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Insect physiology: The mouthparts of moths and butterflies breathe through strategically positioned micropores

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Insects employ a tracheal system to transport oxygen and carbon dioxide to and from the body's cells. A new study discovers a micropore-based mechanism of respiration in the coiling mouthparts of moths and butterflies, which allowed these insects to evolve intricately long mouthparts without also evolving proportionally larger body sizes.

To exchange respiratory gases, insects employ an innovative tracheal system where chitinous tubes that originate in the exoskeleton at the interface with the environment deliver gases to and from every cell in the body[1]. The evolution of the tracheal system allowed insects to colonize land, but this system has limitations. One constraint is that, while the diffusion of gases over short distances is fast, diffusion slows down tremendously as the distance increases. This problematic distance effect is accentuated in long appendages that are far from where the tracheae meet the outside of the body. In this issue of *Current Biology*, Jiang *et al*.[2] make several discoveries that explain how gases are delivered to and from the cells inside the long proboscis, or mouth, of myoglossatan moths and butterflies. Specifically, micropores strategically positioned along the proboscis allow gases to diffuse into and out of superhydrophobic trachea, thereby moving metabolic gases within these vital appendages. This micropore-based mechanism for gas exchange likely allowed moths to evolve intricately long mouthparts without having to evolve a proportionally larger body size.

Mammals utilize an efficient but energetically costly lung-based system that moves gases into and out of the blood, with oxygen transported by the circulatory system as it is bound to hemoglobin within erythrocytes. In a conceptually similar manner, many of the crustaceans, which are the ancestors of insects, utilize a gill-based system that moves gases into and out of hemolymph, with oxygen transported by the circulatory system as it is bound to hemocyanin[3]. In contrast, insects have mostly dispensed with respiratory mechanisms that rely on dedicated cells or molecules to transport oxygen to all cells in the body via a fluid medium, although some ancestral insects still use hemocyanin, and most insects produce hemoglobin that functions intracellularly[1,4]. Instead, insects exchange gases using a series of tubes, called tracheae, that originate in the thoracic and abdominal exoskeleton. These tracheae open to the outside environment, and they divide into thinner tracheoles as they extend into the body. Tracheoles deliver oxygen and remove carbon dioxide — both still in their gas phase — to the insect's cells.

The tracheal system of insects is a bidirectional conduit that requires relatively little energy to operate. Gases move passively via diffusion and actively via convection[5,6]. Diffusion moves gases from areas of higher partial pressure to areas of lower partial pressure, or down their concentration gradient. Because oxygen moves faster in air than in water, its diffusive movement via the tracheal system is higher than it would be if it were dissolved in hemolymph. Convection moves gases down a pressure gradient, which in insects is driven in several ways. The contraction of muscle attached to the body wall decreases the volume of the body cavity, or hemocoel, compressing the trachea and air sacs, thereby expiring air. Conversely, the relaxation of these muscles increases body volume and allows the trachea and air sacs to return to their larger natural shape, thereby inspiring air. In some insects, this process can be seen with the naked eye as abdominal pumping[7]. Contraction of the dorsal vessel, or heart, also changes hemolymph pressure in different regions of the body in a manner that facilitates tracheal expiration and inspiration[8].

But the insect body is three-dimensional, and the appendages pose a conundrum for gas exchange. How is air moved bidirectionally through thin tracheae that extend across the appendages? Appendages are often thin and long, are far from where the trachea interface with the outside environment, and are devoid of muscles attached to the body wall that can drive convection. In the antennae and wings, the rhythmic pumping of the auxiliary hearts or accessory pulsatile organs that deliver hemolymph into these appendages facilitates convection[8,9]. In the legs, gas movement is presumably facilitated either by accessory pulsatile organs or the flexing of muscles during locomotion. Whether these mechanisms are sufficient to fully explain gas exchange in these appendages remains to be determined, but what has been certainly unclear is how air is moved into the long proboscis of some insects. Delivering gases to the cells in the proboscis is important because this appendage is essential for feeding as well as sensory processes such as mechanoreception, gustation and olfaction[10,11].

