaries.

4. Very carefully slip a needle under one of the ovaries and lift it, turning it slightly outwards.

The tracheae which bind and suspend the ovary will now be seen stretched.

5. Break the tracheae with a needle held in the other hand, until the ovary is freed sufficiently to lie over to the side, as shown in the plate.
6. Repeat this operation with the other ovary.
7. Lower the sides of the body wall near the tip of the abdomen.
8. Flush out the body cavity with the pipette.
9. Note:
  - 9.1. the paired oviducts, which converge to the root of the sting, where they join
  - 9.2. the median oviduct;
  - 9.3. the spermatheca;
  - 9.4. the spermathecal gland and investment of silvery tracheae;
  - 9.5. the acid gland of the sting, which is much longer than the worker's;
  - 9.6. the sting apparatus is firmly anchored and cannot be lifted out as it can from a worker dissection. The shaft of the sting is curved, not straight like the worker's.

Queens used for dissection are often discarded ones, a year or two old, sometimes older. In these old queens the fat body and Malpighian tubules are discoloured by the accumulation of waste products (this can be seen in the pericardial fat cells; it is not necessary to spoil a good dissection by removing the viscera to expose the fat cells on the floor of the abdomen).



### 3.5.2.3. Weight of ovaries

The ovaries can be weighed immediately after dissection (wet weight), or after dehydration in an incubator (in separate Eppendorf tubes at 60°C for 24 hours (dry weight)). Dehydration requires time and it does not give an immediate result.

### 3.5.2.4. Number of ovarioles

The number of ovarioles (in one ovary) can vary among honey bee races. For example it has been reported that there are an average of 130-155 ovarioles/ovary in *A. m. macedonica* (Hatjina *et al.*, 2013). In *A. m. ligustica*, there are between 135-175 (Woyke, 1971; Casagrande-Ialoretto *et al.*, 1984) and in *A.m. carnica* they range between 145-160 (Hatjina *et al.*, 2013). The number of ovarioles can be evaluated at any time during the life of a fertilized queen but preferably a few months after mating. Left and right ovaries contain almost the same number of ovarioles, so there is no need to perform the count for both ovaries. It is recommended to always count ovaries on the same side of all queens. The number of ovarioles can be estimated by two main methods: histological preparations or real-time counting. The steps for each method are described below (Table 2; Fig. 46). These two methods have advantages and disadvantages as described in Table 3.

### 3.5.2.5. Diameter of the spermatheca

The diameter of the spermatheca is evaluated with or without the tracheal net which surrounds it. The diameter is a direct estimation of the volume and an indirect estimation of the number of spermatozoa. Digital photographs and measurements with the use of an Image Analysis system give very accurate results. Without this system, measurements of the diameter can be performed using an eye-piece ocular micrometer.

1. Measure, two cross diameters because spermathecae do not always have a perfect spherical shape.
2. Calculate average value in mm.

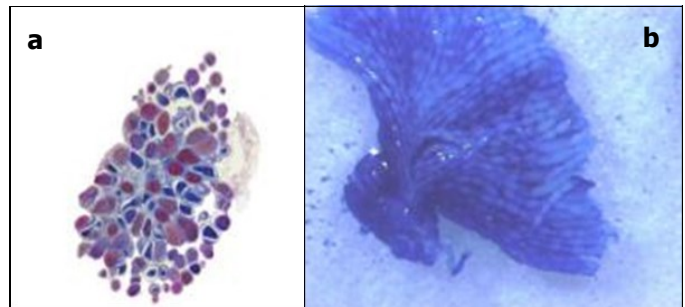
Before the tracheal net is removed, the full spermatheca should have a diameter > 1.2 mm. One can use this measure for spermatheca volume to calculate the theoretical maximum storage capacity and percentage filled for spermatheca (see Tarpy *et al.*, 2011).

3. Remove the tracheal net.

The spermatheca of a mated queen is white milky colour, while the one of an unfertilized queen is transparent (Fig. 47).

