X-Ray Imaging of Living Small Animals: Insights for Biomechanics and Physiology

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Biomechanics and Physiology

Organic Structure

- anatomy and 3D organization of animals

Animal Function:

- how do animals work?
- muscle/skeletal function, basic elements of behavior
- new views of mechanism in tiny animals

Development:

- how are adult bodies constructed during growth?

Evolution:

- functional characters and the search for historical patterns across diverse groups

Approach and Study Systems

Techniques:

- synchrotron phase-enhanced x-ray imaging
- computer tomography: imaging morphology

Insect Respiration:

- novel mechanism of tracheal compression in head

Insect Mouthparts:

- imaging feeding mouthparts with x-rays

Fish Biomechanics:

- respiration, pharyngeal jaw mechanisms

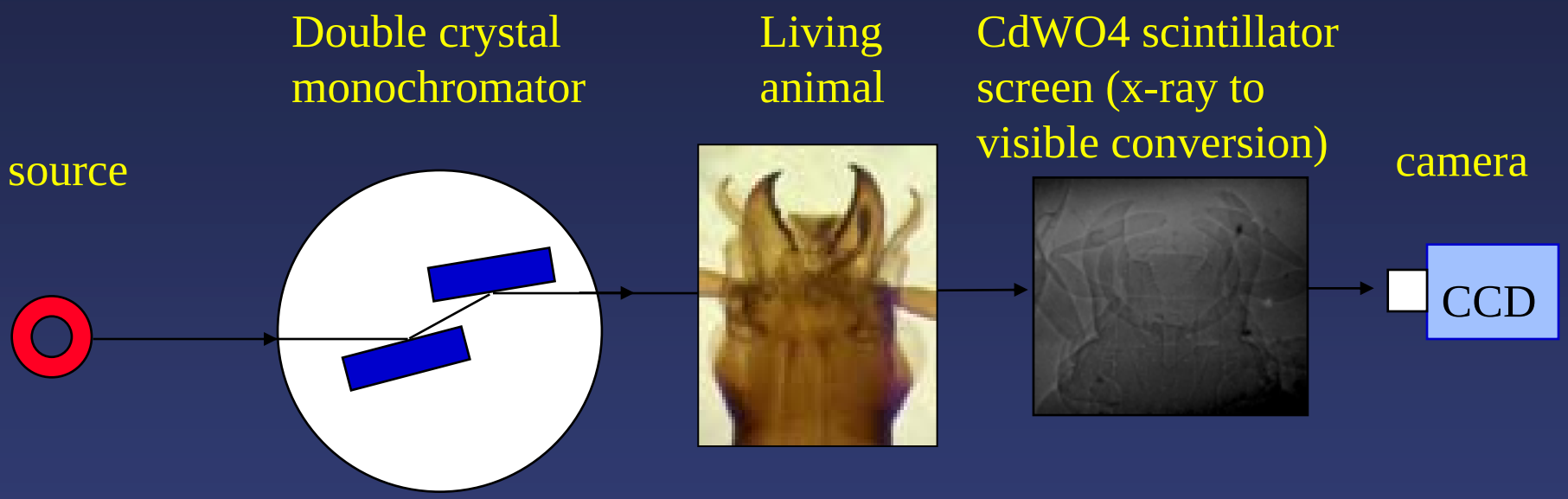
Other Applications:

- drinking mechanisms, cell motility, insect behaviors

Advanced Photon Source:
Beamline XOR 1-ID Station C

station C set up for insect imaging

Phase enhanced x-ray imaging:



X-ray images depend on differences in density within the sample.

Also: Beam coherence produces interference fringes (edge enhancement effects).

Computer tomography (CT)

Procedure:

- Take 2-D images (radiographs) at many sample rotation angles.
- Typically, 500-1000 images (angles). Each radiograph ~ 1 sec.
- Large amount of data: 1K x 1K x 1K x 14bits/pixel = 1.75 GB

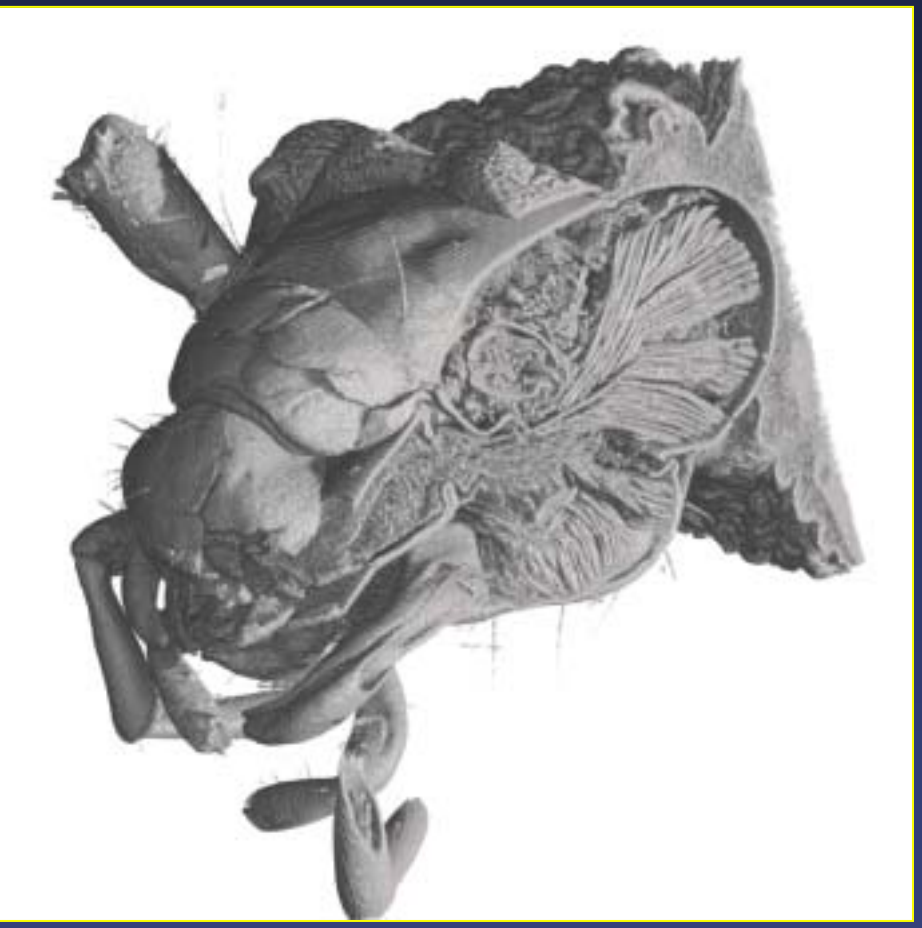
Visualization and analysis software:

- Rendering: makes it look real
- Allows for digital subtraction of high or low density voxels to see minor density differences.

