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Ventilatory Mechanism and Control in Grasshoppers[1]

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SYNOPSIS. **Grasshoppers exhibit a diversity of ventilatory patterns depending on activity status. For each pattern, the mechanism and control of gas exchange is analyzed in terms of a two-step model, consisting of tracheolar and trans-spiracular steps in series. During the intermittent gas exchange that characterizes the most quiescent grasshoppers, spiracles open and close in response to changing carbon dioxide, and trans-spiracular resistance controls gas exchange. In resting but alert grasshoppers, abdominal pumping occurs, and gas exchange is controlled equally by tracheolar and trans-spiracular resistances; tracheal oxygen and carbon dioxide are regulated by variation in abdominal pumping and spiracular opening. During hopping, abdominal pumping does not occur, and bulk gas flow is driven by cuticular deformations associated with locomotion. Increased cellular oxygen consumption depends on use of internal oxygen stores and increased partial pressure gradients. After hopping ceases, abdominal pumping increases dramatically and restores tracheal gas composition; however, the rise in abdominal pumping after hopping is not affected by tracheal gas levels. During flight, bulk flow to the flight muscles is driven by tidal thoracic auto-ventilation, while the remainder of the body is ventilated by abdominal pumping. During both hopping and flight, the greatest resistances to gas transport exist in the tracheolar rather than the trans-spiracular step.**

INTRODUCTION

The tracheal system is one of the defining physiological characteristics of insects. Tracheae provide insects with a light-weight respiratory system which allows them to achieve the highest mass-specific metabolic rates in the animal kingdom. However, despite the ecological, agricultural, and medical importance of insects, the increasing use of insects as model organisms for evolutionary studies, and the central role of the tracheal system in insect physiology, many of the most basic functional aspects of the tracheal system are unknown. This is partly due to technical difficulties imposed by the small size of insects, and perhaps partly due to the perception that the tracheal system is a relatively simple diffusion system. Recent research has overcome many of the technical difficulties associated with the study of insect respiratory physiology, and has demonstrated that tracheal systems are tightly regulated, complex, and diverse.

The significance of the great diversity in the morphology of tracheal systems has not been explored, and tracheal physiology been studied in depth in only a very few insect groups, including lepidopteran pupae, and adult ants and grasshoppers. Due to extensive work on both the neural control of ventilation and on tracheal gas exchange mechanisms by a number of authors, especially Peter Miller and Torkel Weis-Fogh, the physiology of the tracheal system is certainly best understood in grasshoppers. Only for grasshoppers has data been presented which unequivocally demonstrate regulation of tracheal oxygen and carbon dioxide. I will focus in this review on the mechanisms and control of gas exchange in grasshoppers. Detailed studies of respiratory function in this relatively primitive group may provide a model generalizable to many insects, and a basis for comparison for studies of more derived species.

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MECHANISMS OF GAS EXCHANGE

Tracheal morphology

Weis-Fogh (1964*a*,*b*) has provided excellent diagrams of the gross morphology of the tracheal system in *Schistocerca gregaria*. My dissections of *S. americana*, *Romalea guttata*, *Melanoplus bivittatus*, *M. differentialis*, and *Taeniopoda eques* suggest that the general pattern of tracheal morphology described by Weis-Fogh for *S. gregaria* can be applied to grasshoppers generally. Grasshoppers have eighteen spiracles, nine to a side. On each side, a longitudinal trunk (the primary trachea) connects all ipsilateral spiracles. Anastomoses connect the two sides of the system. In resting grasshoppers exhibiting abdominal pumping, inspiration occurs via the thoracic spiracles, and expiration occurs via the abdominal spiracles, primarily the most caudal pair (Weis-Fogh, 1967). Secondary tracheal branches descend to the various tissues, followed by division into tertiary trachea and the tracheoles. The other obvious feature of the tracheal system in grasshoppers are air sacs, which connect to the secondary and primary trachea, and are most numerous surrounding the flight muscle and in the abdomen.

Respiratory pattern and mechanism in relation to activity levels

The gas exchange mechanisms used by grasshoppers depend on their level of activity and metabolism. In very undisturbed states (*i.e.*, 12 hr in the dark at low temperatures), grasshoppers commonly exhibit discontinuous ventilation, in which periods of gas exchange are separated by many minutes of spiracular closure and near-zero carbon dioxide emission and oxygen uptake (Hadley and Quinlan, 1993; Quinlan and Hadley, 1993; Harrison *et al.*, 1995). The mechanism of gas exchange (diffusive or convective) during discontinuous ventilation in grasshoppers is not known.

