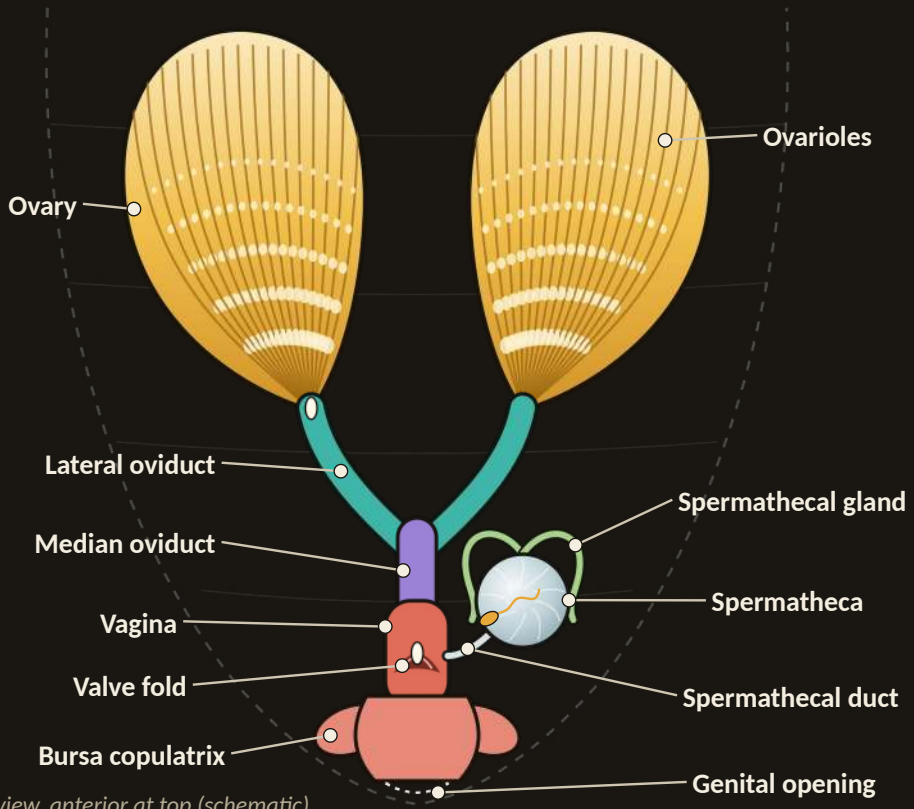
HONEY BEE BIOLOGY

# The queen's reproductive system

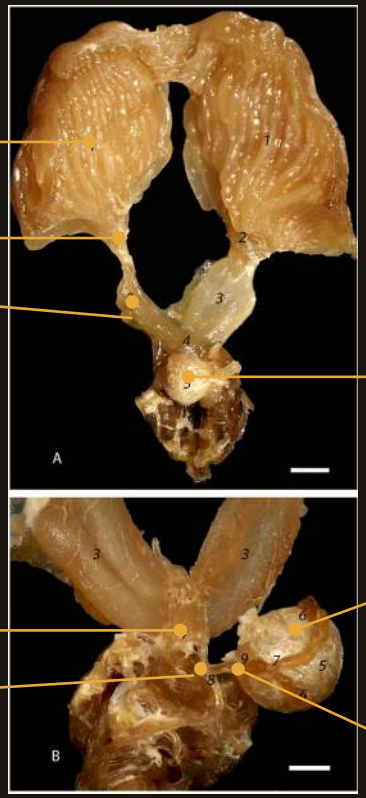



*Dorsal view, anterior at top (schematic)*

1. **Ovaries**
   Paired; each has 150–180 ovarioles. In a laying queen they fill much of the abdomen.
2. **Lateral oviducts**
   Carry mature eggs from each ovary.
3. **Median oviduct**
   The two lateral oviducts unite into one tube.
4. **Spermatheca**
   A sphere c. 1.1 mm across, wrapped in tracheae. Stores millions of sperm for life, fed by the paired glands.
5. **Vagina & valve fold**
   The valve fold presses each egg against the spermathecal duct opening.
6. **Bursa copulatrix**
   Receives the drone's endophallus; only a few per cent of the semen reaches the spermatheca.
7. **Fertilisation**
   A median of just 2 sperm per egg. Fertilised eggs become females; unfertilised, drones.

HONEY BEE BIOLOGY

# Queen reproductive anatomy



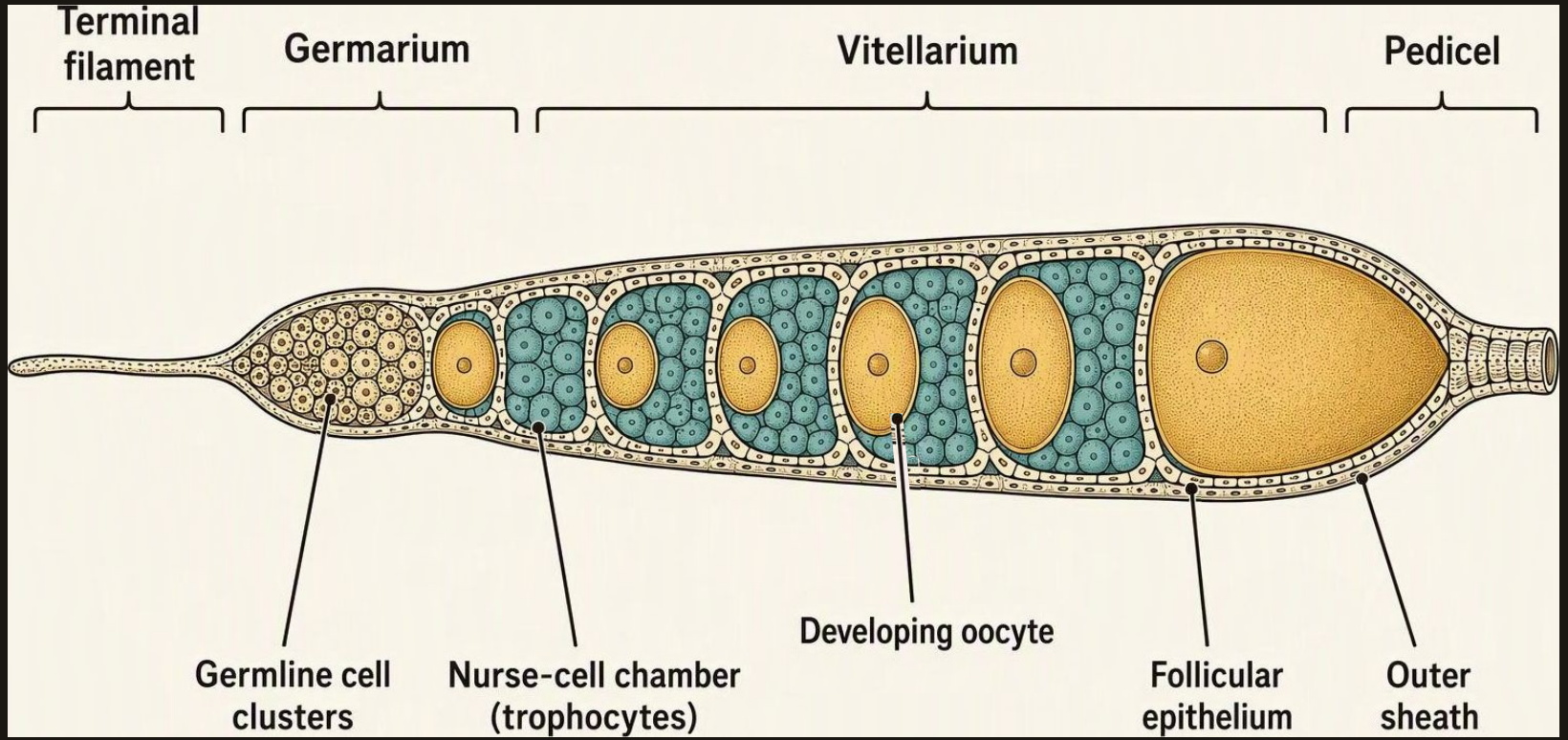

A: upper tract; B: middle region. Source: Journal of Apicultural Research (2021), Histomorphological description of the reproductive system in mated honey bee queens, Fig. 1

# Ovaries

- Paired ovaries fill about two-thirds of the abdomen
- Each ovary is a dense bundle of ovarioles, like a bunch of bananas
- **Terminal filament:** short thread at the front tip
- **Germarium:** germline cells (cystocytes) cluster in rosettes, then line up in a single row
- **Vitellarium:** the longest region; oocytes grow towards the rear, separated by nurse-cell chambers (trophocytes) that feed them
- **Pedicel:** stalk at the rear end
- **Outer sheath:** epithelium enclosing the whole ovariole

HONEY BEE BIOLOGY

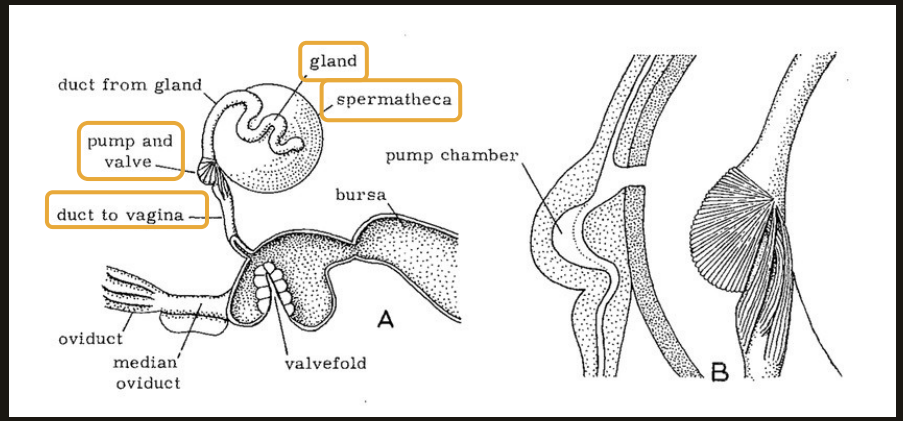
# Inside a queen’s ovariole

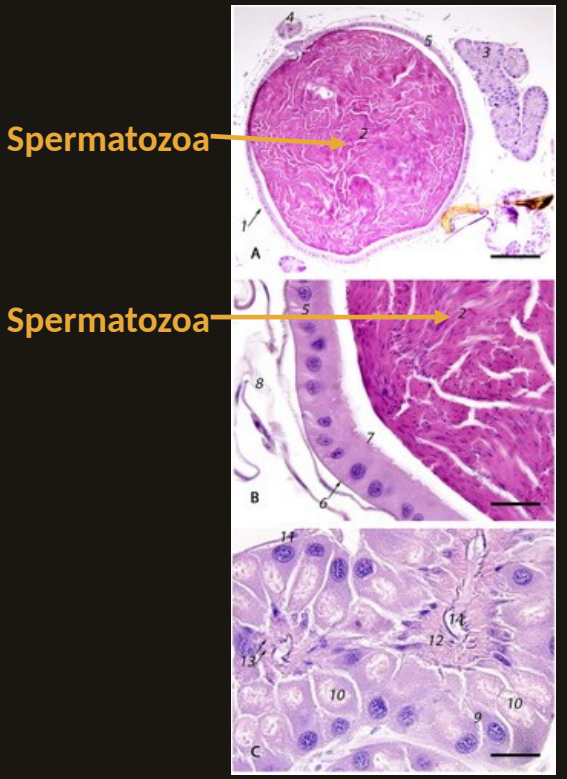



AI-generated teaching schematic, not to scale

# The spermatheca

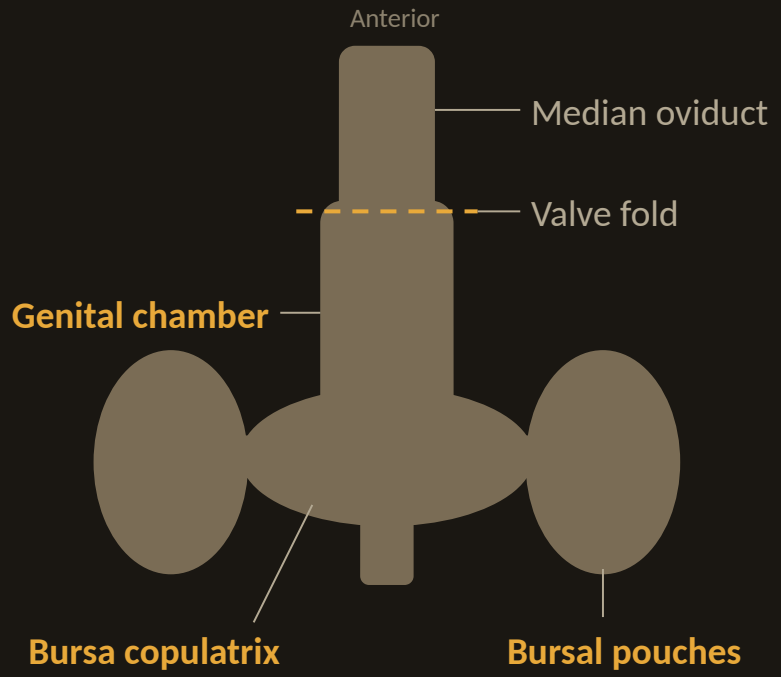
- **Spermatheca:** stores spermatozoa in the mated queen
- **Spermathecal gland:** secretes spermathecal fluid
- **Spermathecal duct:** connects it to the median oviduct
- **Sperm pump:** ring of circular and longitudinal muscles



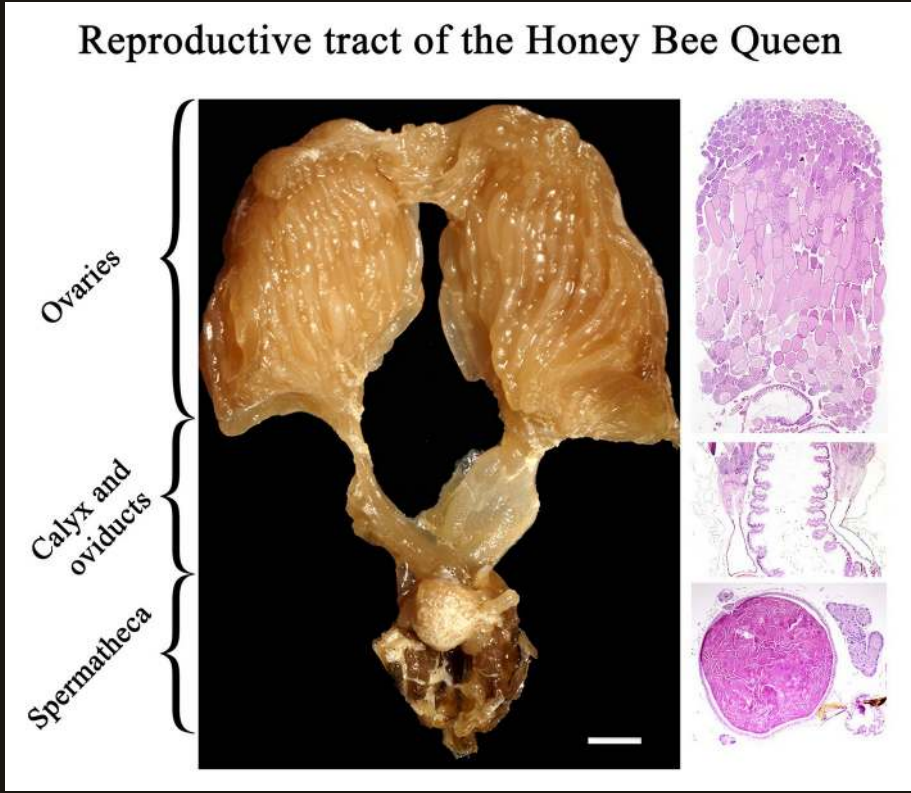
# Genital chamber, bursa copulatrix, and bursal pouches

- **Genital chamber (vagina):** continuation of the median oviduct behind the valve fold
- **Bursa copulatrix:** cleft-like widening at the rear of the genital chamber
- **Bursal pouches:** paired sacs opening either side of the bursa
- All three are lined by a single layer of epithelium
- At the rear, the tract is bounded dorsally by the sting and ventrally by the 7$^{th}$ sternum




Schematic, dorsal view, not to scale. Source: Histomorphological description of the reproductive system in mated honey bee queens, Journal of Apicultural Research (2021).

