Lung morphology of the tarantula, *Eurypelma californicum* Ausserer, 1871 (Araneae: Theraphosidae)

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Summary

The general morphology and fine structure of the book lungs of the North American tarantula, *Eurypelma californicum* Ausserer have been investigated using scanning electron microscopy. In animals weighing between 7.6 and 18.6g, the length of the spiracles is linearly related to body weight. The number of sacculi (air sacs) per mm is almost constant, decreasing only from 38 to 35mm^{-1}. Respiratory surface area increases according to RSA (cm^2) = 8.92·W(g)$^{0.65}$; if related to body weight it decreases according to RSA/W (cm^{2}g^{-1}) = 8.98·W(g)$^{-0.35}$. The minimal air spaces (residual volume) in the sacculi of all four lungs range from 9 to 22mm^3 and are directly related to body weight. Lung haemolymph volume, in contrast, follows the equation HS (mm^3) = 8.30·W(g)$^{0.71}$. About 2.2% of the total haemolymph is contained in the lungs. The haemolymph spaces are traversed by pillars composed of at least four cells. The pillars are regularly spaced at *c.* 65-70μm from each other, and are in the centres of polygonal (mostly hexagonal) fields which are believed to represent the cytoplasmic extent of each pillar. The cuticular struts, which prevent the air space from collapsing, are 5.2-6.3μm high, in the range of animals tested, and hollow. At their tips they are connected by a trabecular network which may occlude between 19 and 29% of the upper sacculus wall.

Introduction

Spiders possess two types of respiratory organs, tracheae and/or book lungs, of which the latter have received most attention. They consist of a series of hollow lamellae in which haemolymph streams laterally towards the efferent sinus and lung vein. In the haemolymph space of the lamellae there are epithelial pillars connecting the dorsal and ventral walls, while the air spaces (the sacculi) are prevented from collapsing by thin cuticular struts. The minimal volume of air (residual volume) in the sacculi is determined by the length of these struts. The cuticular struts are either hair-like with a knob at the end, or they may be interconnected distally by a network of trabeculae. The older literature has been cited by Kaestner (1929) while some recent descriptions and illustrations are found in the papers by Hill (1977), Moore (1976), Anderson & Prestwich (1980), Hexter (1982) and Strazny & Perry (1984, 1987).

There has been a long-standing controversy over whether book lungs are ventilated or not. We do not wish to reiterate the arguments raised since Willem's (1917) work, for or against ventilation of spider lungs. For the tarantula, *Eurypelma californicum* Ausserer, 1871, it has been proved experimentally that the lungs function as diffusion lungs. Although the atria are ventilated, tidal volume represents only *c.* 0.2% of the lung volume in resting animals, and oxygen uptake during the time of one 'breath' is about three times the tidal volume (Paul, Fincke & Linzen, 1987).

In the present study, we have made morphometric measurements of tarantula (*Eurypelma californicum*) lungs, combined with fine structural studies of the cuticular strut network of the lamellae using scanning electron microscopy. This is part of our overall aim to provide a description of gas transport in tarantulas at every level, from the molecular to the organismic.

Materials and methods

Female tarantulas, weighing 7.6-20g, were used in this study. They were obtained from Carolina Biological Supply Co., Burlington, NC, USA. The species was determined as *Eurypelma californicum* Ausserer, 1871, according to Comstock's (1965) key. The animals were kept in 30 × 30 × 15cm plastic bowls on a layer of peat dust, at *c.* 23°C and 50% relative humidity. Drinking water was provided *ad lib.*, and food (crickets or cockroaches) given once a week. Prey animals were removed if they were not captured quickly. Tarantulas approaching a moult often did not accept food for several weeks. We used only those animals which fed regularly and reacted quickly to prey.

For scanning electron microscopy, the lungs were dissected and fixed for 20h at 4°C in a mixture of formaldehyde (2%) and glutaraldehyde (2.5%) in phosphate buffer, pH 7.3 (Millonig, 1961). After dehydration, the specimens were dried by the critical-point method and sputter-coated with gold. Specimens were examined in a Cambridge Stereo Scan instrument, model MkIIA.