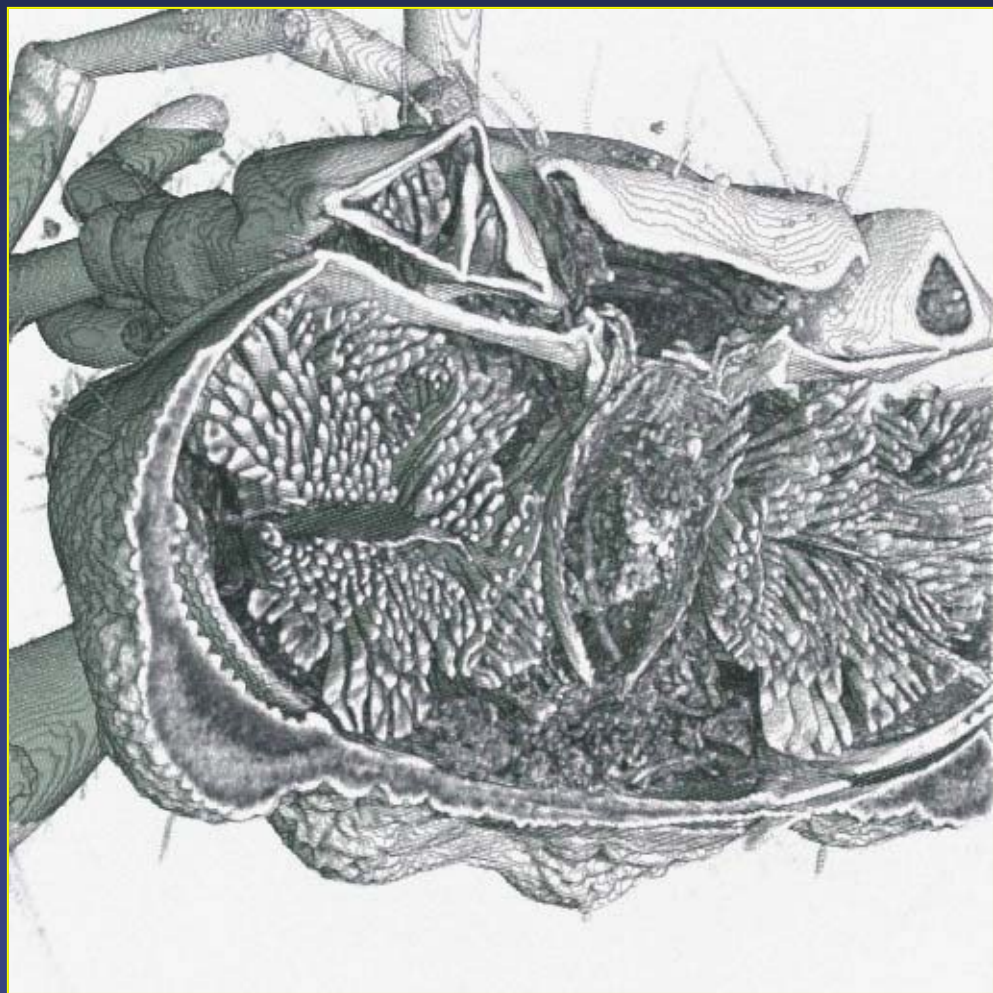
Useful for physiology and biomechanics:

- Fine-scale 3D morphology critical for animal motion studies
- Identify tissue-level changes e.g. muscle-tendon-bone
- Input for biomechanical and functional models

Cephalic Morphology: *Forficula*



Size of head ~ 3 mm
Resolution ~ 7 microns



Reconstruction: O. Betz
Performed at ESRF

Applications:

- Identification of small density differences (~1 percent level), such as between muscle and tendon
- Quantification of details: Porosity, material distributions.
- Visualization of intricate joints and musculature.
- Fiber angles in muscle and connective tissue.
- Developmental morphology.
- Advantage over other techniques (IR, MRI) is RESOLUTION (to < 1 micron).

Respiratory Mechanisms

Ground Beetle *Platynus decentis*

Carpenter ant: *Camponotus pennsylvanicus*

Grasshopper: *Schistocerca americana*

Insect Tracheal Respiration

Diffusion

Krogh 1920 Very slow, dependent on open spiracles.
Largely rejected as sole mechanism.

Circulatory hemolymph pump

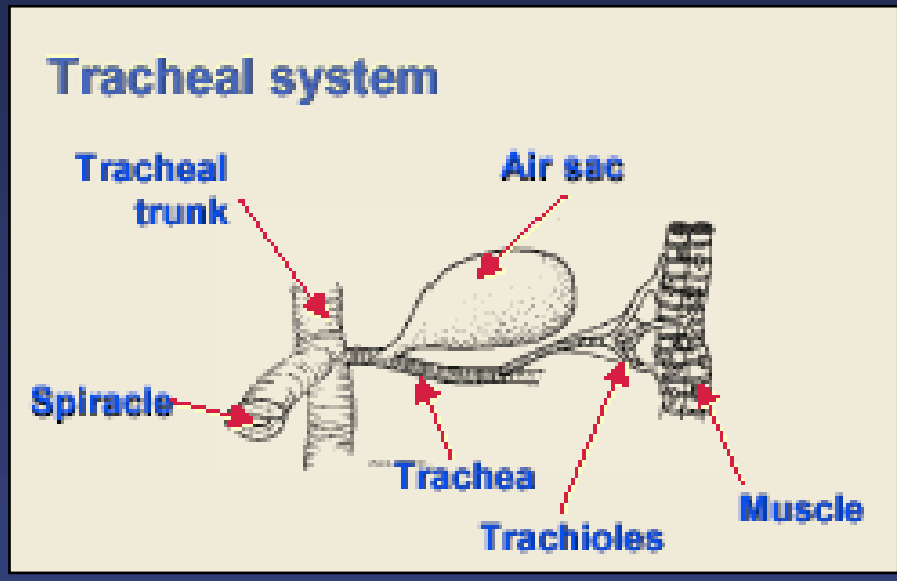
Wasserthal 1996; Slama 1988

Slow, ~ once per minute (at rest).

Abdominal pumping

Miller 1981; Mill 1985

Fast, 0.1 - 3 Hz (stress).



Autoventilation: wing pump, leg pump

Weis-Fogh 1967; Chapman 1998

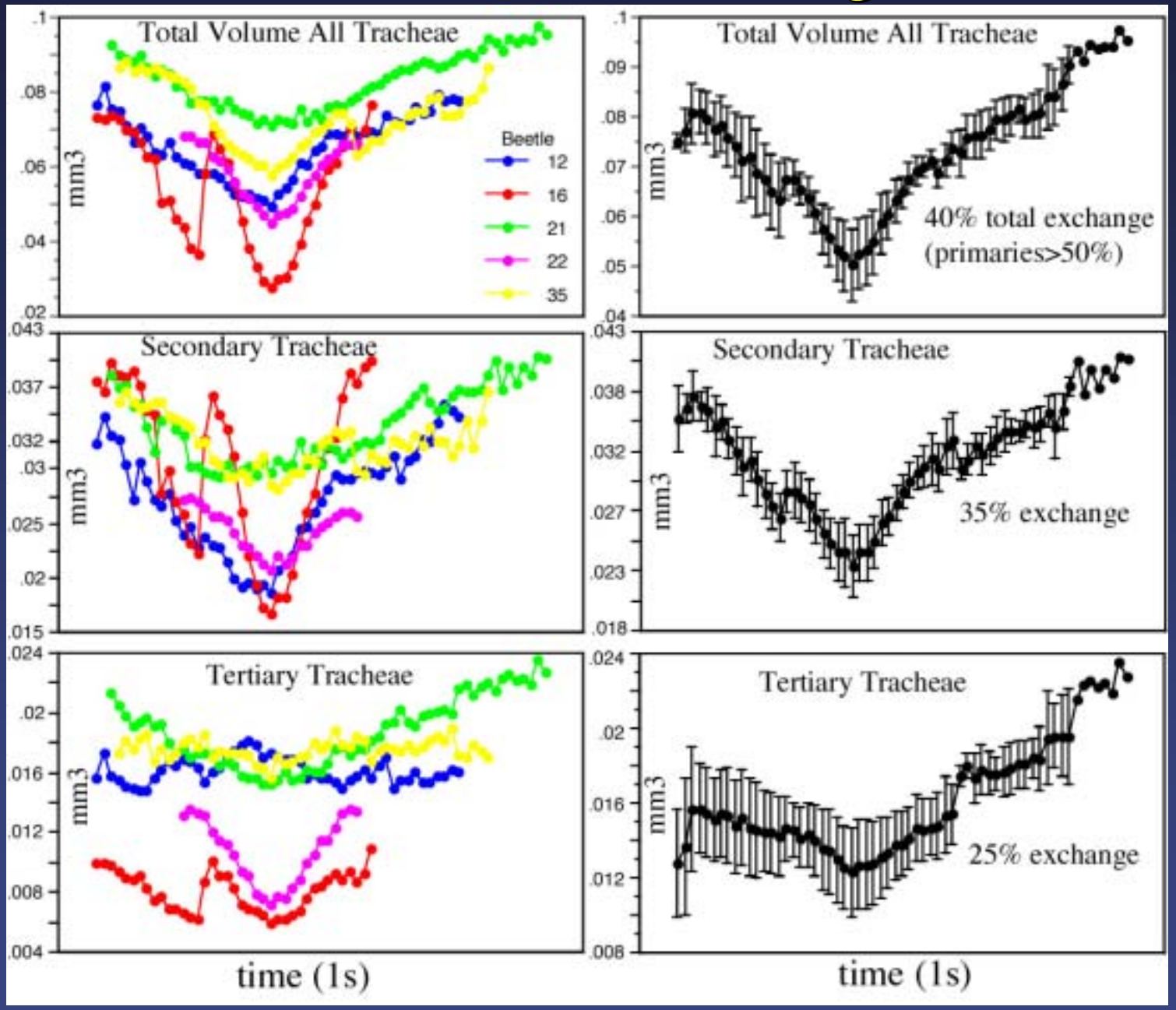
Very fast- at rates of wing beat or limb motion during locomotion.