# Reproductive tract of the honey bee queen



HONEY BEE BIOLOGY

# Sperm production in the drone

1 **Development in the testes**

Germ cells in the testes form sperm during the drone’s immature development.

2 **A modified meiotic process**

Drones are haploid. Their sperm formation avoids halving the chromosome set again.

3 **Maturation and storage**

Sperm move along the vasa deferentia to the seminal vesicles as the young adult matures.

4 **Transfer to the queen**

At mating, the drone transfers semen. The queen stores a small fraction of the sperm in her spermatheca.

HONEY BEE BIOLOGY

# Egg production in the queen

1 **Germarium: the oocyte begins**

Germline cells give rise to an oocyte and associated trophocytes (nurse cells) inside each ovariole.

2 **Vitellarium: the egg grows**

Nurse cells supply material to the oocyte, which accumulates yolk to support the embryo.

3 **Follicle cells: the coverings form**

Surrounding follicle cells contribute to the egg envelopes, including the protective chorion.

4 **Ovulation and laying**

The mature egg enters the oviducts. Stored sperm may fertilise it as it passes towards the genital opening.

HONEY BEE BIOLOGY

# Structure of the honey bee egg



Longitudinal cutaway, schematic

## Chorion

The outer covering protects the egg. Its surface allows gas exchange.

## Vitelline membrane

A thin envelope lies beneath the chorion and surrounds the egg contents.

## Yolk and cytoplasm

Yolk supplies stored nutrients. Living cytoplasm supports early development.

## Micropyle

A small opening at the anterior end provides the route for sperm entry.

HONEY BEE BIOLOGY

# Embryo development within the egg

The embryo develops using nutrients already stored in the egg.

## 01

### Cleavage

The nucleus divides repeatedly. Nuclei move towards the egg surface.

## 02

### Blastoderm

Cells form a surface layer around the yolk. The embryonic region takes shape.

## 03

### Germ band

Cell movements establish the body plan and the layers that form tissues.

## 04

### Organ formation

Body segments, the gut and nervous system develop as the larva takes shape.

The egg stage normally lasts about three days under brood-nest conditions.

HONEY BEE BIOLOGY

# Eclosion (hatching) and the first larval stage

1 **The coverings open**

At the end of embryonic development, the larva emerges from the egg coverings.

2 **A small, legless larva appears**

The pale first-instar larva rests at the base of the open brood cell.

3 **Nurse bees begin feeding**

Workers supply brood food. The larva grows rapidly and later moults through further instars.



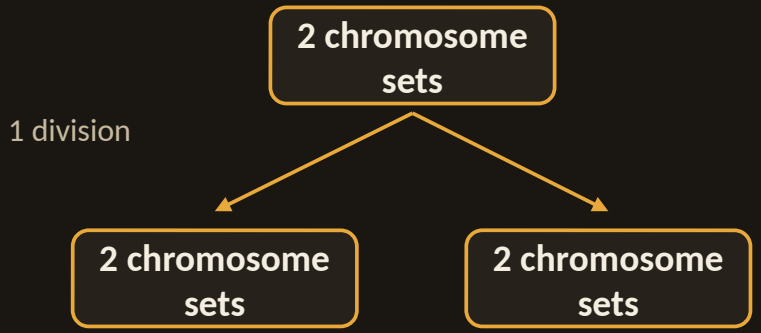
Newly hatched larva, schematic

CELL DIVISION

# Mitosis and meiosis

## Mitosis

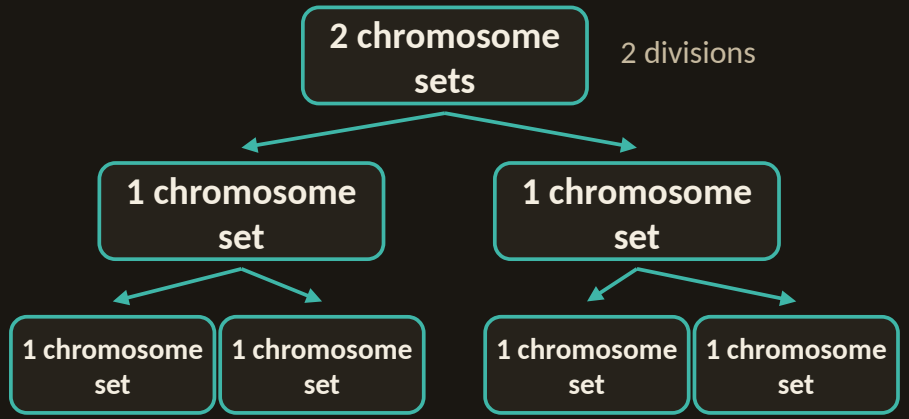
Makes matching cells for growth and repair




**2 matching cells**
**Same chromosome number**

## Meiosis

Helps make eggs or sperm for reproduction

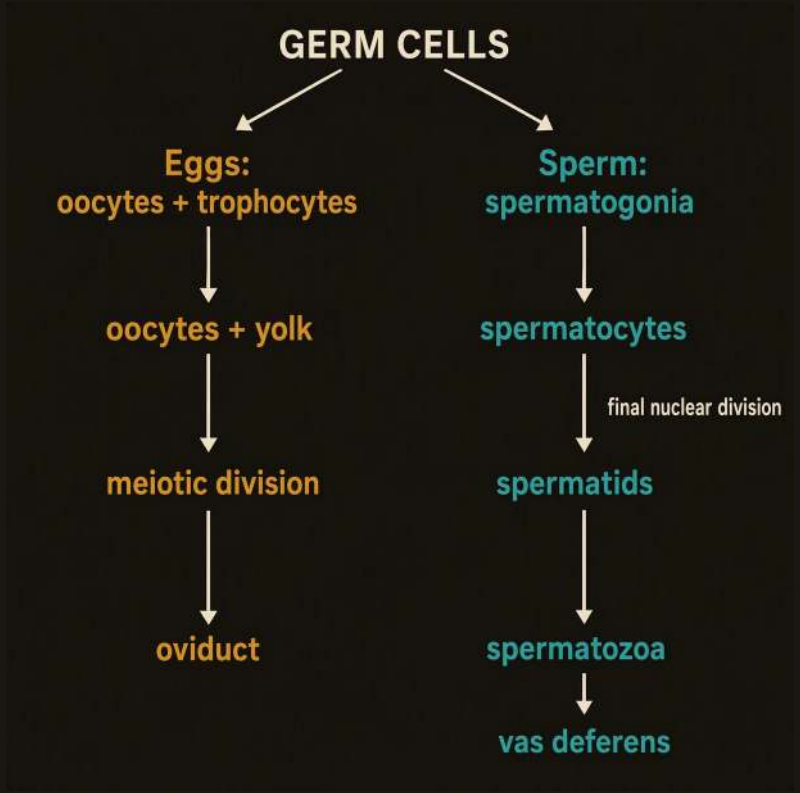



**4 cells with half the chromosomes**

Honey bee drones are different: they already have one set and use modified meiosis.

PRODUCTION

# Egg and sperm development



GENETICS

# Mendelian genetics made simple

## How parents pass on gene versions

In pea plants, an offspring gets one copy of each gene from each parent.

Different versions of a gene are called alleles.

For this flower-colour gene, P is dominant: PP and Pp give purple flowers. The recessive pp gives white flowers.

## Example: two purple parents, Pp × Pp

Each parent passes on P or p at random.

| | P | p |
|---|---|---|
| P | PP | Pp |
| p | Pp | pp |

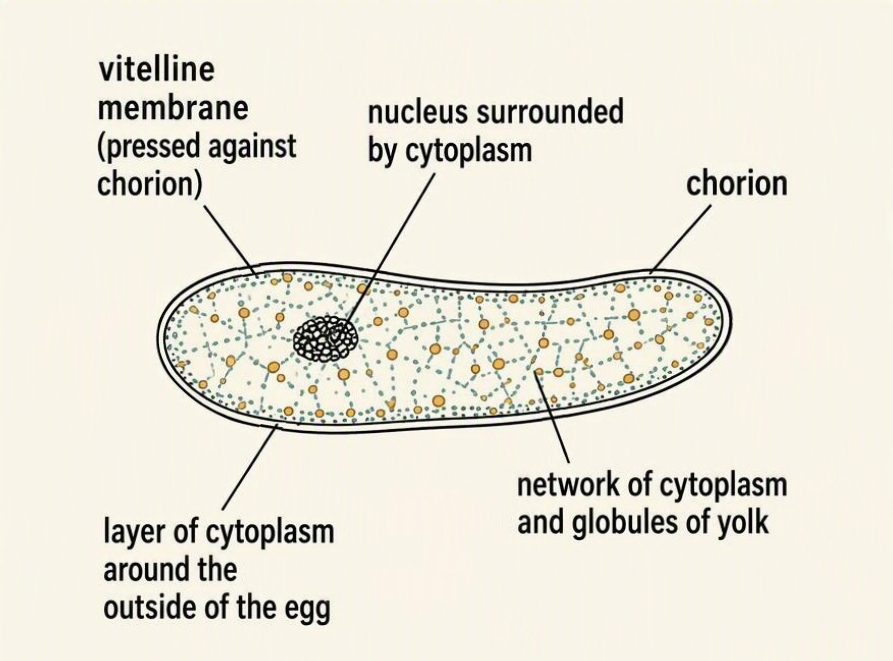
## Each offspring: 75% purple, 25% white

These are chances, not a guaranteed batch of four.

Bee link: queens and workers have two sets of genes. Haploid drones have one.

HONEY BEE BIOLOGY

# Structure of the newly laid egg




**1 Chorion**

The outer shell forms the egg's protective covering.

**2 Vitelline membrane**

This thin membrane lies immediately beneath the chorion.

**3 Nucleus and surrounding cytoplasm**

The nucleus sits within cytoplasm. Its divisions begin development.

**4 Outer layer of cytoplasm**

A layer of cytoplasm lines the outer region of the egg, inside its coverings.

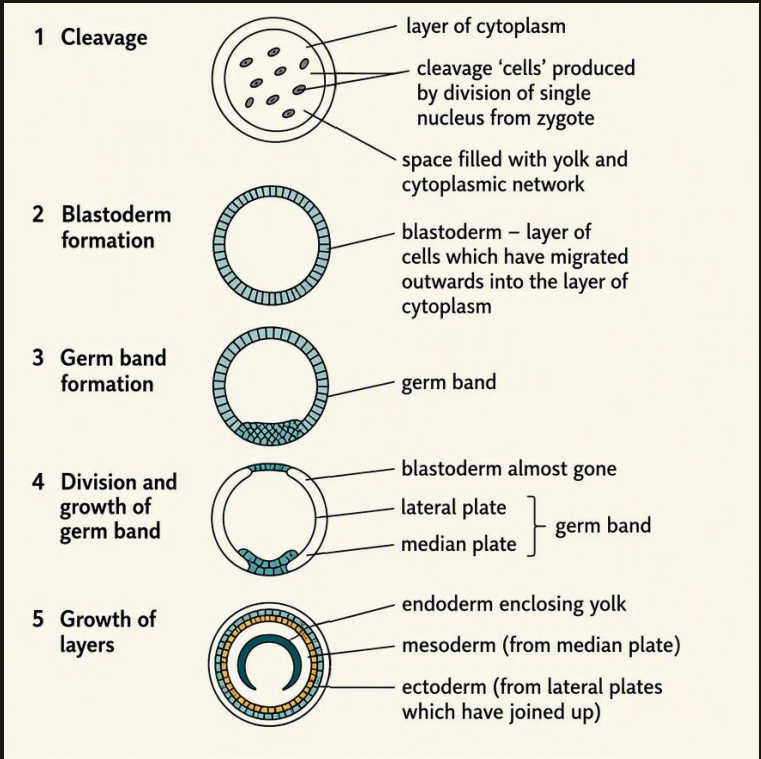
**5 Cytoplasm network and yolk**

A network of cytoplasm extends among yolk globules, which supply nutrients.

Redrawn from the supplied diagram; schematic, not to scale.

HONEY BEE BIOLOGY

# Early embryonic development




Redrawn from the supplied diagram; schematic, not to scale.

## 1 Cleavage

The nucleus divides repeatedly within the yolk-rich cytoplasm.

