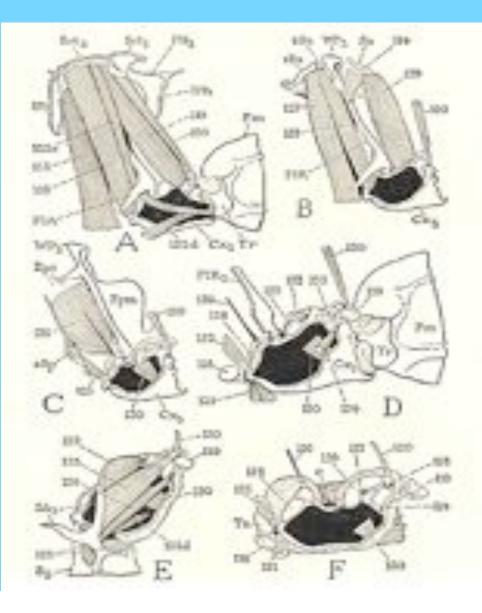
MUSCULAR SYSTEM

MUSCLES ARE THE MOTORS OF THE INSECT

The basic unit is the sarcomere that contains myofibrils inside the cytoplasm known as sarcoplasm. The covering membrane is the sarcolemma.



SCIENCE IS BUILDING ON THE KNOWLEDGE BASE OF OTHER SCIENTISTS AND EXTENDING WHAT THEY HAVE DONE, USUALLY USING DIFFERENT TECHNIQUES AND INSTRUMENTS. THIS GENERALLY RESULTS IN A GREATER UNDERSTANDING OF THE PROBLEM THAN WAS ORIGINALLY DESCRIBED.

Insects possess two to three times the number of muscles than humans

possess.

HUMANS

- 1. Cardiac muscle type
- 2. Smooth muscle type
- 3. Striated muscle type
- 4. Myotendonous connections

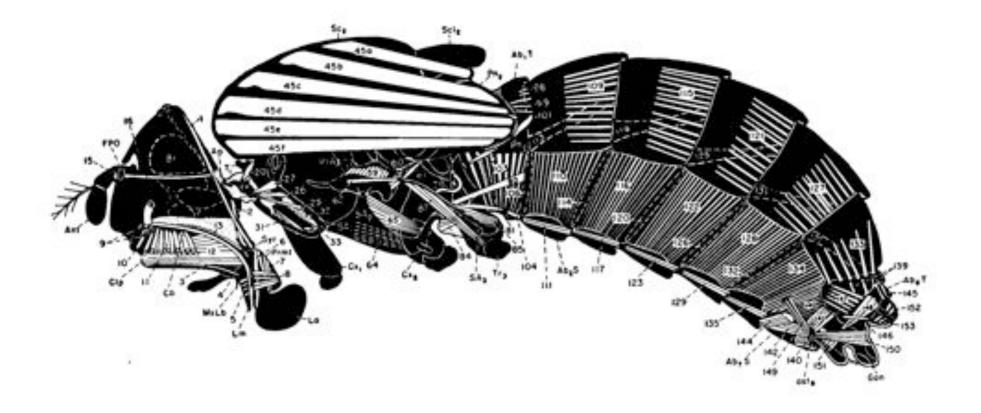
INSECTS

- 1. No cardiac muscle type
- 2. No smooth muscle type
- 3. Yes striated muscle type
- 4. Myocuticular connections

FUNCTIONS OF THE MUSCULAR SYSTEM

- 1. Support of the body
- 2. Helps maintain posture
- 3. Movement of the limbs, including ovipositor
- 4. Movement of the wings-insects are the only invertebrates that fly.
- 5. Movement of the viscera
- 6. Locomotion
- 7. Closure of spiracles
- 8. Operation of various pumps such as cibarial pump and the pumping of the poison glands
- 9. Generation of heat by 'shivering'

Some major muscles in adult Drosophila melanogaster

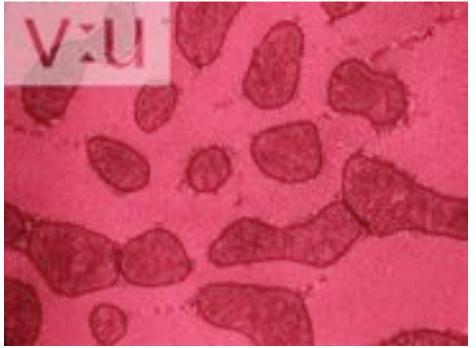


TYPES OF MUSCLE BASED ON MORPHOLOGY

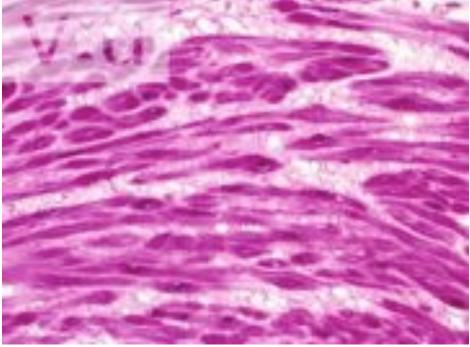
- 1. Cardiac-----not found in insects
- 2. Smooth muscle--not found in insects
- 3. Striated muscle--found in insects

Why is smooth muscle not found in insects? Is it found in other Arthropods? What were the evolutionary constraints for its presence?

Cardiac muscle

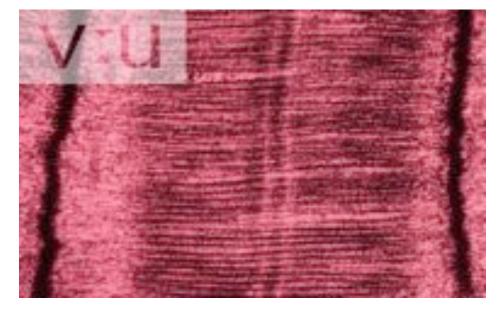


Smooth muscle



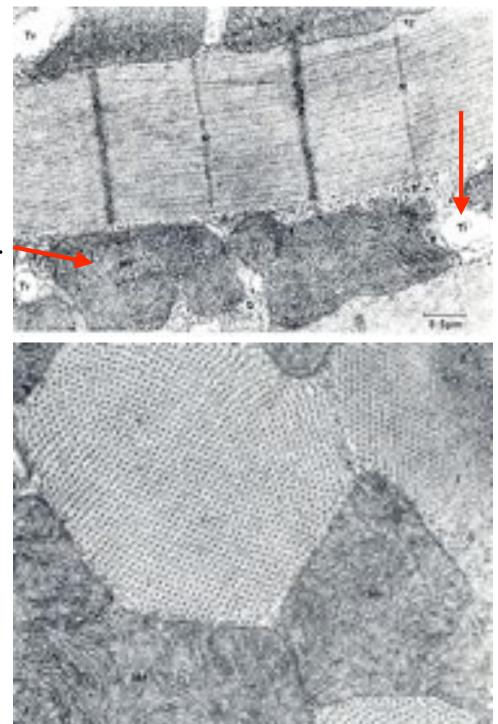
Striated muscle

The only muscle type found in insects is striated muscle. Insects do not have cardiac or smooth muscle types. WHY?



Longitudinal section from the fibrillar flight muscle of the wasp, *Vespa*. Note the presence of Tracheoles (Tr) and the T-tubule system formed by the tracheoles. Darker organelles are mitochondria.

Cross-section of the longitudinal fiber shown above and also from the wasp. Hexagonal array of the fibrils and darker mitochondria. Thin filaments are actin and thick are myosin filaments. It is the organization of the thick and thin filaments that causes this type of muscle to look striated.