Quiescent, alert grasshoppers (between meals in the field, or undisturbed in the lab for minutes to hours) usually exhibit abdominal pumping, with the rate of this pumping depending on temperature (Miller, 1966). Abdominal pumping is driven by contractions of muscles connecting the abdominal sclerites. Expiratory muscles pull the sternites (abdominal sclerites) dorsally (Hustert, 1975; Lewis *et al.*, 1973). Decreasing abdominal volume causes the hemolymph to compress portions of the tracheal system, particularly the air sacs. At this time, thoracic spiracles close and expiratory air flow occurs via the open abdominal spiracles. During inspiration, increases in tracheal volume cause bulk flow inward via the thoracic spiracles. Inspiration is partly passive, due to the natural elasticity of the abdomen, but is assisted by inspiratory muscles (Hustert, 1975; Lewis *et al.*, 1973). In non-flying grasshoppers, about 80% of the air moved by abdominal pumping flows into the thorax and out via the abdomen spiracles; the remaining flow is tidal convection (McCutcheon, 1940; Weis-Fogh, 1967). Convection becomes more tidal during exposure to high ambient carbon dioxide (Weis-Fogh, 1967; Henderson and Prange, 1995).

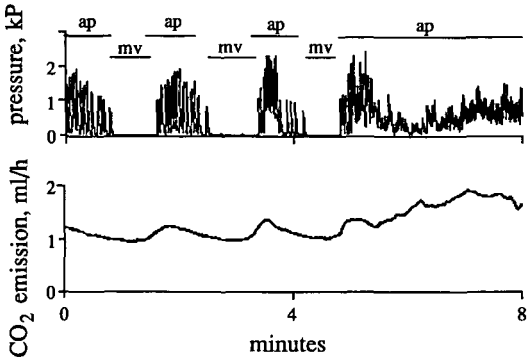


FIG. 1. Tracheal pressures (top) and carbon dioxide emission (bottom) for a *Schistocerca americana* exhibiting intermittent abdominal pumping (ap) and miniature ventilations invisible to the naked eye (mv). The 95% equilibration time for the respirometry system was approximately 1.1 min. Note that abdominal pumping increased the release of carbon dioxide, but only slightly above that observed during miniature ventilations.

In many cases, quiescent grasshoppers intersperse bouts of abdominal pumping with miniature ventilations (Hustert, 1975). These miniature ventilations are invisible to the naked eye, so the grasshoppers appear to be completely motionless. However, tracheal pressure changes and tiny abdominal pumps are detectable (Hustert, 1975; Fig. 1). During miniature ventilations, carbon dioxide emission remains at 50–90% of the level during abdominal pumping (Fig. 1).

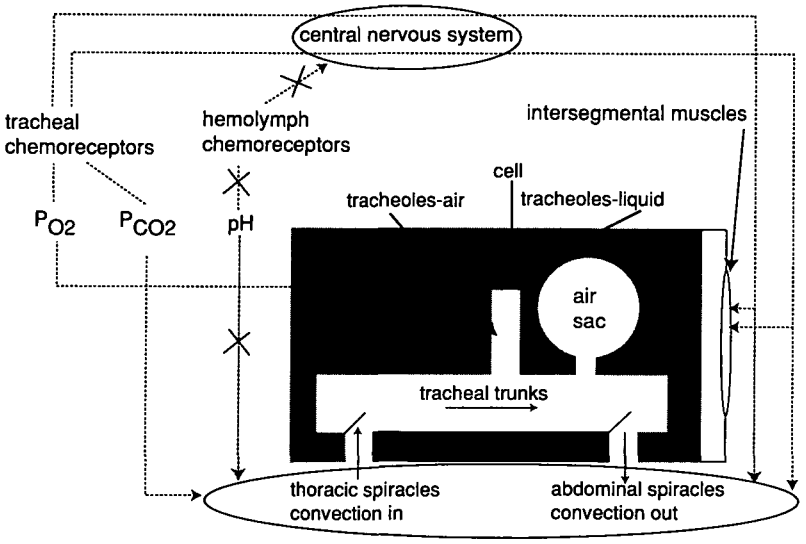


FIG. 2. A schematic model of the mechanisms of gas exchange in a grasshopper exhibiting abdominal pumping, with putative pathways for control of gas exchange (dashed lines). Gas exchange is modeled as a two-step process, having a trans-spiracular step and a tracheolar step. During abdominal pumping for a resting animal, most of the trans-spiracular gas exchange is convective and uni-directional. During flight, hopping, and miniature ventilations, trans-spiracular convection is probably mostly tidal. Tracheal P_{CO_2} affects spiracular opening directly and indirectly via the central nervous system; tracheal P_{O_2} affects spiracular opening via a central mechanism and tracheolar fluid levels by an unknown mechanism. Tracheal P_{O_2} and P_{CO_2} also affect the rate and intensity of abdominal pumping via the central nervous system. Hemolymph pH affects neither spiracular opening nor abdominal pumping.