For light microscopy, animals were fixed in Bouin's solution, dehydrated and embedded by standard methods, and stained with haematoxylin/eosin. Sections were viewed under a Zeiss standard microscope (Achromate 40/0.65 Ph and 100/1.25 oil Ph).

The lung surface was determined by removing individual lamellae at known intervals and measuring their length and average width. The area was multiplied by the number of lamellae per interval. The total lung surface was calculated as twice the sum of all group values.

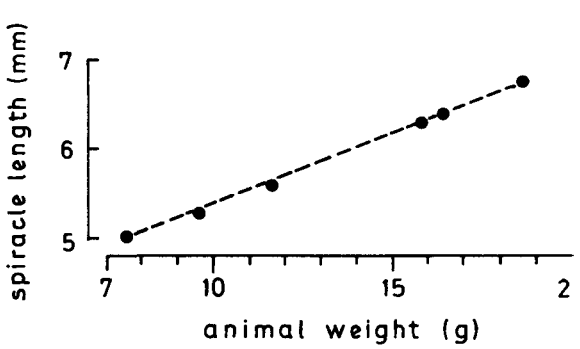

Fig. 1: Relation between body mass and length of the spiracles in six *Eurypelma californicum*.

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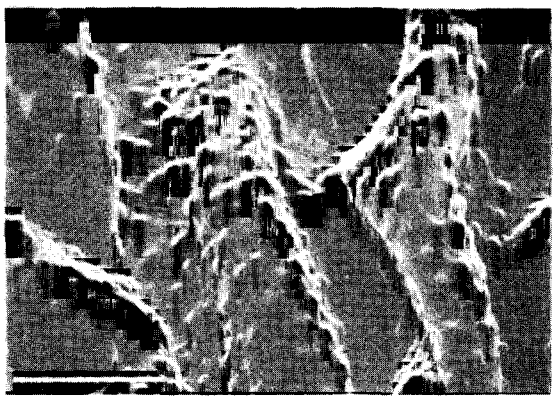

Fig. 2: Structure of the posterior wall of the atrium. SEM. Scale line = 10 μm.

Results

General morphology of the lungs

As is characteristic of the Theraphosidae, *Eurypelma californicum* has two pairs of lungs, each lung being covered by a strongly sclerotised plate. The posterior edge of this plate is bulging and very stiff, forming the anterior lip of the spiracle. The posterior lip of the spiracle is also relatively stiff, followed by a more flexible cuticular region which may bend into a deep fold when the posterior lip is being retracted and the spiracle opened. The length of the spiracles was 5-6.7mm in animals weighing 7-19g, and within this range linearly related to body weight (Fig. 1). Blowing CO_2 at the spiracles stimulated their opening to a width of 0.15-0.4mm, so that one could view the interior of the atrium.

The structure of the posterior wall of the atrium appears slightly grained and strongly folded (Fig. 2). These folds probably impart some stiffness to the posterior wall, which would be important because the retractor muscle inserts rather centrally, but they may also allow for an extension of the wall when the muscle contracts. We did not observe the branched and anastomosing cuticular structures shown by Berteaux (1889).

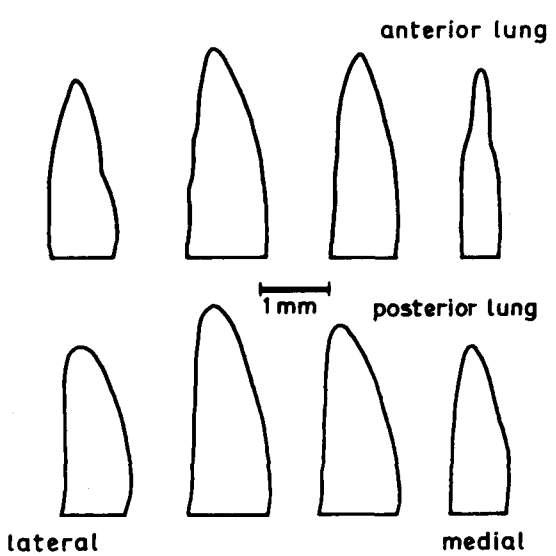

Fig. 3: Outlines of lung lamellae (left lungs) in *Eurypelma californicum* (female, 12.5g). Note the elongated, lancet-like form of the innermost lamella (right) in the anterior lung.

