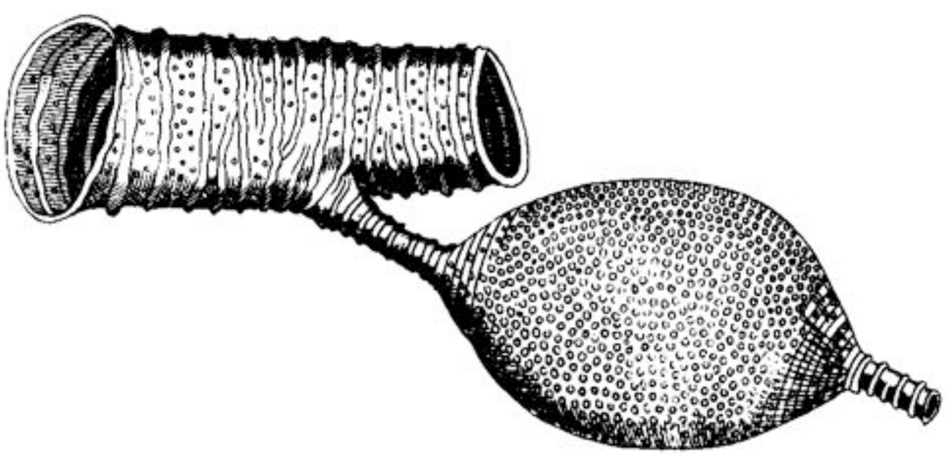
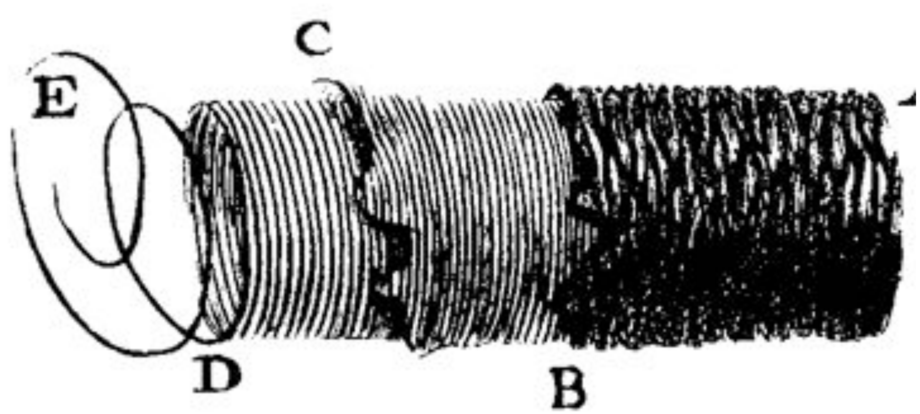
# RESPIRATORY OR VENTILATORY SYSTEM

Based on new evidence, the old tenant that insects don't breathe has been discounted

Swammerdam (1737)



Lyonet (1760)

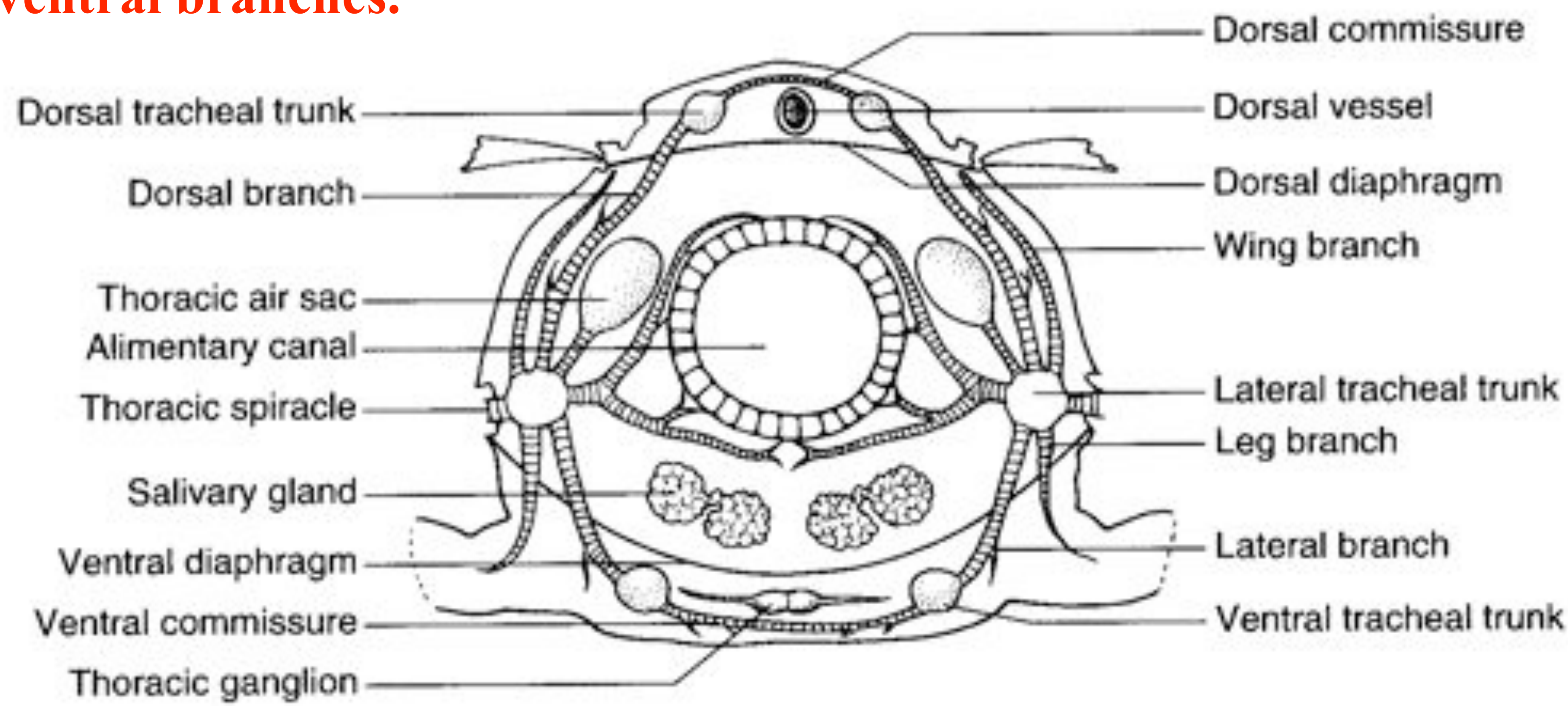


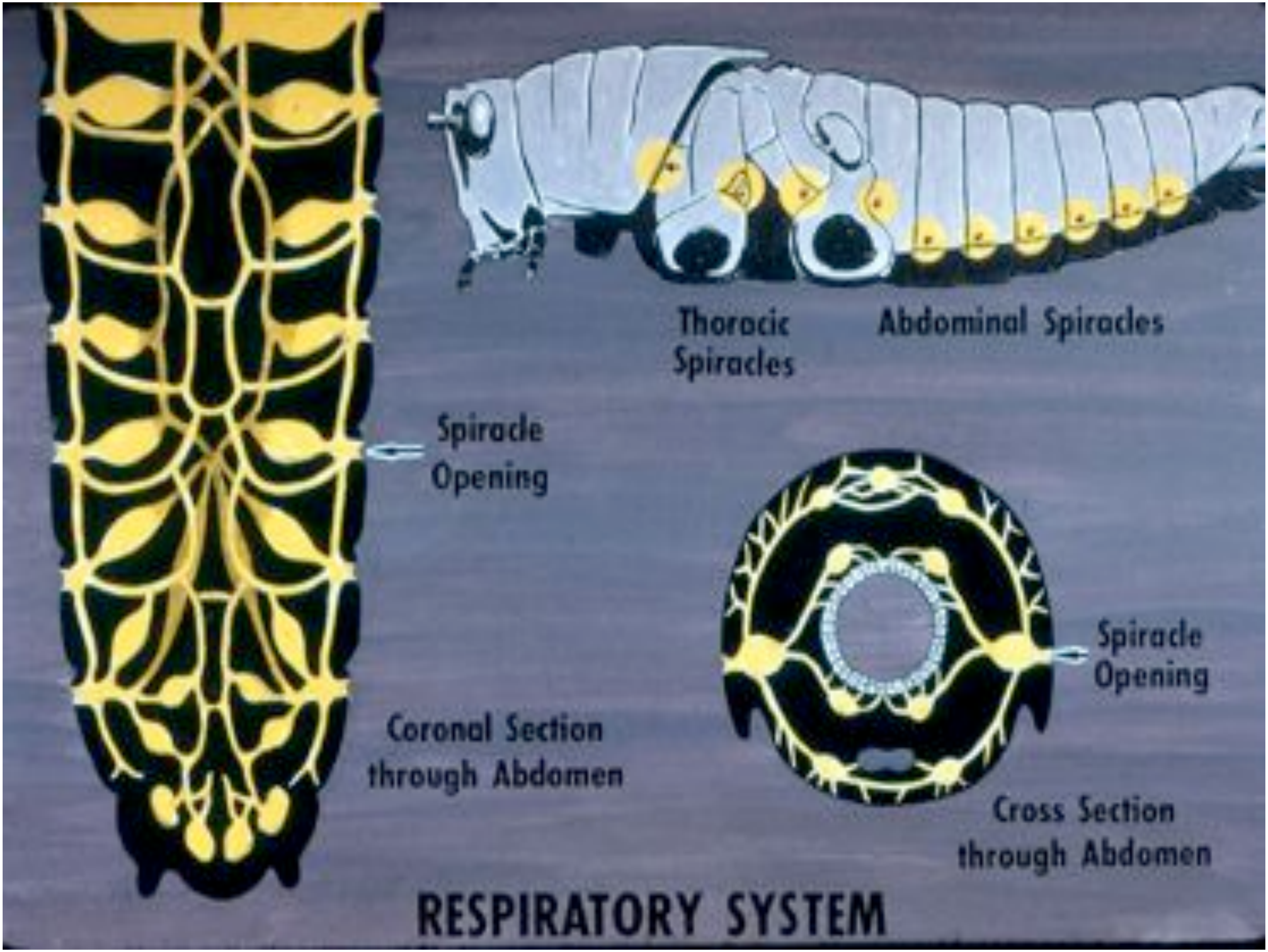

Respiratory System

# Functions of the respiratory system:

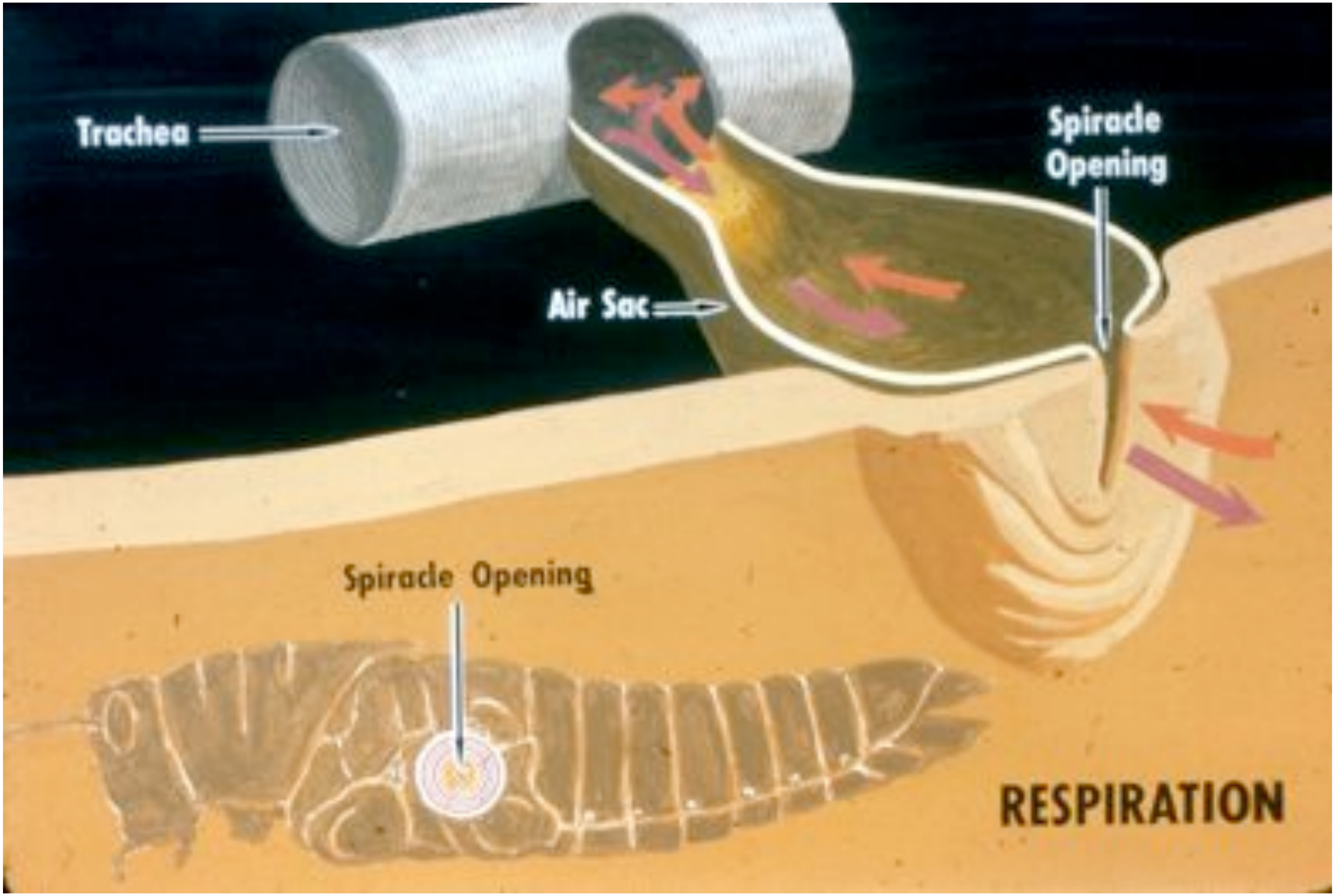
1. Provide the cells and tissues with oxygen
2. To eliminate carbon dioxide a product of cellular respiration
3. To work in conjunction with the circulatory system in providing oxygen to the flight muscle system

Cross-section diagram showing the air intake through the spiracles and the extensive tubular system referred to as the tracheal system. Note that the tracheae service all partitions of the insect and that all insects have expandable areas of the trachea known as air sacs. These are important for ventilatory movements and for reducing the specific gravity of the insect for flight. **System divides into dorsal, visceral and ventral branches.**




Thoracic Spiracles
Abdominal Spiracles
Spiracle Opening
Coronal Section through Abdomen
Spiracle Opening
Cross Section through Abdomen
RESPIRATORY SYSTEM

In the basic insect model, spiracles are found on all of the abdominal segments and each of the thoracic segments. Usually, however there are 10 pairs, 2 on the meso and metathoracic segments and 8 on the abdominal segments.



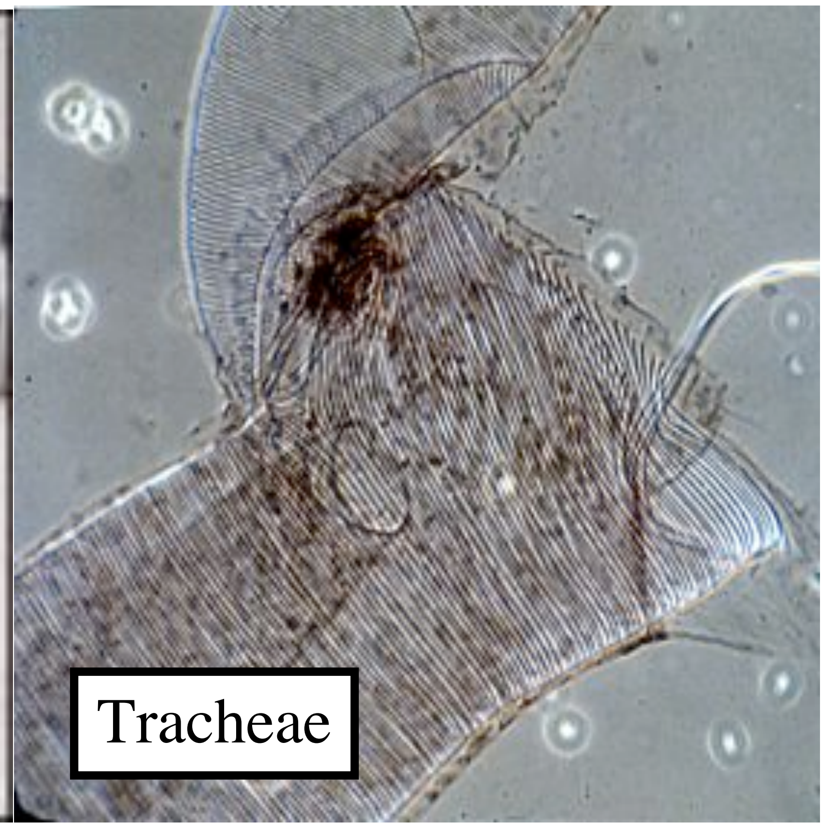
**Why are they not generally found on the prothorax?**

**In some insects, however, the 1st spiracle is on the prothoracic segment but is mesothoracic in origin**

**Most Collembola and Protura have no tracheae at all**

# WHAT DESIGN DO WE USE THAT IS SIMILAR TO THE TRACHEAL TUBE?

- SHOW DEMO

# WHAT LIMITATIONS EXIST ON ANIMALS BECOMING TOO LARGE?

1. SUPPORT OF BODY BY
   A. SKELETON
   B. MUSCLE
2. WATER DIFFUSION
3. **OXYGEN DIFFUSION – REMEMBER THE T-TUBULAR SYSTEM CREATED BY THE TRACHEOLES**

All animals require oxygen to extract energy from their food

and

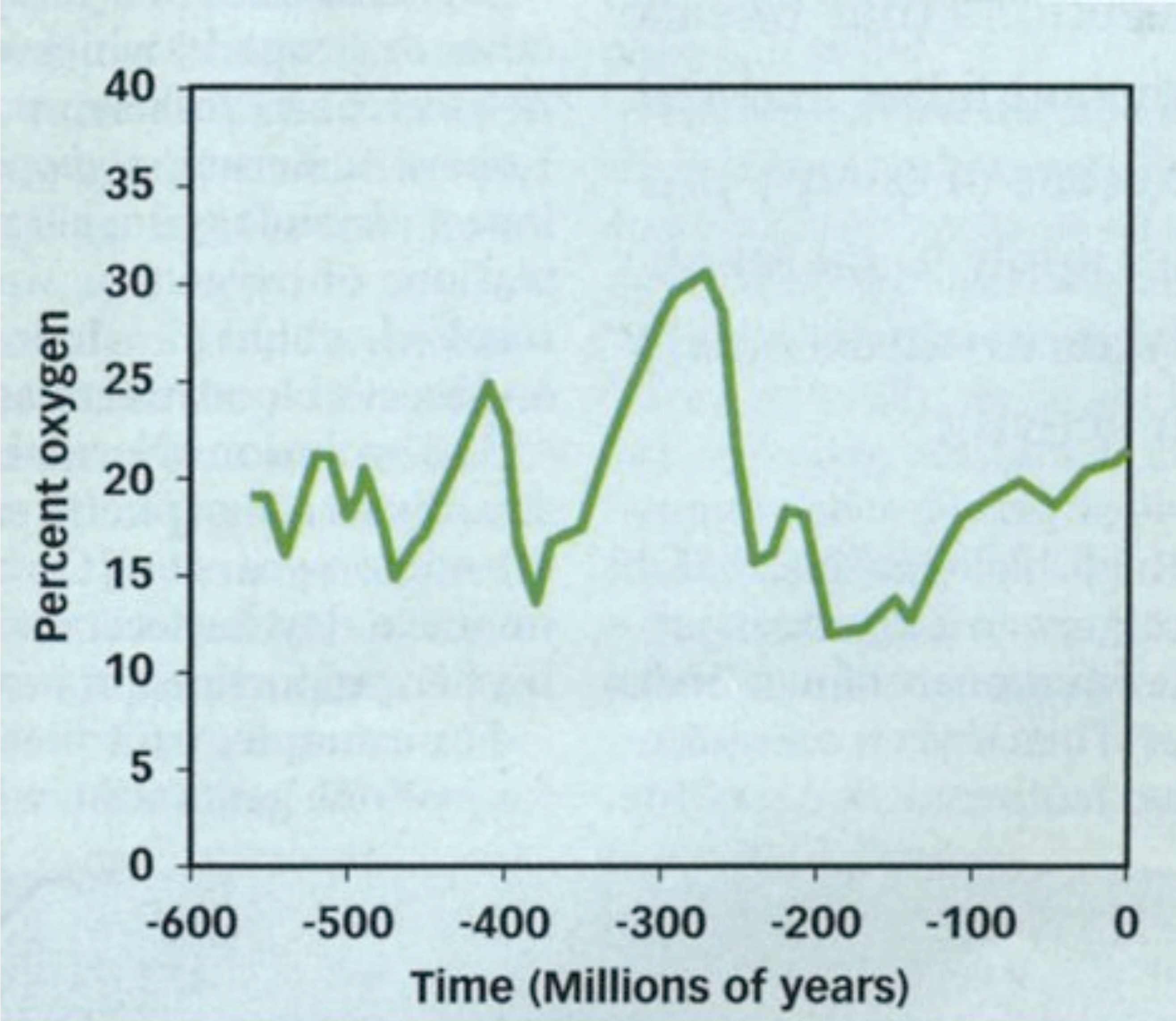
To fuel other activities such as locomotion or flight

**GOSSAMER GIANT** — Immense insects, such as *Meganeura*, a dragonfly with the wingspan of a hawk, thrived 300 million years ago, when the atmospheric concentration of oxygen was much higher than it is today.

**How do we know that such large insects existed?**

Graham, J.B., R. Dudley, N. Aguilar and C. Gans. 1995. Implications of the late Palaeozoic oxygen pulse for physiology and evolution. Nature 375: 117-120.

# Fossil of meganeuron the giant dragonfly




**UPS AND DOWNS** — The atmospheric concentration of oxygen has varied in response to geologic processes, such as the burial of coal and the break-up of continents, as well as to ecological changes.

During periods of high oxygen conc. biological innovations were high.

Current level is 21%

Carboniferous level believed to be 35% This high oxygen level enabled insects and other animals to grow to large proportions.

Higher oxygen levels + increased air pressure would have increased the diffusion rate of oxygen into an insect's bloodstream as much as 67%.

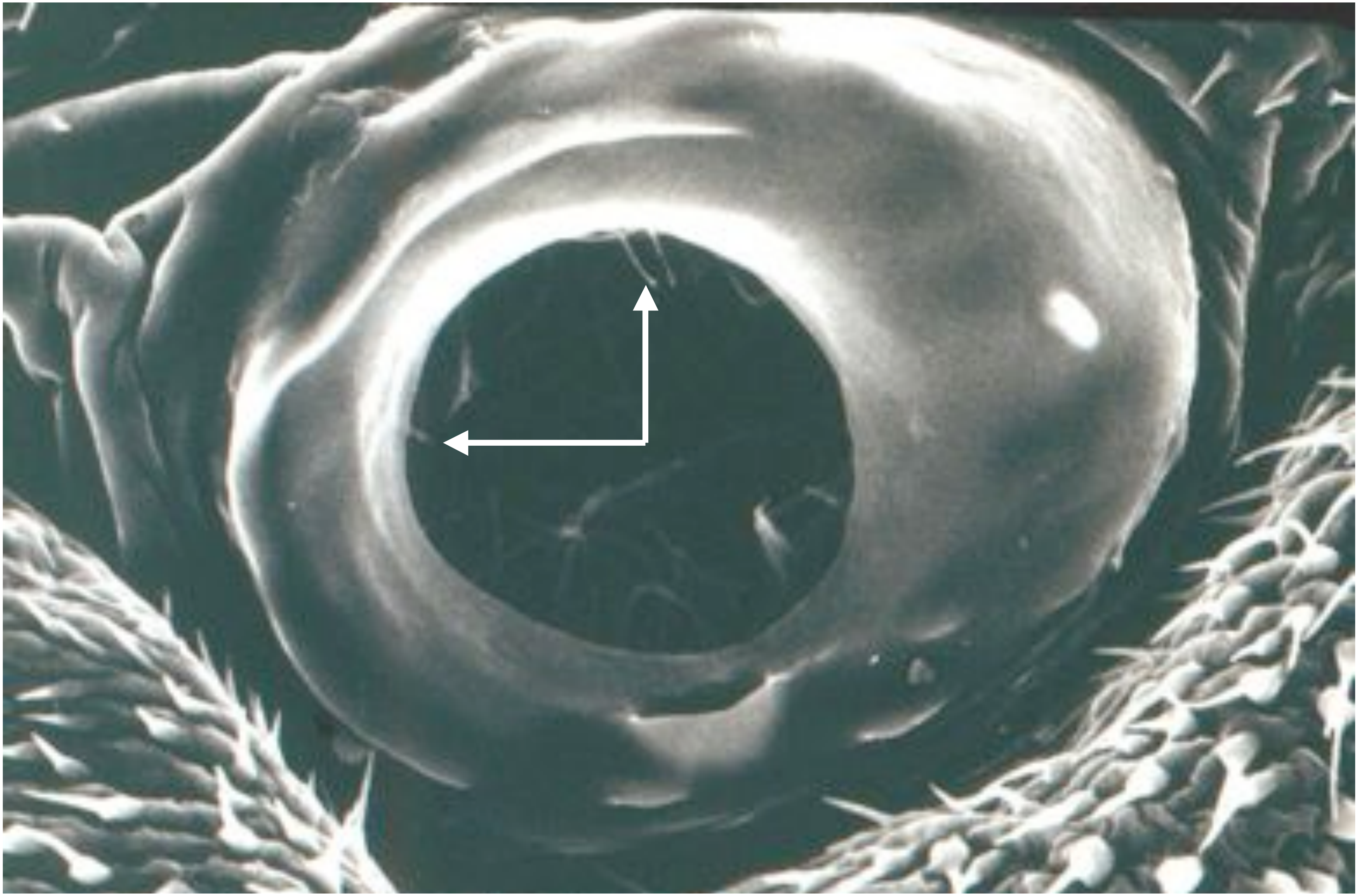
**If you knew that high levels of oxygen permitted larger growth in the past, what experiment could you do?**

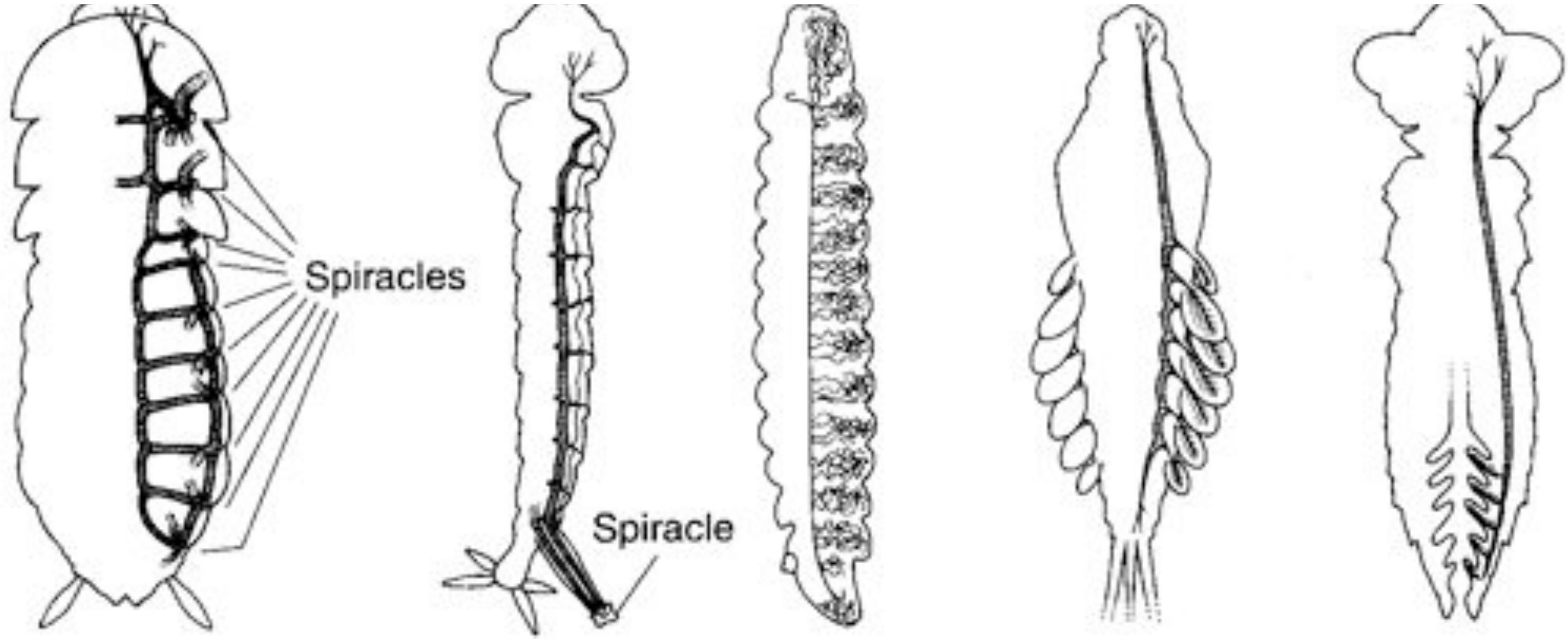
**Jon Harrison of Arizona State Univ. in Tempe raised fruit flies and meal worms with twice the normal amount of oxygen and they grew 3% larger.**

1. Earth's atmosphere had little if any oxygen until 2.5 billion years and the origin of chlorophyll producing plants
2. Oxygen levels have fluctuated throughout the history of the earth
3. High oxygen levels permitted great biological experimentation and greater diversity
4. Low oxygen levels caused mass extinction of those organisms requiring high levels of oxygen

Information taken from Science News, Dec. 17, 2005, vol. 168, pp. 395-6
Title=Changes in the air - variations in atmospheric oxygen have affected evolution in big ways.

Spiracular opening of adult *Phormia regina.* Note cuticular hairs (see arrows) inside opening for filtering out dust particles.