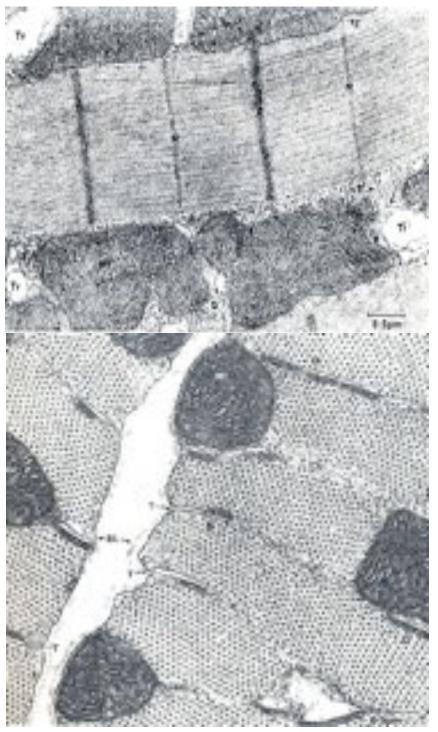


Evolutionary problems with insect flight muscles

- 1. Because of their size (thickness) and mode of oxygen delivery, unlike oxygen delivery in vertebrates via the blood and capillaries, insects would have had a problem in getting oxygen to all of the muscle fibers and problems with the depolarization system.
- 2. How did they solve these constraints?
- 3. The tubular T-system and the tracheal system. This tubular system is created by the tracheoles, which deliver the oxygen deep into the muscle fiber and the T-system that permits the depolarization reaction, taking place on the surface of the cell, to also penetrate deeper into the muscle fibers.

Two structures that evolved in insects to solve the constraints imposed by thick muscles are:

- 1. Tracheoles (Tr) penetrating deep into the muscle (top photo)
- 2. T-system (T) permitting surface depolarization to occur (bottom photo



Contraction of the muscle fiber occurs when the fiber is induced to depolarize by the action or arrival of a nerve impulse from the motoneuron, which causes the depolarization by the release of a transmitter substance.

Acetylcholine is a neuro-neuro transmitter and is **NOT** a neuromuscular transmitter.

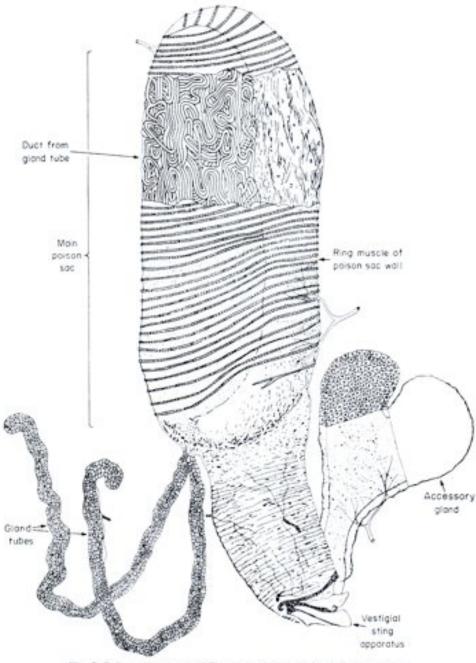
Motoneurons can be myostimulatory or myoinhibitory

Myoinhibitory peptides-Dromysuppressin

Mitochondria provide the power for muscle contractions

TYPES OF MUSCLE BASED ON LOCATION

- 1. Skeletal muscle
- 2. Visceral muscle
 - a. Alary muscle
 - b. Dorsal blood vessel
 - c. Accessory pulsatile organs and various diaphragms
 - d. Alimentary canal, including the crop
 - e. Reproductive organs and ducts
 - f. Venom glands
 - g. Repugnatorial glands
 - h. Organs of defense
 - i. Malpighian tubules

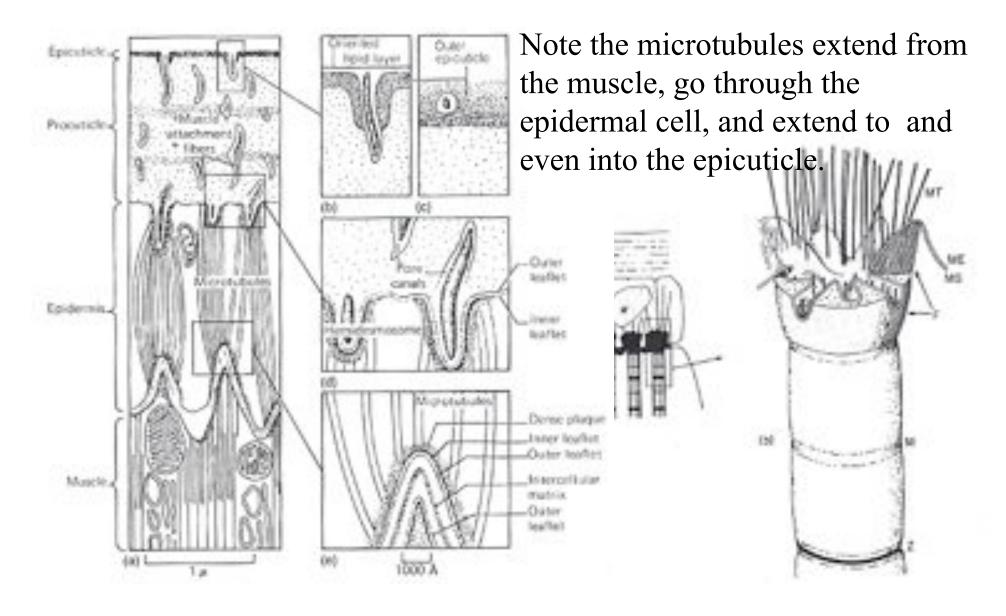


See video showing poison gland contracting following insertion and removal of the stinger.



Fig. 7. Poison apparatus of Formica rufibarbis. (After Wheeler, 1910.)

Schematic representation of cartoon and model showing how the **specialized tendonous epidermal cells** are involved in attaching the muscles to the cuticle via microtubules and thus forming tonofibrillae.



Tonofibrillae forming attachment sties for muscles in house fly larva. Taken from Cantwell, Nappi and Stoffolano. 1976. Embryonic and postembryonic development of the house fly (Musca domestica L.). USDA, Agr. Res. Serv., Techn. Bull. No. 1519. Washington, D.C.

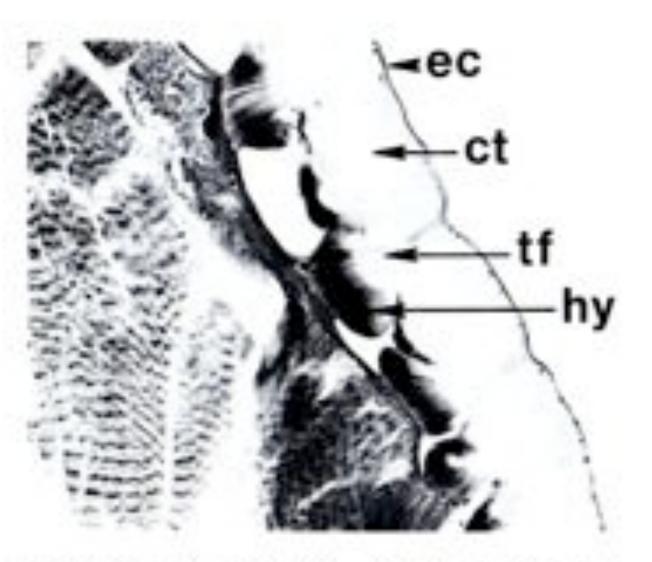


FIGURE 38.—Muscle attachments on body wall with tonofibrillae (tf) traversing hypodermis (hy) and cuticle (ct) and reaching epicuticle (cc).

