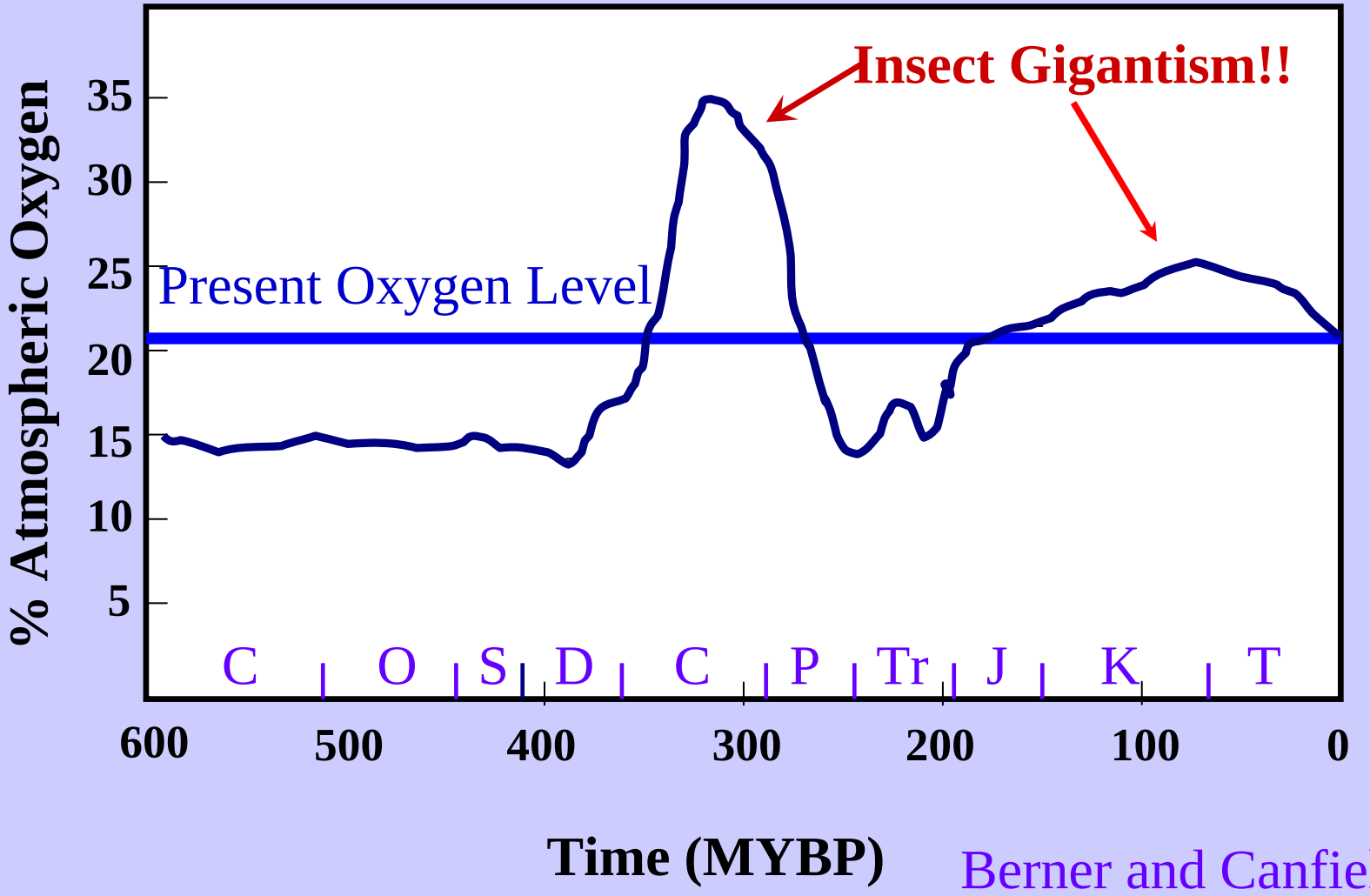
X-ray imaging as a tool to investigate insect gas exchange: questions, initial results, and a wish list

- Jon Harrison
- School of Life Sciences, Arizona State University
- Co-authors: Kendra Greenlee and Scott Kirkton (ASU), Mark Westneat (U Chicago), Wah-Keat Lee (Argonne Nat Labs)

Why study insect respiratory systems?

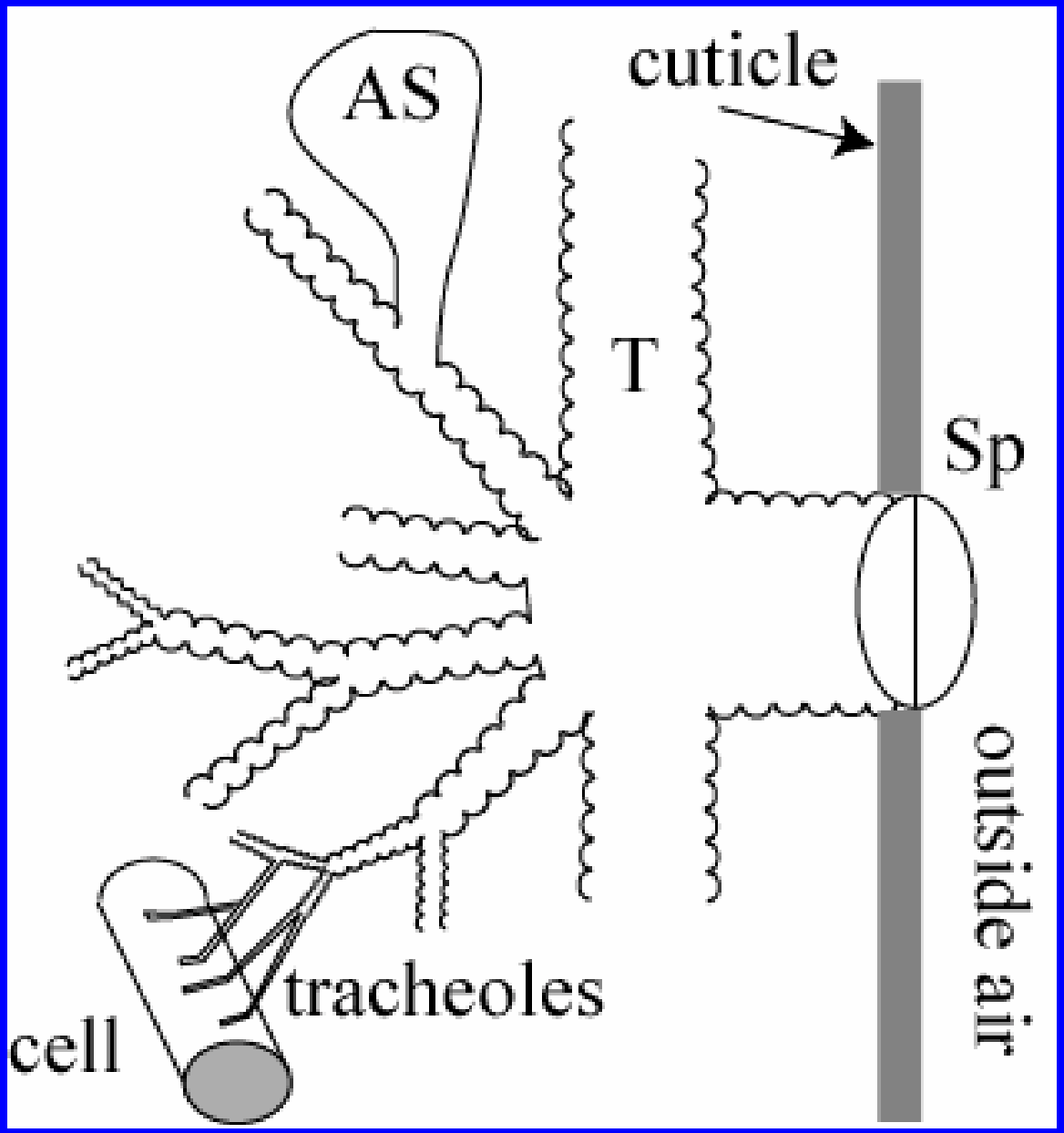
- Great laboratory models for studies of acclimation/adaptation
- Tremendous ecological, agricultural, and medical impact
  - (>90% of described animal species, 200 kg/acre)
- Tracheal respiratory system is light, high-capacity respiratory system that has certainly contributed to insect success
- Relative uniqueness may provide area for pesticides with less effects on vertebrates

Does the tracheal respiratory system limit insect size?



Berner and Canfield,

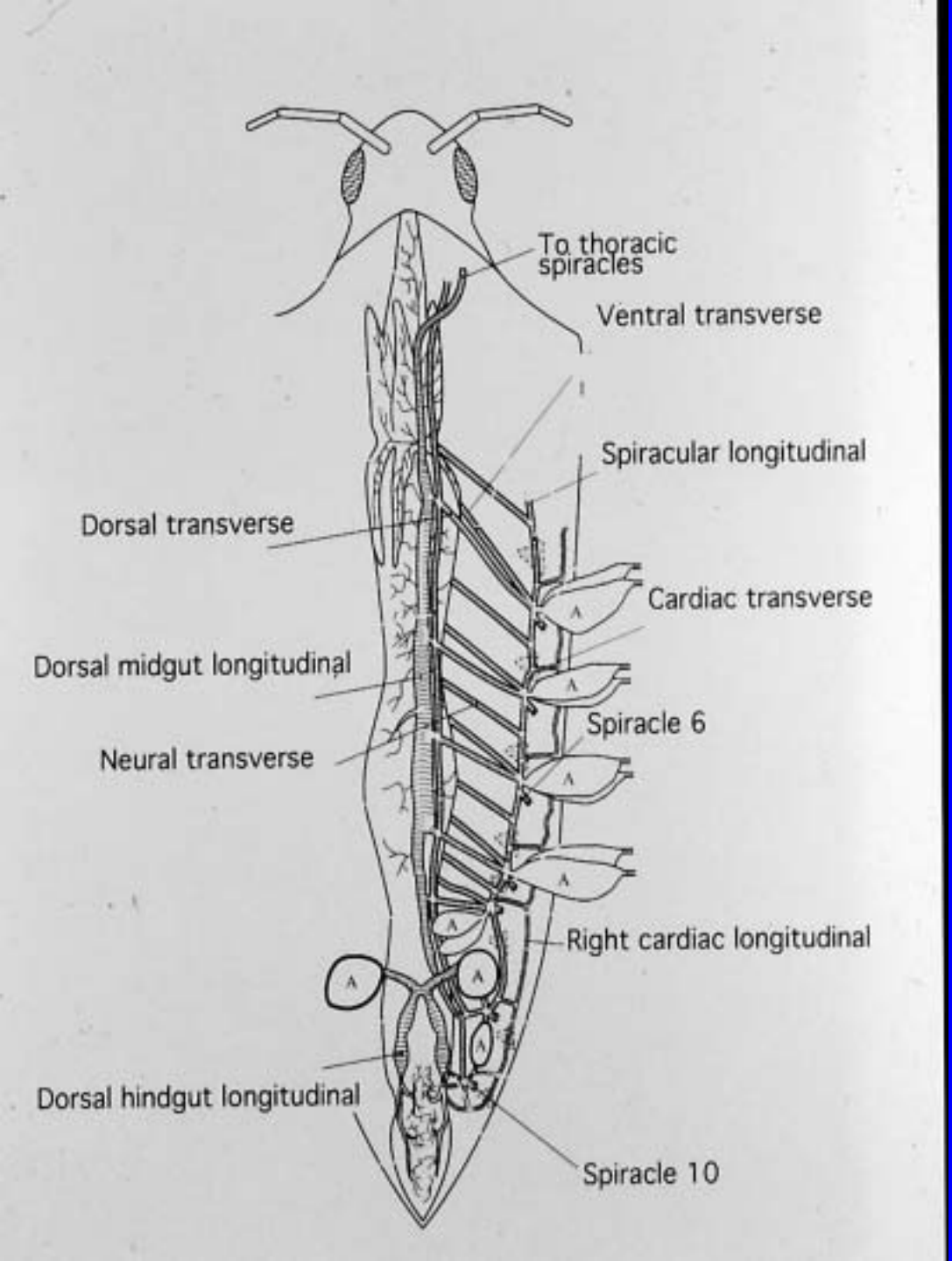
Dudley, Graham et al.



