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Evolutionary biology

The unusual tracheal system within the wing membrane of a dragonfly

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Some consider that the first winged insects had living tissue inside the wing membrane, resembling larval gills or developing wing pads. However, throughout the developmental process of the wing membrane of modern insects, cells and tracheoles in the lumen between dorsal and ventral cuticle disappear and both cuticles become fused. This process results in the rather thin rigid stable structure of the membrane. The herewith described remarkable case of the dragonfly *Zenithoptera lanei* shows that in some highly specialized wings, the membrane can still be supplemented by tracheae. Such a characteristic of the wing membrane presumably represents a strong specialization for the synthesis of melanin-filled nanolayers of the cuticle, nanospheres inside the wing membrane and complex arrangement of wax crystals on the membrane surface, all responsible for unique structural coloration.

1. Background

The origin, development and dynamics of an intriguing subject in biology, insect winged flight, is one of the most exciting topics in organismic biology [1–10]. Some authors have suggested that the first winged insects had living tissue inside wing-like specialized structures and used to skim or row on the surface of water [9,10]. These structures resembled the respiratory system of insect larval tracheal gills and wing pads, which usually contain tracheae and tracheoles forming a network of tubes [11,12]. However, at some point in insect evolutionary history, the primordial wings became much larger, their membranous parts much thinner, and they transformed into the totally different structure that we today know as insect wings.

It is well known that although wings of modern insects are richly permeated by hemolymph, nerves and tracheae, these structures are confined to wing veins [4]. During early development of the wings, the tracheal system is located inside the lumen between the two integumental layers, along with nerves and hemolymph [11]. In recent insects, as the wing reaches its final developmental stage, the dorsal and ventral integumental layers become fused [13]. Throughout this process, tracheoles and cells in the lumen may degrade to ultimately form the wing membrane or migrate into the thorax [14,15]. The areas where the two layers remain separated form veins. This remaining living tissue inside wing veins contain nerves, hemolymph and tracheae [16]. The cuticle of wing veins and the wing membrane sclerotizes, hardens and provides strength and rigidity to the wing, influencing flight performance and fracture resistance [16,17].

Modern dragonflies are known for their fast and manoeuvrable flight [18,19], which is partially due to the corrugated, three-dimensional wing profile [20] and the complex material composition of wings [21–23]. Therefore, the dragonfly wing membrane, an elaborate lightweight rigid structure without living

Table 1. List of dragonfly and damselfly species with the studied wing membrane structure.

species list	wing coloration
Zygoptera	
Amphipterygidae	
Rimanella arcana (Needham, 1933)	no
Calopterygidae	
Matrona basilaris basilaris (Selys, 1853)	yes
Mnesarete pudica pudica (Hagen in Selys, 1853)	yes
Mnais pruinosa pruinosa (Selys, 1853)	yes
Mnais pruinosa costalis Selys 1869	yes
Hetaerina cruentata Rambur 1842	yes
Hetaerina occisa Hagen in Selys, 1853	yes
Calopteryx splendens intermedia Selys, 1887	yes
Calopteryx virgo Linnaeus, 1758	yes
Vestalis gracilis (Rambur, 1842)	partially
Coenagrionidae	
Montonagrion hirosei Asahina, 1972	no
Euphaeaidae	
Euphaea yayeyamana (Matsumura, 1913)	yes
Euphaea ochraccea	yes
Hemiphlebidae	
Hemiphlebia mirabilis (Selys, 1869)	no
Megapodagrionidae	
Philogenia cassandra Hagen in Selys, 1862	no
Perilestidae	
Perissolestes romulus Kennedy, 1941	no
Platycnemididae	
Copera annulata (Selys, 1863)	no
Copera tokyoensis	partially
Platystictidae	
Palaemnema clementia Selys, 1886	no
Polythoridae	
Chalcopteryx rutilans (Rambur, 1842)	yes
Chalcopteryx scintillans McLachlan, 1870	yes
Cora cyane (Selys, 1853)	partially
Euthore fasciata fasciata (Hagen in Selys, 1853)	yes
Protoneuridae	
Psaironeura remissa (Calvert, 1903)	no
Pseudostigmatidae	
Microstigma rotundatum (Selys, 1860)	yes (tip)
Anisoptera	
Aeshnidae	
Aeshna cyanea (Müller, 1764)	no
Gynacantha nervosa Rambur, 1842	yes
Aeshnophlebia anisoptera (Selys, 1883)	yes

(*Continued*.)

