Discontinuous Gas Exchange

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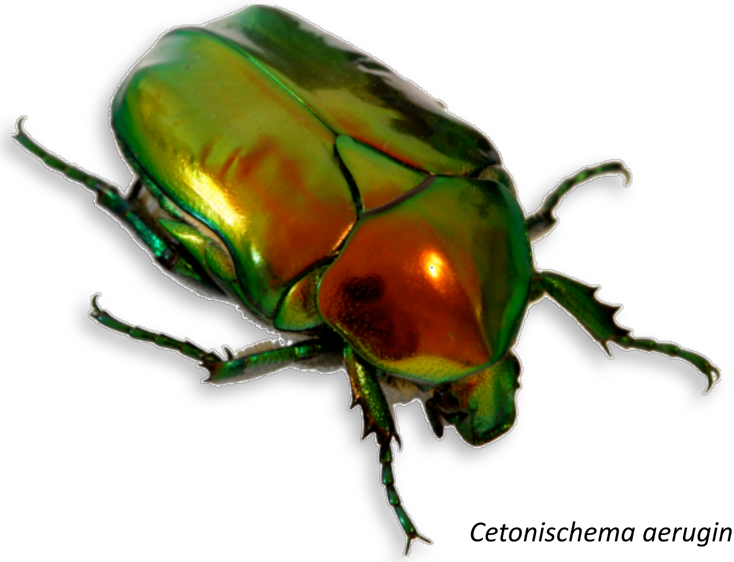


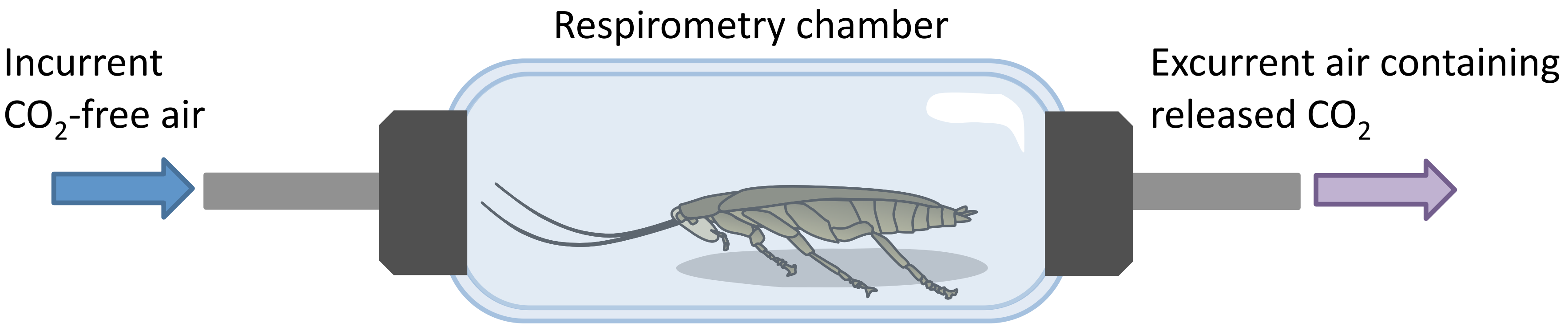
Cetonischema aeruginosa

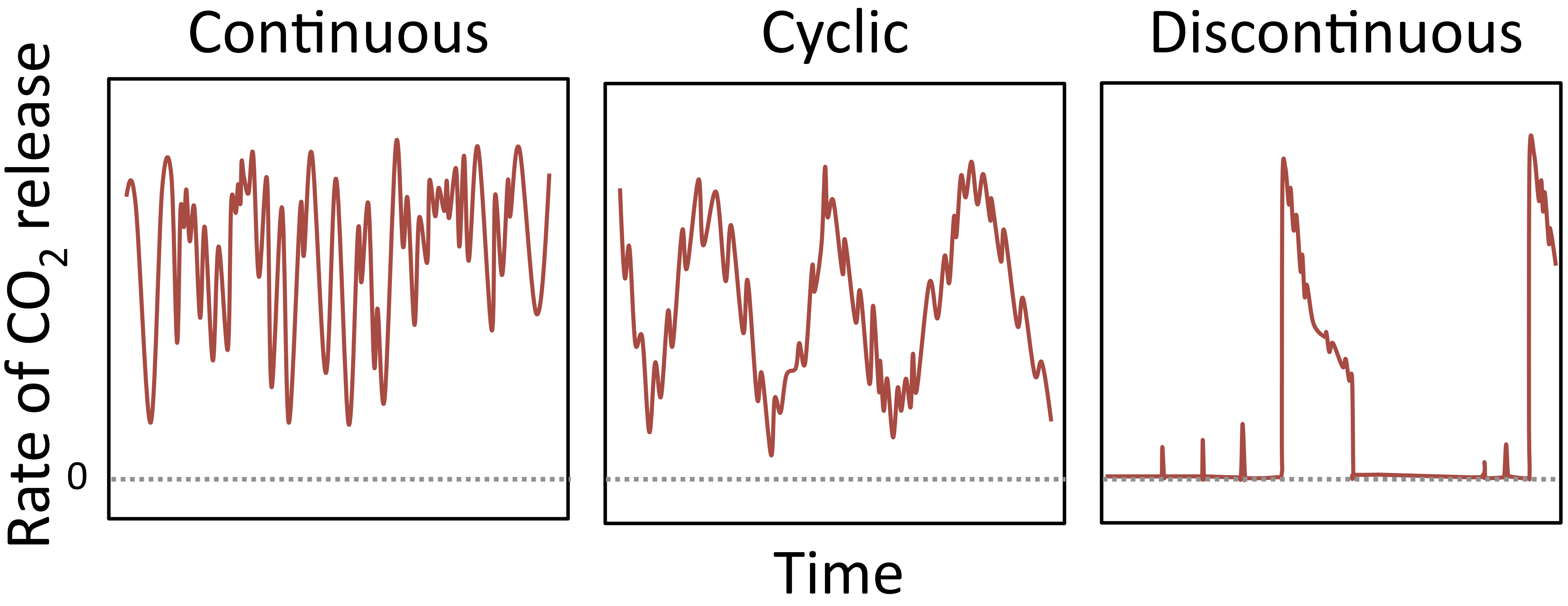
Discontinuous gas exchange

- Outcomes:
  - Be able to identify the different phases of the discontinuous gas exchange cycle (DGC) and describe the changes that occur during each phase
  - Understand the hypotheses which explain the adaptive function of the DGC

In this talk I'll be dealing with patterns of insect respiration, in particular looking at a curious pattern of breathing exhibited by some insects called a discontinuous gas exchange pattern. By the end of this mini lecture you should have a good understanding of what a DGC is, what the various phases of the DGC are, and also what we know, and what we don't know about this gas exchange pattern.

