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How Locusts Breathe

Jon F. Harrison,[1] James S. Waters,[1]
Arianne J. Cease,[1]
John M. VandenBrooks,[1]
Viviane Callier,[1] C. Jaco Klok,[1]
Kimberly Shaffer,[1]
and John J. Socha[2]

[1]Arizona State University, School of Life Sciences Tempe, Arizona; and [2]Virginia Tech, Engineering Science and Mechanics, Blacksburg, Virginia

j.harrison@asu.edu

Insect tracheal-respiratory systems achieve high fluxes and great dynamic range with low energy requirements and could be important models for bioengineers interested in developing microfluidic systems. Recent advances suggest that insect cardiorespiratory systems have functional valves that permit compartmentalization with segment-specific pressures and flows and that system anatomy allows regional flows. Convection dominates over diffusion as a transport mechanism in the major tracheae, but Reynolds numbers suggest viscous effects remain important.

Insect cardiorespiratory systems have evolved over hundreds of millions of years, with natural selection operating on physiological transport systems governing flow at the micrometer scale. Gas exchange in insects occurs primarily through an elaborate air-filled tubular respiratory system: the tracheal system. Tracheae are invaginations of cuticular cells that assemble into branching tubes (tracheae) leading from valved holes in the exoskeleton (termed spiracles). These branching tracheae penetrate organs and tissues and eventually terminate within the tissues as blind-ending tubules (termed tracheoles) that can have submicron diameters. Most tracheoles run outside of cells, with densities within the tissues similar to or higher than observed for mammalian capillaries. Because oxygen and carbon dioxide are transported mostly in the gas phase to the tissues, the system is light weight and has a high flux capacity.

The properties of insect respiratory and cardiac systems differ greatly from both engineered and typical mammalian physiological systems. These differences include the capacity to deliver oxygen from air to cell by either diffusive or convective mechanisms, convection driven by relatively low pressures, and dramatic morphological and structural differences in the components. The high performance of insect fluidic systems, their unique properties, and the ecological success of insects all suggest that insects offer potential bioinspiration for a variety of biomedical engineering processes including microfluidics, nanofabrication, and tissue engineering.

Convective and Diffusive Gas Exchange in Insects

Distinguishing the relative roles of diffusion and convection in insect gas exchange is a major challenge. Gas exchange in anoxia-paralyzed insects must be accomplished primarily by diffusion. Even very large insects weighing multiple grams can recover from exposure to anoxia when returned to room air, demonstrating that they can attain sufficient oxygen delivery to initiate movement by diffusion (24). This is a major benefit of a gas-phase oxygen transport system. Someday we may culture these cells or build synthetic models that allow engineering of artificial tracheal systems that could offer a passive system for supporting gas exchange of cultured tissues of at least a few centimeters in thickness. Study of the morphology of insect tracheal systems (e.g., branching patterns, relative diameters at different branching levels) may aid development of such artificial systems.

Although insects can support life maintenance by diffusive oxygen delivery, many, perhaps most, insects are now thought to utilize convective gas transport. Synchrotron X-ray phase contrast imaging has allowed direct visualization of tracheal compression, and many insects exhibit tidal ventilation associated with rhythmic squeezing of the tracheae (41, 42, 53, 54). Use of such convection is very likely key to achieving dynamic performance and very high fluxes of gases as occurs during flight. However, even resting insects often exhibit such convection, suggesting possible important roles in mixing and equilibration of gases throughout the body. We have only very recently been able to visualize internal tracheal system structures in a manner that enables us to begin to understand the actual air flows and convective mechanisms within insects.

Perhaps the best-understood insects in terms of respiratory mechanisms are the locusts, thanks to pioneering work by Snodgrass (40), Fraenkel (8, 9), Miller (29, 31–33), Weis-Fogh (50–52), McCutcheon (28), and Burrows (3, 4). These seminal early papers have been updated with extensive studies that link measures of internal gas tensions, ventilation, and gas exchange with synchrotron X-ray imaging of tracheal system dimensions and compression (10–15, 19). This review will focus on our present understanding of the structures and function of the

respiratory system of locusts, with a focus on the most commonly observed behavior associated with ventilation, abdominal pumping.

Anatomy of the Locust Tracheal System

The tracheal system of the American locust, *Schistocerca americana*, has a complex morphology, challenging our understanding of the paths and mechanisms of convective flows. Here we focus on the major structures (FIGURE 1). There are 10 pairs of spiracles, 2 on the thorax, and 8 on *abdominal segments I–VIII*. The tracheae are supported by rings of thickened cuticle (taenidia) that resist compression and expansion. The air sacs lack taenidia and are very soft and flexible. The system can be conceptualized as a collection of subsystems

that serve the major regions and tissues of the locust: the head, the thorax/flight muscle, the legs, the ventral nerve cord, the digestive tract/renal system, and the dorsal heart (FIGURE 1).

