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FELLOWSHIP FINAL REPORT

Carbon dioxide and the movement of respiratory gases in caterpillar tracheal systems

H. Arthur Woods[1,3] and Jérôme Casas[2]

1 Division of Biological Sciences, University of Montana, Missoula, MT 59812 USA;

2 LE STUDIUM Institute for Advanced Studies, 45000 Orléans, France

3 Institut de Recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université de Tours, 37200 Tours, France

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Fellow: H. Arthur Woods
From Division of Biological Sciences, University of Montana, Missoula, MT 59812 USA
Host laboratory in region Centre-Val de Loire: Institut de Recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université de Tours, 37200 Tours, France
Host scientist: Jérôme Casas
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ABSTRACT

Insects transport respiratory gases in air-filled tracheal tubes, and a fundamental problem over the past century has been to understand how insects transport gases rapidly enough to support their metabolic rates under highly variables regimes of supply and demand. Here, using a mix of mathematical and physical models, we evaluate the roles that carbon dioxide plays in shaping tracheal structure and function in caterpillars. We first evaluate whether CO_2 trapping by midgut fluids drives convective flows of oxygen toward tracheal tips. We then evaluate whether and how CO_2 is recycled, via the tracheal system, between the highly alkaline midguts of caterpillars and their acidic hindguts. We conclude that, for caterpillars, there is only weak evidence for CO_2-driven convective gas flows. Nevertheless, the models suggest that caterpillars can use convection driven by oxygen absorption to move CO_2 rapidly from posterior to anterior parts of the tracheal system. Our results also suggest new approaches to key problems in microfluidics – namely, how to control the movements of gases and liquids within small channels.

1- Introduction

Insects transport respiratory gases in tracheal systems, and a key problem over the past century has been to understand mechanisms that generate and coordinate flows so that gas exchange (uptake of oxygen and elimination of carbon dioxide) is well matched to metabolic demand from tissues, at least over intermediate time scales.

Here we construct and evaluate mathematical and physical models to examine the coupling of tracheal gas transport to unusual acid/base chemistry occurring in the alimentary canals of caterpillars. In particular, many caterpillars generate extremely high pH levels (> 11) in their midguts, which then are acidified to below neutrality (< 5) in the hindgut (Dow, 1984). We hypothesize that such swings in pH interact strongly with CO_2 produced from metabolism. Specifically, we expect that metabolic CO_2 is trapped in the midgut as bicarbonate and carbonate and transported posteriorly dissolved in the gut contents. Then, upon acidification in the hindgut, the dissolved carbon compounds are reconverted to gaseous CO_2, which may escape into the posterior tracheal system.

We use mathematical and physical models to evaluate two questions about gas transport in caterpillar tracheal systems. First, could CO_2 trapping by the midgut drive *convection* of gases toward the midgut tissues, or is transport primarily driven by *diffusion*? Second, how

likely is it that CO_2 generated in the hindgut is recycled to the midgut, and what are the most likely mechanisms of transport?

2 - Overview of tracheal structure and function

Insects take up oxygen and rid themselves of carbon dioxide via air-filled tubes that ramify finely thought their bodies (Harrison et al., 2012). Exchange with the outside air occurs via gated portholes called spiracles. When a molecule of gas enters a spiracle, it moves first into large primary tracheae, and possibly along the longitudinal tracheae that connect spiracles. The primary tracheal tubes branch repeatedly into finer tracheal tubes, all of which are lined with cuticle and relatively impermeable to gases. By contrast, the finest branches, the tracheoles, are much more permeable to gases.

It is now well known that many, perhaps most, insects can drive convective flows within larger branches of their tracheal systems (Weis-Fogh, 1964). They accomplish this primarily with muscular contractions. Bulk pressure gradients can then be coordinated with control over spiracular openings to generate one-way flows through larger branches (Harrison, 1997, 1997; Miller, 1966; Wasserthal, 2001).

3 – Convective flows from CO_2 absorption?

We start by examining a novel possible mechanism of transport: that net absorption of oxygen, carbon dioxide, and nitrogen in caterpillars drives convective flows of tracheal gases into fine tracheal and tracheolar tips. The midgut of caterpillars is highly aerobic and accounts for perhaps 20 or 30% of the overall metabolic rate (Dow & Peacock, 1989); it thus consumes significant amounts of oxygen. In addition, as argued above, it absorbs traps CO_2 in the alkaline midgut. The dynamics of N_2, which is inert and therefore does not participate in chemical reactions, are less well known. It may nevertheless be driven out of tracheae by consistent, large differences in partial pressure between the tracheal system and the gut.