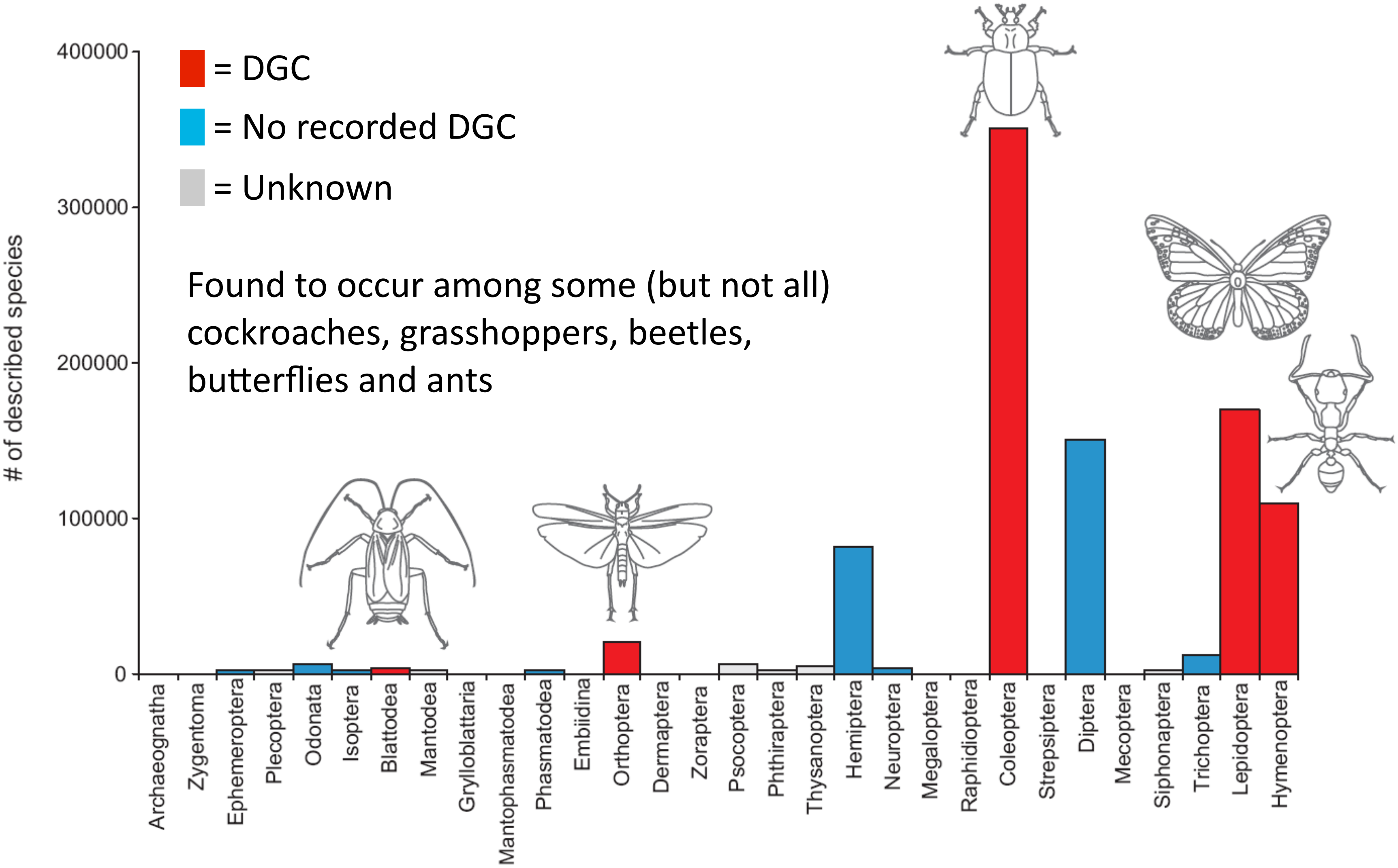
Respiratory gas exchange patterns





So firstly, what do I mean when I talk about a respiratory gas exchange pattern? Since most insects possess spiracles, they have the ability to restric or permit the exchange of oxygen and carbon dioxide between their tracheal system and their environment. So by controlling when they open and close their spiracles they can produce regular patterns of gas exchange. Researchers are able to measure insect gas exchange patterns using a process called flow-through respirometry. This is achieved by placing an insect in a small container (as shown in the diagram above) which is then flushed with carbon dioxide-free air. The air leaving the chamber is then passed through a gas analyser so that changes in carbon dioxide or oxygen can be monitored, and this can be used to determine when and how the insect is breathing. The patterns of gas exchange which have been observed using this method can be very broadly classified into three groups. Continuous respiration, as the name suggests, is the continual release of carbon dioxide and uptake of oxygen. This pattern is used when insects are active and therefore have higher metabolic rates. Some insects are unable to completely close their spiracles, while others lack spiracles entirely, and so these insects also show a continuous pattern of gas exchange regardless of their activity levels. Other insects display a cyclic pattern, where there are clear alternating periods of high and low gas exchange, but again gas exchange never stops completely. Finally there is the discontinuous gas exchange pattern or DGC in which periods of very low or no gas exchange are regularly punctuated by large bursts of carbon dioxide release and oxygen uptake. DGCs are only displayed by insects when they are inactive and their metabolic rates are very low. The non-respiratory periods of the DGC can be very long, with some moth pupa and beetles effectively holding their breath for hours. Performing such an impressive feat as this has lead many researchers to study discontinuous gas exchang cycles in an attempt to understand how and why they occur.