Within the abdomen, longitudinal tracheal trunks connect the abdominal segments. Pairs of longitudinal trunks run along the dorsal heart and the ventral nerve cord, whereas others run dorsally, laterally, and ventrally along the gut or connect the abdominal spiracles. Regular transverse tracheae connect the spiracles of each segment to these longitudinal tracheae. The dorsal and lateral gut longitudinal tracheae connect anteriorly to *spiracle 3*, whereas the ventral gut longitudinal tracheae connect to *spiracle 4*. Finer tracheae branch from both the main transverse tracheae and the longitudinal tracheae into the tissues where gas exchange
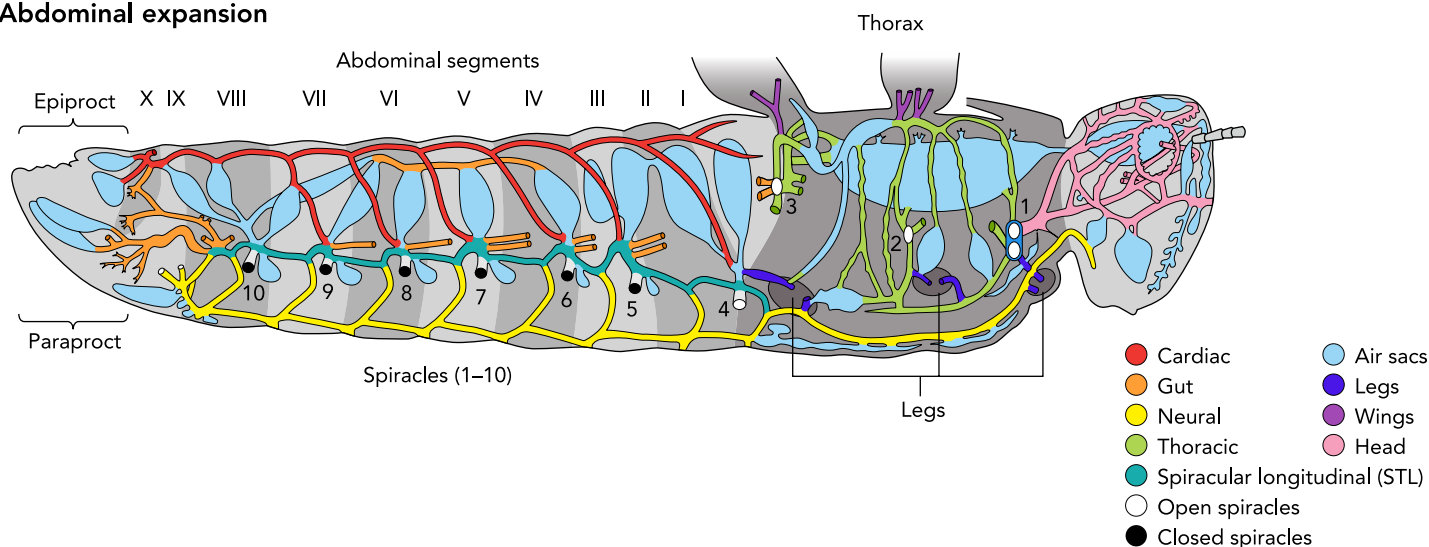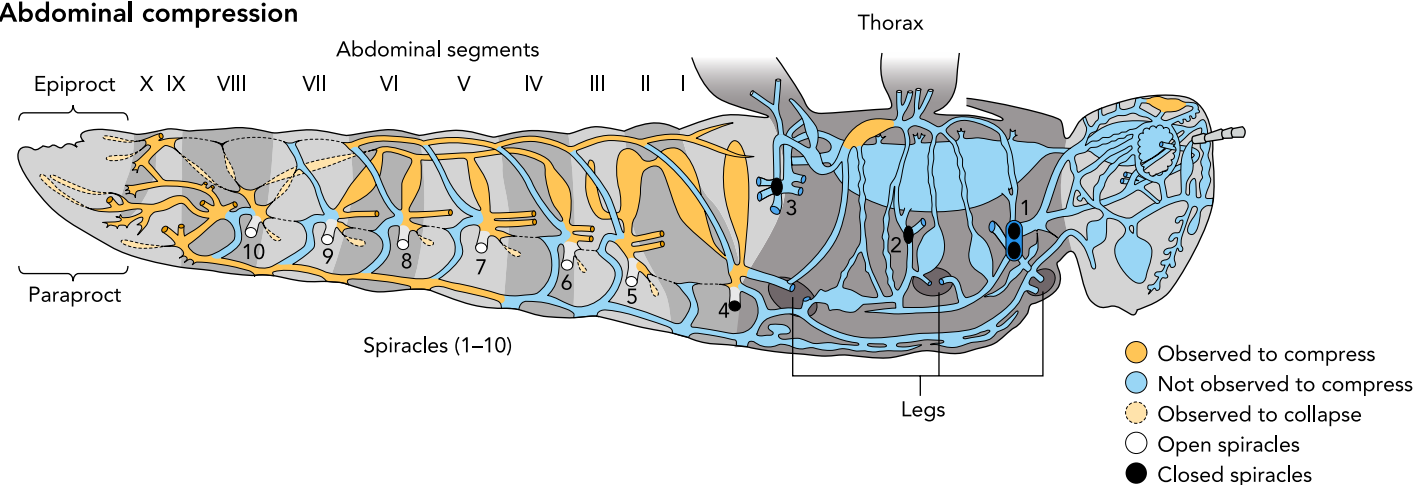

FIGURE 1. **American locusts, *Schistocerca americana***
American locusts, *Schistocerca americana*, were reared at Arizona State University and transported to the Advanced Photon Source at Argonne National Laboratory for imaging at beamline 32-ID as previously described (13, 43). Drawings were made by Kim Shaffer based on these X-rays, many light-microscope dissections, and consultation with prior sources (including Refs. 1, 33, 44, 51). The abdominal dorsal air sacs are shown smaller than their normal inflated size, and some tracheae, such as the cardiac transverse tracheae, are shifted slightly to facilitate illustration of the connections between components of the tracheal system.