-Ocludable spiracles

-air sacs/bellows

-most gas exchange via tracheoles



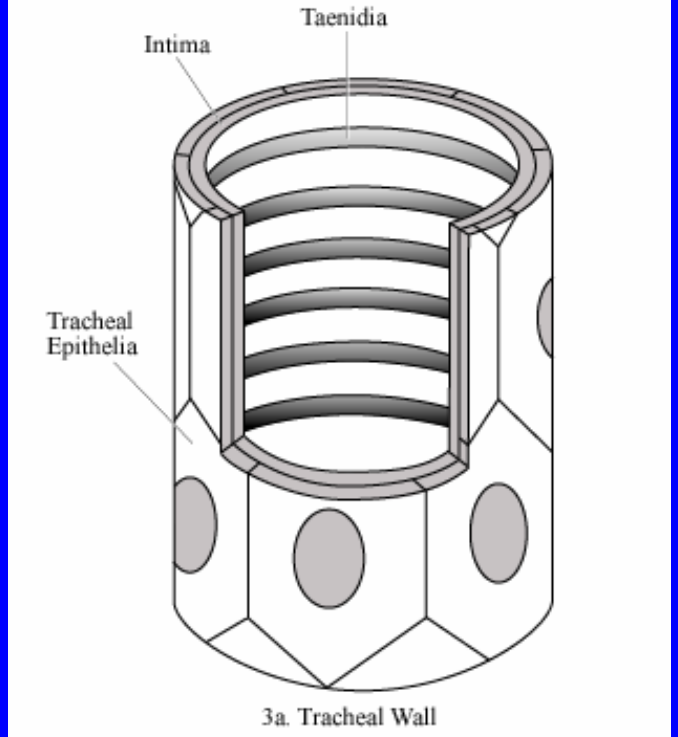
Tracheal system:

-O_2 transported in gas phase to tissues

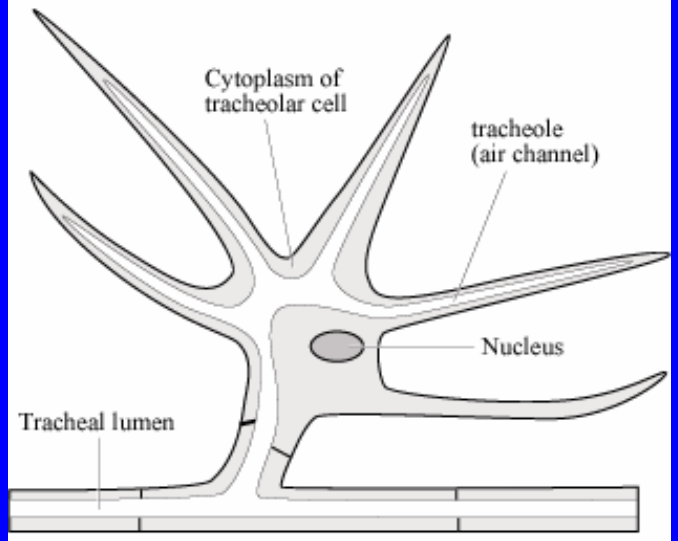
-tracheal tubes lead inward from spiracles

-2-20 spiracles/insect

-Many longitudinal trachea/anastomosis



3a. Tracheal Wall



3b. Tracheolar Cell Connected to Trachea

Tracheae:

-2 – 1000 microns

-Multicellular

-Taenidia can prevent collapse

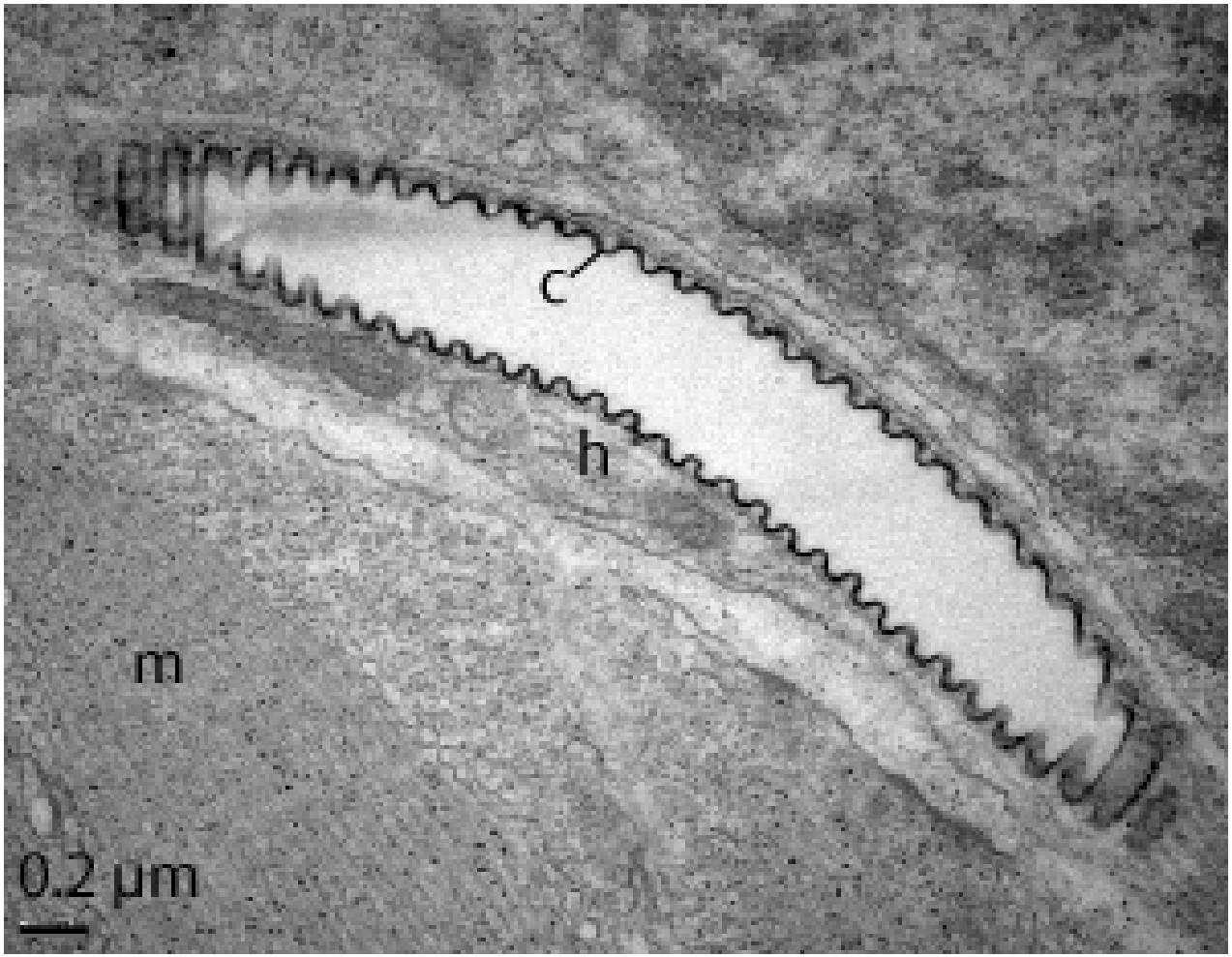
Tracheoles:

-air-filled channels within projections of single cells

-0.5-2 microns

Major tracheae (T) within grasshopper leg (light microscopy).

C. 2nd instar, 31,000x, class I trachea (tracheole)



Tracheole (electron microscopy)

C = cuticle

H = hypodermal layer

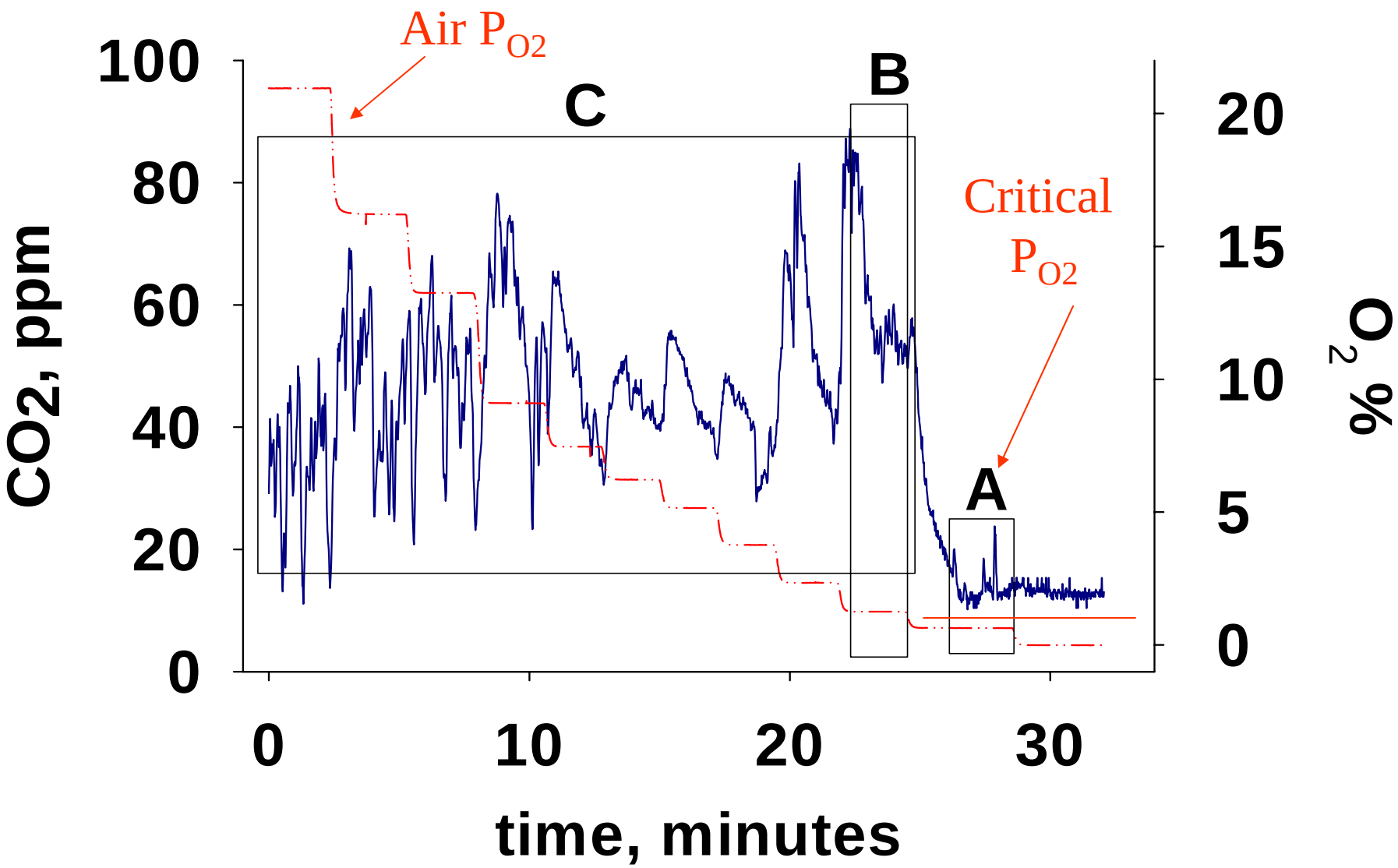
M = mitochondria

American locust *(Schistocerca americana)*

140x mass change during ontogeny

Does oxygen delivery become more challenging with growth?

