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COMMENTARY

CENTENARY ARTICLE

Air sacs are a key adaptive trait of the insect respiratory system

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ABSTRACT

Air sacs are a well-known aspect of insect tracheal systems, but have received little research attention. In this Commentary, we suggest that the study of the distribution and function of air sacs in tracheate arthropods can provide insights of broad significance. We provide preliminary phylogenetic evidence that the developmental pathways for creation of air sacs are broadly conserved throughout the arthropods, and that possession of air sacs is strongly associated with a few traits, including the capacity for powerful flight, large body or appendage size and buoyancy control. We also discuss how tracheal compression can serve as an additional mechanism for achieving advection in tracheal systems. Together, these patterns suggest that the possession of air sacs has both benefits and costs that remain poorly understood. New technologies for visualization and functional analysis of tracheal systems provide exciting approaches for investigations that will be of broad significance for understanding invertebrate evolution.

KEY WORDS: Tracheal system, Insect, Flight, Ventilation, Evolution

Introduction

Air sacs in insects can be defined as enlarged, irregularly shaped structures connected to and developmentally derived from the tracheal system (Fig. 1). Their large size and reduced (or absent) taenidia (see Glossary) enable them to compress more easily in response to a pressure gradient than most tracheae. In a half-page 1963 article in Nature (Wigglesworth, 1963), V. B. Wigglesworth outlined the function of air sacs in the insect tracheal system. Paraphrased into modern scientific language, these include: (1) aiding flight by lowering the density of the insect body (akin to the role of air sacs in the bones of birds); (2) aiding flight by reducing mechanical damping of the wings; (3) increasing advection (see Glossary) in the tracheal system, enabling high rates of gas exchange; (4) displacing hemolymph in the body, improving circulatory delivery of nutrients; (5) allowing for changes in dimensions of other organs associated with growth, consumption or reproduction; and (6) contributing to buoyancy regulation in aquatic insects. The most up-to-date textbook of insect physiology (Nation, 2022) lists most of these same functions in a single short paragraph on the respiratory system, adding that air sacs can also increase the freedom of movement of sound-producing tympanic membranes (Yager and Spangler, 1995). The theme of this article is that the function of air sacs, and variation in their form and occurrence across insects, is a key innovative trait – likely to be under significant selection – that is critical to many aspects of insect physiology and life history. We begin by more precisely defining air sacs and describing what is known about their development. We then discuss: (1) the association between air sacs and flight across insect orders; (2) compressible tracheae as an alternative or additional mechanism to generate advection in tracheal systems; (3) likely effects of the possession of air sacs on other aspects of respiratory function; (4) other evolutionary innovations associated with air sacs; and (5) how air sacs are linked to life history in aquatic insects.

Air sacs: definition and development

Air sacs are prominent structures in many insects (Fig. 1), and are highly variable in occurrence across species, age and environments. Air sacs in insect tracheal systems have been documented since at least since 1737 (Swammerdam, 1737), and have been classified into three types: (1) taenidial sacs, in which the general shape of the trachea is maintained but dilated, and regular taenidia are present but widely spaced; (2) reticulate sacs, with criss-crossing taenidia and irregular shapes; and (3) punctate sacs, with irregular shape and lacking taenidia (Faucheux and Sellier, 1971; Faucheux, 1972). For the purpose of this Commentary, because most relevant literature has not determined the presence or pattern of taenidia, we will discuss two types based on shape: (1) trachea-like sacs, which are structures that maintain the cylindrical tracheal shape but are often enlarged and slightly misshapen (likely to be equivalent to the 'taenidial sacs' of Faucheux, 1972), and (2) air sacs, which are irregularly shaped, easily compressible structures (likely to be equivalent to combining the reticulate and punctate sacs of Faucheux, 1972). Like many biological parameters, tracheal and air sac classifications occupy a continuum from ringed tracheae to smooth air sacs, requiring future quantitative analysis. Air sacs are thin walled and may exchange some gases with the hemolymph, but most gas exchange is thought to occur in the tracheoles because of their large surface-to-volume ratio. Air sac compression is caused by various indirect muscle-driven mechanisms, most commonly by reducing the volume of the local hemocoel compartment.

Air sac development has been best studied in *Drosophila*. The air sacs associated with *Drosophila* flight muscles are formed from distinct stem cells (tracheoblasts) that migrate to sites on the transverse tracheae of the second body segment during the third larval instar; these tracheoblasts form epithelial cells that morph into a primordial air sac that expands within the thorax and around the developing flight muscles in the pupae (Guha and Kornberg, 2005; Sato and Kornberg, 2002). The air sac cells express fibroblast growth factor (FGF) receptors and grow toward tissues expressing FGF, as has been shown for other parts of the tracheal system (Hayashi and Kondo, 2018; Sato and Kornberg, 2002). Air sacs grow from an existing tracheal branch as an enlarging epithelial sheet that forms an apical lumen, mostly due to an increase in cell number controlled by signaling of epidermal growth factor (Cabernard and Affolter, 2005).

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Glossary

Advection
Pressure-driven bulk flow of fluid (air within the tracheal system).

Discontinuous gas exchange
A three-phase pattern of gas exchange often observed in adult and diapausing pupal insects. The three phases are: closed (spiracles are closed, no gas exchange occurs); flutter (spiracles are closed except for brief periods of spiracular opening during which oxygen uptake occurs and variable amounts of carbon dioxide and water vapor are emitted); and open (spiracles are open and gas exchange rates are high).

Hemocoel
Body compartment through which hemolymph flows.

Primary, secondary and tertiary tracheae
Primary tracheae connect directly to spiracles; secondary tracheae branch from primary tracheae and tertiary tracheae branch from secondary tracheae.

Resilin
Exceptionally resilient cuticular protein.

Spiracle
Opening of the tracheal system through the body wall, usually gated.

Taenidia
Ring-like thickenings of tracheal wall thought to reduce compressibility.