Holopneustic
All open

Metapneustic
only 1 open

Apneustic
cuticular

Apneustic
plate gills

Apneustic
rectal gills

OPEN

CLOSED

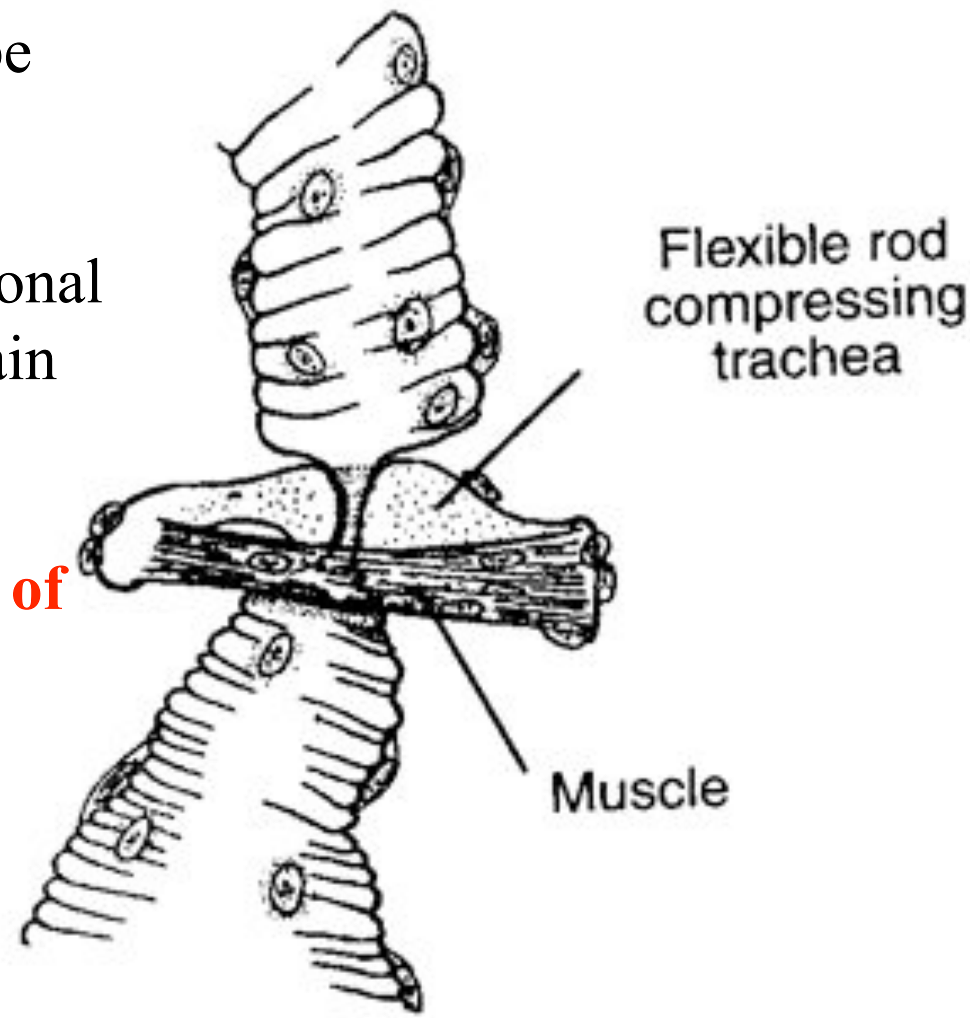
Basic variations in open and closed spiracle types

Insects having open spiracles are able to close them either with

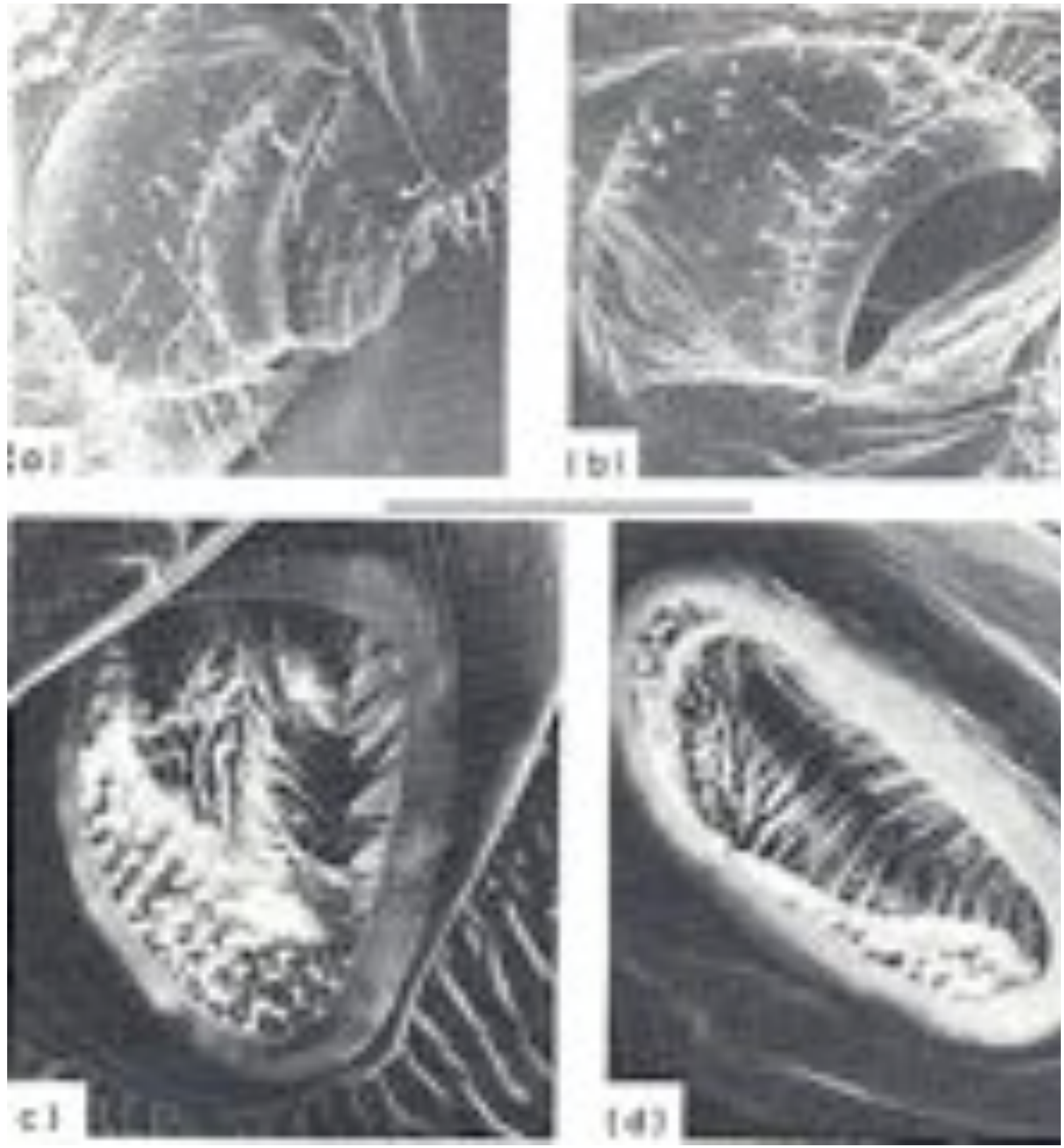
1. Valves on outside or on outer part of the atrium
2. Muscles behind the atrium

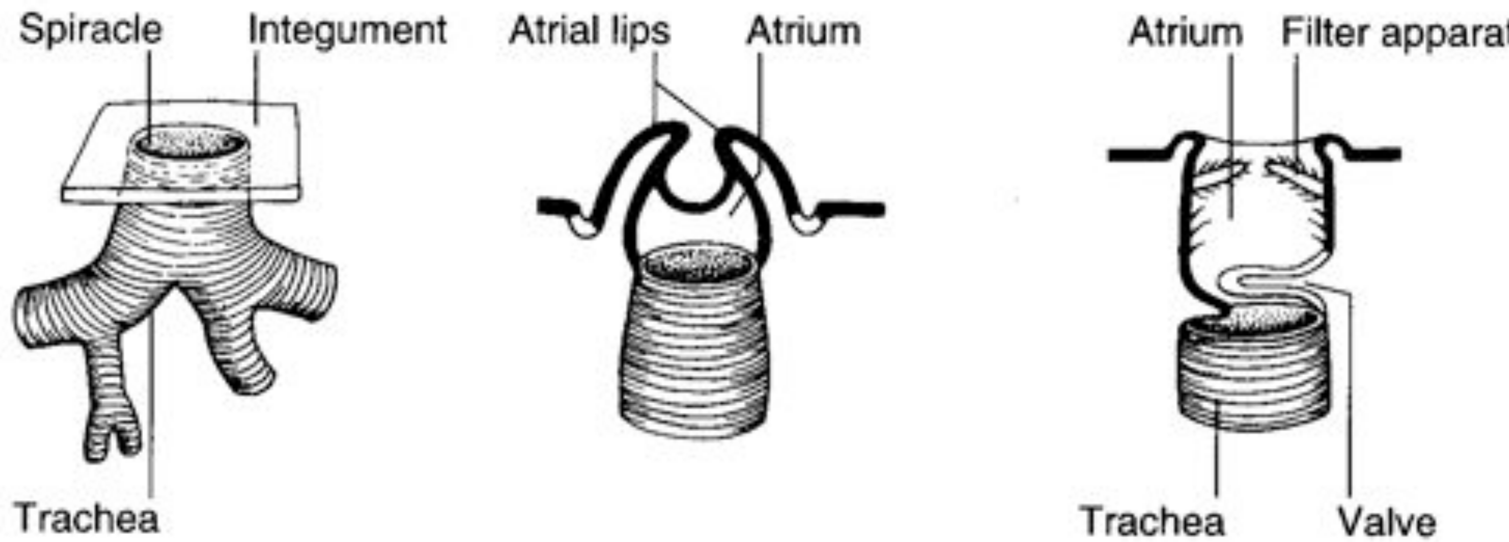
It is important for the insect to be able to close the spiracles for:

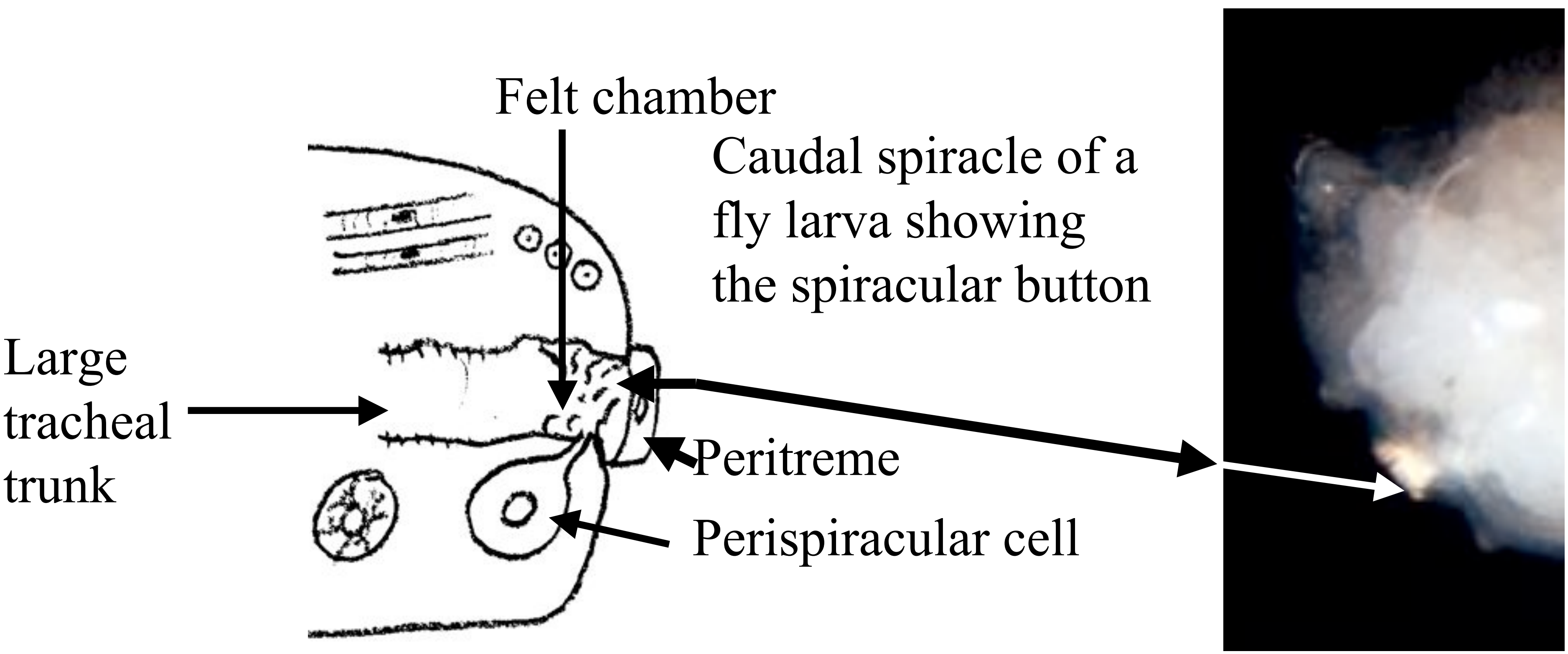
1. Prevent loss of water
2. Provides basis for unidirectional airflow that occurs in the main tracheal trunks
3. **New hypothesis is that it prevents the accumulation of toxic oxygen molecules**

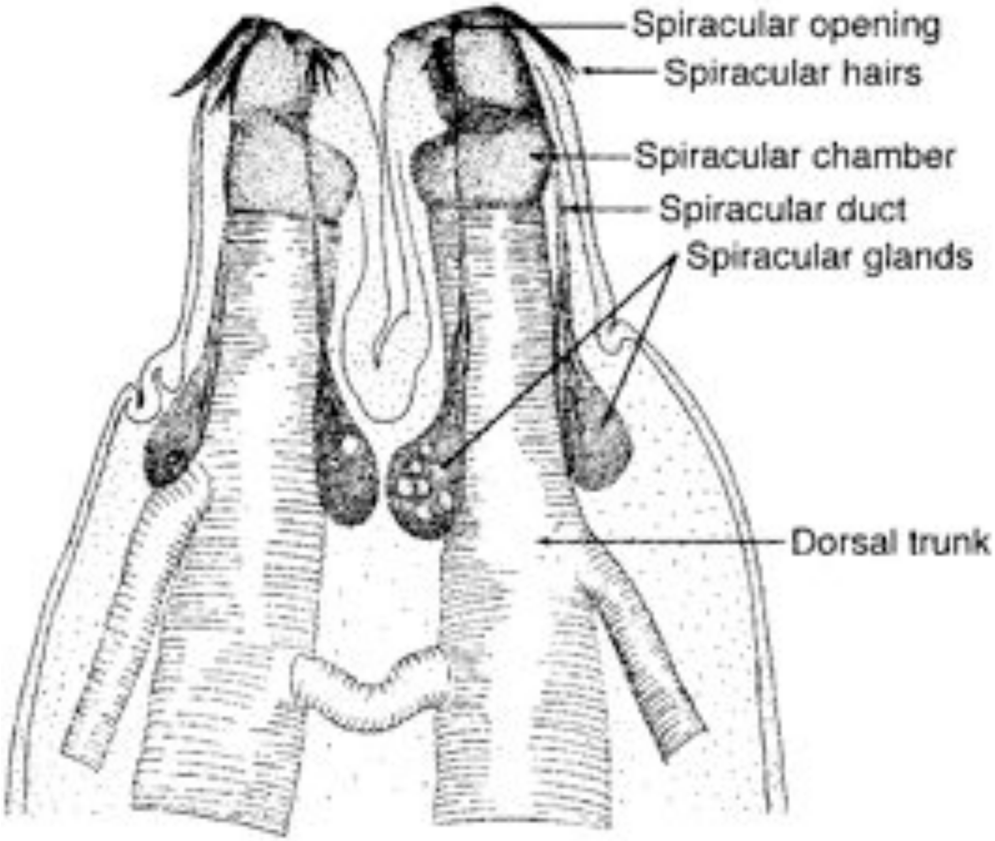


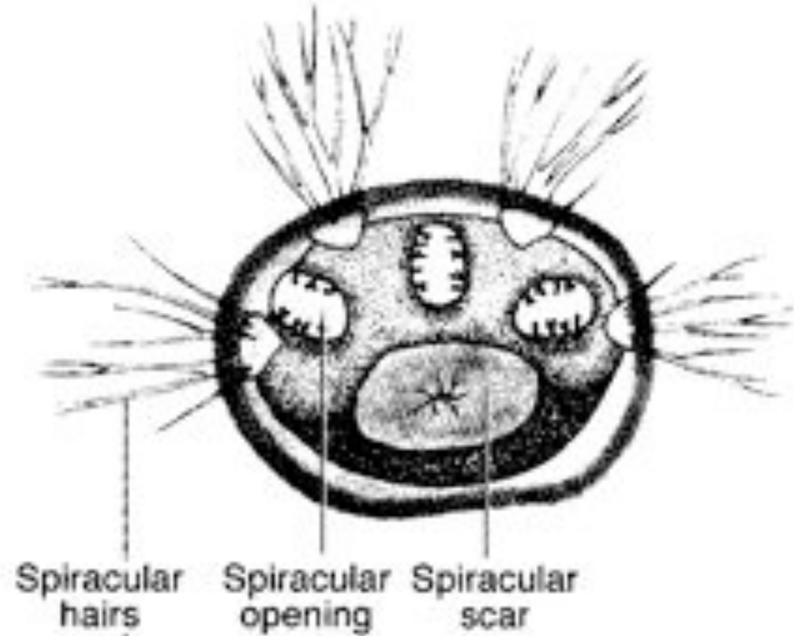
SEM showing spiracle 2 in top photos of cockroach *P. americana*. Note in **a** of spiracle 2 that the valves are closed while in **b** they are open. Below in **c** is spiracle 10 showing the honeycomb structure and cuticular hairs. In **d** showing the spiracle 7 note the hairs but, below and inside note the valves below the outer atrium

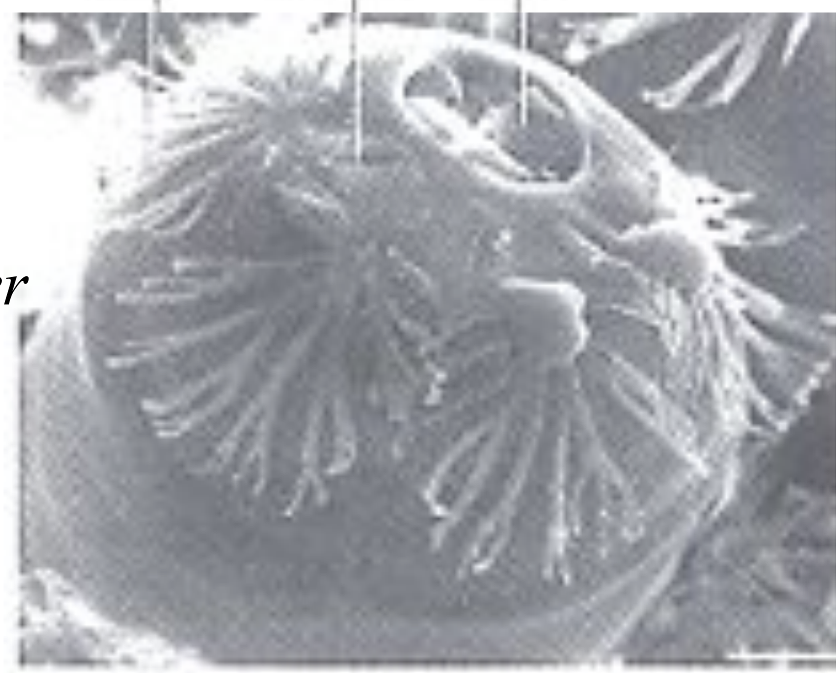



Spiracle
Integument
Trachea
Atrial lips
Atrium
Atrium
Filter apparat
Trachea
Valve


Felt chamber
Caudal spiracle of a fly larva showing the spiracular button
Large tracheal trunk
Peritreme
Perispiracular cell

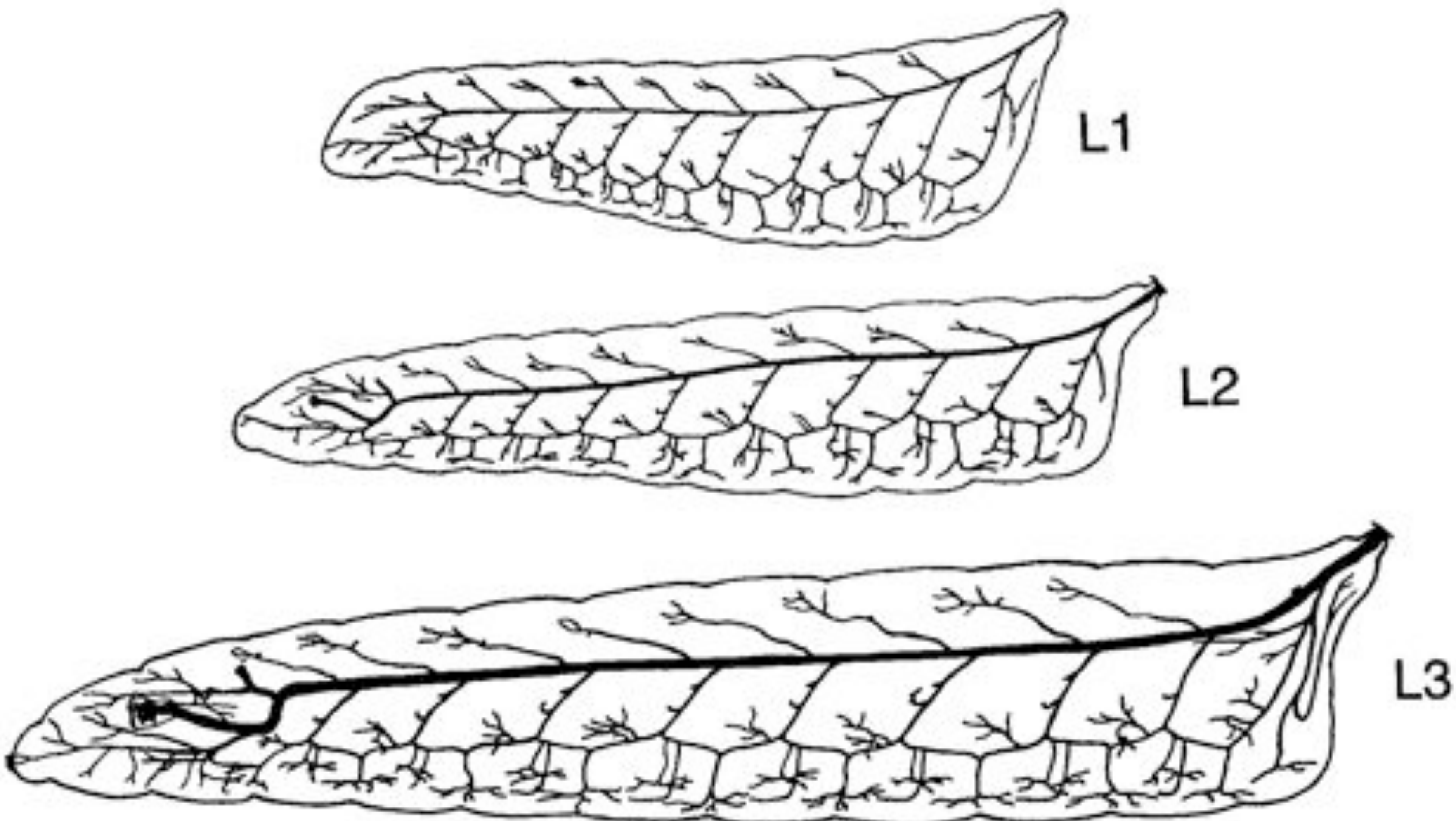


Larval, caudal spiracle of *D. melanogaster*

The larvae of Cyclorrhaphous dipterous larvae can be determined by the position of the spiracle(s). Note L1 only has caudal spiracles, L2 has caudal and anterior spiracles while L3 has both but, the spiracular button is of different shape.



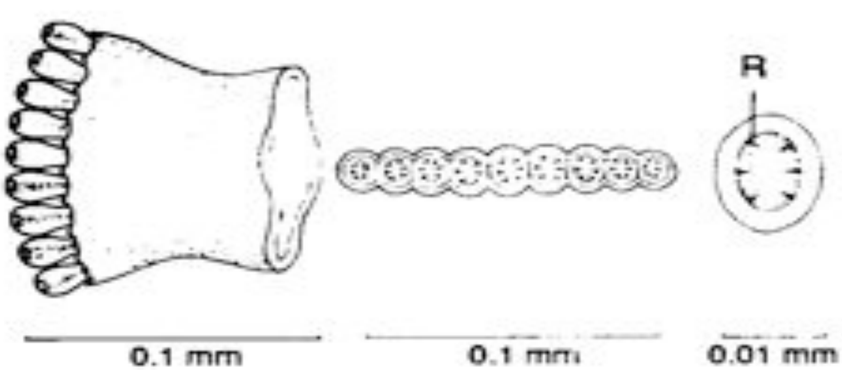
Trachea are formed by the invagination of the ectoderm and are lined by a cuticular intima of the cuticle. Thus, this intima consists of an epicuticle with a protein/chitin layer beneath it.

The distribution of trachea in any insect reflects and tells you something about the demands of the tissue they are supplying. Thus, if you see a tissue with lots of trachea going to it, you can be assured that it has need for a lot of oxygen to conduct its metabolic processes.

One idea is that areas of oxygen debt stimulate tracheal cells to produce more trachea.

Notice the large number of trachea going to this encapsulated nematode. Wigglesworth thought this was due to the oxygen deficit created by the dying parasite.

The diagram below shows the anterior spiracle of a 2nd or 3rd instar fly larva. First instars lack one. On the right are two caudal buttons, that are used two stage the instars. The one on the left is a 2nd instar and the one on the right is a 3rd instar. Used in staging dipterous larvae.



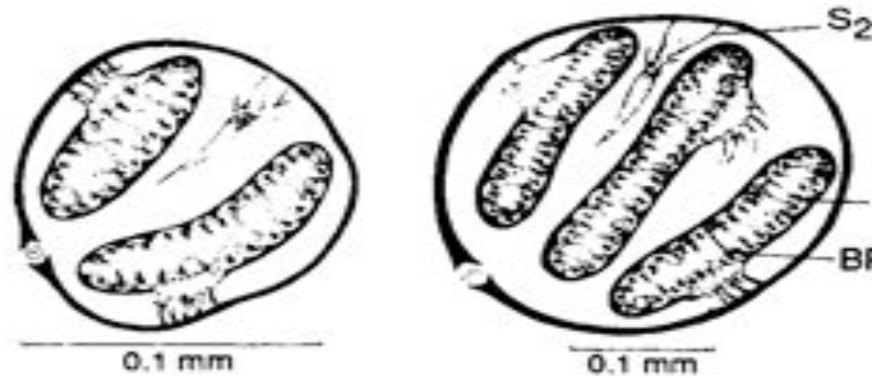
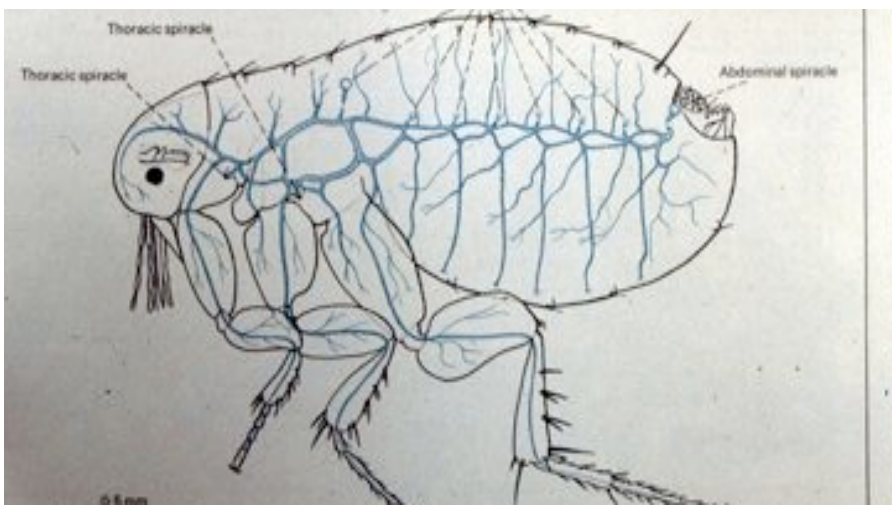
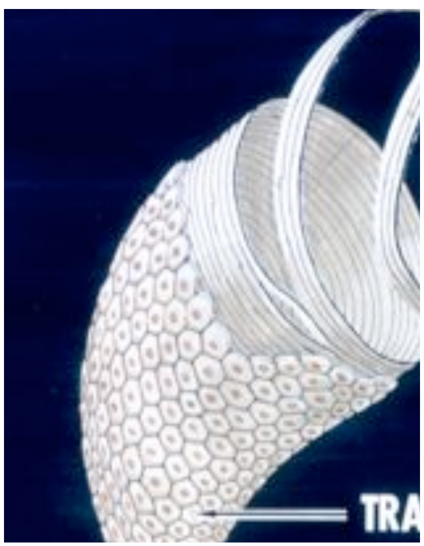



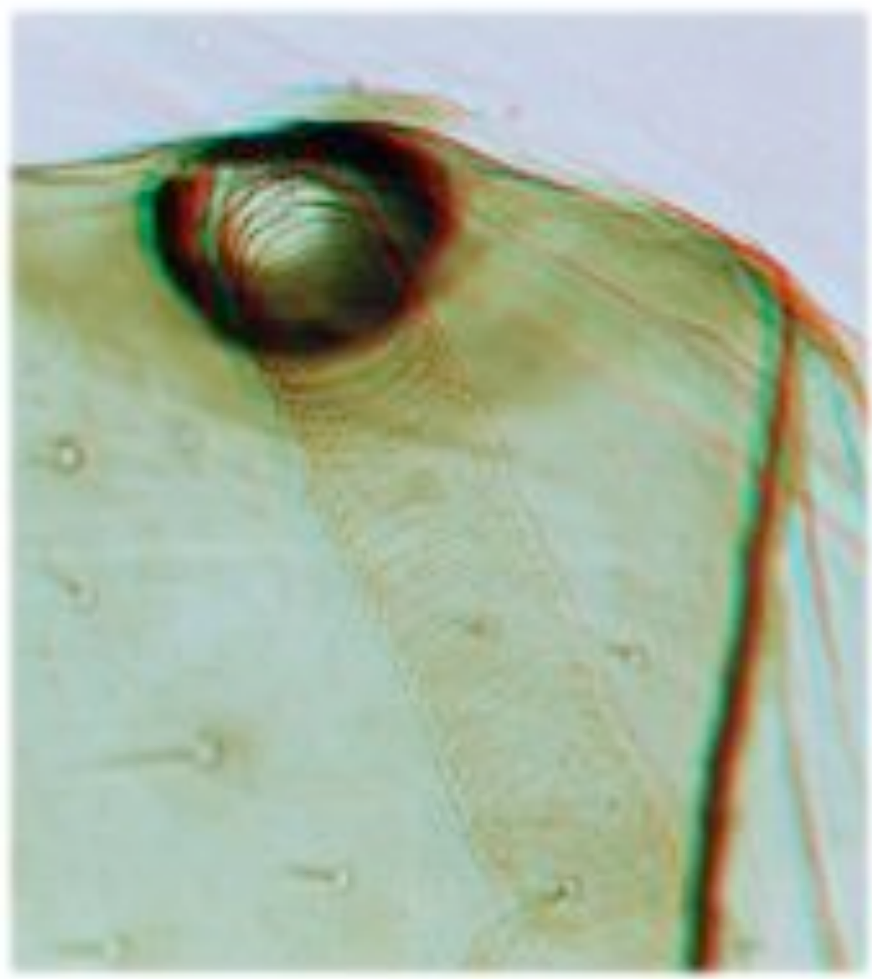

Diagram on the left showing the extensive tracheal system of an adult flea. The photo on the right is a dipterous larva that is clear but beautifully shows the looping and extensive tracheal system in white.

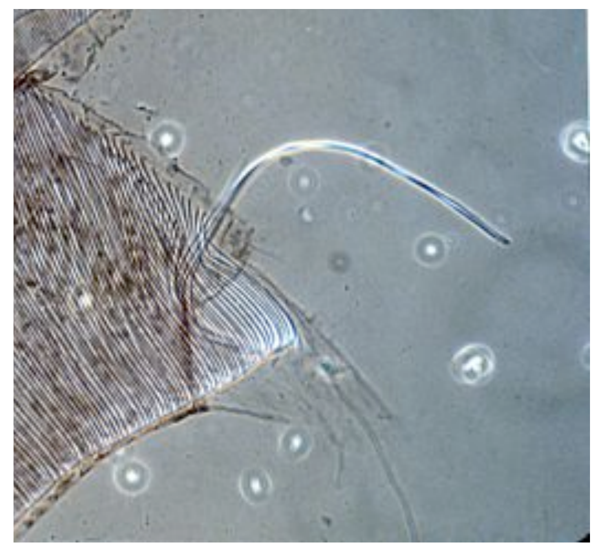



Apneustic tracheal system

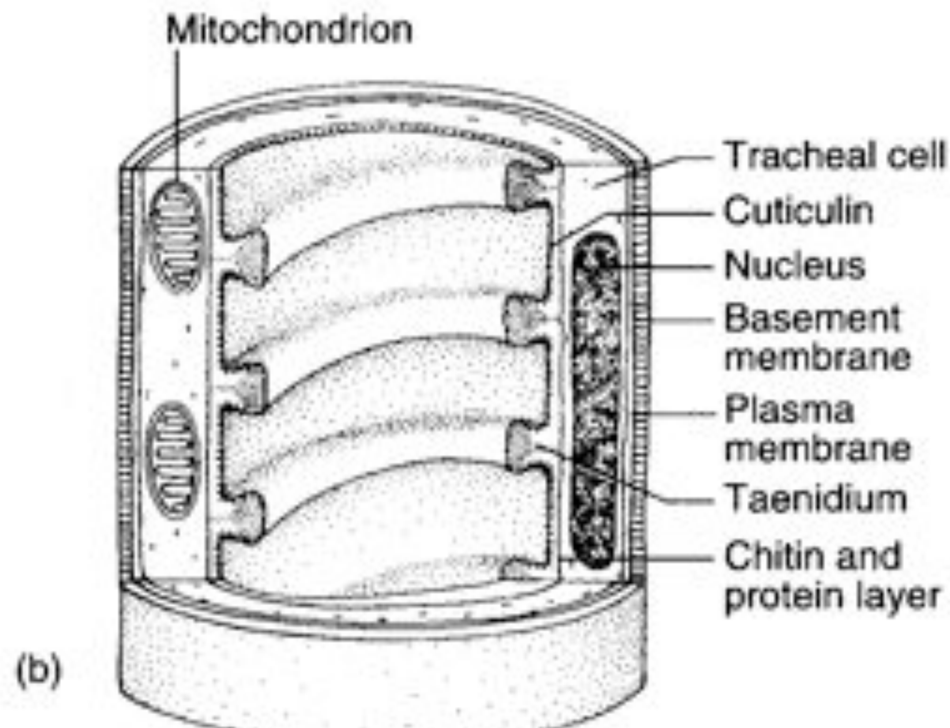
# TRACHEAE ARE INVAGINATIONS OF THE CUTICLE

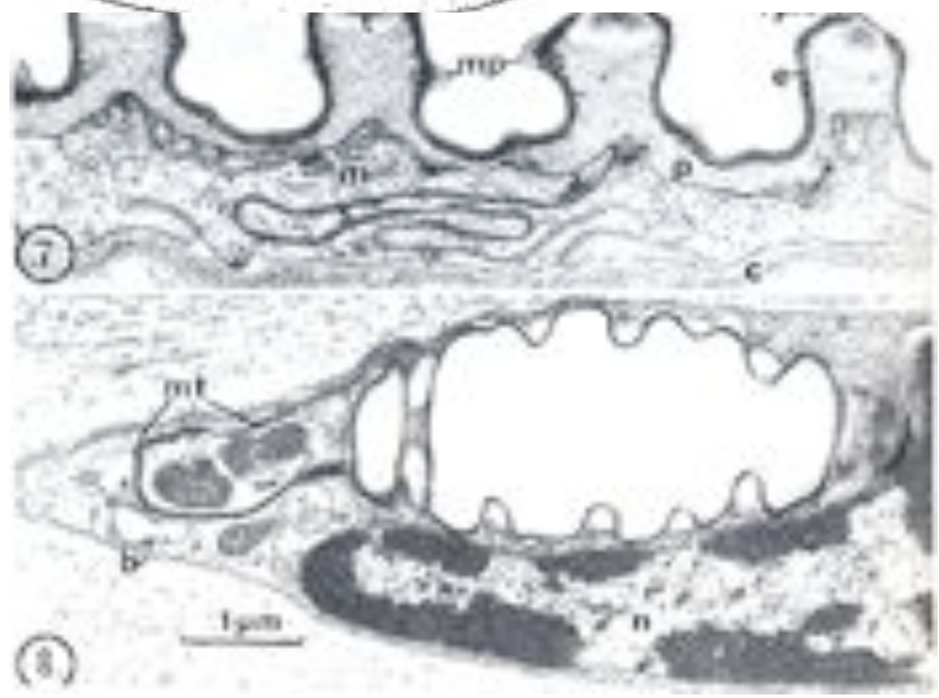


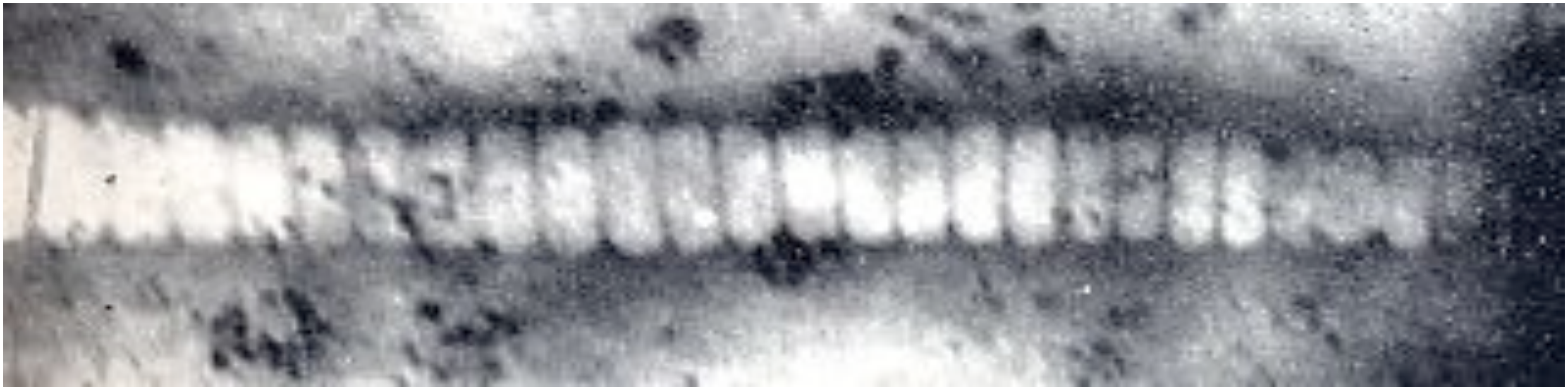


# Freeze fracture-SEM showing the taenidia

TEM showing the end (on the right) of a tracheole of ***Rhodnius*** showing that it ends blindly and they have annular corrugations.