Tracheal diameter and volume change

-segment length (h)
-diameter (2r)

Volume= $\pi r^2 h$

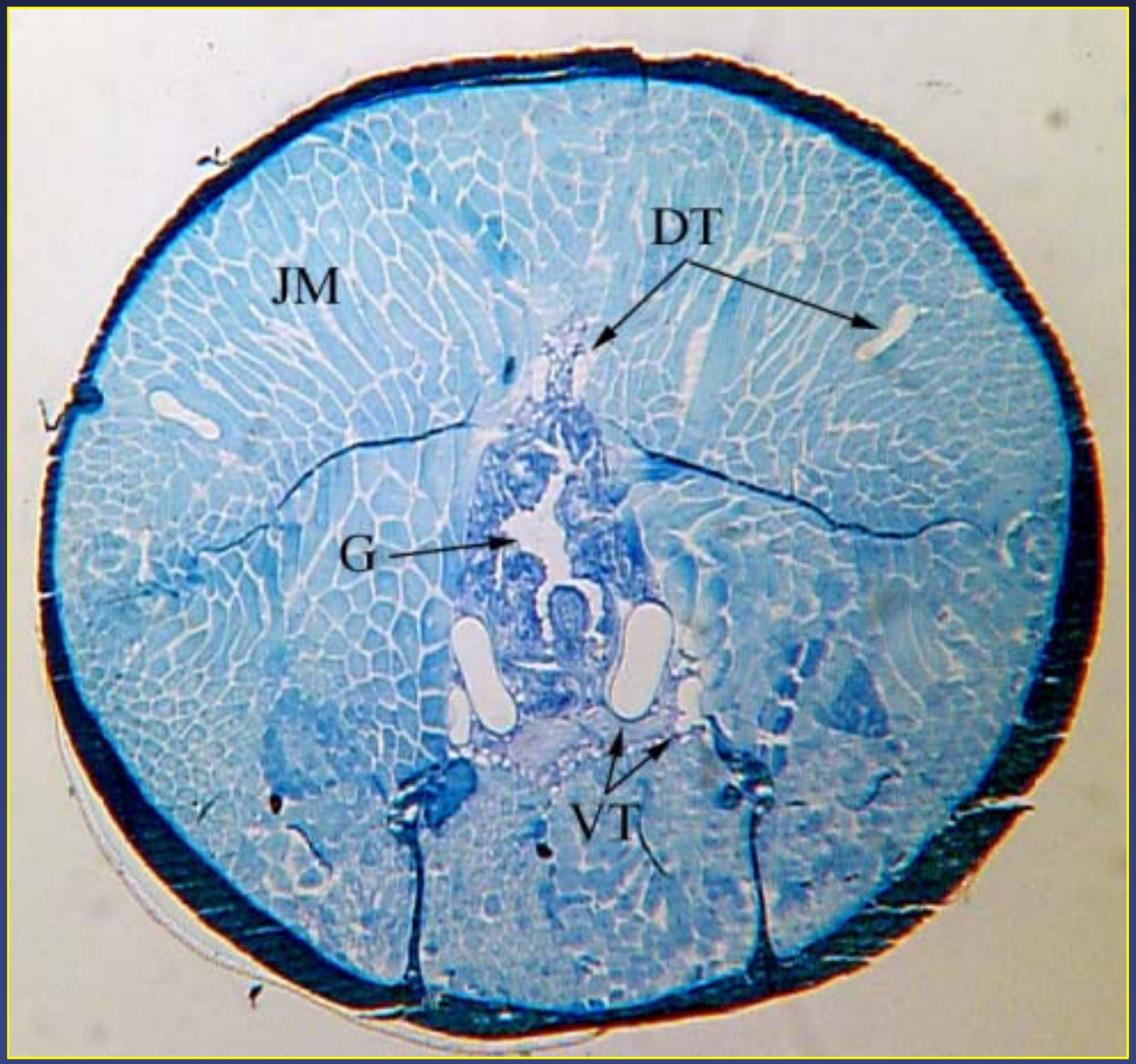
Tracheal volume change



Tracheal Morphology: Head

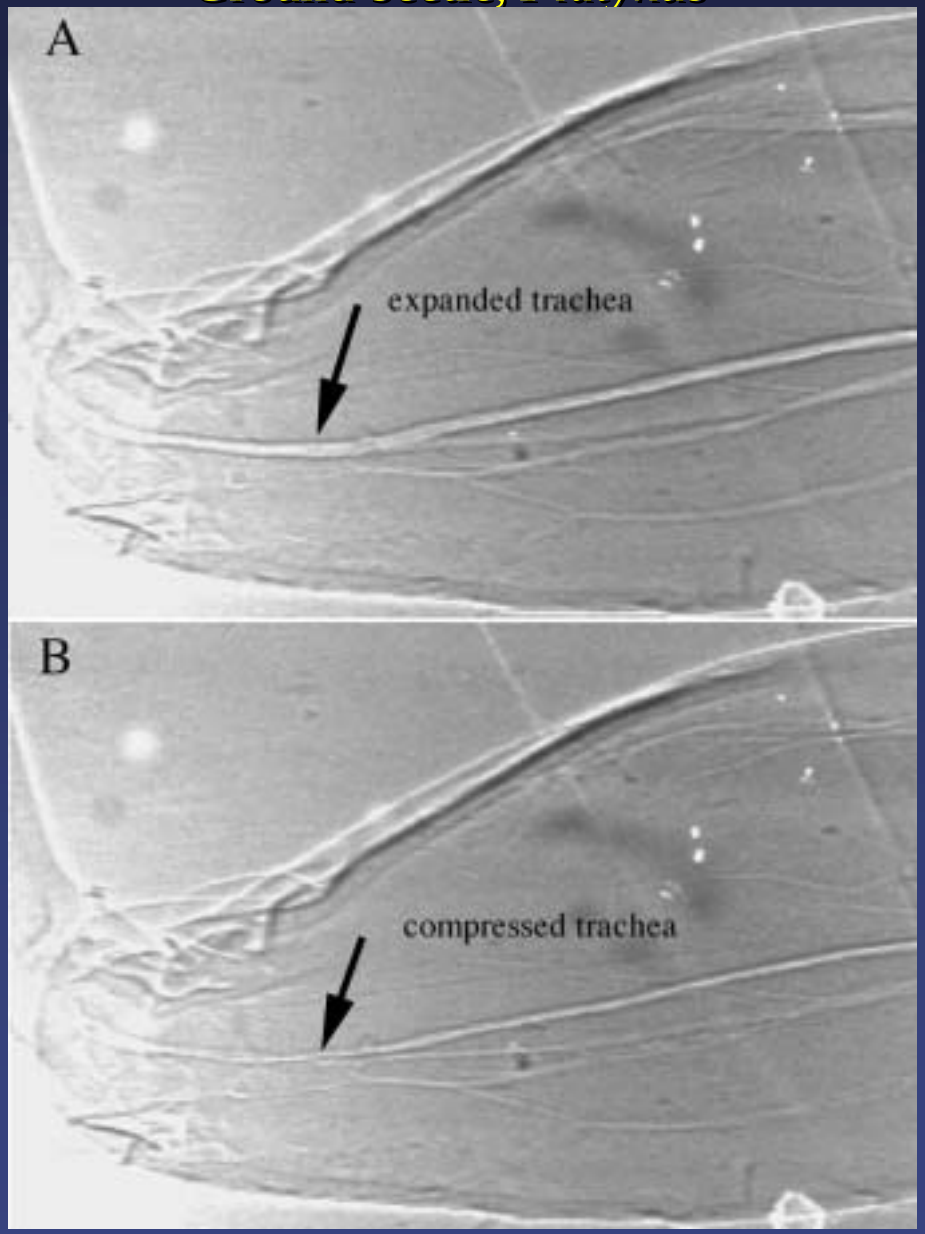
Ground beetle, *Platynus*

Taab resin
Stevenel's Blue



Tracheal Compression: Leg

Ground beetle, *Platynus*



Socha et al. In Prep.