Air sacs are associated with phylogeny and strong flight

Air sacs have not been reported within the basal, apterygote orders of Hexapoda (Fig. 2; Table S1). Outside Hexapoda, within other tracheate arthropods, including Onychophora, Myriapoda and Chelicerata, air sacs have only been reported in the chelicerae of two relatively large camel spiders (Chelicerata: Solfugidae; Franz-Guess et al., 2016). Within the basal winged insects, Paleoptera, we have found no reports of air sacs other than air-filled alimentary canals (Herhold et al., 2023) in adult Ephemeroptera (mayflies), which are generally weak fliers (Dittrich and Wipfler, 2021; Wisely, 1965); note, however, that air sacs have been observed in the head of a burrowing larval ephemopteran (Landa, 1948). By contrast, air sacs are widespread in Odonata (dragonflies), which are considered as strong fliers. Within more derived winged insects (Neoptera), air sacs occur mostly in adults of orders in which many species have the capacity for strong flight (Fig. 2; Table S1). The fact that air sacs have been observed in a chelicerate and in a single larval ephemeropteran suggests that the genes that enable air sac development are widespread and perhaps ancient in tracheated arthropods. The occurrence of air sacs across the hexapod orders is closely associated with strong flight (Fig. 1; chi-square test, χ^2_3=25.3, P<0.001), suggesting that air sacs only develop and are utilized in response to specific selection pressures. However, this analysis is weakened by challenges in classifying flight (e.g. are adult ephemeropterans weak or strong fliers?), and the possibility of previously unidentified air sacs in poorly examined species. The occurrence of air sacs is also associated with the general capacity for flight across hexapod orders, but more weakly (chi-square test, χ^2_3=8.2, P=0.042).

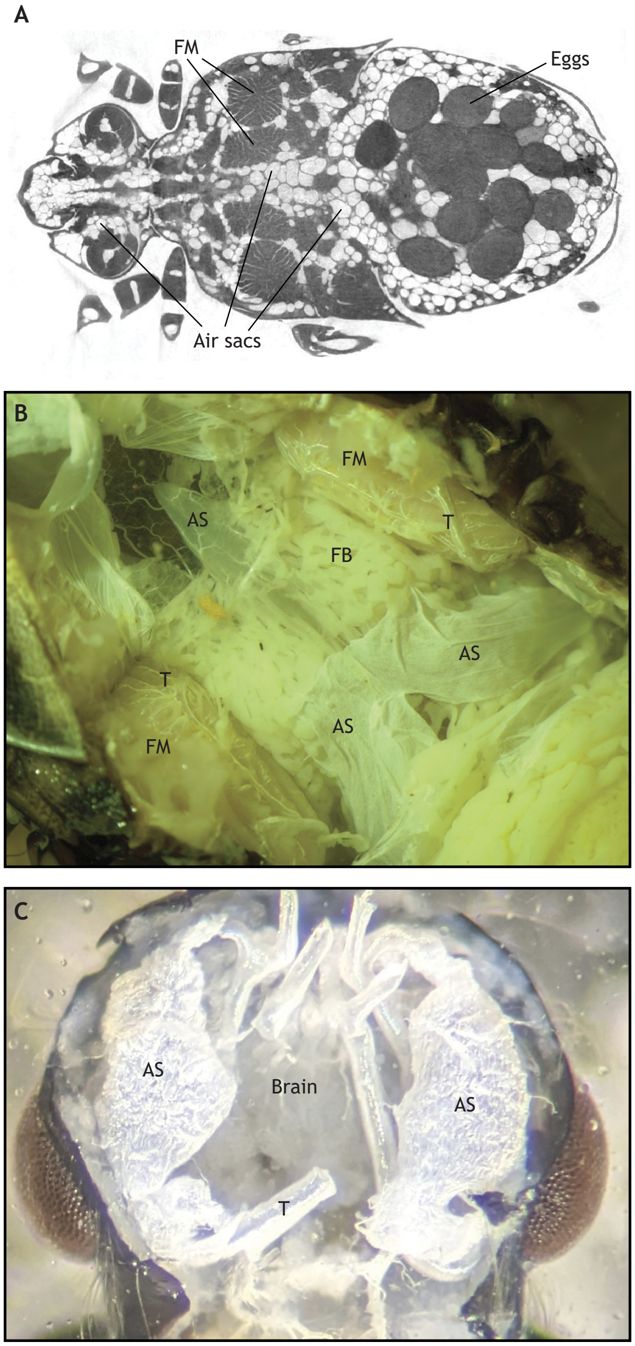

Fig. 1. Images of air sacs. (A) Microcomputed tomography (microCT) of a scarab beetle (*Dicronorrhina derbyana*). (B) Dissected thorax of a locust (*Locusta migratoria*). (C) Head of a male ant (*Veromessor pergandei*). AS, air sacs; FM, flight muscle; T, trachea; FB, fat body.

The link between possession of air sacs and the capacity for strong flight can also be seen within orders, families and even species. Coleoptera (beetles), adult Scarabaeidae and Buprestidae are well supplied with air sacs and are generally capable of strong flight (Dittrich and Wipfler, 2021; Taylor et al., 2010), whereas adult Tenebrionidae and Carabidae are usually non-flying or poor fliers and lack air sacs. However, air sacs occur in the thorax and abdomen of a tiger beetle, which are rare strong fliers in the family Carabidae, emphasizing the link between flight and air sacs (Yager and Spangler, 1995). Within Blattodea, we used synchrotron X-ray imaging (Socha et al., 2007) to visualize the tracheal system of 11 cockroach species from diverse clades, and found that only one species, *Panchlora nivea*, had air sacs, and it is the only one reported to be a strong flier (Table S1). In most holometabolous species in which flying adults have air sacs, larval non-flying forms lack them. Together, these across- and within-order data suggest that possession of air sacs is a key trait that is beneficial to strong flight in insects – possibly through aiding a high delivery of oxygen to flight muscles (an idea which is discussed further below) – but also that air sacs may be disadvantageous for non-fliers.