TEM, using negative staining. Note the helical folds (below)

## Tracheoles-

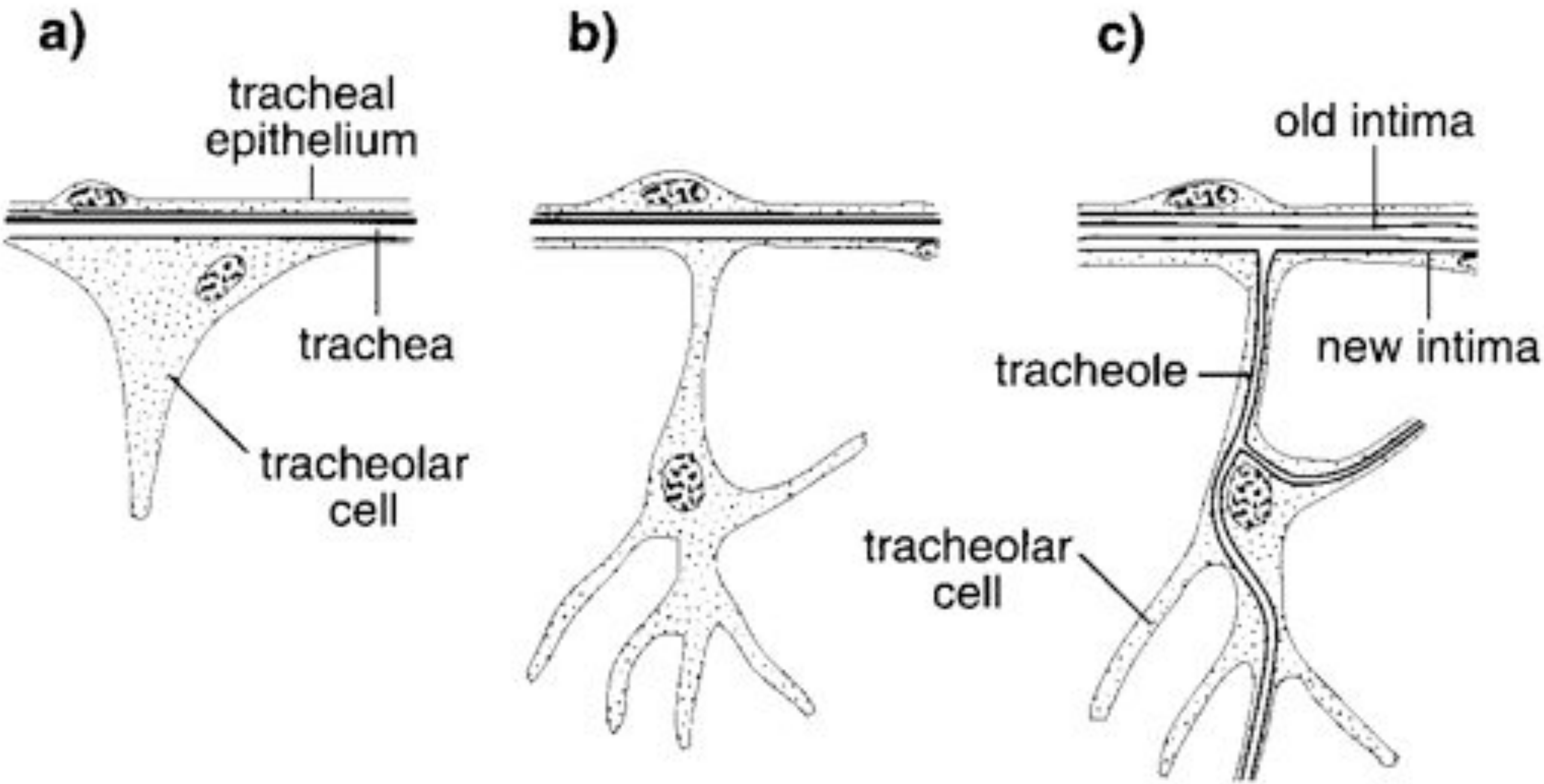
Are are not shed at the molt

The tracheolar extremities are filled with a liquid.

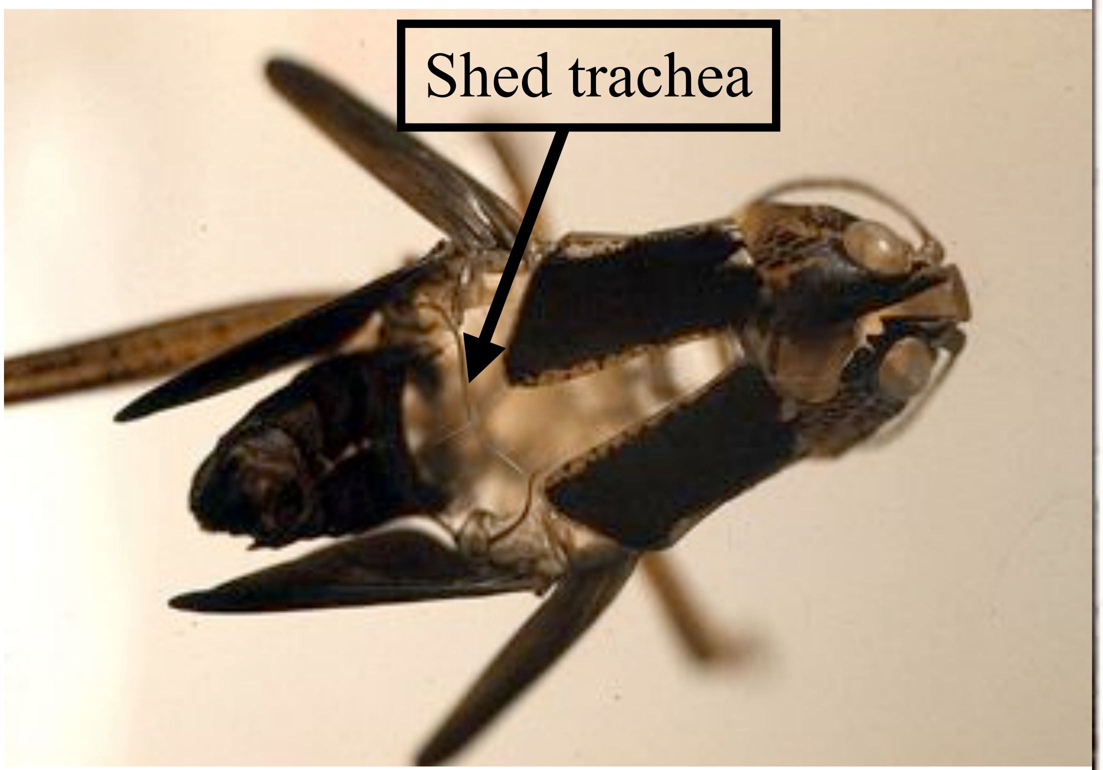
They are very small, 1µm in diameter or 0.1µm or less. A cell may be 30 µm.

If the epidermis is damaged, they send out cytoplasmic threads that connect to the nearest tracheoles. The threads then contract and pull the tracheoles to the area. A similar phenomenon occurs when a foreign objec is being encapsulated.

Tracheoles are formed from cells known as tracheolar cells, which are derived from the epidermal cells lining the trachea.

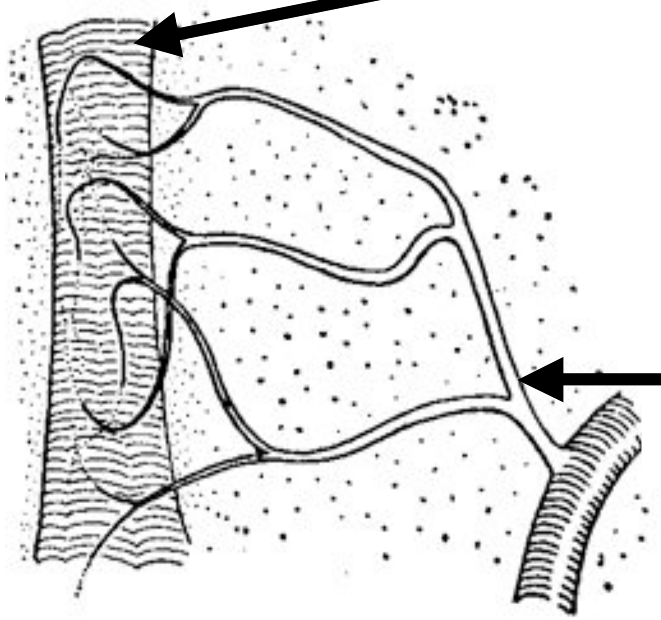


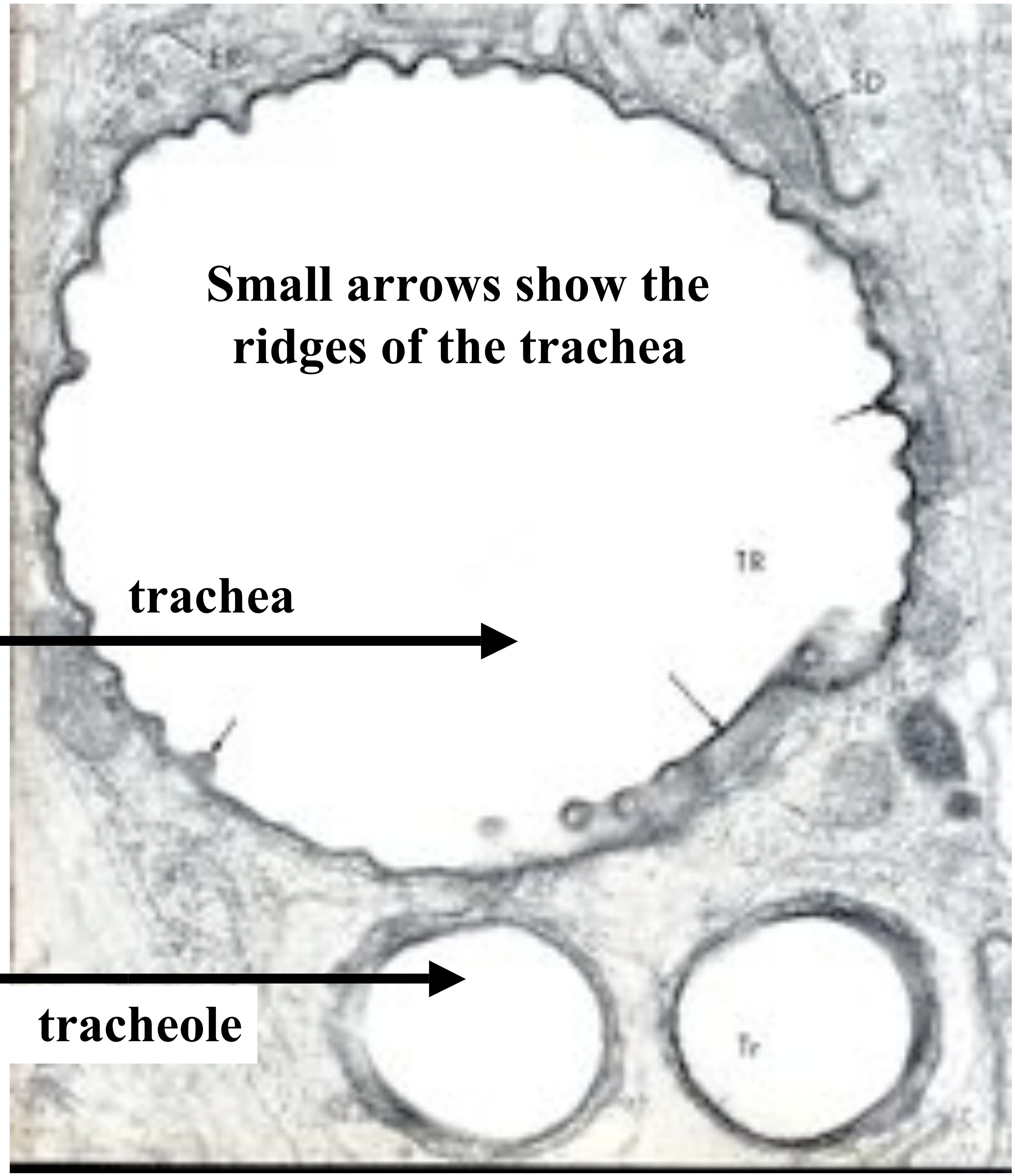
The trachea are surrounded by one or two epithelial cells that produce the new trachea, which is shed at each molt because it contains an epicuticular lining. The tracheoles are not shed at the molt.




Air is taken in at the molt to assist in getting rid of the old exoskeleton and expanding the new.

Note in the TEM at the right the large trachea and the two smaller tracheoles. Note that the large trachea has the ridges, which show the taenidia while the tracheoles lack taenidia and their inner surface is smooth.

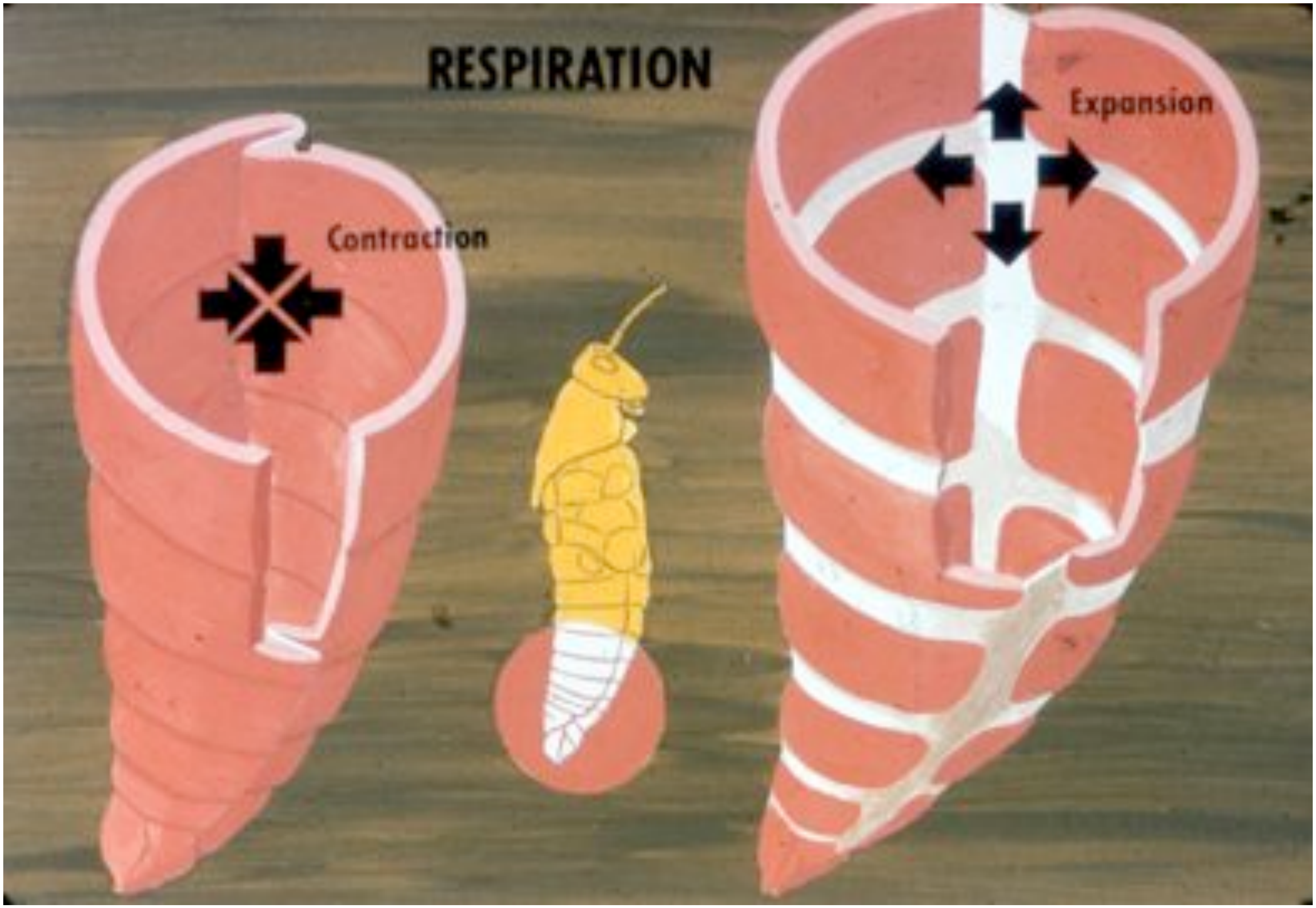




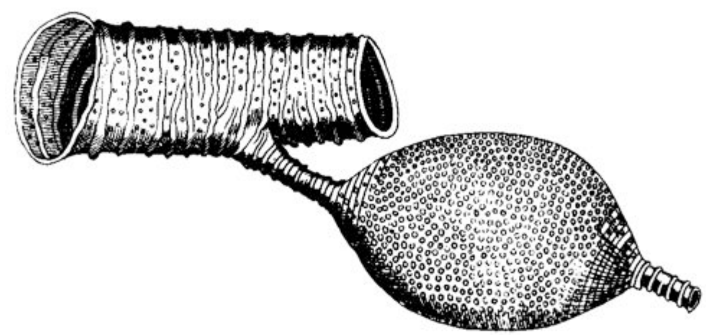
## Tissues or organs requiring a lot of oxygen (i.e. good tracheal supply)
## Any tissue or organ requiring a lot of metabolic energy.

1. Muscle, especially flight muscle (meso and metathoracic spiracles)
2. Ovaries
3. Light organs
4. Cymbal of cicada
5. Expiratory dorsoventral muscles of the dragonfly naiad rectal system
6. Ganglia are usually heavily tracheated

Oxygen enters the spiracle and goes throughout the entire tracheal system by a combination of diffusion along a concentration gradient and the process known as ventilation.



**Air sacs**-Dilated and expandable regions of the tracheal system where the taenidium is reduced or lacking, thus permitting expansion. Are large in bigger insects, especially those that fly (Diptera, Hymenoptera and some Coleoptera).



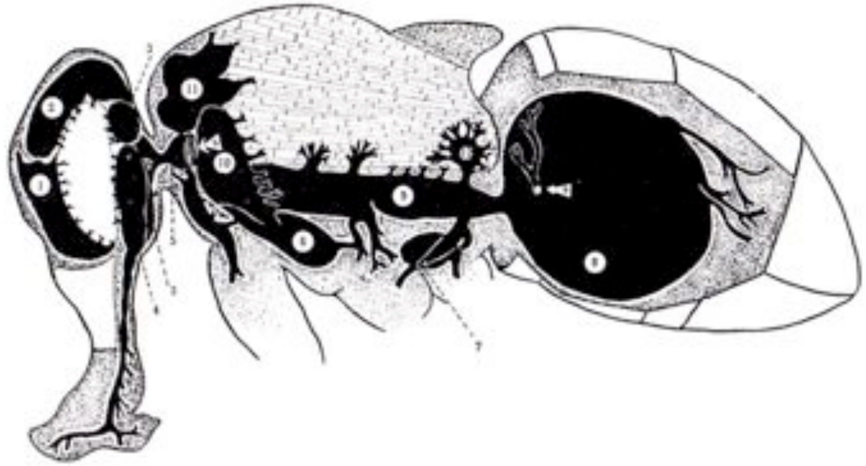
Swammerdam, 1937

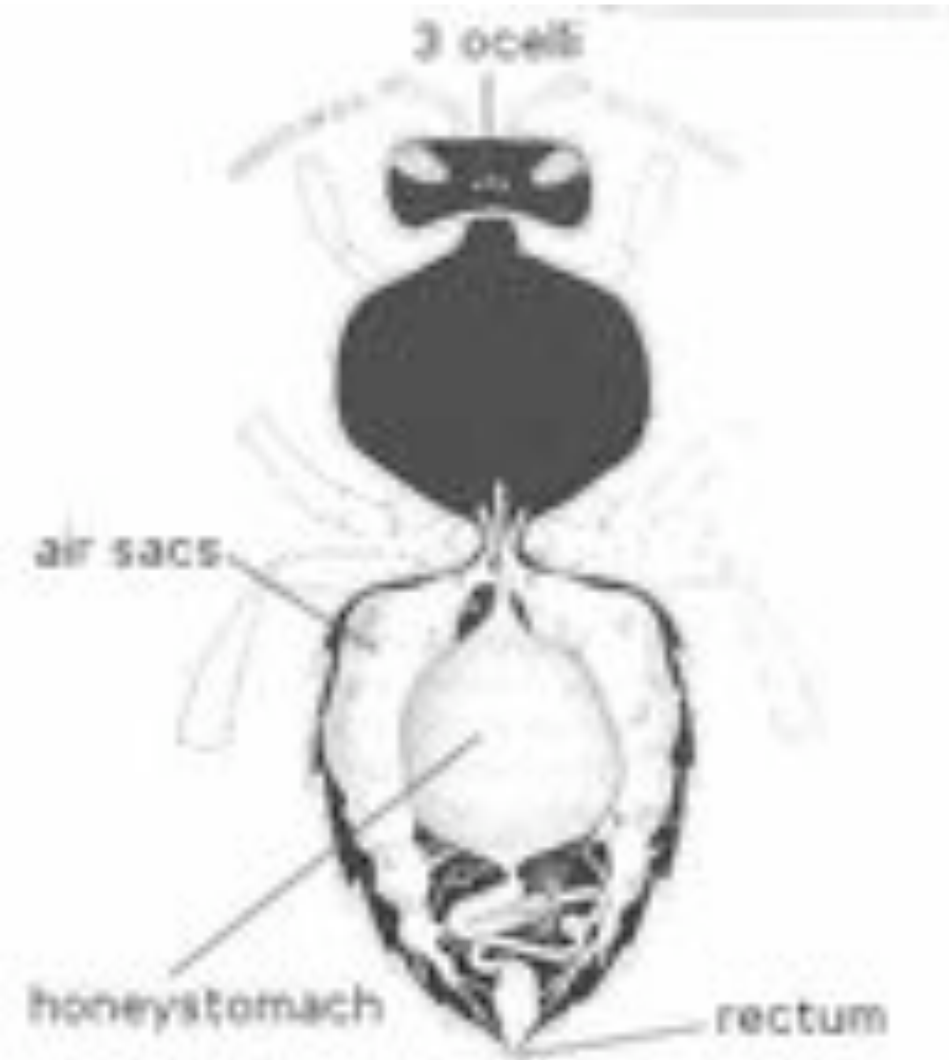
FUNCTIONS

1. Increase the volume of tidal air
2. Reduce the diffusion path to the tissues
3. Aid in ventilation of the tracheal system
4. Can be used to provide sectioning of the thorax from the abdomen
5. Form tympanic cavities for hearing organs and sound producing organ
6. Lower the specific gravity of insect, thus aiding in flight
7. In some chironomids, the air sac can determine at what depth they are found depending on how much air is in the sac.

In locusts and dragonflies each flight muscle receives a primary tracheal trunk with an associated air sac.

Tracheal system of honeybee and house fly showing air sacs

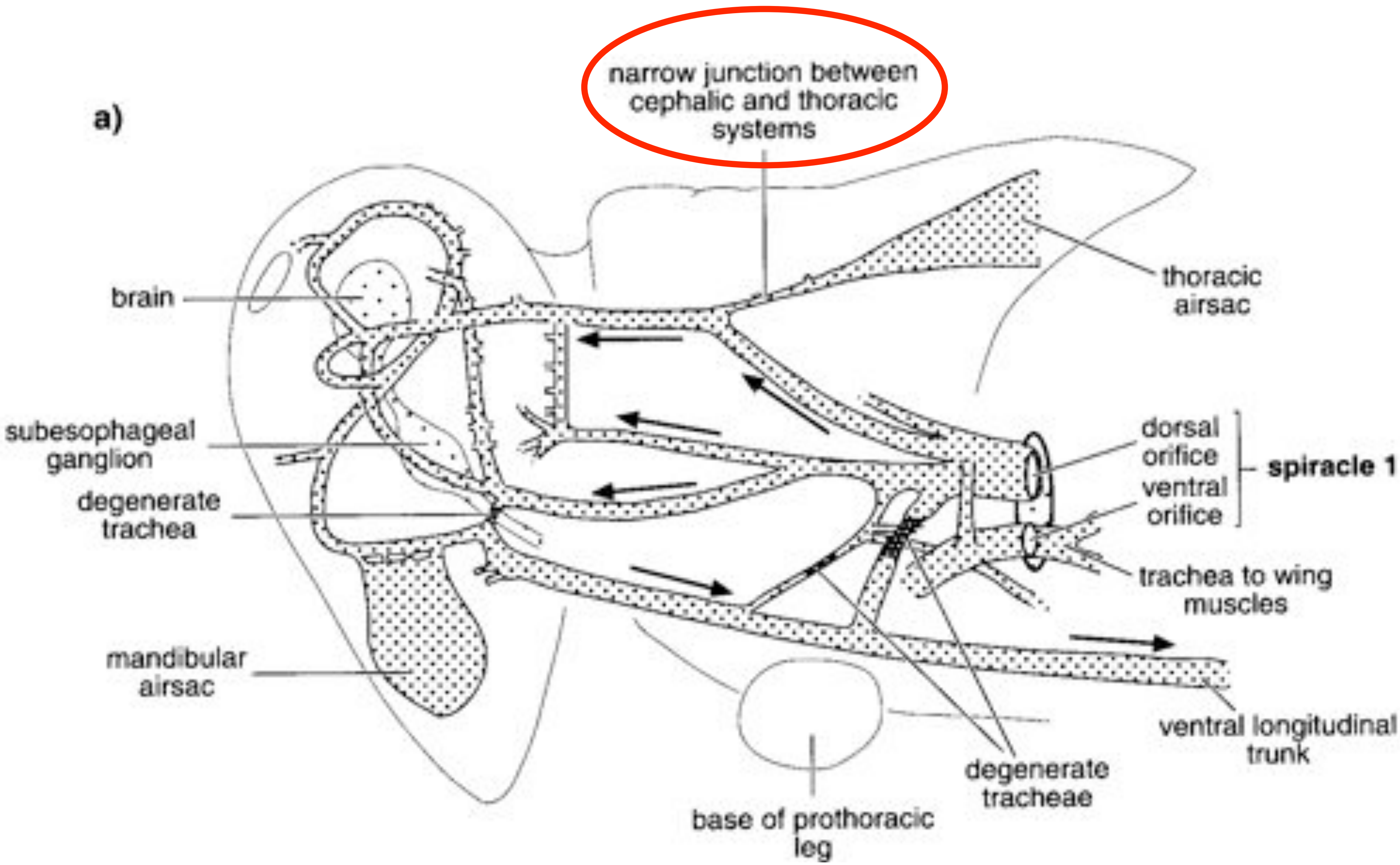




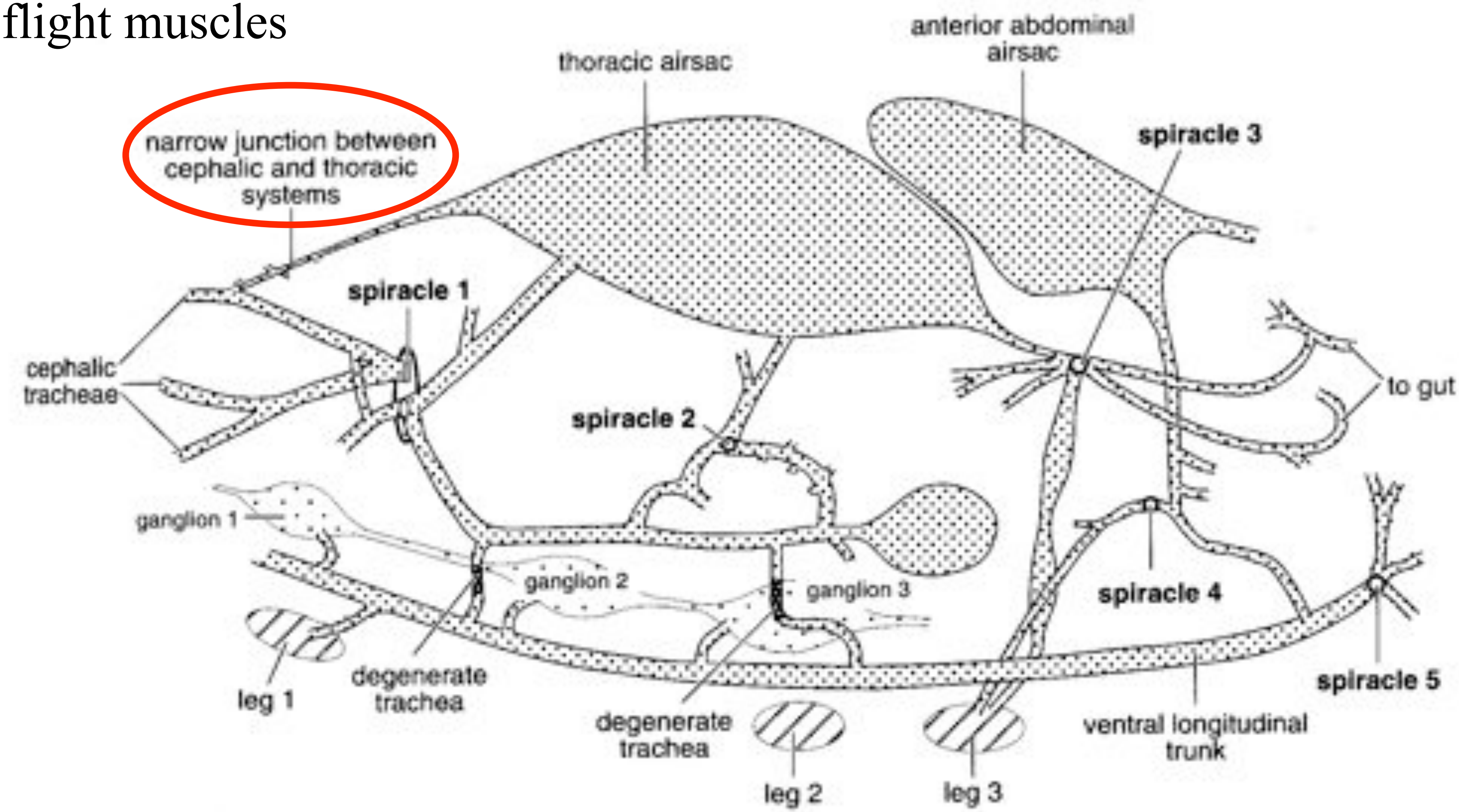
**Ventilation** or the use of muscles to assist in air movement varies and depends on the insect:

1. Flying insects
   a. Thoracic pumping in locusts, large moths and beetles, and dragonflies
   b. Abdominal pumping in hymenopterans and dipterans

**Isolation of air supply to head and to thorax**-Under non-flying circumstances, air from spiracle 1 and abdominal pumping puts air to the head and rest of the body.



**Keeping the air supplies to the head and thorax separate** -The demands of the flight muscles in a large insect like the locust are great. They need air (i.e., oxygen). In flight they conduct thoracic pumping. Because of the narrow junction between the cephalic and thoracic parts, oxygen from spiracles 2-4 provide the air to the thoracic air sacs, thus the flight muscles



## Opening and closure of the spiracles

1. When closure muscle contracts they are closed
2. When closure muscle relaxes they open

## What regulates their opening and closing?

1. Under the control of the autonomic nervous system in response to the local chemical stimuli of the area

## Factors regulating the nervous signal going to the spiracle muscles

1. High levels of carbon dioxide and low levels of oxygen in tissues
2. This causes a **reduction** in the action potential frequency of the nerves going to the spiracle muscles.
3. This causes relaxation of the closure muscles and spiracles open
4. Water balance can also affect this. Less water, they remain shut.