occurs. The abdominal segments have large dorsal air sacs on each side that connect near the spiracles within that segment. In anterior regions of the abdomen, these dorsal air sacs are interconnected, in the posterior regions these connect to the dorsal gut longitudinal trunk. The dorsal air sacs of *abdominal segment I* and *II* are particularly large and paired. *Abdominal segments III–VIII* also have smaller air sacs that connect near the spiracles and lay blind-ended between the gut and lateral abdominal wall. Multiple air sacs extend beyond the longitudinal trunks into the most posterior region of the abdomen, *segments IX* and *X,* and the epiproct and paraproct. Anastomoses connect the left and right sides between the paired neural longitudinal tracheae, between the gut longitudinal tracheae, and between the large air sacs in *abdominal segments I* and *II,* but not between the paired cardiac longitudinal tracheae.

The tracheal system of the thorax is substantially separated from that of the abdomen (33, 52). The cardiac longitudinal tracheae and spiracular tracheae do not extend into the thorax. The spiracular longitudinal tracheae bypasses *spiracle 3,* connecting to the ventral longitudinal tracheae anterior to *spiracle 4.* The ventral neural longitudinal trachea runs through the thorax between the head and the abdomen but has minimal connections with the thoracic tracheal system. Thus the primary connection between the abdominal and thoracic tracheal systems occurs where longitudinal tracheae of the dorsal and lateral gut meet tracheae from the thorax at *spiracle 3.*

In the thorax, tracheae branch from *thoracic spiracles 1* and *2* and *abdominal spiracle 3* to the flight muscles, as well as to the various air sacs, of which the largest are the mesial tergo-pleural air sacs that occur ventral to the dorso-longitudinal flight muscles (51). There are also multiple pleuro-coxal air sacs connected to tracheae that feed the dorso-ventral flight muscles and the legs. There are two major tracheae that feed each leg, and these arise from different branches and often different spiracles. For example, the metathoracic (jumping leg) receives one trachea directly from *spiracle 4* and is also serviced from the neural longitudinal tracheae.

The head is supplied dorsally by large tracheae branching from dorsal atrium of the first spiracle and ventrally by the neural longitudinal. Tracheae branch to the brain, mandibular muscles, and alimentary canal, and anastomose. There are many air sacs, with the largest in the mandibles. These accommodate movements of the mandibles during chewing, allowing the mandibles to move in and out of the rigid head capsule and likely providing autoventilation to support the extra oxygen need associated with chewing.

Abdominal Pumping and Pressures

The exoskeleton of the locust, and many other insects, consists of hardened, sclerotized plates, which can be fixed rigidly together (as in most of the head) or connected by soft, flexible membranes (as in the abdomen). Compression of the abdomen is accomplished primarily by contraction of dorso-ventral intrasegmental muscles that pull the sclerotized dorsal and ventral segments (terga and sterna) of the abdomen together, reducing abdominal and air sac volumes, thus increasing hemolymph and tracheal pressures (25, 37, 40). These compressions reduce abdominal dimensions primarily in the dorsoventral and lateral dimensions, with little consistent variation in abdominal length, at least in the American locust *S. americana* (10). During expiration, longitudinal intersegmental muscles prevent abdominal extension (22). In locusts, and perhaps other insects, expansion of the abdomen can be passive, due to elasticity of the structures, or active. During active expansion, muscles attached to tall spurs within the abdomen (the apodemes) can lift upward on the tergum (roof of the abdomen), expanding abdominal volume (28). At rest at body temperatures of 25°C, abdominal pumping frequencies average 20–30 pumps/min with tidal volumes in the range of 40 μl, and volume changes (ventilation) of ~1 ml/min (10). Abdominal pumping increases during flight, higher temperatures, hypoxia, and hypercapnia by two- to fivefold (10, 17, 32, 52).

The abdominal compressions produce positive pressures in the tracheal system. These pressures, which have been measured via cannula placed through the second spiracle into the thoracic tracheae, average ~0.3 kPa but range up to 7 kPa (23). McCutcheon reported similar values in a different species of locust [0.3 kPa average and 3 kPa maximum pressure (28)]. Positive pressures peak during the "compression phase" when expiratory muscles are contracting and all spiracles are closed (28). Weis-Fogh reported that, during the hyperventilation induced by CO_2 narcosis, peak positive pressures in the thoracic tracheae were ~2 kPa, and negative pressures as low as 0.5 kPa below atmospheric were generated. At 15°C, during discontinuous gas exchange, pressures are lower, averaging 0.06 kPa and peaking at 1.2 kPa (15).

Spiracular Timing and Direction of Airflow

Locusts and some other insects (bees, cockroaches, beetles) have been shown to have unidirectional air flow (2, 7). In locusts, inspiration often occurs via the first four pairs of spiracles, which open during expansion of the abdomen, whereas expiration usually

occurs via the posterior six pairs of spiracles that open during abdominal compression (9, 27, 31, 32, 52). During flight, *spiracles 1* and *4–10* remain synchronized with abdominal pumping, but *spiracles 2* and *3* are kept wide open, allowing tidal ventilation of the flight muscles due to autoventilation associated with thoracic volume changes driven by the flight muscles (33, 51).