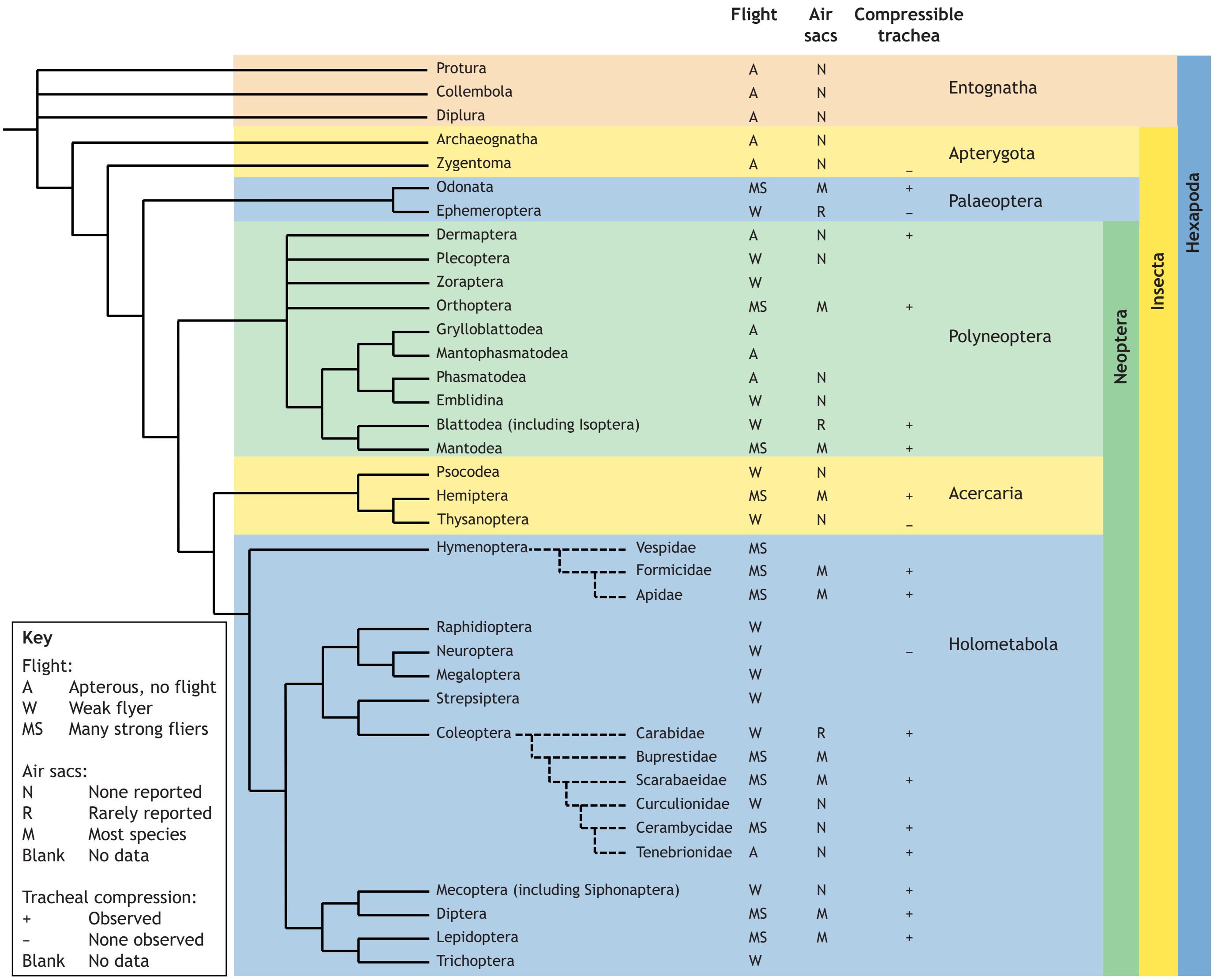

Fig. 2. Phylogenetic patterns of air sacs and compressible tracheal systems among adult Hexapoda. Air sacs are associated with strong flight capacity, though we lack data for many groups. Compression of tracheal structures is widespread, occurring in many insects examined. This phylogenetic tree of Hexapoda was modified according to Misof et al. (2014) and Tihelka et al. (2021).

It is important to note that diversity may be the most important hallmark of insects, and alternative mechanisms of achieving high oxygen delivery and flight capacity occur within some clades that lack air sacs. For example, large Cerambycidae (Coleoptera) such as *Petrognatha gigas* lack thoracic air sacs but achieve high levels of gas exchange during flight by using a form of 'ram ventilation', made possible by giant forward-facing spiracles (see Glossary) and huge thoracic primary tracheae (Amos and Miller, 1965; Miller, 1966). It is likely that other insect species display similar innovations, given the diversity of insect forms.

Although these data support an evolutionary link between flight and air sacs, many questions remain that cannot be answered with our dataset, partly because very few species have been assessed in many orders. For example, it is unclear whether air sacs evolved multiple times within Insecta, or evolved early, in ancestors of the Odonata, and then were lost in insect clades in which they do not occur. The observation that many species within an order are similar in having or lacking air sacs suggests that ancestral species in these orders evolved air sacs, enabling later radiations of descendant species able to occupy niches that require strong flight, such as acting as pollinators or being capable of long-distance, powered migration. The observation that some flightless Scarabidae (Passalidae, bessbugs), as well as worker ants, possess extensive air sacs (Waters et al., 2013) also supports the hypothesis that possession of air sacs is associated with clade and inheritance, rather than always being an adaptation to strong flight.