Occurrence of DGCs

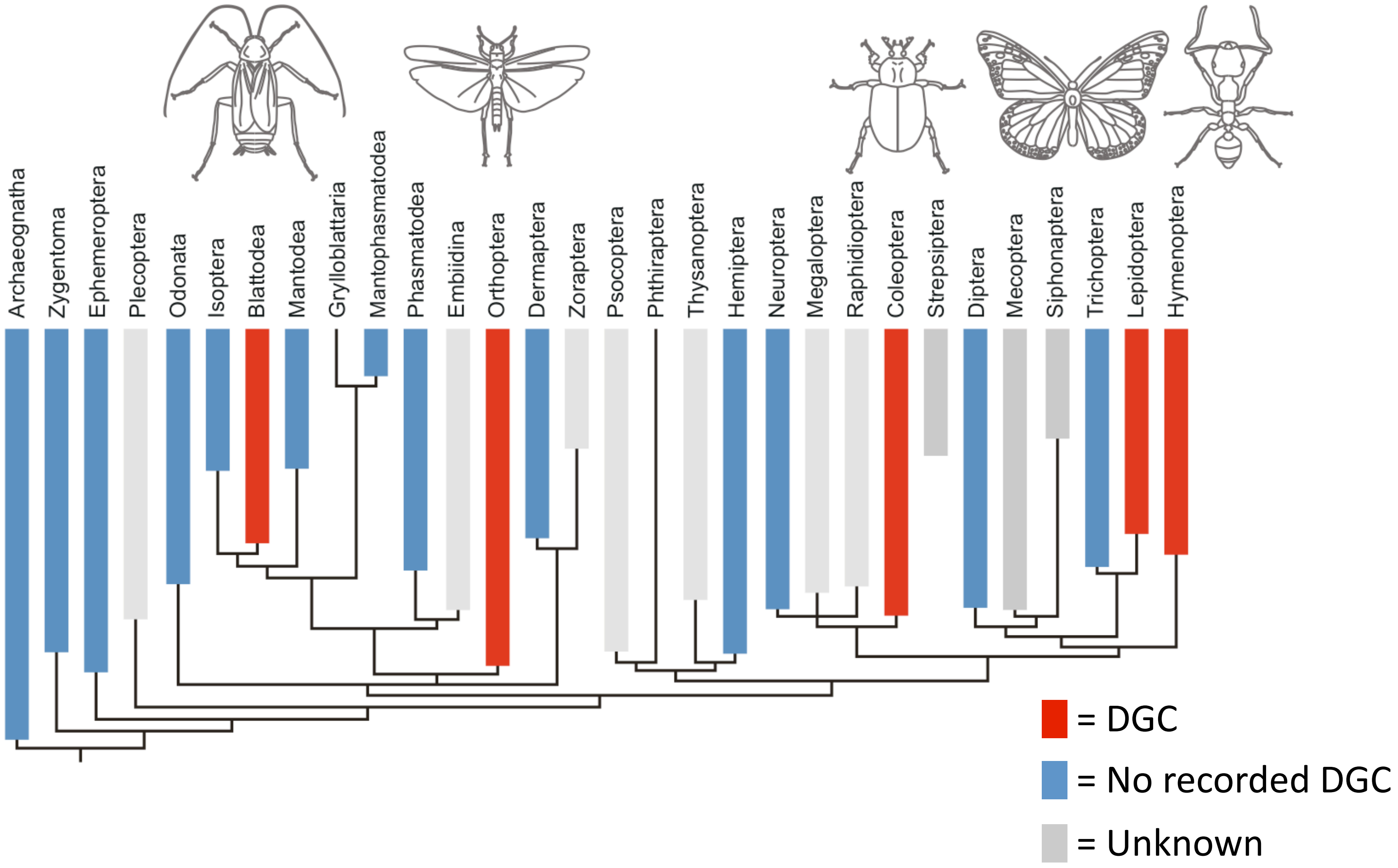


Found to occur among some (but not all) cockroaches, grasshoppers, beetles, butterflies and ants

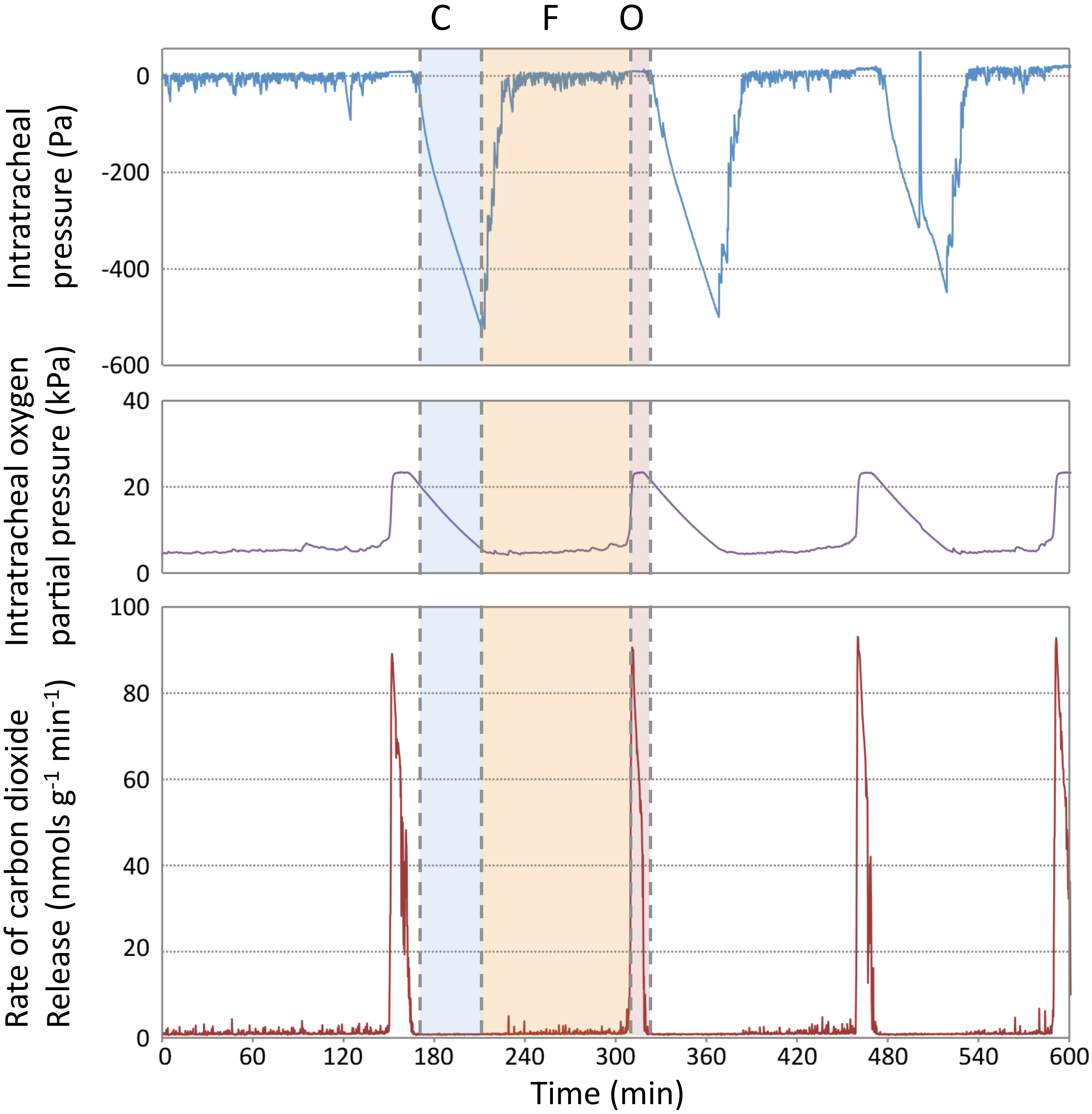
Firstly, not all insect orders have members which breathe using a DGC. But DGCs have been recorded from cockroaches (Blattodea), grasshoppers (Orthoptera), beetles (Coleoptera), butterflies and moths (Lepidoptera) and ants (Hymenoptera). These are the orders marked in red. But other orders don’t seem to have any individuals which perform a true DGC, so flies and bugs, for example haven’t been shown to periodically hold their breath at rest. And the respiratory patterns of many other insects are completely unknown. Looking at this graph, you can see that DGCs occur among a diverse range of insects – it encompasses both hemimetabolus insect and holometabolus orders, as well as incorporating flightless and flying insects