Thus, at least two of the three main gases, and possibly all three, disappear into the midgut. Net disappearance will lower the bulk pressure in tracheal tips, which should drive convective transport toward the tips. If such flows are large enough, they could provide a mechanism for delivering oxygen via just a small total investment in tracheal tubes.

How fast would convective flows have to be to matter? An approach to this problem is available via the Sherwood number (Denny, 1993), a dimensionless quantity that represents the ratio of transport by convection to transport by diffusion:

$$Sh = \frac{ul_c}{D}$$

where u is speed of flow (m s^{-1}), l_c is a characteristic length (m), and D is the diffusion coefficient (m^2 s^{-1}). Transport is dominated by convection when $Sh \gg 1$ and by diffusion when $Sh \ll 1$. We can get a sense of what a meaningful value of u is by setting $Sh = 1 = ul_c/D$ and rearranging as $u = D/l_c$.

D of oxygen in air at 25 °C is 2 x 10^{-5} m^2 s^{-1}. Appropriate values of l_c are uncertain but should capture something about the linear distance within the tracheal system between spiracle and tracheole and probably vary between 100 µm (= 10^{-4} m) in small or early-stage caterpillars and 5 mm (= 5 x 10^{-3} m) in large or late-stage caterpillars like *Manduca sexta*. These values give calculated threshold flow speeds of u = 0.2 m s^{-1} (= 200 mm s^{-1}) in small caterpillars and u = 4 x 10^{-3} m s^{-1} (= 4 mm s^{-1}) in large caterpillars. Thus, flow-dominated transport is unlikely to occur at any size, as these flows are very high. It is nevertheless worth stepping through some additional calculations to estimate how fast actual flows might be.

These calculations rely on developing a more sophisticated and complete model of gas movement in tracheal tubes. If we assume that all metabolically produced CO_2 is trapped in

the midgut, then we reduce the problem to analyzing movements of O_2 and N_2. Interestingly, the dynamics of N_2 will turn out to control the overall dynamics of the system.

Specifically, we model gases moving within three spaces – the external environment, the tracheal system, and the midgut (Fig. 1).

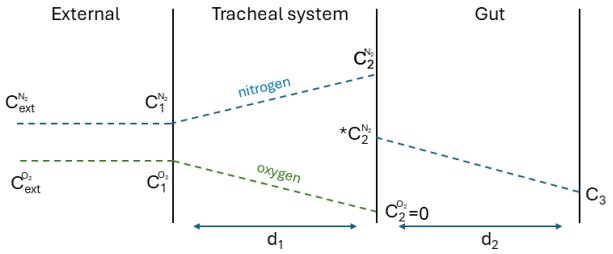


Fig. 1. Model schematic of simultaneous fluxes of nitrogen and oxygen in the tracheal system. We assume that oxygen disappears at the gut epithelium, so there is no need to keep track of its flux inside the gut.

In the external atmosphere, nitrogen occurs at a partial pressure of 0.79 atm and oxygen at 0.21 atm. At steady state, the nitrogen gradient goes from 0.79 atm to 1 atm (we use concentrations, C, in the model, which are related to partial pressures, P, by the ideal gas law $C = \frac{n}{V} = \frac{P}{RT}$), rising as it goes inward because oxygen is gradually being depleted by metabolism. At the same time, the oxygen gradient goes from 0.21 to 0 atm. That is, the oxygen concentration at the gut wall is zero (see Johnson & Barbehenn, 2000), which is equivalent to assuming that all oxygen that hits the gut wall disappears as it is consumed by metabolic processes. We also assume that CO_2 produced by metabolism disappears into the gut immediately and does not reappear in the tracheal system.

The first thing to see is that nitrogen disappears from the tracheal system in two ways – into the gut but also by diffusing down its gradient within the tracheal system and exiting via the spiracles. This raises the question of which of these is more important; if diffusive fluxes within the tracheal tube are much greater than into the gut, we would be able to simplify the model by neglecting the latter.