Abdominal pumping is not visible in hopping grasshoppers, but hopping is accompanied by erratic pressure fluctuations, possibly due to cuticular deformations associated with locomotion (Krolikowski and Harrison, 1996). During a several minute bout of hopping, much of the oxygen is consumed from internal tracheal stores, and increased gas exchange depends on increased partial pressure gradients (Harrison *et al.*, 1991). High-frequency abdominal pumping commences immediately after cessation of hopping (Harrison *et al.*, 1991; Krolikowski and Harrison, 1996). During flight, the primary ventilatory mechanism for the flight muscles is autoventilation, in which changes in thoracic volume created by the flight muscles create tidal flow equivalent to about 7% of the volume of the tergo-pleural air sacs per wing stroke (Weis-Fogh, 1967). Abdominal pumping also increases during flight, and serves to ventilate the central nervous system and remainder of the body (Miller, 1960*b*; Weis-Fogh, 1967).

A two-step model for gas exchange in grasshoppers

A simple, two-step model of gas exchange for a grasshopper exhibiting abdominal pumping is presented in Figure 2. The total respiratory conductance between cells and air consists of trans-spiracular and tracheolar steps in series. The mechanisms for gas exchange at the trans-spiracular step depend on the respiratory pattern, potentially ranging from pure diffusion during discontinuous ventilation to primarily unidirectional, bulk air flow during abdominal pumping. During abdominal pumping, the conductance of the trans-spiracular step is primarily affected by the rate of flow of air through the spiracles, which is a function of the rate and intensity of abdominal pumping, and the degree of spiracular opening. Trans-spiracular conductance may also be affected by the proportion of the bulk flow which is uni-directional.

The tracheolar step in gas exchange is between the primary tracheal trunks and the cells via the secondary and tertiary tracheae

and tracheoles. Most, but not all, of this step will occur in the gas phase. The final distance in the liquid phase within the tracheoles and cells could potentially provide the majority of the resistance to gas exchange (Wigglesworth, 1983; Kestler, 1985). Gas exchange during the tracheolar step may be primarily diffusive given the blind-ended morphology of the tracheoles. However, hemolymph pressure pulsations associated with abdominal or other muscular activity may drive some bulk flow in the tracheoles. Over short time periods, within an individual, the conductance of the tracheolar step will be affected by the fluid levels in the tracheoles (Wigglesworth, 1983), and the amount of bulk flow. Between individuals, and within individuals over periods of time long enough for developmental changes in the tracheoles to occur, tracheal morphology will also affect tracheolar conductance.

Pressures, flows and resistances associated with trans-spiracular bulk flow

The relationships between pressures and flow are quite different in insects than in lung-breathers, partly because the tracheolar system (1) can have uni-directional flow, (2) has multiple apertures, and (3) can modulate the resistance to flow over a wide range. Just as blood pressures are quite low in "open" relative to "closed" circulatory systems due to the relatively low resistance to blood flow in open circulatory systems, the pressures generated within tracheal systems appear to be quite low yet are accompanied by relatively high rates of flow.

Resting grasshoppers at 25°C use abdominal pumping to move a volume of air approximately equal to one tracheal volume per minute. Weis-Fogh (1967) estimated the volume of air moved by abdominal pumping in resting *S. gregaria* as 0.7 ml g^{-1} min^{-1} by measuring the rate of flow via the caudal spiracles with all the other abdominal spiracles sealed. Ventilatory flow could be increased about eight-fold by exposing the animal to elevated carbon dioxide. We (Harrison and Krolikowski, unpublished) have recently measured ventilation volumes by an optical method for animals with all spiracles intact, and found similar values for the closely related and similarly-sized *S. americana*.

For resting grasshoppers, the pressures accompanying each abdominal stroke are a few kPa or less. McCutcheon (1940) and Watts (1951) have both reported that tracheal pressures can increase 7–10 fold with "forced ventilation." However, Weis-Fogh (1967) reported only small increases in hemolymph pressures when *S. gregaria* were exposed to elevated carbon dioxide. Similarly, we have found that either post-activity (Krolikowski and Harrison, 1996), or during exposure to high carbon dioxide or low oxygen (Harrison, Krolikowski, and Waclawski, unpublished data), increases in ventilation rate and volume are accompanied by constant or decreasing tracheal pressures. Therefore, relative increases in spiracular convective conductance and tidal volume are likely to be similar, allowing greater bulk flow at similar pressures per breath.