## 2 Blastoderm formation

Cells move outwards and form a layer around the yolk.

## 3 Germ band formation

One region of the blastoderm thickens to form the germ band.

## 4 Division and growth of germ band

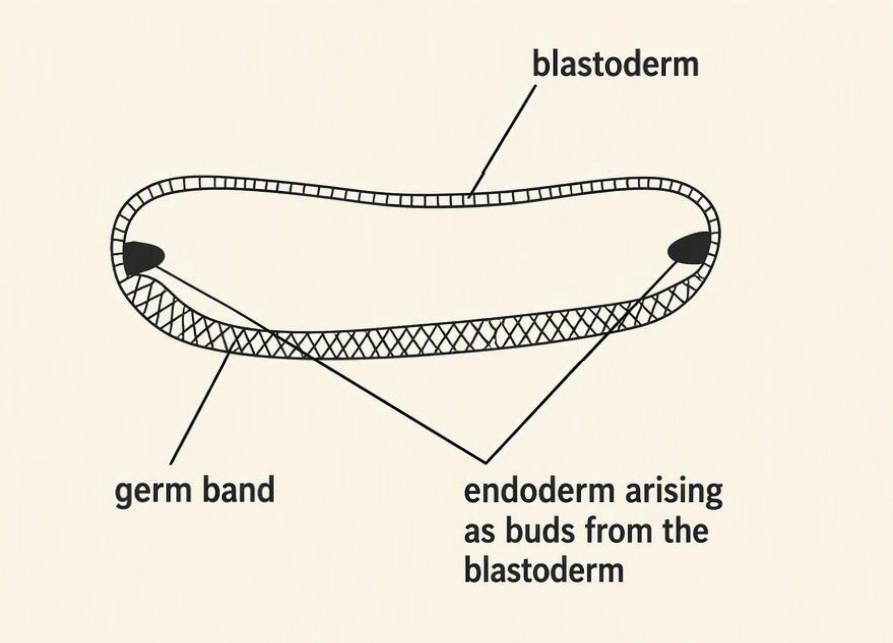
The germ band develops median and lateral plates.

## 5 Growth of layers

Ectoderm and mesoderm develop from the plates. Endoderm encloses the yolk.

HONEY BEE BIOLOGY

# Endoderm formation within the egg




## A longitudinal view

This section shows the length of the egg, complementing the previous cross-sections.

## The labelled regions

The blastoderm surrounds the egg contents. The germ band occupies one side.

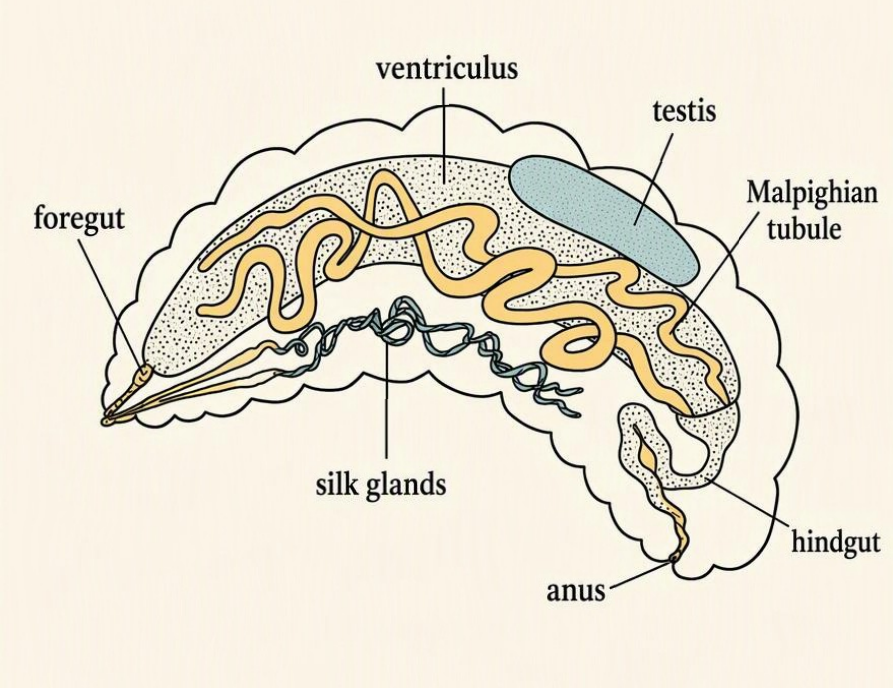
## Endoderm begins

The diagram shows endoderm arising as buds near the two ends of the blastoderm.

Redrawn from the supplied diagram; schematic, not to scale.

HONEY BEE BIOLOGY

# Larval internal anatomy




## A body built for growth

The ventriculus, or midgut, occupies much of the larval body.

## Internal organisation

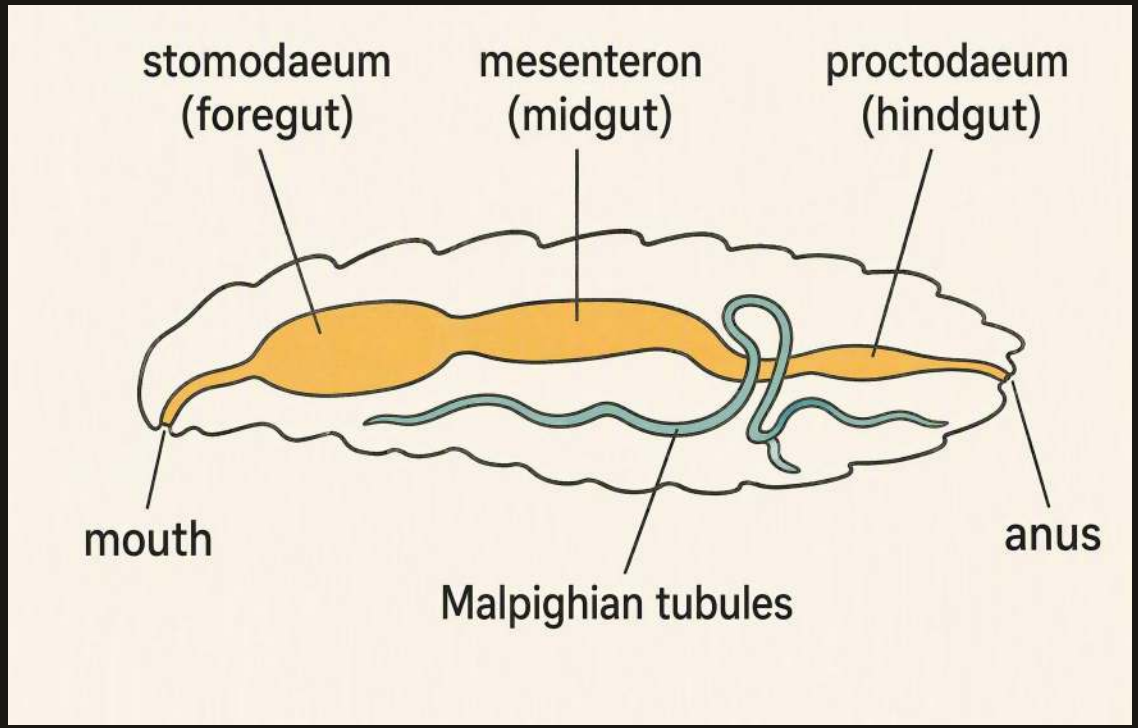
The illustration also labels the foregut, hindgut, silk glands and Malpighian tubule.

## Reading this specimen

The labelled testis identifies a male larva. This is an anatomical schematic, not a hatching sequence.

Redrawn from the supplied diagram; schematic, not to scale.

# Embryo: notebook comparison




Notebook sketch redrawn for comparison; schematic, not to scale.

## The same three regions

The sketch labels foregut, midgut and hindgut, as in the earlier version.

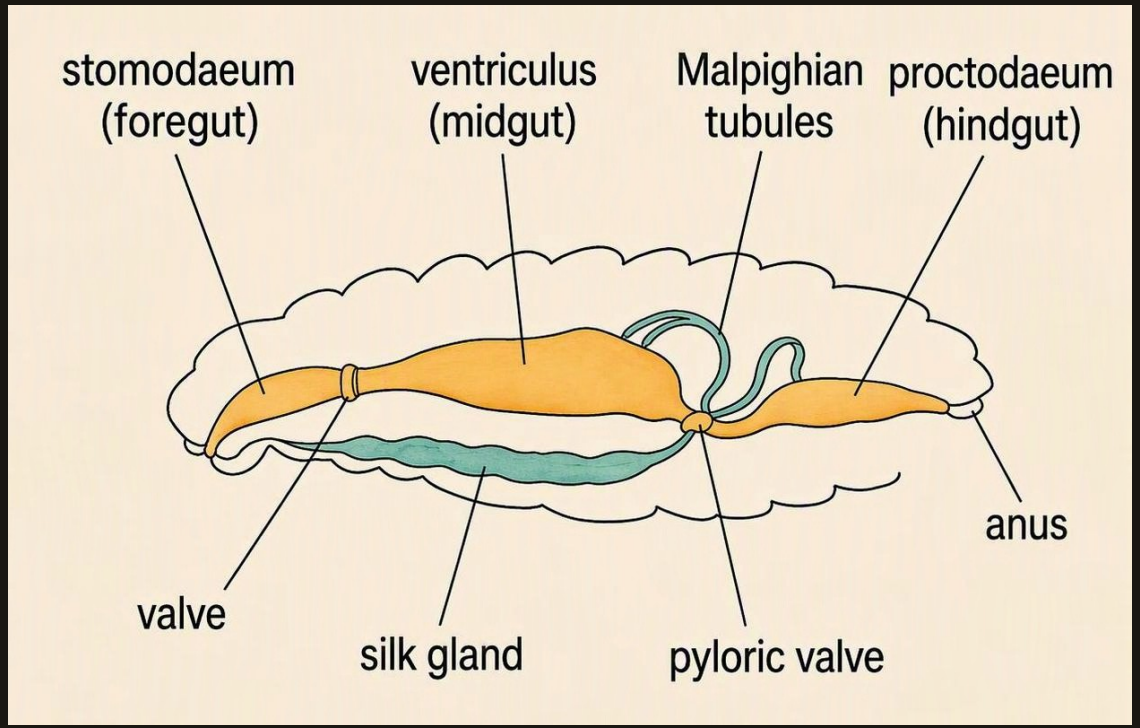
## Shared terminology

Mesenteron names the midgut. Stomodaeum and proctodaeum name the end regions.

## A simpler view

Heart and nerve-cord labels are omitted here. This does not imply absent organs.

# Larva: notebook comparison




Notebook sketch redrawn for comparison; schematic, not to scale.

## Shared landmarks

Both versions identify the ventriculus, silk gland and Malpighian tubules.

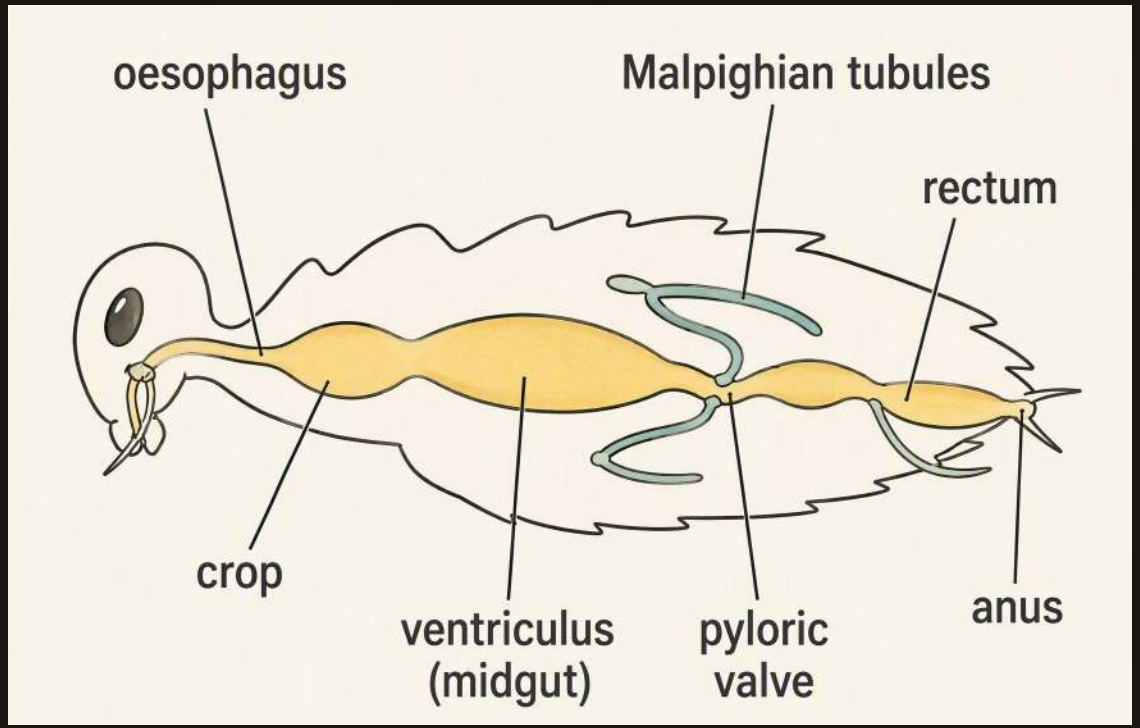
## Additional valve labels

This sketch labels an anterior valve and a pyloric valve near the posterior gut.

## Compare the labels

Use corresponding structures to compare the drawings, not their proportions.

# Adult: notebook comparison




Notebook sketch redrawn for comparison; schematic, not to scale.

## A familiar gut layout

Both drawings identify the oesophagus, crop, ventriculus and rectum.