In a previous analysis (not shown), I calculated that nitrogen moved into the gut from a single tuft at a rate of $J_{tuft} = 1.51 \times 10^{-11}$ mol s^{-1}. It is straightforward to calculate the outward N_2 flux in the tracheal tube itself with the equation

$$J^{N2} = \frac{D^{N2}_{air}}{d_1}(C^{N2}_2 - C^{N2}_1)$$

which, because $C^{N2}_1 = 0.79\ C^{N2}_2$, becomes

$$J^{N2} = \frac{D^{N2}_{air}}{d_1} 0.21 C^{N2}_2$$

Appropriate parameter values are D^{N2}_{air} = 1.98 x 10^{-5} m^2 s^{-1}, and $C^{N2}_2 = 1/RT$ = 40.9 mol m^{-3} at 25 °C. In addition, we need the radius and length of the tracheal tuft, which for a 5-g caterpillar are r = 1.3 mm and length, d_1 = 4.6 mm (Helm & Davidowitz, 2013). From the equation above, we calculate that J^{N2}= 0.037 mol m^2 s^{-1}, meaning that the molar flux through the tuft is $J^{N2}_{tuft} = J^{N2}\pi r^2$= 1.98 x 10^{-7} mol s^{-1}. This value is nearly 10,000x higher than the flux into the gut and suggests that N_2 transport within the gut is too small to matter and should be dropped from the model. The simplified model is shown in Fig. 2.

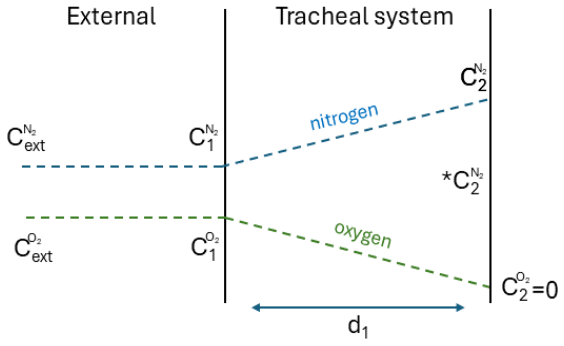


Fig. 2. Simplified model of simultaneous fluxes of nitrogen and oxygen in the tracheal system. Because fluxes of nitrogen in the tracheae are so large compared to fluxes into the gut, we omit the gut section from further analysis.

The next question is how nitrogen and oxygen affect each other's transport. What we show below is that differences in their rate of diffusion (arising from differences in their diffusion coefficients) can result in *convective* inward flow in the tube. This effect is what Buck (1958) called 'flow-diffusion' (also analyzed by (Kestler, 1984)). These authors analyzed flow-diffusion in terms of complex gas mixtures (oxygen, nitrogen, carbon dioxide, water vapor) interacting to induce flows across insect spiracles. Similar arguments have been developed for induced flows across leaf stomata (Leuning, 1983). Here we apply these ideas to gas transport within the tracheal tufts themselves.

The starting observations are that the total barometric pressure is the sum of the partial pressures of all component gases (Dalton's law: $P_{tot} = P_{N2} + P_{O2} + P_{CO2} + P_{H20}$) and that P_{tot} is the same (= 1 atm) everywhere in the tracheal system. This will not be strictly true, but the tracheal system has high enough conductance to convective flows that P_{tot} is likely to rapidly equilibrate everywhere in a tracheal tuft.

The implication of equal P_{tot} everywhere is that any net *diffusive* fluxes of gases are immediately offset by *convective* flows. Applying this logic to the model above means calculating the steady-state diffusive fluxes of nitrogen and oxygen, which will differ, and then calculating how much convective flow is needed to offset this difference.

Above, we calculated tracheal outward fluxes of nitrogen (and going forward, we drop the subscripts *tuft* and *gut* since we're now considering only air phase transport in the tracheae). To get the net effects of diffusion, we need a pair of equations, one for nitrogen and one for oxygen:

$$J^{N2} = \frac{D_{air}^{N2}}{d_1}(C_2^{N2} - C_1^{N2})$$

$$J^{02} = \frac{D_{air}^{O2}}{d_1}(C_2^{O2} - C_1^{O2})$$

Which in the context of the model above (nitrogen 100% at wall and oxygen 0%) can be simplified by recognizing that the gradients of nitrogen and oxygen are exactly symmetric, i.e., $\Delta C^{N2} = \Delta C^{O2} = \Delta C$, so that adding together the equations above gives