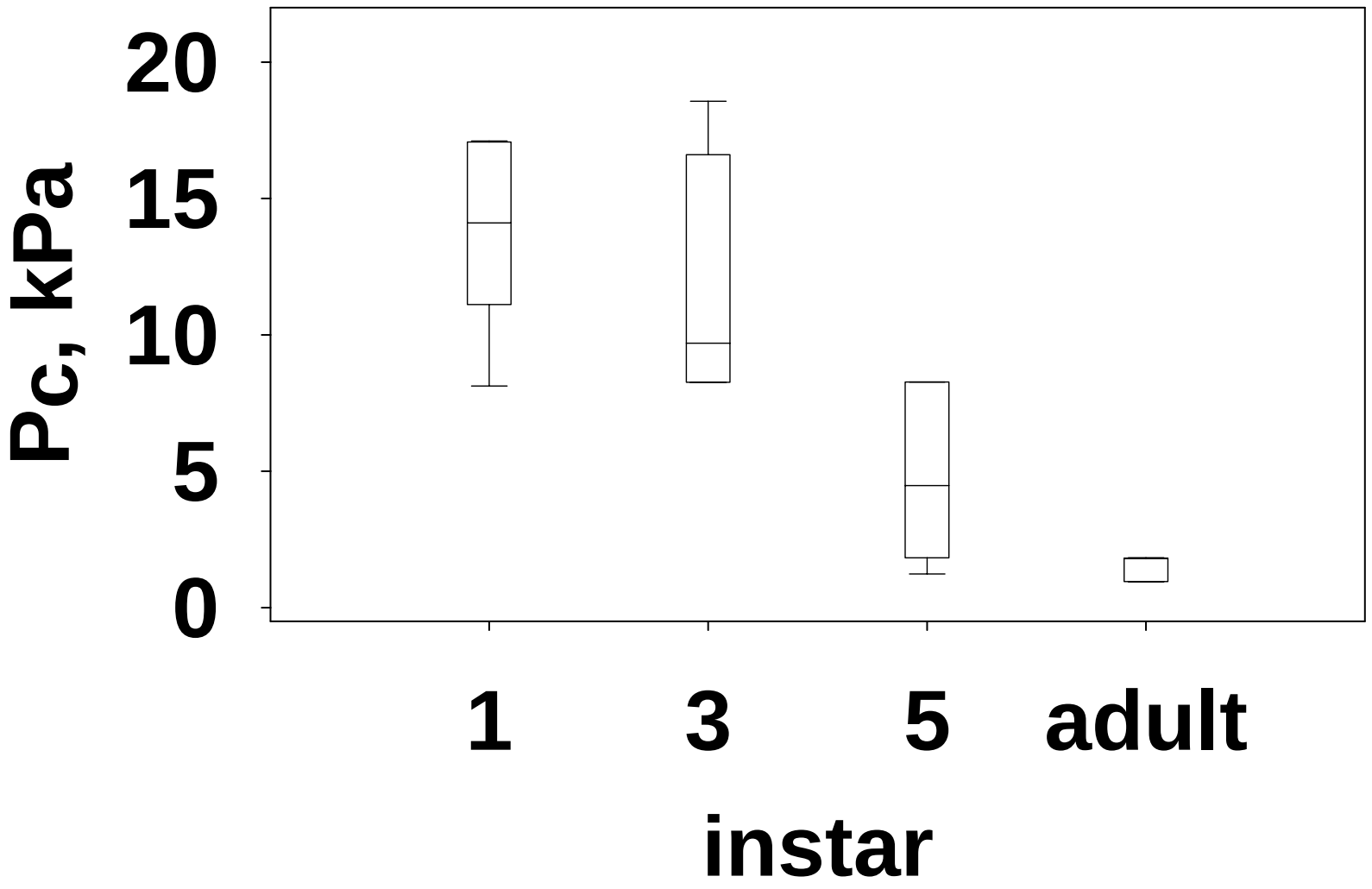
Measurement of the critical P_{O2} for gas exchange



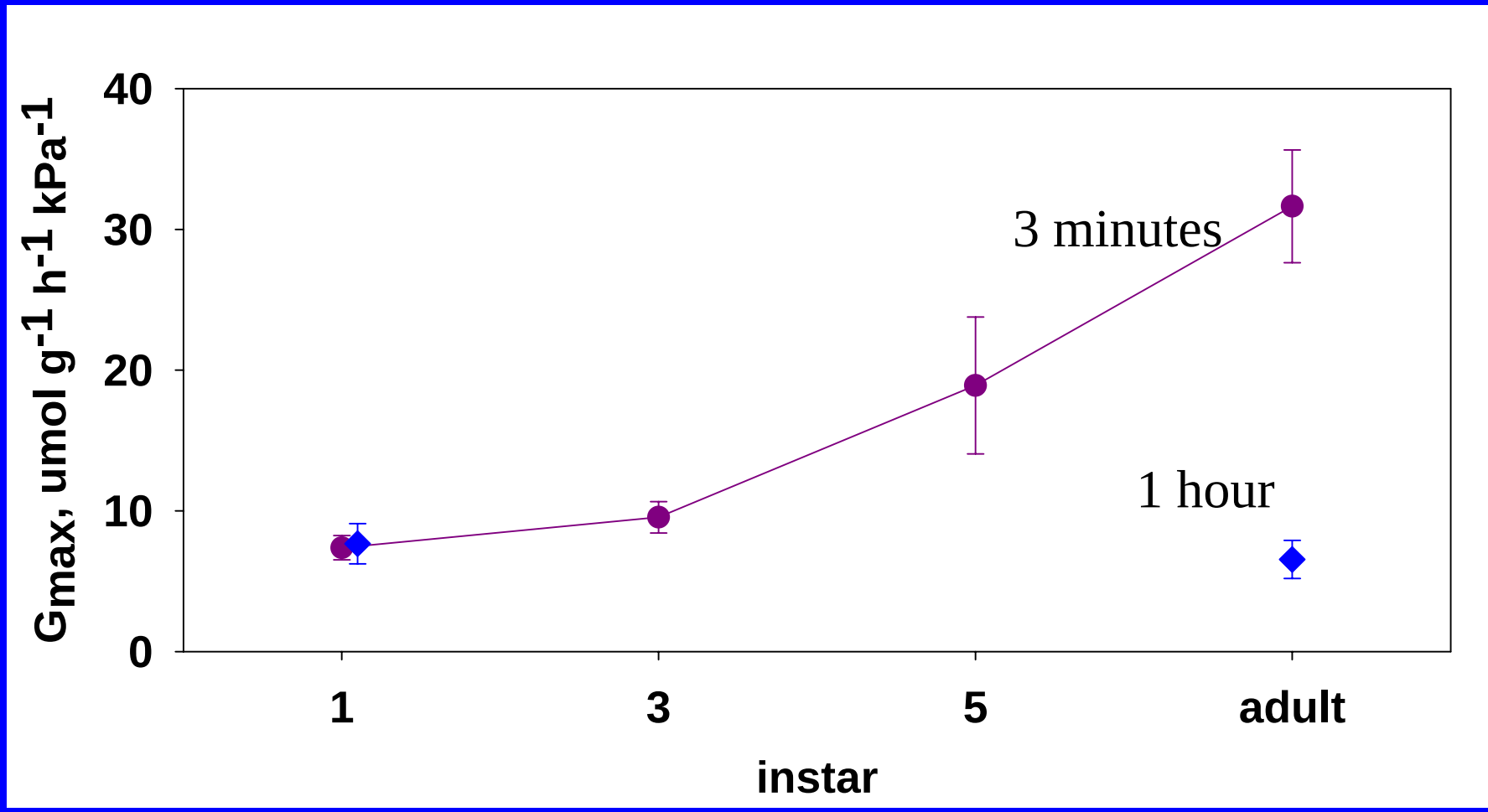
From measurements of gas exchange during progressive hypoxia we calculate:

- Critical P_{O2} = below this, oxygen-limited
- G = tracheal conductance at critical P_{O2}
- M_{O2} = G * (air P_{O2} – mitochondrial P_{O2})
- At critical P_{O2}, mitochondrial P_{O2} = 0.1 kPa
- At critical P_{O2} , G = M_{O2} / (air P_{O2} – 0.1)

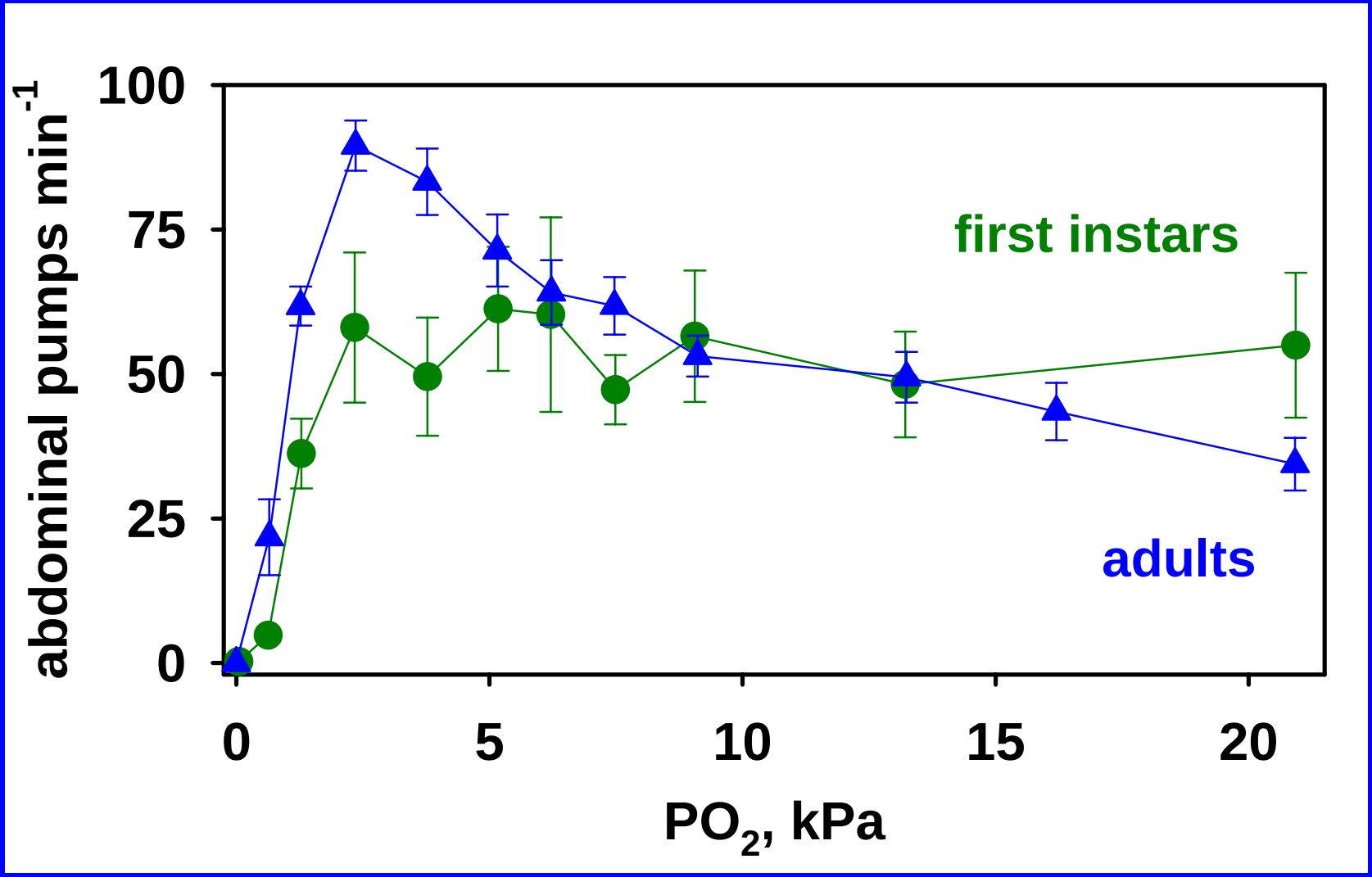
The critical P_{O2} for metabolic rate FALLS dramatically during ontogeny.