However, this basic pattern can be altered. Some variation is graded. The number of open spiracles (both inspiratory and expiratory) increases with activity and respiratory drive, as does the percent of time during each respiratory cycle that each spiracle is open (31). Spiracular patterns also vary substantially. Many locusts exhibit discontinuous gas exchange when very quiescent, closing spiracles and ceasing external gas exchange for periods of time (16, 18, 26). *Spiracles 3*, *4*, and *10* have been reported to exhibit both inspiratory and expiratory functions (27). If the abdominal spiracles are sealed, locusts can both inspire and expire from their thoracic spiracles (27). Coordination of spiracular timing depends on central interneurons, but local effects of O_2 and CO_2 on spiracles also influence opening time (29, 30, 35, 36).

Functional Valving Between Body Segments Allows Segment-Specific Air Sac Action

How are these changes in abdominal volume coupled to volume changes in the tracheal system? Given the open circulatory system of insects and the lack of obvious constrictions between the body segments of locusts, the simplest model predicts that decreases in abdominal volume would lead to near-simultaneous increases in hemolymph pressure throughout the body, leading to simultaneous compression of all air sacs ("syringe model"; FIGURE 2A). In contrast to predictions of the syringe model, X-ray observations demonstrate that tracheal volume changes are primarily limited to the abdomen during abdominal pumping (FIGURE 1).

Why does hemolymph not flow from the abdomen into the thorax and head, causing air sac compression when the abdomen compresses? One hypothesis is that there is functional valving of the open circulatory system. Evidence for functional hemolymph valves can be obtained by experiments with anesthetized locusts. Our X-ray observations show that, normally, the size of air sacs in the locust does not vary appreciably whether the animal is horizontal or vertical. However, if the locust is anesthetized with ketamine in a head-up position, the air sacs in the head dramatically expand while the air sacs in the caudal regions of the abdomen collapse (FIGURE 3). The converse occurs if the animal is anesthetized with the head down (FIGURE 3). Together, these data suggest that, in anesthetized animals, the functional valves fail, allowing a continuous fluidic column of hemolymph within the body, with the bottom end of the column experiencing the highest hydrostatic fluid pressures (theoretical, ~0.5 kPa) and the top the lowest pressures. In the living animal, functional valves may seal to create smaller chambers, resulting in smaller fluid columns with correspondingly smaller hydrostatic pressures throughout the body. This type of separation is well known for insects with constrictions between their thorax and abdomen (47, 48), but has not been previously recognized for insects with no obvious "waist" be-
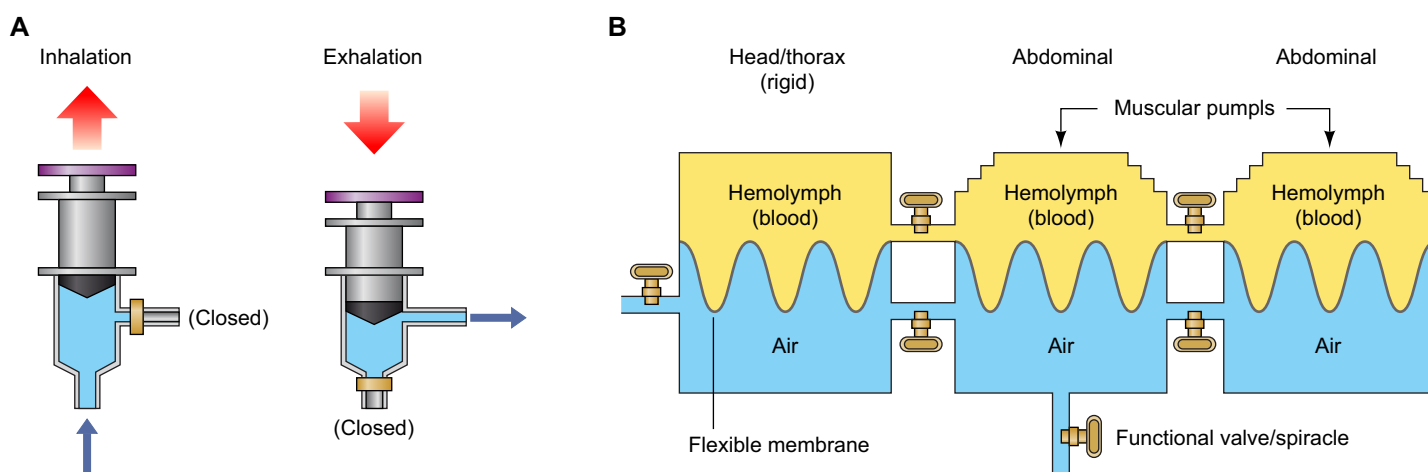

FIGURE 2. **Two models for abdominal pumping**
A: the "syringe model" suggests that, due to the open circulatory system of insects, abdominal pumping would displace abdominal volume like a syringe plunger, producing uniform elevations and depressions of air pressure throughout the locust, with unidirectional flow created by spiracular timing. *B*: the "segmental multi-fluid model" of abdominal pumping suggests that functional valves limit hemolymph and fluid pressure pulses between segments. Muscular pumping of a particular segment can drive air flow to or from adjacent segments. Segments contain air and hemolymph, separated by a flexible membrane. Proper spiracular timing produces unidirectional flow. Our recent experiments support the segmental multi-fluid model.

tween the thorax and abdomen like a locust. How might such functional valves work? The most likely explanation is that tonic contractions of intersegmental muscles maintain a tight fit of the exoskeleton around the digestive tract. Positional changes in the gut could also play a role in such functional valves.