$$J^{N2} + J^{02} = \frac{\Delta C}{d_1}(D_{air}^{N2} - D_{air}^{O2})$$

In other words, the net diffusive flux is proportional to the *difference* in the diffusion coefficients of the two gases. Using D_{air}^{N2} = 1.98 x 10^{-5} m^2 s^{-1} and D_{air}^{O2} = 1.78 x 10^{-5} m^2 s^{-1}, which differ by the amount 0.2 x 10^{-5} m^2 s^{-1}, we calculate for a 5th instar caterpillar that the net diffusive flux within a tracheal tube of radius 1.3 mm is 2 x 10^{-8} mol s^{-1} outward. In other words, because N_2 is lighter than O_2 and has a higher diffusion coefficient, it diffuses more rapidly down the same magnitude concentration gradient. This means that N_2 leaves the tube by diffusion faster than O_2 enters. In turn, this sets up a minor difference in total pressure, with lower pressure inside (despite our assumption that $P_{tot} = 1$ everywhere; this works because the pressure differences necessary to drive bulk flow are trivially small), which causes bulk flow of outside air into the tracheal tube.

How fast is this flow? We can convert the molar flux into a linear flow speed by again using the ideal gas law and the area of the tracheal tube $u = J^{net}RT/AP$. The flow speed in a tube of radius 1.3 mm is 92 μm s^{-1}, which gives a Sherwood number for these flows, calculated for oxygen, of $Sh = 0.024$. *Thus, the convective inflow is small enough that overall gas transport is likely to be dominated by diffusion.*

Interestingly, when we redo this analysis in a scaling context (for caterpillars between 5 mg and 10 g body mass), flow speeds are highest in the smallest individuals but the Sherwood number is the same across all sizes (not shown). This represents that tracheal tufts that are smaller magnify the effects of the differential diffusion coefficients of N_2 and O_2 (which speeds up flow) but also magnify the

importance of diffusion directly on transport in exactly offsetting ways.

Circling back to the question that motivated the analysis in Section 3: does absorption of metabolic CO_2 by alkaline midguts generate inward convective flows of respiratory gases in the tracheal system? The answer is a qualified 'yes', but the flows appear to be small enough that they largely do not matter; inward transport of oxygen still is dominated by diffusion. This also implies that inward movement of CO_2 (analyzed in greater detail below) is also primarily due to diffusion.

4 – Is metabolic CO_2 recycled between hindgut and midgut in caterpillars? Mathematical model

Recall that caterpillar alimentary tracts are divided into two areas: a larger, alkaline midgut that we think traps metabolic CO_2 as bicarbonate and carbonate; and a small, acidified hindgut that we think regenerates CO_2 from the dissolved bicarbonate and carbonate. Total levels of bicarbonate + carbonate in the *Manduca* midgut exceed 70 mmol/L (Harvey, 2009), which is quite high and difficult to explain without invoking recycling of CO_2 from the hindgut. Indeed, our calculations (not shown) suggest that the midgut would need to capture about a third of the total metabolic CO_2 production *and likely more than the entirety of the metabolic CO_2 produced by the midgut epithelium itself*.

In this section, we use both mathematical and physical models to evaluate the probability of CO_2 recycling via the tracheal system. We start by asking whether diffusion alone is sufficient to move significant quantities of CO_2 from posterior to anterior.

Is diffusion alone sufficient? To analyze this, we calculate the magnitude of the CO_2 gradients necessary to drive a significant fraction of metabolically produced CO_2 from the hindgut to the midgut via the longitudinal tracheal tubes. The logic is that we have reasonable evidence already that CO_2 accumulates in the midgut and then is off-gassed in the hindgut. We imagine two scenarios – one in which all off-gassed CO_2 is recaptured by the midgut and another in which just a quarter is recaptured. Because we know the metabolic rates of larvae and the approximate dimensions of their bodies and tracheal systems, we can calculate how large the gradient *must be* to drive the fluxes we assume.