Diversity

Several ground beetles: Carabidae

Centipede: Chilopoda

Earwig:

Dermaptera

Large milkweed bug
Hemiptera: Lygaeidae *Oncopeltus*

Silverfish: Thysanura

Beetle Tracheal Compression: Conclusion:

Respiratory compression in the head and thorax:

- previously undescribed mechanism of tracheal compression for oxygen exchange to cephalic tissues.
- NOT air sacs, hemolymph pumping, abdominal compression, autoventilation, larval pumping, or PSV

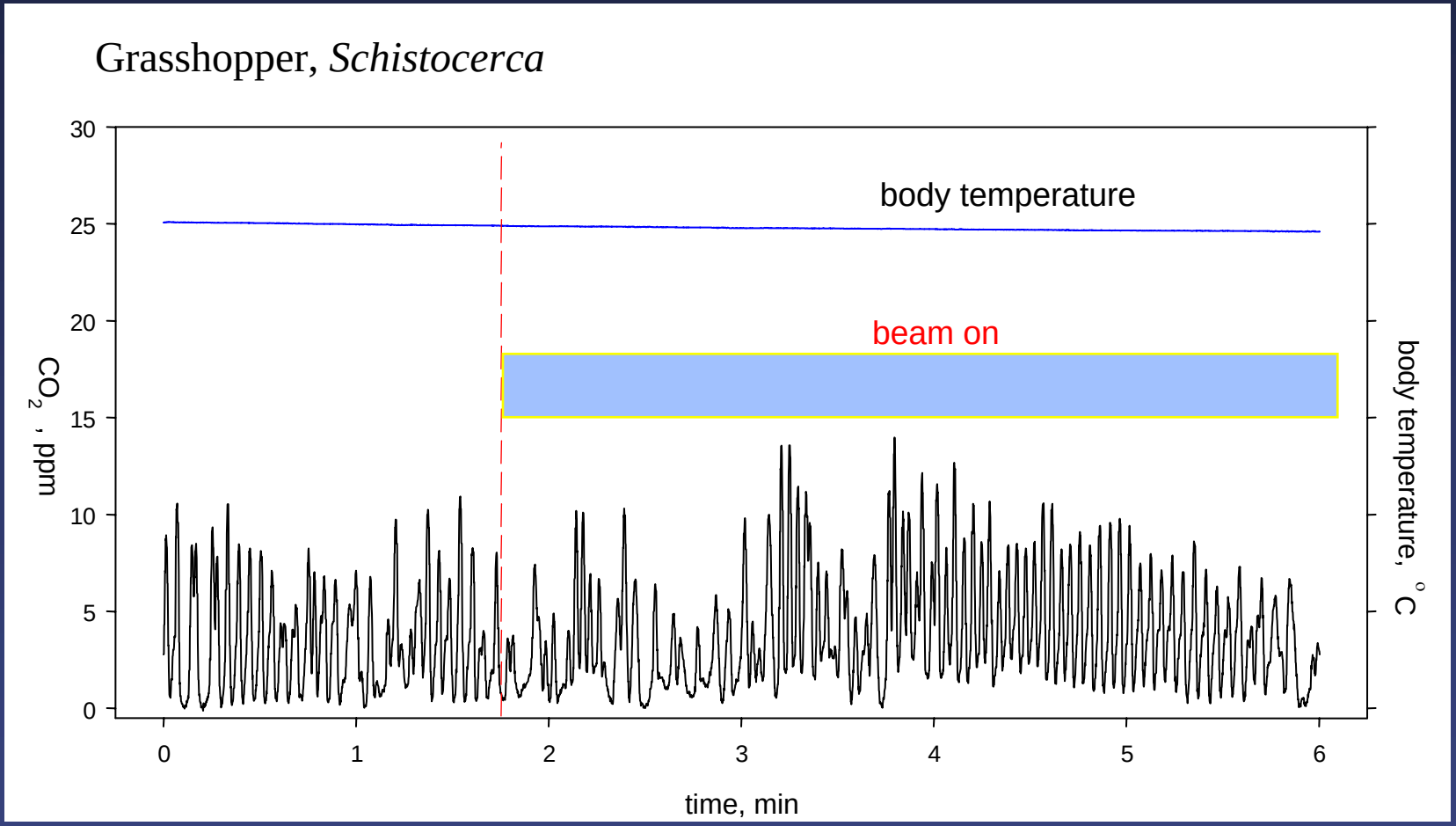
Proposed mechanisms:

- intramuscular pressure
- thoracic or cephalic muscular diaphragm

Proposed functions:

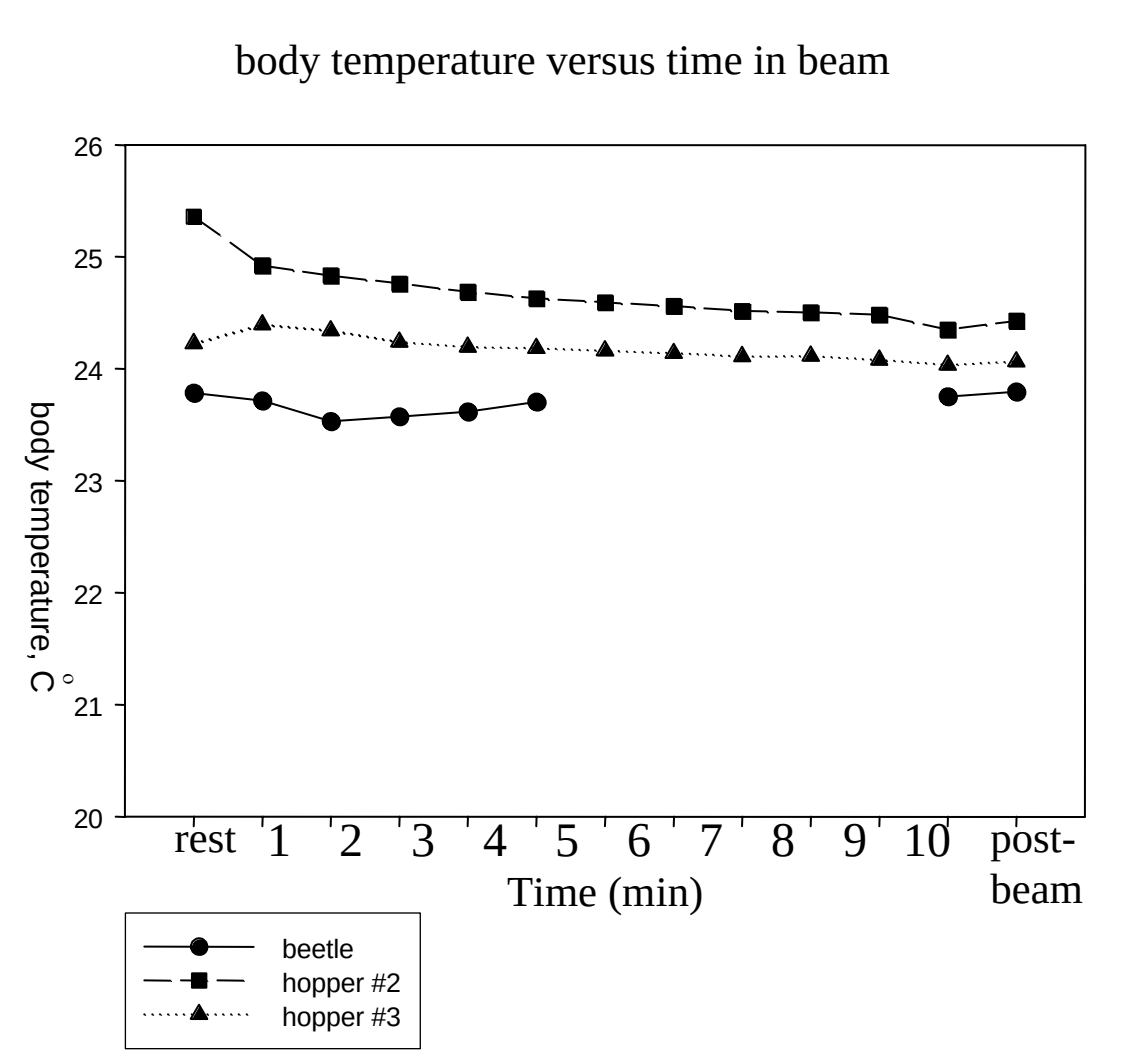
- spiracle open: 30-50% convective air exchange
- spiracle closed: increase internal PO2 for diffusion