Further evidence for functional valving between segments comes from "window experiments" in which holes are cut in the exoskeleton of a single segment. If this is done in a single abdominal segment, the dorsal air sacs in that segment exhibit inflation and deflation that is out-of-phase with the adjacent segments. In other words, as the abdomen compresses, the air sac in the segment with the window expands, sometimes pushing out through the window; as the abdomen expands, the air sac in the windowed segment collapses. X-ray observations indicate that, in the abdominal segments adjacent to the window, the air sacs continue to exhibit the normal pattern, compressing as the abdomen compresses and expanding as the abdomen expands. Together, these observations suggest that functional valves in the hemocoel allow independent pressures and air sac function in adjacent abdominal segments, whereas the tracheal connections between segments remain open and allow air flow between segments (FIGURE 2B). The capacity to separate hemolymph pressures

across segments may be at least partially responsible for the observed dramatic regional variation in air sac action within the abdomen, with more posterior air sacs and tracheae compressing more completely (FIGURE 1).

Within-Segment Variation in Tracheal System Compression

Within a given segment of the abdomen, compression of the tracheal system is often non-uniform (FIGURE 1). Most obviously, some tracheae (e.g., the transverse tracheae leading from the cardiac longitudinal to the spiracles) do not compress and seem to function as conducting elements for air within the system. It should be noted that compression of tracheae in locusts, as in other insects, is often localized, with some regions within a tracheae compressing strongly, suggesting that specific locations in the tracheae have weak points that reduce resistance to compression (49). Collapsing tracheae may function as valves to regulate flow between segments and regions, in addition to serving as air pumps. Another striking within-abdominal segment variation is that the blind-ended lateral air sacs often compress completely, whereas the dorsal air sacs only partly compress. This could be due to the lateral air sacs being more compressible, but it also seems possible that the lateral air
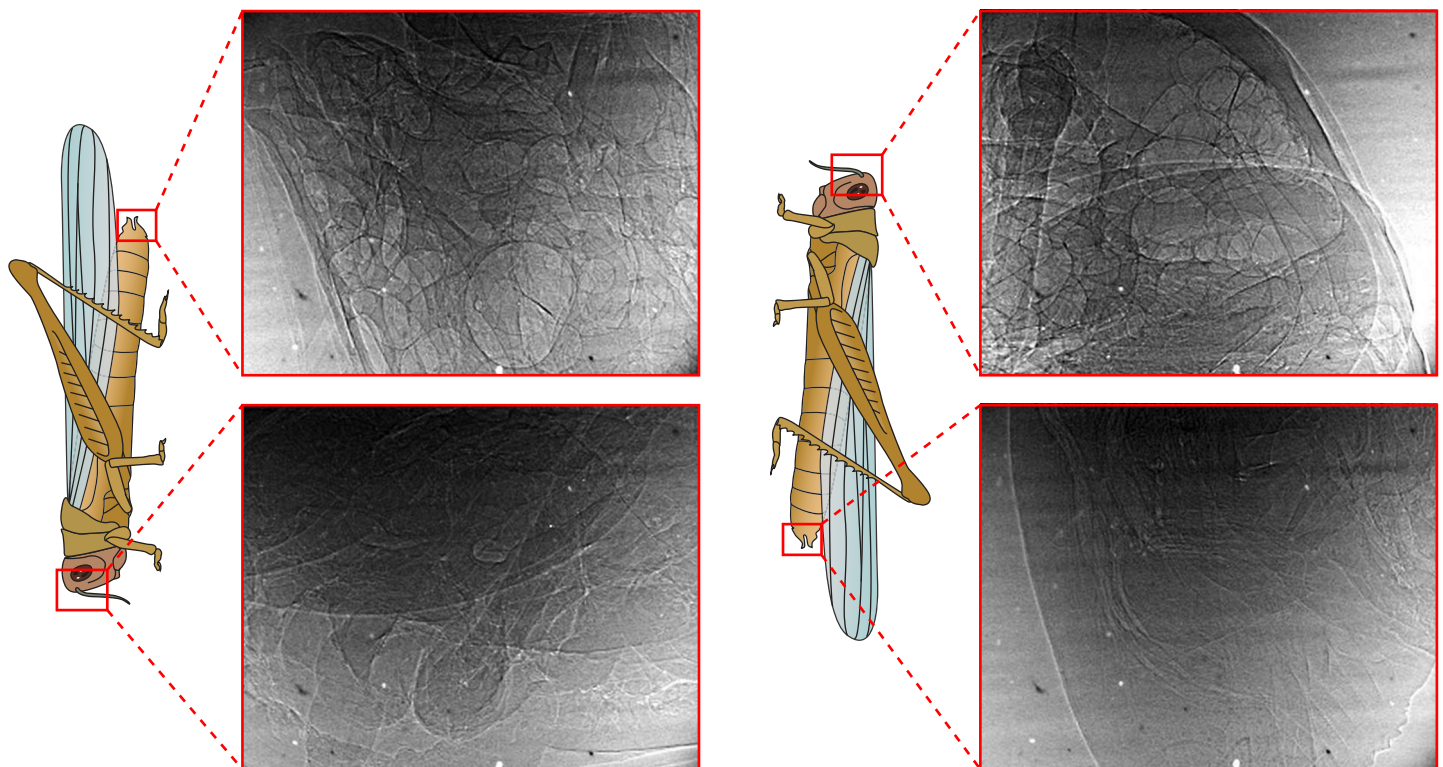

FIGURE 3. Ketamine anesthesia allows hemolymph communication throughout compartments within locusts
When the animal is anesthetized head down, air sacs in the abdomen expand (*top left*) and air sacs in the head compress and disappear (*bottom left*). When animals are anesthetized head-up, air sacs in the head appear and expand (*top right*) and air sacs in the terminal abdomen compress and disappear (*bottom right*), as shown by X-ray imaging.

sacs experience higher local fluid pressures as these air sacs appear tightly packed between the abdominal wall and the apodemes/gut wall. Together, the non-uniformity of air sac and tracheal compression suggest that flow patterns within the system can be complex and perhaps regionally directed.