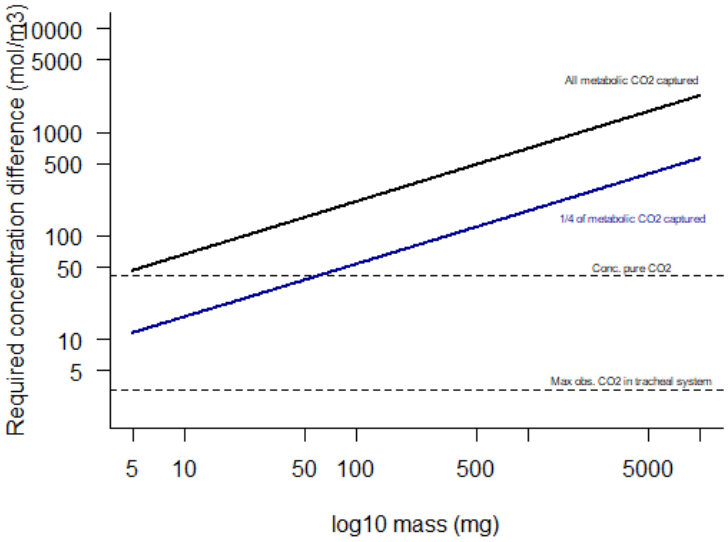


Fig. 3. Concentration gradients of CO_2 required to drive either all or ¼ of the observed metabolically produced CO_2 half the length of the body (i.e., from hindgut to midgut) through the longitudinal tracheae. The upper dotted line represents the concentration of pure CO_2 and the lower dotted line the approximate value of the maximal measured concentrations in living insects.

Here, we also do this analysis across body sizes, as we have enough information to estimate the relevant parameters for all larval stages of *M. sexta*. We use measured scaling relationships for total larval CO_2 production (Woods, unpubl.). In addition, we use the scaling of larval lengths and widths available from (Lin et al., 2011). The parameter that is least well known is the scaling

coefficient of tracheal radius. Here we use the one found by (Harrison et al., 2005) of 0.33, which is also the expectation from geometric isometry. The data of Harrison et al. come from a range of body sizes in a single species of grasshopper.

This analysis (Fig. 3, above) suggests strongly

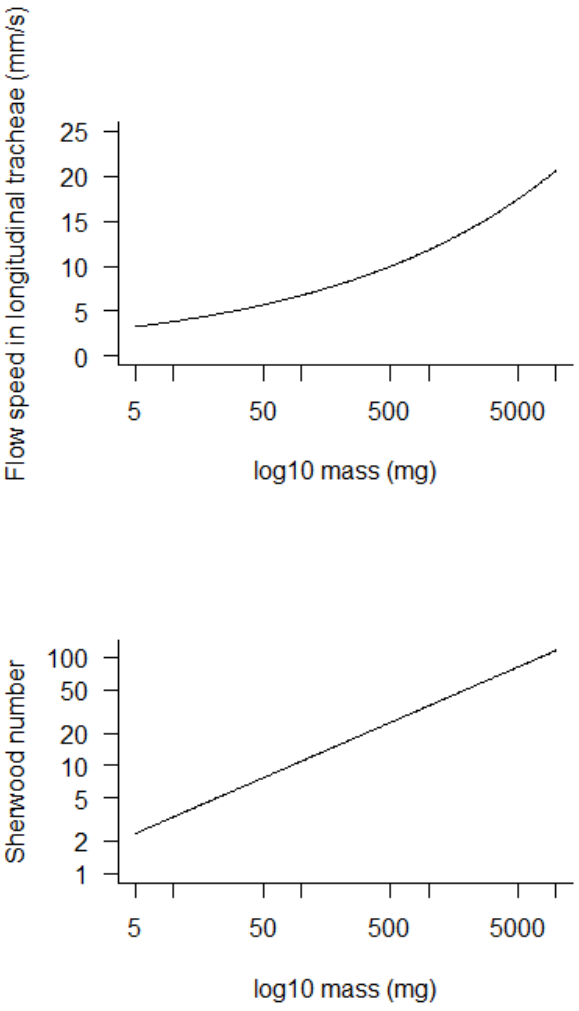


Fig. 4. (A) Predicted flow speeds of gas from the posterior to the anterior tracheal system in the longitudinal tracheae as oxygen is depleted from tracheal stores by metabolism. (B) Associated Sherwood numbers calculated using the diffusion coefficient of carbon dioxide in air.

that diffusion alone is inadequate across all larval stages, but especially in larger larvae. The maximum possible gradient arises when CO_2 occurs as a pure gas in hindgut tracheae (concentration ~ 40 mol m^{-3}) and at zero in anterior tracheae. Actual posterior concentrations (and therefore gradients), however, must be much lower (lower dotted line), as CO_2 never occurs as a pure gas but rather at maximal values of 5 – 10%. *The conclusion is that carbon dioxide is not recycled in the gas phase by diffusion alone*.

Convective flows from posterior to anterior in the longitudinal tracheae? Given that caterpillars have multiple ways of inducing convective flows (especially via body compressions of different kinds, Greenlee et al., 2013), perhaps CO_2 is transported instead by convection. Here we analyze a potential mechanism: transient closure of the anterior spiracles while leaving open the last pair (or last two pairs) of spiracles associated with the hindgut. This pattern of closure could in principle draw gases rapidly toward the anterior end as the caterpillar uses up oxygen in the tracheal volume.