### 3.5.3. Dissection of the fertile queen from the ventral aspect

This more difficult operation will demonstrate that part of the reproductive tract which was concealed by the sting apparatus in the preceding dissection. This part of the tract, including the median oviduct, vagina, and bursa, together with the sting apparatus which lies above them, are firmly attached to the floor of the abdomen, from which they can be detached only by careful, patient use of the point of the knife.



**Fig. 46.** Two different methods of seeing the ovarioles:

**a.** the histological preparation (transversal section from Jackson *et al.*, 2011), each ellipse represents an ovariole; and **b.** real-time stereomicroscopy counting (from Hatjina, 2012).



**Fig. 47.** Spermatheca with the tracheal net.

Photo: Fani Hatjina.

1. Mount the insect with the ventral surface upwards.
2. Cut away the floor of the abdomen using scissors, starting at the anterior end, and working backwards, removing the sternites until the lateral oviducts are uncovered.
3. Continue cutting through the body wall at the extreme sides of the abdomen, towards the tip.
4. Grip the floor with forceps, at the forward edge.
5. Insert the point of the knife and very carefully separate the sternites from the soft parts beneath them.
6. Remove the floor piecemeal, as it becomes possible to cut away parts of it to facilitate the work.

When the dissection is finished, it will be possible easily to identify the bursa and lateral pouches; the latter, in this position of the queen, are uppermost. The remaining structures are more difficult to identify, but will become clearer after soaking in glycerol.

### 3.5.4. Dissection of the virgin queen (Plate 17)

Dissect from the dorsal aspect, and compare with the fertile queen. The ovaries are small and undeveloped, the ovarioles containing no eggs. The spermatheca contains fluid, and collapses easily. Compare also with the laying worker.



**Table 2.** Estimating the number of ovarioles using histological preparations or real-time counting.

Steps of procedure	Histological preparations	Real-time counting
1	The ovaries are dissected in saline solution and then are immediately transferred to alcoholic Bouin's fixative prepared by combining 150 ml 80% ethanol, 60 ml commercially prepared formaldehyde, 15 ml glacial acetic acid, and 1 g of saturated picric acid solution for fixation. Jackson <i>et al.</i> (2011).	The ovaries are dissected in saline solution.
2	After overnight fixation at room temperature, dehydration is completed by transferring the ovaries to 100% ethanol (two changes, 15 minutes each). Dehydrated ovaries are then immersed into a 1:1 solution of 100% ethanol: xylene for one hour, then transferred to xylene for storage until infiltration with paraffin wax in a 60°C oven (two changes of paraffin, approximately 60 minutes each in duration).	Ovaries are removed, dyed by electrophoresis gel stain, and then rinsed with alcohol and transferred to a microscope slide. (Rodes and Somerville, 2003). A transverse section cuts the ovary into two parts. Most ovarioles contain eggs at the same stage of maturation.
3	Finally they are embedded in paraffin wax and the wax blocks containing the embedded ovaries are sectioned using a rotary microtome. Sections of 10 µm-thickness should be mounted in a pool of water at 48°C and collected on slides. The slides can stay on a slidewarmer overnight and then stored at room temperature in a covered box until staining. Immediately prior to staining, paraffin is removed from sections by immersion in xylene (three changes, 5 minutes each) and rehydrated in a graded series of ethanol of descending concentrations, 5 minutes per change (100%, 100%, 95%, 70% with lithium carbonate, 50%, and 30%). After a brief dip in distilled water, sections can then stained for 2 minutes in trichrome stain prepared by adding phosphotungstic acid (1 g), orange G (2 g), aniline blue WS (1 g), and acid fuchsin (3 g) to 200 ml distilled water and differentiated in 95% ethanol prior to dehydrating in 100% ethanol, clearing in CitriSolv and coverslipping with Permount (Fisher Scientific). Slides are stored flat for a week at room temperature prior to microscopy to ensure adequate drying of the Permount mounting medium.	The upper part of the ovary is then used for separation and counting of the ovarioles under a stereomicroscope at 200x or 250x magnification (Hatjina, 2012).
4	Ovarioles in several tissue sections from each ovary are counted and the average number is noted.	Ovariole numbers are noted only once per ovary (left and right).

**Table 3.** Advantages and disadvantages of estimating the number of ovarioles using histological preparations or real-time counting.