The lamellae are roughly triangular, the lateral and posterior edges being almost at right angles; the medial edge is rounded. Figure 3 shows some typical outlines, and Fig. 4 the relation between length, width, and position relative to the medial (i.e. ventral) edge of the lungs, in a 16g specimen. In this figure, the lateral edge of each lamella is drawn as a straight line, for reference. The smallest lamellae are the lateral (i.e. dorsal) ones, in the horn-like space of the atrium projecting beyond the outer end of the lung slit. The largest lamellae are near the centre of each lung while the innermost (medial, i.e. ventral) ones become narrow and lancet-like in the anterior lung. In the posterior lung the outlines vary much less.

Fine structure of the lamellae

Both haemolymph and air spaces are traversed by supporting elements — cellular pillars on the haemolymph face of the lamellae and minute struts on the cuticular face (Fig. 5). The pillars appear to be composed of at least four cells, containing the nuclei, while their cytoplasm extends into an extremely thin layer. SEM pictures show spurs of cytoplasm radiating from the pillars (Fig. 6). The pillars show a fairly regular arrangement (Fig. 7), their distance apart being estimated at 60-80 μm from SEM photography, and minimal distance 54-65 μm from sections examined by light microscopy. Near the lateral edges of a sacculus and especially towards its tip (i.e. the edge of the lamella away from the atrium), the density of pillars increases.

The cuticular struts, which prevent the air space from collapsing, originate from the ventral surface of each sacculus wall. The dorsal surface is only in loose contact and becomes easily detached (Fig. 5). The struts are tree-like, forming several spurs at their thickened base. Distally the struts are interconnected

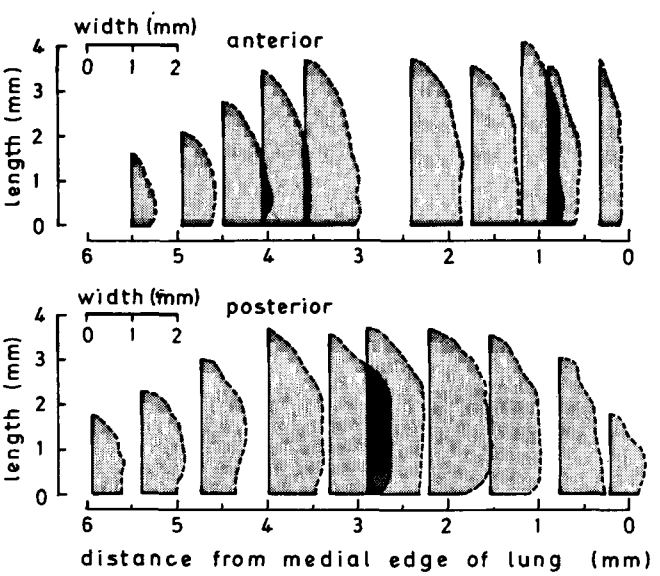

Fig. 4: Length and width of the lamellae in the anterior and posterior lung in relation to position. For reference, the lateral edge of each lamella is drawn as a straight line. Scale on the abscissa starts at the medial (i.e. ventral) edge of each lung.

by a network of trabeculae which are somewhat flattened and often branched and curved, forming twin connections (Figs. 8, 9). In addition to this distal meshwork, the struts are sometimes ramified forming a Y or H. The network is more dense near the atrium, while it is absent in the anterior tip region of a sacculus.

The struts and trabeculae are hollow as shown in Fig. 10. It is not clear whether they are filled or only lined with cytoplasm, so that haemolymph might enter. If the ventral sacculus wall is viewed from the haemolymph side, the bases of the struts appear as funnel-shaped structures (Figs. 10, 11).