To do this calculation, we imagine that the caterpillar switches between two states: (1) spiracles open everywhere with the tracheal system mostly flooded with air (at the extreme, we can imagine 21% oxygen and 79% nitrogen everywhere in the anterior parts of the tracheal system); and (2) all spiracles closed except the posterior-most pair. We then calculate the total metabolic rate of the anterior 7/9 of the caterpillar (corresponding roughly to the sections containing the midgut) and calculate what that volumetric flow must occur through the longitudinal tracheae as the caterpillar uses up the oxygen in the anterior tracheal system. Here, we assume that gas transport is fast enough that we can ignore movement of N_2 by diffusion (either in the tracheal system or into the gut) or accumulation of CO_2 (all of it is trapped in midgut or dissolves into tissues).

The results (Fig. 4) suggest that flow could be a rapid, effective way of moving gases anteriorly. In the smallest caterpillars (1^{st} instars weighing 5 mg), flow speeds are predicted to be a little over 3 mm s^{-1}. For 5^{th} instars weighing 10 g, the speeds rise to over 200 mm s^{-1}. These correspond to Sherwood numbers (calculated for CO_2) of between 2 and 100, all in the range indicating that transport is dominated by convection.

The related question is how long such convective transport could be supported (before the oxygen stores run out), and how far a packet of tracheal gas would travel during that time. We calculated this by calculating the time until the oxygen stores run out, and then combining that time with the flow speed to calculate flow distances (Fig. 5, below).

Although times rise slightly with increasing caterpillar size, they are all around 1 minute.

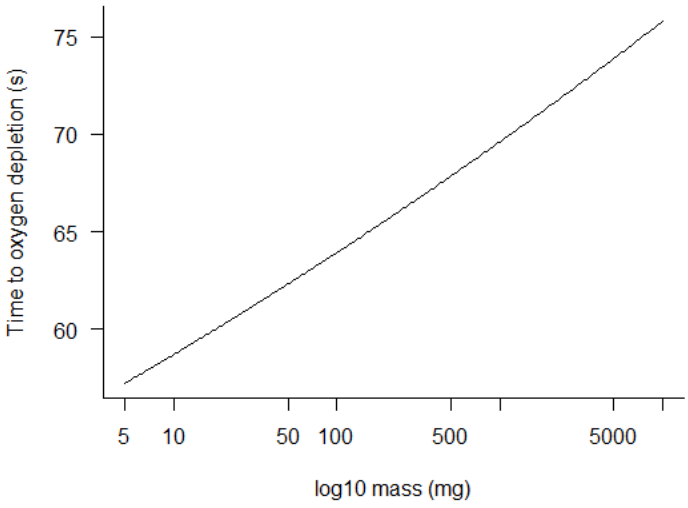


Fig. 5. Total time until oxygen stores in the anterior part of the tracheal system are depleted by metabolism. This calculation assumes that metabolism isn't ever limited by oxygen transport from the tracheae.

And during that time, the gas packages would have time to travel 18 – 20x the length of the caterpillar.

There are significant potential errors in the parameters that go into this analysis. The least well known are tracheal morphologies. Nevertheless, the overall message is clear*: convection can completely dominate diffusion and result in long-distance movements within the longitudinal branches of the tracheal system*.

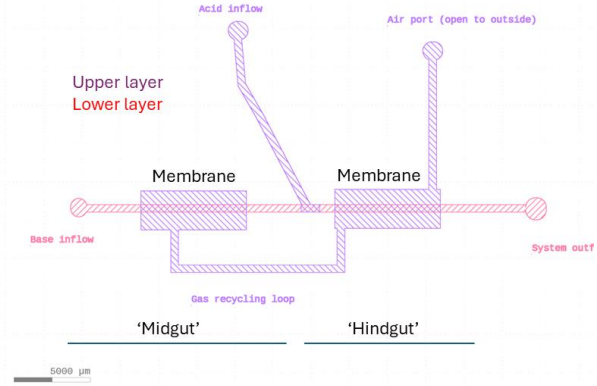
5 – Is metabolic CO_2 recycled between hindgut and midgut in caterpillars? Physical model

Another way to approach the CO_2 recycling problem is to build a physical model of the hypothesized chemistry and structure and to assess whether it accumulates carbon compounds. We built such a device by leveraging a system, already in place in the Casas lab, for fabricating microfluidic devices. The idea was to flow liquids through a central channel simulating the caterpillar alimentary tract – with high alkalinity (pH 11) in the anterior part and acidity (pH 4 – 5) in the posterior part, with a coupled gas circuit simulating the insect tracheal system.