Occurrence of DGCs

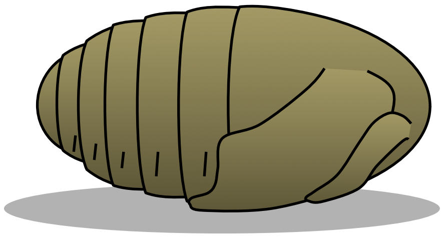
Appears to be polyphyletic in origin (i.e. DGCs evolved independently multiple times)



From an evolutionary perspective it looks like the ability to DGC evolved independently among these different groups – So looking at this evolutionary tree you can see that the insects that use DGCs are not all closely related, so it looks like DGCs didn't just evolve once in a common ancestor and was then passed on to its descendants. Each origin of DGCs appears to be independent, so we say that DGCs are polyphyletic in origin. Because it has evolved at least 5 times, this suggests that breathing periodically must provide some advantage to those individuals who can do it. This would explain why it has occurred among such a diverse range of insects. But before we'll cover what DGC might provide, lets look at what actually occurs during a discontinuous gas exchange cycle

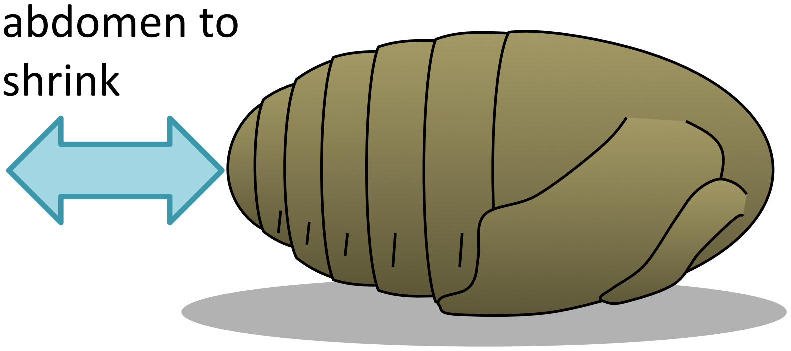


Traces showing changes in intratracheal pressure, oxygen level, and rate of carbon dioxide release during DGCs. Recorded from a moth pupa (*Samia cynthia*)



This graph is showing how rates of CO2 release, intratracheal oxygen level and intratracheal pressure vary during three complete cycles of discontinuous respiration. This data was collected from a moth pupa by implanting a tiny oxygen sensor in to the pupa through a spiracle to measure oxygen inside the trachea, wile another spiracle was intubated and connected to a very sensitive pressure sensor. The moth pupa was then placed in an air tight respirometry chamber so that carbon dioxide release could be recorded. So at the top in blue you can see intratracheal pressure. So this is the air pressure inside the pupa's tracheal system in units of Pascals. Looking at this graph you can see that the pressure within the pupa's tracheae regularly fall below atmospheric pressure. Below this in purple is the level of oxygen in the pupa's tracheal system. The level of oxygen in the atmosphere is around 21 kilopascals, and looking at the oxygen trace you can see it regularly rises to atmospheric levels before falling to a plateau. And the bottom graph in red shows exhaled carbon dioxide leaving the pupa. These large spikes in CO2 release in between long periods of no gas exchange are a key feature of the DGC. So, there are three phases to a DGC, and they are designated according to what the insects spiracles are doing: so there is a closed phase, a flutter phase and an open phase. During the closed phase you can see that the pressure in the tracheal system drops continually, as does the oxygen level. And from the co2 trace you can see that no carbon dioxide is released. In this pupa, there was absolutely no gas exchange for around an hour. Then you can see intratracheal pressure rises rapidly, and this indicates the start of the flutter phase, where the spiracles open very briefly. During the flutter phase you can see that the intratracheal oxygen level stabilizes, while on very small amounts of CO2 are released with each brief opening. Finally there is the open phase, where both intratracheal pressure and oxygen levels return to atmospheric levels, and there is a large burst of CO2. So those are the phases of a classic DGC, but what is happening to cause these changes? I'll go through the phases one at a time.

DGC phases



Reduced tracheal system pressure causes abdomen to shrink

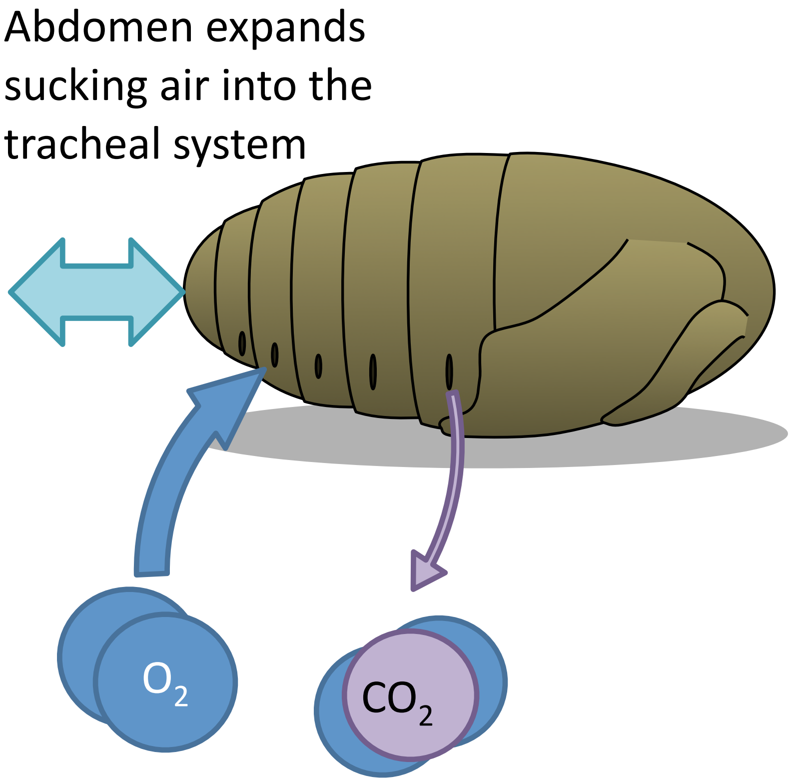
No gas exchange

Closed Phase:

- Spiracles tightly closed
- Intratracheal O_2 levels drop
- Intratracheal CO_2 levels rise but most CO_2 is dissolved in the haemolymph
- Total volume of gas in the tracheal system drops
- Total pressure in the tracheal system drops

So lets start with the closed phase immediately after an open phase. At the beginning of the closed phase the tracheal system contains near atmospheric levels of oxygen, low levels of carbon dioxide and the pressure in the tracheae is also the same as atmospheric pressure. But the spiracles are held tightly shut. So the insect is still respiring aerobically, its tissues are consuming oxygen and producing carbon dioxide. So the oxygen in the tracheal system will diffuse into the tissues where it is respired, and as a by product carbon dioxide is produced. So oxygen is being removed from the tracheal system causing the intratracheal oxygen level to start to drop. You'll also remember that intratracheal pressure begins to drop, also. Why should this be? Well respiration is consuming oxygen from the tracheal system, reducing the total number of oxygen molecules in the tracheal system. If a carbon dioxide molecule was released into the tracheal system for each oxygen molecule consumed the total number of gas molecules in the tracheal system would remain constant and so would the pressure. But carbon dioxide is very soluble in water, so the carbon dioxide being produced by the respiring tissues tends to remain dissolved in the insects body fluids, its haemolymph. This means that more oxygen molecules are removed from the tracheal system that carbon dioxide molecules are returned, slowly reducing the amount of gas in the tracheal system, causing its pressure to drop below atmospheric. As the pressure in the tracheal system drops below that of the atmosphere, the atmospheric pressure actually begins to compress the insects tracheal system, causing the pupa's abdomen to shrink in length!