Grasshopper: CO2 cycling and body temp in the x-ray beam



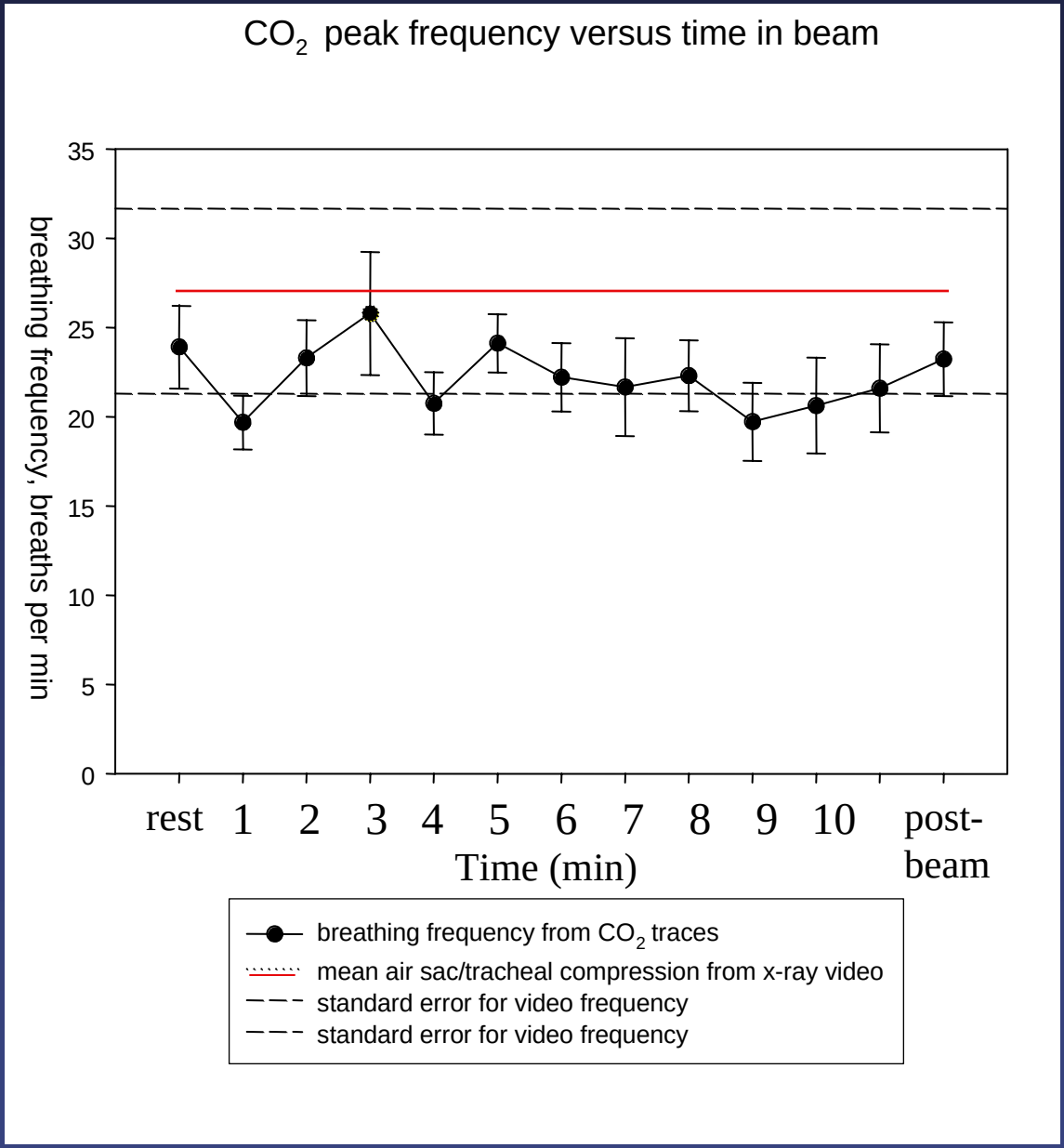
Greenlee, Harrison, et al. data

Hoppers and beetles: body temp in the x-ray beam



Greenlee, Harrison, et al. data

Grasshoppers: CO2 cycling and compression frequency



Greenlee, Harrison, et al. data

Respirometry Conclusions

Respiratory behavior in the x-ray beam is not high stress, but typical of previous work with insects in respiratory chambers.

- no significant difference between beam and no beam.

Beetles and Grasshoppers show tight correlation between x-ray video compression frequency and CO_2 emmissions.

- in different body regions and with different air-exchange mechanisms.

Ability to directly calculate tracheal and air sac volume change will allow more detailed physiological and biomechanical calculations of respiratory function.

Other mechanisms

Mouthpart function during feeding:
using X-rays for imaging internal mouthparts, and mechanics of mandible and maxilla.

Drinking mechanisms in insects.

Joint function: limb joints, tagmata joints
- click beetle

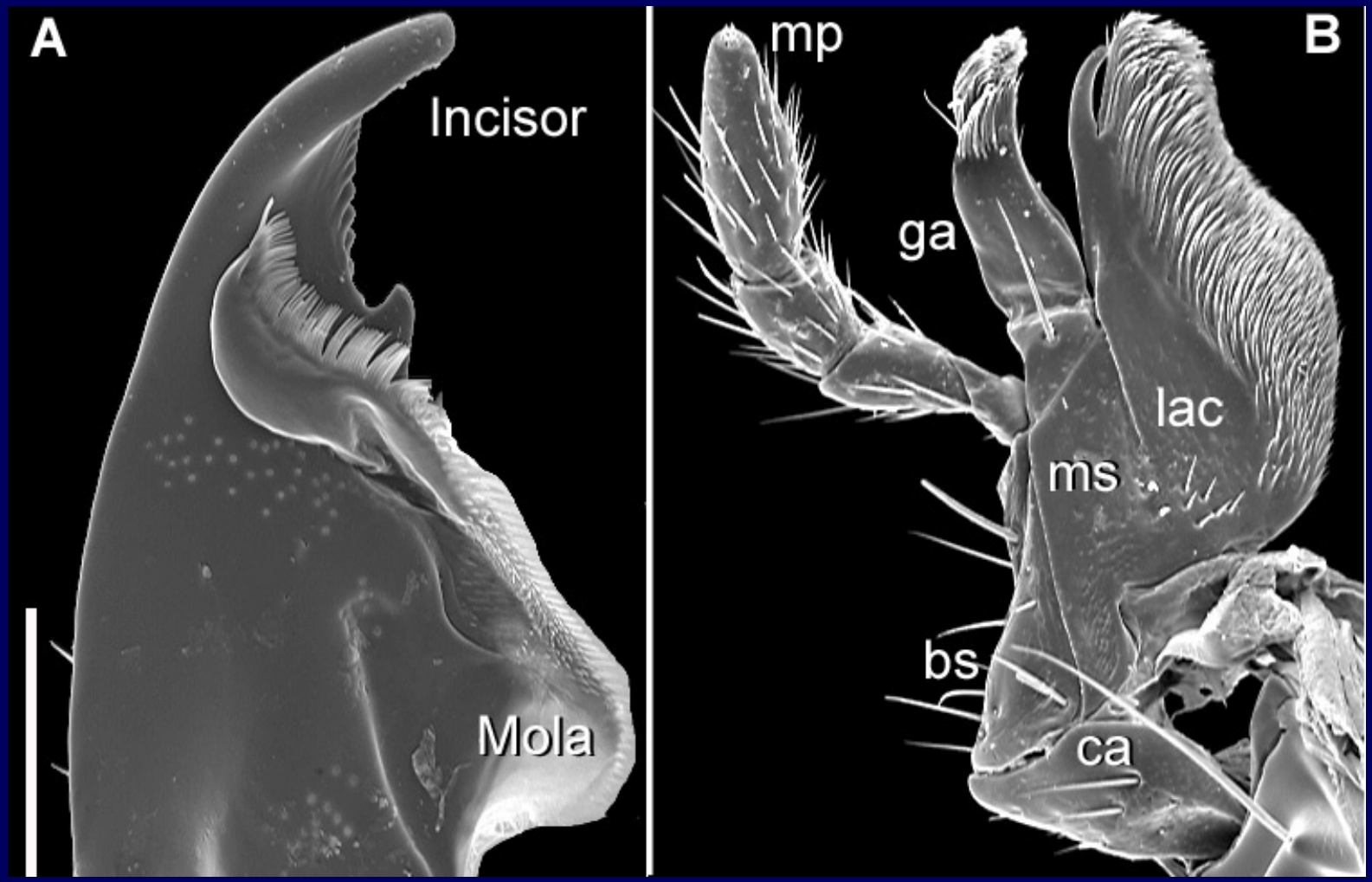
Copulation: x-ray study of complex genital coupling in beetles and flies