## The posterior junction

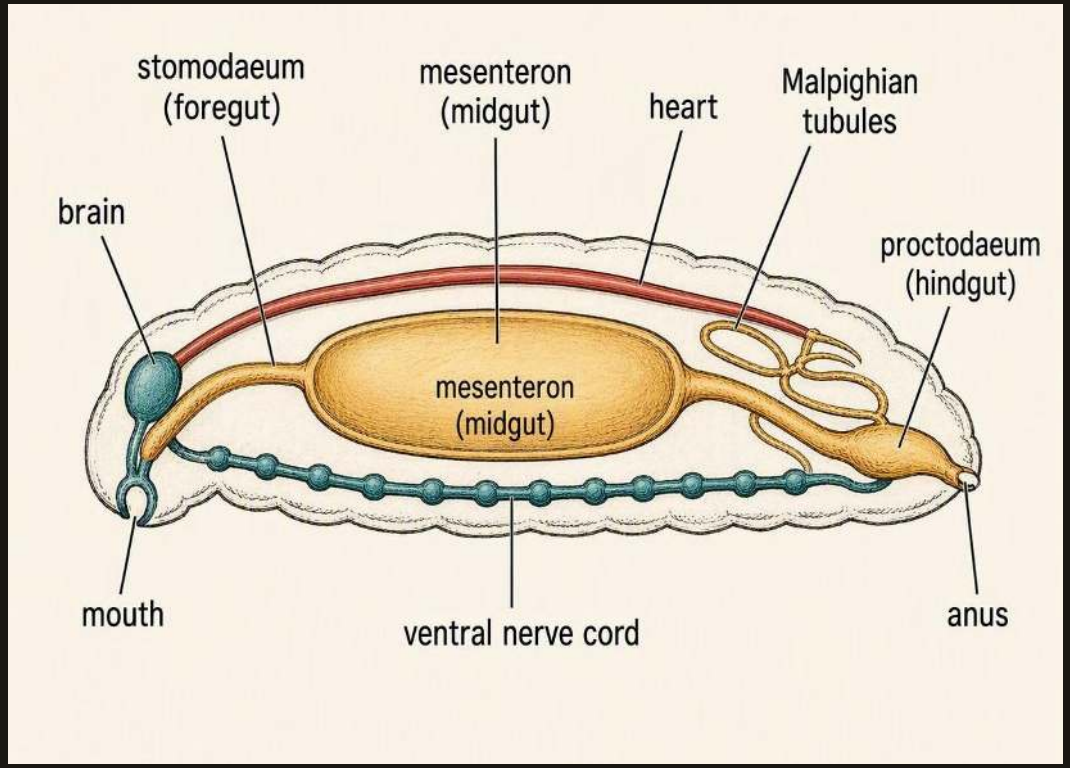
The pyloric valve and Malpighian tubules mark the region behind the midgut.

## Less anatomical detail

This sketch omits the brain and nerve-cord labels shown in the earlier version.

HONEY BEE BIOLOGY

# The developing alimentary system




Redrawn from supplied study notes; schematic, not to scale.

## Three gut regions

The embryonic diagram identifies the foregut, midgut and hindgut.

## Names used in the notes

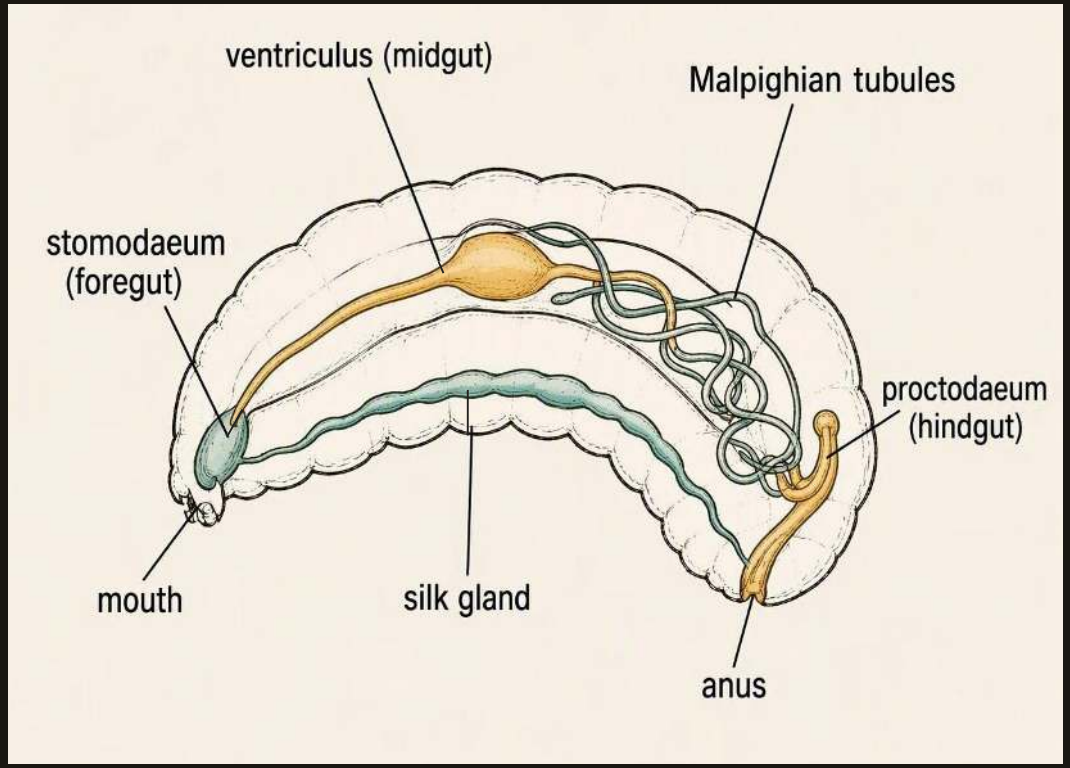
Stomodaeum = foregut.
Mesenteron = midgut.
Proctodaeum = hindgut.

## Other developing organs

The heart lies above the gut, with the ventral nerve cord below.

HONEY BEE BIOLOGY

# The larval alimentary system




Redrawn from supplied study notes; schematic, not to scale.

## A prominent midgut

The ventriculus occupies much of the curved larval body.

## Associated structures

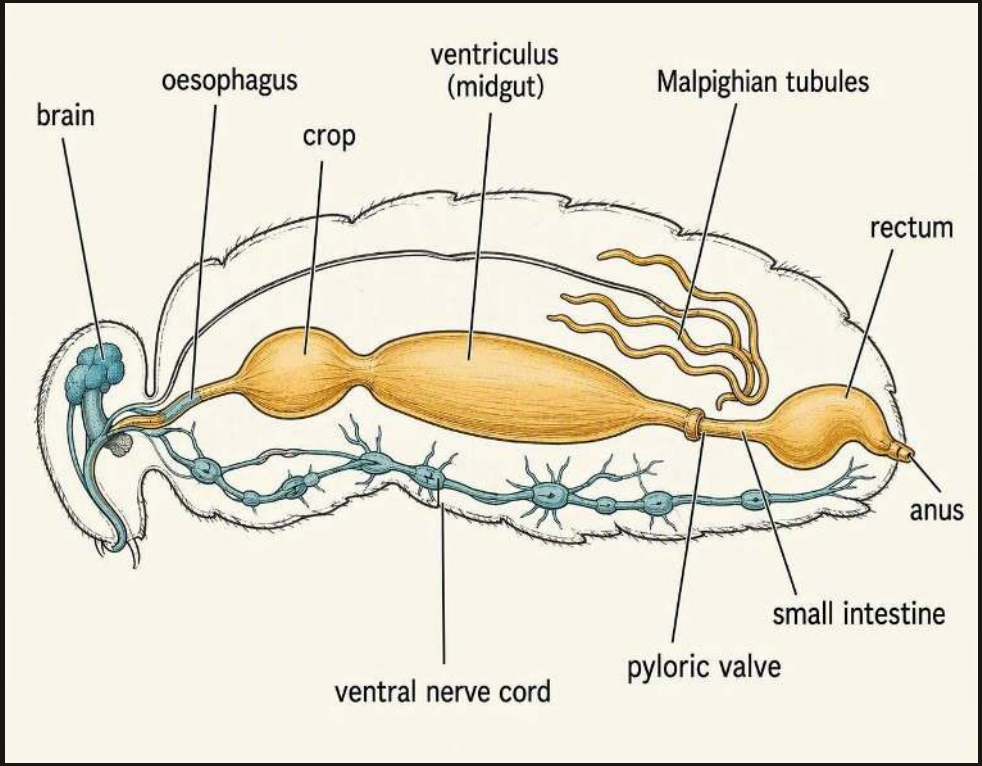
The diagram also identifies the Malpighian tubules and silk gland.

## Feeding and growth

After hatching, the larva relies on nurse-bee food and grows rapidly.

HONEY BEE BIOLOGY

# The adult alimentary system




## Anterior regions

The oesophagus leads towards the crop, ahead of the ventriculus.

## Posterior regions

The source labels the pyloric valve, small intestine, rectum and anus.

## Anatomical landmarks

The brain and ventral nerve cord help orient this side view.

Redrawn from supplied study notes; schematic, not to scale.

# Summary: reproduction and development

## 1 Reproductive anatomy

Paired ovaries of 150–180 ovarioles feed the oviducts; the spermatheca stores sperm for life.

## 2 Making eggs and sperm

Eggs form in ovarioles with nurse cells; haploid drones make sperm by a modified meiosis.

## 3 Inheritance

Queens and workers carry two gene sets and drones one; alleles pass on by Mendel’s rules.

## 4 The egg

A chorion and vitelline membrane enclose yolk and cytoplasm; sperm enter at the micropyle.

## 5 Embryo to larva

In about 3 days, cleavage, blastoderm and germ band form the body before hatching.

## 6 The gut

Foregut, midgut and hindgut form in the embryo; the midgut (ventriculus) fills the larva.

# Any questions?



HONEY BEE BIOLOGY

# Caste determination and larval food

How feeding and other factors decide whether a female larva becomes a queen or a worker, and how royal jelly differs from brood food



HONEY BEE BIOLOGY

# Queen or worker from the same egg

**Fertilised egg**

Diploid female with the same genes either way

## Queen

**Egg to adult**
16 days

**Ovarioles per ovary**
150–180

**Adult life**
Up to several years

**Specialised organs**
Large ovaries, spermatheca

## Worker

**Egg to adult**
21 days

**Ovarioles per ovary**
About 2–12

**Adult life**
About 6 weeks in summer

**Specialised organs**
Pollen baskets, wax glands

Values are typical for Apis mellifera; ovariole numbers and worker lifespan vary with colony and season.

HONEY BEE BIOLOGY

# Brood food and royal jelly

**Brood food** is any food that nurse bees, about 5–15 days old, prepare for larvae. **Royal jelly** is its richest form, fed to queen larvae.

## INGREDIENTS

**Hypopharyngeal glands**
Clear secretion rich in protein

**Mandibular glands**
White secretion: lipids, 10-HDA

**Honey and pollen**
Sugars; pollen for older larvae

## THREE KINDS OF BROOD FOOD

**Royal jelly**
Queen larvae, throughout larval life, in excess

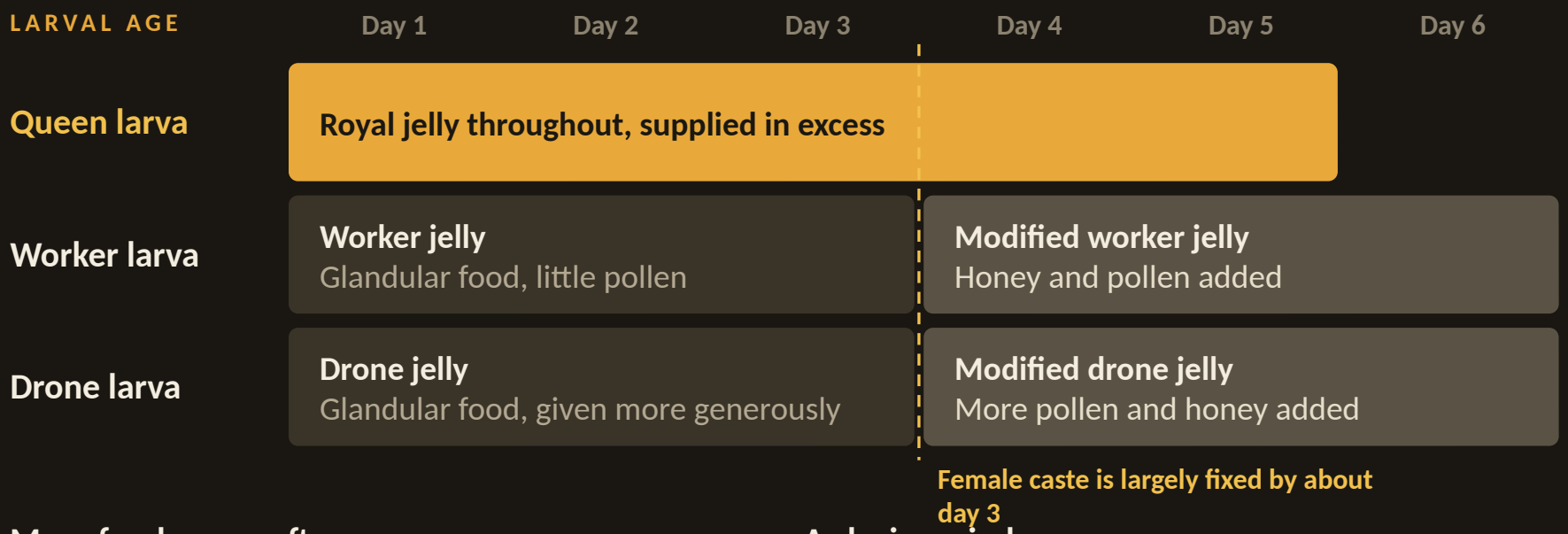
**Worker jelly**
Worker larvae; diet changes after about day 3

**Drone jelly**
Drone larvae; more pollen and honey in later days

10-HDA: 10-hydroxy-2-decenoic acid, a fatty acid characteristic of royal jelly.

HONEY BEE BIOLOGY

# How larval feeding diverges




## More food, more often

Nurses visit queen larvae far more often. The sealed queen cell still holds surplus jelly.

## A closing window

Larvae up to about 3 days old can still be reared as queens; older ones become intercastes.

Schematic; timings approximate. Feeding lasts about 5 days in queens, 6 in workers and 6–7 in drones, which come from unfertilised eggs.