SYNCHRONOUS MUSCLES VERSUS ASYNCHRONOUS

Synchronous muscles or nonresonating muscles-Non-fibrillar type of muscle. When stimulated by a single nervous impulse there is usually a single muscle contraction.

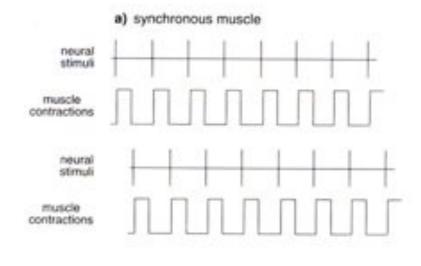
1 nervous impulse to 1 muscle contraction

Asynchronous muscles or resonating muscles-Is of the fibrillar type of muscle. When stimulated by a single nervous impulse it can undergo successive contractions. Muscle that must contract rapidly.

1 nervous impulse to many muscle contractions

Examples: rapidly contacting muscles sets

- a. flight
- b. halteres
- c. sound producing mechanism of cicada



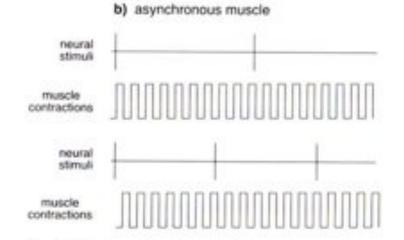


Fig. 10.22. Contraction of antagonistic muscle pairs (as in flight muscles) in relation to neural stimulation. (a) Synchronous muscle. Each contraction results from the arrival of an action potential. The stimuli to antagonistic muscles are in antiphase. (b) Asynchronous muscle. Muscle oscillations occur independently of the arrival of action potentials which serve only to keep the muscle in an activated state. The rate of neural input to the antagonistic muscles may differ, as in this example. Muscle contraction is due to depolarization and is regulated by or modulated by:

- a. Myogenic contraction-Thus, all non-innervated muscles are probably controlled by blood borne factors.
 (1) Stretch-activated ion channels
- **a.** Neurogenic contraction-Contractions stimulated by the release of a neurotransmitter, which in the case of muscles appears to be L-glutamate.

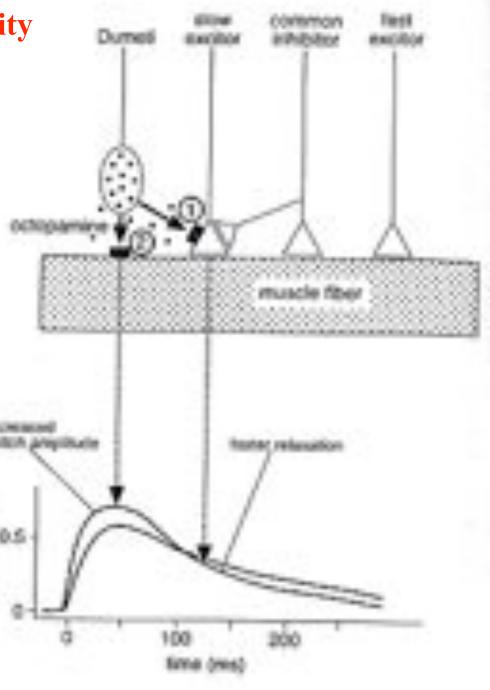
Neuromuscular junctions

Abdominal intersegmental muscle fiber of cockroach. P. americana. Fast skeletal muscles of vertebrates have only one motor nerve terminal for each fiber. Insects have many as shown in this slide. It also shows a polyneuronal junction. **Ax=axons; Tr=tracheoles**



Neuromodulation of muscle activity by

- a. Octopamine--biogenic amine
- b. Serotonin----biogenic amine
- c. Proctolin----peptide
- d. Slow excitor neuron
- e. Inhibitor neuron
- f. Fast excitor neuron



Neurotransmitters:

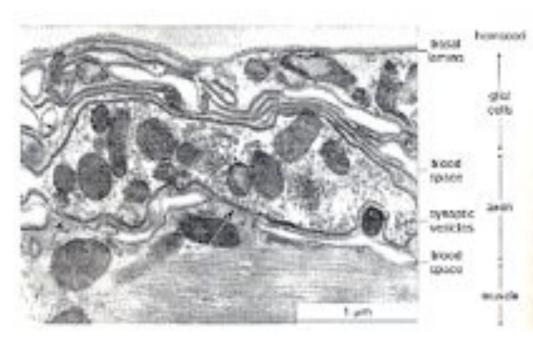
- a. Inhibitory neurotransmitter is GABA (γ-aminobutyric acid)
- b. Stimulatory neurotransmitter is L-glutamate

Myostimulatory and cardioactive peptides:

a. Proctolin-effect on visceral muscles of the hindgut. Is a neurotransmitter and neurohormone.

Myoinhibitory peptides:

a. Dromysuppressin on crop and probably heart

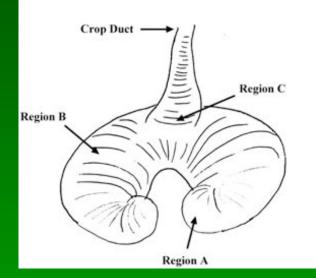


Muscle innervation takes place at neuromuscular junctions (see arrow above). At these junctions the glial or surrounding cells are lacking and there is direct contact at the synapse between the nerve and the muscle. Right-Nerve/muscle synapse delimited by two arrows in body wall of locust. Note synaptic vesicles within neuron.



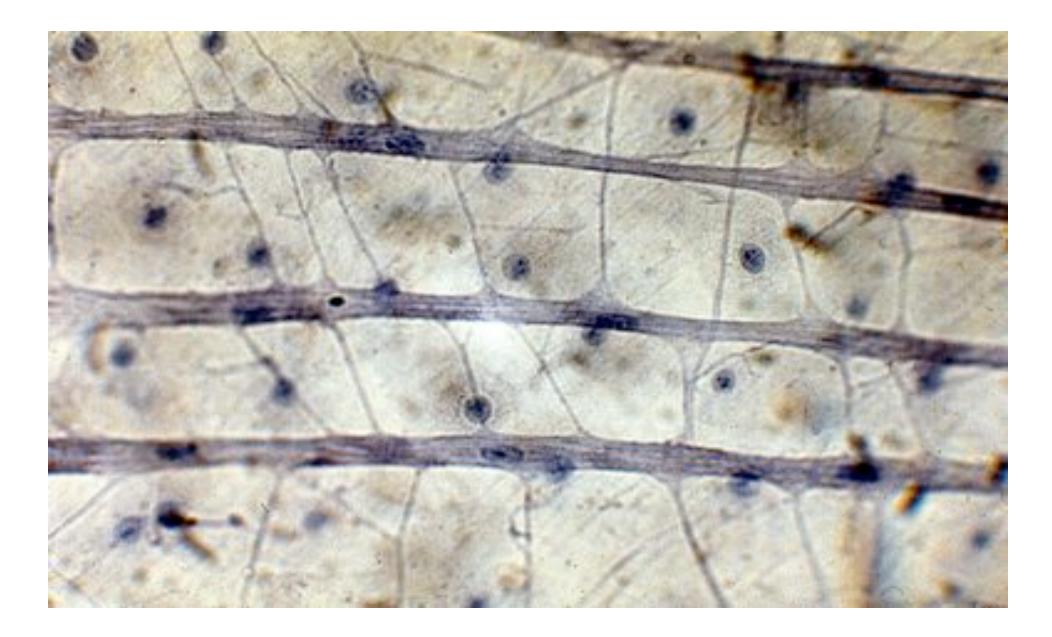
The Dipteran crop

- as crop fills, it is constantly contracting (myogenic)
- contraction frequency increases with volume ingested probably due to stretch activated ion channels