The mechanisms responsible for the association between air sacs and flight remain unclear and little tested. A nearly 100 year old idea is that air sacs enable high ventilation rates that are essential for flight (Lee, 1929). In support of this hypothesis, tracheal volume is positively correlated with flight capacity in grasshoppers (Huang et al., 2015). Air sacs may also enhance flight metabolic power by providing thermal insulation, aiding endothermy (Church, 1960). However, alternative hypotheses revolve around the idea that air sacs reduce the cost of flight. As noted by Wigglesworth (1963), air sacs within the thorax may reduce the cost of flight because air would provide less mechanical damping than tissue within the thorax. Air sacs have also been hypothesized to reduce the cost of flight by reducing insect body density (Newport, 1851; Wigglesworth, 1963). The possession of air sacs will reduce flight costs if air sacs are replacing weighty tissue or hemolymph, because added weight requires added power; this makes sense when air sacs fill a structure such as a horn, which has been observed in many scarab beetles. However, if air sacs increase volume rather than displacing mass, this will not reduce the induced power requirements of hovering, and may even increase drag during forward flight. Thus, experimental and comparative tests are required to determine whether the primary evolutionary advantage of air sacs for flight is enhancement of aerobic capacity or reduction of flight costs.

The converse question is also interesting: what are the disadvantages of having air sacs that result in their absence in many clades? The most obvious answer is that, at a given volume of exoskeleton, possession of air sacs reduces the internal space for water and nutrient storage. Selection for desiccation tolerance increases the water content of insects, which would likely reduce tracheal and air sac volume (Bradley et al., 1999; Talal et al., 2016). This disadvantage should become increasingly important as insect body size decreases and surface-to-volume ratio increases, as a result of increasing mass-specific water loss rates and metabolic rates. The compressibility of air sacs could also be a disadvantage if spiracles open during locomotion of insects with hydrostatic skeletons, such as many larval Holometabola.

Compressible tracheae as alternative and additional mechanisms for generating advection

Historically, many entomologists considered tracheae with taenidia to be incompressible, and therefore thought that air sacs were necessary for insects to transport gases by advection (Lee, 1929). However, for many years there has also been evidence that insects can ventilate by advection without the involvement of air sacs (reviewed by Sláma and Santiago-Blay, 2017). Many insects that have been examined with synchrotron X-ray imaging exhibit tracheal compression (Fig. 2; Table S1). Pressure pulses, often generated by abdominal pumping, cause regular compression of the tracheal system. This compression may occur locally in the head, thorax or legs, or may occur in the tracheal system more widely (Westneat et al., 2003). For at least one carabid beetle, each pressure pulse increases spiracular gas exchange by about 20% (Socha et al., 2008). The compressibility of tracheae can be associated with tracheal shape; for example, the compressible tracheae of fleas are oval in shape (Herford, 1938), with those of beetles modeled as a collapsing elliptical cylinder (Westneat et al., 2003). However, even tracheae that are round in cross-section (Socha and DeCarlo, 2008) can be compressed (Hochgraf et al., 2018), given sufficient pressure (Adjerid, 2019).

Although the evidence is far from conclusive, data suggest that compression of the tracheal system evolved early in the evolution of insects and is likely to occur in most Neoptera. Only one apterygotan (Zygentoma) has been observed using synchrotron X-ray imaging; these insects only survived briefly, but tracheal compressions were not observed. However, many of the neopterans studied using synchrotron X-ray imaging do exhibit tracheal compression (Fig. 2; Table S1). The insects that have not been observed to exhibit tracheal compression during synchrotron imaging are mostly small and slow moving (Fig. 2), and can possibly exchange gases purely by diffusion. The finding that insects can achieve substantial ventilation by using tracheal compression challenges the assumption that air sacs are necessary for insects to achieve high rates of advective gas exchange. We need more comparisons of ventilatory capacity in insects with and without air sacs to rigorously test whether air sacs expand the capacity of insects to ventilate or appreciably reduce the cost of ventilation.

Possession of air sacs may alter tracheal system function

Beyond a simple association between air sacs and strong flight, inclusion of highly compliant air sacs in the tracheal system has the potential to alter the function of the remainder of the tracheal system. Because air sacs tend to be much more compliant than tracheae, tracheae in insects with air sacs may not exhibit compression until the air sacs are completely compressed, potentially altering ventilation patterns in secondary and tertiary tracheae (see Glossary; Fig. 3A). By contrast, for insects without air sacs, decreases in body volume can drive compression throughout the primary, secondary and tertiary tracheae (Fig. 3B). However, at least some insects can create sub-regions in the hemocoel (see Glossary) with different pressures, which may allow simultaneous compression of air sacs and distal tracheae (Fig. 3C; Harrison et al., 2019; Pendar et al., 2015). More experimental tests are needed to determine which (if any) of these models predominates.

Air sacs may also alter tracheal system function during discontinuous gas exchange (see Glossary). Air sacs that increase tracheal volume are likely to allow longer closed and flutter periods as result of larger compartments for internal oxygen and carbon dioxide stores. Independently of effects on tracheal system volume, air sacs will usually increase the compliance of the tracheal system, allowing greater changes in tracheal volume as negative pressures develop during the closed phase. During spiracular fluttering, the highly compliant air sacs will minimize the development of negative pressures during brief periods of spiracular closure, suggesting that variation in air sac presence and compliance may explain why some insects but not others can take up oxygen without loss of carbon dioxide during the flutter phase (Lighton, 1996). Finally, compression of air sacs may enhance the mixing of gases during the flutter phases and speed gas exchange during the open phase.

Air sac links to other evolutionary innovations

Air sacs may be important for many other evolutionary innovations within Insecta. Air sacs fill the horns of scarab beetles, allowing them to be large but light, reducing the cost of generating lift (McCullough and Tobalske, 2013). Among beetles, horns occur predominantly in scarab beetles (Kijimoto et al., 2013); is this partly because the capacity to develop air sacs reduces the cost of these sexually selected traits? Similarly, the alimentary canal is air filled in the non-feeding adults in Ephemeroptera and Plecoptera (Herhold et al., 2023). The tibiae of many orthopterans are filled mostly with air sacs (Hartung et al., 2004; Ruan et al., 2018), which may reduce the cost of locomotion.