	Histological preparations	Real-time counting
<b>Advantages</b>	<ul style="list-style-type: none"> <li>The preparations are permanent.</li> <li>Photographs can be taken and the ovarioles can be counted at any time.</li> <li>Greater accuracy in the estimation can be achieved.</li> </ul>	<ul style="list-style-type: none"> <li>The procedure is very quick and simple.</li> <li>It does not require special chemicals or equipment such as a microtome.</li> <li>You count each ovary only once.</li> </ul>
<b>Disadvantages</b>	<ul style="list-style-type: none"> <li>The method requires special chemicals and equipment such as a microtome.</li> <li>The preparation of the samples is very long.</li> <li>Handling the ovary so many times can be destructive.</li> </ul>	<ul style="list-style-type: none"> <li>The method is destructive for the ovary.</li> <li>The ovariole count needs to be done at the time of the dissection.</li> <li>You cannot have more than two different estimations of the same ovary as the ovarioles are destroyed.</li> </ul>

### 3.5.5. Spermatozoa in the spermatheca

To obtain spermatozoa or check their presence:

1. Anaesthetize the queen, and kill her (see section 2.1.2. on immobilising, killing and storing adult *Apis mellifera* in the *BEEBOOK* paper on miscellaneous methods (Human *et al.*, 2013)).
2. Dissect quickly in 0.15% salt solution.
3. Remove the spermatheca.
4. Put the spermatheca into a drop of the salt solution in a watch glass or on a microscope slide.
5. Tear open the spermatheca with needles.

If spermatozoa are present they will emerge as a creamy mass with something of the appearance of a tuft of cotton wool.

6. Spread this out with needles and examine under a microscope with 16 mm objective or higher power. See Fig. 30.

Alternatively:

1. Pinch off the top of the abdomen.
2. Pick out the spermatheca (easily seen with the naked eye).
3. Burst it under a coverslip.
4. Examine under a microscope with 16 mm objective or higher power. See Fig. 30.

## 3.6. Dissection of the juvenile forms

### 3.6.1. Dissection of the larva (Plate 19)

1. Choose large coiled larvae.

In the fresh state they are too fragile to handle, while if they are fully hardened the fat cells form a solid mass which cannot be broken up without destroying the viscera. They must therefore be hardened:

2. Immerse in formol-alcohol for only two or three days before dissection.

In any case, the removal of the fat is a rather tedious process, requiring care and patience to take it away piecemeal.

3. Lay the coiled larva on the side since it cannot be straightened out for dissection.
4. Remove the body wall using scissors.
5. Disengage the fat in small fragments.

If this is done successfully, the structures shown in Plate 19 will be revealed. The plate shows a drone larva, more convenient for this work on account of its larger size. The ovaries are very small in worker larvae, much larger in queen larvae; they occupy the same position as the testes, which are still larger.

### 3.6.2. Dissection of the prepupa

Prepupae show the same structures as larvae, but after the faeces are discharged the ventriculus is reduced to a narrow, flat strap, hardly recognizable as the same organ as the enormously distended ventriculus of the feeding larva.

### 3.6.3. Pupae

In the early stages pupae may be dissected like larvae, but from the dorsal aspect. Older pupae can be treated like adult insects.

## 4. Acknowledgements

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer. The COLOSS (Prevention of honey bee Colony LOSSes) network aims to explain and prevent massive honey bee colony losses. It was funded through the COST Action FA0803. COST (European Cooperation in Science and Technology) is a unique means for European researchers to jointly develop their own ideas and new initiatives across all scientific disciplines through trans-European networking of nationally funded research activities. Based on a pan-European intergovernmental framework for cooperation in science and technology, COST has contributed since its creation more than 40 years ago to closing the gap between science, policy makers and society throughout Europe and beyond. COST is supported by the EU Seventh Framework Programme for research, technological development and demonstration activities (Official Journal L 412, 30 December 2006). The European Science Foundation as implementing agent of COST provides the COST Office through an EC Grant Agreement. The Council of the European Union provides the COST Secretariat. The COLOSS network is now supported by the Ricola Foundation - Nature & Culture.

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