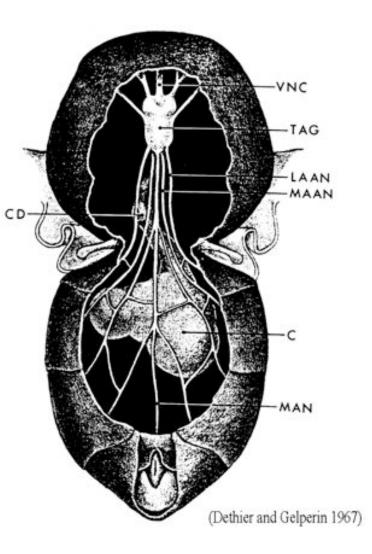
- filled crop is detected by stretch receptors in the abdominal nerve net
- stretch receptors send inhibitory feedback to brain and feeding ceases – food is later forced into midgut

MUSCLES OF THE CROP OF ADULT PHORMIA REGINA

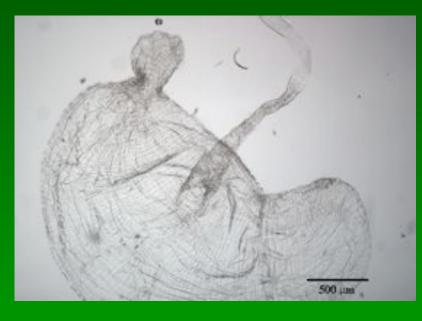


Dromyosuppressin (DMS) and crop contractions

- myosuppressins isolated from several insects, including *Drosophila*
- myotropic myoinhibitors
- dromyosuppressin (DMS) structure: TDVDHVFLRFamide
- CNS, alimentary tract with DMS IR cells and processes
- DMS applied to *in vitro* crop preparation

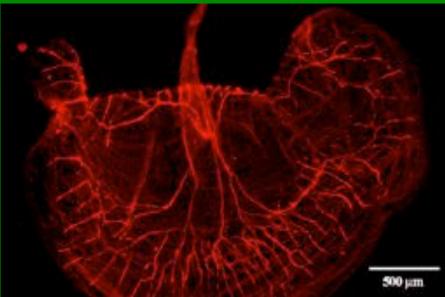


Musca DMS-like IR



Musca domestica crop (light micrograph)

Musca domestica crop Probed with anti-Dromyosuppressin (DMS) antiserum



DMS and crop contractions



Application of 10-6 M DMS reduced crop contractions by 95% (from 46 to 2 contr./min)

At the next stop sign turn right: the metalloprotease Tolloid-related 1 controls defasciculation of motor axons in Drosophila. <u>Meyer F</u>, <u>Aberle</u> H.

Max-Planck-Institute for Developmental Biology, Department III/Genetics, Spemannstrasse 35, 72076 Tubingen, Germany. Navigation of motoneuronal growth cones toward the somatic musculature in Drosophila serves as a model system to unravel the molecular mechanisms of axon guidance and target selection. In a largescale mutagenesis screen, we identified piranha, a motor axon guidance mutant that shows strong defects in the neuromuscular connectivity pattern. In piranha mutant embryos, permanent defasciculation errors occur at specific choice points in all motor pathways. Positional cloning of piranha revealed point mutations in tolloid-related 1 (tlr1), an evolutionarily conserved gene encoding a secreted metalloprotease. Ectopic expression of Tlr1 in several tissues of piranha mutants, including hemocytes, completely restores the wild-type innervation pattern, indicating that TIr1 functions cell non-autonomously. We further show that loss-of-function mutants of related metalloproteases do not have motor axon guidance defects and that the respective proteins cannot functionally replace Tlr1. tlr1, however, interacts with sidestep, a musclederived attractant. Double mutant larvae of tlr1 and sidestep show an additive phenotype and lack almost all neuromuscular junctions on ventral muscles, suggesting that Tlr1 functions together with Sidestep in the defasciculation process.

PMID: 16971470 [PubMed - indexed for MEDLINE]

German cockroach proteases regulate matrix metalloproteinase-9 in human bronchial epithelial cells.

•Page K, Hughes VS, Bennett GW, Wong HR.

Division of Critical Care Medicine, Cincinnati Children's Hospital Medical Center and Cincinnati Children's Research Foundation, Cincinnati, OH 45229, USA.

BACKGROUND: Matrix metalloproteinases (MMPs) digest extracellular matrix proteins and may play a role in the pathogenesis of bronchial asthma. MMP-9 levels are increased in the bronchoalveolar lavage fluid and sputum of asthmatics compared with that of controls. As exposure to cockroaches is an environmental risk factor for asthma, we sought to investigate the role of German cockroach fecal remnants (frass) on MMP-9 expression. METHODS: Human bronchial epithelial cells (16HBE14o-) and primary normal human bronchial epithelial cells were treated with cockroach frass in the absence or presence of tumor necrosis factor (TNF)alpha. MMP-9 mRNA, protein levels and pro-MMP-9 activity were determined using real-time polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and zymogram assays. Pretreatment of frass with aprotinin abolished protease activity. PD98059, a chemical inhibitor of extracellular signal regulated kinase (ERK), and SLIGKV, an activator of proteaseactivated receptor (PAR)-2 were also used. AP-1DNA binding was determined by electrophoretic mobility shift assay (EMSA) and ERK phosphorylation by Western blot analysis. RESULTS: Cockroach frass augmented TNFalpha-mediated MMP-9 mRNA and protein expression by a mechanism dependent on active serine proteases within frass and not on endogenous endotoxin. Frass increased ERK phosphorylation, and chemical inhibition of ERK attenuated cockroaches' effects on MMP-9. Serine proteases are known to activate the PAR-2 receptor. We found that selective activation of PAR-2 using the peptide SLIGKV augmented TNFalpha-induced MMP-9 protein levels and increased ERK phosphorylation. Frass and SLIGKV each increased AP-1 translocation and DNA binding. CONCLUSIONS: These data suggest that German cockroach frass contains active serine proteases which augment TNFalpha-induced MMP-9 expression by a mechanism involving PAR-2, ERK and AP-1.PMID: 16867053 [PubMed - indexed for MEDLINE]

Muscles associated with molting:

1. To prevent the insect from 'ballooning', or expanding beyond normal, at times when the cuticle is soft. Example: *Rhodnius* has ventral intersegmental muscles in the abdomen that are fully functional only at the molt. A few days after the molt they no longer function. This prevents over expansion of the plasticized cuticle at the time of feeding.

Regressive changes in flight muscles:

Insects that migrate or lose muscles for various reasons

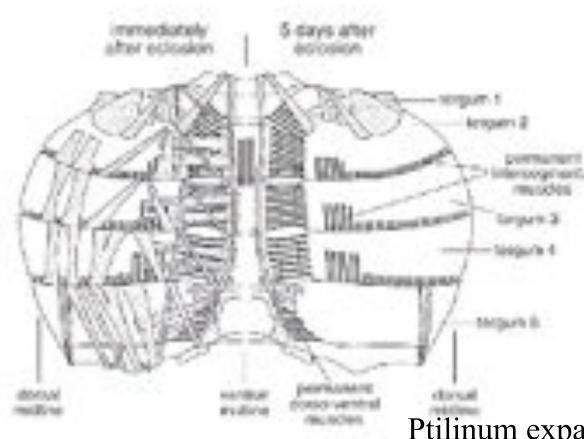
- 1. Flight muscles of reproductive castes of termites and ants during nuptual flight. Other insects that become alates following migration such as aphids. These insects use the histolyzed muscle material for egg development. Histolysis is brought about by **increased JH titer**, which is correlated with egg development and behavior.
- Diapause-Mitochondria degenerate or decrease in size due to lack of JH, which is associate with the diapause. In the spring, when day length increases, JH titer goes up, migratory behavior is stimulated, and reproductive development and behavior is turned-on. Demonstrated in the Colorado Potato Beetle, *Leptinotarsa decemlineata* by Jan de Wilde of Holland.