Table 1. (*Continued*.)

species list	wing coloration
Austropetaliidae	
Phyllopetalia apicalis Selys, 1858	partially
Corduliidae	
Hemicordulia okinawensis Asahina, 1947	partially
Idionyx saffronata Fraser, 1924	partially
Gomphidae	
Stylogomphus suzukii (Matsumura, 1926)	no
Chlorogomphus brunneus costalis (Oguma, 1926)	partially
Libellulidae	
Erythrodiplax juliana Ris, 1911	partially
Trithemis annulata Palisot de Beauvois, 1807	yes
Brachythemis fuscopalliata Selys 1887	yes
Neurothemis tullia (Drury, 1773)	yes
Sympetrum vulgatum (Linnaeus, 1758)	no
Petaluridae	
Tanypteryx pryeri (Selys, 1889)	no

cells and tracheae [24], is quite different from the original form of gills or larval wing pads. However, this study suggests that in some highly specialized wings, the wing membrane can be supplemented by tracheae. Here, we present the remarkable case of the libellulid dragonfly *Zenithoptera lanei*, which is the first report of an adult insect with a tracheal system inside the wing membrane or fully functional flying wing.

2. Material and methods

The wings of *Z. lanei* (and other species) were air-dried, fractured, mounted on scanning electron microscope (SEM) stubs, sputter-coated with gold–palladium (thickness 10 nm), and examined in a scanning electron microscope (SEM) (Hitachi S-4800; Hitachi Ltd., Tokyo, Japan) at 3 kV. Other samples were prepared following a previously described protocol [24] and then analysed in a transmission electron microscope (TEM) (Tecnai Bio TWIN, FEI, Eindhoven, The Netherlands). Images of light microscopy (LM) were used to show the tracheal system in the horizontal plane view. In addition, wings of a further 40 dragonfly and damselfly species (table 1) with coloured and transparent wings were studied for comparison (with LM and SEM).

3. Results and discussion

Zenithoptera lanei have extremely bright-blue–coloured wings, and therefore are often called *Morpho* among dragonflies (figure 1*a*) [25]. The present study shows for the first time that their wing membranes are supplemented with extremely fine taenidia-bearing trachea, with diameters of typical insect tracheoles (0.3–2.0 µm) [11]. High-resolution microscopy showed that tracheae with diameters ranging from 0.3–0.5 µm in males and 0.3–1.0 µm in females (figure 1*b–f*) are widespread in the wing membrane. The wing tracheae emanate