New evidence suggests that there is also neurohormonal control over insect breathing. (see Slama, K. 1999. Active regulation of insect respiration. Ann. Entom. Soc. Amer. 92: 916-929.

# Types of 'respiration' or gaseous exchange in insects

**1. Cutaneous respiration**-gaseous exchange directly through the cuticle.
Occurs to a limited extend in all insects. Occurs in Protura and those Collembola that lack a tracheal system

Wax layer- Impermeability to water loss
Epicuticle- Generally impermeability to oxygen but not due to the wax layer

**2. Gaseous exchange in terrestrial insects**

**a. air-tube diffusion**

**b. tissue diffusion**

**oxygen diffuses in air 100,000 times faster than it does in water**

carbon dioxide travels much faster through tissues than oxygen

**3. Gaseous exchange in aquatic insects**

## 2. Gaseous exchange in terrestrial insects

In many species of insects, air movement, plus spiracular opening and closing, is coordinated with the ventilatory movements of the abdomen so that air is pushed out when the abdominal muscles contract and sucked in when they relax.

### DISCONTINUOUS VENTILATION

**The spiracles remain closed for a period of time. Movement of gases occurs in discrete bursts.**

**This type of ventilation usually occurs when the insect is at rest and it also occurs in pupae. In diapausing pupae of *Hyalophora* the interburst period may be 8 hours in which time the spiracles remain closed. During closure, relatively little gaseous exchange takes place.**

Hetz, S.K. and T.J. Bradley. 2005. Insects breathe discontinuously to avoid oxygen toxicity. Nature 433: 516-519.

# SUGGESTED FUNCTIONS FOR DISCONTINUOUS BREATHING IN INSECTS:

**1. Avoid water loss**

**2. Prevent hypoxia or low oxygen**

**3. Avoid oxygen toxicity**

Fridovich, I. 1977. Oxygen is toxic! Bioscience 27: 462-466.

Westneat, M.W. et al. 2003. Tracheal respiration in insects visualized with synchrotron x-ray imaging. Science 299: 558-560.

Orr, W.C. and R.S. Sohal. 1994. Extension of life-span by over-expression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science 263: 1128-1130.

insect breathing


http://www.sciencemag.org/feature/data/bioimaging/bug.html

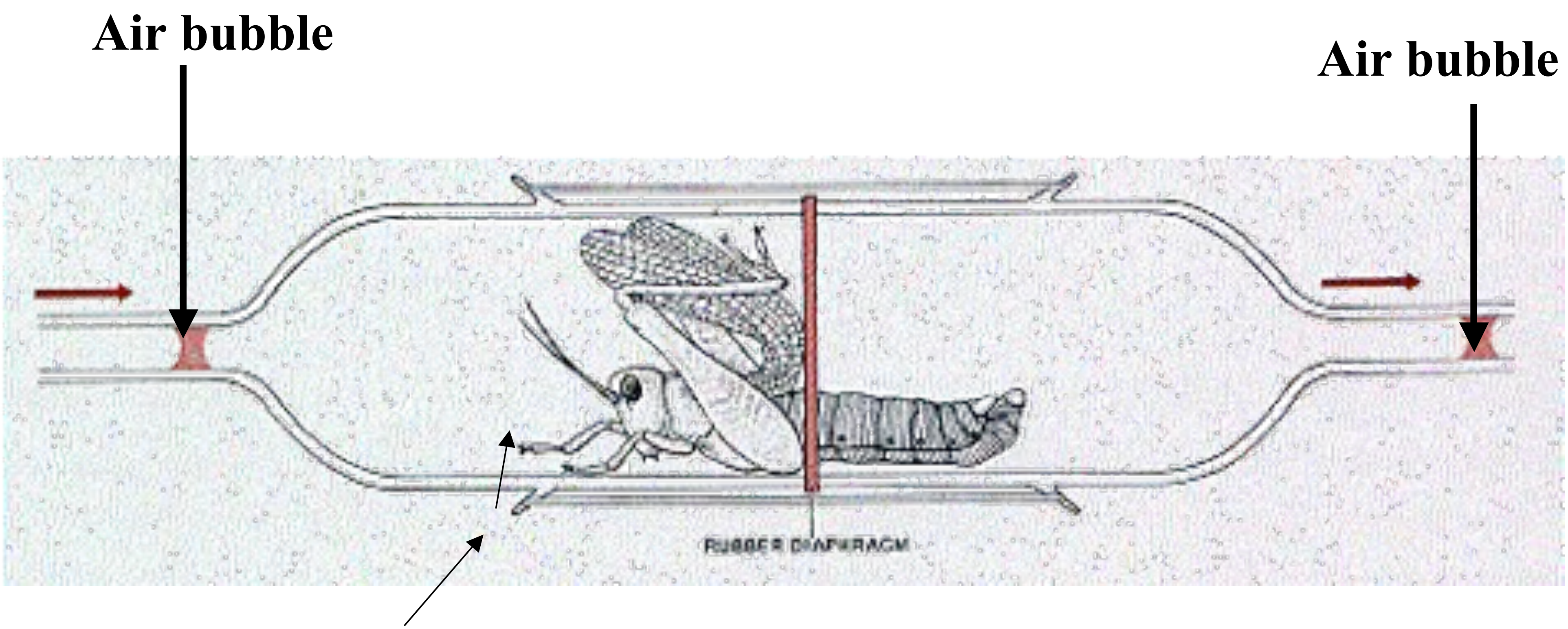
For years it was known that heart reversal occurred in the adult Diptera

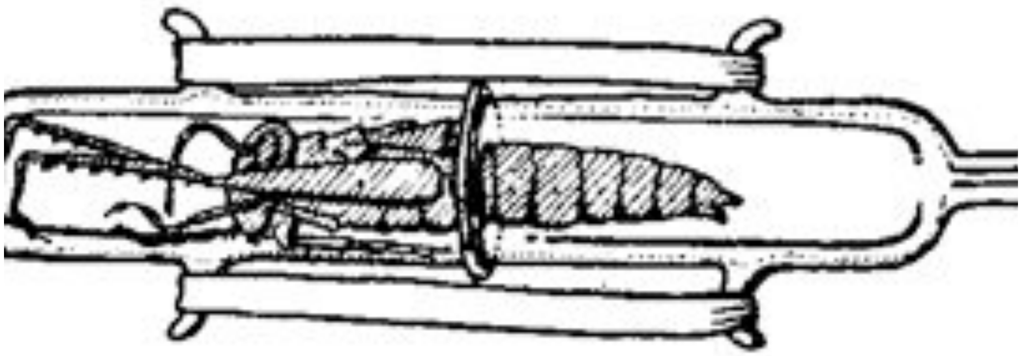
Angioy, A.M. and P. Pietra. 1995. Mechanism of beat reversal in semi-intact heart preparations of the blowfly *Phormia regina* (Meigen). J. Comp. Physiol. B165: 165-170.

**What is the function of heart reversal??**

Wasserthal. 1996. Interaction of circulation and tracheal ventilation in
Holometabolous insects. Adv. Insect Physiol. 26: 297-351.

This apparatus developed by Fraenkel separated the air compartments of the insect into two areas, thoracic and abdominal. By checking the movement of the bubble, he could measure air volume changes in each half of the device.

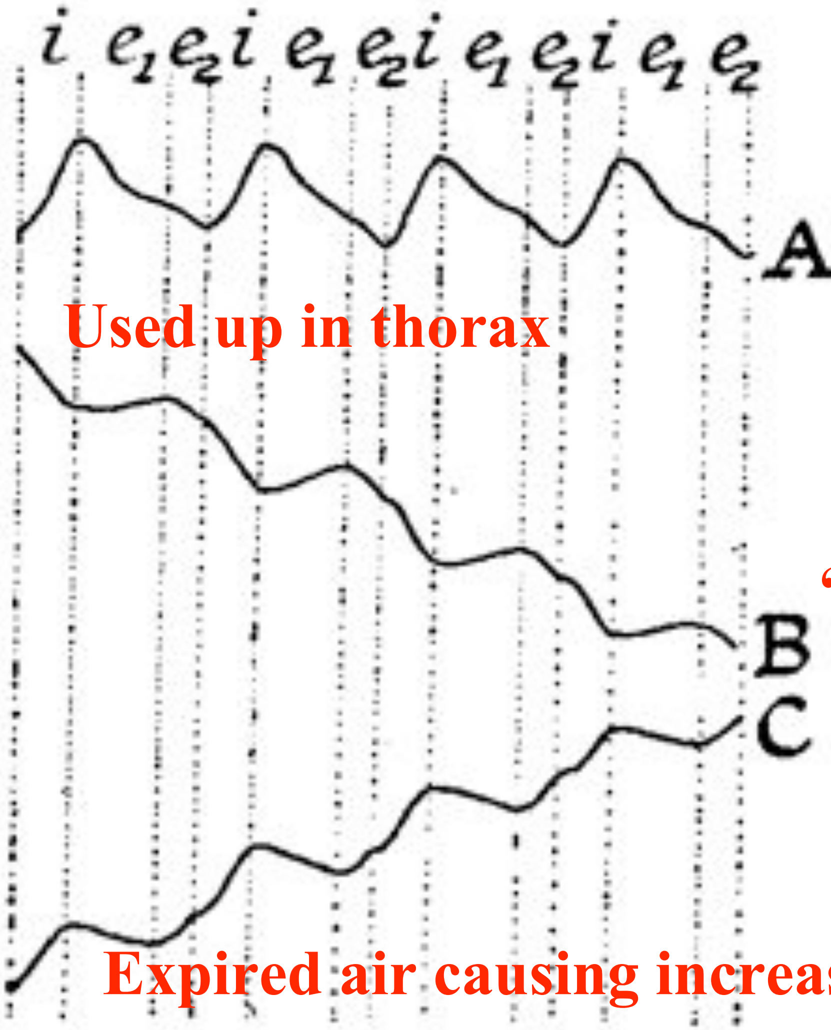




i=inspiratory phase; thoracic spiracles open; abdominal closed

$e_1$=expiratory phase;all spiracles closed

$e_2$=2nd part of expiratory phase; abdominal spiracles open; air streams into posterior part




Used up in thorax

Expired air causing increase

‘A’ shows the respiratory movements in the two regions of the insect as measured in the 2 halves of the gas chamber

‘B’ shows the fall in air volume in the thoracic half of the gas chamber

‘C’ shows the increase in volume in the abdominal half of the chamber

This simple apparatus set the stage for ideas concerning separate control over the two major regions of the insect (i.e., thoracic versus the abdominal).

Thus, the separate opening and closing of spiracles in different parts of the insect is so timed that inspiration occurs predominately in one region, expiration in another, thus producing directed air flow through the tracheal system.

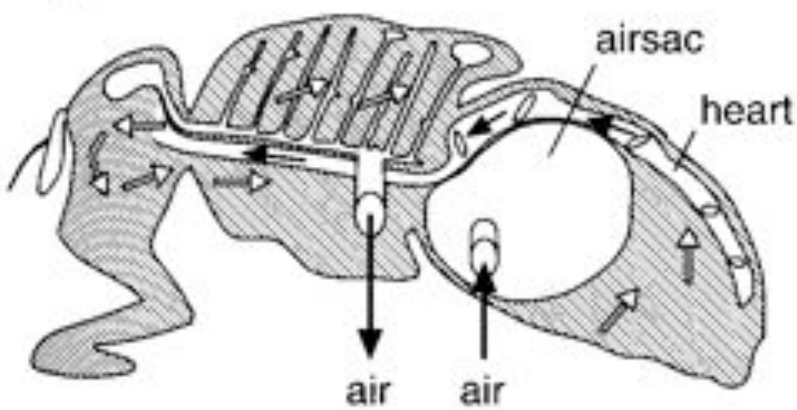
**Thus, air enters via the thoracic spiracles and leaves via the abdominal**

**This experiment demonstrated that insects might have separate control over air in the thoracic versus the abdominal segments**

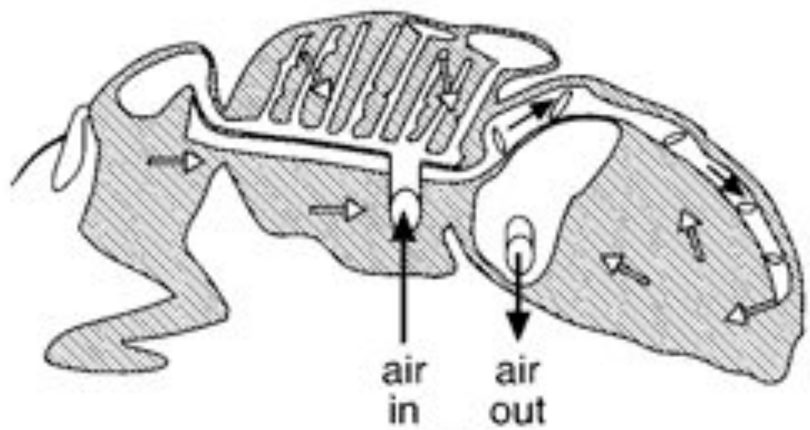
Thus, in locust flight there is a two-way ventilation system that is independent of one another.

1. A two-way system that ventilates the flight muscles through the open spiracles of 2 and 3.
2. A one-way system that ventilates primarily the central nervous system and is pumped by the abdomen in through spiracle 1 and out through spiracles 5-10.

a) Forward movement



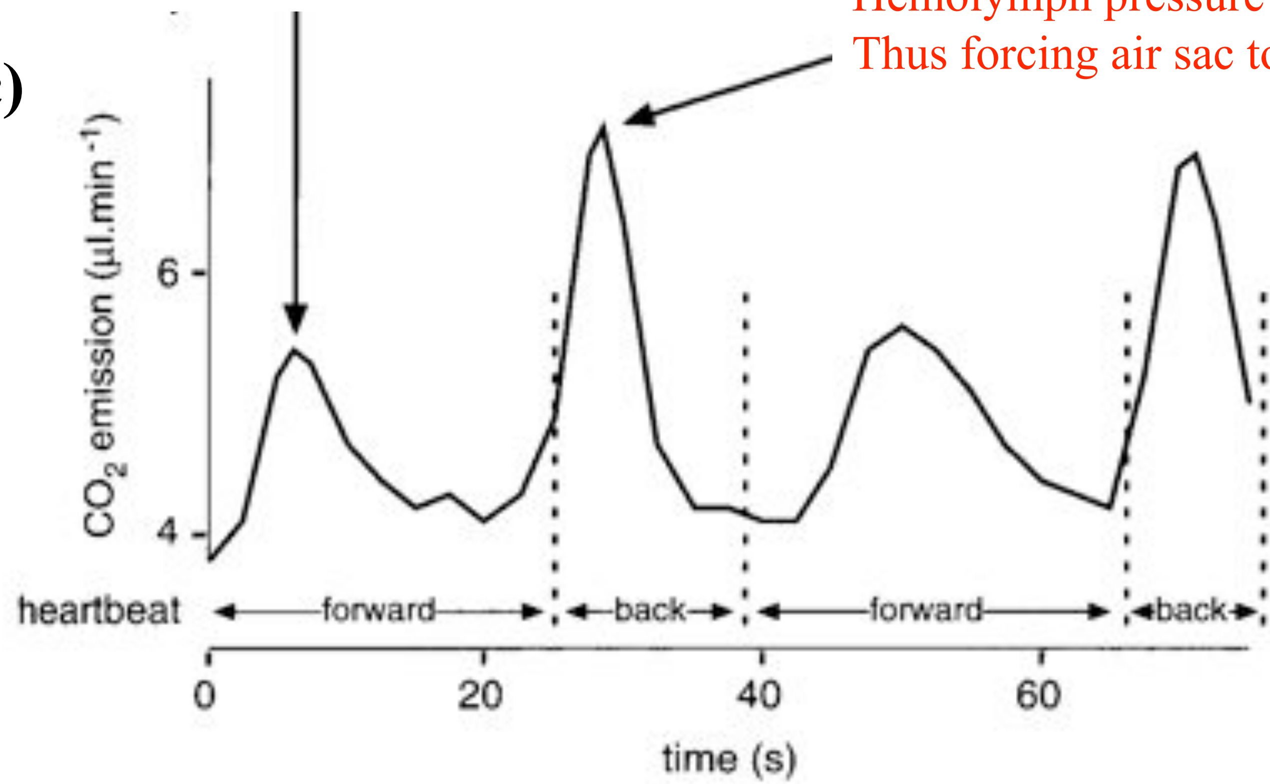

b) Backward movement




Hemolymph pressure greater in anterior, thus
Air sac can inflate and take in air

Hemolymph pressure greater in abdomen,
Thus forcing air sac to compress and air out

c)



# Respiratory System

Locke, M. 1997. Caterpillars have evolved lungs for hemocyte gas exchange. J. Insect Physiol. 44: 1-20.

Since insect blood usually lacks oxygen-carrying pigments it has always been assumed that respiratory needs are met by diffusion in the gas-filled lumen of their tracheal systems. Outside air enters the tracheal system through segmentally arranged spiracles, diffuses along tubes of cuticle secreted by tracheal epithelia and then to tissues through tracheoles, thin walled cuticle tubes that penetrate between cells. The only recognized exceptions have been blood cells (hemocytes), which are not tracheated because they float in the hemolymph. In caterpillars, anoxia has an effect on the structure of the hemocytes and causes them to be released from tissues and to accumulate on thin walled tracheal tufts near the 8th (last) pair of abdominal spiracles. Residence in the tufts restores normal structure. Hemocytes also adhere to thin-walled tracheae in the tokus compartment at the tip of the abdomen. The specialized tracheal system of the 8th segment and tokus may therefore be a lung for hemocytes, a novel concept in insect physiology. Thus, although as a rule insect tracheae go to tissues, this work shows that hemocytes go to tracheae.

# Caterpillars have lungs

Figure 1 Alice confronts one caterpillar that clearly has lungs.

(Mill 1998 after Carroll 1866)

Inside view of the "lung"

(Locke 1998)

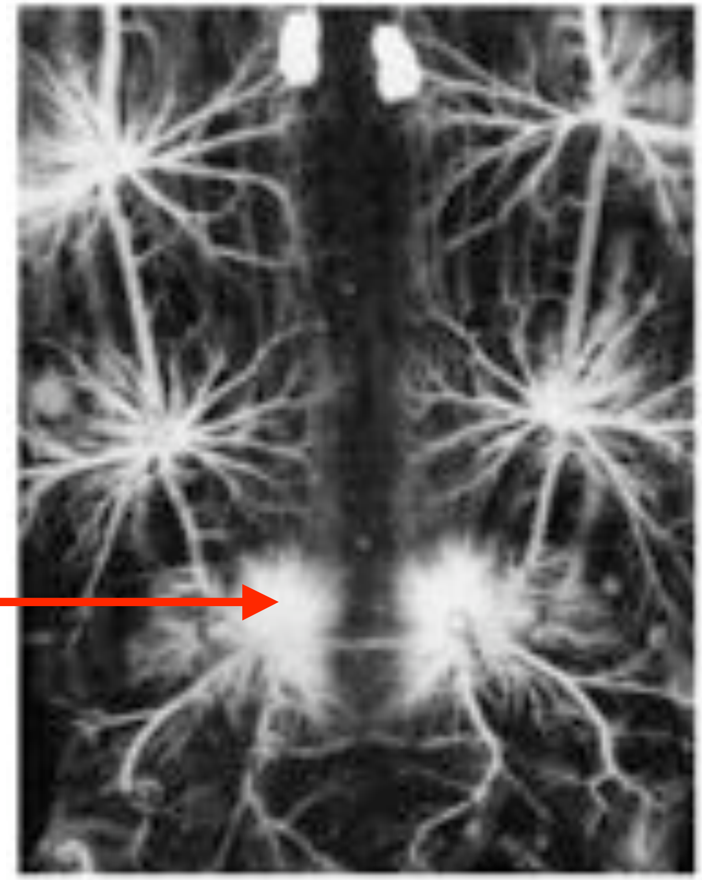
# Locke's caterpillar of choice

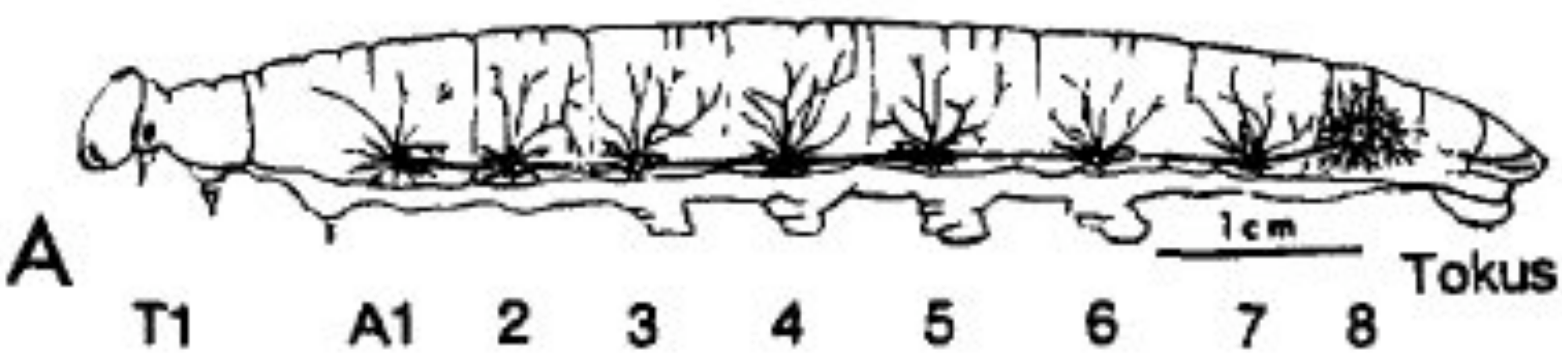

**Brazilian skipper or Canna leafroller, *Calpodes ethlius* (Stoll) (Lepidoptera; Hesperiidae)**

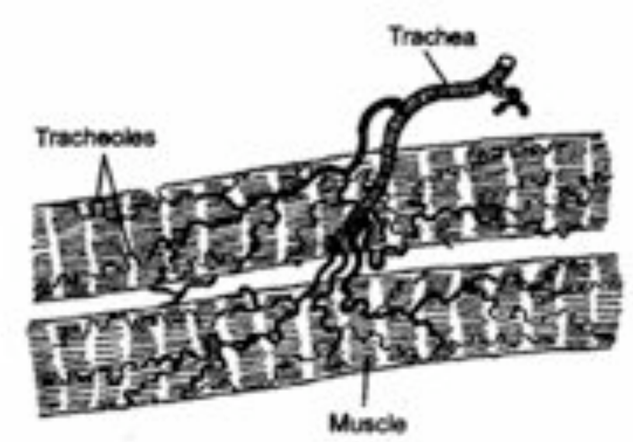
Larval Photo by Drees. http://insects.tamu.edu/fieldguide/cimg264.html
Adult Photo by Paul Opler http://www.npwrc.usgs.gov/resource/distr/lepid/bflyusa/sc/461.htm

In *Calpodes* and larvae from 13 other families of Lepidoptera

- Most have spiracle 8 large than spiracle 7
- All have tufts associated with 8th spiracle
- All have a distinct pattern of tracheation in the telson





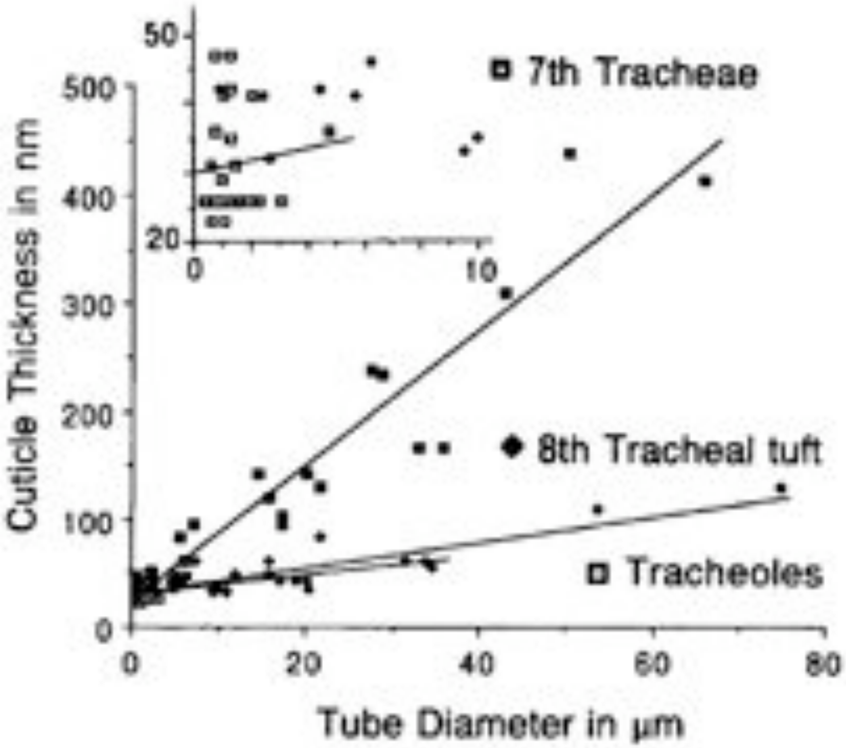


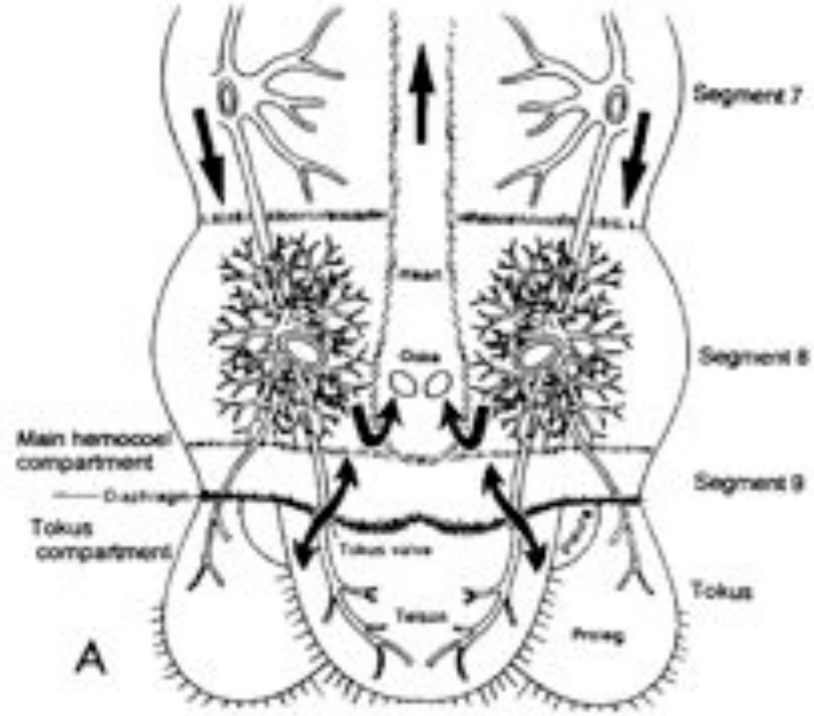

The tracheal system in the last three segments of a live caterpillar.

**Gaseous exchange occurs through tracheoles that penetrate between cells.**

**Not all tissues are permanently tracheated (i.e. hemocytes)**

**Not all trachea supply cellular tissues (i.e. tufts at spiracle 8)**



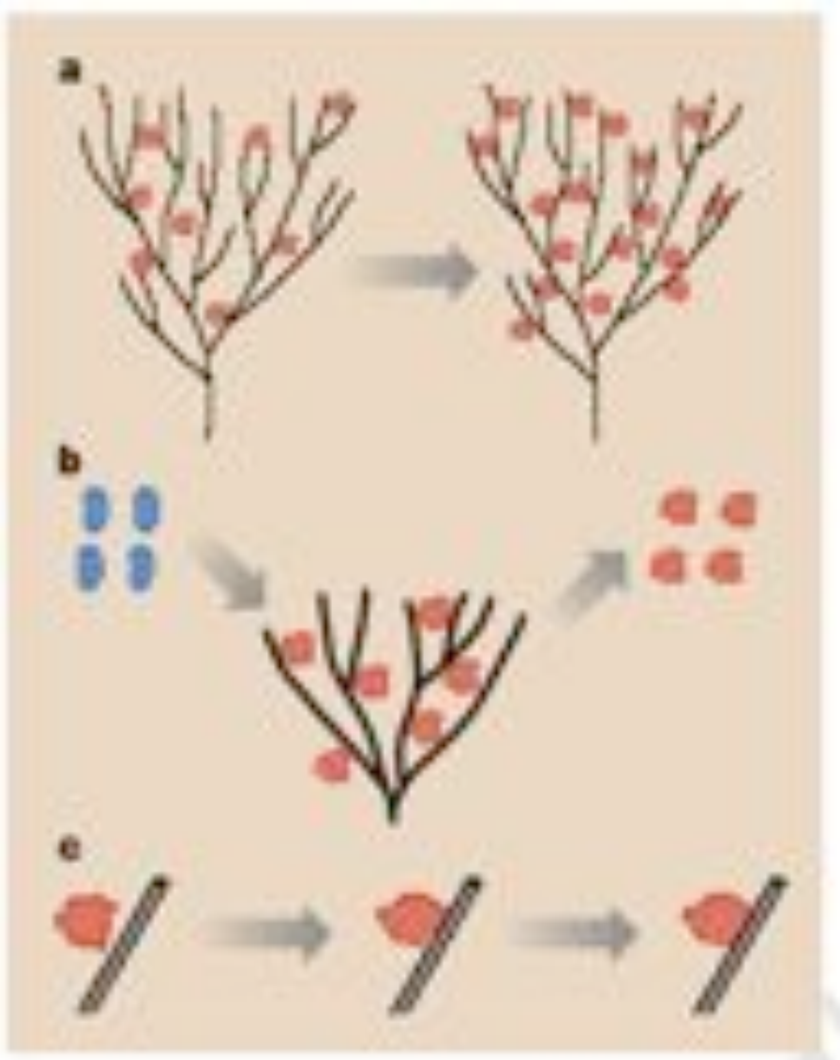



## How do tufts differ from trachea in other segments?

- Terminal tracheoles turn back on themselves and end in knots in hemolymph
- Cuticle between the taenidia is very thin
- Attachment to muscle and connective tissue strings suspending from the heart keep tufts in constant motion
- Aerating trachea

(Locke 1998)

# The branched tufts of trachea and tracheoles that provide blood cells with oxygen




(Mill 1998)

a) **The number of hemocytes (red) in a tuft increases when a caterpillar is subjected to oxygen starvation.**

b) **Oxygen-starved granulocytes (blue) entering a tuft resume the characteristics of those in a well-oxygenated environment (red).**

c) **In the tokus – a 'lung'-like compartment — the hemocytes become closely apposed to the thin-walled tracheae and tracheoles.**

## Common misconception:

Insect tracheal system is inefficient at transport of gases

## Reality:

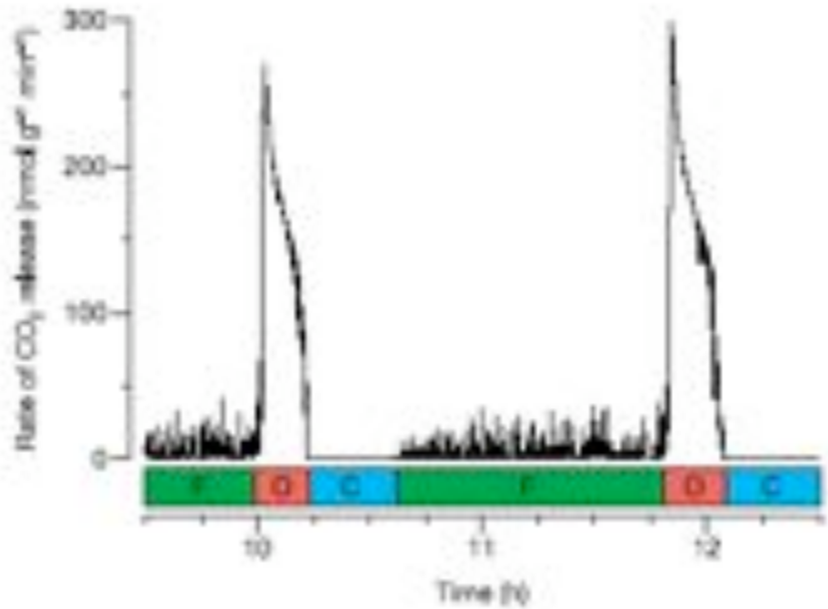
In air, oxygen is delivered 200,000 times faster and carbon dioxide 10,000 faster than in blood.

The largest insects known to exist would get adequate oxygen supply and carbon dioxide removal through simple diffusion (e.g *Meganeura monyi,* ancient dragonfly with a wingspan of 70cm, lived 280 mya)

Burmester 2005

# Discontinuous gas-exchange cycle:

Spiracles remain closed for hours or days and open occasionally for a few minutes




The rate of release of $CO_2$ from a pupa of *Attacus atlas* over time.

Hetz and Bradley 2005

- A burst of $CO_2$ release is observed during the open phase (O, red bar). Open phase is initiated by critically high $CO_2$.

- During the closed phase (C, blue bar), the spiracles are closed and $CO_2$ release is low.

- The closed phase is followed by a flutter phase (F, green bar) during which $CO_2$ release occurs in brief intervals. Flutter phase is initiated by critically low levels of $O_2$.