Air sacs can provide other diverse functions in insects, with many potential interactions with other traits. For example, air sacs provide space for tissue growth. Possession of air sacs may be crucial for insects with rigid cuticle to be able to accumulate large volumes of eggs; conversely, female insects without air sacs may need to have flexible, expandable abdomens to accommodate reproduction. In addition, air sacs can be used as a hydrostatic pressure system to facilitate molting and the remarkable expansion of the abdomen during oviposition into the ground (Rose et al., 2000). Sensory neurons on these compressible structures may provide feedback to coordinate respiratory rhythms or volume expansions during molting (Miller, 1960).

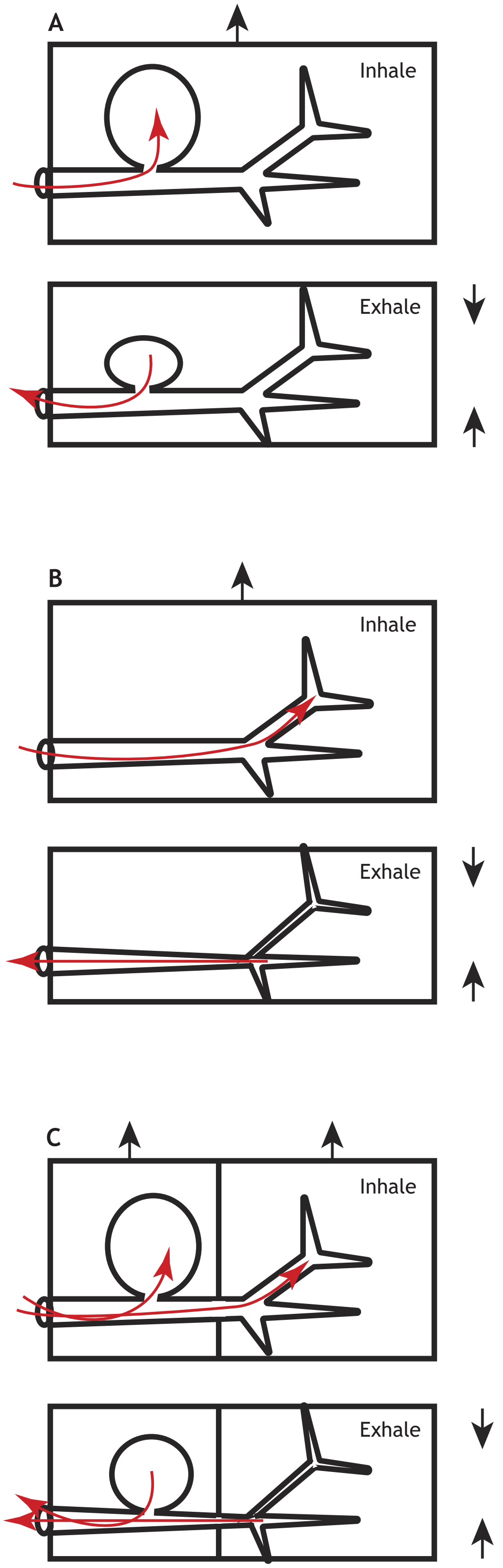

Fig. 3. Hypotheses of how air sacs affect tracheal system function. (A) With a single-compartment hemocoel and a tracheal system with large air sacs that are more compliant than the tracheae, body volume changes drive advection through the air sacs and primary tracheae, but not through the distal tracheae. (B) In tracheal systems without air sacs, body volume changes drive advection throughout the tracheal system. (C) Subregions created by functional valving (creating multiple compartments) may allow compression of both highly compliant air sacs and less-compliant tracheae in response to body volume changes.

The capacity to develop air sacs may be important for the evolution of large insects. Large body size increases the need for strong advective gas exchange, which is likely to be assisted by possession of air sacs. Miniaturized insects usually have simplified tracheal systems and lack air sacs (Polilov, 2005; Randolf and Zimmermann, 2019). Most very large adult insects, with the exception of the cerambycid beetles already mentioned, have air sacs. Giant stick insects do not, perhaps because these are usually slow moving (Staruss, 2021). One comparative study has shown that the tracheal system occupies a larger fraction of body volume in larger tenebrionid beetle species (which lack air sacs; Kaiser et al., 2007), but a similar study has not been performed for a clade with air sacs. However, there is good evidence that air sacs enlarge with body size during ontogeny in the locust *Schistocerca americana* (Greenlee et al., 2009). First instars of this species lack air sacs, and air sacs increase in relative size within the juvenile stages, and then dramatically in the flying adults.

Linkage of air sacs and life history in aquatic insect larvae

Air sacs are encountered less frequently in aquatic insects, tending to be absent even from aquatic juvenile life stages that ultimately develop into flying adults with well-developed air sacs (e.g. Odonata). Their absence can be attributed to two challenges posed by the aquatic environment: buoyancy and hydrostatic pressure. The air-free density of an insect's body is only slightly greater than that of fresh water (e.g. 1.078 g cm^{-3} in the diving bug *Anisops deaneii*), indicating that an insect lacking an internal or external air volume will sink. However, the addition of an air volume greater than 7.8% of the insect's body volume (using values from *Anisops*) will make it positively buoyant, forcing it to actively swim or cling to submerged material to remain underwater. Although some aquatic insects carry air bubbles on the outside of their bodies while diving, the volume of air carried by most water-breathing insects is contained entirely within their tracheal system. Thus, the reduction or elimination of air sacs, and the associated decrease in internal air volume and buoyancy, is an advantage for aquatic insects that seek to remain submerged. In the many aquatic insects that have closed tracheal systems, compressible air sacs could not drive advective gas exchange with water. Studies on aquatic dragonfly nymphs support this view, with investigations showing that their tracheal system is without air sacs (Tillyard, 1917) and undergoes minimal compression during the hemolymph pressure pulsations that occur when they ventilate their rectal gill (Krogh, 1920).