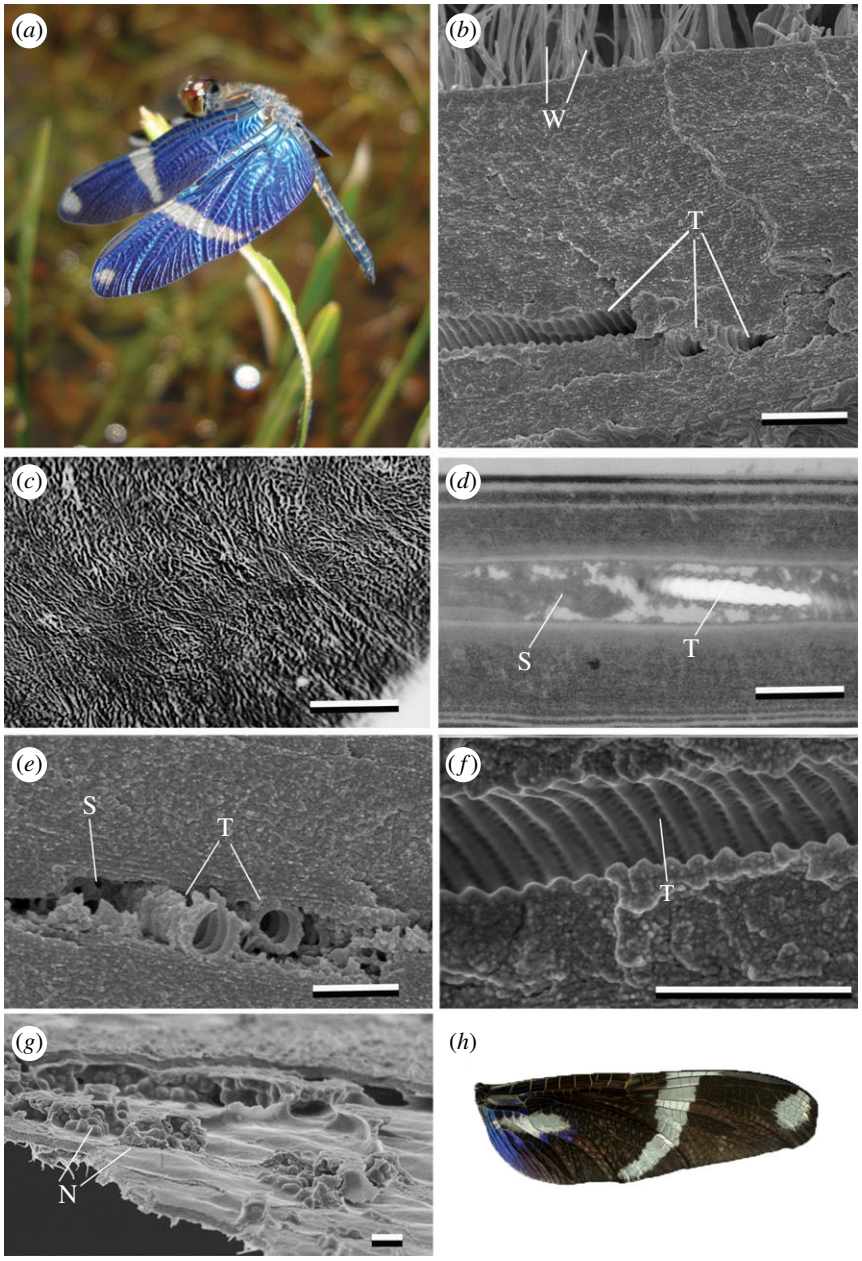

Figure 1. Wings of *Z. lanei*. (*a*) Photograph showing the dorsal blue-coloured side of wings. (*b*) Tracheae are shown in both cross and longitudinal sections; long wax filaments cover the dorsal surface of the wing. (*c*) LM image of a part of a single membrane cell with tracheae emanating from a vein (right lower corner), elongating and branching towards the cell centre. (*d*) TEM image of a cross-section of the wing membrane, showing trachea and the spongy tissue between the dorsal and ventral cuticle. (*e*) SEM image of taenidia-bearing tracheoles and spongy tissue within the wing membrane. (*f*) SEM micrograph of a tracheole embedded in a spongy matrix. (*g*) SEM micrograph of nanospheres inside the wing membrane. (*h*) Photograph showing the coloration pattern on the dorsal surface of female wings. N, nanospheres; S, spongy material; T, tracheae and tracheoles; W, wax filaments. Scale bars: 1 µm (*b*,*d*–*g*); 50 µm (*c*). Dorsal surface on top and ventral surface on the bottom of all electron microscopic images. (Online version in colour.)

from longitudinal and cross veins (electronic supplementary material, file S1*a*–*d*), elongate and branch in the direction of the membrane cell centre (figure 1*c*). The distance between insertion points of tracheae on wing veins may range from 0.2 to 6 µm (figure 1*c*, electronic supplementary material, file S1*b*–*d*). This distance between tracheae narrows towards the cell centre due to high ramification of tracheoles (figure 1*b*,*c*,*e*, electronic supplementary material, file S1*b*–*d*). This pattern can be found throughout the whole wing membrane. The tracheae are embedded in a spongy matrix of presumably dead elongated columnar hypodermal cells between the two cuticle layers (figure 1*d*,*e*, electronic supplementary material, file S2*a*,*b*), resembling larval wing pads [4,12]. These cells and tracheae are located in a lumen between the two cuticle layers and seemingly enter the vein's inner cavity by passing its endocuticle (electronic supplementary material, file S1*a*). In the whitish-coloured wing areas, the taenidia-bearing tracheoles (figure 1*f*) may be accompanied by nanospheres located between the two cuticular layers (figure 1*g*). The same pattern can be found in the less strikingly coloured female wings (figure 1*h*). Comparison with a further 40 dragonfly and damselfly species, studied as references, did not reveal the presence of tracheae in the wing membrane (figure 2). However, a recent study on another libellulid, *Rhyothemis resplendens*, also shows that a spongy matrix can be found between the two wing membrane layers, but so far there was no evidence of tracheation [26].

Why can this unique tracheal pattern be found within the wings of this particular species? Although our results do not answer this question, we suggest three hypotheses explaining the function of the wing tracheal system in adult *Z. lanei*. First,