Degeneration of muscles associated with molting:

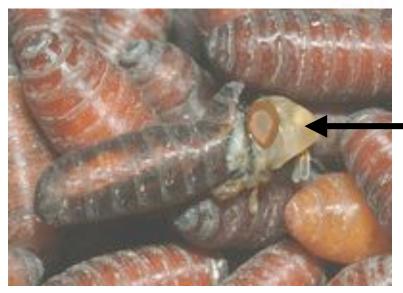
- 1. Muscle used at one stage or for a specific function, but not in the next stage because they no longer need that function
 - a. Muscles that breakdown in this case do so because of two processes:
 - (1) Loss of contractility-due to declining concentration of molting hormone or ecdysone
 - (2) Muscle degeneration-

Manduca-Lack of ecdysone causes muscles to degenerate *Antheraea*-eclosion hormone provides signal for degeneration.

***Hormones act on a switch that in some way switches on genetically preprogrammed cell death.



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.

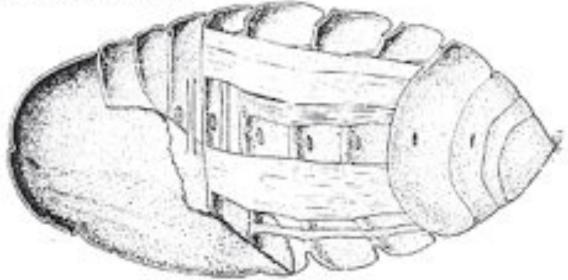


Ptilinum expanded. This structure aids the adult in escaping from the pupal case. Also, the muscle sets on left and above help increase hemolymph pressure that facilitates ptilinal extrusion from the ptilinal suture. 5 days after eclosion these muscles degenerate. Probably bursicon sensitive but not proven. Muscle development and maintenance in hemimetabolous insects differs greatly from that in holometabolous insects. In the former, the transition is gradual and the same muscle sets are present in the adult that were in the larva. In the holometabolous insects, however, most immature muscles degenerate (i.e., histolyzed) and new ones are developed. Basically there are four ways in which muscles undergo modifications in holometabolous insects:

- 1. Larval muscles may pass unchanged into the adult
- 2. Existing larval muscles are reconstructed
- 3. Larval muscles may be destroyed and not replaced
- 4. New muscles, not represented in the larva such as flight muscles and leg muscles, are formed from imaginal discs.



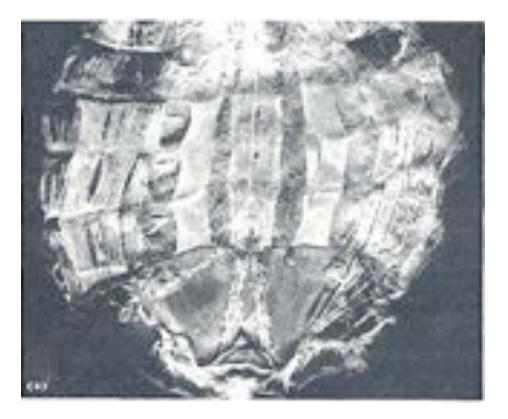
Muscles are resistant to molting fluids, thus they remain functionally attached to the cuticle at apolysis and ecdysis



Antheraea polyphemus moth-newly emerged adults still have the 4-6th abdominal longitudinal muscles, while in an adult 4 days following emergence these muscles are absent. Where did they go?

From Finlayson, 1956. Quart. J. micros. Sci. 97:215-233 This was a morphological study that reported that something happened to the muscle sets in abdominal segments 4-6.

Newly emerged adult



Adult 4 days after emergence

In 1960, ligation experiments showed that a factor from the brain and thorax influences degeneration of the intersegmental muscles in segments 4-6

Ligated the head and thorax from the abdomen at different times

Before adult eclosion

After adult eclosion

Muscles did not degenerate

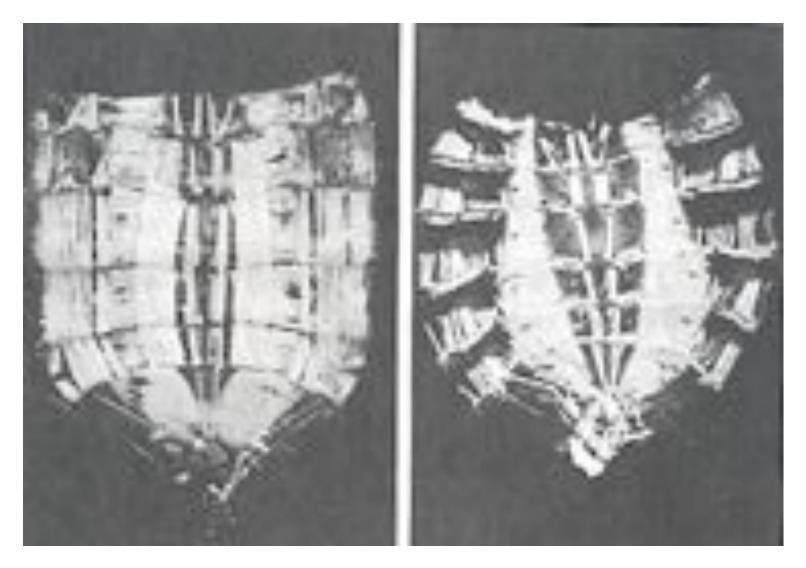
Muscles degenerated within 30 following eclosion

CONCLUSION: A factor from the brain and also one from the thorax are released at eclosion and are involved in muscle degeneration

Schwartz and Truman-Manduca sexta-

Before adult eclosion

36 hrs after adult eclosion

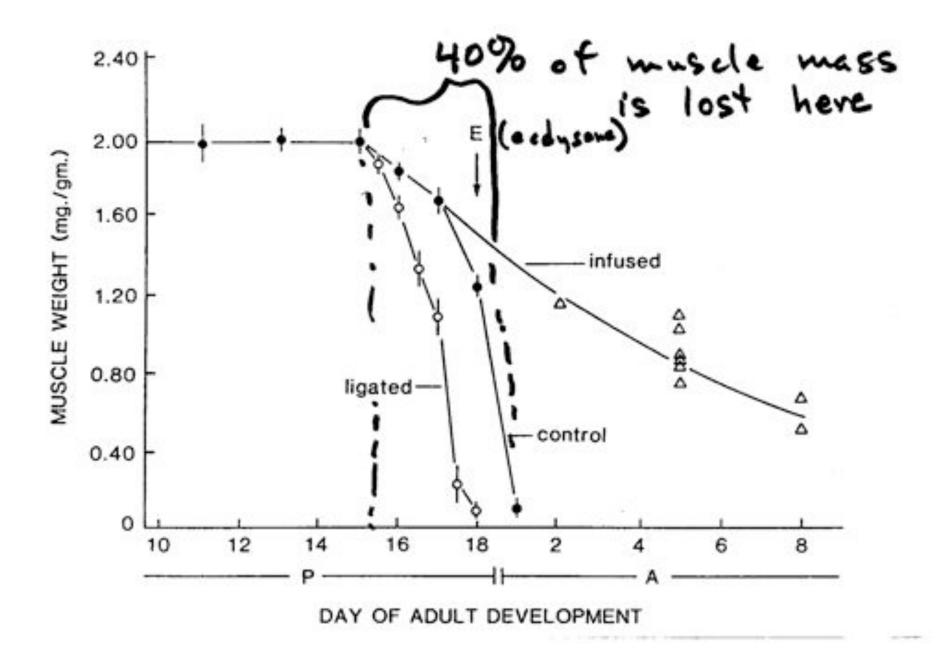


Truman's lab. showed that two hormones were involved in muscle degeneration at the time of eclosion and that there were two types of muscle degeneration, each under different control

Slow took about 6 days-Ecdysone hormone from the ecdysial glands activates this process and starts the molt

Fast took about 30 hrs-Involves both eclosion hormone and a new peptide eclosion hormone called the ecdysis-triggering hormone.