DGC phases

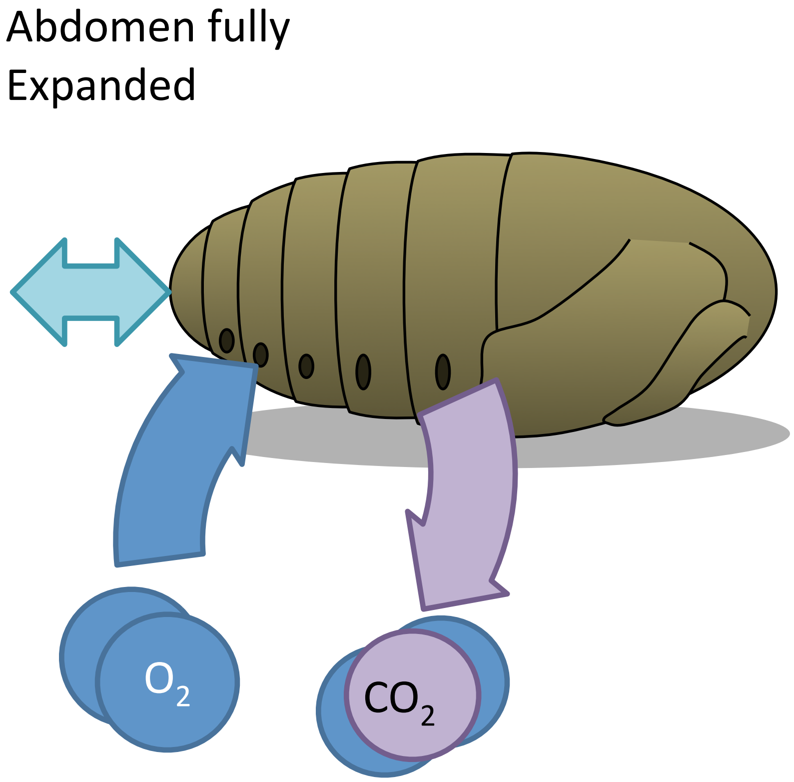


Flutter Phase:

- Low intratracheal O_2 levels trigger the spiracles to flutter open and closed
- Small amounts of O_2 enter tracheal system keeping O_2 levels approx. constant
- Suction prevents CO_2 release. CO_2 still accumulating in haemolymph
- Total pressure in the tracheal system returns to near atmospheric

Eventually the oxygen level in the tracheal system drops to a critically low level and this initiates the start of the flutter phase. So the flutter phase is triggered by hypoxia – low oxygen level. The spiracles respond to hypoxia by rapidly fluttering open and closed. Because the intratracheal pressure has dropped below that of the atmosphere due to co2 dissolving in the haemolymph, when the spiracles do briefly open, air is convectively sucked into the tracheal system. This is called passive suction ventilation. So air containing ambient levels of oxygen is sucked into the tracheal system, and the spiracles open and close often enough to ensure the oxygen level stabilises at this low level, and doesn't drop any lower. The first couple of times the spiracles open following the closed phase, almost no CO2 escapes from the tracheal system to the atmosphere, as the large pressure gradient sucks air in so quickly that carbon dioxide can't diffuse quickly enough in the opposite direction through the convective flow of gas and escape to the atmosphere. But the intratracheal pressure gradually rises back to equilibrium with the atmosphere with each flutter of the spiracles. When the spiracles open in the absence of a subatmospheric intratracheal pressure, small amounts of CO2 then begin to escape.

DGC phases

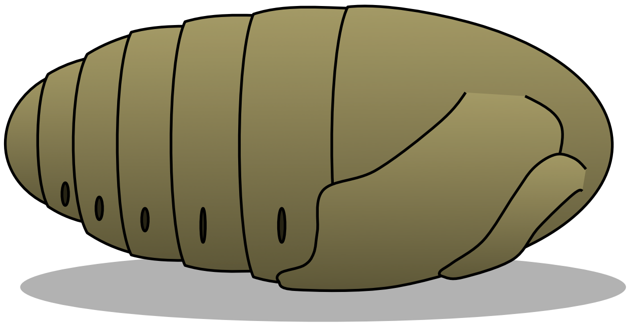


Open Phase:

- CO_2 concentration in the haemolymph reaches a threshold level causing the spiracles to open widely
- Intratracheal O_2 levels rise to atmospheric levels
- CO_2 from haemolymph and tracheal system released in a burst

So during the closed and flutter phase, carbon dioxide hasn't been able to escape to the atmosphere and has been building up in the haemolymph and tracheal system. So finally CO2 builds up to a level, a threshold point which trigger the pupa's spiracle to open widely and stay open. The high levels of co2 diffuse out of the haemolymph into the tracheal system and out to the atmosphere in a big burst. As the spiracles are widely open, oxygen is also free to diffuse in to the tracheal system where it rises to near atmospheric levels once more.

DGC phases



No gas exchange

Closed Phase:

- Spiracles close once CO_2 released
- Cycle begins again

At this point the spiracles shut and the whole cycle begins once more.

Why breathe discontinuously?

Adaptive Hypotheses

Hygric Hypothesis:
Reduce water loss
(Buck *et al* 1953)

Chthonic (underground) Hypotheis:
Enhance gas exchange in low O_2/high CO_2
(Lighton & Berrigan 1995)

Oxidative Damage Hypothesis:
Reduce oxidative damage
(Bradley 2000)

Non-adaptive Hypothesis

Emergent Property Hypothesis:
An emergent property of the system
(Chown & Holter 2000)

So why would an insect want to prevent gas exchange for hours on end, suffer low intratracheal oxygen levels and a build up of carbon dioxide? Several hypotheses have been proposed which try to make sense of this pattern, and most hypotheses presume that breath holding must be beneficial to the insect. So in the scientific literature at the moment there a three hypotheses that try to explain the benefit of a DGC to the insect, these are the adaptive hypotheses, and one hypothesis which considers that DGCs don't necessarily confer any special adaptive advantage, but instead arise from how oxygen and carbon dioxide trigger the spiracles to open and close.