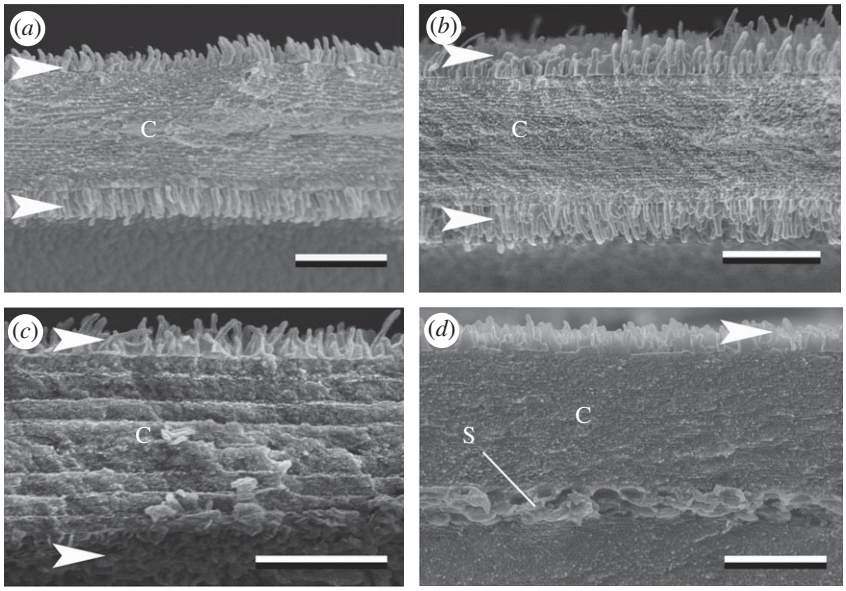

Figure 2. SEM micrographs of cross sections of the wings of four different Odonata species: (*a*) *Erythrodiplax juliana* (Anisoptera: Libellulidae), (*b*) *Mnesarete pudica* (Zygoptera: Calopterygidae), (*c*) *Chalcopteryx rutilans* (Zygoptera: Polythoridae), and (*d*) *Stylogomphus suzukii* (Anisoptera: Gomphidae). Arrowheads indicate wax crystals of the epicuticle. C, wing cuticle; S, spongy matrix in *S. suzukii* without tracheae. Scale bars: 1 µm.

it is known that tracheae are more abundant in insect tissues of high metabolic activity, such as glands [27]. Odonate wings exhibit an epicuticular layer of wax secreted by a system of pore channels in the membrane [24,28]. Usually, cuticular wax crystals have a similar size, shape and abundance on the dorsal and ventral wing surfaces, resembling filaments and rods [24]. While *Z. lanei* males exhibit normal crystals on the ventral surface of the wings, they have two hierarchically organized layers of wax crystals on the dorsal surface: a lower layer of extremely long filaments and an upper layer of leaf-shaped crystals [25]. Females also exhibit thick wax coverage on the dorsal surface of the wings, but do not have such a complex hierarchical structure of wax crystals as males. Possibly, the combination of such elaborate hierarchical wax structure, which generates striking light-blue coloration, in concert with dark-blue interference colour of the wings [25] and nanospheres, requires strong synthesis of melanin, chitin and waxes. This synthesis may be not only intensive, but also prolonged during adult lifetime, as indicated by the presence of cell remnants in the membrane (electronic supplementary material, file S2*a*).

Strong and long activity of hypodermal cells needs stronger oxygen supply when compared with transparent or just melanized wings. Our study supports this hypothesis, since other odonate species with colourful wings did not exhibit the tracheolar system in the wing membrane. The main difference between *Z. lanei* and other dragonflies is the multicomponent wing coloration described above, which is mainly influenced by complex wax crystals [25]. Therefore, this set of evidence allows us to suggest that both unique traits in this species may be linked by the physiological demands of the wing coloration. Therefore, the tracheated wing membrane may be an adaptation to extensive oxygen demand of hypodermal cells in the wing membrane responsible for the generation of nanospheres and the secretion of melanin and thick complex wax coverage. Here, further studies are necessary to examine whether the hypodermal cells are active for a longer time than in other species.

An additional hypothesis is that tracheoles form an interference film resulting in structural coloration as previously reported from the tapetum in the eye of butterflies [29]. Our third hypothesis is based on the fact that *Zenithoptera* have the habit of folding their wings like damselflies, a unique trait among the Anisoptera [30]. This behaviour is probably involved in intraspecific communication and thermoregulation, and the latter may have a direct relationship with the presence of tracheae in the wings to distribute heat or cool down body temperature.

4. Conclusion

We conclude that *Z. lanei* exhibits a wing tracheal system that presumably supports the prolongation of the secretory function of hypodermal cells. Dragonfly wings often bear pigment-based and structural coloration [25,26], but to the best of our knowledge none with such an elaborate combination of melanin-filled nanolayers of the cuticle with complex arrangement of wax crystals on the membrane surface and internal nanospheres. The presence of tracheation of the wing membrane may promote further research to unravel their role and also start the search for similar cases in Insecta.

Ethics. This study complies with ethical guidelines in Brazil and Germany.

Data accessibility. All data are presented in the manuscript.

Authors' contributions. All authors contributed to conception and design, or acquisition of data or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors gave final approval for publication and agreed to be held accountable for the content.

Competing interests. We have no competing interests.

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