**Why do insects stop breathing?**

**1) Reduce water loss through the spiracles**

**2) Adapt to an under ground life style-hypoxia**

**3) To avoid oxygen toxicity**

**Oxygen is a double-edged sword.**

- Reactive oxygen species can damage proteins, DNA, and lipids.
- Sufficient oxygen levels are required for efficient mitochondrial respiration.

The insect respiration system has been designed to function most efficiently at high levels of $O_2$ consumption.

The DGC respiratory pattern is the insect's attempt to use a high capacity system during periods of "metabolic idling".
DGC is observed only in resting insects.
DGC disappears when insects increase their metabolic rate when cells use oxygen at a faster rate.

DGC=discontinuous gas-exchange cycle

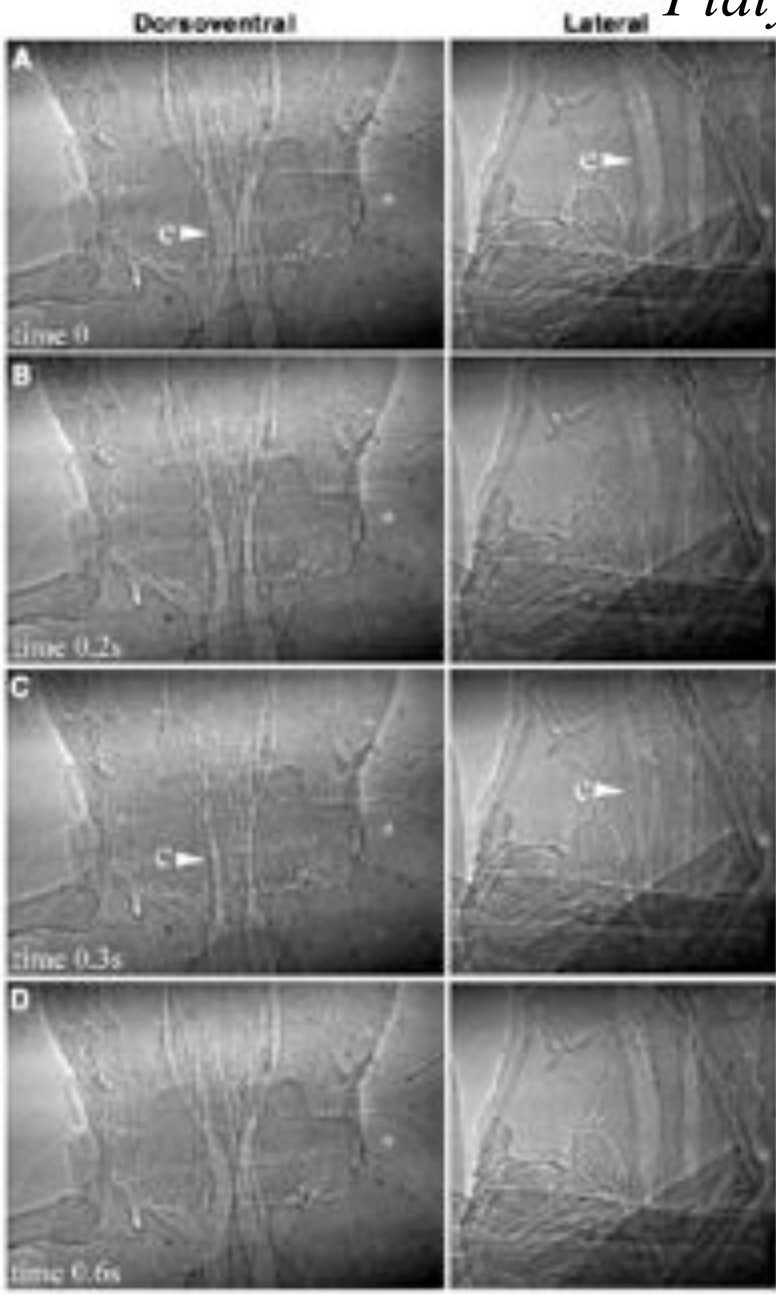
**Mechanisms for insect respiration:**

- Passive gas diffusion (Krogh 1920)
- Changes in internal pressure due to hemolymph pumping by heart or by muscle contraction (Wasserthal 1996)
- Autoventilation- body movements change volume of tracheal tubes or air sacs (Slama 1999)
- **Compressing and expanding the trachea (like the way vertebrates fill their lungs)**

**Misconception:**
Insect cannot breathe

**Use technology to visual insects breathing**
A synchrotron, a circular particle accelerator that can generate x-rays was used to look inside living insects.
Videos of the movements can be created.

# Respiration by tracheal compression in the head and thorax of beetle *Platynus decentis*




A- tracheal tubes expanded at rest arrowhead e
B-compression occurs throughout the anterior region of the insect
C- maximal compression
Arrowhead c
D- compression followed quickly by expansion of the trachae

Entire cycle takes less then 1 second

Westneat *et al.* 2003

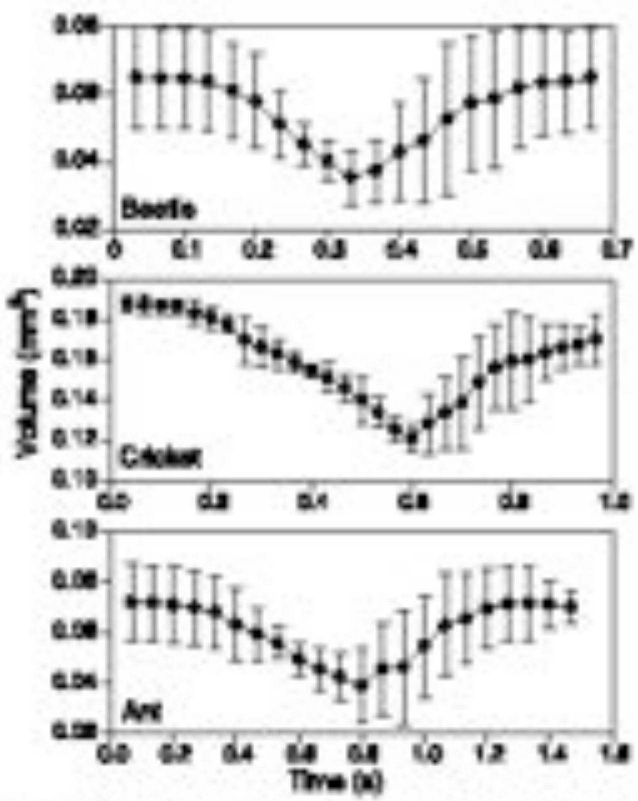



Fig. 3. Volume change in the main tracheae of the anterior thorax and head of the wood beetle, house cricket, and carpenter ant during respiratory cycles. Error bars, standard deviation of the mean of three respiratory pumping events in different individuals.

# Advantages of rapid, active breathing mechanism

- Rapid conduction of gases when insects are respiring at high rates (e.g. stress, flight, locomotion)- 50% volume change

- Aide oxygen diffusion to tissues- increased pressure will raise the diffusion gradient of oxygen across the tracheole-tissue boundary when spiracles are closed

Westneat *et al.* 2003

## Breathing observed in:

**Endopterygotes (beetles, butterflies, flies)**
**Hemiptera, Orthoptera, Dermaptera, Blattodea, and Odonata**

## Mechanism of tracheal compression

- Contraction of jaw or limb muscles cause elevated pressure inside the exoskeleton
- When muscles relax the tracheae expand due to support from rings of taenidia in the tracheal wall

**Active tracheal breathing may have played an important part in the evolution of terrestrial locomotion, running performance, and flight in insects, and it may be a prerequisite for oxygen delivery to complex sensory systems and active feeding mechanisms.**

**Burmester, T. 2005. A welcome shortage of breath. Nature. 433: 471-472**

**Hetz, S.K. and T.J. Bradley. 2005. Insects breathe discontinuously to avoid oxygen toxicity. Nature. 433: 516-519.**

**Locke, M. 1998. Caterpillars have evolved lungs for hemocyte gas exchange. Journal of Insect Physiology. 44(1):1-20.**

**Mill, P.J. 1998. Caterpillars have lungs. Nature. 391:129-130.**

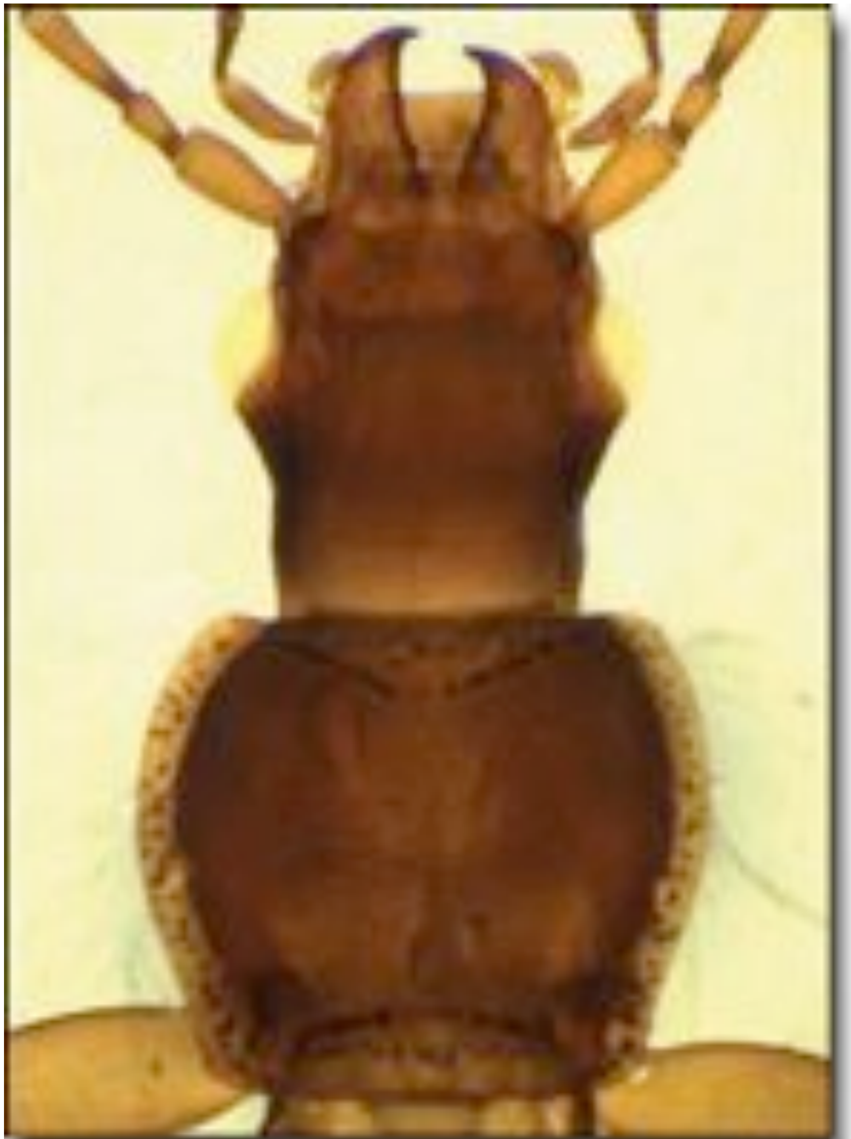
**Slama, K. 1999. Active regulation of insect respiration. Annals of the Entomological Society of America. 92 (6): 916-929.**

**Westneat, M.W., Betz, O., Blob, R.W., Fezzaa, K. Cooper, W.J., and W-K. Lee. 2003. Tracheal respiration in insects visualized with synchrotron x-ray imaging. Science. 299:558-560.**

Locke, M. 1998. Caterpillars have evolved lungs for hemocyte gas exchange. J. Insect Physiol. 44:1-20.
Hemocytes accumulate under anoxia conditions on thin-walled tracheal
tufts near the 8th (last) pair of abdominal spiracles. Stoff's idea (This may also be the reason why they accumulate around the caudal spiracle area in fly larvae).

Slama, K. 1999. Active regulation of insect respiration. Ann. Entom. Soc. Amer. 92: 916-929.

Respiratory function of the hemolymph in insects



X-rays shot through this wood beetle revealed an unknown insect breathing mechanism.
*Image courtesy of the Field Museum.*
http://www.anl.gov/Media_Center/Frontiers/2004/c3facil.html

**Living insects reveal breathing mechanism**

Scientists from The Field Museum in Chicago and Argonne, using Advanced Photon Source (APS) X-ray beams, discovered a surprising new insect breathing mechanism that is similar to lung ventilation in vertebrates.

"The discovery of this fundamental aspect of respiratory biology for insects could revolutionize the field of insect physiology," said Mark Westneat, associate curator of zoology at The Field Museum.

Insects—the most numerous and diverse group of animals—don't have lungs. Instead, they have a system of internal tubes called tracheae that are known to exchange oxygen through slow, passive mechanisms, including diffusion. But this new study demonstrates that beetles, crickets, ants, butterflies, cockroaches, dragonflies and other insects also breathe through the use of rapid cycles of tracheal compression and expansion in their head and thorax.

Tracheal compression was not found for all types of insects studied, but for those where it was found, the compression patterns varied within individuals and between species. The three species most closely studied—the wood beetle, house cricket and carpenter ant—exchange up to 50 percent of the air in their main tracheal tubes approximately every second. This is similar to the air exchange of a person doing moderate exercise.

Until now, it has not been possible to see such movement inside living insects. This problem has been solved by using the brilliant X-rays at the APS to obtain videos of living, breathing insects.

"This is the first time anyone has applied this technology to obtain highly detailed, real-time video images of the internal organs of living insects," said Argonne physicist Wah-Keat Lee.

One aspect of the technique that makes the videos so revealing is edge enhancement, which highlights the edges of some internal organs.

This effect is due to the special properties of the APS's X-ray beams. "It's almost as if parts of the anatomy have been outlined in pencil, like a drawing in a coloring book," Lee explained. This work may lead to powerful new techniques for studying how living animals function, he added.

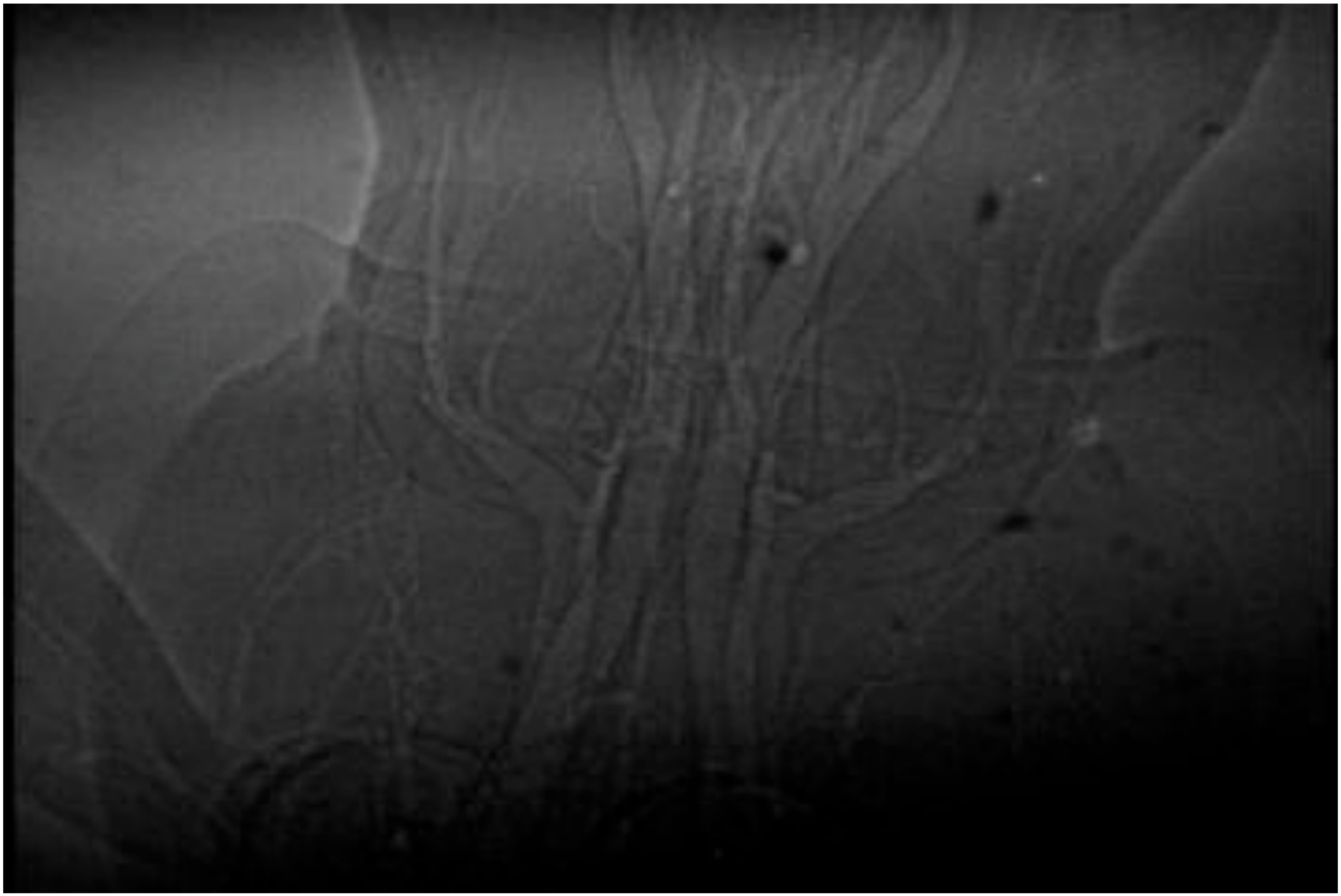
Indeed, Westneat, Lee and their collaborators are already aiming the synchrotron at the jaws of insects to see how they chew. "Most of the 12 moving parts in an insect's jaw mechanism are internal, so our inability to see inside living, moving insects has prevented us from understanding how these parts work together," Lee said.

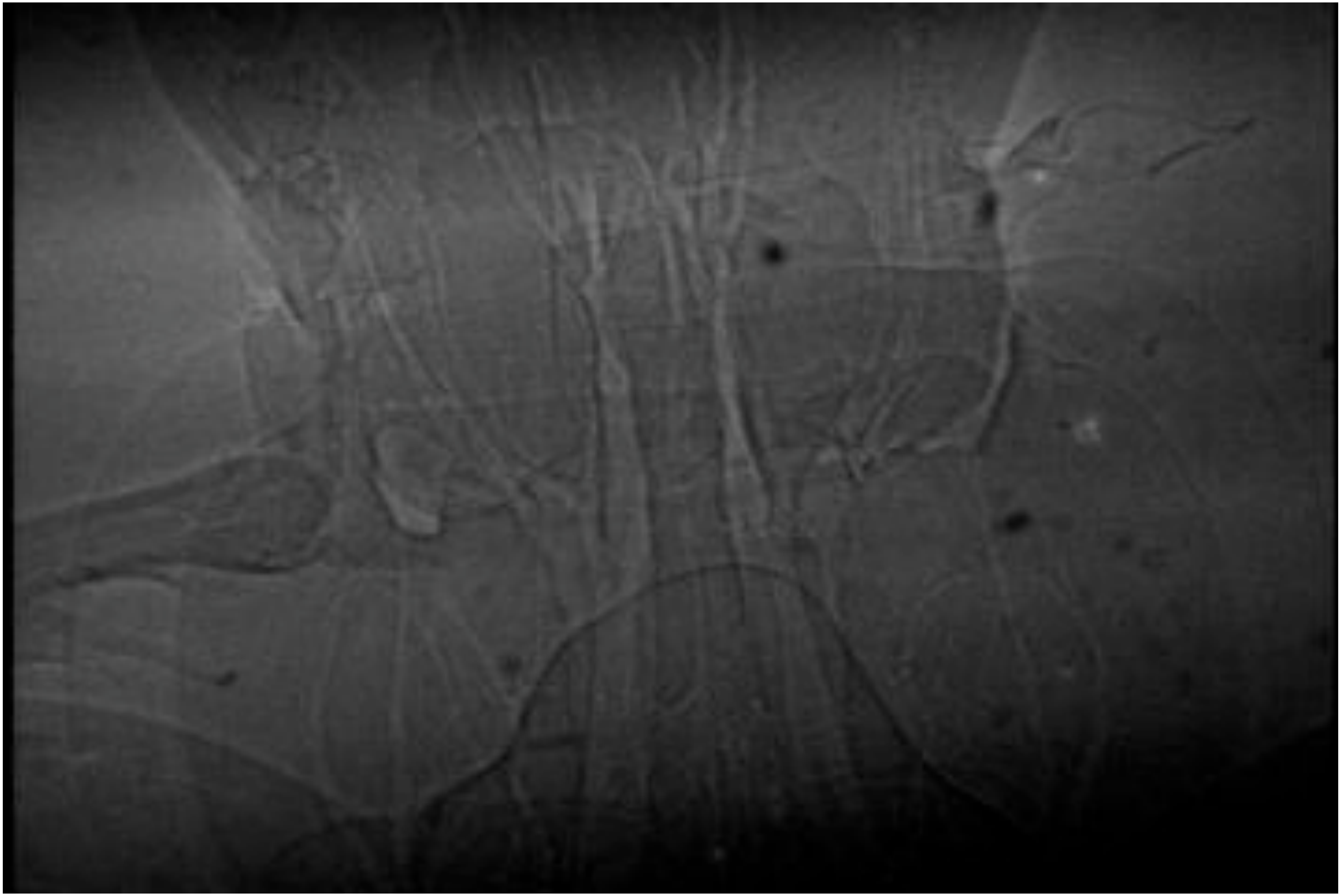
Westneat envisions using similar videos to study animal functions, biomechanics and movements. New discoveries about animal function can have broad implications. For example, active tracheal breathing in the head and thorax among insects may have played an important role in the evolution of terrestrial locomotion and flight in insects, and be a prerequisite for oxygen delivery to complex sensory systems and the brain.

This research could not only help scientists learn more about the animals studied but could also provide insights into human health. For example, studying how larval fish move their backbones could shed light on how to treat spinal cord injuries in humans. Likewise, studying the walls of blood vessels in mice and the tiny hearts in beetles—each beetle has eight to ten hearts—could shed light on high blood pressure.

"Basic principles of mammal, fish or insect physiology and function could have important implications for health care," Westneat says. "We intend to develop this novel technique for a range of applications that will greatly improve our knowledge of how tiny animals live and function."

Inserted and on the CD are the following 3 video clips showing the action of the tracheal system and breathing. Go to the file and change the format to insert into the powerpoint.





1078008S1.mov

1078008s3.mov

1078008s2.mov

# 3. Gaseous exchange in aquatic insects

## Aquatic insects obtain oxygen from the air, that dissolved in the water, or from both

**For those that must come to the surface of the water, they face two major problems. What are they?**

1. **Breaking the surface tension (hydrofuge hairs)**
2. **Preventing water from entering the spiracle (perispiracular glands) in dipterous larvae**

a. Cutaneous respiration
b. Tracheal gills
c. Plastron respiration
d. Respiratory siphons

**Table 4A.** Respiratory options with open and closed tracheal systems. (The life stages known or inferred to use a particular respiratory option are indicated by the following: L = larvae/nymphs; P = pupae; A = adults.)

| Respiratory Option | Tracheal System | Oxygen Source | Examples | |
|---|---|---|---|---|
| Atmospheric Breathers | open | atmosphere | Diptera: Culicidae (L, P), Dolichopodidae (L), Ephydridae (L, P), Psychodidae (L), Stratiomyidae (L, P), Syrphidae (L, P), Tabanidae (L, P), Tipulidae (L, P), Ptychopteridae (L, P)<br>Coleoptera: Amphizoidae (L), Dytiscidae (L, A), Hydrophilidae (L, A)<br>Hemiptera: Nepidae (L, A) | |
| Plant Breathers | open | plants | Coleoptera: Chrysomelidae (L, P, A), Curculionidae (L)<br>Diptera: Culicidae (L, P), Ephydridae (L, P), Syrphidae (L) | |
| Temporary Air Store | open | atmosphere and dissolved | Coleoptera: Dytiscidae (A), Gyrinidae (A), Haliplidae (A), Helodidae (A), Hydraenidae (A), Hydrophilidae (A)<br>Hemiptera: Belostomatidae (L, A), Corixidae (L, A), Naucoridae (L, A), Notonectidae (L, A), Pleidae (L, A) | |
| Permanent Air Store | | | | |
| Plastrons | open | dissolved | Coleoptera: Curculionidae (A), Dryopidae (A), Elmidae (A), Hydraenidae (A), Hydrophilidae (A)<br>Hemiptera: Naucoridae (L, A)<br>Lepidoptera: Pyralidae (L, P) | |
| Spiracular Gills | open | dissolved | Coleoptera: Psephenidae (L)<br>Diptera: Blephariceridae (L, P), Canaceidae (L, P), Deuterophlebiidae (L, P), Dolichopodidae (L, P), Empididae (L, P), Simuliidae (L, P), Tipulidae (L, P) | |
| Tracheal Gills | closed | dissolved | Ephemeroptera (L), Odonata (L), Plecoptera (L), Megaloptera (L), Neuroptera (Sisyridae) (L), Coleoptera (several families), Diptera (several families), Trichoptera (L), Lepidoptera (Pyralidae) (L) | 677, |
| Cutaneous | closed | dissolved | Diptera: Chaoboridae (L, P), Chironomidae (L, P), Simuliidae (L, P) | |
| Hemoglobin | open or closed | atmosphere or dissolved | Hemiptera: Notonectidae (L, A)<br>Diptera: Chironomidae (L, P) | 4, 2 |

**Table 4B.** Ventilation methods for insects utilizing dissolved oxygen.

| System Ventilated | Ventilation Method | Taxon |
|---|---|---|
| Cutaneous | Undulation | Chironomidae |
| | | Trichoptera (gills lacking) |
| | | Lepidoptera (gills lacking) |
| | Swimming | Chaoboridae |
| | | Chironomidae |
| | Natural water flow | Trichoptera (caseless, gills lacking) |
| | | Plecoptera (gills lacking) |
| | | Simuliidae |
| Tracheal Gills | Beating gills | Ephemeroptera |
| | | Psephenidae |
| | | Corydalidae |
| | | Gyrinidae |
| | Undulation | Trichoptera |
| | | Lepidoptera |
| | | Chironomidae |
| | Leg contractions which move body (push-ups) | Plecoptera |
| | | Lestidae |
| | Rectal pump | Anisoptera |
| | Natural water flow | Heptageniidae |
| | | Plecoptera |
| | | Zygoptera |
| | | Trichoptera (caseless) |
| | | Trichoptera (with case) |
| | | Blephariceridae |
| Temporary and Permanent Air Stores | Leg movements | Notonectidae |
| | | Naucoridae |
| | | Corixidae |
| | Swimming | All taxa that swim with exposed air bubble |
| | Natural water flow | Simuliidae (pupae) |
| | | Dryopidae (adult) |
| | | Lepidoptera (larvae and pupae) |

**Table 4C.** Demonstrations of respiratory processes (*superscripts* refer to Section C. *Useful Equipment*). Table 4B contains relevant literature.

A. *Closed Respiratory System* (larvae only)

1. Ventilation Methods and Behavior
   a. Beating gills[1,2,8] (e.g., burrowing, climbing, sprawling Ephemeroptera; Corydalidae)
   b. Push-ups[1,8] (e.g., Plecoptera, Lestidae)
   c. Undulation[1,2,5] (e.g., Trichoptera, Lepidoptera, Chironomidae)
   d. Muscular rectal pump[1,8] (Anisoptera)
   e. Swimming[1] (e.g., Chaoboridae, Chironomidae)
   f. None (other than possible position change) (e.g., Blephariceridae, Simuliidae, fast-water Ephemeroptera, Plecoptera)
2. Respiratory Currents Produced by Insect (Section A.1.a–d)[8]
3. Micro-areas from which Respiratory Water Obtained (Section A.1.a–d)[8]
4. Environmental Effects on Ventilation Frequency and Volume of Respiratory Flow (Section A.1.a–d)
   Vary: $O_2$ concentration[10]
   water current[7]
   temperature[11]

B. *Open Respiratory System* (larvae and adults)

1. Ventilation Methods and Behavior
   a. Leg movements[5] (e.g., Corixidae, Naucoridae, Notonectidae)
   b. Swimming[5] (any species with exposed air store)
2. Environmental Effects on Diving Time (any species with temporary air store)
   Vary: dissolved $O_2$ concentration[5,10]
   dissolved $CO_2$ concentration[5,10]
   temperature[5,11]
3. Diving Stimulus (any species with temporary air store)
   Provide: air atmosphere[5,10]
   $O_2$ atmosphere[5,10]
   $CO_2$ atmosphere[5,10]
   $N_2$ atmosphere[5,10]
4. Need for Surface Tension to Establish Atmospheric Connection[5,9] (e.g., Culicidae, Tipulidae, Syrphidae, Notonectidae, Dytiscidae)
5. Plastron (any species using plastron respiration only)
   Vary: dissolved $O_2$ concentration[1,10]
   current velocity[1,7]
   temperature[1,11]

### 3. Gaseous exchange in aquatic insects

a. Cutaneous respiration-occurs directly through the cuticle and insects are usually in a liquid medium. Probably the way most endoparasitic hymenopterans get oxygen.

b. Probably accounts for some oxygen uptake in most aquatic insects

## 3. Gaseous exchange in aquatic insects

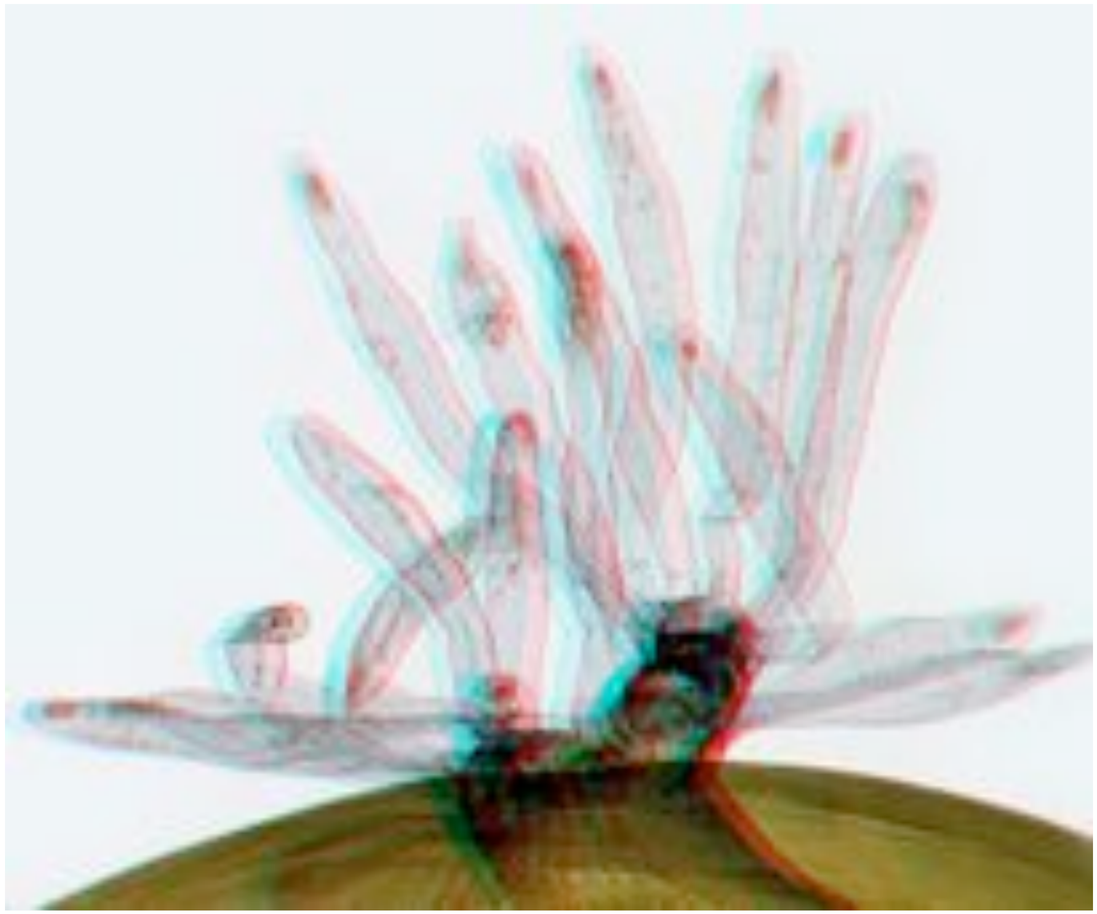
b. Tracheal gills

Are found in immatures of:

1. Odonata
2. Plecoptera
3. Trichoptera
4. Megaloptera that are aquatic
5. Neuroptera
6. Some aquatiac Coleoptera
7. Some Diptera
8. Some pyralid Lepidoptera
9. Ephemeroptera

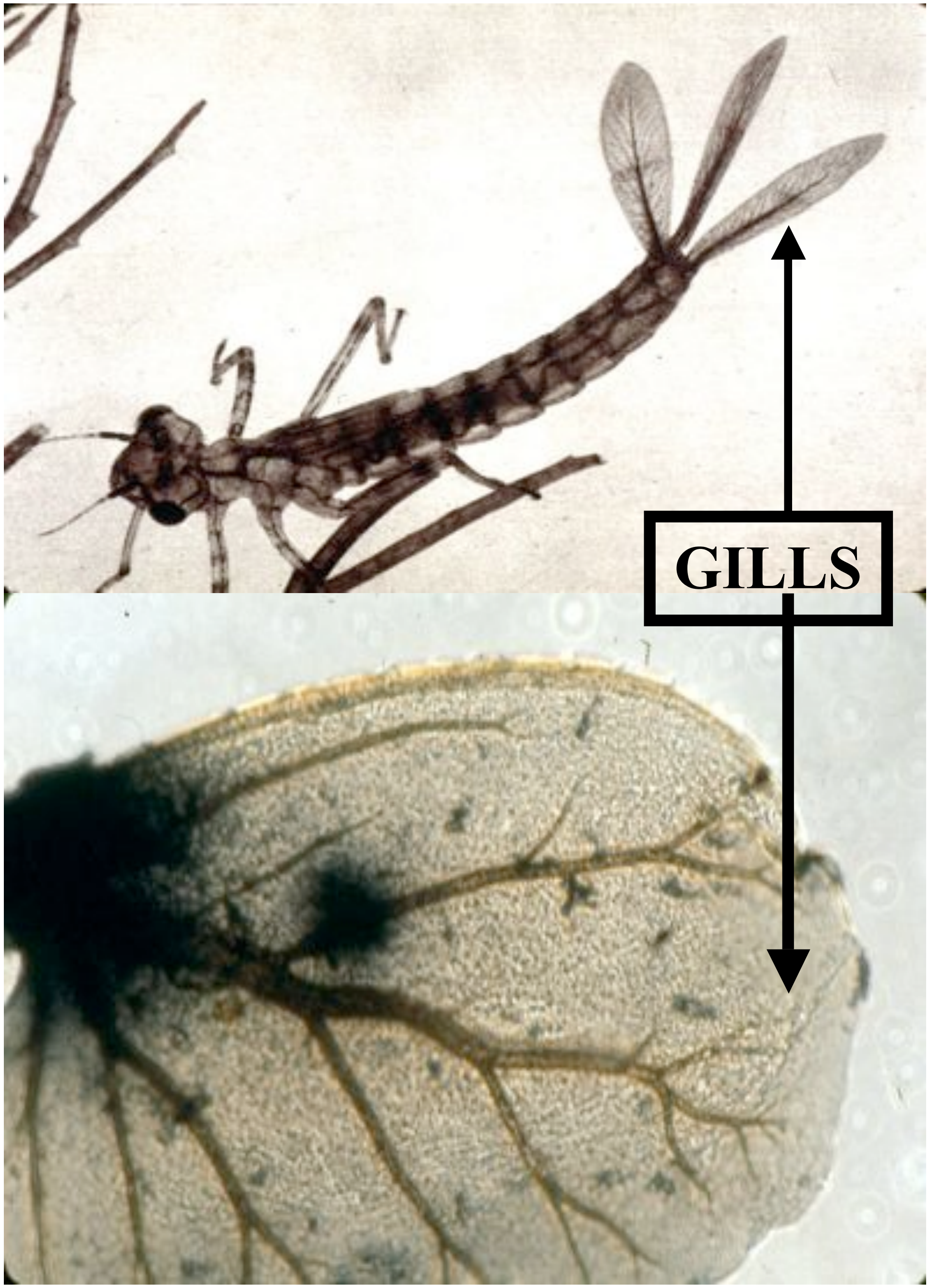
In insects with leaf-like or plate-like gills they are usually moveable by muscles. This permits greater oxygen supply by stirring up the water.