Relative conductances of the trans-spiracular and tracheolar gas exchange steps

Understanding the relative conductances of the trans-spiracular and tracheolar steps is essential to understanding gas exchange and its control in insects. Conductance (including diffusive plus convective conductances) can be defined as the magnitude of gas exchange achieved for a given partial pressure gradient. At steady state, the gas transport rates of the trans-spiracular and tracheolar steps will be similar. Under these conditions, the ratio of the conductance of the trans-spiracular step relative to the tracheolar step (R) can be calculated from:

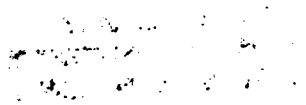
$$R = (P_{CO_2}\ \text{trunk} - P_{CO_2}\ \text{air}) \cdot (P_{CO_2}\ \text{cell} - P_{CO_2}\ \text{trunk})^{-1}$$

where P_{CO_2} trunk represents the P_{CO_2} measured in the primary tracheal trunks, P_{CO_2} air the P_{CO_2} of ambient air, and P_{CO_2} cell the average cellular P_{CO_2}.

I have estimated R for quiescent and post-hopping grasshoppers from:

$$R = (P_{CO_2}\ \text{trunk} - P_{CO_2}\ \text{air}) \cdot (P_{CO_2}\ \text{hemo} - P_{CO_2}\ \text{trunk})^{-1}$$

where P_{CO_2} hemo is the P_{CO_2} of hemolymph. I have also estimated R for gas ex-



change to the leg muscle during jumping, by substituting the P_{CO_2} measured within the leg for P_{CO_2} hemo. With a R value greater than one, the spiracles offer the majority of the resistance to gas exchange; with a R value below one, the tracheoles offer the major resistance to gas exchange.

The first potential technical problem in the estimation of *R* is the assumption that hemolymph P_{CO_2} is similar to cellular P_{CO_2}. Obviously, cellular P_{CO_2} should exceed hemolymph P_{CO_2} to some extent, because the cells are the source of most carbon dioxide production. To the extent that equilibrium between cellular and hemolymph P_{CO_2} is violated, *R* will be overestimated. However, because carbonic anhydrase is found in cells but not hemolymph for most insects (Darlington *et al.*, 1985), the majority of carbon dioxide exchange between hemolymph and trachea probably occurs via the cells, ensuring a close match between hemolymph and cellular P_{CO_2}. Across a range of temperatures, the average whole body P_{CO_2} is similar to that calculated for hemolymph in quiescent grasshoppers (Harrison, 1988), providing empirical support for the assumption used here that hemolymph P_{CO_2} approximates cellular P_{CO_2}. A second potential problem with this measure of *R* is that tracheal air is sampled via an inspiratory spiracle (Gulinson and Harrison, 1996; Krolikowski and Harrison, 1996), so it is possible that this measure of the P_{CO_2} of the tracheal trunks underestimates expiratory P_{CO_2}, producing an underestimate of the true *R*. However, the expiratory P_{CO_2} calculated from the metabolic rate and ventilation volume of *S. americana,* are quite similar (within 0.4 kPa) to those measured by sampling tracheal gases via the metathoracic spiracle, suggesting that this error is small (Harrison, unpublished data).

During the long spiracular closures of discontinuous ventilation, trans-spiracular conductance must be very low, R very high, and gases must approach equilibration within the insect. During the spiracular open phase of discontinuous ventilation, the spiracles open widely and R must fall. However, washout patterns of inert gases during discontinuous ventilation for diapausing pupae suggest that internal resistances are minor and that R remains above one under these conditions (Scheid *et al.*, 1981). In contrast, for resting and post-active locusts exhibiting abdominal pumping, whole-body *R* has been estimated as 0.6–1.4, suggesting that tracheolar and trans-spiracular resistances are similar (Table 1). For the leg, *R* decreases from 1 at rest to 0.3 post-activity (Table 1), indicating that the primary resistance to gas exchange occurs in the tracheolar rather than the trans-spiracular step during hopping. This finding is consistent with the long diffusion path for this enlarged limb and the dependence of hopping on anaerobic metabolism in grasshoppers (Zebe and McShaw, 1957; Harrison *et al.*, 1991). In flying *S. gregaria,* tracheal P_{CO_2} is 5 kPa, and the partial pressure gradient across the tracheolar step calculated from tracheal morphology is 10 kPa (Weis-Fogh, 1964*b;* Weis-Fogh, 1967), suggesting a *R* of about 0.5 ([5–0]/[15–5]). The limited data to date support the hypothesis that *R* varies with ventilatory pattern and activity, with complete control of gas exchange by the spiracles during discontinuous ventilation, and the tracheoles providing the primary resistance to gas exchange during activity.