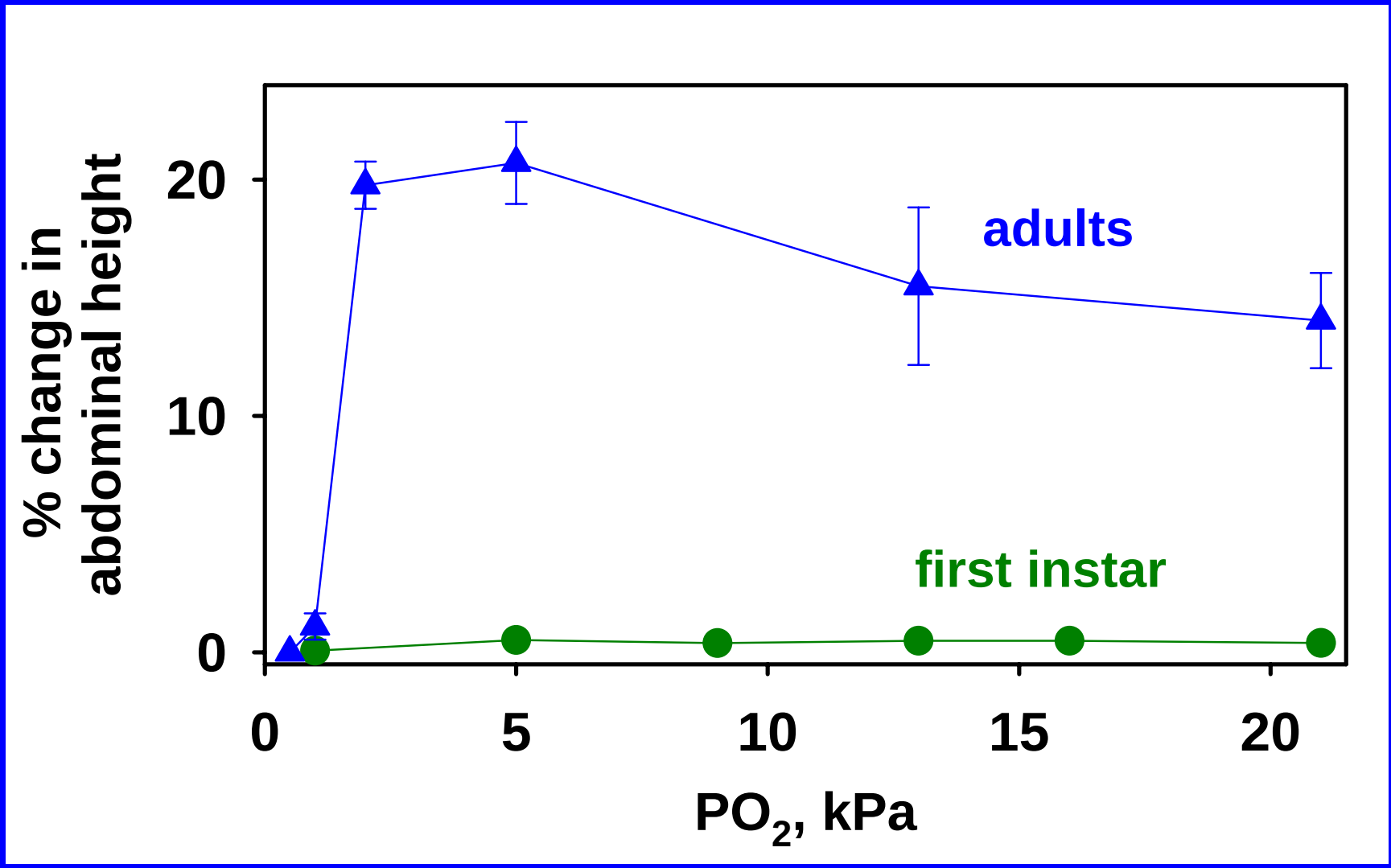
Maximal tracheal conductance increases 4-fold (3 min exposure) or No Change (1 hour exposure).



An increase in ventilatory response to hypoxia provides increased tracheal conductance in adults.



Adults also have a greatly increased tidal volumes.

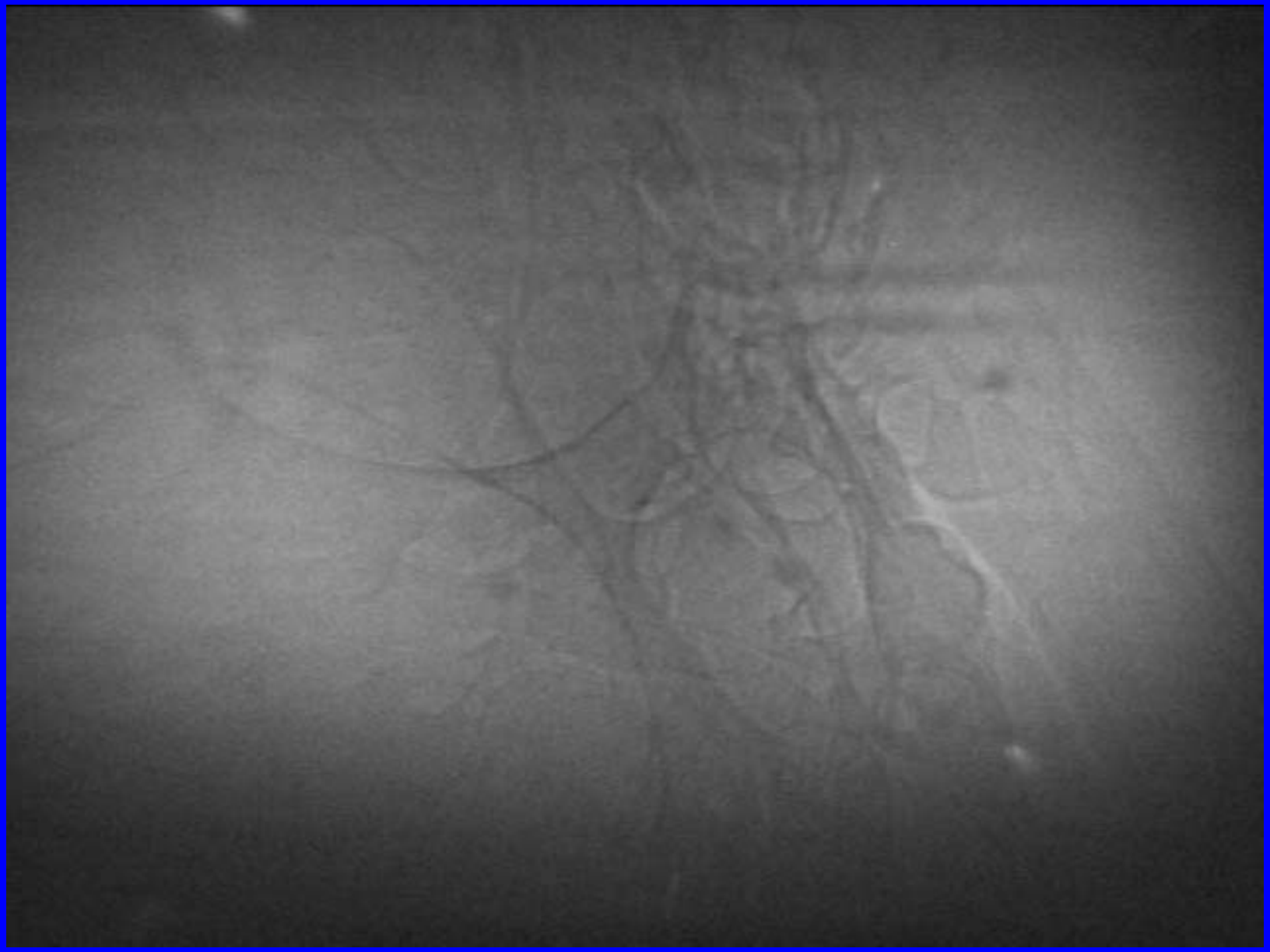


Goals for x-ray imaging study, summer 2003

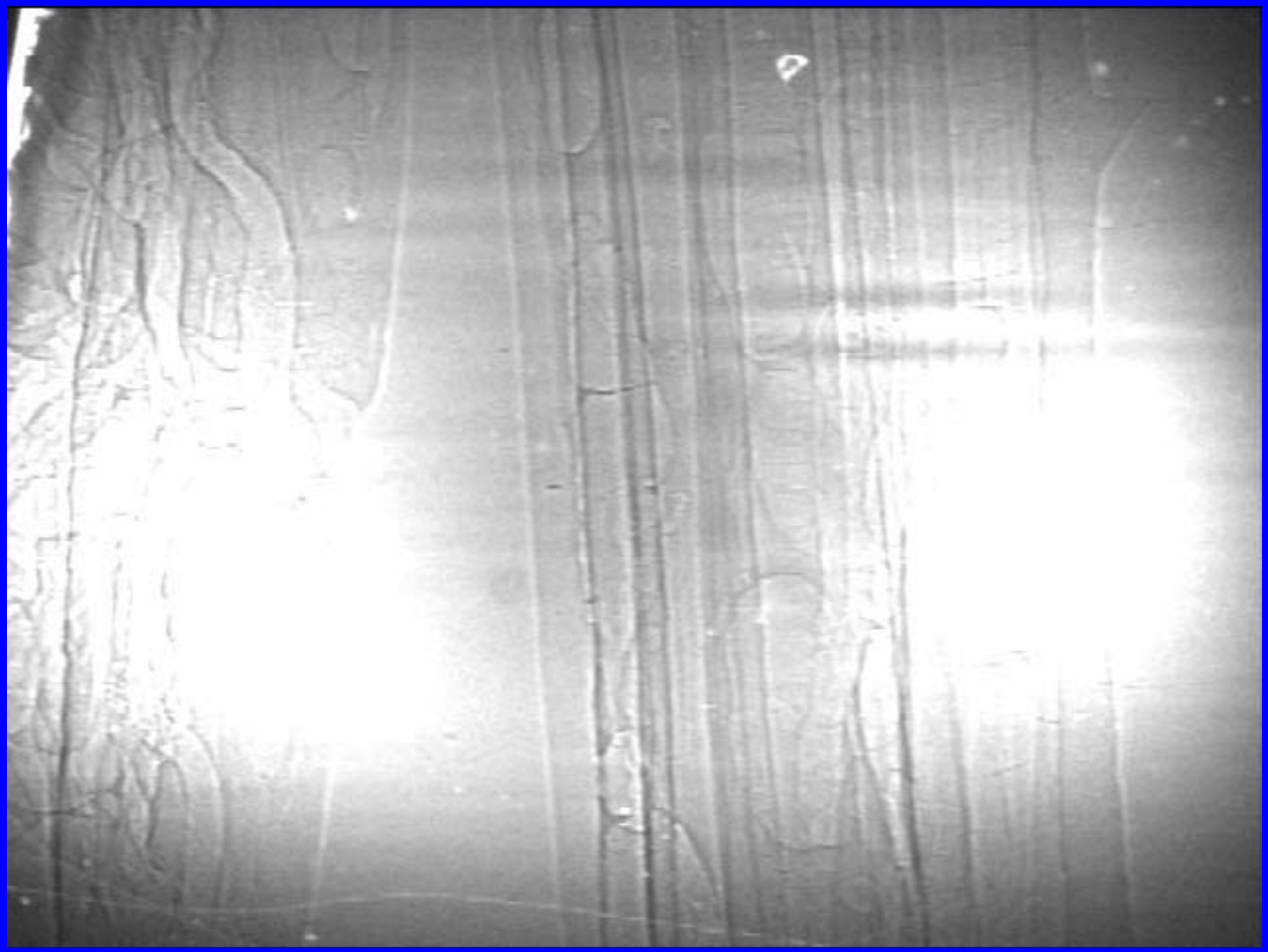
- What morphological changes account for the greater tracheal conductances of larger grasshoppers?
- A. More tracheae?
- B. More air sacs?
- C. Greater compression of air sacs/tracheae
- D. A, B and C together?
- Can we directly measure convection?