HONEY BEE BIOLOGY

# Royal jelly versus worker and drone food

| | Royal jelly | Worker food | Drone food |
|---|---|---|---|
| **Fed to** | Queen larvae and queen | Worker larvae | Drone larvae |
| **Gland mix** | More mandibular | Mostly hypopharyngeal | Similar to worker food |
| **Sugar, days 1–3** | About 3–4× higher | Lower; rises later | Lower than royal jelly |
| **Pollen** | Almost none | Added after day 3 | More, after day 3 |
| **Amount** | Supplied in excess | Rationed | More than workers get |
| **Water and 10-HDA** | Less water, more 10-HDA | More water, less 10-HDA | Less studied |

Sugar is for larval food in days 1–3. Sources: Asencot & Lensky (1988); Wang et al. (2016); Bees for Development; Winston (1987).

HONEY BEE BIOLOGY

# What in the diet decides caste

No single ingredient makes a queen. The evidence points to the overall amount and balance of the diet.

SUPPORTED

### Sugar content

Adding sugar to worker jelly made larvae develop more queen-like traits.

SUPPORTED

### Quantity of food

In laboratory rearing, the amount of diet a larva eats shifts it towards queen or worker.

SUPPORTED

### What workers are given

p-Coumaric acid and microRNAs from pollen in worker food hold back ovary development.

DISPUTED

### A single “royal” protein

Royalactin (MRJP1) was proposed as the trigger in 2011; later tests could not reproduce this.

Sources: Asencot & Lensky (1988); Kaftanoglu et al., J. Insect Sci. (2011); Mao et al., PNAS (2015); Zhu et al., PLoS Genet. (2017); Kamakura, Nature (2011); Buttstedt et al., Nature (2016).

HONEY BEE BIOLOGY

# From diet to queen development

1

### Richer diet

More food and more sugar in the first 3 days of larval life.

2

### Nutrient sensing

Insulin and TOR signalling pathways respond to the extra nutrients.

3

### JH levels rise

Queen larvae carry more JH; applying JH to worker larvae induces queen traits.

4

### Genes switch

Gene expression and DNA methylation change; silencing Dnmt3 gives queen-like bees.

**Result:** large ovaries, faster development (16 days) and none of the worker's foraging organs

JH: juvenile hormone. TOR: target of rapamycin. Sources: Patel et al., PLoS ONE (2007); Kucharski et al., Science (2008).

HONEY BEE BIOLOGY

# The endocrine route to queen or worker

1

## Food sensed

Insulin and TOR signalling in the fat body respond to the larva's diet.

2

## Brain signals

Neurosecretory cells in the brain control the corpora allata.

3

## JH released

Queen larvae show a JH peak in the 4th and early 5th instars; workers stay low.

4

## Ovaries respond

High JH keeps ovarioles alive; with low JH most worker ovarioles die.

**Moulting:** ecdysteroids from the prothoracic glands time each moult; levels differ by caste.

JH: juvenile hormone. TOR: target of rapamycin. Sources: Rachinsky & Hartfelder (1990); Patel et al. (2007); Mutti et al. (2011).

HONEY BEE BIOLOGY

# Juvenile hormone in queen and worker larvae

| | Queen larva | Worker larva |
|---|---|---|
| **JH level, instars 4–5** | High peak | Stays low |
| **Corpora allata** | Very active | Less active |
| **JH breakdown** | Slower, less JH esterase | Faster |
| **Ovarioles at emergence** | 150–180 per ovary survive | Most die; about 2–12 remain |
| **Adding JH** | Not needed | Larvae develop queen-like traits |
| **Adult JH** | Stays low for life | Low as nurses, rises in foragers |

JH: juvenile hormone. Sources: Wirtz (1973); Rachinsky & Hartfelder (1990); Schmidt Capella & Hartfelder (1998); Bomtorin et al. (2014).

HONEY BEE BIOLOGY

# Other factors in caste determination



**Fertilisation**

Only fertilised eggs can become queens or workers; unfertilised eggs give drones.



**Larval age**

Queen cells are started over larvae under about 3 days old; the youngest make the best queens.



**Cell type**

Large, vertical queen cells leave room for a pool of jelly and a bigger body.



**Colony signals**

Queen loss, swarming or a failing queen trigger queen rearing; queen pheromone suppresses it.



**Food income**

Pollen and nectar supply limit how much jelly nurses make, and so queen size and quality.

HONEY BEE BIOLOGY

# Summary

1 **Diet decides caste**

A fertilised egg can become either caste; food in the first 3 larval days sets the path.

2 **Royal jelly is a special brood food**

It has more mandibular secretion and sugar, little pollen, and is given in excess throughout.

3 **Balance matters more than one ingredient**

Sugar, quantity and pollen compounds all play a part; the royalactin claim is disputed.

4 **Diet acts through hormones and genes**

Nutrient signalling raises juvenile hormone and changes gene expression and DNA methylation.

# Any questions?

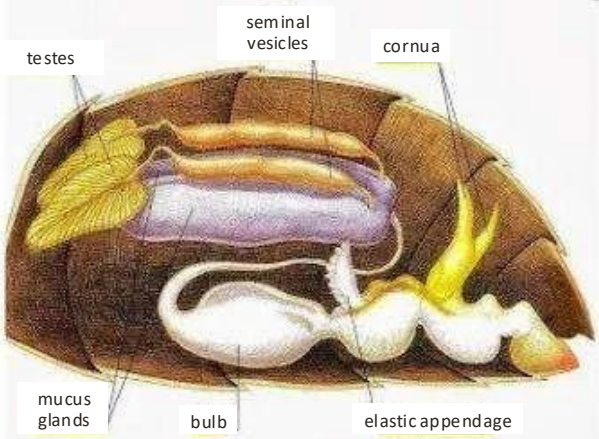


HONEY BEE BIOLOGY

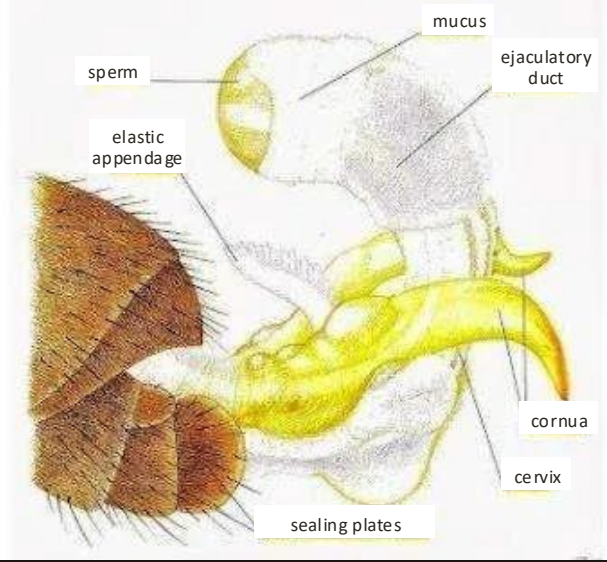
# The reproductive system of the drone

*Anatomy, sperm production and the mechanics of mating in Apis mellifera*

Alan Baxter · Education Officer, FDBKA




***Drone endophallus***



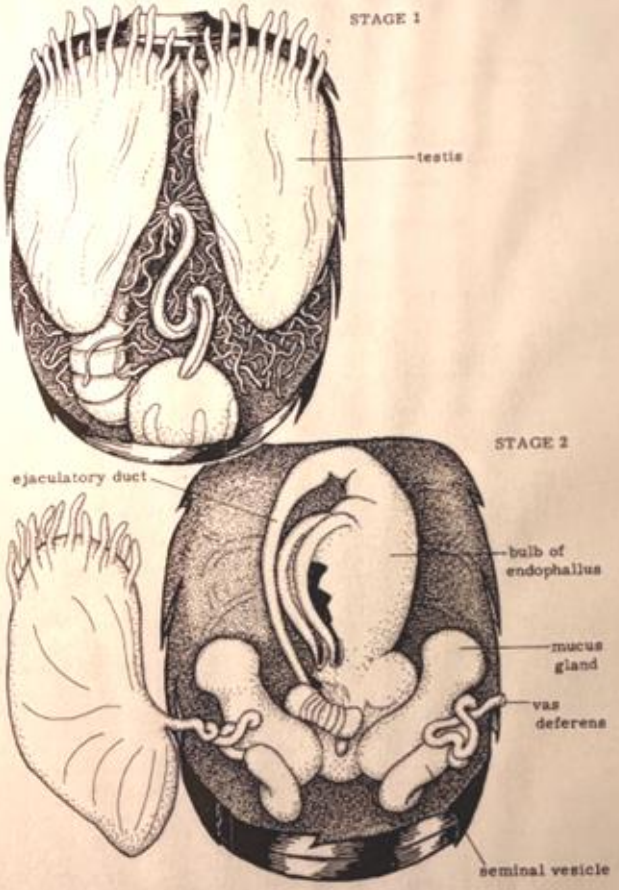



PLATE 14. Dissection of immature (newly emerged) drone. Stage 1, viscera undisturbed. Stage 2, testes laid out and alimentary Canal removed to expose the complete reproductive apparatus.

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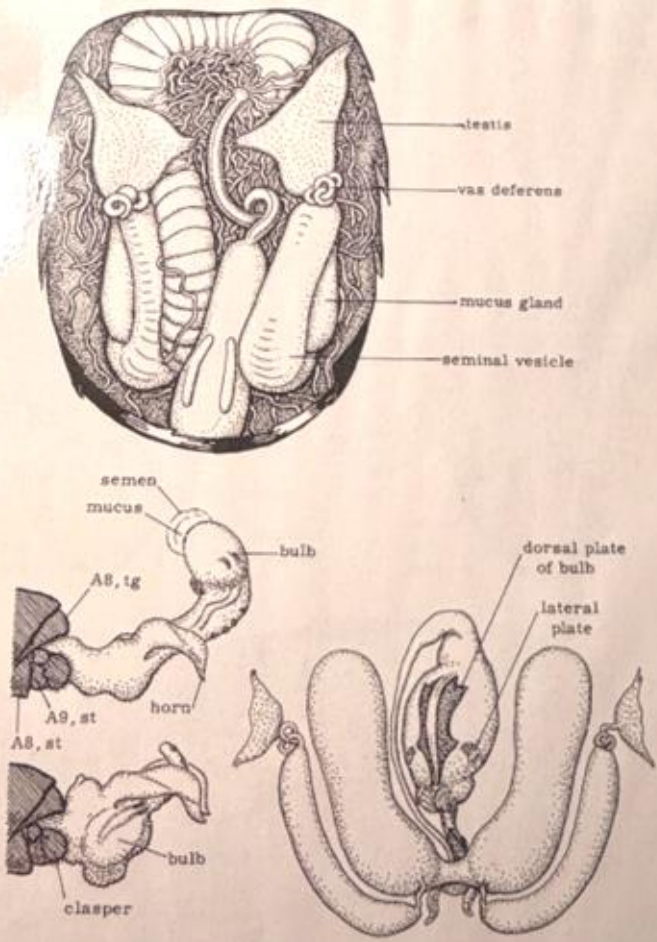



PLATE 15. Dissection of maturing drone, viscera undisturbed. Below, right: the reproductive apparatus removed and laid out. Below, left: two stages in induced eversion of the endophallus.

# Six key reproductive structures form the reproductive tract

**A** Testis

**B** Ejaculatory duct

**C** Vas deferens

**D** Seminal vesicle

**E** Mucus gland

**F** Endophallus

A–F anatomy key

PRODUCTION & TRANSPORT

# Testes make sperm; ducts move it into storage

## 01 TESTES

- Very large in newly emerged drones, then shrink into two smaller triangular organs by about day 12.
- Produce spermatozoa, which pass rapidly towards storage.

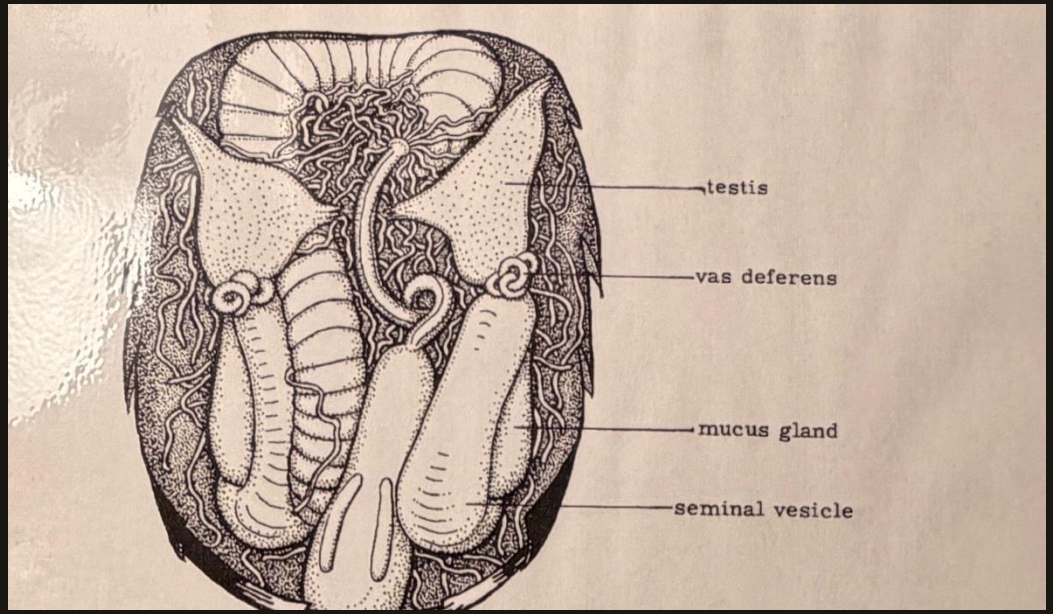
## 02 VASA DEFERENTIA

- Short, coiled muscular tubes connecting the testes to the seminal vesicles.
- Transport sperm to the seminal vesicles for storage.

Production → Transport → Storage

STORAGE & SECRETION

# Seminal vesicles and mucus glands




## Seminal vesicles

- Enlarged sections of the two vasa deferentia
- Receive sperm from the testes on days 3–8
- Sperm and seminal fluid keep maturing here (~days 6–14)
- Contribute proteins to the ejaculate

## Mucus glands

- Large paired glands whose bases unite in the ejaculatory duct
- Fill with mucus during maturation; their protein pattern is ecdysteroid-controlled
- At ejaculation the mucus drives semen out ahead of it
- Coagulates to form the bulk of the mating sign

TRANSFER

# The ejaculatory duct combines sperm and mucus

SEMEN + MUCUS → EJACULATORY DUCT

## A single muscular route

Connects the seminal vesicles and mucus glands with the bulb of the endophallus.