The belastomatid bug *Sphaerodema rusticum* has two morphs that could provide an excellent test system for investigating the trade-off that adult aquatic insects face between the potential use of air sacs to support flight versus eliminating air sacs to decrease buoyancy. The adults of this species occur as two morphs: a winged morph with air sacs and a body density of 0.88 g cm^{-3}, or a wingless morph which lacks air sacs and has a body density of 0.92 g cm^{-3} (Presswalla and George, 1935). The greater buoyancy of the winged morph is likely to contribute to an increased energetic cost associated with remaining submerged.

Increased hydrostatic pressure across the tracheal system walls is also likely to favor aquatic insects that do not possess air sacs or easily compressible tracheae. First, every meter of depth in fresh water adds an additional 9.78 kPa of external pressure. Second, total air pressure within an aquatic insect's closed tracheal system is sub-atmospheric because respiration reduces the P_{O_2} within the tracheal system below ambient levels while highly soluble CO_2 is rapidly lost into the surrounding water. Thus, a dragonfly nymph in air-equilibrated sea-level water (total pressure of dissolved gases:

101.3 kPa) will have a tracheal P_{N_2} of 79 kPa, but a P_{O_2} of 2–15 kPa, and a P_{CO_2} of 2 kPa (Lee et al., 2018), resulting in an additional pressure difference of 5.3–18.3 kPa across the walls of the tracheal system. If the tracheal system contained compliant tracheae and air sacs, the resultant narrowing of their cross-sectional area would restrict diffusion of respiratory gases within the insect.

A few aquatic larval dipterans do possess air sac-like structures, but these do not assist in respiration as they are not connected to the tracheal system (Krogh, 1911). One member of this group are the aquatic larvae of the genus *Chaoborus*, which have four large air sacs with thickened walls and prominent taenidia that resist collapse (Fig. 4; Damant, 1924; von Frankenberg, 1915). These air sacs function as hydrostatic organs, enabling *Chaoborus* larvae to control their buoyancy and position within the water column. The volume of *Chaoborus*' air sacs is adjustable, such that larvae can regulate their density and ascend or descend. They achieve this control by altering the pH, and therefore the swelling state, of bands of resilin (see Glossary) within the air sac wall. The change in the resilin, in turn, causes the volume of the air sac to increase or decrease (McKenzie et al., 2022). Thus, unlike all other tracheae, which rely on their stiffness to respond to forces imposed on them, these air sacs can dynamically produce force.

Larvae of *Mochlonyx*, a sister genus to *Chaoborus*, live in shallow vernal pools and possess an open tracheal system with a posterior respiratory siphon and air sacs (Fig. 4). *Mochlonyx* also maintains neutral buoyancy and surfaces infrequently (P.G.D.M.,