Just prior to adult eclosion the ecdysone titer declines This decline sets the stage for the muscles to respond to the ecdysis triggering hormone.

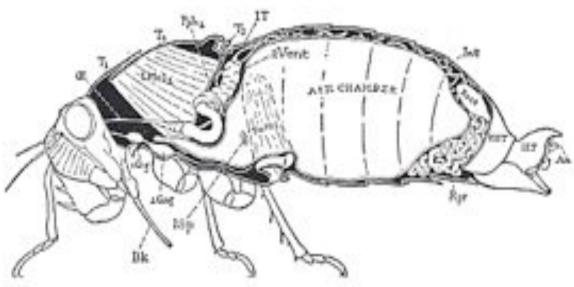






How does the fly larva ensure it is the only larva in the host?



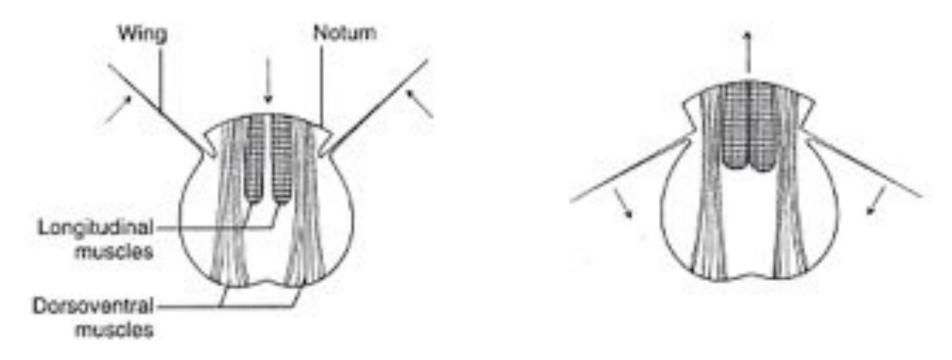


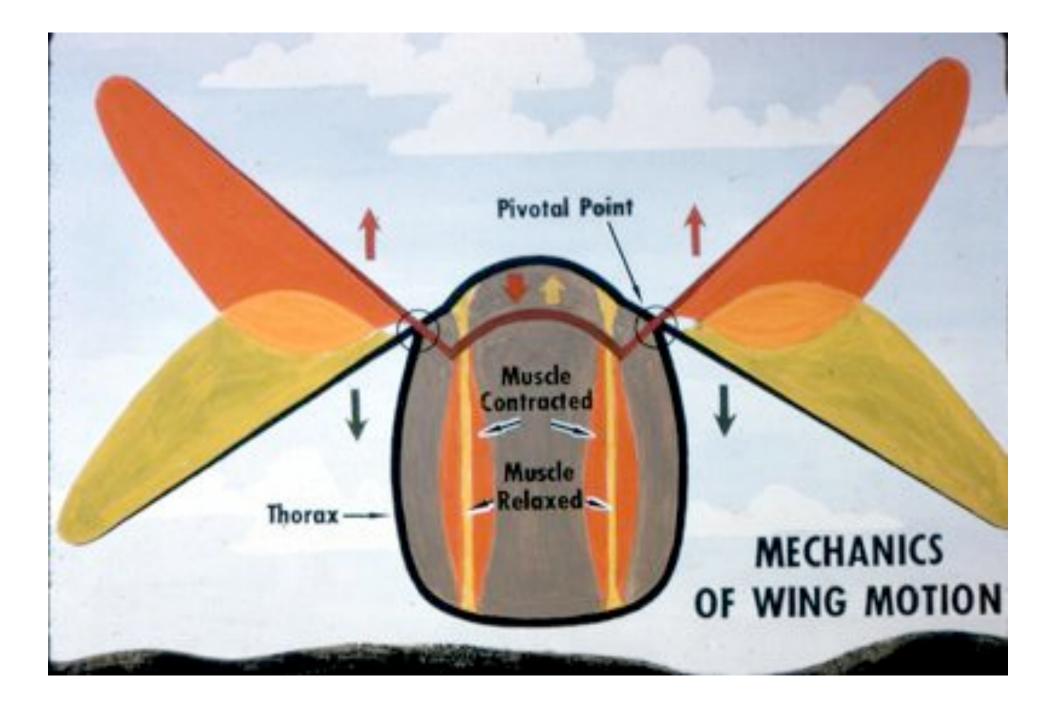
Make note of the tymbal or sound producing mechanism. The muscles that vibrate the tymbal and the tympanum or 'ear'.

How a fly has intercepted the message and parasitizes only the males and what it does to them.

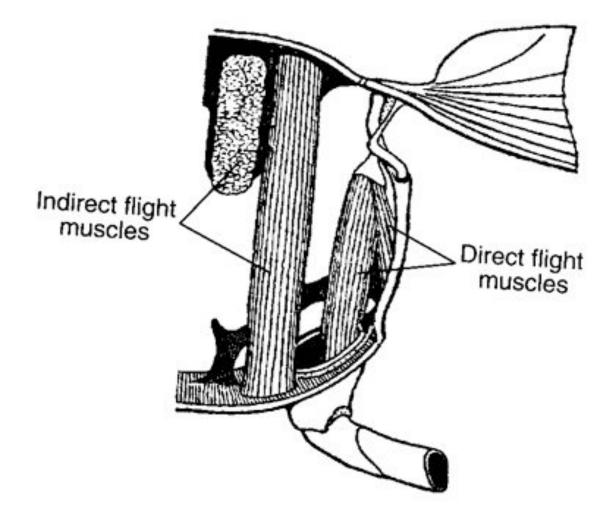


Wing movement, and most of flight, is controlled by **indirect flight muscles**. They are called this because the longitudinal and dorsoventral muscles do not connect directly to the wing but, control flight by affecting the dorsal surface of the thorax. When the dorsoventral muscles contract, as shown in the figure on the left, it causes a depression of the tergum, causing the wings to go up. Contraction of the longitudinal muscles causes an arching (see fig. on the right) of the notum and the wings go down. It is a pivotal movement based on the arrangement of the cuticle and wings.