## Its job at mating

Transports semen and mucus to the endophallus for deployment during mating.

**Seminal vesicles + mucus glands → duct → bulb**

DELIVERY

# The endophallus is a four-part delivery system

| | | |
|---|---|---|
| 01 | BULB | Large extension of the ejaculatory duct with cuticular plates. |
| 02 | CERVIX | Narrow section leading from the bulb. |
| 03 | VESTIBULE | Chamber bearing the paired horn-like cornua. |
| 04 | PHALLOTREME | Opening to the exterior. |

AT MATING

## It everts to deliver sperm to the queen.

**One structure. One deployment.**

TRANSFER

# The endophallus — an inside-out penis



Partially everted



Fully everted



Partially everted endophallus showing the vestibule and two horns

MATING

# Eversion: a one-shot mechanism



**1 Pressure builds**

Mounted on the queen in flight, the drone contracts his abdomen; haemolymph pressure and inflating air sacs push the endophallus outwards.



**2 Partial eversion**

Vestibulum and cornua turn out first. The cornua anchor the drone as his genitalia enter the queen's open sting chamber.



**3 Full eversion**

The bulb is forced through the cervix. Semen is ejaculated into the queen's oviducts, pushed from behind by mucus.



**4 Separation**

The paralysed drone falls backwards; the endophallus tears. Bulb and mucus stay in the queen as the mating sign. He dies soon after.

MATING

# The mating sign



*Queen's abdomen after mating*

## What it is made of

- **White mucus**
  from the mucus glands — the bulk of the sign
- **Chitinous plates**
  of the bulb, left behind as the endophallus tears
- **Orange cornual secretion**
  sticky layer from the cornua gland cells
- **Transparent membrane**
  torn mucus-gland epithelium at the distal end

### Not a plug

The next drone removes it easily, and drones prefer to mate with queens bearing one — so it is now read as a signal, not a barrier. Orange membranes on a sign can come from a later drone that failed to dislodge it.

HONEY BEE BIOLOGY

# Sperm production in the drone

1 **Development in the testes**

Germ cells in the testes form sperm during the drone’s immature development.

2 **A modified meiotic process**

Drones are haploid. Their sperm formation avoids halving the chromosome set again.

3 **Maturation and storage**

Sperm move along the vasa deferentia to the seminal vesicles as the young adult matures.

4 **Transfer to the queen**

At mating, the drone transfers semen. The queen stores a small fraction of the sperm in her spermatheca.

PRODUCTION

# Testes: every sperm made before adulthood



Spermatogonia Spermatocytes Spermatids Spermatozoa

*Apical end → basal end: maturation runs along every testiolar tubule towards the vas deferens (teal)*

**200+**

long testiolar tubules make up the testes

**Pre-adult**

meiosis starts when the cell is capped; spermatids appear by the red-eyed pupa

**~Day 14**

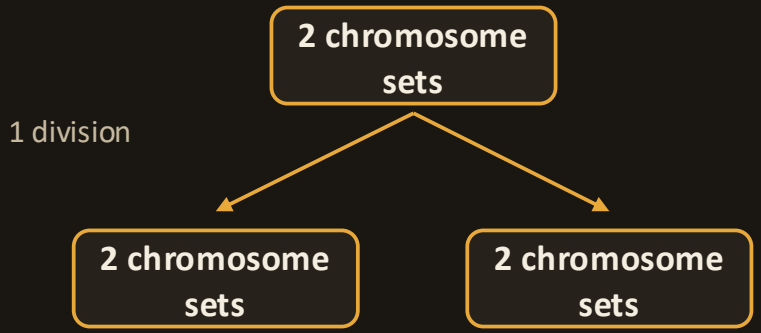
the adult testes have shrunk and sperm production has all but stopped

CELL DIVISION

# Mitosis and meiosis

## Mitosis

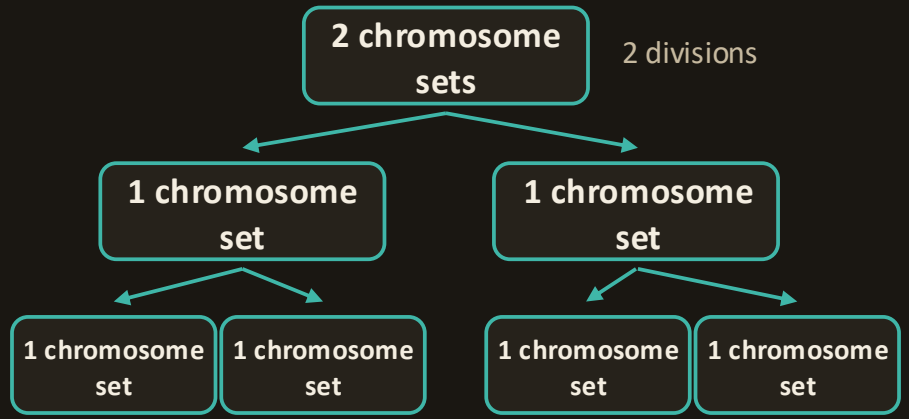
Makes matching cells for growth and repair




**2 matching cells**
**Same chromosome number**

## Meiosis

Helps make eggs or sperm for reproduction




**4 cells with half the chromosomes**

Honey bee drones are different: they already have one set and use modified meiosis.

PRODUCTION

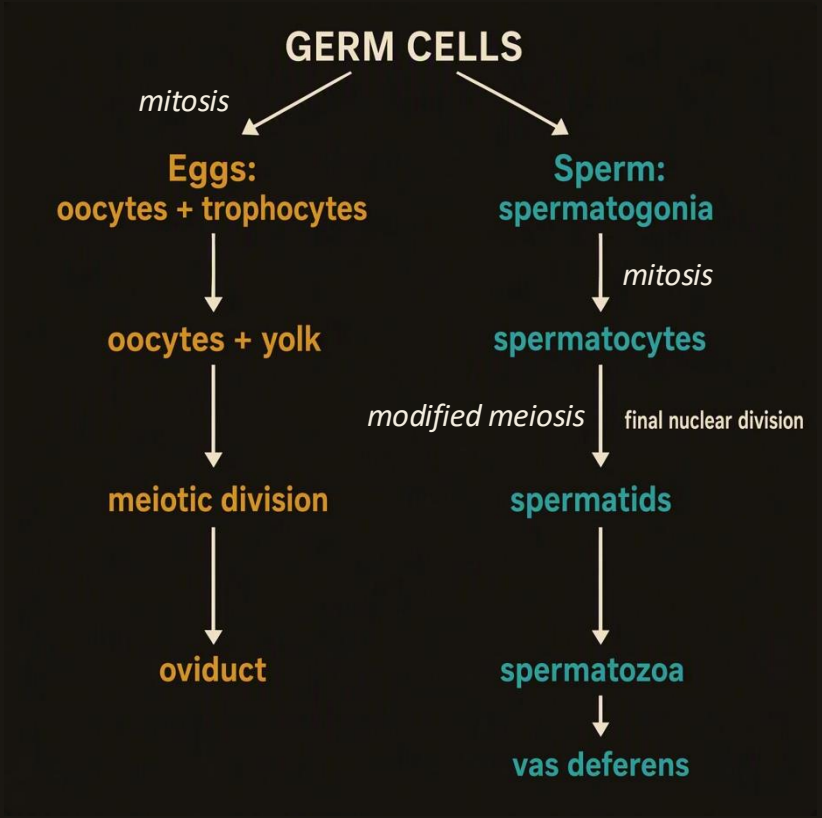
# Egg and sperm development

## Mitosis

Germ cells multiply. Each new cell keeps the same number of chromosomes.

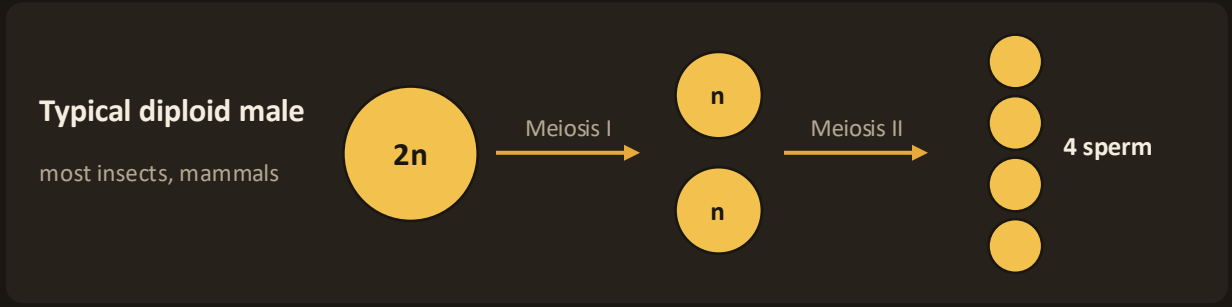
## Meiosis

Halves the chromosome number in eggs. Drones are already haploid, so their sperm form by a modified meiosis that keeps the single set.



PRODUCTION

# Meiosis in a haploid male




**n** = one set of chromosomes (16 in honey bees)  **2n** = two sets

## No reduction

The first division fails: only a small bud of cytoplasm is shed, so the single chromosome set is kept intact.

## Two cells, not four

The second division splits cytoplasm unequally — one full spermatid and one small one, whose fate is still debated.

## Clonal sperm

Every sperm a drone carries is genetically identical: a copy of the queen's egg that made him.

GENETICS

# Haploid drone development

Arrhenotokous parthenogenesis in Apis mellifera

### 1 Diploid queen

**2n = 32**

chromosomes

Meiosis reduces the chromosome number from 32 to 16.

### 2 Unfertilised egg

**n = 16**

chromosomes

Without fertilisation, the egg receives no paternal chromosome set.

### 3 Haploid drone

**n = 16**

chromosomes

Mitosis and development preserve the haploid chromosome number.

Arrhenotoky: male development from an unfertilised egg.

GENETICS

# Diploid drone development

Sex is set by one gene, complementary sex determiner (csd): two different alleles make a female, two matching alleles make a male

### 1 Fertilised egg

**2n = 32**

chromosomes

The queen and her mate share a csd allele, so the egg receives two matching copies.

### 2 Diploid male larva

**2n = 32**

chromosomes

Matching alleles switch on male development, even though the egg was fertilised.