So the first hypothesis proposed to explain DGCs is the hygric hypothesis, and it is based on the fact that when insects respire, they necessarily lose water to the environment. This is because the tracheal system is gas-permeable – oxygen and CO2 can diffuse across the walls of the tracheae – and any membrane which is permeable to gas is also permeable to water vapour. The tracheal system is saturated with water vapour, and any time the insect opens its spiracles, unless the ambient air is completely water saturated, some water will be lost to the environment. As ins insects are small, they can't afford to lose much water, so perhaps by keeping their spiracles shut for as long as possible and restricting gas exchange to short bursts, they can reduce this respiratory water loss. The second hypothesis is the chthonic or underground hypothesis. It is based on the assumption that DTGCs enhance gas exchange in a high carbon dioxide and low oxygen atmosphere, such as might be found in subterranean insect burrows. The logic behind this is that by holding its breath an insect reduces the concentration of oxygen in its tracheal system and increases its co2 concentration. They would increase the diffusion gradient driving oxygen into the tracheal system and the carbon dioxide out, when the insect finally opened its spiracles. Having a long breath hold period between breaths may also give the CO2 released during the last breath time to disperse away from the insect. The last adaptive hypothesis is called the oxidative damage hypothesis. So although animals need oxygen to respire, oxygen is actually toxic. Oxygen can react with chemicals in the cell to produce reactive oxygen species which can damage cell functions. So the oxidative damage hypothesis is based around the idea that by holding its breath insects are in fact limiting the entry of oxygen to protect their tissues from damaging levels of oxidative damage. The flutter phase is the key to this hypothesis as it is during this phase that oxygen is admitted at a slow constant level which could thus keeps the insects tissues protected.

Hygric Hypothesis

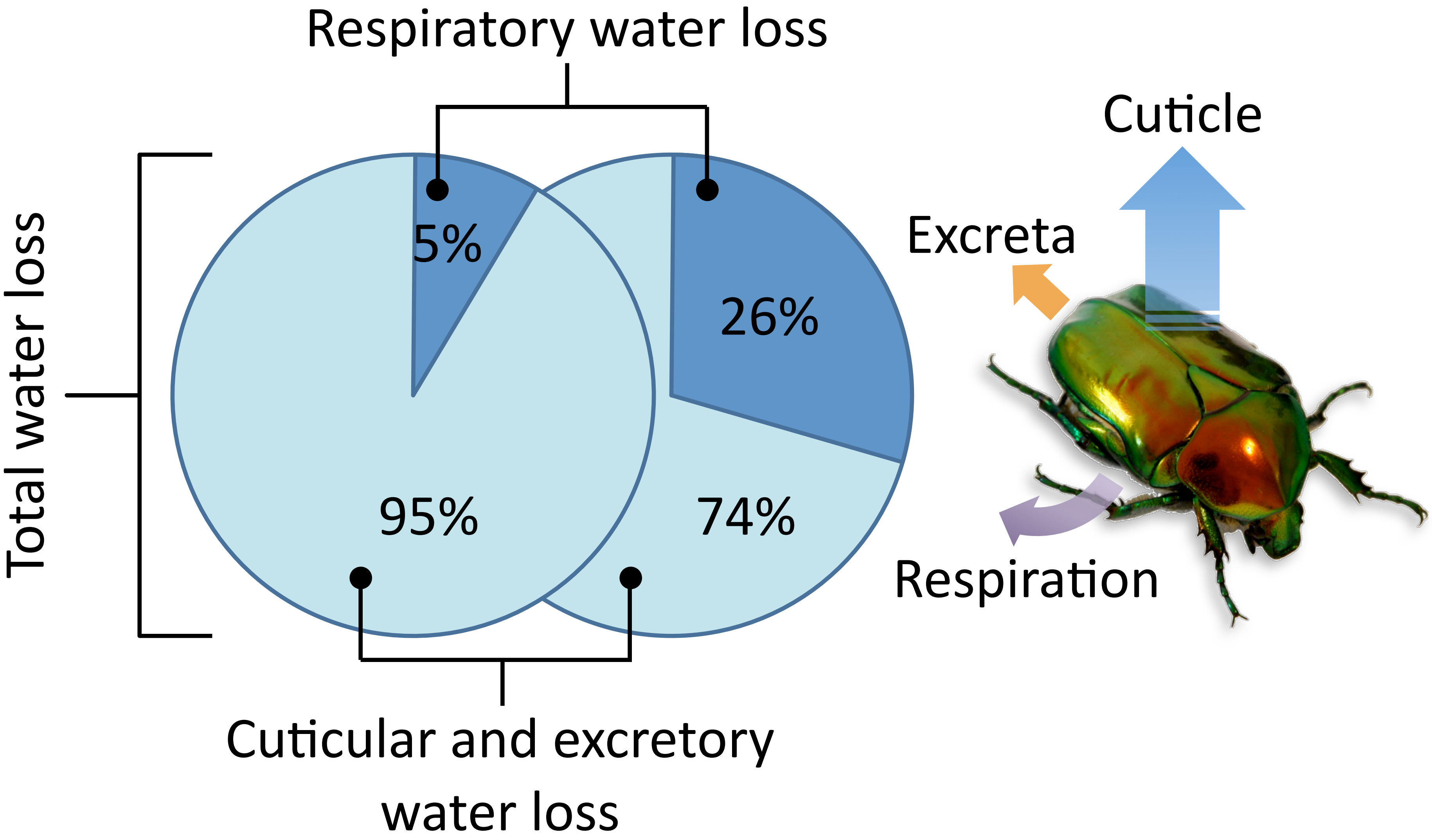
Arid

Mesic

Tropical

Now lets go through these hypotheses and see how they stack up with what we see in nature. So firstly – the hygric hypothesis. Water loss from an insect is likely to be a big problem for insects from arid environments where it can be both hot and extremely dry. Under these conditions it would be expected that all avenues of water loss should be minimised. In more mesic environments where water is more available, temperatures may be milder, so perhaps dehydration isn't such a problem. And finally in tropical environments water is abundant and the air is very humid. So looking at these landscapes, if DGCs were vital for reducing respiratory water loss, you'd expect the tenebrionid from Namibia to DGC, while it would be less likely that the flower beetle or scarabid from the mesic and tropical environments would. But as it turns out, they all breathe using DGCs! And in fact, there are many desert beetles which don't use DGCs. While tenebrionids from African deserts appear to breathe discontinuously at rest, tenebrionids living in American deserts don't!