Figure 1. Illustration of the adult and larva of *Manduca sexta*.
The adult, known as the Carolina sphinx moth and the tobacco hawkmoth, has a proboscis that is longer than the rest of its body. The larva is an agricultural pest known as the tobacco hornworm. Illustration by John Curtis[17].

Lepidopteran insects, which include the moths and butterflies, have a proboscis that ranges from less than 0.1 cm to greater than 28 cm. This largest proboscis belongs to *Xanthopan praedicta*, or the Darwin's sphinx moth from Madagascar, which has a forewing that is ~7 cm long (and a body sans proboscis that is ~6 cm long) yet feeds on the orchid, *Angraecum sesquipedale*, which has a spur that can be deeper than 27 cm[12]. A more common sphinx moth, the Carolina sphinx moth, has a ~9.5 cm proboscis that is also significantly longer than the rest of its body[13] (Figure 1). Jiang and colleagues[2] examined the proboscis of the Carolina sphinx moth, *Manduca sexta*, and uncovered that it contains rows of micropores along its length. After applying droplets of water to this proboscis, they discovered that these micropores are directly connected to the internal trachea. This is a critical finding because it demonstrates that environmental gases delivered to the proboscis do not need to enter the trachea where it originates in the thorax. Instead, air can also diffuse into the trachea through micropores on the proboscis surface. But importantly, these micropores are major contributors for gas exchange — diffusive exchange through the micropores is higher than through the length of the trachea. Even when considering that *M. sexta* can coil and uncoil its proboscis[14], the diffusive exchange through micropores is an order of magnitude greater than the convecting exchange from coiling and uncoiling. Altogether, these discoveries change our view of how gases are exchanged within the proboscis of this insect. That is, these previously unknown micropores are a greater contributor to gas exchange than the major trachea that enters the appendage.

The ability to coil and uncoil the proboscis is a trait of the lepidopteran clade Myoglossata[14]. Jiang *et al.*[2] investigated whether micropores are unique to the Carolina sphinx moth. They found micropores in the proboscis of species in all eight families within the myoglossatan lineage that they examined. This included the painted lady, *Vanessa cardui*, which has a much shorter proboscis that is ∼1.3 cm long. However, micropores were not detected in the proboscis of the Chinquapin leaf-miner (a non-myoglossatan moth) or in the antennae of the Chinese praying mantis (a member of the order Mantodea, and not a lepidopteran). In the insects that have micropores, these structures are not randomly scattered throughout the proboscis. They are ordered and with higher densities toward the base of the proboscis, which is where the intrinsic musculature that drives coiling and uncoiling is located, and presumably where metabolic gases are most needed. Interestingly, the diameter of the micropores is similar in all species, and this diameter appears to have physical properties that optimize both oxygen intake and carbon dioxide exit. These findings suggest that micropores evolved concurrently with the evolution of the intrinsic proboscis musculature that is characteristic of the myoglossatan lineage, and as a result of these micropores, the proboscis of many members of this clade evolved to be longer without an accompanying larger body size with larger diameter trachea.

The discovery of this previously unknown mode of gas exchange is another indication of how underexplored the field of respiration in the class Insecta is. It also opens additional questions. For example, fruit flies ventilate their trachea by pulsating their proboscis[15] — do lepidopterans, and more specifically myoglossatans, also pulsate their proboscis to enhance gas exchange through the micropores? Likewise, could micropores have also evolved in other insect orders? Probosces that are far longer than body length have evolved multiple times in the insect lineage, occurring in bees (Hymenoptera), flies (Diptera), and other families of moths and butterflies (Lepidoptera)[13]. How respiratory gases are efficiently exchanged in the proboscis of these insects is not well understood. Likewise, many insects have antennae that are thin and longer than the rest of the body, or have a complex architecture[16]. Whether gas exchange in these antennae is facilitated by means other than convection actuated by the auxiliary hearts remains to be determined.

The study by Jiang and colleagues[2] adds another critical piece to the puzzle of how insects exchange gases with their environment. In a large clade of lepidopterans, the investigators uncovered an adaptation that facilitates gas exchange in a physiologically critical appendage. This study highlights the structural and physical complexity of gas exchange in insects, and opens new questions into one of the physiological systems of insects that is least understood.

DECLARATION OF INTERESTS

The author declares no competing interests.

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