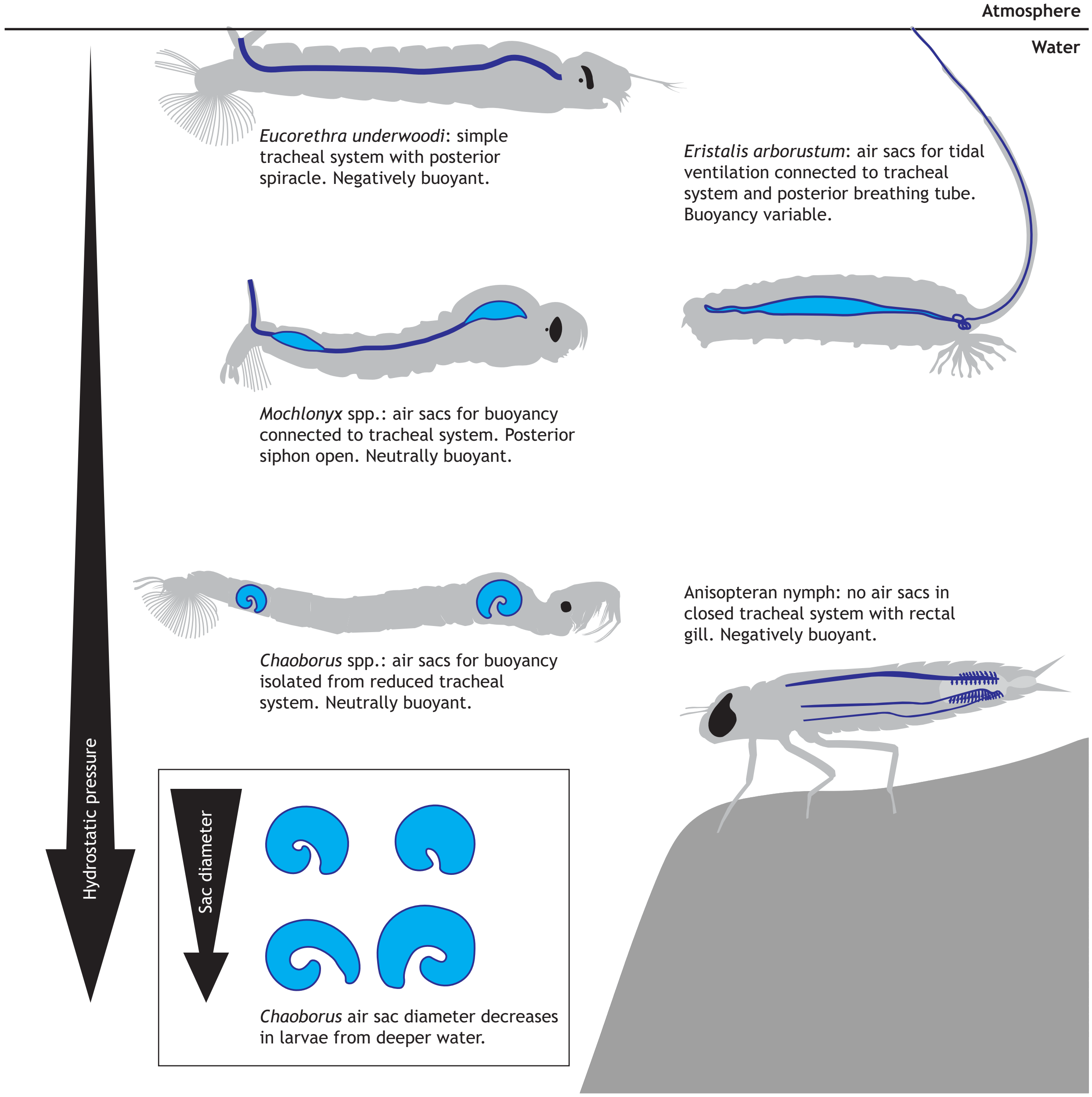

Fig. 4. The presence and function of air sacs within tracheal systems of aquatic insect larvae. Light blue represents air sacs, dark blue represents major tracheae. Adjustable air sac volumes in *Mochlonyx* and *Chaoborus* enable them to achieve neutral buoyancy to float mid-water. By contrast, both *Eucorethra* and Anisopteran nymphs lack air sacs and are negatively buoyant. *Eristalis arbustorum* possess air sacs that function to advectively move air through their long posterior breathing tube. Inhaling or exhaling air allows them to vary their buoyancy at will. *Chaoborus* air sacs based on Bardenfleth and Ege (1916); *Eristalis* silhouette based on Dunavan (1929).

personal observation), although they can maintain contact with the surface using their respiratory siphon in much the same way as a mosquito larva (O'Connor, 1958). The contrast between the open and closed tracheal systems of *Mochlonyx* and *Chaoborus* can be attributed to the different hydrostatic pressures these insects experience. Unlike the surface-dwelling *Mochlonyx* larvae, lake-dwelling *Chaoborus* larvae routinely descend to the bottom during the day to escape fish predation, and have been recorded at over 200 m depth in Lake Malawi (Irvine, 1997). Air sac diameters in *Chaoborus* larvae are smaller than those in *Mochlonyx* larvae (Bardenfleth and Ege, 1916), probably because smaller air sacs are harder to collapse.

Achieving neutral buoyancy using air sacs enables both *Chaoborus* and *Mochlonyx* larvae to feed on zooplankton suspended in the water column. In the absence of their hydrostatic air sacs, the larvae would sink to the bottom. This condition is exemplified by the most basal genus within the Chaoboridae, *Eucorethra* (Fig. 4). These larvae entirely lack tracheal dilations or air sacs and are indeed negatively buoyant. They attach themselves to the surface of the water by their respiratory siphon, feeding on insects that fall onto the water's surface. But when they are disturbed, they break contact with the surface of the water and sink rapidly into the debris on the bottom of their shallow pools (Rossignol, 1978).

The second group of aquatic dipterans with air sacs are the larvae of hover flies from the subfamilies Eristalinae and Syrphinae (Miranda and Rotheray, 2018). These larvae possess a very long tail-like breathing tube that can extend some four times the length of the body, giving them their common name of rat-tailed maggots. A pair of spiracles at the end of the breathing tube open into fused tracheal tubes that connect with the paired air sacs (Dunavan, 1929). Driven by muscles, these compressible air sacs collapse and expand to drive a tidal flow of air through the long respiratory siphon, which also has the added effect of allowing them to alter the density and buoyancy of their body at will (Fig. 4; Buckton, 1895).

Aquatic insects can reveal much about the trade-offs associated with possessing air sacs, particularly as aquatic forms are usually the juvenile life stage of a terrestrial, winged imago. As such, these insects transition from an environment where air sacs may be a liability, or are at least constrained in volume, into a winged adult where air sacs are advantageous for strong flight. Investigating those few aquatic insects that have air sacs may reveal unusual structure–function relationships, as these air sacs are likely to operate with a larger static pressure difference across their walls compared with those found in their terrestrial counterparts.

Conclusions and perspectives

Air sacs are clearly important components of insect tracheal systems, and arguably among the most adaptable components in terms of links with aspects of life history such as flight, large body size, evolution of weapons, and location and movement strategies of aquatic insects. The broad and 'scattered' distribution of air sacs across the arthropod phylogeny suggests that air sacs are easily evolved; strong associations with life history are likely to be driven by selection on the trade-offs associated with the costs and benefits of air sacs. Air sacs are not required for insects to achieve substantial advective gas exchange, as this can also be accomplished by tracheal compression. Based on the literature presented here, we hypothesize that: (1) tracheal compression evolved early in insect evolution and was critical for the high metabolic rates of active insects; (2) air sacs have evolved multiple times, facilitating the evolution of strong flight; (3) air sac elaboration supports the evolution of giant insects; (4) air sac form and function have differentiated among aquatic clades in association with distinct life-history strategies; and (5) air sacs and compression of tracheae have disadvantages that select against their presence in many species. For air sacs, a primary disadvantage is reduced stores of water and nutrients per unit volume, and for compression of tracheae, a primary disadvantage is collapse under high pressures. In-depth comparative analyses will be required to rigorously test these hypotheses.

There are multiple exciting future directions for studies of air sacs in insects. Most obviously, we need more comparative data across and within orders of hexapods and tracheate arthropods to test the association of air sacs and tracheal compression with different life histories, body size and phylogeny. The increased availability of table-top microtomography and synchrotron X-ray imaging will make obtaining such data much easier, and will allow data to be more quantifiable. Physiological tests, both comparative and experimental, should enable determination of the functional benefits of air sacs for flight, and the relative benefits of air sacs versus tracheal compression for ventilation. Biomechanical tests of the compressibility of the tracheal system and the relationship to shape and taenidial properties have the potential to greatly improve our understanding of the relationship between tracheal form and function. Elucidating the roles of different air sac forms and resilin properties seems particularly promising for improving our understanding of how insects control their buoyancy. In general, new technologies and appreciation of a comparative approach have opened up the study of insect air sacs, with wide implications for the study of insect physiology, life history and evolution.

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Competing interests

The authors declare no competing or financial interests.

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Table S1. Evidence for distribution of air sacs and tracheal compression in adults across hexapod orders. In most cases, there are only data for a few species in each order. Only one or two representative publications are listed, though often more could be shown.