Hygric Hypothesis



There is also the argument that respiratory water loss may not be an overly important component of an insects water budget, as it has been shown that most insects lose 95% of their water through their cuticle or through their waste. But desert adapted insects with very thick impermeable cuticles lose proportionally more water through respiration, closer to 26% of total water loss, since they have greatly reduced water loss through the cuticular pathway. But a recent study has found that insects from arid environments which are known to DGC do have longer closed and flutter phases when they DGCs than insects from more mesic environments. As the closed and flutter phases of the DGC minimise water loss by keeping the spiracles closed or mostly constricted, this may be evidence for the DGC playing a role to reduce respiratory water loss. A recent study on the pupae of a Canadian moth looked at rates of water loss form the pupae when they were breathing continuously and when they switched to a DGC. This study showed that, consistant with the hygric hypothesis, the pupae lost less water if they breathed discontinuously than continuously.

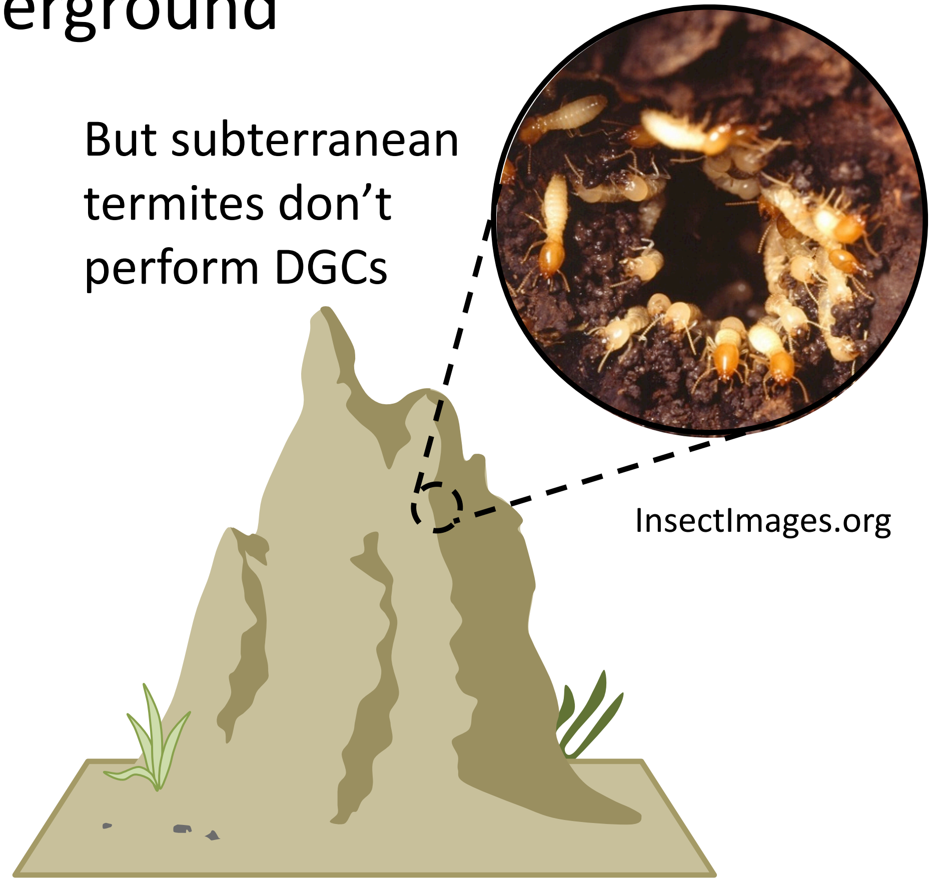
Chthonic (Underground) Hypothesis

- Both ants and termites spend a significant portion of their lives underground

Some (but not all) ants do perform DGCs

Camponotus sp. InsectImages.org

But subterranean termites don’t perform DGCs



InsectImages.org

What about evidence for the chthonic hypothesis? This hypothesis assumes that the DGC is beneficial when the insect is in a high carbon dioxide or low oxygen atmosphere and so needs to hold its breath in order to make a steeper gradient to drive oxygen into its tracheal system and CO2 out. Some of the evidence presented to support this viewpoint is that many ants perform DGCs, with ant queens, which spend their entire lives underground, known to breathe using a DGC. It as also been shown that if an ant is exposed to increasing levels of hypoxia, so if the oxygen level is steadily dropped, it will begin to extend the length of its flutter phase. Since the intratracheal oxygen level is lowest during the flutter, this would provide the steepest concentration gradient to drive the diffusion of oxygen into their tracheal system. It is also important to note that ants don’t seem to use convection to ventilate their tracheal system –they rely more on diffusion. So this is compelling evidence in support of this hypothesis – at least in ants. However, there are many insects which spend their entire lives underground which don’t DGC, termites being just one example. Also many worker ants don’t display a DGC while they are at rest, even though they often live below ground. There is also a problem with knowing what the oxygen and carbon dioxide levels are like underground. There aren’t many measurements. But it has been shown that in cow pats, CO2 levels can get very high and oxygen very low. It would seem then that if a DGC was useful under these conditions dung beetles would be using this repsiratory pattern. And while dung beetles do use DGCs while at rest, they abandon this pattern of respiration when exposed to high CO2!

Chthonic (Underground) Hypothesis

- Speckled feeder roaches show a DGC – but don't live underground
- Giant burrowing cockroaches live underground – but don't display a DGC!

Speckled feeder roach (*Nauphoeta cinerea*)