Jaws and Mouthparts

Beetle- *Platynus* ventral view

Ant- *Campostoma* dorsal view

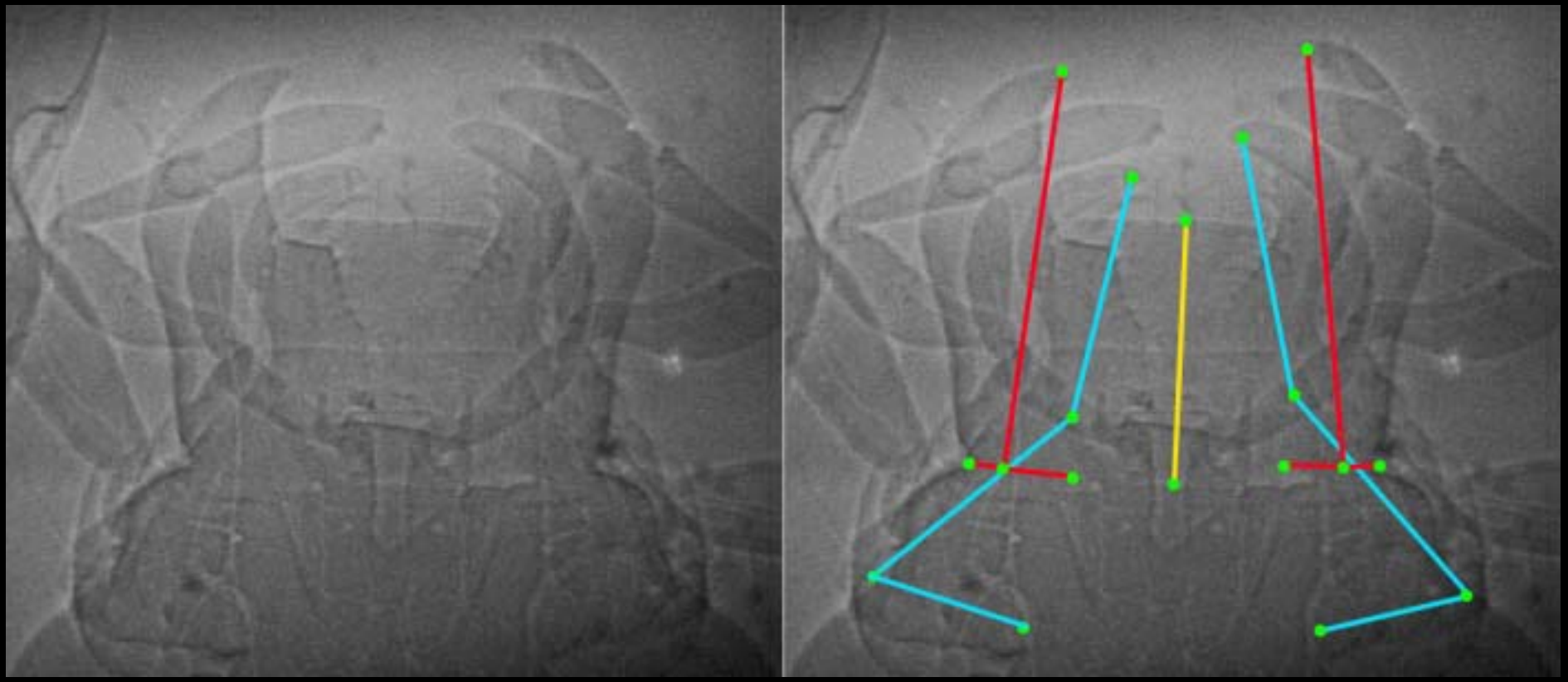
Electron micrograph- mandible and maxilla