TABLE 1. *The P_{CO_2} at three internal sites and the calculated ratio of spiracular conductance to tracheolar conductance* (R) *measured at rest, after two min of forced hopping, and after two min of recovery from hopping in grasshoppers at 37°C.**

	Rest	Hopping	Two min recovery
Hemolymph P_{CO_2}	3.3	4.9	3.5
Metathoracic tracheal P_{CO_2}	1.9	1.9	1.9
Leg tracheal P_{CO_2}	3.7	9.5	NM
R (whole body)	1.35	0.63	1.18
R (leg)	1.06	0.25	NM

* (Harrison *et al.*, 1991; Krolikowski and Harrison, 1996). NM = not measured.

VENTILATORY CONTROL

Neural control of gas exchange

The rhythm that drives abdominal pumping is initiated by the metathoracic and cranial abdominal ganglia in grasshoppers (Miller, 1960*a*, 1966; Lewis *et al.*, 1973; Hustert, 1975). It can be modified by feed-

back from stretch receptors in the abdomen (Hughes, 1952; Farley and Case, 1968).

Spiracular opening can be controlled locally or by the central nervous system. In general, control of each spiracle resides in the local ganglia (Miller, 1960*c*, 1966). In spiracles with single control muscles, *i.e.*, the metathoracic spiracle which has only a closer muscle, local control by carbon dioxide levels dominates, and central control of spiracular opening serves to grade the response of the spiracle to carbon dioxide (Hoyle, 1960; Miller, 1960*c*). In spiracles with two control muscles (*i.e.*, the prothoracic and first abdominal spiracles), peripheral control by carbon dioxide is unimportant, and the frequency of action potentials to the opener and closer muscles determines spiracle behavior. In these nerves, response to carbon dioxide depends on central effects of this gas (Miller, 1960*c*, 1966).

Synchronization of spiracular action with abdominal pumping is brought about by interneurons between the local ganglia and the metathoracic ganglia (Miller, 1966). As carbon dioxide increases, all spiracles tend to open, and spiracular opening and abdominal pumping may become less synchronized (Miller, 1960*c*; Weis-Fogh, 1967).

Potential mechanisms for the control of internal gases

A schematic of some of the possible mechanisms for the control of internal gases is presented in Figure 2. If metabolic rate is constant, any mechanism that increases respiratory conductance will decrease internal carbon dioxide and increase internal oxygen. Because both the trans-spiracular and tracheolar steps appear to influence respiratory conductance under most conditions, levels of internal gases could potentially be affected by regulation of either step in the gas exchange process.

Short-term regulation of tracheolar conductance could potentially occur by variation of fluid levels in the tracheoles, or by variation in the amount of convection occurring in the secondary tracheoles. Fluid levels in the tracheoles decrease in response to decreasing oxygen tensions (Wigglesworth, 1983), and this is potentially an important mechanism for regulation of cellular oxygen levels. Control of fluid levels is generally thought to be controlled by local metabolic effects of oxygen or pH (Wigglesworth, 1983), but could also be affected by circulating or local neurohormones.

The conductance of the trans-spiracular step could be affected by the degree of spiracular opening, the rate and magnitude of pressure generation from abdominal pumping, and the degree of synchronization between the spiracles and the abdominal pump. As noted above, the degree of spiracular opening is affected by carbon dioxide and to a lesser extent by oxygen (Wigglesworth, 1935; Case, 1957; Miller, 1960*c*). High ambient carbon dioxide or low ambient oxygen increase the rhythmic output of the metathoracic ganglia and the rate and depth of abdominal pumping (Miller, 1960*a*).

Control of the spiracles during discontinuous ventilation

In lepidopteran pupae, the end of the period of complete spiracular closure (closed phase) is triggered by a fall in tracheal oxygen levels to less than 5 kPa, with oxygen deprivation acting directly on the ganglia (Levy and Schneiderman, 1966; Burkett and Schneiderman, 1974). The subsequent flutter phase is ended when a rise in internal carbon dioxide to approximately 8 kPa triggers the spiracular open phase, with carbon dioxide acting directly on the spiracular muscle (Levy and Schneiderman, 1966; Burkett and Schneiderman, 1974). Decreases in oxygen to a threshold value also appear to regulate the duration of the closed phase in ants (Lighton and Garrigan, 1995).