What Are the Patterns of Air Flow During Abdominal Pumping?

Air flows within insects have not yet been visualized, so the actual patterns of air flow remain conjectural and can only be inferred from tracheal system morphology and spiracular timing. In one common pattern, *spiracles 1–4* open during inspiration, and *spiracles 5–10* open during expiration (8, 31). How might air flow during this behavior?

Miller proposed that air flow from *spiracle 1* is primarily directed in a forward direction, through the head and then backward through the neural longitudinal trunk (33). Major morphological evidence for this hypothesis is that functional tracheal connections between the tracheae, leading from the first spiracle to the head, and the neural longitudinal tracheae only occur where these systems meet within the head, and also that the neural longitudinal tracheae lack functional connections with the thoracic tracheal system. To partly test this hypothesis, we cut windows in the head cuticle and observed head air sacs. With an opening cut in the head, head air sacs often showed compression and expansion at the same frequency as abdominal pumping. Synchronized video recordings were made of the pumping abdomen and the head air sacs under the window cut in the head exoskeleton. In some animals, the head air sacs compressed as the abdomen expanded. This conformed to our expectation that negative pressures created in the abdomen would pull air from the first spiracle through the head and the neural longitudinal tracheae.

In other animals, the air sacs expanded as the abdomen expanded and compressed as the abdomen compressed. One possible explanation for this behavior is that there are channels that permit hemolymph pressure pulses from the abdomen to be transmitted into the head. Alternatively, such ventilation could be associated with head pumping caused by intersegmental muscles that protract and retract the head relative to the thorax (32). We found that the head pumping occurred at the same frequency as abdominal pumping but slightly out of phase. Head retraction caused the neck membranes to bulge outward and the head air sacs to collapse, suggesting that head retraction elevates local hemolymph pressures, which compress the head air sacs. Cutting a window in the neck elim-

inated the fluctuations in head air sac volumes, supporting this model.

Air inspired from *spiracle 1* thus is hypothesized to travel through the head and continue through the thorax and into the abdomen via the neural longitudinal tracheae. Air could then flow all the way to fill the paraproct air sacs or travel via transverse tracheae to fill the lateral or dorsal abdominal air sacs.

The thoracic tracheae from *spiracle 2* do not directly connect to any abdominal tracheae, and it could be suggested that the central flight muscle exchanges gases by diffusion since the thoracic air sacs and tracheae do not compress or expand when the locust is at rest. However, correlation of *spiracle 2* opening with abdominal expansion and Weis-Fogh's measurement of negative pressures in the thorax suggest that air does flow from this spiracle toward the abdomen. Pathways from *spiracle 2* via air sacs and tracheae to *spiracle 3*, and then to the dorsal gut longitudinal tracheae, could allow such flow (FIGURE 1). Alternatively, the region served by *spiracle 2* could exchange air flow with the abdomen via the leg tracheae (FIGURE 1).

Spiracle 3 has tracheae leading to the dorsal and lateral gut longitudinal tracheae; thus air inspired via *spiracle 3* could travel to the abdomen via this route. During expiration when *spiracle 3* is closed, positive air pressures could be transmitted from the abdomen to the thorax via this route. *Spiracle 4* has connections to the lateral and ventral gut longitudinal tracheae as well as the spiracular longitudinal; air could flow through these and fill the lateral air sacs, epiproct air sacs, and perhaps the dorsal abdominal air sacs via the transverse tracheae. Inspiration for the cardiac longitudinal tracheae seems most likely to come via *spiracle 4*, with air likely filling the air sacs in *abdominal segment VIII* and more posterior segments.

During expiration, the abdominal air sacs compress, as well as many of the tracheae, especially in the posterior regions (FIGURE 1). Air is forced out through open tracheae and the abdominal spiracles. Since positive pressures are recorded in the thoracic tracheal system, it is likely that some air moves forward into the thorax, and perhaps the head.

Evidence for Alternative Patterns of Air Flow When Abdominal Length Changes Occur

Normally the length of the abdomen does not change appreciably during abdominal pumping. However, in some animals, especially during stressed breathing, as the abdomen compresses, the abdomen lengthens and vice versa (FIGURE 4). This could occur due to hemolymph being pumped from

the central abdomen to the terminal abdominal segments or to air being pumped from the mid-abdomen to the terminal segments. X-ray observa-tions show that, when the central abdomen is compressed and the abdomen is lengthening, there is an expansion of the air sacs and tracheae in