X-ray synchotron imaging of air sac compressions

Some images too dark (3rd instar abdomen and leg)



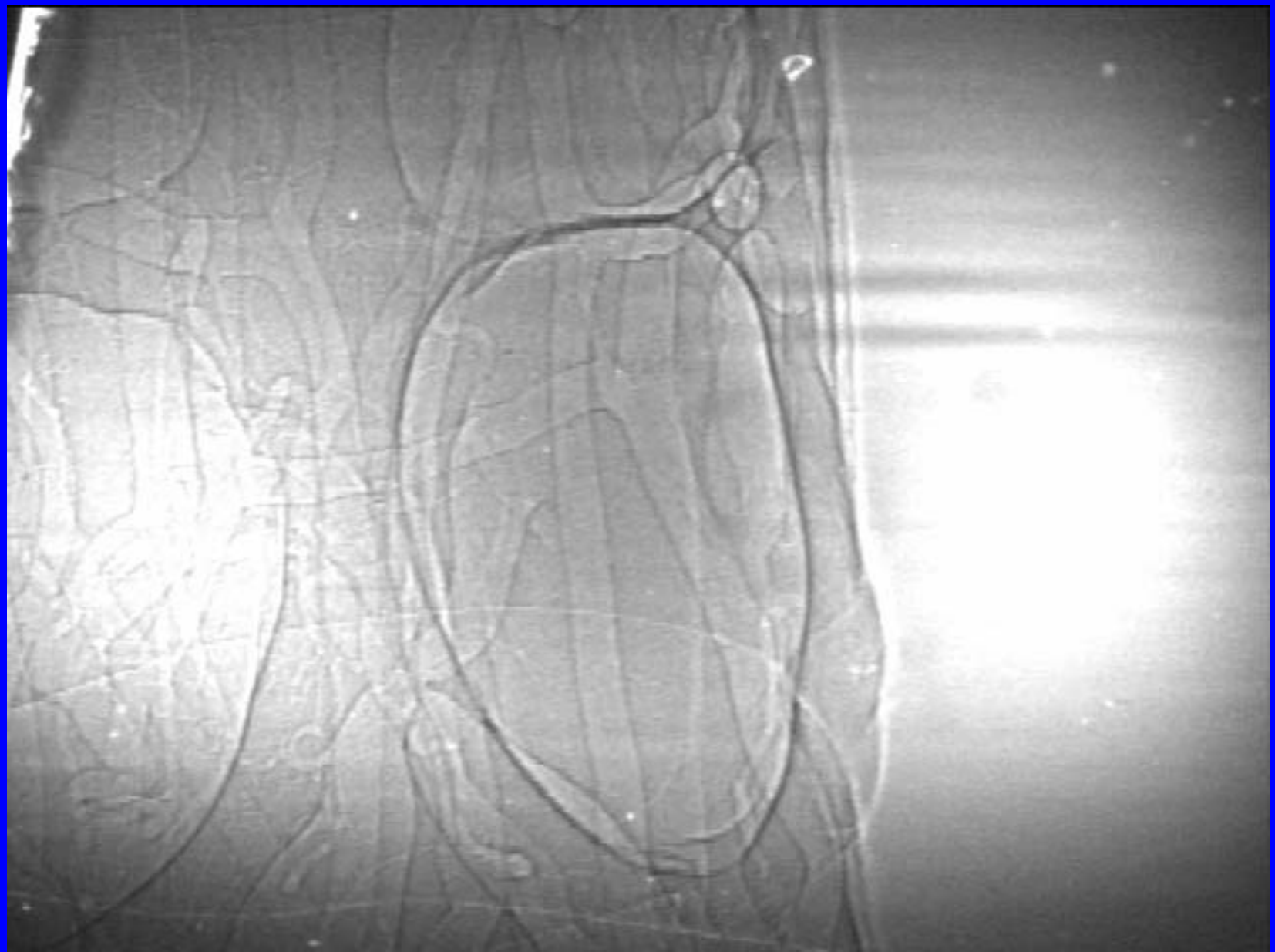
Some images to light (overall, 50% of images analyzable)



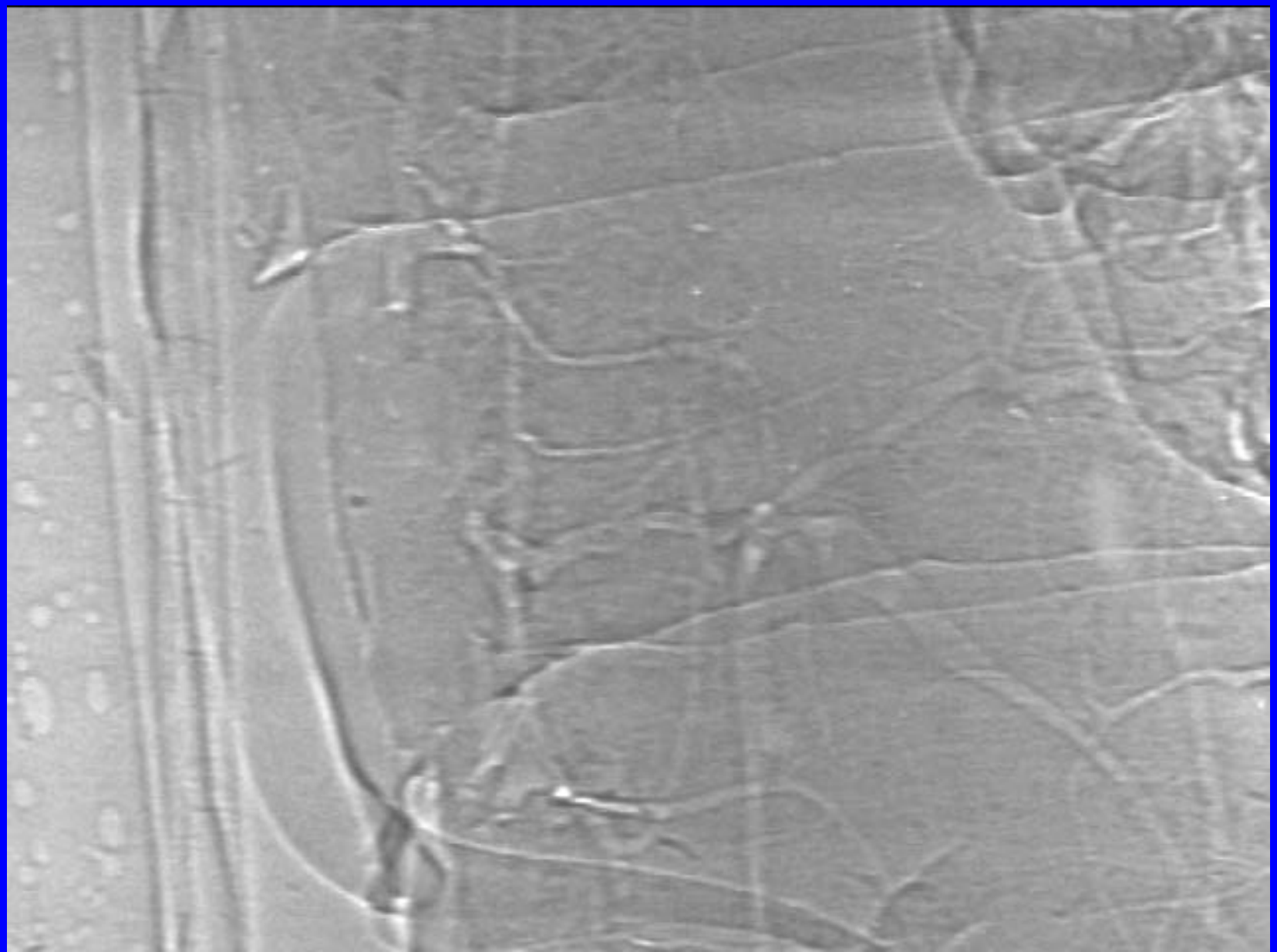
Quantitating tracheal system morphology

- Printed images from video
- Analyzed 50% of animal across all segments
- Overlaid acetate printed with high density points
- Used point counts to estimate cross-sectional area of body, tracheae, air sacs