Fig 6. Schematic of PDMS device for simulating acid/base chemistry and gas flows in caterpillar tracheal systems.

We built the system from two layers of polydimethylsiloxane (PDMS), a silicone-based elastomer that is commonly used in microfluidics (Kiran & Chakraborty, 2020). The layers of PDMS were separated by an additional very thin (100 micron) custom-built membrane that separated liquid and gas spaces. The design is shown in Fig. 6 (below). An alkaline solution (pH 11, sodium hydroxide with sodium sulfite to act as an oxygen scavenger, and copper sulfate to catalyze the oxygen reaction) is directed into the red port on the left and the liquid flows toward the right. When it arrives at the halfway point, it is neutralized (and made slightly acidic) by a solution of 1N hydrochloric acid. The upper layer of PDMS also has a gas circuit built into it, which allows movement of oxygen and carbon dioxide between the gas and liquid phases.

If our hypothesis is supported, we expect outside air to be drawn convectively into the system via the 'air port' (upper right) as oxygen and carbon dioxide are absorbed



into the alkaline liquid flowing on the left. As it dissolves into the alkaline liquid, the CO_2 is rapidly converted to bicarbonate and carbonate, which is then entrained to flow to the right. Once the flowing liquids are acidified, the bicarbonate and carbonate are converted back into CO_2, which can then escape across the second membrane (on the right) and reenter the gas circuit. Thus, in principle CO_2 can accumulate as it recycles between liquid and gas phases, with transport from right to left occurring either via diffusion or convection.

By the end of Woods's time in France, in spring 2024, we had (after much trial and error) fabricated a working PDMS device and run the alkaline and acidic fluids through it (Fig. 7). However, there was not enough time to assess whether CO_2 was recycling via the air circuit and accumulating in the alkaline anterior section. Those measurements await Woods's next trip to Tours.

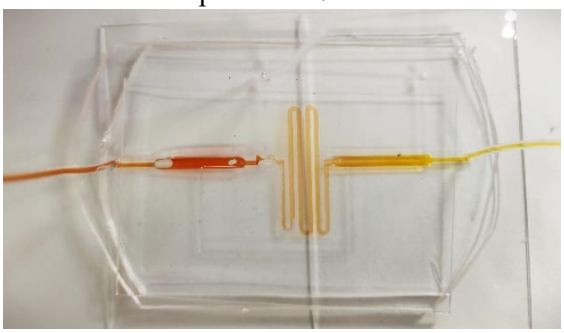


Fig. 7. Photograph of working PDMS device. In this run, alkaline fluid containing phenol red (which is dark red when alkaline but orange/yellow when acidified) is pushed in from the left at a volumetric rate of 1 µl per s. At the same time acid (1N HCl) is pushed in from the upper port, and it neutralizes and slightly acidifies the flowing solution, which then passes through a serpentine mixing section before moving beneath the second membrane, where CO_2 can escape back into the gas circuit.

We intend to measure the accumulation of carbon compounds in the alkaline section by sampling that fluid and acidifying it in the gas stream of a sensitive carbon dioxide analyzer (Licor LI7000). From the area under the CO_2 pulse curve, we will be able to calculate total amounts of CO_2 released.

6 - Discussion

The basic structure and function of insect tracheal systems has been understood for much of the past century (Krogh, 1920; Weis-Fogh, 1964; Wigglesworth, 1930). The astounding diversity of tracheal systems across taxonomic groups, however, raises many unanswered questions about the identities and importance of the ecological and physiological factors that drive tracheal evolution. One way to approach this problem is to identify insects with unusual physiologies and then to examine those tracheal systems in detail using a combination of experiments and mathematical models.

Our project emerged from the observation that caterpillars have unusual acid/base chemistry in their guts – with an extraordinarily alkaline anterior section but slightly acidic hind section. These pH swings should have important consequences for CO_2 movement within the tracheal system. Specifically, we hypothesized that metabolic CO_2 is absorbed and trapped in the midgut and then liberated in the hindgut. In other words, the unusual gut chemistry may act as *a CO_2 pump*. What are the consequences for how gases move within caterpillar tracheal systems?