The relationship between tracheal gases and discontinuous ventilation differs in several respects between lepidopteran pupae and grasshoppers, at least based on studies of *Taeniopoda eques*, the western lubber grasshopper (Harrison *et al.*, 1995). In *T. eques*, hemolymph P_{CO_2} increases from 1.8 kPa immediately after spiracular closing to 2.3 kPa immediately preceding the next spiracular opening. Changes in internal carbon dioxide levels are less than 10% of those observed in pupae. Elevation of am-

bient carbon dioxide to 3 kPa or above abolishes periods of spiracular closure, while grasshoppers exposed to ambient carbon dioxide levels of 2 kPa or below continue to exhibit discontinuous ventilation. Thus, despite the relatively small changes in internal gases, elevation of carbon dioxide levels to a threshold appears to trigger the spiracular open phase. In contrast, increasing ambient oxygen to 30 kPa, or decreasing it to 10 kPa, has no effect on the duration of the discontinuous ventilatory cycle, indicating that internal oxygen levels do not decrease sufficiently to trigger a change in spiracular behavior during discontinuous ventilation in grasshoppers. Gas exchange in discontinuously ventilating *T. eques* may be independent of ambient oxygen because: (1) the low threshold level at which P_{CO_2} triggers spiracular opening ensures that oxygen levels stay high relative to pupae, and (2) the higher mass-specific tracheal volume (3–4 fold greater than in lepidopteran pupae or ants) cause similar changes in tracheal carbon dioxide to be accompanied by smaller changes in tracheal oxygen.

Ventilation and the regulation of extracellular pH

Extra-cellular pH does not affect ventilation in grasshoppers as it does in air-breathing vertebrates. Manipulation of hemolymph pH by injections of HCl or NaOH (changing hemolymph pH by 0.3 to 0.5 pH units) does not affect ventilation rate in resting or post-active grasshoppers (Gulinson and Harrison, 1996; Krolikowski and Harrison, 1996). Decreasing pH could potentially increase tracheal conductance by some mechanism other than by affecting ventilation rate. However, *S. gregaria* injected with HCl recover from the induced acidosis without reducing hemolymph P_{CO_2} indicating that acidosis does not affect respiratory conductance in grasshoppers (Harrison *et al.*, 1992). In grasshoppers, the excretory system appears to be completely responsible for recovery from non-volatile acid-base challenges (Harrison and Phillips, 1992; Phillips *et al.*, 1994; Harrison, 1995).

The relationship between hemolymph P_{CO_2} or $[HCO_3-]$ and ventilation is less clear. Ventilation rate increases after $NaHCO_3$ injection, but depression of hemolymph bicarbonate (HCl injection) has no effect (Gulinson and Harrison, 1996). Possibly extracellular bicarbonate affects the respiratory centers. Alternatively, the stimulatory effect of the $NaHCO_3$ injection may be due to elevation of tracheal P_{CO_2}. Because we (Gulinson and Harrison, 1996) were not been able to independently manipulate hemolymph and tracheal P_{CO_2}, we cannot eliminate the possibility of chemoreceptors which sense hemolymph P_{CO_2}.

Regulation of tracheal oxygen and carbon dioxide levels

Resting grasshoppers regulate oxygen and carbon dioxide levels in their longitudinal trunks at approximately 18 kPa and 2 kPa respectively (Gulinson and Harrison, 1996). This can be demonstrated by perfusing the tracheal system with gases with varying carbon dioxide at constant oxygen level, or vice versa. Under these conditions, ventilation rate is positively correlated with tracheal P_{CO_2} and negatively correlated with tracheal P_{O_2}. Because decreasing tracheal P_{CO_2} below normal levels, or elevating tracheal P_{O_2} above normal levels decreases ventilation rate, we conclude that both tracheal P_{CO_2} and P_{O_2} stimulate ventilation in normal, resting grasshoppers, and that there is a true setpoint for regulation of tracheal oxygen and carbon dioxide.

Why grasshoppers regulate their tracheal oxygen levels at such a high level is unclear, since metabolic rates of resting grasshoppers do not decrease until atmospheric oxygen levels fall to 5 kPa (Arieli and Lehrer, 1988). High internal oxygen stores might be important in enhancing jump performance during burst locomotion. In *M. bivittatus*, 40% of the oxygen used during a few minutes of burst locomotion is consumed directly from air within the tracheae rather than from freshly inspired air (Harrison *et al.*, 1991).