A

Abdomen dorsoventrally expanded

Air sacs compressed

Abdomen dorsoventrally compressed; tip expanded
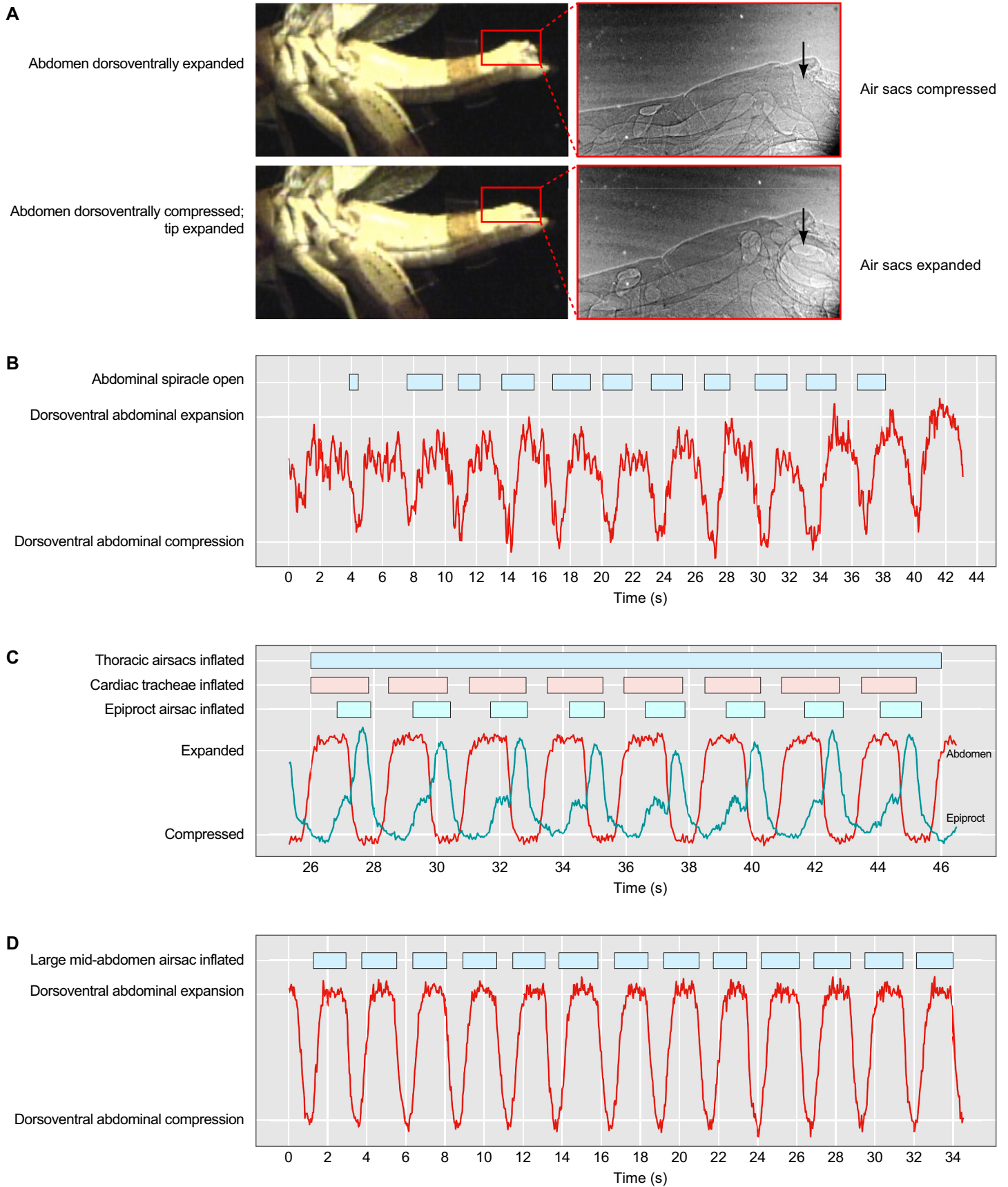
Air sacs expanded

B

C

D

Table 1. Estimated velocities, Reynolds numbers, and Péclet numbers in the longitudinal tracheae of *Schistocerca americana* during resting ventilation at 25°C

Parameter	Units	Value	Notes/Refs.
Summed diameter of all longitudinal tracheae	m	0.00086	Measured from X-rays
Summed areas of longitudinal tracheae	m^2	0.000000033	
Tidal volume	m^3	0.00000004	10
Time for half a respiratory cycle	s	1.2	10
Volume flow	m^3/s	0.000000033	Tidal volume/time
Air velocity	m/s	0.1	Volume flow/area sum
Kinematic viscosity	m^2/s	0.00001568	
Reynolds number		5	Velocity·diameter/viscosity
Abdomen length	m	0.03	
Oxygen diffusion coefficient	m^2/s	0.0000209	6
Péclet number		144	Velocity·length/diffusion coefficient

the terminal abdominal segments. Conversely, when the central abdomen is expanded and the abdomen shortens, the air sacs and tracheae in the terminal segments collapse. Furthermore, windows cut in the exoskeleton of *abdominal segment VIII* (releasing hemolymph pressure) do not affect the capacity of the abdomen to lengthen or the behavior of the terminal air sacs observed under X-rays. Together, these observations indicate that abdominal lengthening is at least partially driven by air flow from the middle abdomen into terminal segments. Under these conditions, *abdominal spiracle 10* opens as the central abdomen expands and closes as the central abdomen compresses, functioning as an inspiratory spiracle (FIGURE 4). *Spiracle 10* closes during abdominal compression, allowing air to be pushed posteriorly past the spiracle into the terminal segments. This behavior may also function specifically to increase air flow to the hindgut, one of the most metabolically active insect tissues (5). Interestingly, elevated air pressures in the abdomen also help enable abdominal lengthening during oviposition in females (39).