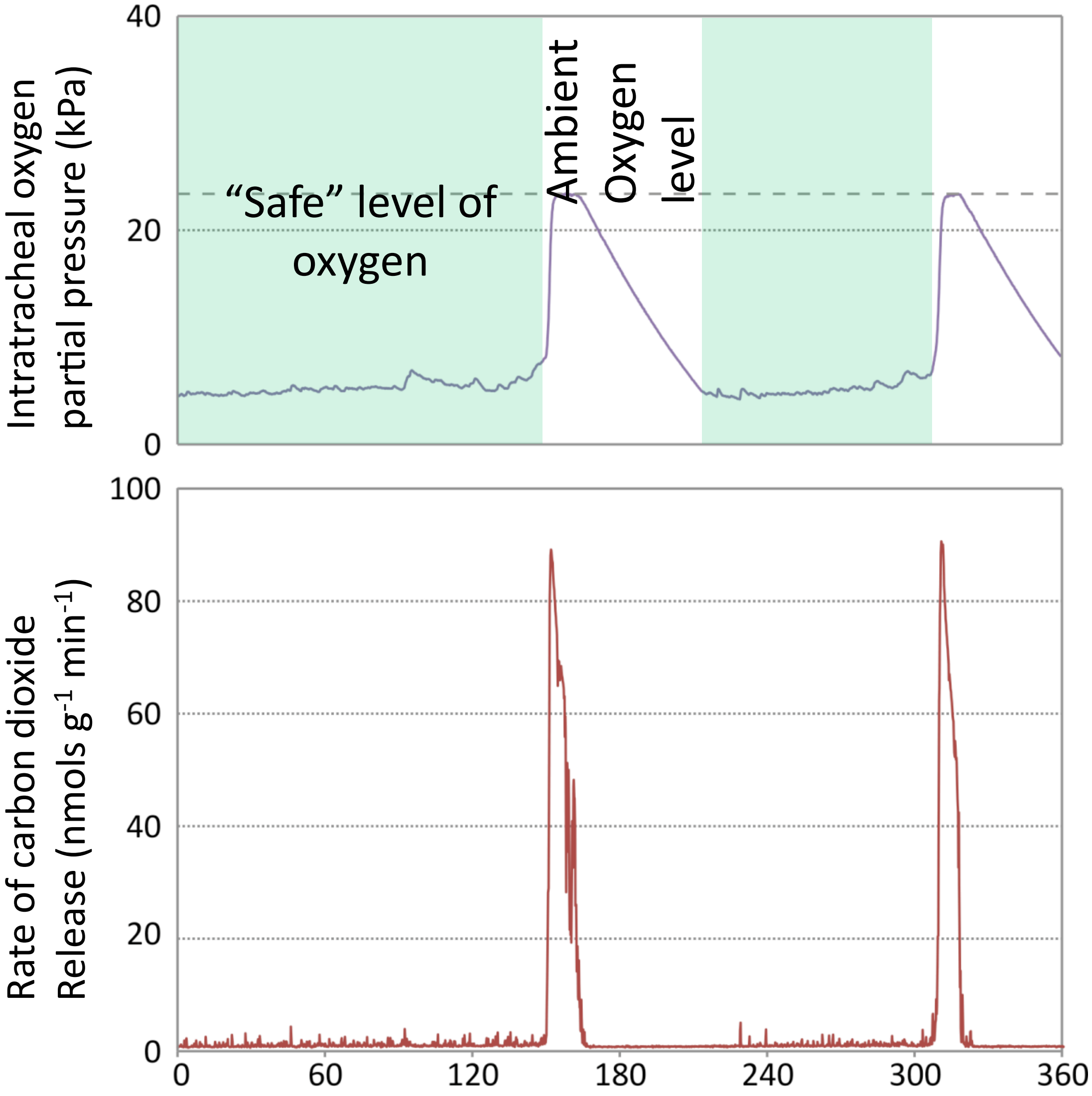
Giant burrowing roach (*Macropanesthia rhinoceros*)
The Daily Telegraph

Another example where the chthonic hypothesis doesn't hold up is found in cockroaches. The speckeld feeder roach, which is a species I work with, will quite happily breathe discontinuously while it is resting. But its relative the giant burrowing roach, which spends most of its time in a subterranean burrow, breathes cyclically while at rest, and never has periods of breath holding.

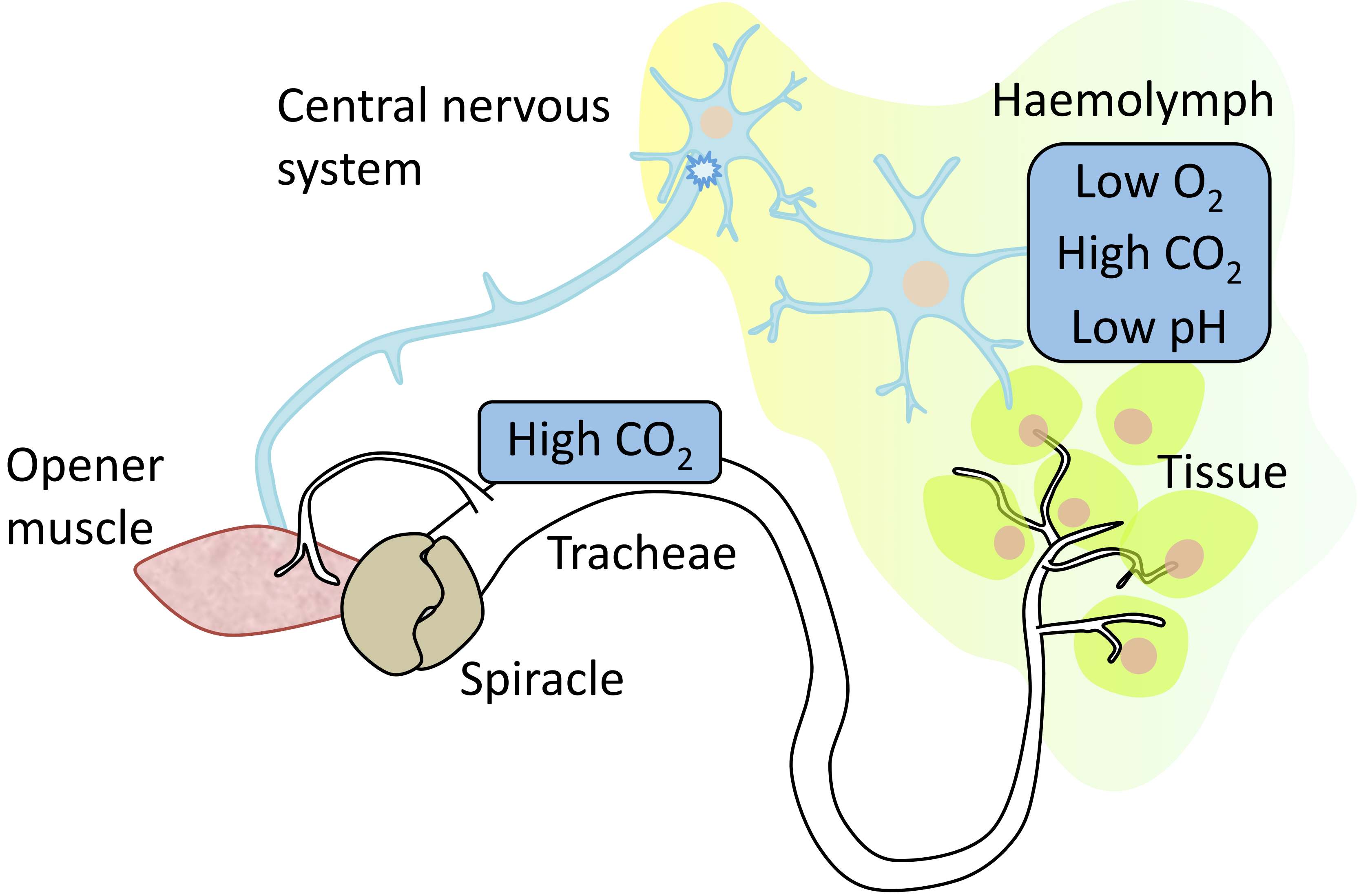
Oxidative Damage Hypothesis

Oxygen is toxic: it can form reactive oxygen species (ROS) which damage cells

Moth pupa regulate a low and constant oxygen level during the flutter phase, potentially protecting against excess ROS



Emergent Property Hypothesis



DGC summary

- DGCs have evolved among a wide range of insects from many different habitats
- DGCs typically have three phases: Closed, Flutter and Open
- Several adaptive hypotheses proposed to explain the function of DGCs – all with equivocal support
- DGCs may be the result of a carbon dioxide and oxygen control system oscillating in the absence of high metabolic demand