Order or Family	Air Sacs	Tracheal Compression	Link to Image or video
Protura	None. (Dittrich and Wipfler, 2021; Herhold et al. 2023)	No data	
Collembola	None (Dittrich and Wipfler, 2021)	No data	
Diplura	None (Dittrich and Wipfler, 2021)	No data	
Archaeognatha	None (Dittrich and Wipfler, 2021)	No data	
Zygentoma	None (Dittrich and Wipfler, 2021; Herhold et al. 2023)	No. Synchrotron x-ray; n=1	
Odonata	Extensive. (Weis-Fogh, 1964; Herhold et al. 2023)	Yes. Synchrotron x-ray; 2 species dragonfly (n=4 each) and 1 damselfly (n=2) (Westneat et al., 2006)	X-ray Video Link FigShare: https://figshare.com/articles/media/Insect_Respiration_X-ray_Videos/22082384?file=39223100
Emphemeroptera	Alimentary air sac (Herhold et al. 2023)	No. Synchrotron x-ray; n=1	
Dermaptera	None. (Jaglarz et al., 2019; Herhold et al. 2023)	Yes. Synchrotron x-ray; 1 species, n=3 (Westneat et al., 2006)	
Plecoptera	Aliimentary air sac (Herhold et al. 2023)	No data	
Zoraptera	no data	No data	
Orthoptera	Extensive. (Harrison et al., 2013;Herhold et	Yes. synchrotron x-ray; 3 species n>4, grasshoppers and crickets	X-ray Video Link FigShare: https://figshare.com/articles/media/Insect_Respiration_X-ray_Videos/22082384?file=39220226

	al. 2023)	Westneat et al. 2003 (Harrison et al., 2013)	
Grylloblattodea	None (Herhold et al. 2023)	No data	
Mantophasmatodea	No data	No data	
Phasmatodea	None. (Strauß, 2021; Herhold et al. 2023)	No data	
Emblidina	Alimentary air sac (Herhold et al. 2023)	No data	
Blattodea	No air sacs observed in 10/11 species (7 of 8 species shown in FigShare); also (Dittrich and Wipfler, 2021; Herhold et al. 2023)	Yes. Synchrotron x-ray; *Blattella germanica* (Westneat et al., 2006)	X-ray Video Link FigShare: https://figshare.com/articles/media/Insect_Respiration_X-ray_Videos/22082384?file=39223763 X-ray images of 8 cockroach species FigShare link: 10.6084/m9.figshare.22121633
Mantodea	Dittrich and Wipfler 2021 report no air sacs; Herhold et al. report air sacs in head of one species. *Hierodula membranacea*, a large flying species, has air sacs (Kerry et al., 1987)	Yes. Synchrotron x-ray; 1 species (Westneat et al., 2006)	X-ray Video Link FigShare: https://figshare.com/articles/media/Insect_Respiration_X-ray_Videos/22082384?file=39223766
Psocodea	None. (Mukerji and Sen-sarma, 1955)	No data	
Hemiptera	Air sacs in a flying but not a nonflying species.	Yes. N > 3 animals *Oncopeltus* sp.	

	(Miller, 1961) (Wigglesworth, 1954)		
Thysanoptera	None. (Polilov and Smakov, 2016)	No data	
Hymenoptera (Vespidae)	No data	No data	
Hymenoptera (Formicidae)	Air sacs in each tagma (Keister 1963) (see synchrotron images, also (Meyer, 1989))	Yes. Synchrotron x-ray; head and thorax of *3* ant species. Westneat et al. 2003	Air sacs in head of male ant, Link FigShare: 10.6084/m9.figshare.22089179 X-ray Video Link FigShare: https://figshare.com/articles/media/Insect_Respiration_X-ray_Videos/22082384?file=39220211
Hymenoptera (Apidae)	(Meyer, 1989)	Yes. Synchrotron x-ray; *Bombus* and *Apis* sp. (Westneat et al., 2006)	X-ray Video Link FigShare: https://figshare.com/articles/media/Insect_Respiration_X-ray_Videos/22082384?file=39220220
Raphidioptera	No data	No data	
Neuroptera	None in *Chrysopa pallens* (Zhao et al., 2022) But reported for large fliers (Newport, 1851)	No data	
Megaloptera	No data	No data	
Strepsiptera	No data	No data	
Coleoptera (Carabidae)	None. See synchrotron videos Thoracic and abdominal ais sacs were reported in tiger beetles (Yeger and Spangler, 1995)	Yes. Synchrotron x-ray; *4* carabid species. Westneat et al. 2003 Socha et al. 2008 Hochgraf et al. 2019	X-ray Video Links FigShare: https://figshare.com/articles/media/Insect_Respiration_X-ray_Videos/22082384?file=39220214 https://figshare.com/articles/media/Insect_Respiration_X-ray_Videos/22082384?file=39220217
Coleoptera (Buprestidae)	Extensive. (Miller, 1966)	No data	
Coleoptera	Extensive. (Miller,	Yes. 2 species.	https://askabiologist.asu.edu/sites/default/files/

(Scarabaeidae)	1966)		games/beetle-dissection/play.html
Coleoptera (Curculionidae)	None. (Miller, 1966)	No data	
Coleoptera (Cerambycidae)	None. (Miller, 1966)	No data	
Coleoptera (Tenebrionidae)	None. (Pendar et al., 201 al., 2018)	Yes. (Pendar et al., 2015)	
Mecoptera	None. (Herford, 1938; Wigglesworth, 1935)	Yes. (Herford, 1938)	
Diptera	In head and thorax. (Manning and Krasnow, 1993)	Yes. Multiple animals and species. (Westneat et al., 2006) (Hale et al., 2006)	X-ray Video Link FigShare: https://figshare.com/articles/media/Insect_Respiration_X-ray_Videos/22082384?file=39220229
Lepidoptera	Extensive. (Lowe et al., 2013; Newport, 1851)	Yes. Synchrotron x-ray; 2 species. N=6 each.	
Trichoptera	No data	No data	

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