Velocities, Reynolds, and Péclet Numbers

What are the flow conditions within the tracheae during abdominal pumping? These will vary dramatically with location. In the tiny, blind-ended tracheoles (~0.5 μm in diameter), it is likely that little convective flow occurs and all transport is by diffusion. In contrast, through the spiracles and the longitudinal tracheae that conduct the air flow, velocities should be relatively high. To provide an order-of-magnitude characterization of flow within the longitudinal tracheae, we made simplifying assumptions based on known tracheal anatomy and respiratory behavior. We assumed that the entire tidal volume moves uni-directionally from thorax to abdomen via the 10 major longitudinal trunks, and calculated velocities assuming distribution of flow among those tubes according to their cross-sectional area, and that the flow through the abdomen occurred within one-half of the respiratory cycle (Table 1). Calculated average air velocity within the longitudinal tracheae was 0.1 m/s, suggesting that air would flow from the front to the back of a locust (~0.05 m) in 0.5 s. This velocity could be underestimated since the inspiratory spiracles are only open for ~20% of the respiratory cycle; also, tidal volumes can more than double during heavy breathing. This velocity could be overestimated if a large fraction of the flow moves elsewhere or if a significant part of the tidal volume is tidal rather than unidirectional. However, Weis-Fogh measured air flows out the 10th spiracle of locusts that were very similar to the estimated flows used here, supporting the idea that most air moves uni-directionally rather than tidally during abdominal pumping (52).

FIGURE 4. Air sac and 10th spiracle actions during abdominal lengthening observed during excessive, stressed breathing
A: when the abdomen is dorsoventrally expanded during inspiration, the air sacs in the terminal segments of the abdomen are compressed. When the abdomen is compressed, the abdomen lengthens and the terminal air sacs expand. *B*: red line shows the compression (down) and expansion of the abdomen (up). Boxes show that the 10th spiracle (observed using simultaneous X-ray video) opened during inspiration and closed during expiration. *C*: red line shows the compression (down) and expansion (up) of the middle portion of the abdomen, whereas the blue line shows that the terminal abdomen (epiproct) expands (lengthens) when the middle abdomen compresses. Rectangles above show that the epiproct air sacs and posterior cardiac tracheae were expanded as the middle abdomen compressed and the abdomen lengthened; meanwhile, thoracic air sacs were still. Air sacs were observed with simultaneous X-ray video. *D*: in the middle of the abdomen, air sacs inflate when the abdomen expands and compress when the abdomen compresses. For simultaneous visible light and X-ray video, animals were restrained using X-ray transparent film and positioned for imaging on a series of translational and rotational stepper-motor stages. Video data (both X-ray and visible light) were recorded at 30 frames/s on MiniDV magnetic tapes. The recordings were digitized and synchronized using Final Cut Pro X (Apple, Cupertino, CA). A light-flash within the experimental hutch at the beginning of each recording made it possible to synchronize the X-ray and visible light recordings with a synchronization error estimated at ±2 frames or 0.067 s. The two synchronized recordings (720 × 480 pixels) were positioned adjacent to each other and exported as a single movie (1,440 × 480 pixels). External movements of the locust abdomen and internal dynamics of tracheal compression and airsac inflation were quantified using ImageJ software (38).

Despite the relatively high air velocities, calculated Reynolds numbers were relatively low (~5), indicating that viscous forces on flow are more important than inertial ones (6, 46). Péclet numbers, which indicate the potential relative importance of convection to diffusion (45), were relatively high (~144). Because there should be only a small diffusion gradient for oxygen from the anterior to posterior animal, the role of diffusion must be trivial in oxygen transport through the longitudinal trunks.

What Type of Pump?

In some ways, the abdominal pump of locusts acts like a simple syringe-type displacement pump; during abdominal pumping, the ventral sternites slide up along the dorsal tergites, reducing abdominal volume. In other ways, the function is more like a diaphragm pump; contraction of the intersegmental muscles bends the dorsal tergites inward. However, the observation that parts of the tracheal system may compress while other parts remain open within a segment suggests that other mechanisms for generating air flow may be at play. Aside from actuation by direct muscular displacement, the abdominal pump may help to create a differential impedance pump within the tracheal system, in which pumping of tubes with variable impedance drives directional flows (20, 21).

A Segmental Multi-fluidic Pumping Model

Synthesis of these data suggests a new segmental multifluidic model for abdominal pumping in locusts (FIGURE 2B). The locust can be modeled as multiple independent segments: head, thorax, and eight abdominal segments. Our observations suggest that these segments may be functionally separated by hemolymph valves, permitting independent pressures. Each segment contains two fluids: air and hemolymph, separated by a flexible membrane (the air sac wall). Fluid pressure differences created by muscular compression in one segment will compress the local air sacs and push air out, either via the spiracles or via tracheae into adjacent segments. If the adjacent segment is sufficiently flexible, the air sac in that segment will expand and increase the volume of that segment (like the lengthening abdomen in FIGURE 4). Muscular expansion of a segment (during inspiration) will expand the air sac and reduce local air pressure. This will tend to pull air into that segment, either through open spiracles or via tracheae from adjacent segments. Functional valving may also explain why the air sacs in the head and thorax do not collapse or expand during abdominal pumping. A negative pressure within the head tracheal system will tend to collapse the head air sacs. However, if hemolymph cannot move into the head and the head is rigid, collapse of the head air sacs would produce expansive forces on the hemolymph in the head fluid compartment, producing negative hemolymph pressures that will resist collapse of the head air sacs. As predicted by this model, cutting windows in the head capsule that permit the local hemolymph to equilibrate with air pressure allows head air sacs to expand and compress. Definitive tests of this model await measurement of segment-specific and air vs. hemolymph pressures. ■

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