We first examined whether CO_2 absorption results in convective flows of gases toward the

tracheal tips that supply midgut tissues. The idea is that midgut tissues, because they have high metabolic rates, consume oxygen at high rates. They also produce CO_2 from metabolism (~ 1:1 ratio of O_2 consumed:CO_2 produced), but all of that CO_2 does not emerge back out into the tracheal system. Thus, it is possible that net gas consumption occurs at the tissues, which may draw gases inward through the tracheal tufts. The main potential block to such transport is the accumulation of N_2 in the system.

We analyzed these dynamics by building and analyzing a mathematical model of the simultaneous transport of O_2 and N_2 (we did not analyze CO_2 explicitly because we are assuming it all disappears into the midgut) by both diffusion and by convection. The model showed clearly that although the gases interact in ways that generate small inward convective flows, those flows are small and transport is almost completely dominated by diffusion.

We then turned to the problem of whether and how CO_2 is recycled from the hindgut to the midgut via the tracheal system. Some recycling is likely to occur, as measured levels of bicarbonate and carbonate in midgut fluids are high enough that it is difficult to account for their origins otherwise. We first assessed whether CO_2 is likely to move by diffusion, through longitudinal tracheae that that run along each side of the gut, from the posterior to the anterior parts of the tracheal system. The logic was that we know both the overall rates of CO_2 production are (from measured metabolic rates) and the approximate dimensions of the tracheal tubes. With this information, we can calculate the *required* concentration gradients to move the necessary amounts of CO_2. Those gradients turned out to be enormous (Fig. 3) – much larger than is biologically realistic. This means that longitudinal CO_2 transport cannot occur by diffusion alone.

We then assessed whether caterpillars could drive rapid convective movement of gases along the longitudinal tracheae. Here the hypothesized mechanism is by spiracular closure (common in insects) and metabolic drawdown of oxygen by respiring tissues, as happens during a kind of respiratory gas exchange known from other species, called discontinuous gas exchange cycles (Chown et al., 2006; Levy & Schneiderman, 1966; Lighton, 1996). If the anterior spiracles are all closed and the posterior spiracles open, we showed that caterpillars could entrain rapid anterior flows. These flows could easily move CO_2 from its posterior sites of production to anterior parts of the tracheal system, where it would then move (by diffusion) down the tracheal tufts and enter into and be trapped by the midgut fluids.

A powerful way to test these theoretical predictions is with empirical measurements of gas movements in living caterpillars or in physical simulations of them. We chose to develop a microfabricated device that strips down the proposed mechanisms to their simplest forms. Although we successfully built the device, there was not enough time to assess how rapidly it accumulates CO_2.

7- Conclusion

Insect tracheal systems are fascinating physiological systems, and understanding them requires integrating ideas and approaches from biology, physics, chemistry, and ecophysiology. The work presented here is focused on ‘just’ one set of insects (caterpillars, the larvae of Lepidoptera). Nevertheless, the principles we uncovered may be more broadly applicable. Indeed, although not outlined in the document above, the set of models that we developed during the past three months hints at the broader importance of carbon dioxide as a driver of tracheal structure and function. We will be pursuing these broader ideas and hope to develop some robust theory that applies to all insects.

8- Perspectives of future collaborations with the host laboratory

We have made a strong start on the main problems envisioned at the beginning. Toward the end of the research period, we discussed and agreed that there still were many productive aspects of this project to explore together, and we have already started to plan future visits by Woods to Tours. These visits may be supported in part by additional funds provided by Prof. Casas. In addition, Woods intends to apply to Le Studium again, either for a one-moth follow-up to his 2024 visit or a new one-year fellowship.

A first future visit will focus on getting data from the PDMS device, and on exploring more thoroughly the possibility of using acid/base driven changes in CO_2-bicarbonate phases to drive liquids within microfluidic devices. Another exciting possibility is for Casas to engage a Masters student in this research in Spring 2025 (for which Woods would also serve as an advisor). Beyond these specifics, we anticipate writing at least two articles together and possibly more.

9- Articles published in the framework of the fellowship

This work is only several months old now, so it is too early to report any publications. Nevertheless, we anticipate writing two articles this year for submission to peer-reviewed journals. One of them will focus on the problem of how gas fluxes are coupled to the unusual chemistry of caterpillar alimentary tracts. The other will focus on more general theory about how the problem of CO_2 disposal drives the evolution of tracheal morphology and patterns of ventilation.

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