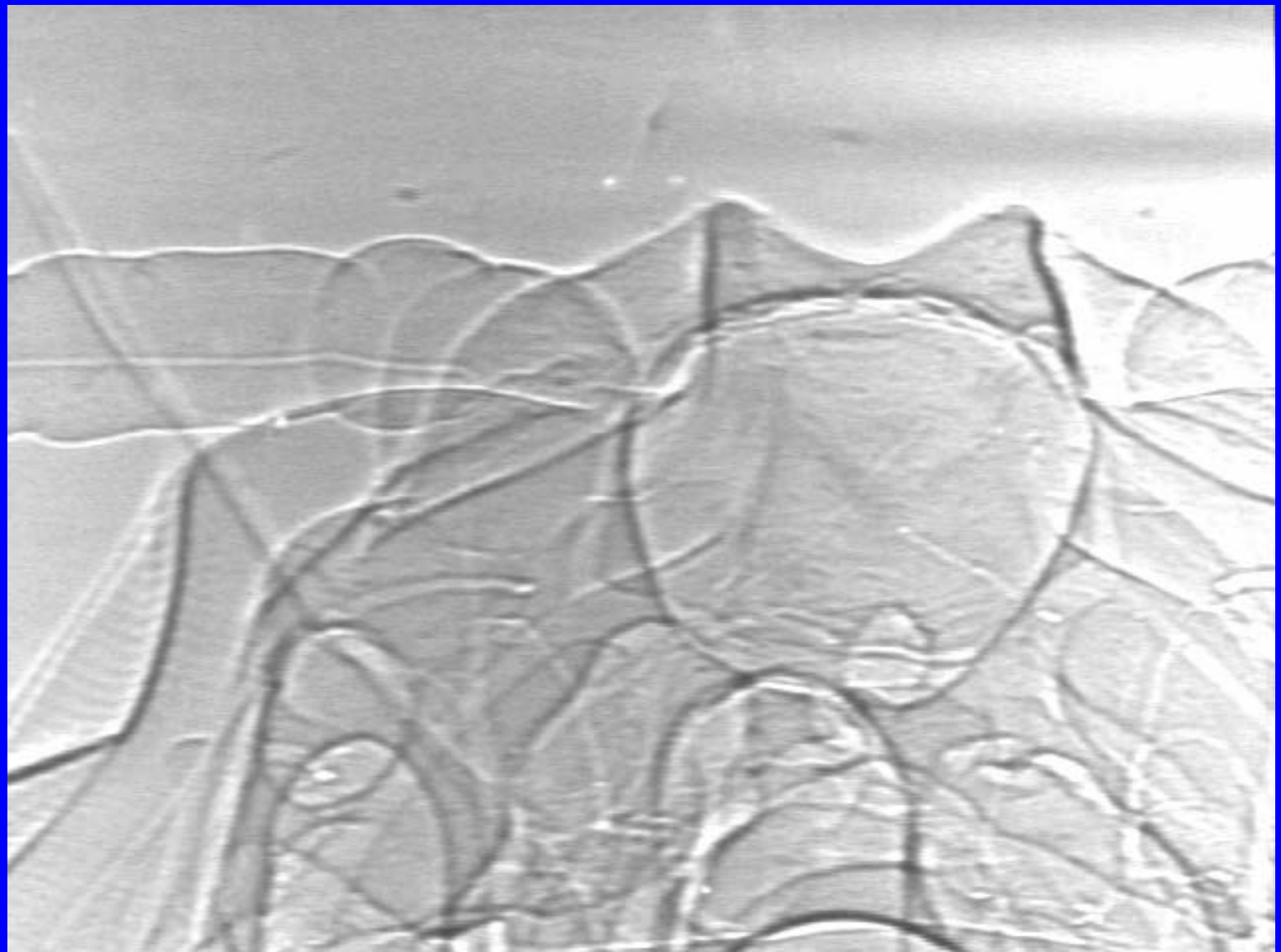
Adult abdomen: large air sacs overlay tracheae



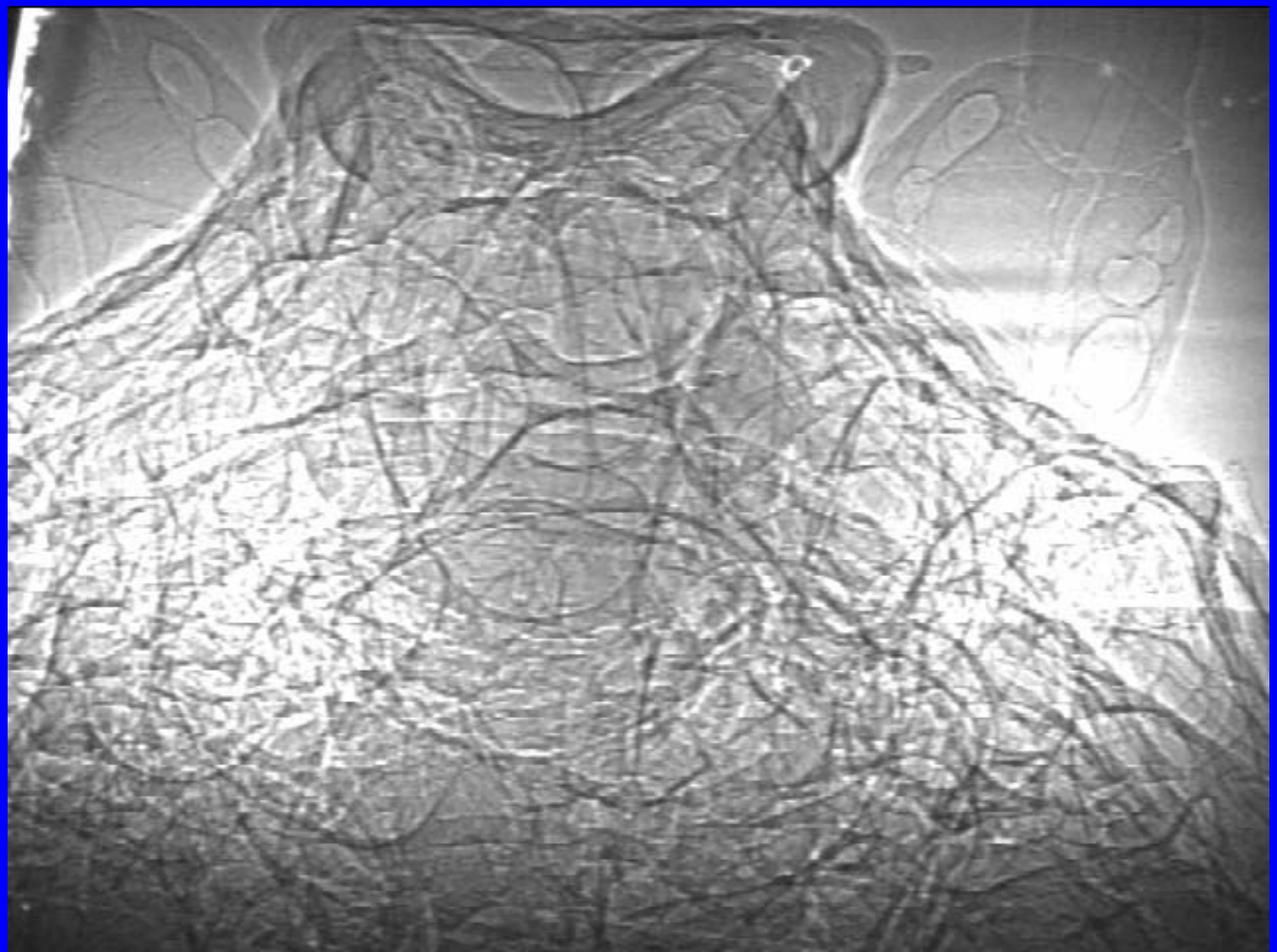
1st instar abdomen: some collapsible tracheae but no air sacs



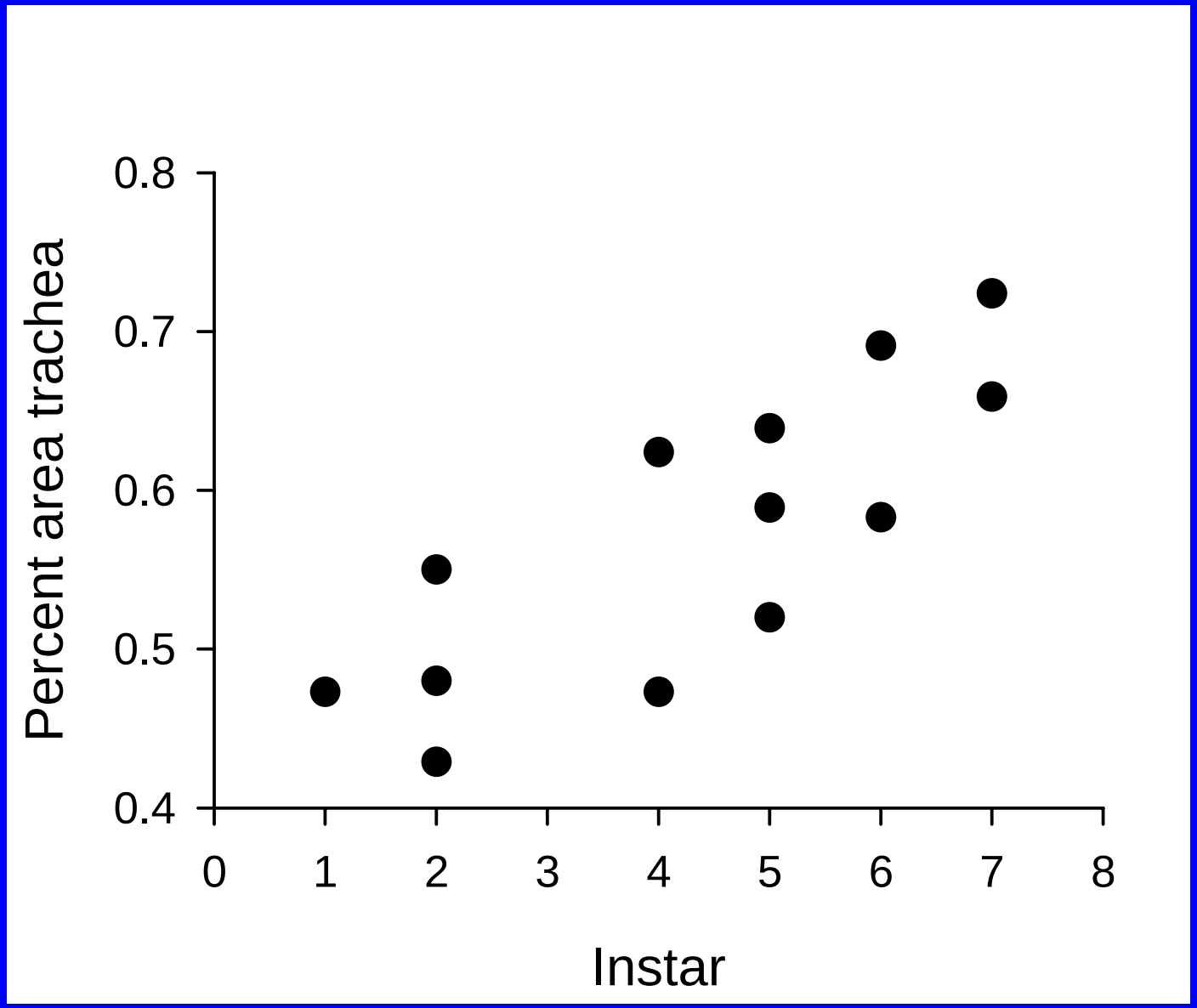
1st and 2nd instar: some air sacs in head