In contrast to the situation observed at rest, active (hopping) grasshoppers do not regulate tracheal P_{O_2} or P_{CO_2} (Krolikowski and Harrison, 1996). Manipulations of tracheal P_{O_2} and P_{CO_2} in post-active grasshoppers have no systematic effect on ventila-

tion rate. Although tracheal gases change substantially during hopping (Krogh, 1913; Harrison *et al.*, 1991), tracheal P_{O_2} and P_{CO_2} do not appear to be used as a signal to match ventilation to metabolic need in grasshoppers. Changes in tracheal P_{O_2} and P_{CO_2} near the respiratory control ganglia are very small relative to those occurring in the metathoracic leg (Krolikowski and Harrison, 1996), and therefore would serve as a poor signal of muscle activity level. The mechanism responsible for the rise in ventilation after hopping is unknown, but may be a feed-forward neural mechanism as shown for the control of abdominal pumping during flight (Ramirez and Pearson, 1989*a*, 1989*b*).

Future directions

Many important questions remain concerning the mechanisms of gas exchange in grasshoppers and other insects. The finding that tracheolar conductance is similar in magnitude to spiracular conductance in active insects indicates that variation in tracheolar fluid levels might be important in the regulation of gas exchange, but no direct test of this possibility has been made. In general, the relative importance of convection and diffusion in gas exchange remain unclear. How do tracheolar and transspiracular conductances compare when analyzed for oxygen rather than carbon dioxide? Why do grasshoppers exhibit such a diversity of respiratory patterns, ranging from discontinuous ventilation to miniature ventilations to abdominal pumping? What is the significance of variation in the degree of uni-directional bulk flow during abdominal pumping?

Similarly, a variety of fundamental information is required to arrive at a basic understanding of ventilatory control. Studies of the regulation of oxygen and carbon dioxide setpoints need to be performed with identified chemosensory cells to determine the neuronal mechanism of homeostatic regulation. The finding that the degree of spiracular opening matches the intensity of abdominal pumping to allow relatively constant tracheal pressures raises many questions about how the spiracular and ventilatory control systems are integrated. Is tracheal or hemolymph pressure regulated? Finally, why is the setpoint for tracheal oxygen so high, and do the setpoints vary with physiological state or among animals in different environments?

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REFERENCES

Arieli, R. and C. Lehrer. 1988. Recording of locust breathing frequency by barometric method exemplified by hypoxic exposure. J. Insect Physiol. 34:325–328.

Burkett, B. and H. A. Schneiderman. 1974. Roles of oxygen and carbon dioxide in the control of spiracular function in *Cecropia* pupae. Biol. Bull. 147:274–293.

Case, J. F. 1957. Differentiation of the effects of pH and CO_2 on spiracular function of insects. J. Cell. Comp. Physiol. 49:103–114.

Darlington, M. V., H. J. Meyer, and G. Graf. 1985. Carbonic anhydrase in the face fly, *Musca autumnalis* (DeGreed) (Diptera: Muscidae). Insect Biochem. 15:411–418.

Farley, R. D. and J. F. Case. 1968. Sensory modulation of ventilative pacemaker output in the cockroach, *Periplaneta americana*. J. Insect Physiol. 14:591–601.

Gulinson, S. L. and J. F. Harrison. 1996. Control of resting ventilation rate in grasshoppers. J. Exp. Biol. 199:379–389.

Hadley, N. F. and M. C. Quinlan. 1993. Discontinuous carbon dioxide release in the eastern lubber grasshopper *Romalea guttata* and its effect on respiratory transpiration. J. Exp. Biol. 177:169–180.

Harrison, J. M. 1988. Temperature effects on intra- and extracellular acid-base status in the American locust, *Schistocerca nitens*. J. Comp. Physiol. B 158:763–770.

Harrison, J. F. 1995. Nitrogen metabolism and excretion in locusts. *In* P. J. Walsh and P. A. Wright (eds.), *Nitrogen metabolism and excretion*, pp. 119–131. CRC Press, New York.

Harrison, J. F., N. F. Hadley, and M. C. Quinlan. 1995. Acid-base status and spiracular control during discontinuous ventilation in grasshoppers. J. Exp. Biol. 198:1755–1763.

Harrison, J. F. and J. E. Phillips. 1992. Recovery from acute haemolymph acidosis in unfed locusts II. Role of ammonium and titratable acid excretion. J. Exp. Biol. 165:97–110.

Harrison, J. F., J. E. Phillips, and T. T. Gleeson. 1991. Activity physiology of the two-striped grasshopper, *Melanoplus bivittatus:* Gas exchange, hemo-

lymph acid-base status, lactate production, and the effect of temperature. Physiol. Zool. 64:451–472.

Harrison, J. F., C. J. H. Wong, and J. E. Phillips. 1992. Recovery from acute haemolymph acidosis in unfed locusts I. Acid transfer to the alimentary lumen is the dominant mechanism. J. Exp. Biol. 165:85–96.