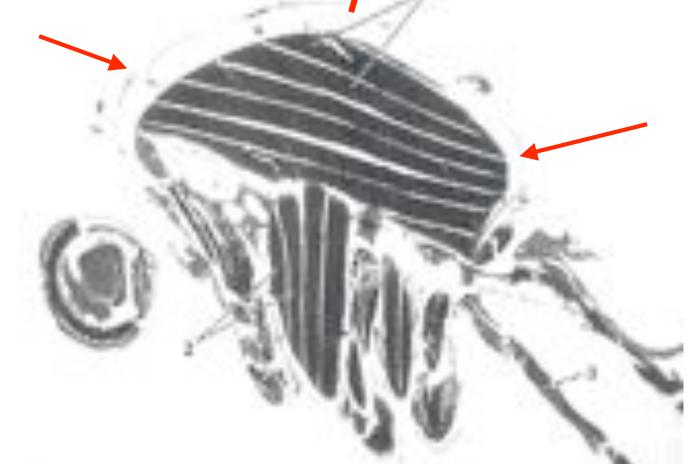




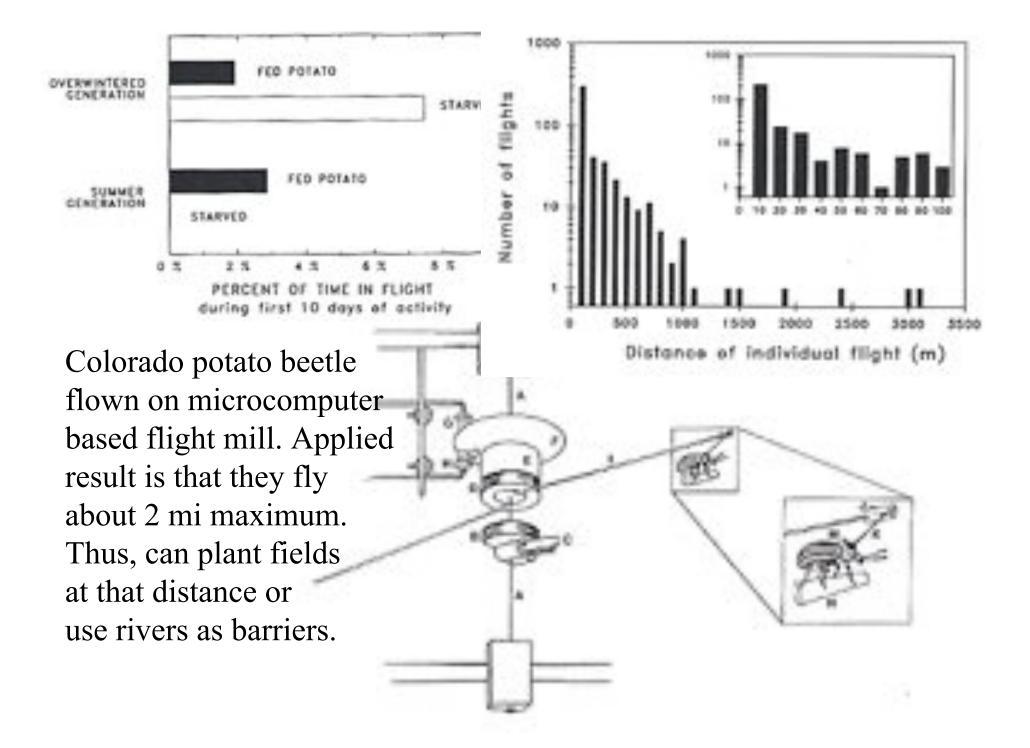
Also involved in flight are **direct flight muscles** that connect directly to the wing base and are involved in canting the wing and producing other fine movements that cause the finer movements of flight. Without the direct flight muscles the insect would just go up and down.



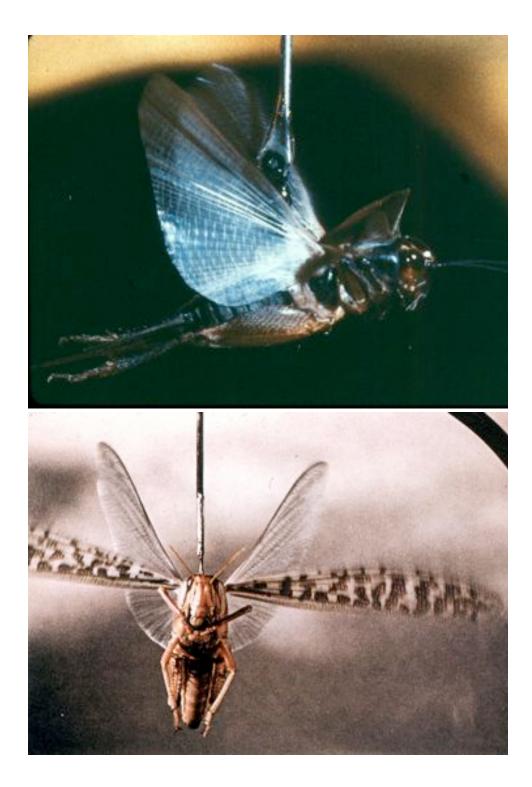
- shows longitudinal indirect flight muscles in mosquito
 shows tergosternal or dorsoventral indirect flight muscles
- 3 shows intersegmental muscles of abdomen



When the longitudinal indirect fllight muscles contact one can see that they cause the notum to go up, thus the wings to go down.



By using tethered insects, wind tunnels and computers, insect physiologists are able to provide a lot of information about sensory input and flight manipulation as wind changes directions. They can also manipulate visual input into the flight system.

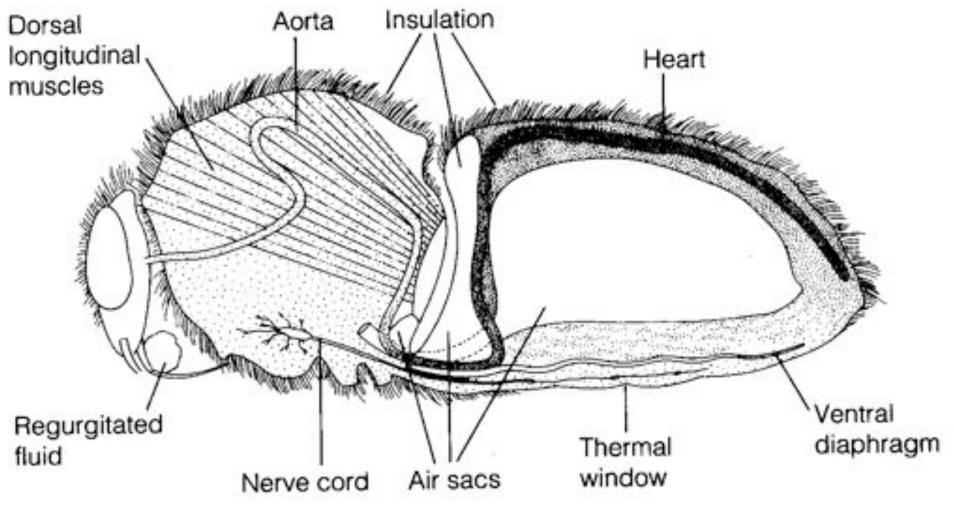


Muscles and shivering by bees, bumblebees, and some moths to produce cellular respiratory heat

- 1. Increase temperature above ambient in order to fly
- 2. Bees maintain colony temperature during winter at a constant temperature
- **3. Bumblebees producing heat so they can incubate their eggs and/or larvae**
- 4. Japanese honeybees producing enough heat to kill the predatory wasp

Many insects have heat exchangers that permit blood to be either heated or cooled. Some insects, like the bumblebee and wasp queens over winter in a diapause condition but, in the spring are ready to produce a brood. The queens first priority is to rear a group of helpers. Temperatures, however, in early spring may be near zero. In order to successfully hatch eggs and develop brood in temperate and arctic areas, the queen must 'incubate' the eggs and brood, just like a chicken does. To do this they have a special heat exchange unit (the dorsal blood vessel) that picks up the heat they generate by shivering (contracting of the dorsal and longitudinal flight muscles). As the blood goes through the thoracic region it picks up heat, which is then deposited into the head. This warmed blood then travels ventrally into the abdomen where there is a heat exchange 'thermal window' on the abdomen. The queen presses this area over the eggs and brood and is able to generate enough heat that they can now produce eggs and brood within a 2 week period, even at these cold temperatures.