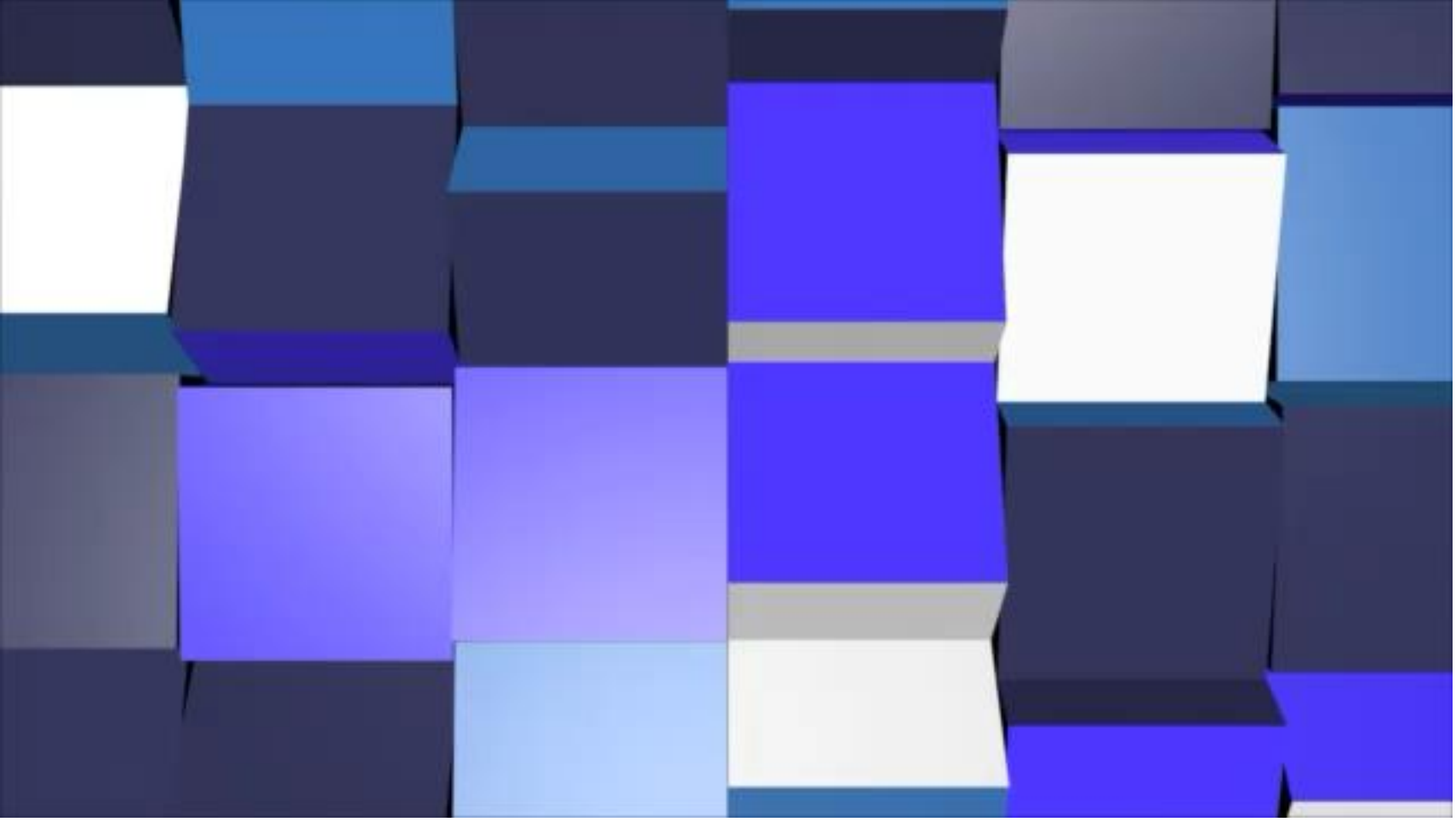
### 3 Removed by workers

**~6 hours**

after hatching

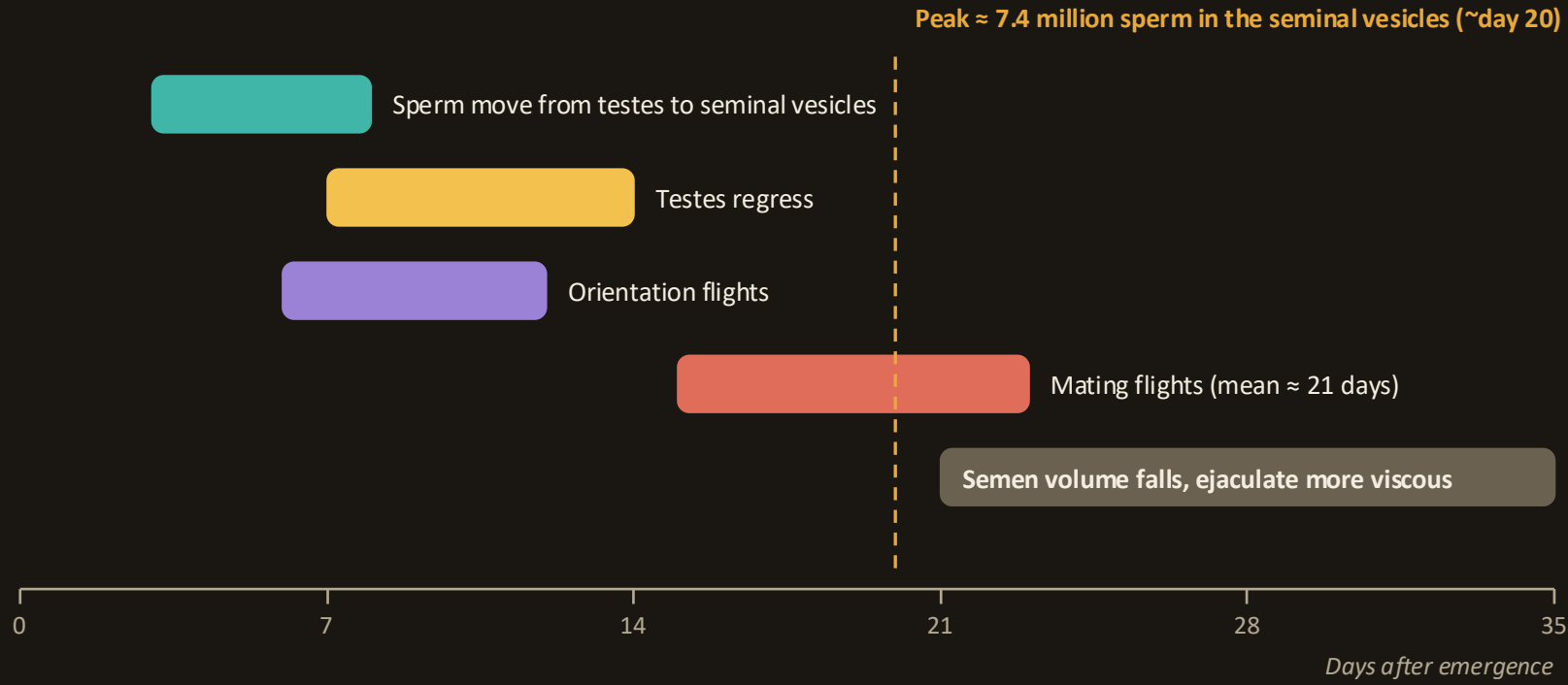
Nurse bees detect the larva and eat it, leaving an empty cell in the brood.

Each drone sharing a csd allele with the queen turns half the eggs he fertilises into diploid males. Inbreeding raises the odds and shows as spotty brood.



MATURATION

# From emergence to sexual maturity




Day 0: the drone emerges with empty seminal vesicles and cannot mate for roughly 9–12 days.

OUTCOME

# From drone to spermatheca

**3.6–12 M** sperm in one drone's ejaculate (0.9–1.7 µl); about 7 M is typical

**10–20** drones mate with one queen over one or a few flights

**≈ 5–7 M** sperm finally stored in her spermatheca — a few percent of what she received

*Only around 2.5% of all sperm a queen receives reaches the spermatheca by one estimate; the rest is expelled.*

## Viability on the way in

**98%**

live sperm in the drone's seminal vesicles

**89%**

still alive in the queen's lateral oviducts

FOR THE BEEKEEPER

# What shapes drone fertility

### Age

Sperm stores peak at about three weeks; older drones produce less, more viscous semen.

### Body size

Drones reared in worker cells are ~13% smaller (wing length) and carry fewer sperm.

### Brood temperature

Drones reared at 32 °C had larger testes, seminal vesicles and mucus glands than at 35 °C.

### Nutrition & season

Drones are reared when resources are plentiful; heavier drones matured by day 15 and lived longer.

### Miticides & pesticides

In-hive miticides and some agrochemicals have been linked to reduced sperm viability.

### Pathogens

Viruses including DWV and CBPV occur in semen — a venereal route into queens.

SUMMARY

# Key points



1. **Drones are haploid.** They develop from unfertilised eggs and carry one chromosome set (n = 16).
2. **All sperm are made before emergence.** Modified meiosis keeps the single set, so every sperm is identical.
3. **Testes make, ducts move, vesicles store.** Sperm pass along the vasa deferentia to mature in the seminal vesicles.
4. **Maturity takes 9–12 days.** Sperm stores peak at about 7 million around day 20.
5. **Mating is a one-shot event.** Mucus drives semen out as the endophallus everts; the drone dies, leaving the mating sign.
6. **Rearing shapes fertility.** Age, size, brood temperature, nutrition, chemicals and pathogens all affect sperm.

SUMMARY

# References




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**Woyke (1963)** J. Apic. Res. — diploid drone larvae removed by workers

**Hoage & Kessel (1968)** J. Ultrastruct. Res. — drone spermatogenesis

**Beye et al. (2003)** Cell — the csd sex-determination gene

**Woyke (2008)** Apidologie 39 — partial eversion

**Woyke (2010)** Apidologie 41 — three ejected substances

**Rangel & Fisher (2019)** Apidologie — drone reproductive health review

**Metz & Tarpy (2019)** Insects — reproductive senescence

**Apidologie (2020)** — testis development atlas

**PLoS Genetics (2023)** — honey bee spermatogenesis roadmap

MODULE 5 HONEY BEE BIOLOGY

# Honey bee metamorphosis

Larva, pro-pupa, pupa and imago



Scientific illustrations, not to scale

# The developmental sequence

Complete metamorphosis in the worker honey bee

01

## Egg

Embryonic development

02

## Larva

Feeding and growth through five instars

03

## Pro-pupa

Straightened, non-feeding larva

04

## Pupa

Adult body form becomes visible

05

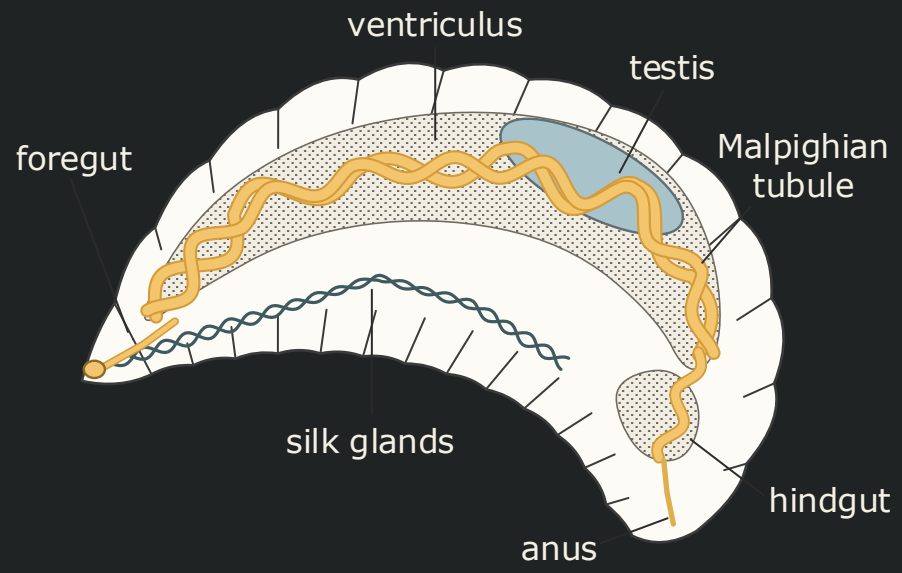
## Imago

Adult bee leaves the brood cell

**About 21 days**

Egg to adult emergence in a worker. Timing varies with caste and conditions.

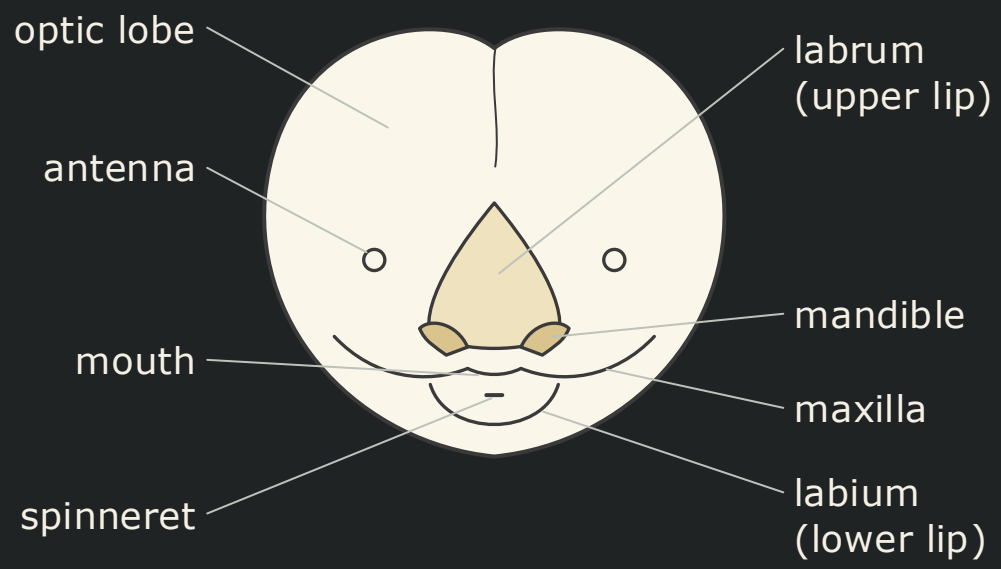
# Larval internal anatomy




Redrawn from the supplied diagram; schematic, not to scale.

Divided into a head and 13 segments:

3 x thoracic and 10 x abdominal




Although visible in the larva, the various ‘adult’ mouthparts are not functional

# Larval development

**Moulting** the larva sheds its skin 4 times and starts to spin a cocoon using silk from the silk glands.

**Digestive system** most important as the larva needs to eat and grow.

Mid gut and hind gut not connected at this stage. Rudimentary mouth parts not developed only to suck in semi liquid food

**Excretory system** only 4 Malpighian tubules that thicken as they fill with waste. Connection to the intestine is closed

**Respiratory system** clearly segmented with 10 pairs of spiracles permanently open and connect to one of two lateral tracheal tubes

**Nervous system** has same segmental structure. Brain and sub oesophageal ganglion already formed. Ganglia from A8,9,10 fused but otherwise each segment has its own ganglion and functions independently

**Circulatory system** heart ends in A9 and leads to T2. Has 11 chambers and 10 ostia. Short aorta in inverted U open at the bottom. Dorsal and ventral diaphragms present but break down in older larvae

**Fat body** creamy white colour and fills most of the space. Contains oenocytes, fat cells and urate cells to store waste.

**Silk glands** long coiled structures lying near the ventral surface and opening at the spinneret.

**In queen & worker** ovaries begin to develop, in queen >2mm long. **Drones** have larger testes. All lie between A4-A6

Two days after cell capping the larva undergoes a partial 5th moult and becomes a pro-pupa.

# Cocoon spinning

## Silk glands

Paired labial glands run the length of the larva and open at the spinneret below the mouth.

## What starts spinning

When feeding ends, juvenile hormone falls and ecdysone from the prothoracic glands rises.

## Lining the cell

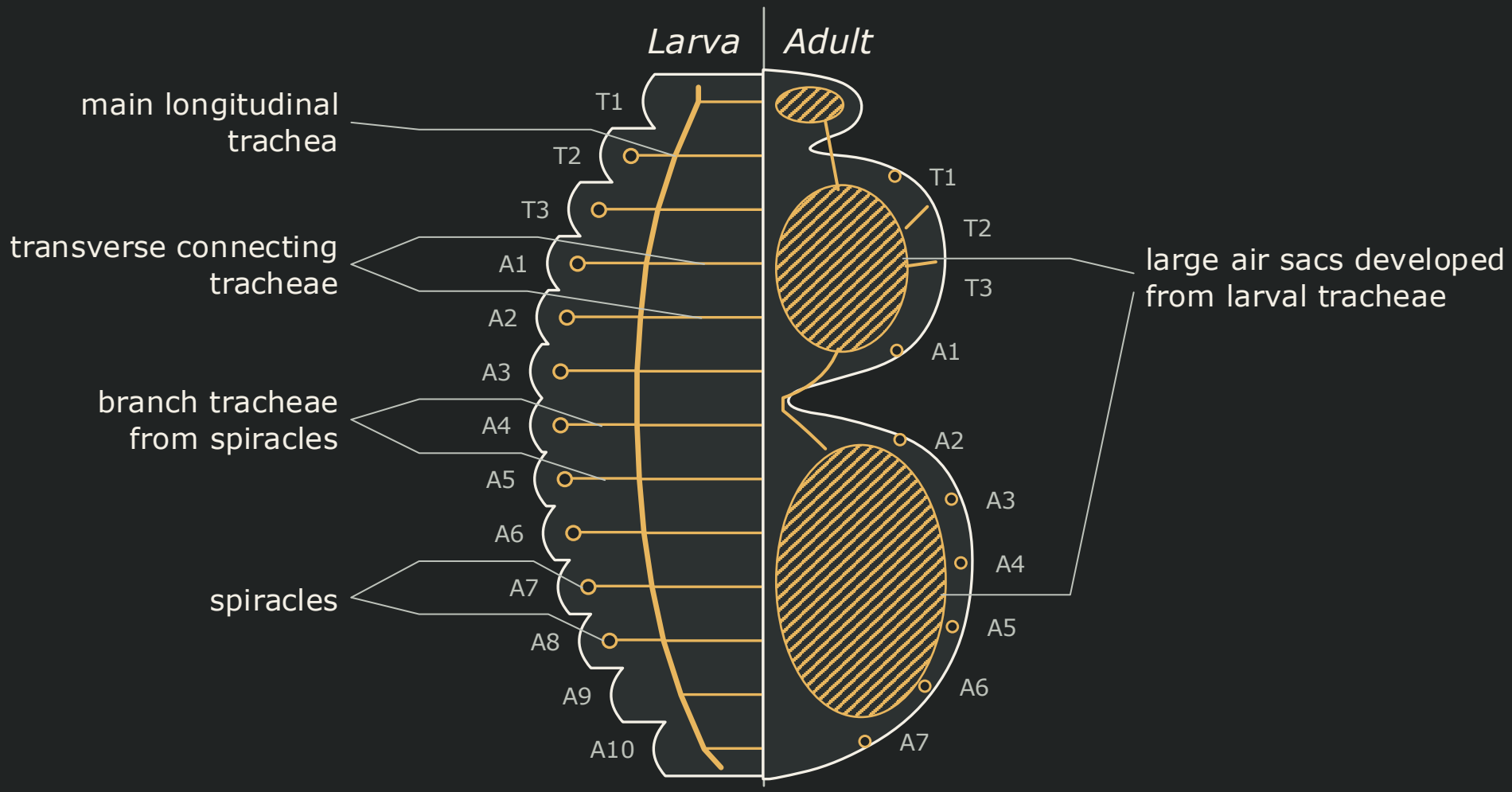
Head and body movements lay silk against the cell walls, enclosing the developing bee.