The length of the struts is 5-6 μm, and they are spaced at intervals of 2.5-4 μm. The connecting trabeculae are between 0.25 and 0.75 μm wide. The meshwork of trabeculae may occupy a considerable area of the dorsal sacculus wall (Figs. 8, 9), which becomes important when the respiratory surface is to be estimated. In a few preparations we have estimated the area covered by the trabeculae and the end plates of the struts to be between 19 and 29%.

There are about 15 cuticular struts in the air space between each pair of cell pillars in the haemolymph space, but there is considerable variation. Especially in the central regions of a lamella preparation somewhat irregular polygonal, mostly hexagonal, fields can be observed (Fig. 12), along the edges of which the struts are placed closer to each other. The diameter of these fields is about 50-60 μm, and *c.* 11-15 struts were counted between parallel edges. This corresponds with the distance between cell pillars and number of struts between them (see above). It is suggested that each group of pillar cells is associated with one hexagonal field.

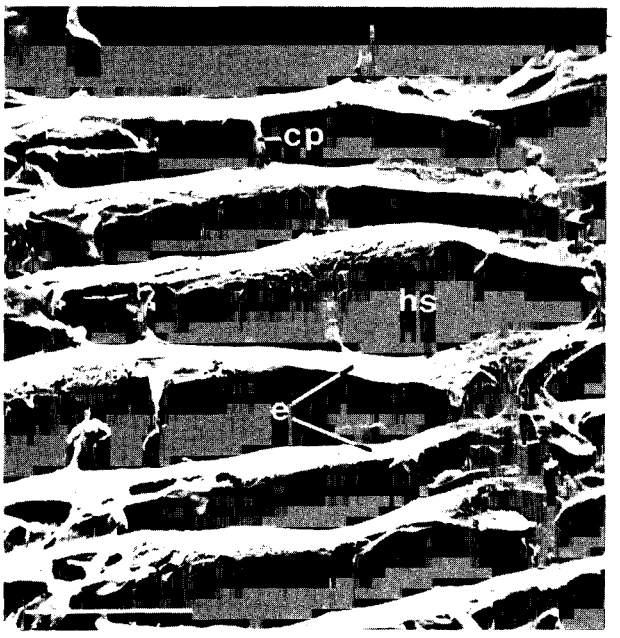

Fig. 6: SEM view of the haemolymph spaces (hs) and the lateral edges (e) of the sacculi (hence they appear "closed"). Note the spacing of cellular pillars (cp) and spurs radiating from lower and upper base. Scale line = 50 μm.

Lung morphometry

Various lung parameters are shown in Table 1. The number of sacculi per mm decreases only slightly (from 38 mm^{-1} to 35 mm^{-1}) over a nearly 2.5-fold weight range. Respiratory surface area (RSA) increases according to RSA (cm^2) = 8.92·W $(g)^{0.65}$ ($r = 0.91, p < 0.01$). If related to body weight (W) there is a decrease which can be described by the equation RSA/W (cm^2 g^{-1}) = 8.98·W $(g)^{-0.35}$. The minimal volume of the air space contained by the sacculi (= residual volume) is determined by 0.5 RSA times the length of the

Fig. 5: Cross-section through lung of *Eurypelma californicum*, showing pillar cells (p) and haemocyte (h) nearby in the haemolymph space, and the cuticular struts (cs) in the air space. There are 3 nuclei in the top half of the pillar, and 2-3 in the bottom half. The cuticular struts are interconnected distally, and the dorsal (upper) walls of the sacculi are detached from the supporting struts. Note the extreme thinness of the diffusion barrier (cytoplasm + cuticle) which is estimated here at less than 1 micron. Phase contrast. Scale line = 10 μm.