INCUBATION OF EGGS AND BROOD IN COLD ENVIRONMENTS Bumblebee and heat production and transfer from Heinrich.Dorsal and longitudinal muscles produce the heat. Hot blood goes ventrally into the abdomen. Heat produced is lost through the thermal window and the cooled blood is pumped back into the heart.



Honeybee worker drinking sugar must shiver to keep warm. Sugar water in black; Thorax is at the highest temp. due to muscle shivering (in white) while the abdomen (in green) stays cool. From Heindrich



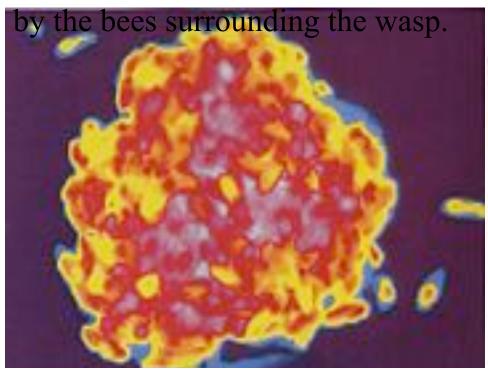
Unusual thermal defense by a honeybee against mass attack by hornets

Masato Ono, Takeshi Igarashi, Eishi Ohno & Masami Sasaki THE giant hornet Vespa mandarinia japonica (Hymenoptera: Vespidae) is the only hornet species known to have evolved *en masse* predation of other social bees and wasps. Here we show that hornets is initiated by secretion of a foraging-site marking pheromone from the van der Vecht glands (metasomal sternum VI glands) by a single foraging hornet. The lone hornet rubs the basal tuft of the terminal gastral sternite around a prey food resource, such as a honeybee colony, and the hornet nestmates then congregate and attack the marked site *en masse*. The sympatric Japanese honeybee Apis cerana japonica (Hymenoptera: Apidae) can detect the hornet marking pheromone, and responds by increasing the number of defenders at the nest entrance. When an invading hornet is captured by a defending bee, more than 500 other bees quickly engulf the hornet in a ball which contains isoamyl acetate. Thermography showed that the ball temperature is very high (~47 °C), which proves lethal to the hornet but not to the bees. Defenders patrolling the nest entrance also generate high temperatures. These findings suggest that aspects of the interaction between V. mandarinia japonica and A. cerana japonica are specifically coevolved. letters to nature

If a colony of 30,000 European honeybees (*Apis mellifera*) is attacked by 30 giant hornets (*Vespa mandarinia japonica*) they'd be wiped out in 3 hours.

... The defense by *Apis japonica* is to form a tight ball of living bees around the wasp and generate a lot of muscle heatenough to kill the wasp. Temp. can be as high as about 47 degrees Centigrade.

Infrared photo showing heat produced

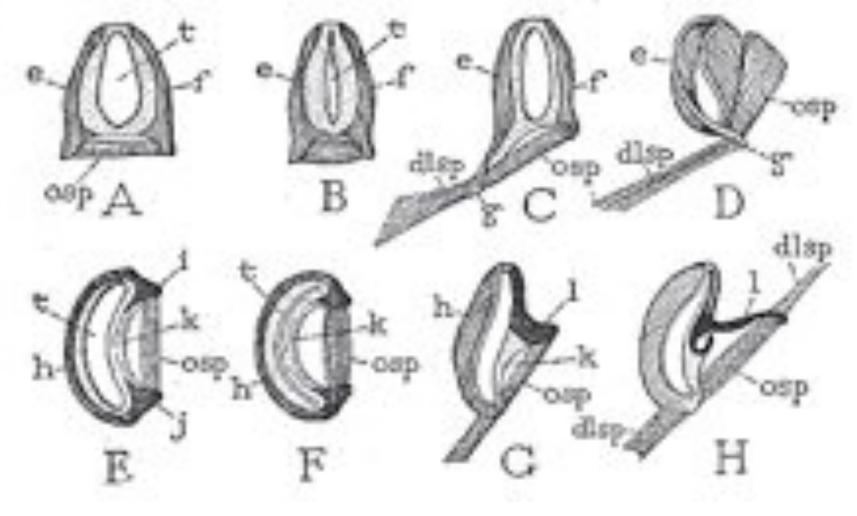




Demonstrative half of Aprix services performent with source 400 tightly appropried from (Country of Maccio Origi)



Insects are able to close their spiracles, thus avoiding water loss through them. They remain closed until carbon dioxide builds up and then their is a mechanism that causes the muscles to relax and the spiracle opens allowing oxygen uptake. What would be a technique used in grainaries to facilitate the action of fumigants to kill grain insects?



APPLIED AND BASIC RESEARCH

1. Applied

a. Flight mills and how far insects can really flyb. Robotics

c. Stop crop and/or cibarial muscles – thus intake

- 2. Basic
 - a. Programmed cell death or apoptosis
 Schwartz, L.M. 1991. Insect muscle as a model for programmed cell death. Journal of Neurobiology 111: 13-30.

ROBOTICS

• **The Cricket Robot**-Use of input from antennae to the mechanisms ("muscles") to control locomotion.



http://home.earthlink.net/~henryarnold



The Virtual Hexapod, created by Anne Torres, demonstrates the power of Virtual Model Control, which is used to allow the hexapod's body to be controlled as if it had force and torque thrusters, even though it actually has 6 legs with non-linear kinematics including 18 controlled and 18 uncontrolled degrees of freedom. In this video, the hexapod walks smoothly in various directions, then balances an inverted pendulum (connected to the body via a universal joint) while walking over rough terrain. Despite the complexity of the emergent behavior, Virtual Model Control allows the controller to be specified very simply. The controller is switched off at the end of the video to demonstrate that the pendulum can, in fact, fall down. http://www.ai.mit.edu/projects/leglab/mpeg_vcd/ J Insect Sci. 2004; 4: 34. Published online 2004 October 22. <u>Copyright</u> © 2004. Open access; copyright is maintained by the authors.

Impact of pymetrozine on glassy-winged sharpshooter feeding behavior and rate of *Xylella fastidiosa*

transmissionB.R. Bextine,^{1,2} D. Harshman,¹ M.C. Johnson,¹ and T.A. Miller¹

¹University of California, Riverside; Department of Entomology; Riverside, California 92521, USA²Email: <u>blake.bextine@ucr.edu</u> Pymetrozine is a compound that interferes with insect feeding and interrupts transmission of plant pathogens. The glassy-winged sharpshooter, *Homalodisca coagulata* Say (Hemiptera, Cicadellidae), is a vector of *Xylella fastidiosa*, the foregut-borne, propagative bacterium that causes Pierce's disease of grapevine. Pymetrozine, a novel compound sold under the trade names Fulfill[™], Endeavor[™], and Chess 250 WP[™], is a systemic antifeedant, belonging to the class of chemicals known as pyridine-azomethines (MSDS 2001).

•Harrewjin P, Kayser H. Pymetrozine, a fast-acting and selective inhibitor of aphid feeding. In-situ studies with electronic monitoring of feeding behavior. Pesticide Science. 1997;49:130–140.0031-613X(1997)049<0130:PAFASI>2.0.CO;2

We hypothesized that the inactivation of the **cibarial muscles** after ingestion of pymetrozine (Harrewjin and Kayser 1997) would reduce the rate of *X. fastidiosa* transmission by *H. coagulata*. Unexpectedly, the rate of *X. fastidiosa* transmission to grapevines was not decreased when vines were treated with pymetrozine; in fact, a higher incidence of disease resulted in vines treated with pymetrozine compared to untreated control vines.