Under low oxygen, plecopteran naiads do ‘push-ups’ to move more water over the gills.

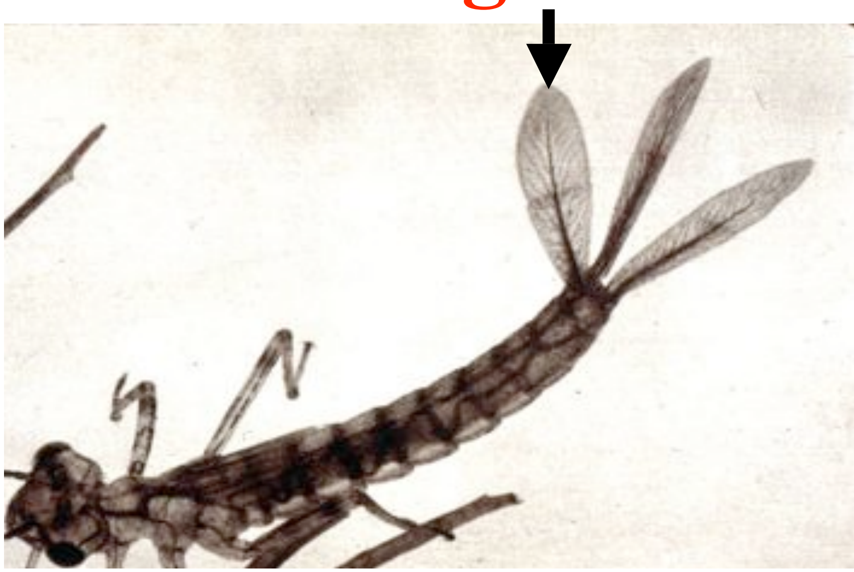


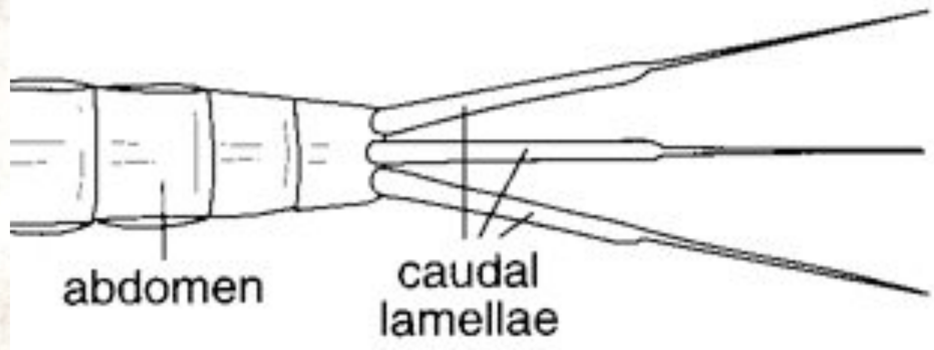
TRAHEAL GILLS OF CADDISFLY

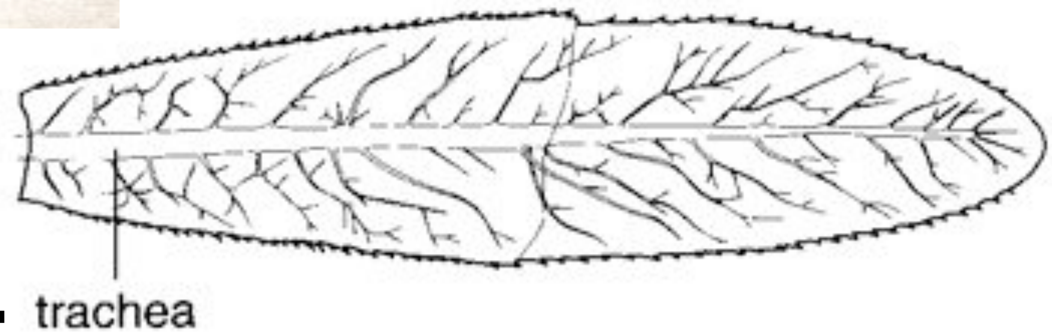
# tracheal gills in aquatic insects

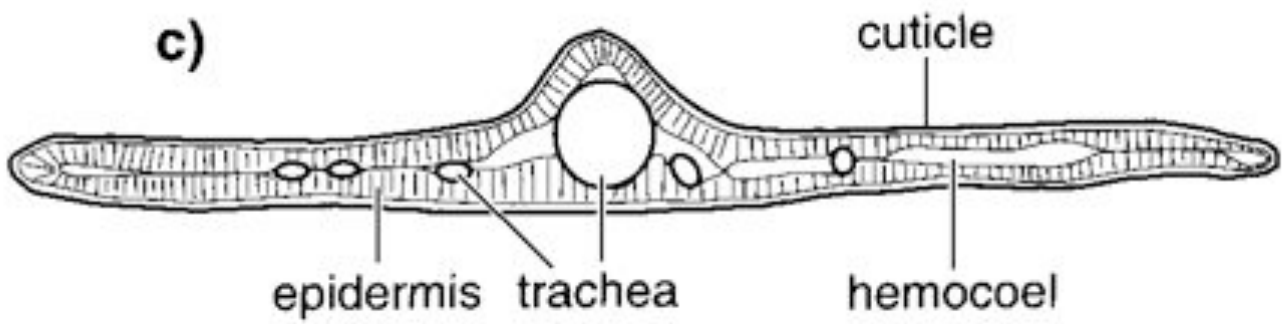



# Tracheal gills of the damselfly larva

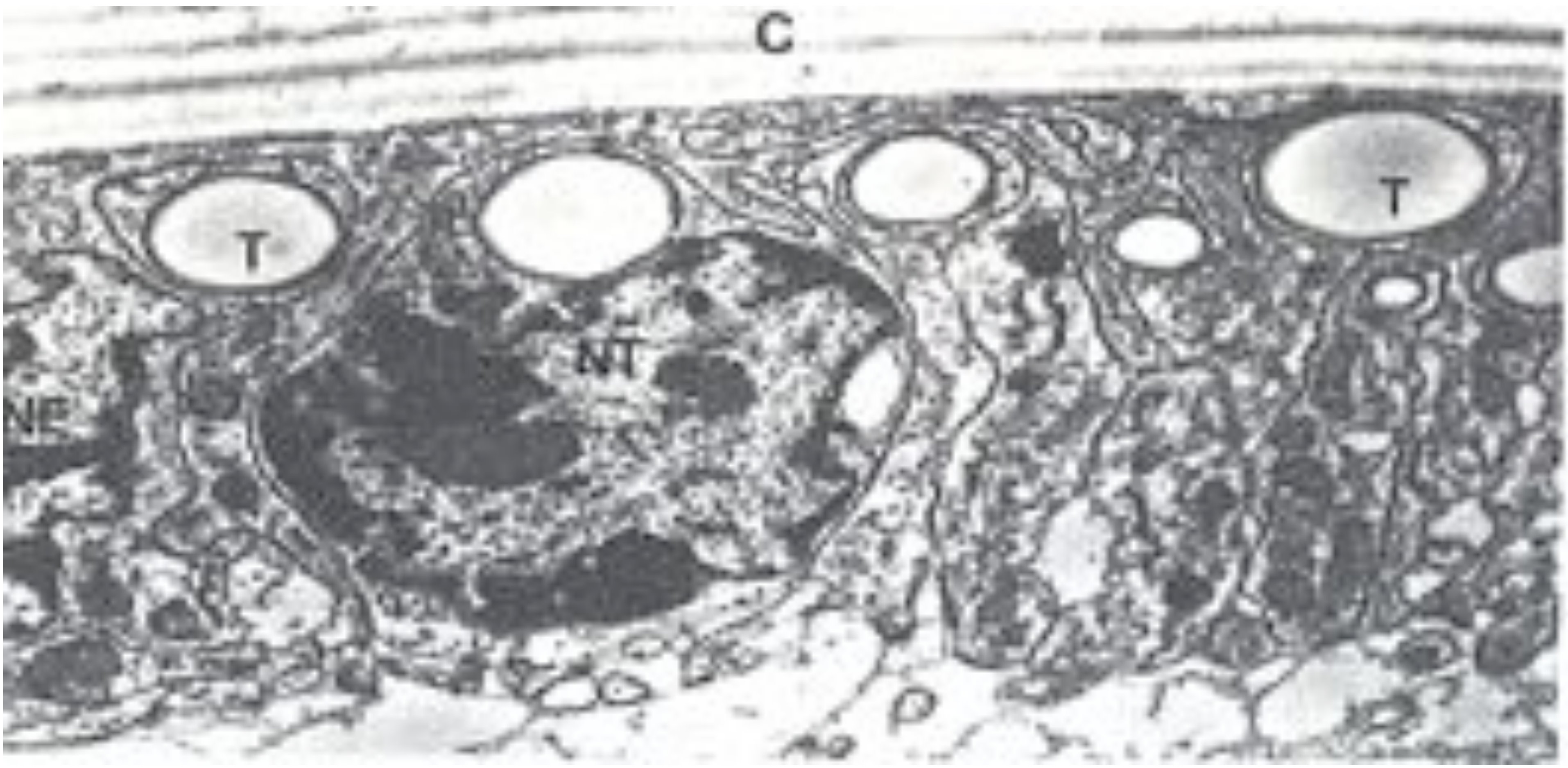




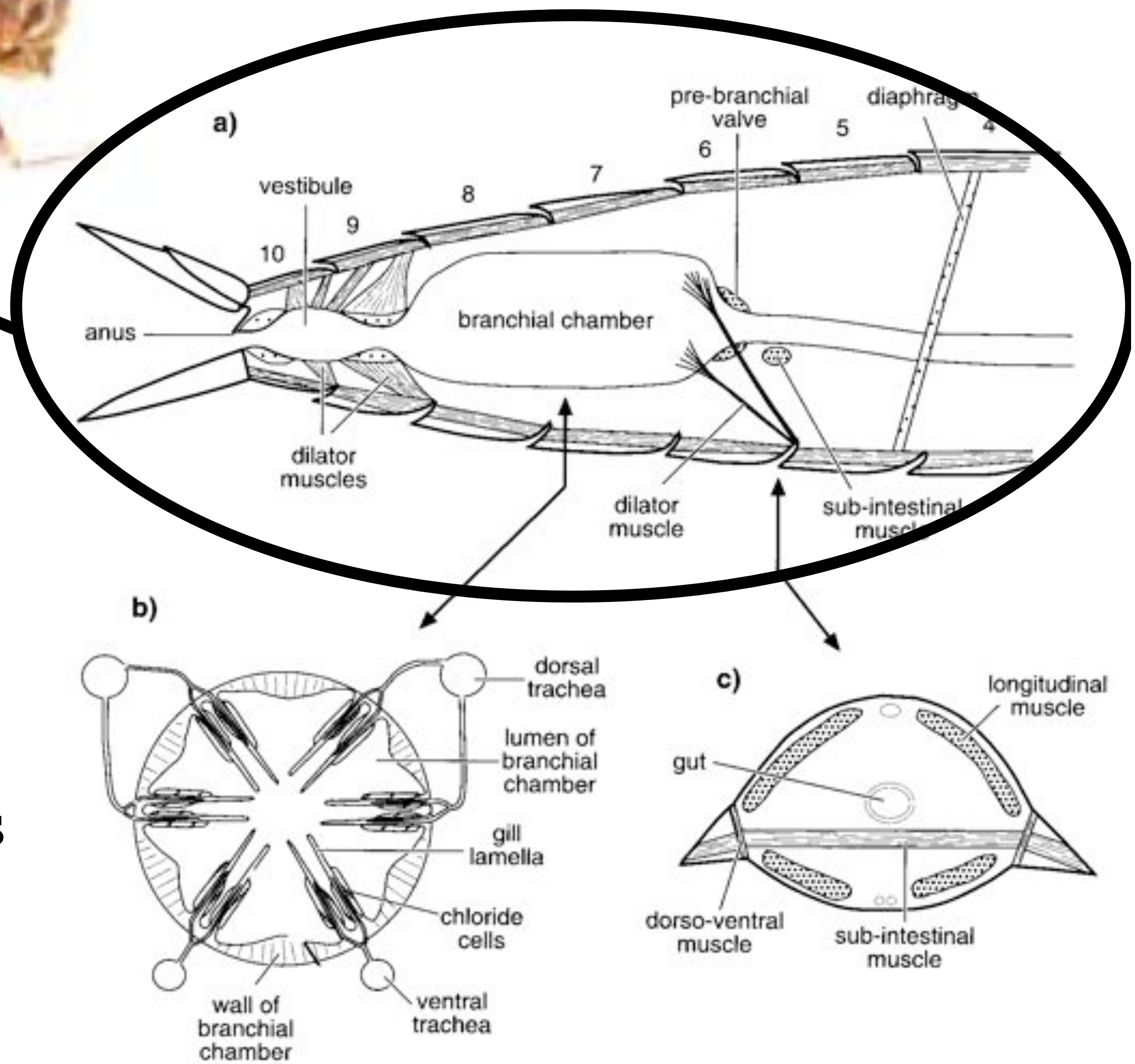



TEM cross-section through caudal gill of larva of damselfly.
C=cuticle
T=tracheoles
NE=nucleus of epithelial cell
NT=nucleus of tracheoblast



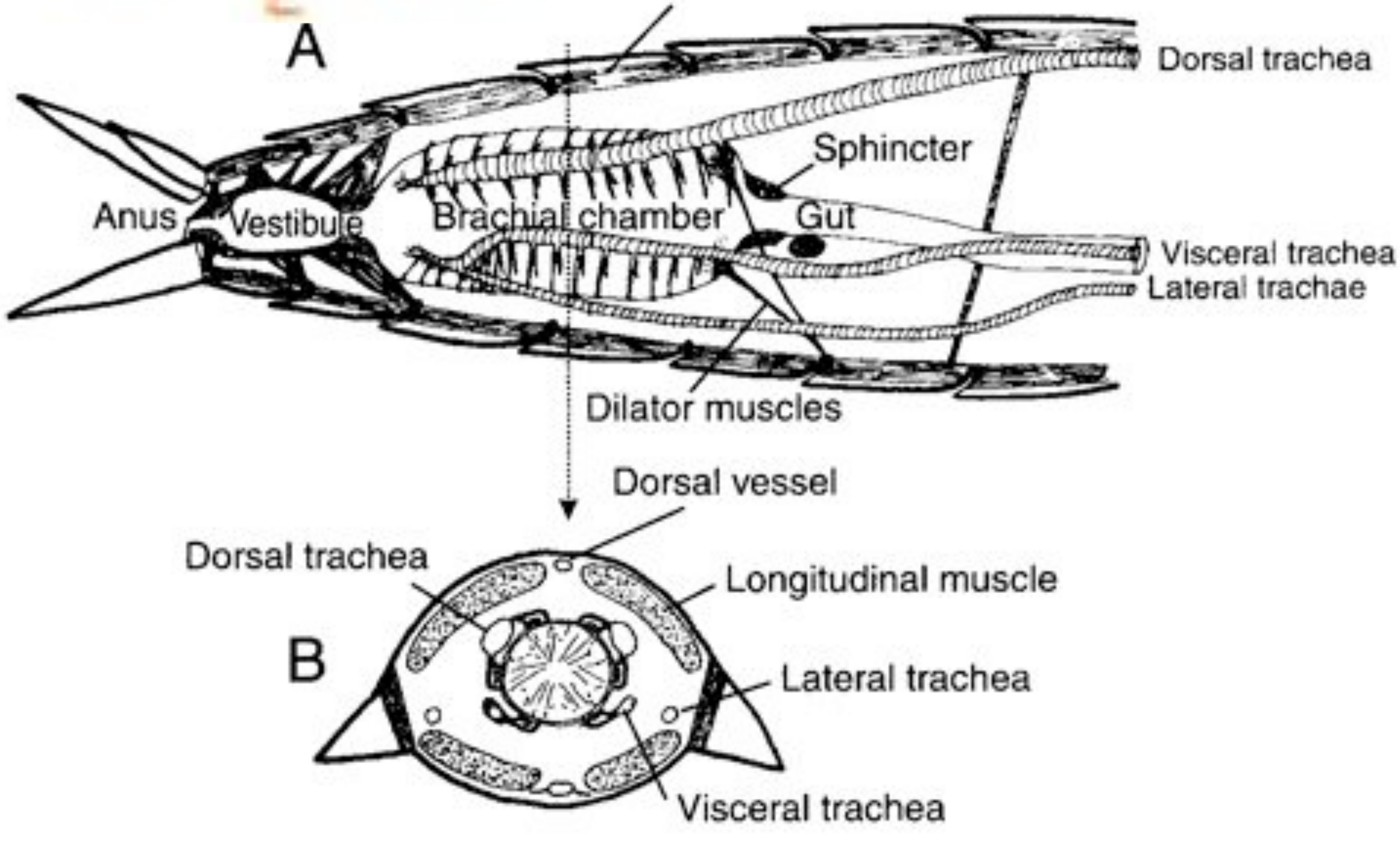
Brachial chamber also houses tracheal gills. Remember, this is a modified hindgut.




Chloride cells in the rectal chamber of dragonfly naiad for retrieval of salt ions. Also in the brachial chamber are tracheal gills that take oxygen to all parts of the naiad.

Systems involved in rectal respiration in dragonfly naiad:

1. Muscular
2. Respiratory
3. Nervous
4. Circulatory



## 3. Gaseous exchange in aquatic insects

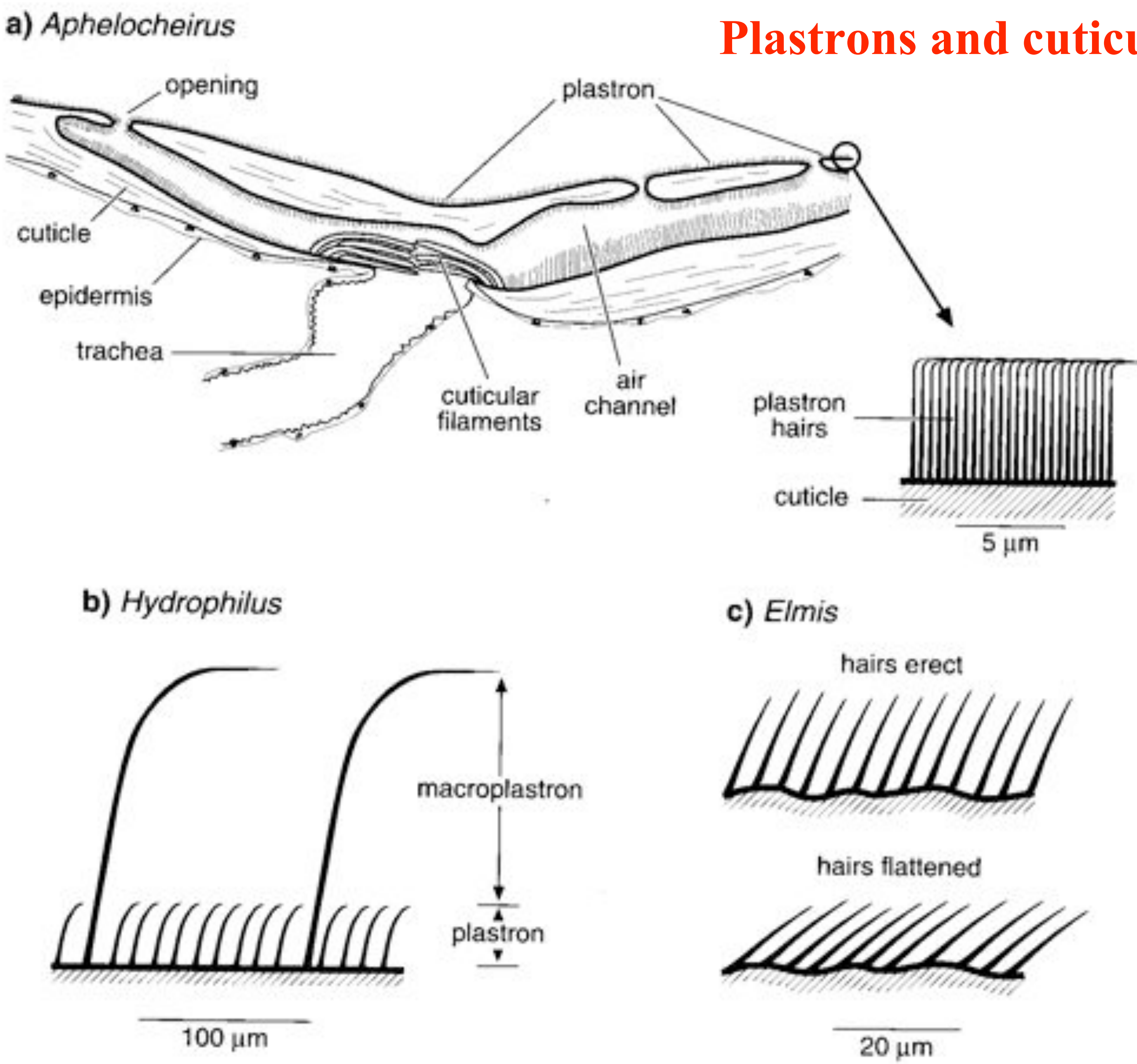
a. Temporary (compressible) gas gills-present in those insects that store air in bubbles or use hairs to store air but in both cases the air is depleted, thus compressible.

b. Permanent (incompressible) gas gills-

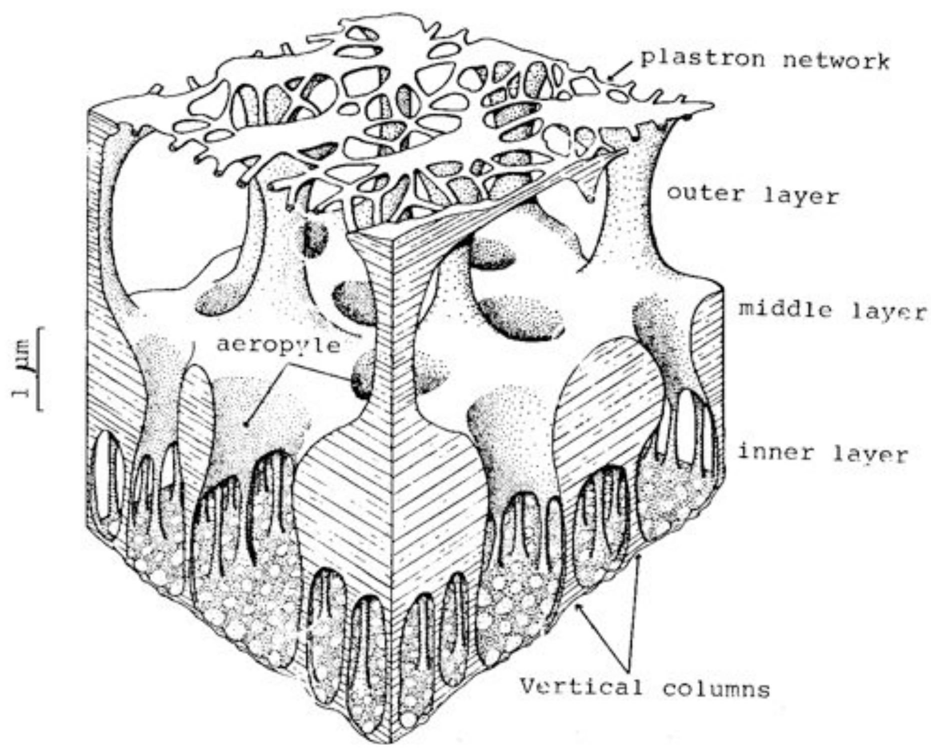
(1). **PLASTRONS**

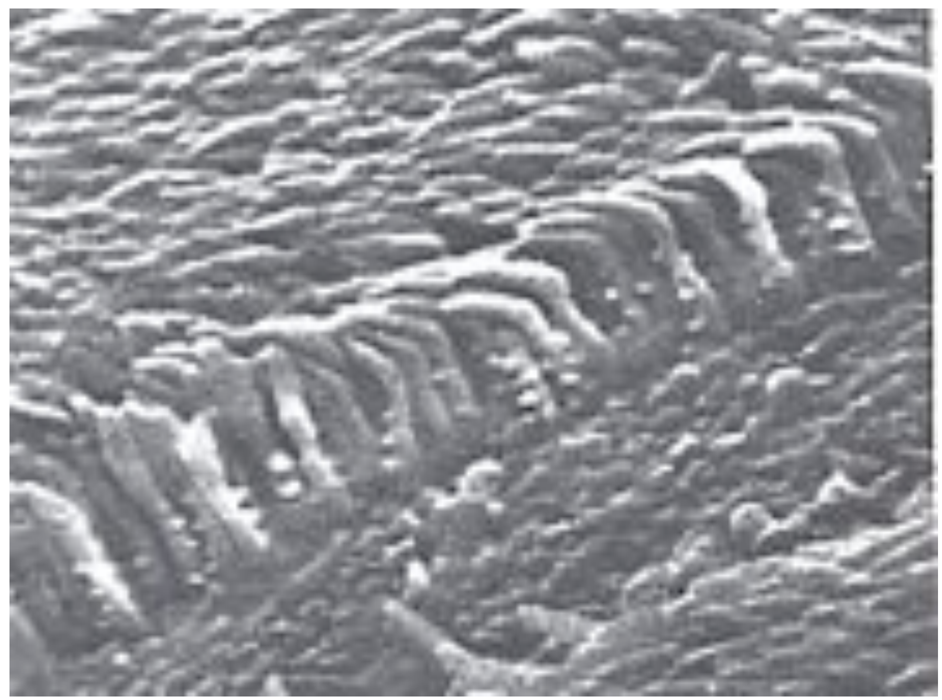
Gas is held in place either by hairs or cuticular modifications that permanently trap air. This trapped air then serves as a physical gill

# Plastrons and cuticular hairs

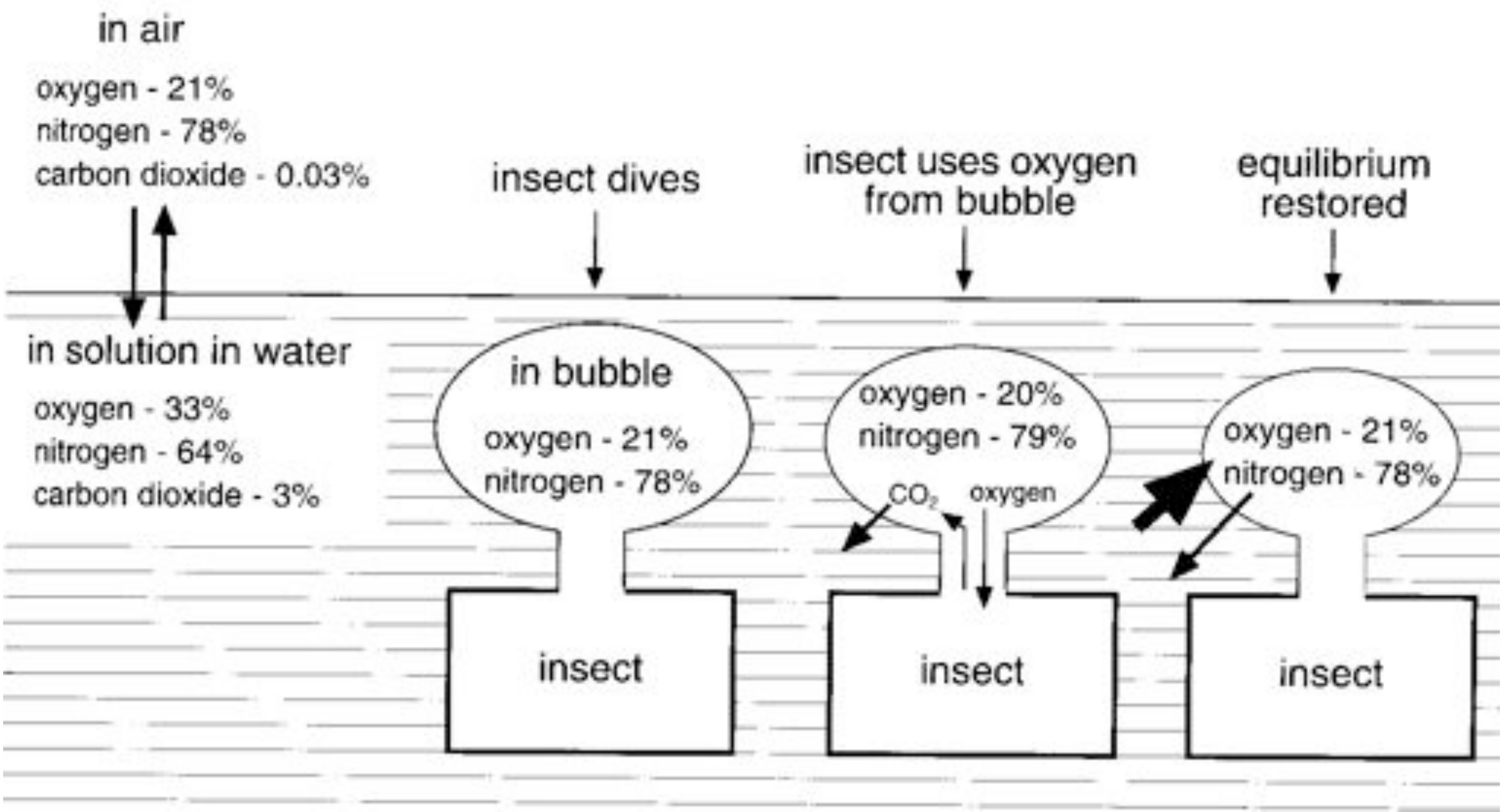


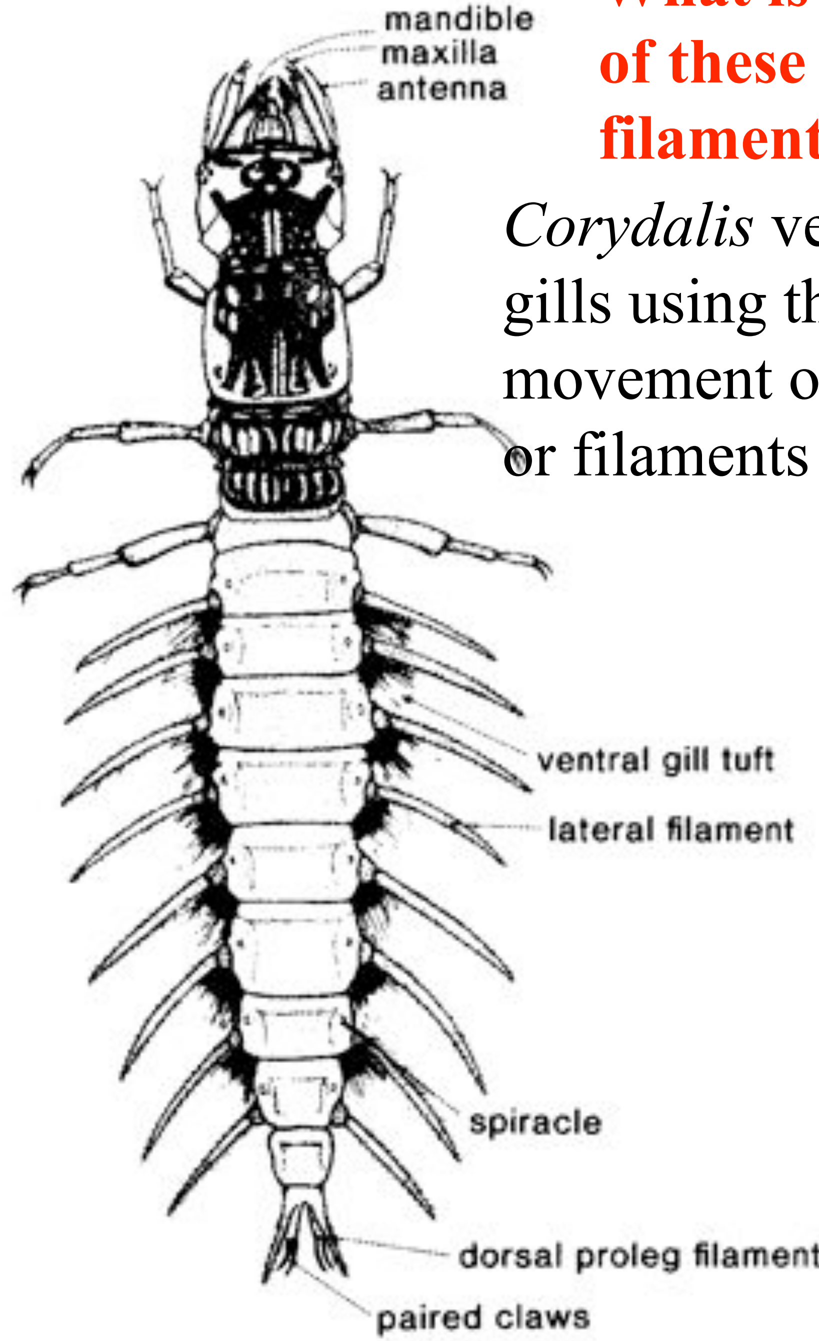
Plastrons of various aquatic larvae on the right showing the physical corrugations or pitting that makes the plastron that captures air in its interstitial cuticular areas and holds it there. Below is a cutaway showing these interstitial spaces of an egg plastron of a fly.