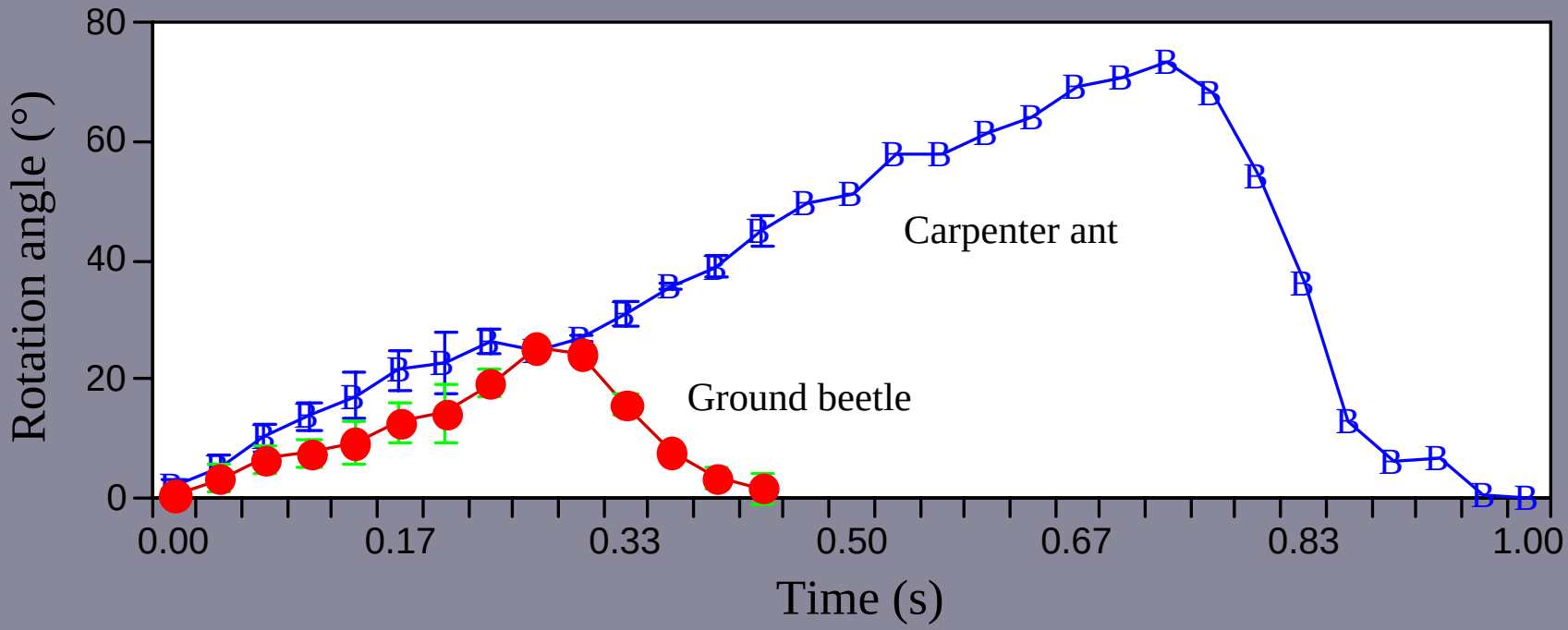
Beetle mandible

Beetle maxilla

Jaw mechanics- digitizing protocol



Mandible kinematics: beetle and ant



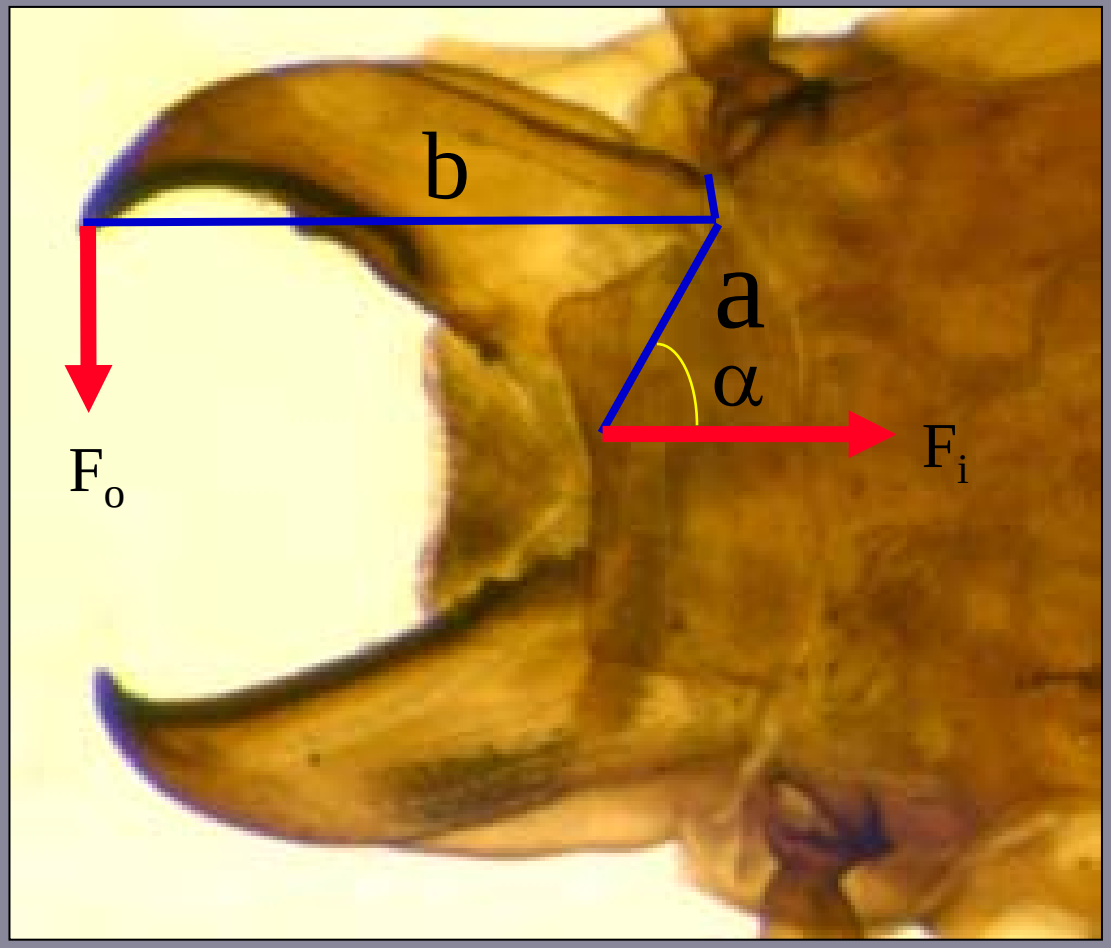
Frequency low in ant (1Hz), high in beetle (2-3 Hz).

Angle low in beetle (30°), high in ant (75°).

Closing velocity high in ant.