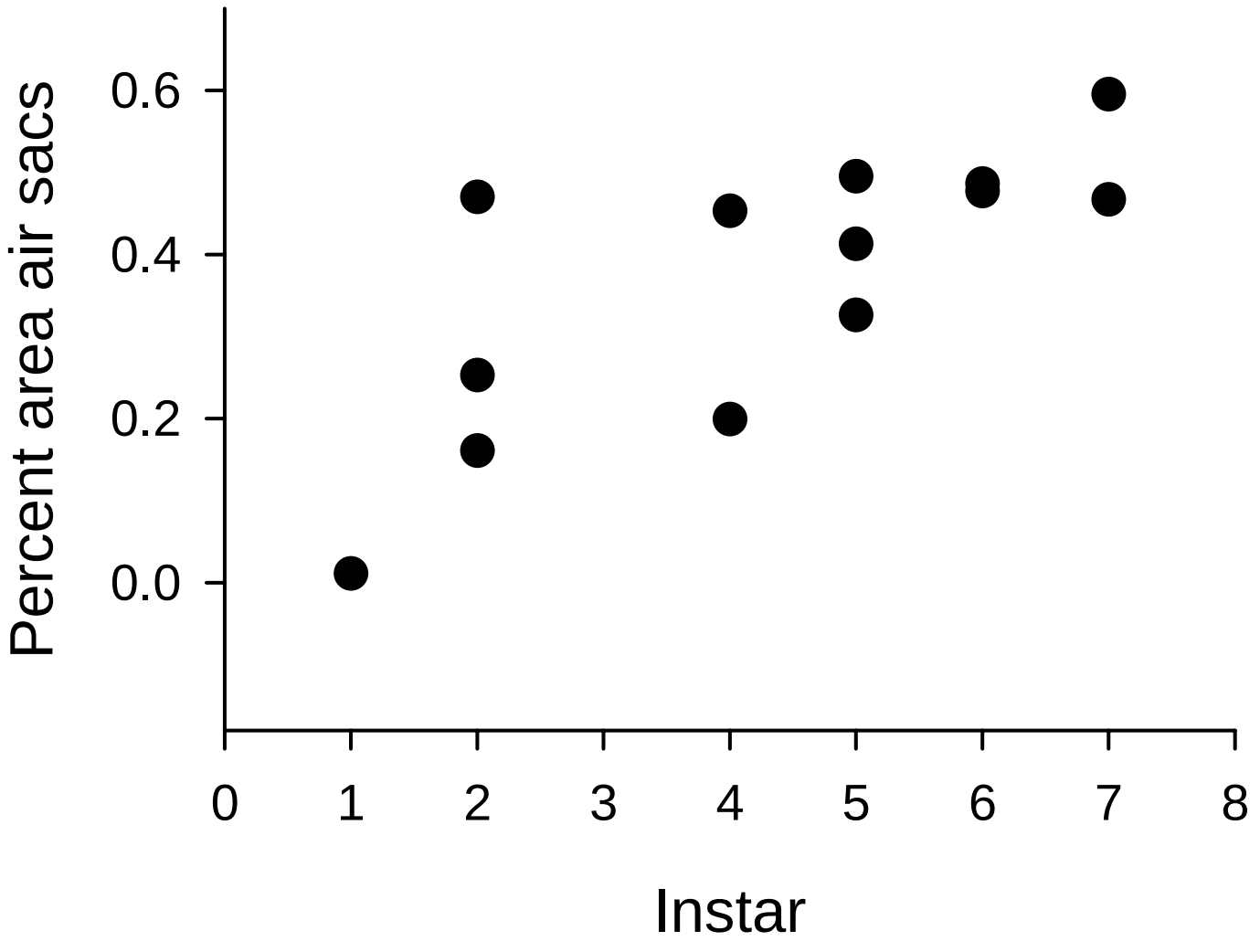
Overlaid maze of air sacs and tracheae in adult head



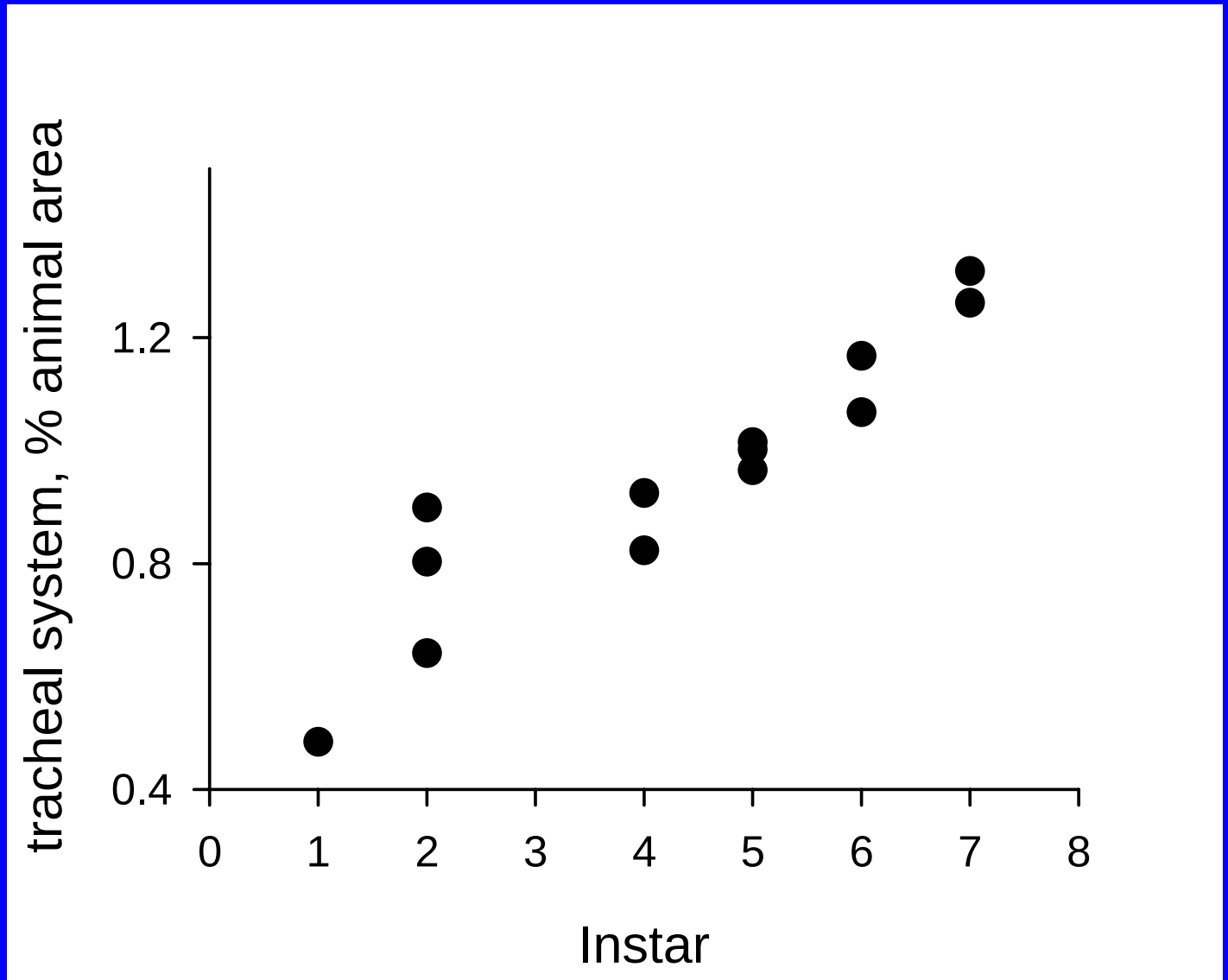
Larger/older grashoppers have more tracheae



Larger/older animals have more air sacs



Total tracheal system area increases strongly with size/age



Tracheal volumes (not % areas), and change in volumes/time will relate directly to convection

- Can do this for single, identifiable air sacs or tracheae
  - Choose a geometric model
  - Measure required distances
- How to do this for a whole animal?
  - Insect on a rotating pole?/split beam with image taken in 2D?

Does atmospheric oxygen level limit insect size?

- Evidence against:
  - Larger insects do not have more difficulties attaining oxygen (also comparative studies)
  - Larger insects use convection to overcome oxygen delivery challenges
- Evidence for: In some species, rearing oxygen level strongly affects body size