# Physical gills






**What is the function of these lateral filaments?**

*Corydalis* ventilates the gills using the rhythmic movement of the tubercles or filaments

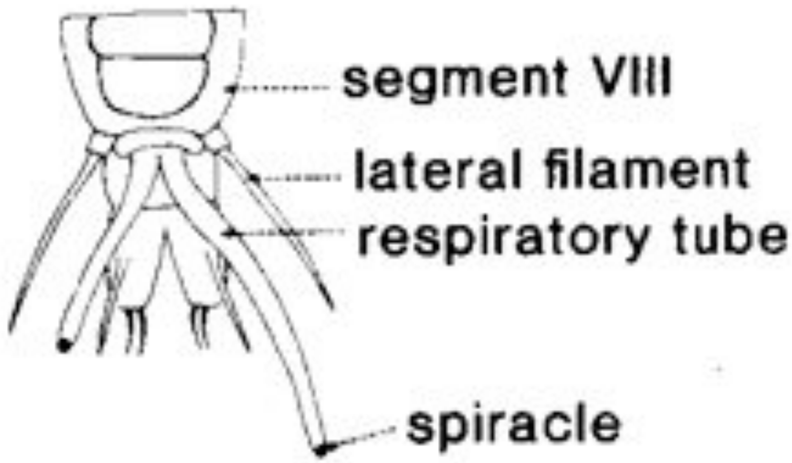



Figure 15.18

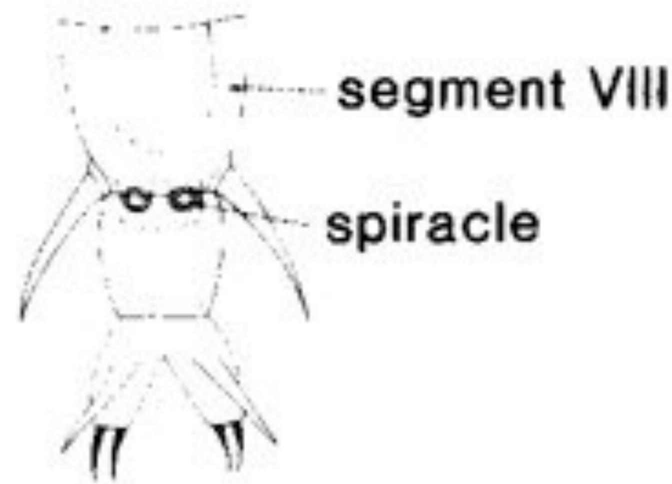



Figure 15.19

Different views of the posterior, segment 8 of various larvae of different species of hellgrammites. Note spiracle in addition to lateral filaments and gills

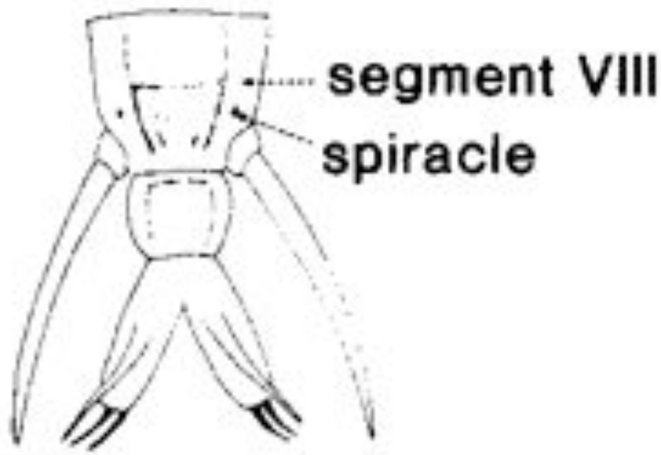



Figure 15.20

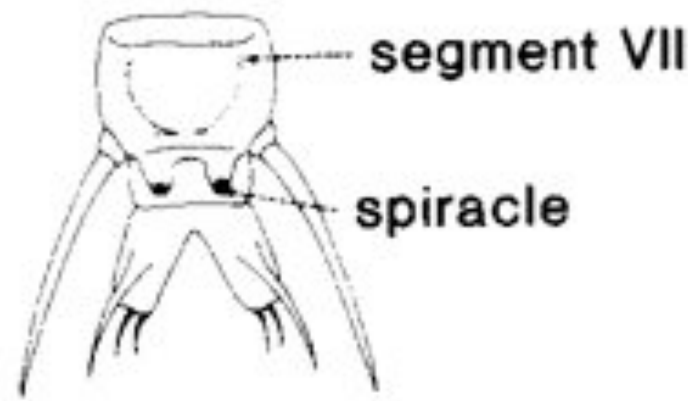
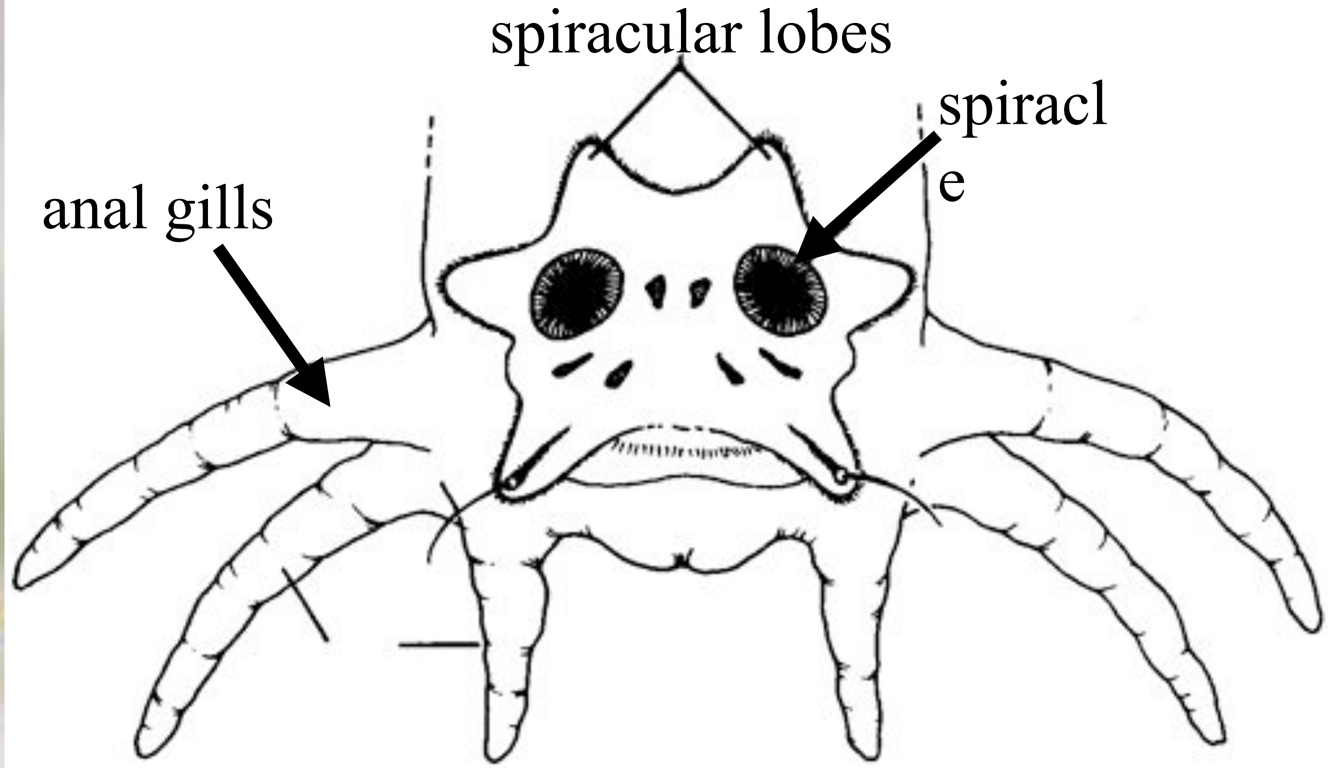



Figure 15.21




Anal gills and caudal spiracles of the larva of the crane fly or Tipulidae.

# Air stores for aquatic insects-**open directly into the spiracle.**

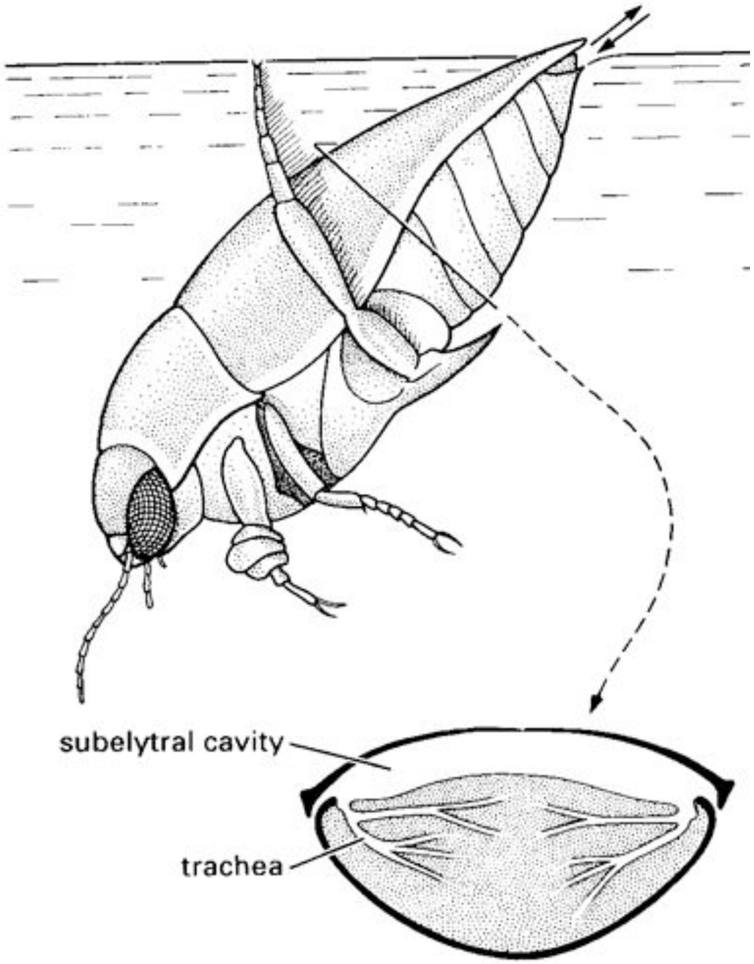
**Hydrofuge hairs cover the spiracle.**

*Dytiscus* beetles carry a bubble or store of air at their posterior ends. They can control how much air is carried by filling the rectal ampulla with water. They can control how much air is carried in the sub-elytral space. This sub-elytral air is also important in diving in addition to supplying some oxygen. Oxygen content of the elytral air falls from 19.5 percent at the moment of the dive to about 1 percent or less 3 to 4 minutes into the dive.

A similar thing also occurs in *Notonecta, Corixa, Nepa and Hydrophilus*. This stored air in hydrofuge hairs serves two purposes:

1. Respiration
2. Making dives and returning to the surface in the correct position

# Air stores for aquatic insects




Cross-section showing the air store cavity or subelytral cavity

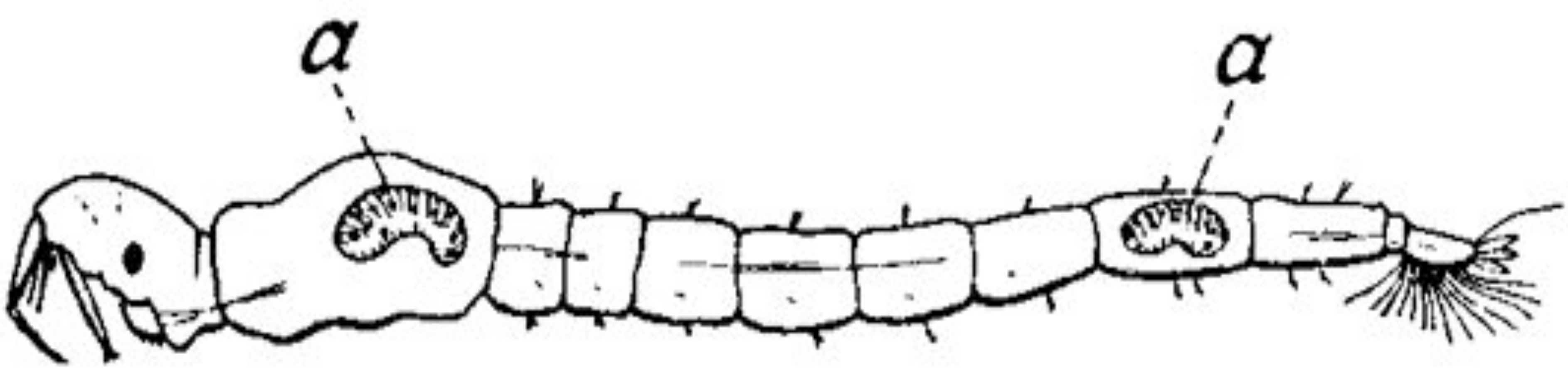
Whirligig beetle with air store

Diving beetle with air store

# Air sacs and hydrostating function of tracheal system

***Chaoboridae - Phantom Midges-*** ***Corethra*** larvae have two air sacs (a, below) that are able to increase in size or decrease. If they increase they can expand to 120% of their size and they can inflate to 90%. If they have less air, they sink while if they have more air they move towards the surface. Little is known about how this mechanism works. Read about Professor Krogh and his early experiments with *Corethra* and Winning the Nobel Price in Medicine. See website below: http://nobelprize.org/medicine/laureates/1920/krogh-bio.html

Experientia. 1974 Sep 15;30(9):1076-7.
**[Innervation of the air sac Wemhoner K, Weber W.**
**epithelium in the tufted gnat *Corethra plumicornis* (Chaoborus)]**



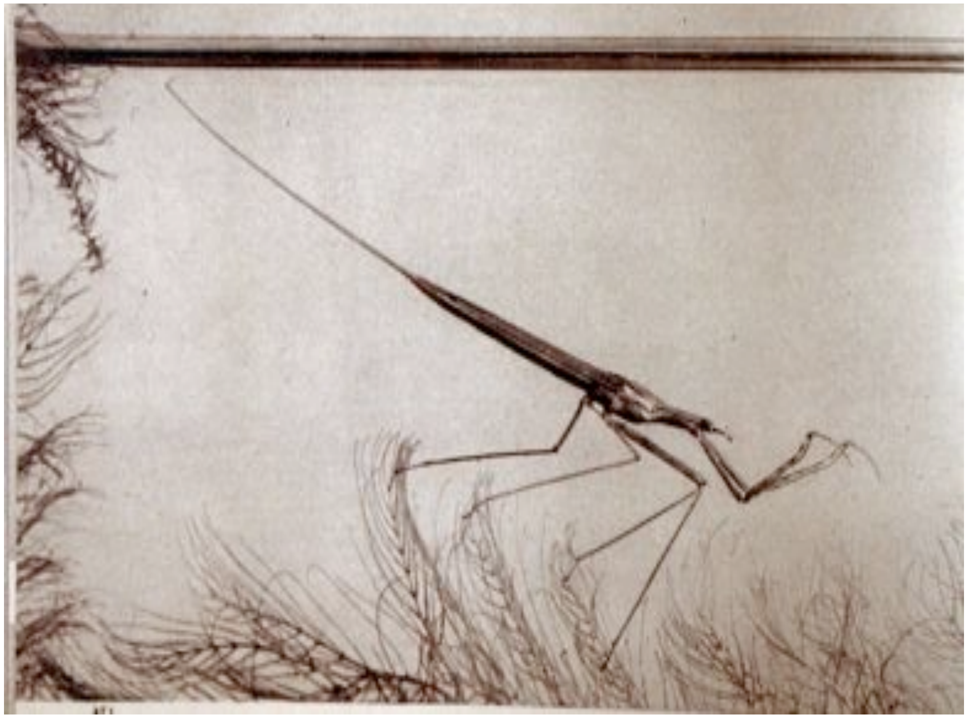
# Air stores for aquatic insects

In the large Belostomatidae, the first two pairs of spiracles are not in contact with the subelytral air store; but spiracle 3, the most permeable of all spiracles opens directly into the store which thus supplies the main trachea to the thoracic muscles. All abdominal spiracles likewise communicate with the air store.

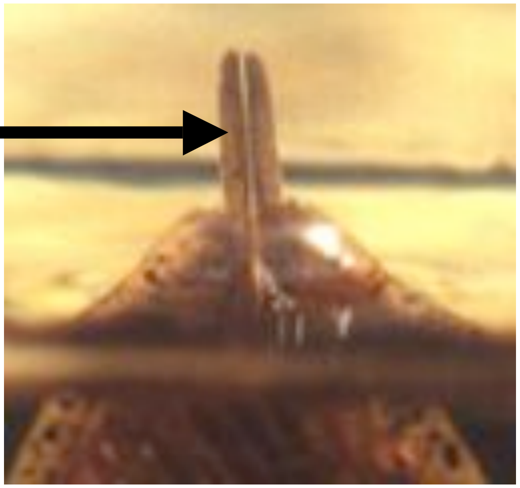
These large bugs also use a respiratory siphon to obtain air??

## 3. Gaseous exchange in aquatic insects

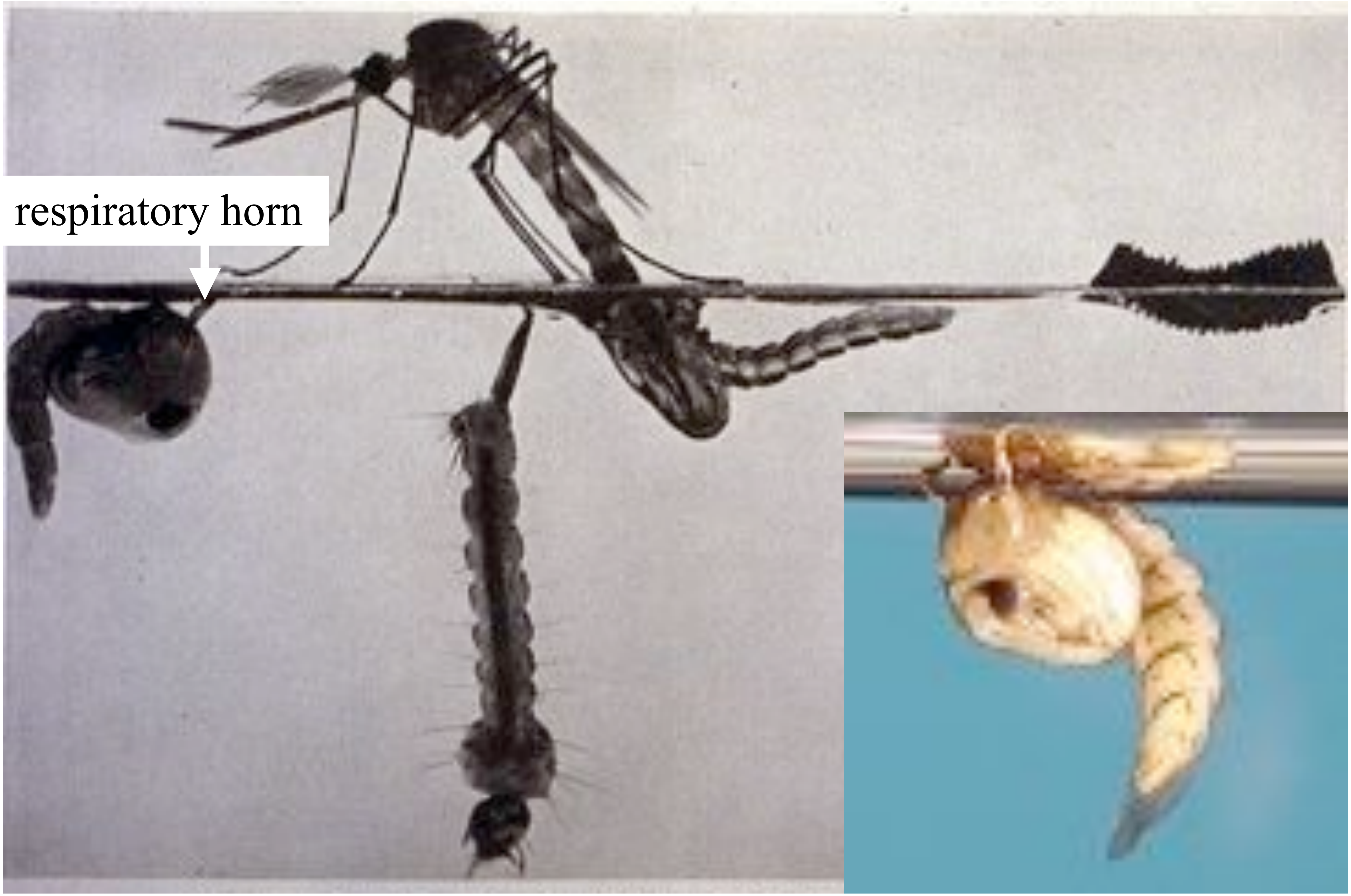
d. Respiratory siphons



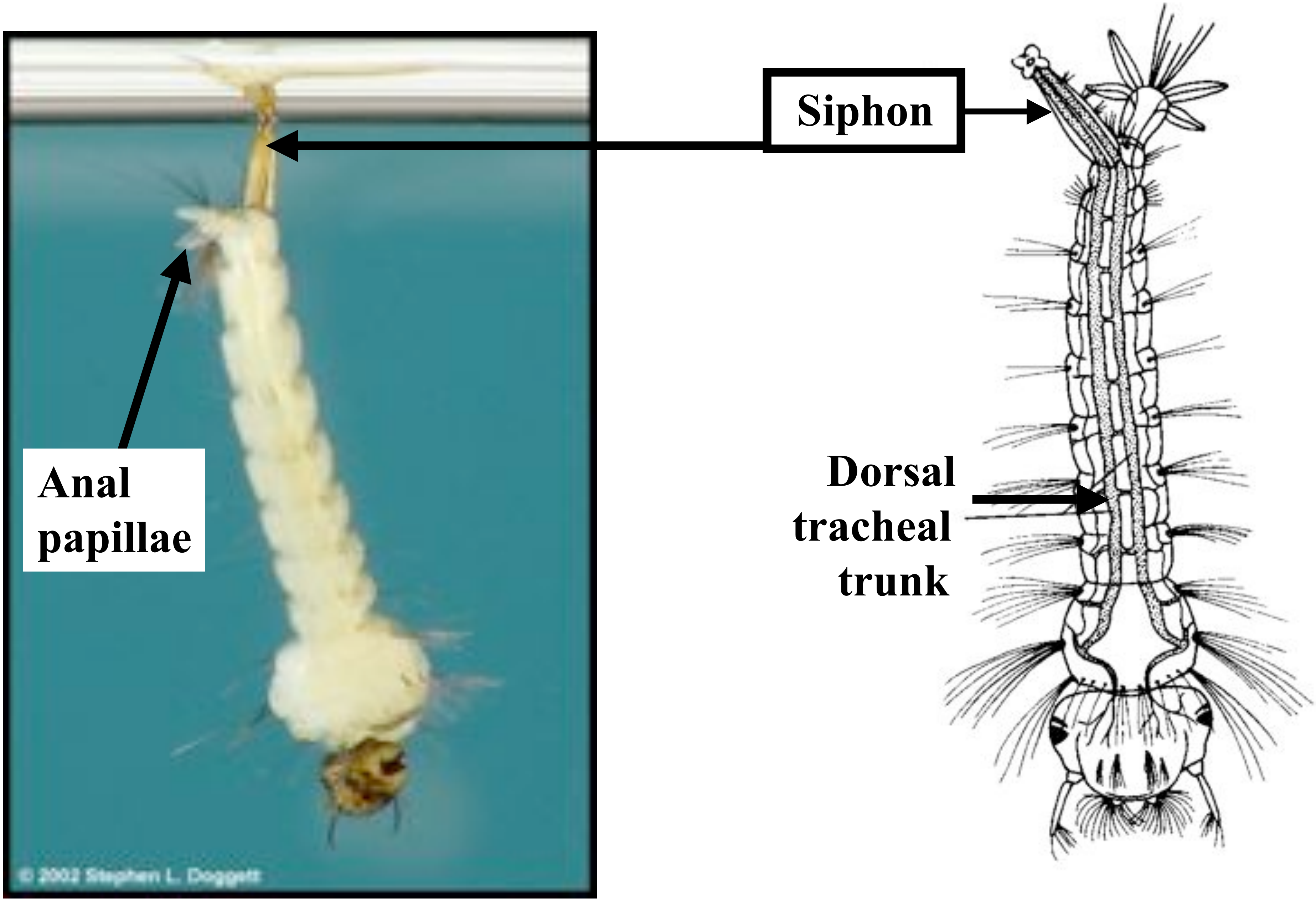
Is this a respiratory siphon at the tip of the giant water bug or is it a device that facilitates air being delivered to the subelytral air space??

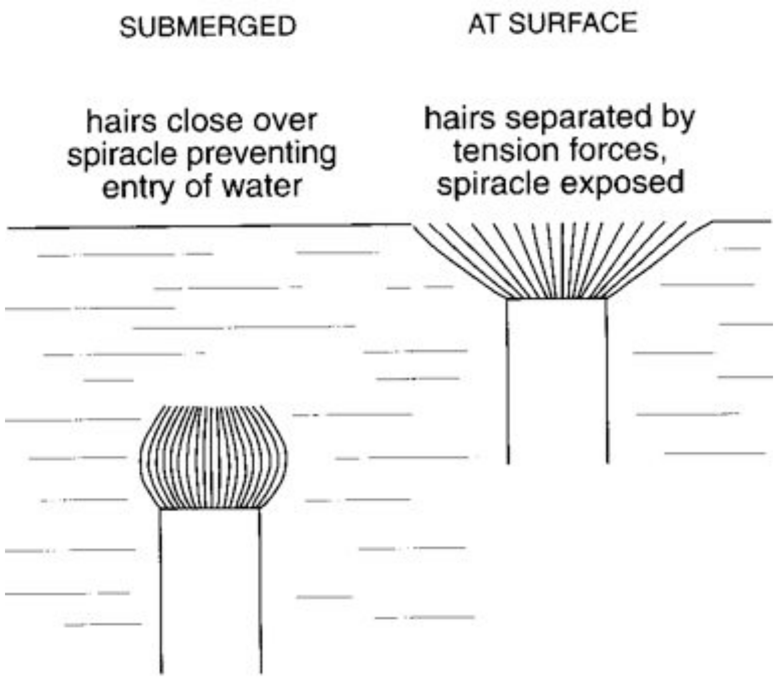


# Mosquito respiratory adaptations to aquatic life



# Mosquito respiratory adaptations to aquatic life






The role of hydrofuge hairs in breaking surface tension and preventing water from entering the spiracle of the siphon of various aquatic insects.

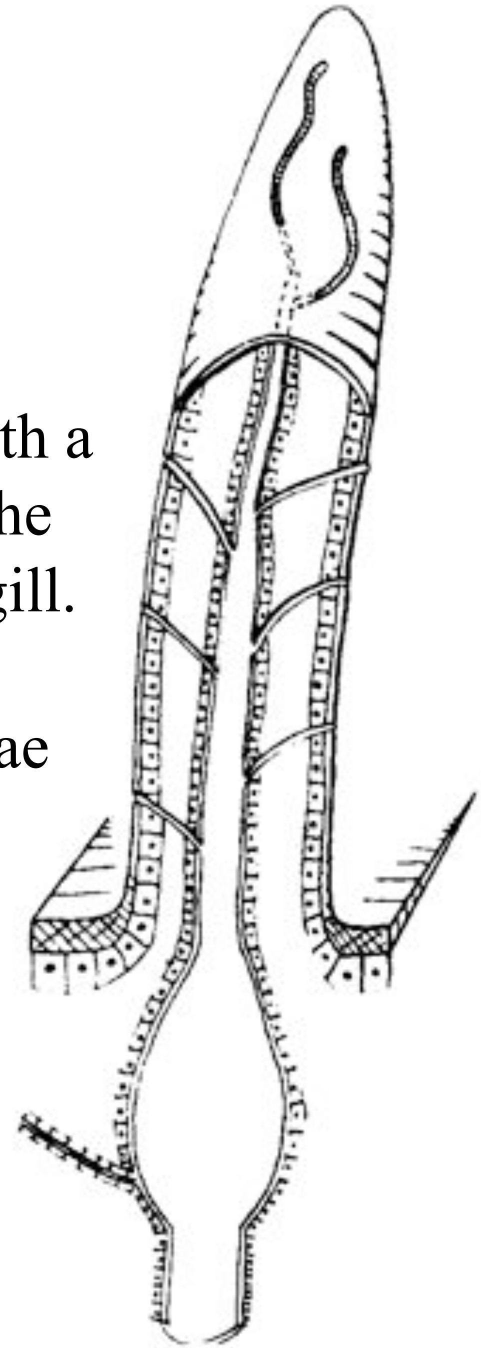
## 3. Gaseous exchange in aquatic insects

e. Spiracular gills

Found in insects that inhabit running water but water that is subject to periodic drying out. These are found in the pupal stage of several dipteran and coleopteran species.

They are outgrowths of the cuticle but are covered with a plastron that allows for greater surface area when in the water. When dry, oxygen is taken up by the spiracle gill.

Found in blackfly pupae



# Behavioral ventilation in aquatic insects

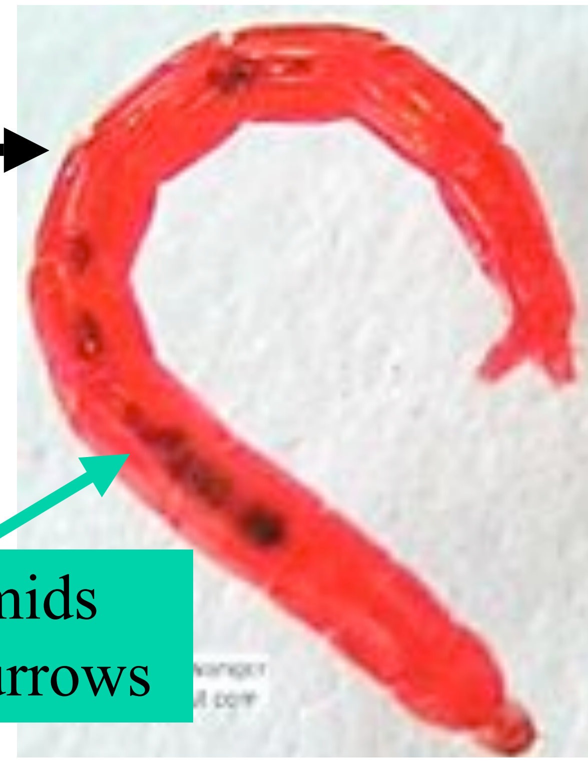
1. Many aquatic insects orient themselves in water so that they get the greatest oxygen available.
2. Others, are able to increase certain behaviors, such as moving the gills more rapidly, increasing how fast they take water into and out of the rectal gills, or do ‘push-downs’ such as Plecoptera.

# RESPIRATORY PIGMENTS

The great majority of insect do not have respiratory pigments. A few do and those that do have the pigments in solution in the blood or in special cells called hemoglobin cells (even here it is in solution).