Lever mechanics: insect mandible as 3rd-order lever

Force out (F_o)= F_i * a/b* cosα

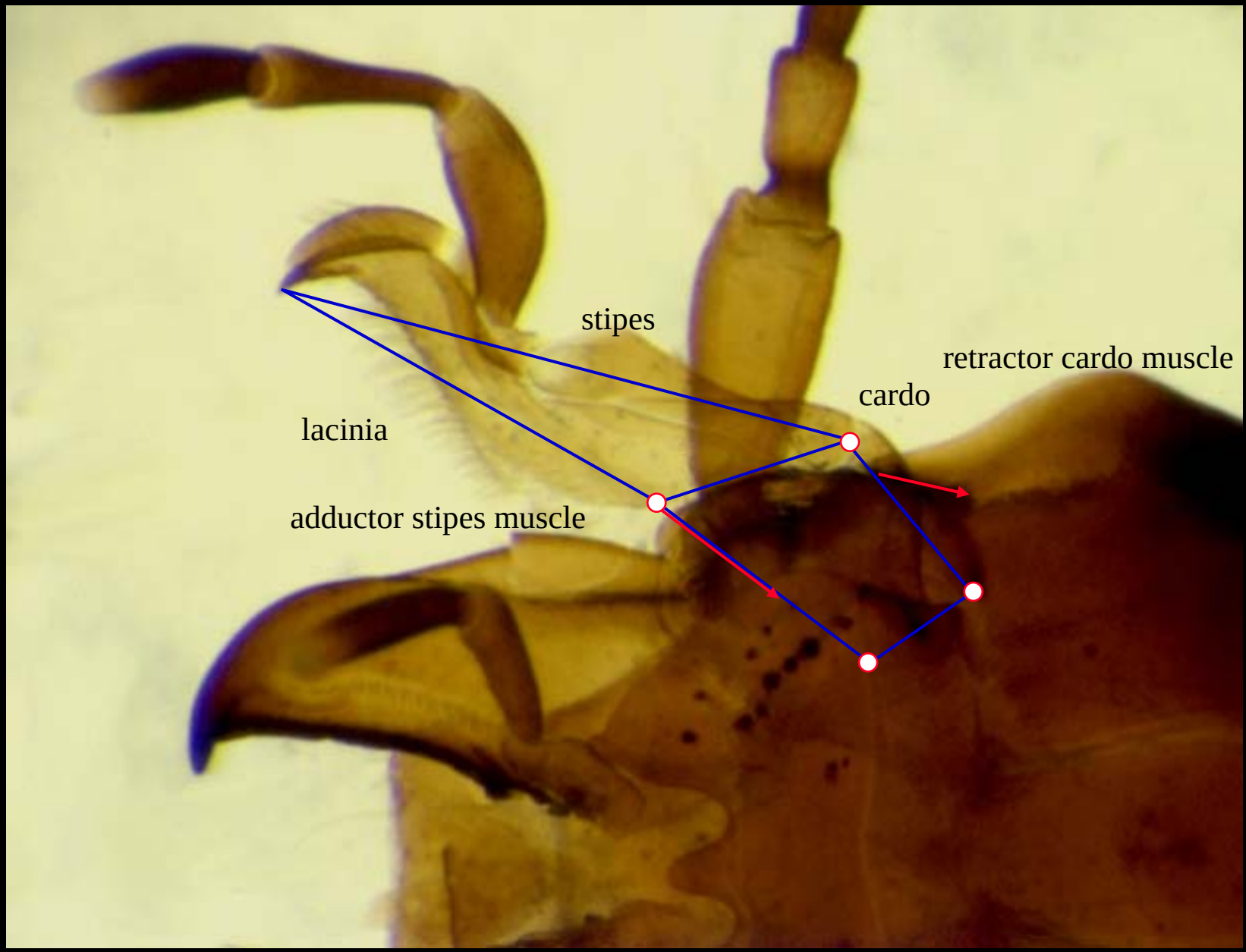


a= Inlever
Mandibular closing muscles

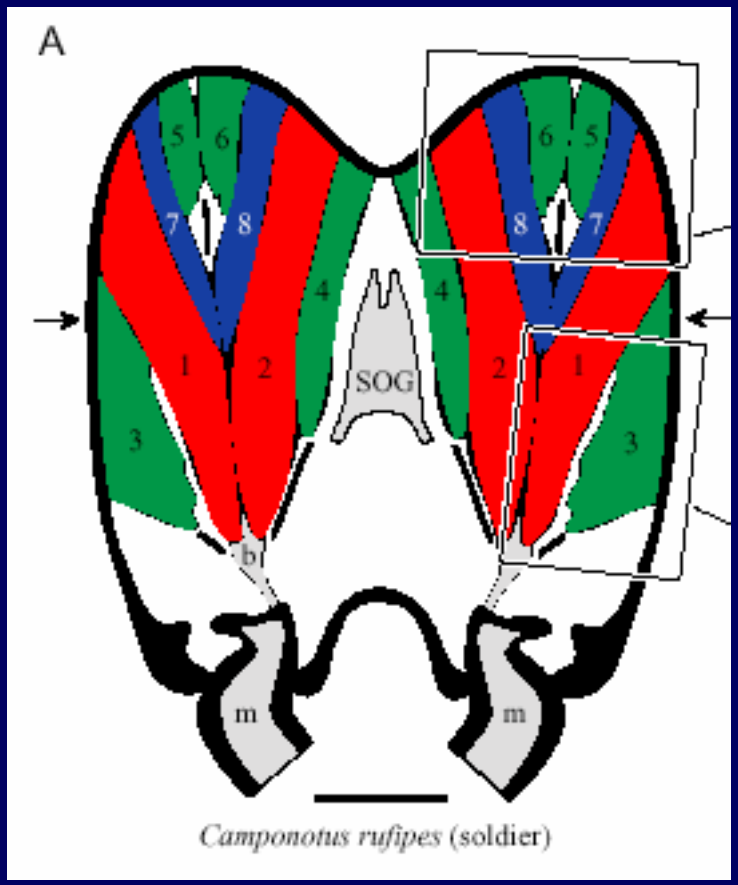
b= Outlever
Mandible length

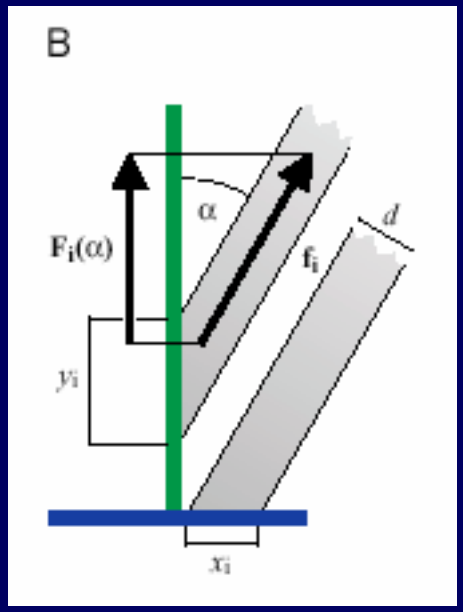
Closing Mechanical Advantage = a/b

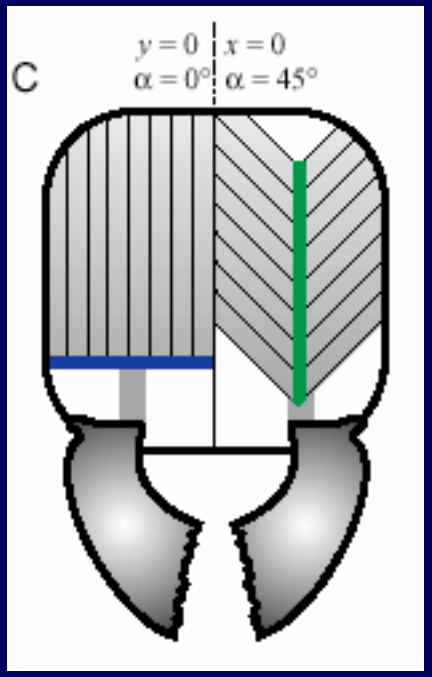
Maxillary mechanics- engineering linkage models



Models of Mouthpart Function

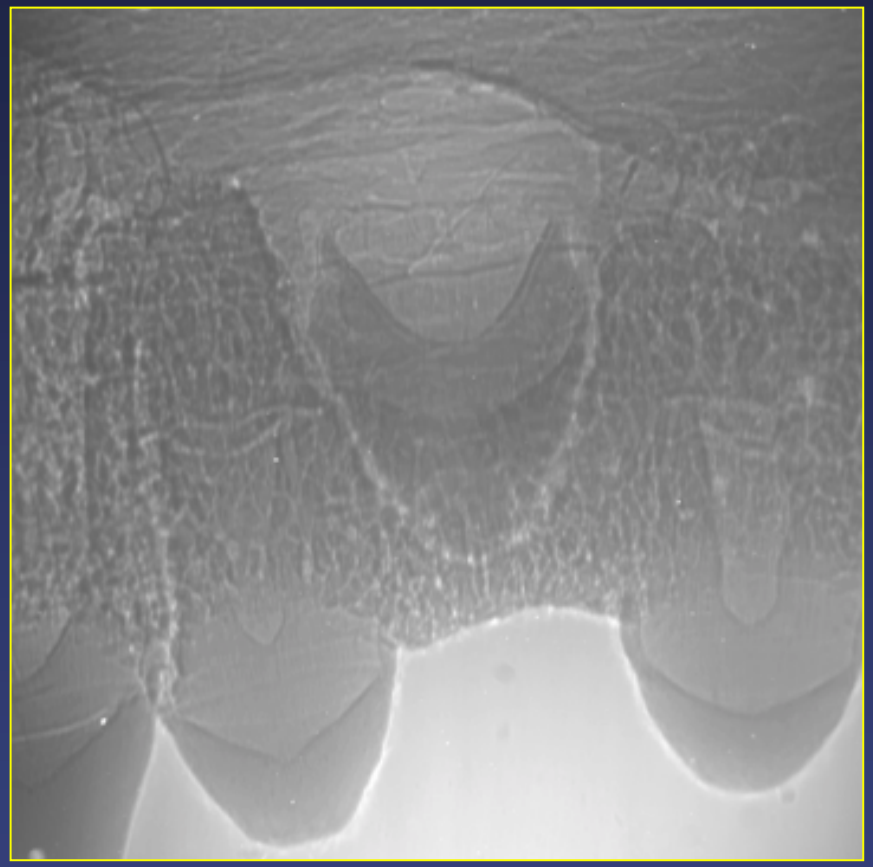


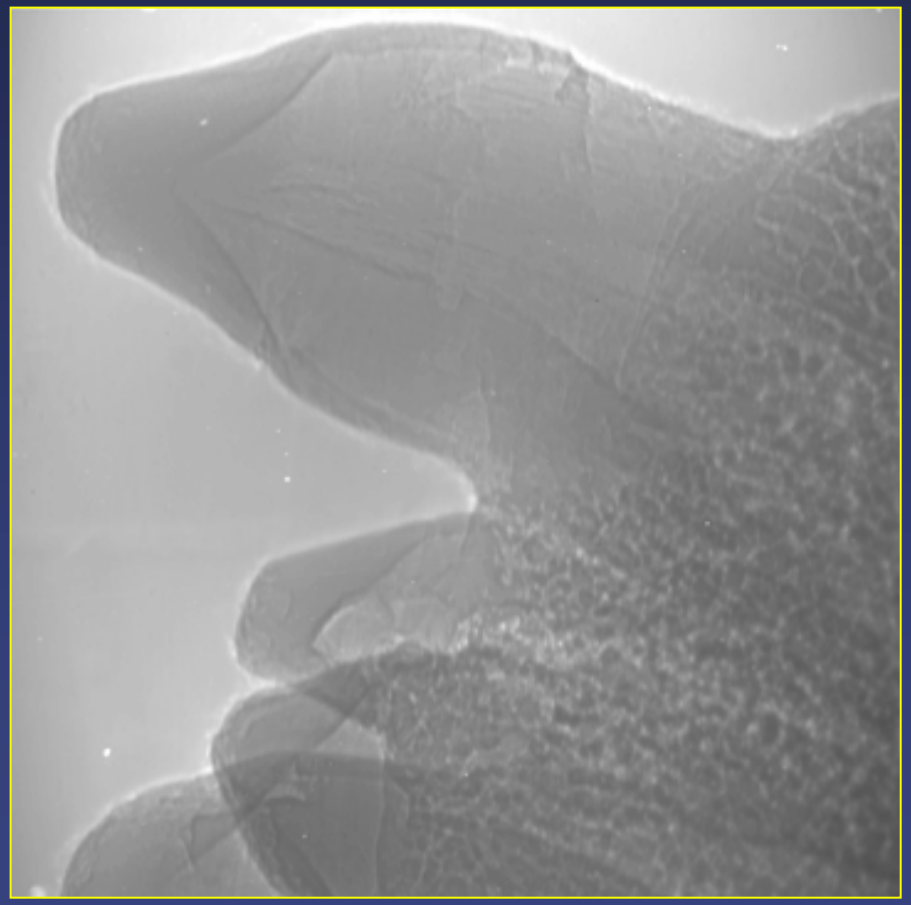




From J. Paul and W. Gronenberg JEB 1999

Fish Jaw Morphology





Conclusions

Insect Respiration:

- new mechanism of tracheal pumping in head and pro-thorax of beetles and many other taxa.
- new high resolution view of morphology and mechanical design of insect breathing.
- respirometry key to joining imaging and mechanics with respiratory physiology

Insect Mouthparts:

-feeding mechanisms- first high-resolution view of internal mouthpart mechanisms.

Future Work:

- drinking, limb joint mechanics, jumping, vertebrate functional morphology.

Challenges/Goals

Imaging and Beamline:

- brighter field, allowing higher frame rates to 1 KHz
- larger field of view- up to 1cm square
- beam stability, eliminate “flicker”
- cameras, optics and resolution enhancement
- zoom capability

Develop Collaborators:

- other insect applications
  spider silk, sperm function, flight, limb joints
- vertebrates
  develop experimental preps for fishes, heart function, backbone bending analysis

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