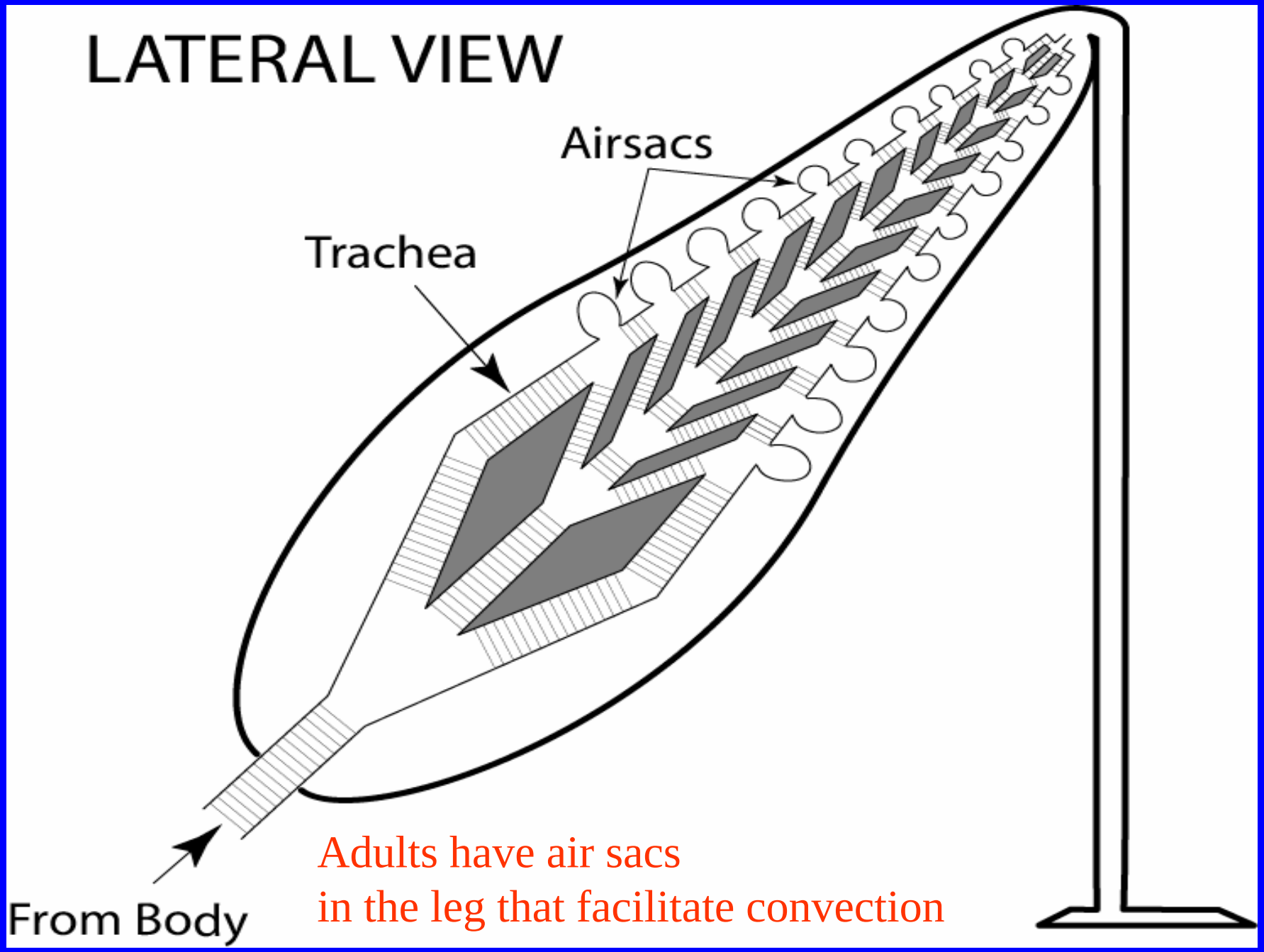
Questions and tools wish list

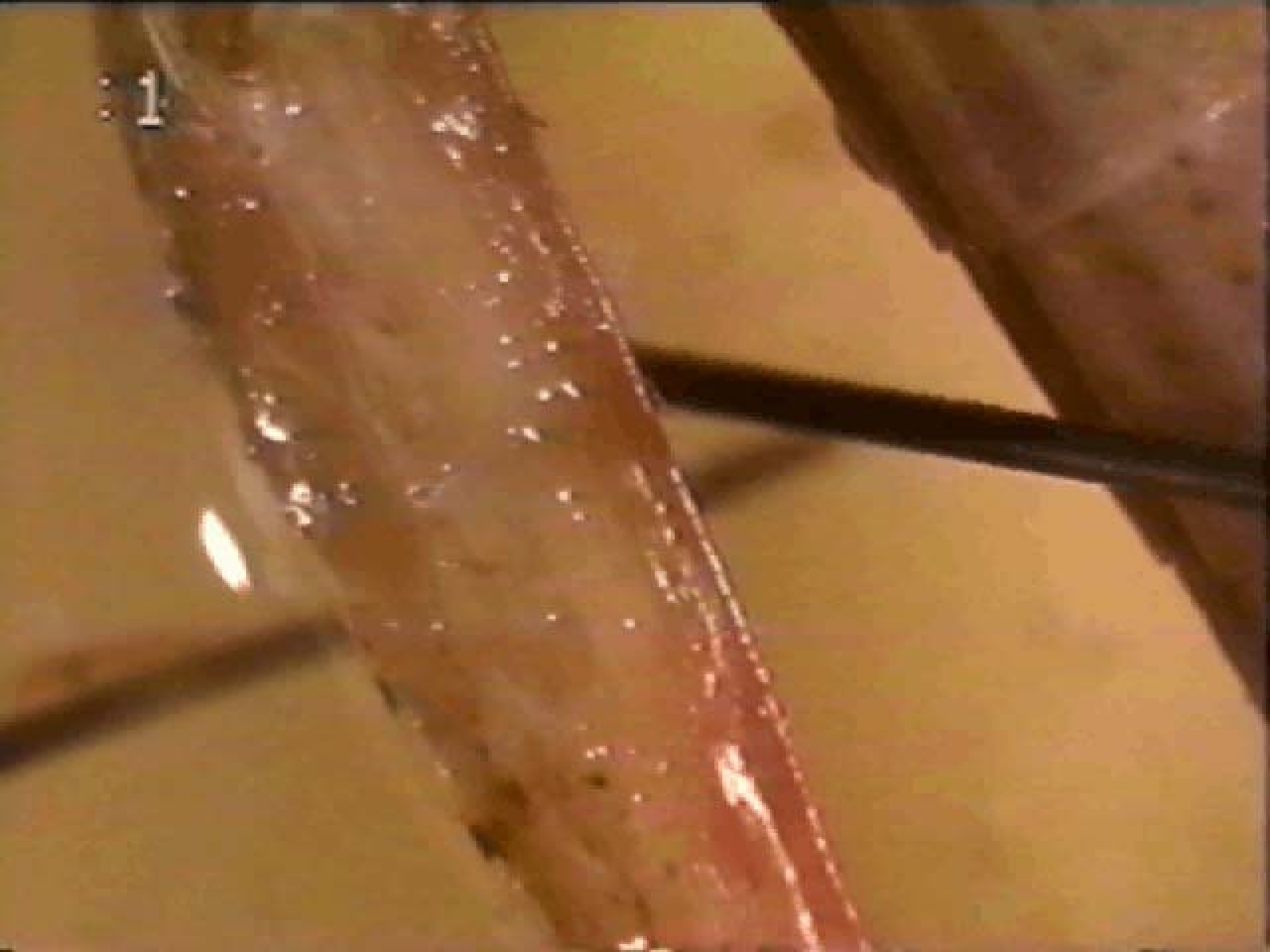
small enhancements of current system

- How does the tracheal system scale across insect species? 3-D tomography of tracheal systems for insects ranging from <1 mm to 10 cm
- How does air flow within segments, to legs, antennae, etc? Improved, repeatable image quality, high speed video

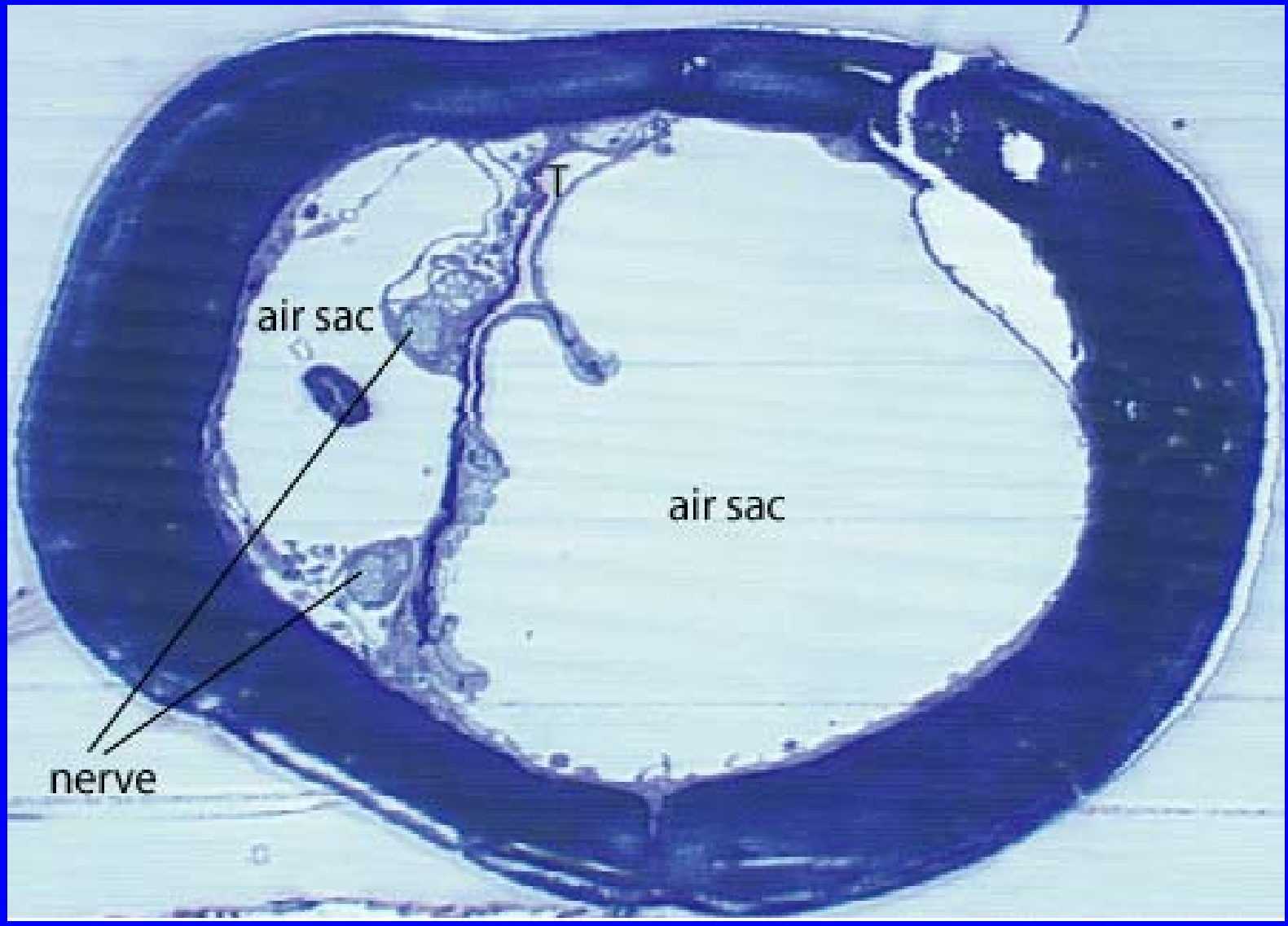
Dream Questions/Experiments

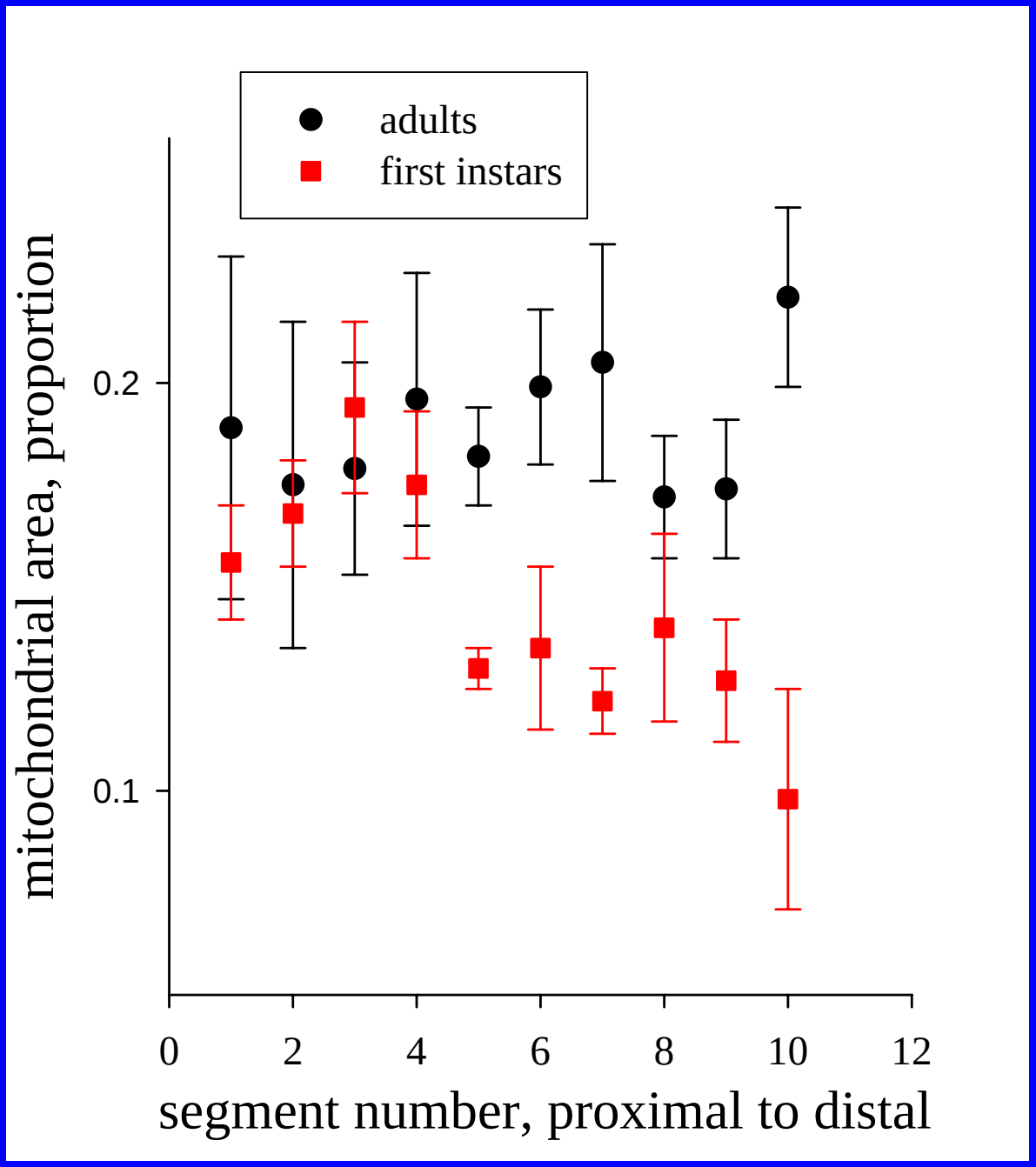
- How and under what conditions is variation in tracheolar fluid level important? Resolution to measure fluid levels in tracheoles (0.5 micron) over 0.1 sec time scales
- How much convective air flow occurs and where does it go? Radio-opaque gases or suspended particles
- What's the P_{O2} in every location of the body? Measure the oxidation state of iron in hemoglobin and myoglobin with high spatial resolution in 3D in a living animal.


LATERAL VIEW
Airsacs
Trachea
From Body
Adults have air sacs
in the leg that facilitate convection



Tibia interior all air!





In juveniles but not adults, mitochondrial content decreases distally along the leg.

Adults average nearly 2x the mitochondrial volume.