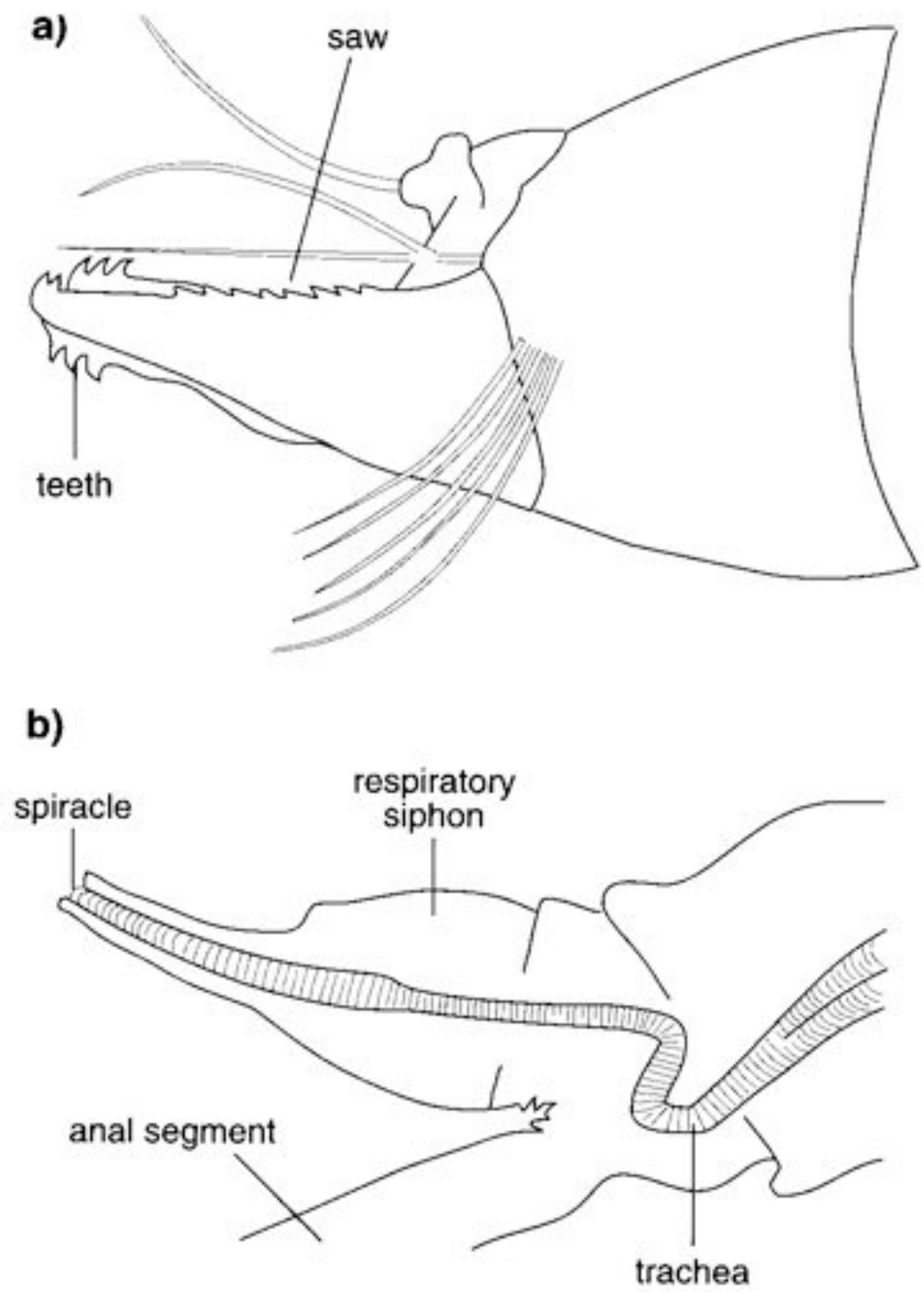
## EXAMPLES

1. *Chironomus* or blood worms
2. *Anisops* or backswimmer
3. *Gasterophilus*



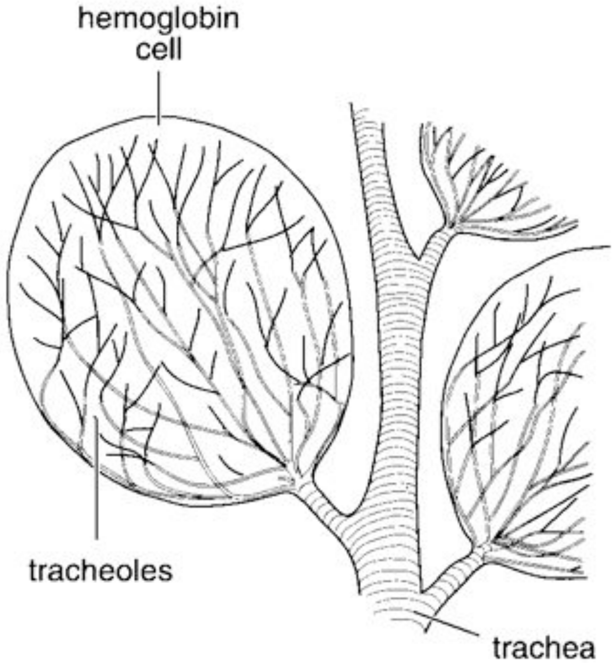

Hemoglobin in these 2 larvae has a much higher affinity for oxygen than does the vertebrate hemoglobin

The mosquito larva of *Mansonia* gets its oxygen from aquatic plants by using a special device, which is part of the respiratory siphon to penetrate into the air tissues or aerenchyma of the aquatic plants.



# Hemoglobin & oxygen supply in insects

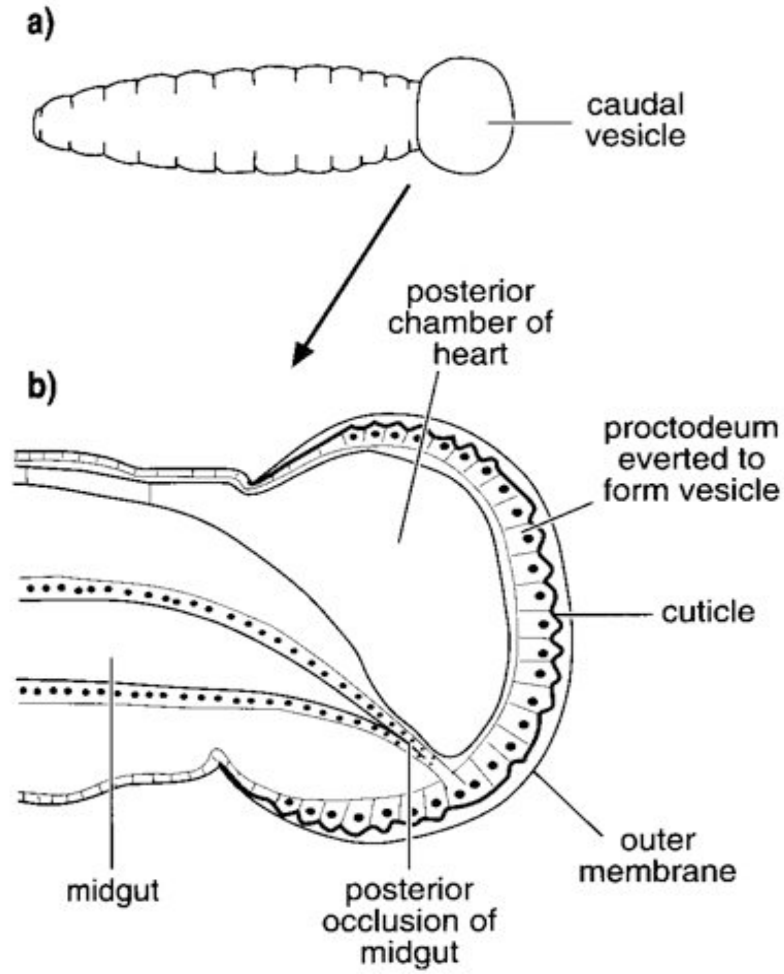
In *Anisops* (Heteroptera), adults have ventral and subelytral stores supplemented by oxygen loosely associated with hemoglobin in large hemoglobin cells just inside the abdominal spiracle. Hemoglobin becomes oxygenated when the insect is at the surface. They can remain submerged for 5 mins. Also found in *Gasterophilus* larvae. 1st two instars hemoglobin is in the blood but last instar it is in the hemoglobin cells.



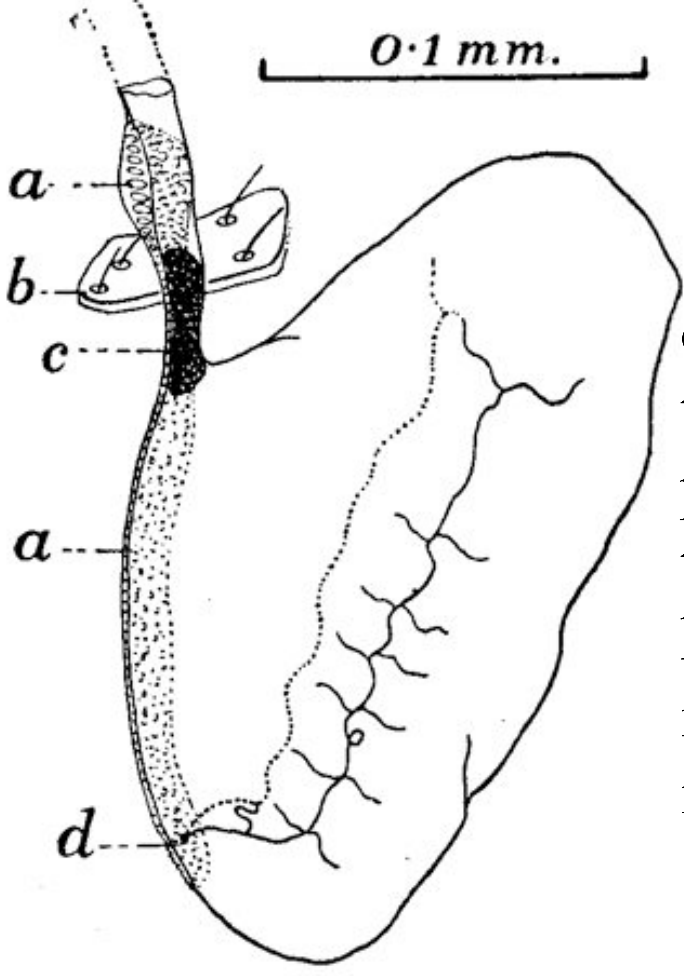

# Respiration in parasites-

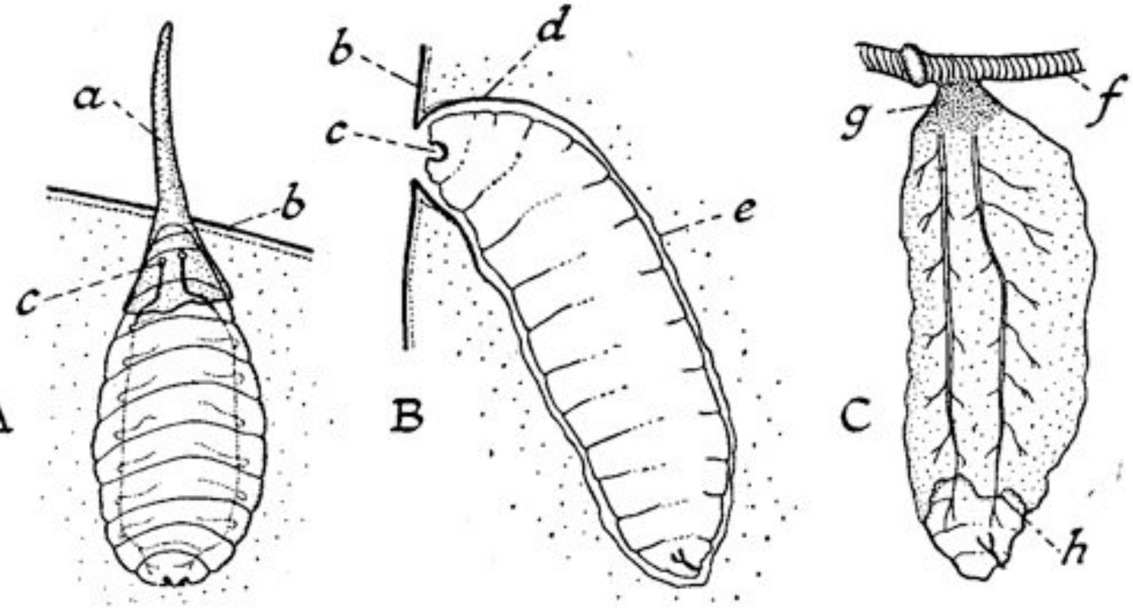
1. In adults it is mainly spiracular
2. In endoparasitic larva
   a. Cuticular with a special caudal vesicle in braconid larvae
   b. Direct contact through host's cuticle using siphons, etc.

In these larva the hindgut is everted through the anus to form a caudal vesicle. This becomes in close apposition to the heart. As oxygen comes in it goes directly to the heart and from there to all of the tissues



A recently hatched (below) larva with its posterior spiracles (d) plugged into the aerospace of the egg whose pedicel is outside the host's cuticle.




Some parasites during embryonic development or as first instars obtain oxygen directly from the air by using their own egg pedicel that protrudes through the host's cuticle (A). In (B) the larva has made an entrance hole in the host's cuticle while in (C) the tachinid larva is enclosed in a tracheal sheath and has plugged into the tracheal system of the host.

# Respiratory system and defense-

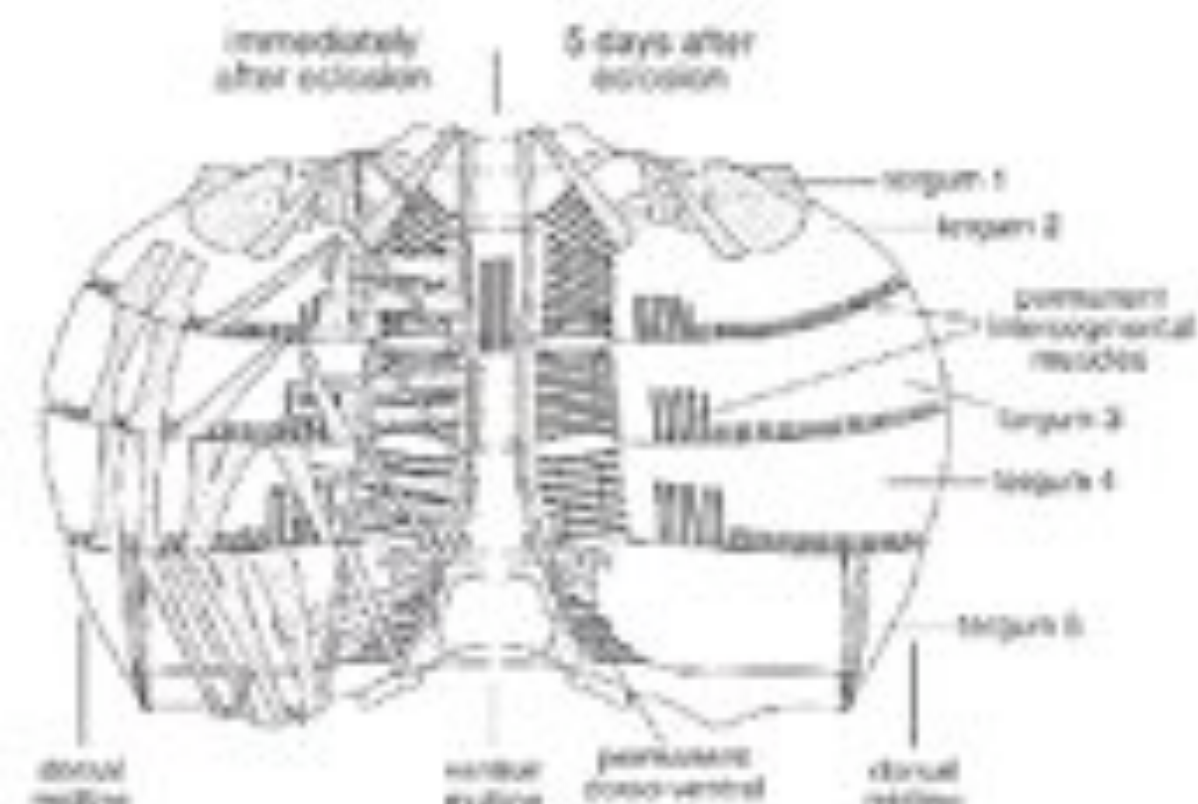
## 1. Hissing cockroach

**How does it hiss?** Insects breathe through holes on the side of their body called spiracles. To hiss, the roach forces air out of a pair of modified spiracles on its abdomen. Air sacks in the body work like bellows to squeeze the air out. Hissers are the only insects to make sounds this way, a method usually used by vertebrates (snakes, angry people). Other insects usually make sounds by rubbing body parts together, vibrating a membrane, or banging something.

**Repugnant glands or defensive glands of this species of grasshopper**

# Respiratory system and molting-

Important for breaking out of the exoskeleton and also in expanding the wings. Important in ptilinal extrusion and emergence of the Cyclorrhaphous dipterans from their pupal case and in digging.



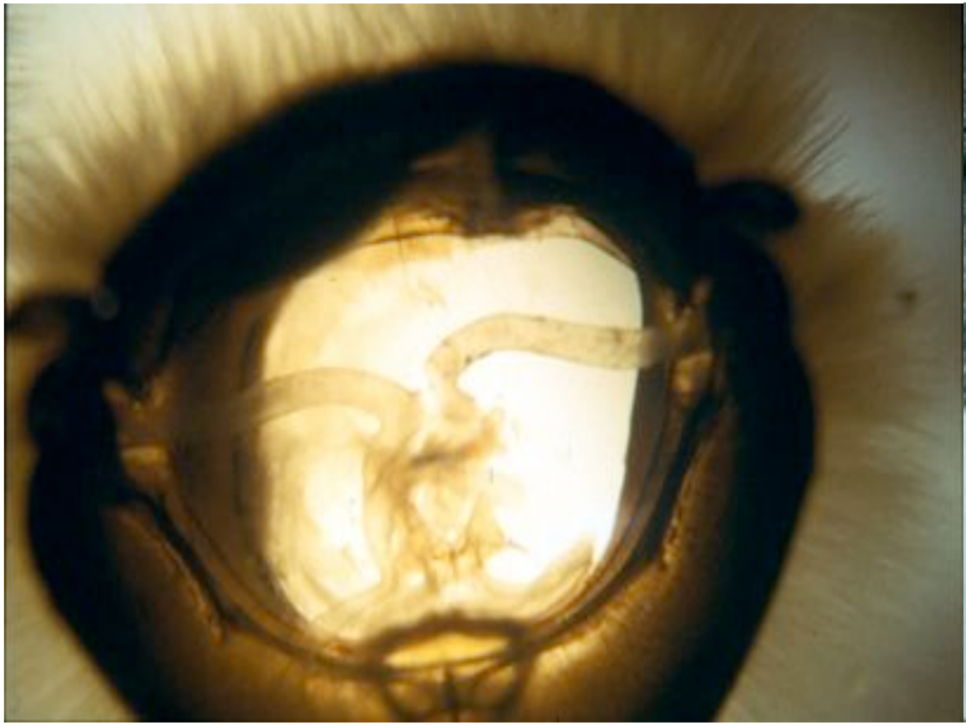

**Ptilinum** is only used at the time of emergence from the puparium. It is used to pop-off the cap of the pupal case and also aids in digging through the soil.

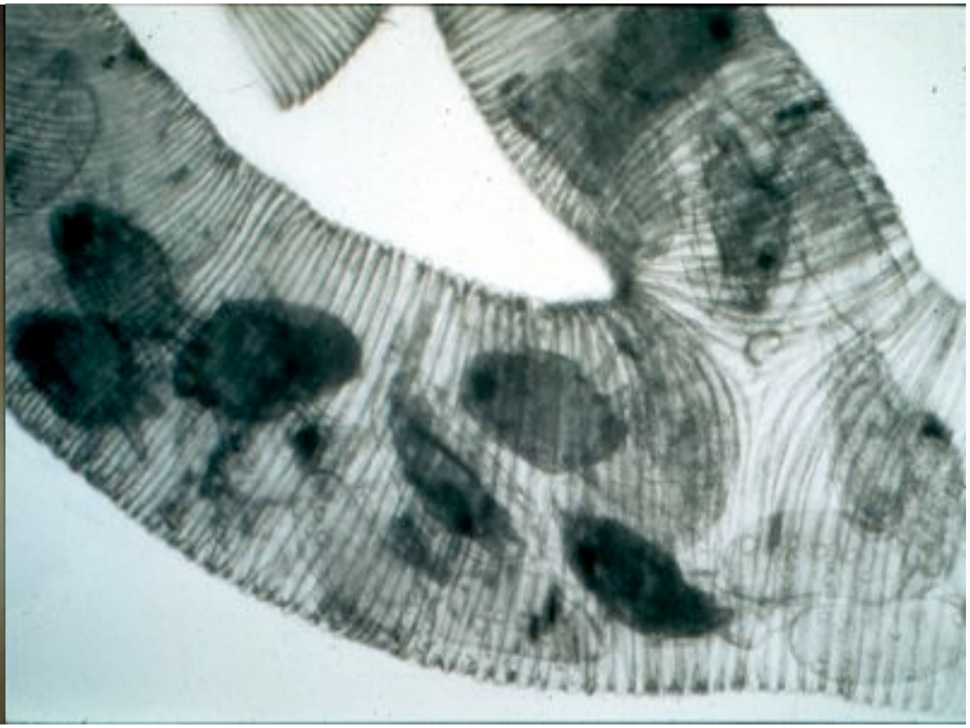
Ptilinum expanded. This structure aids the adult in escaping from the pupal case. Also, the muscle sets on left and above help increase hemolymph pressure that facilitates ptilinal extrusion from the ptilinal suture. 5 days after eclosion these muscles degenerate. Probably bursicon sensitive but not proven.

**The ptilinal mechanism also involves the respiratory system where the fly takes in air into its digestive tract and air sacs. This helps in increasing the hemolymph pressure when the muscles contract thus aiding in escaping from the pupal case and also in spreading the wings.**

# Parasites of the tracheal system of honeybees-

The tracheal mite, ***Acarapis woodi*** **(Rennie) (Arachnida: Acarina: Tarsonemidae)** is causing tremendous damage to the honey bee industry in this country. The mites penetrate the trachea to reach the hemolymph where they feed. Why no host response??

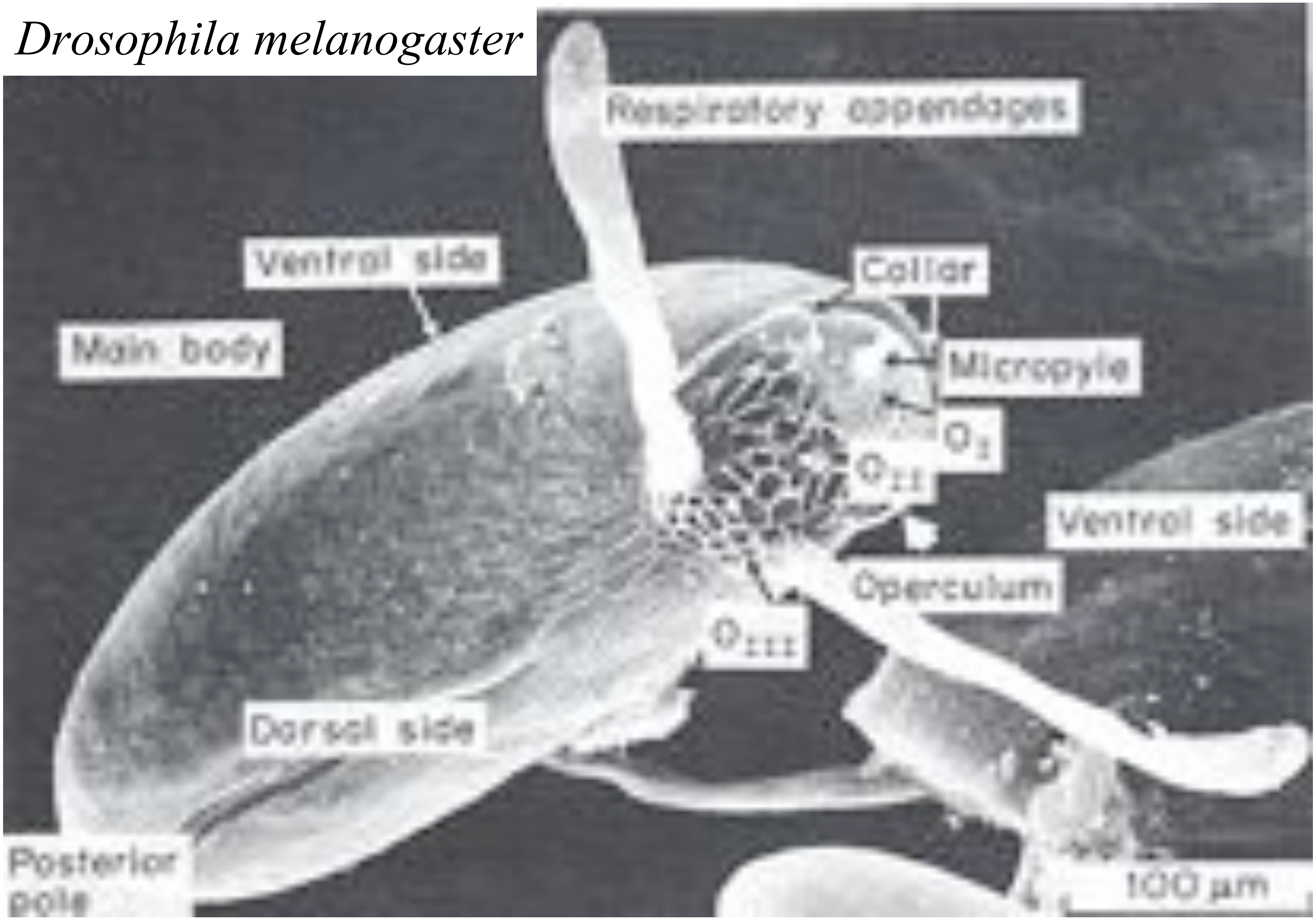




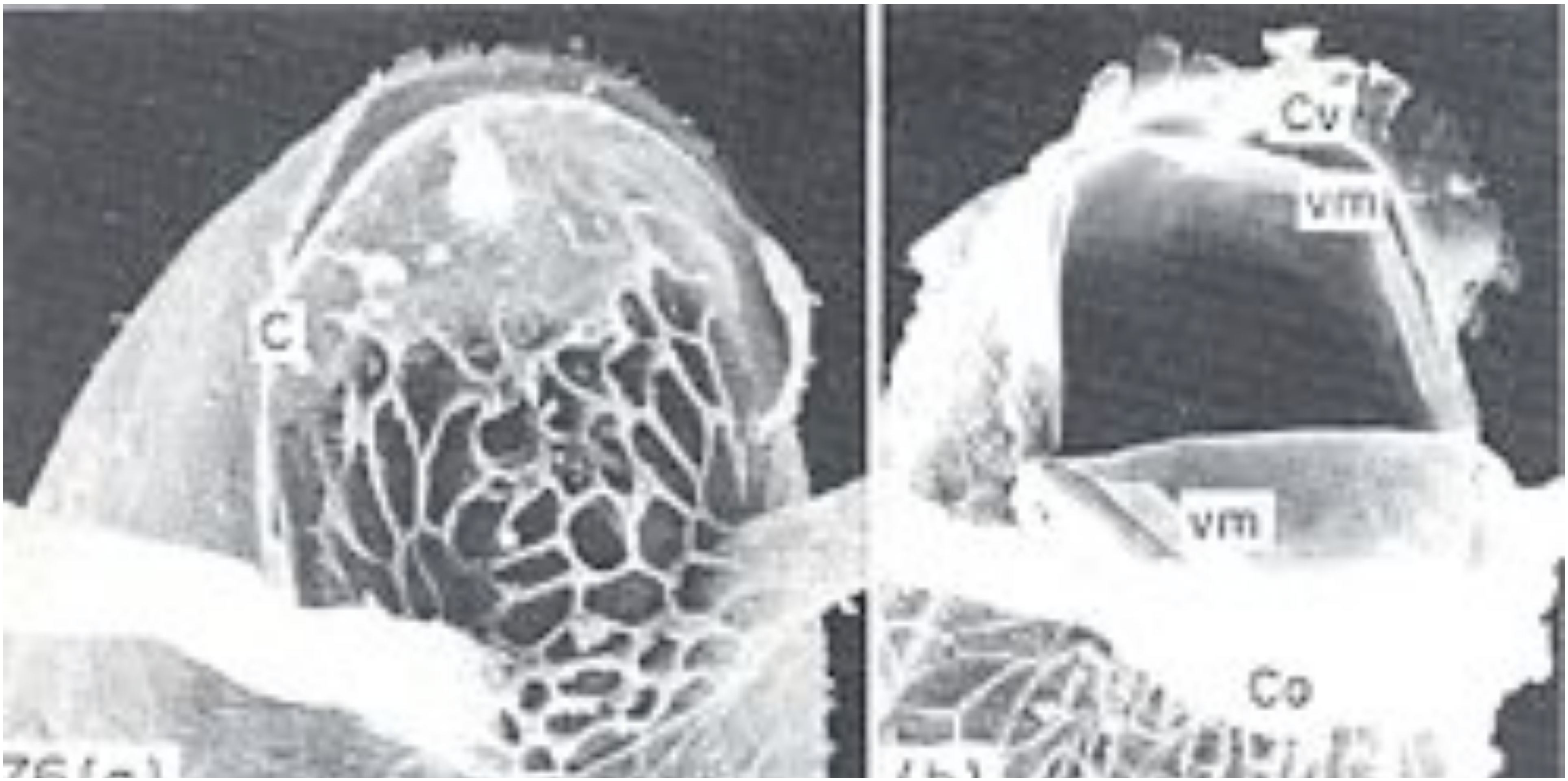
# Respiration for embryos-

## How are eggs modified to do this?

*Drosophila melanogaster*

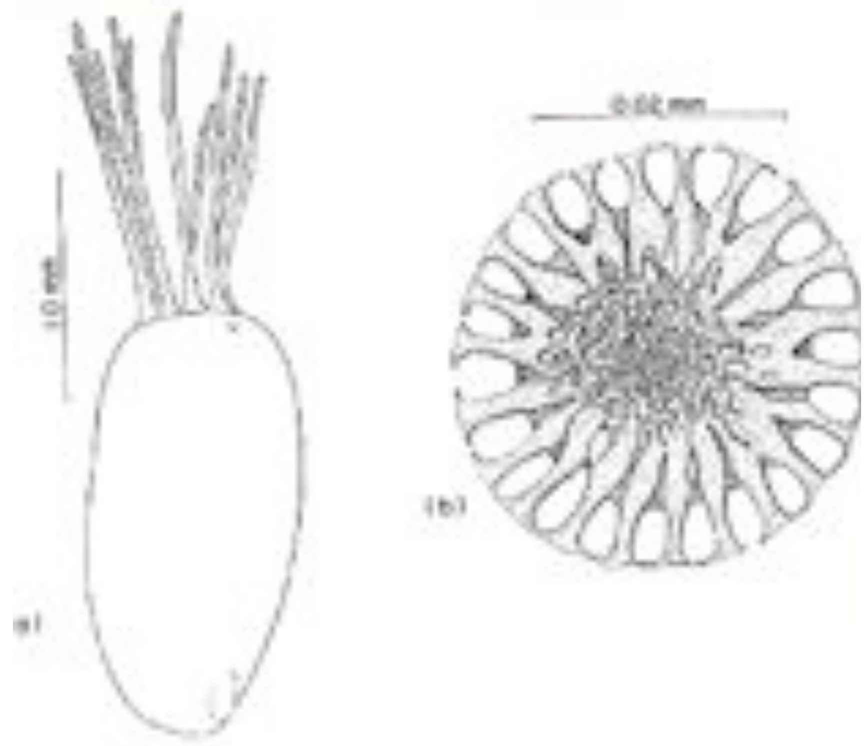


Egg of *Drosophila melanogaster* showing unhatched egg on the left and the remaining eggshell after the larva has hatched out on the right.



**Respiratory horns on the eggshells of some insects where contact with the air is important, usually because the eggs is deposited below a substrate the would prevent the embryo from getting oxygen**

Below eggs of face fly, *M. autumnalis* laid in wet manure. Eggs of *Ranatra* laid in leaf. Lower eggs of *Nepa cinerea*

## BASIC & APPLIED ASPECTS OF THE RESPIRATORY SYSTEM

### BASIC RESEARCH

1. Neural control or orchestration of several ganglia in producing ventilatory movements

### APPLIED RESEARCH

1. Putting various substances on surface of liquids to kill mosquitoes. Remember the talk by Elnaiem and use of polymers
2. Fumigants-add carbon dioxide to stored grain silos thus increasing the effectiveness of the fumigant.
3. Some aquatics are excellent pollution indicators or index species
4. Use of dormant oils and light oils for scale insects, etc.
5. New methods for controlling tracheal mites in honey bees

# SUMMARY

1. Contrary to belief, insect do actively breathe
2. New evidence suggests that discontinuous breathing or ventilation protects the insect from the toxic effects of oxygen
3. New ideas concerning the interaction between the circulatory and the respiratory system have now been used to help explain heart reversal in some insect species
4. Breathing is under the control of the stomatogastric or stomodael nervous system
5. Invasion of the aquatic environment has led to insects exploiting and developing numerous and diverse strategies in obtaining oxygen
6. Insect flight, especially in fast flyers and large insects, has led to various mechanisms that assure an oxygen supply to the flight muscles but, doesn’t shut-off the supply of oxygen to the rest of the tracheal system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