Henderson, D. R. and H. D. Prange. 1995. Contributions of spiracle movement and airway resistance to unidirectional flow in grasshoppers *Melanoplus differentialis*. FASEB J. 9:A642.

Hoyle, G. 1960. The action of carbon dioxide gas on an insect spiracular muscle. J. Insect Physiol. 4:63–79.

Hughes, G. M. 1952. Abdominal mechanoreceptors in *Dytiscus* and *Locusta*. Nature 170:531.

Hustert, R. 1975. Neuromuscular coordination and proprioceptive control of rhythmical abdominal ventilation in intact *Locusta migratoria migratorioides*. J. Comp. Physiol. 97:159–179.

Kestler, P. 1985. Respiration and respiratory water loss. *In* K. H. Hoffman (ed.), *Environmental physiology and biochemistry,* pp. 170–175. Springer-Verlag, Berlin.

Krogh, A. 1913. On the composition of the air in the tracheal system of insects. Skandinav. Arch. Physiol. 29:29–36.

Krolikowski, K. and J. F. Harrison. 1996. Haemolymph acid-base status, tracheal gas levels and the control of post-exercise ventilation rate in grasshoppers. J. Exp. Biol. 199:391–399.

Levy, R. I. and H. A. Schneiderman. 1966. Discontinuous respiration in insects. II. The direct measurement and significance of changes in tracheal gas composition during the respiratory cycle of silkworm pupae. J. Insect Physiol. 12:83–104.

Lewis, G. W., P. L. Miller, and P. S. Mills. 1973. Neuro-muscular mechanisms of abdominal pumping in the locust. J. Exp. Biol. 59:149–168.

Lighton, J. R. B. and D. Garrigan. 1995. Ant breathing: Testing regulation and mechanism hypotheses with hypoxia. J. Exp. Biol. 198:1613–1620.

McCutcheon, F. H. 1940. The respiratory mechanism in the grasshopper. Ann. Entomol. Soc. Am. 33:35–55.

Miller, P. L. 1960*a*. Respiration in the desert locust. I. The control of ventilation. J. Exp. Biol. 37:224–236.

Miller, P. L. 1960*b*. Respiration in the desert locust. III. Ventilation and the spiracles during flight. J. Exp. Biol. 37:264–277.

Miller, P. L. 1960*c*. Respiration in the desert locust. II. The control of the spiracles. J. Exp. Biol. 37:237–263.

Miller, P. L. 1966. The regulation of breathing in insects. Adv. Insect Physiol. 3:279–344.

Phillips, J. E., R. B. Thomson, N. Audsley, J. L. Peach, and A. P. Stagg. 1994. Mechanisms of acid-base transport and control in locust excretory system. Physiol. Zool. 67:95–119.

Quinlan, M. C. and N. F. Hadley. 1993. Gas exchange, ventilatory patterns, and water loss in two lubber grasshoppers: Quantifying cuticular and respiratory transpiration. Physiol. Zool. 66:628–642.

Ramirez, J. M. and K. G. Pearson. 1989*a*. Alteration of the respiratory system at the onset of locust flight I. Abdominal pumping. J. Exp. Biol. 142:401–424.

Ramirez, J. M. and K. G. Pearson. 1989*b*. Distribution of intersegmental interneurones that can reset the respiratory rhythm of the locust. J. Exp. Biol. 141:151–176.

Scheid, P., C. Hook and C. R. Bridges. 1981. Diffusion in gas exchange of insects. Fed. Proc. 41:2143–2145.

Watts, D. T. 1951. Intratracheal pressure in insect respiration. Ann. Entomol. Soc. Am. 44:527–538.

Weis-Fogh, T. 1964*a*. Functional design of the tracheal system of flying insects as compared with the avian lung. J. Exp. Biol. 41:207–227.

Weis-Fogh, T. 1964*b*. Diffusion in insect wing muscle, the most active tissue known. J. Exp. Biol. 41:229–256.

Weis-Fogh, T. 1967. Respiration and tracheal ventilation in locusts and other flying insects. J. Exp. Biol. 47:561–587.

Wigglesworth, V. B. 1935. The regulation of respiration in the flea, *Zenopsyla cheopis,* Roths. (Pulicidae). Proc. Roy. Soc. London, Ser. B 118:397–419.

Wigglesworth, V. B. 1983. The physiology of insect tracheoles. Adv. Insect Physiol. 17:85–149.

Zebe, E. C. and W. H. McShaw. 1957. Lactic and alpha-glycerophosphate dehydrogenases in insects. J. Gen. Physiol. 40:779–790.