Workers make the wax capping. The larva spins the silk cocoon inside it.

## What the silk is made of

- **Protein:** four small fibroins (AmelF1–F4), each about 30–35 kDa
- **Rich in alanine**, with serine, glutamic acid and leucine
- **Coiled coils:** α-helical, unlike the β-sheet silk of silkworms and spiders
- **No sericin** gum coat
- Each generation's silk and faeces add a new lining to the cell

# Respiratory system of the larva and adult






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# Apolysis and ecdysis

Two distinct events within a moult

## APOLYSIS

Process of moulting initiated by secretion of ecdysone by prothoracic glands and reduction of JH in haemolymph

## ECDYSIS

The insect sheds the old cuticle and the new form emerges

## Between these events

A new cuticle forms beneath the old one. Moulting fluid digests part of the old inner cuticle.

There are **6 moults** before emerging as an adult

# Larva in its cell



- Larva stretched out in its cell
- Segments clearly visible

# Development of the pro-pupa

Stretched out on back with head near cell cap

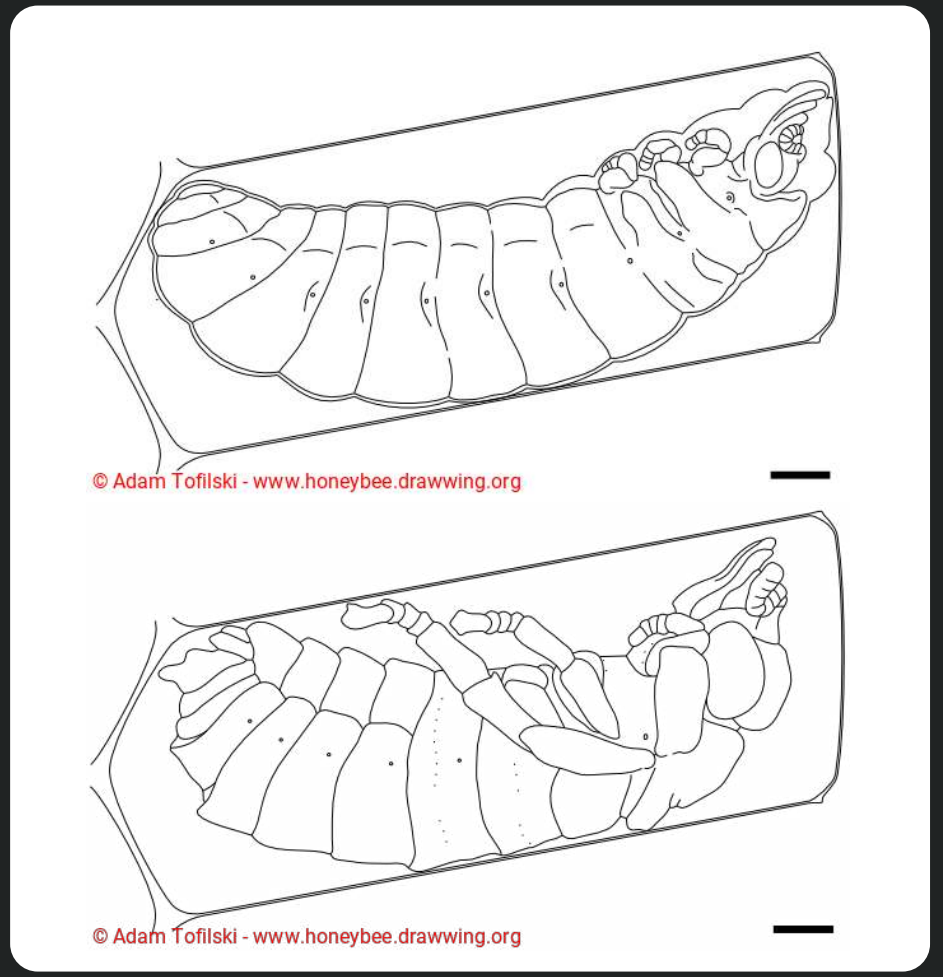
Connection between proctodaeum and ventriculus made

**Contents of Malpighian tubules and ventriculus voided into cell**

New cuticle, protected by the old larval skin, remains soft and pliable

## External features formed

- Head and mouthparts
- Compound eyes
- Thoracic segments change shape
- Petiole forms
- Rudimentary wings and legs appear
- Sting begins to form
- Ventriculus reduces in size





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# Larval defaecation

## The feeding larva retains its faeces

Its midgut initially has no open connection to the hindgut. Waste remains separate from the food in the cell.

### 01 Feeding ends

The mature larva stops taking food as it prepares for metamorphosis.

### 02 The gut connects

The midgut and hindgut form a continuous passage. The larva voids the retained faeces.

Defaecation occurs near the end of larval life, before cocoon completion.

# Development of the pupa

## Internal features

- Alimentary tract and reproductive systems develop
- Reproductive system takes adult form
- Drone testes shrink
- Drone sperm transferred to seminal vesicle

## External features

- Cuticle hardens
- External hairs grow
- Wings develop
- Exoskeleton colour changes from white to brown
- Eyes change from pink to purple





# The imago and cell emergence

## Adult ecdysis

The bee sheds the pupal cuticle. Its adult appendages become functional.

## Cell emergence

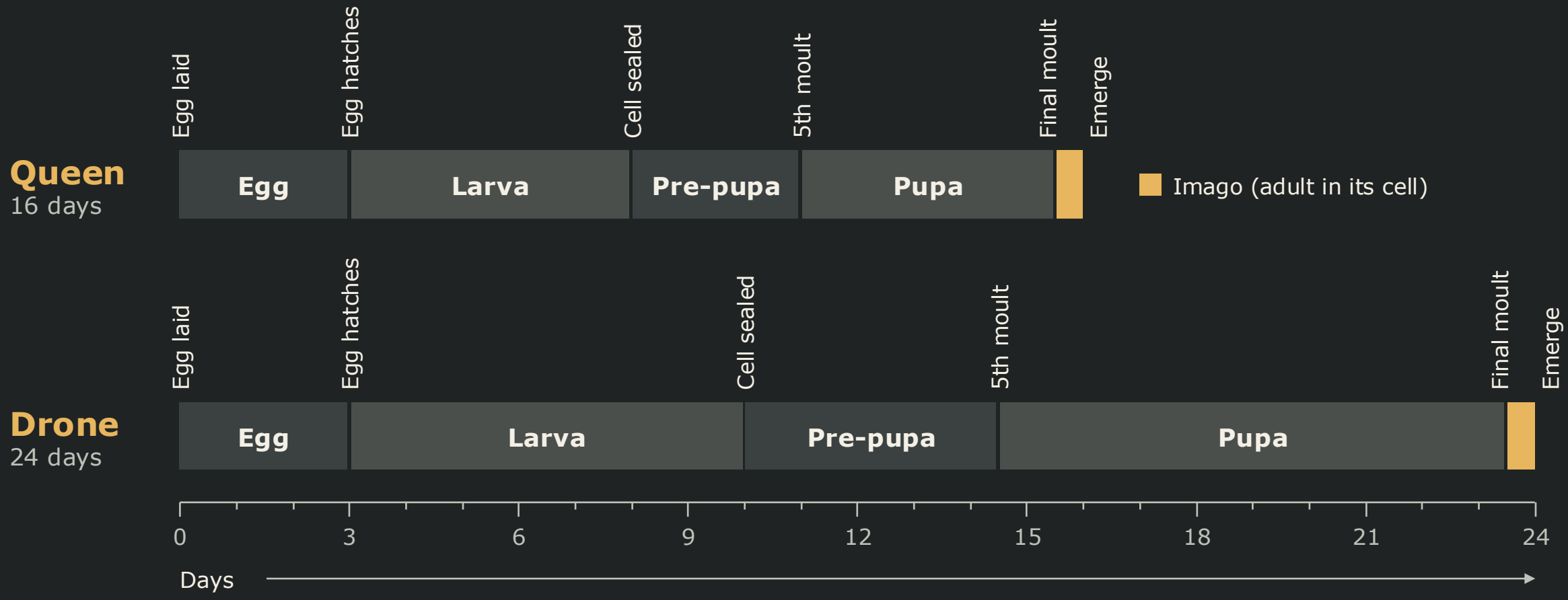
The worker chews through the capping and climbs out as a pale, soft, hairy adult.

**Imago means adult. A newly emerged worker is a callow bee.**



Adult worker, schematic illustration

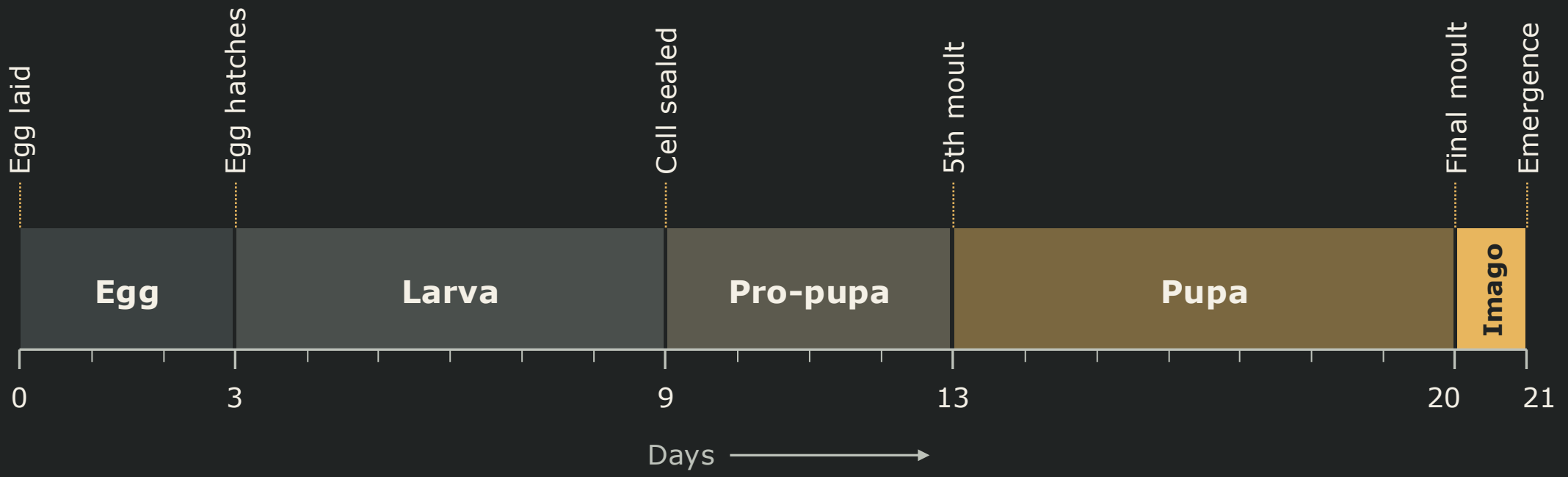
# Development of the queen and drone






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# Development and metamorphosis of the worker honey bee





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# External anatomy of the pro-pupa

Also called the prepupa

## Head

Small larval head directed towards the cell capping.

## Body outline

Pale, elongated and segmented. No narrow adult waist.



Pro-pupa: a non-feeding phase of the final larval instar

## Surface

Larval cuticle remains. Spiracles open along the sides.

## Appendages

No free adult legs or wings yet. Pupal structures develop beneath.

# The change to a pupa

## Pupal ecdysis

The bee sheds its last larval cuticle, revealing a soft, white pupa.



## The adult body plan appears

Head, thorax and abdomen become distinct. Antennae, legs and wing sheaths are visible.

Exarate pupa: appendages lie free of the body rather than fused to it.

# Pupal development beneath the cap



Pigmentation helps distinguish developmental stages

## Visible colour changes

Eyes darken through pink and purple. Body pigmentation develops later.

## Internal remodelling

Larval tissues reorganise and adult structures mature, using stored reserves.

Pharate adult: the adult develops beneath the pupal cuticle before the final ecdysis.

# Lesson summary

**01**

## Metamorphosis

Egg, larva, pro-pupa, pupa and imago. About 21 days from egg to adult worker.

**02**

## Moulting

Apolysis: ecdysone rises, JH falls and a new cuticle forms. Ecdysis: the old cuticle is shed. 6 moults in all.

**03**

## The larva

A head and 13 segments. It feeds, grows and moults 4 times. Midgut and hindgut are not yet connected.

**04**

## The pro-pupa

The cell is sealed around day 9. The larva spins its cocoon, its gut connects and waste is voided.

**05**

## The pupa

The adult body form appears. Eyes darken from pink to purple and the cuticle hardens.

**06**

## The imago

After the final moult, the adult worker chews through the capping on day 21.



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