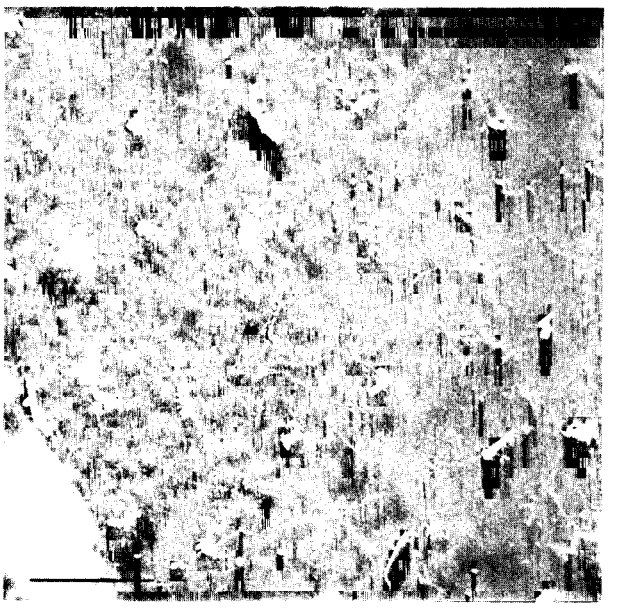

Fig. 7: Dissected lamella seen from its haemolymph face to show regular spacing of cell pillars. SEM. Scale line = 100 μm.

W (g)	No. lamellae (mm^{-1})	RSA (cm^2)	RSA/W ($cm^2\,g^{-1}$)	Strut length* (μm)	AS (μl)	AS/W ($\mu l\,g^{-1}$)	HS (μl)	HS/W ($\mu l\,g^{-1}$)
7.6	38	36.0	4.75	5.22	9.4	1.24	37.9	4.99
8.5	38	33.2	3.91	5.35	8.9	1.05	34.8	4.09
9.6	38	40.0	4.17	5.49	11.0	1.15	41.6	4.33
15.9	36	45.0	2.83	6.10	13.7	0.86	48.6	3.06
16.4	36	58.8	3.58	6.14	18.1	1.10	63.4	3.86
16.5	36	52.8	3.20	6.15	16.2	0.98	56.9	3.45
18.6	35	68.4	3.68	6.31	21.6	1.16	76.2	4.10

Table 1: Morphometric data of tarantula (*Eurypelma californicum*) lungs. W = weight, RSA = respiratory surface area (all 4 lungs), AS = air space (residual volume), HS = haemolymph space.
*calculated from the formula given by Anderson & Prestwich (1980) and using the factor 0.8 for four-lunged spiders.

cuticular struts. According to Anderson & Prestwich (1980) strut length is given by the formula, strut length (μm) = 0.8·W $(mg)^{0.21}$ in four-lunged spiders. We have used this formula and calculated the minimal air spaces to range from *c.* 9-22mm^3; these values are directly related to body weight. The haemolymph space (HS = 0.5·RSA·[(No. of lamellae)$^{-1}$–strut length]) on the other hand increases less than body weight, the regression equation being HS (mm^3) = 8.30·W $(g)^{0.71}$. If related to an average blood content of 18.1% (v/w) (Stewart & Martin, 1970), the fraction of haemolymph contained in the lungs is 2.20 ± 0.31% (n = 7) of the total haemolymph, calculated from the data in Table 1.

It must be remembered that the cuticular struts occupy relatively little space on the floor of each sacculus, but that the trabeculae, when in contact with the ceiling, occupy from 20-30% of the area. Hence, the RSA as estimated above, may be too high by 10-15%. However, if the sacculi widen by ventilatory movements, and hence the ceiling lifts off the trabeculae, the argument holds only during the period of expiration, i.e. as long as the struts and trabeculae support the sacculus ceiling.

Discussion

Fine structure

At regular intervals throughout the haemolymph space of the book lung, cellular pillars extend between the lamellae. The number of cells forming these pillars may vary from species to species. While Berteaux (1889) described one or two only, we observed four nuclei or more. The primary role of these perikarya is probably to provide the necessary elements for the metabolism of the hypodermis, but their grouping into pillars suggests an additional function. A contractile action was dismissed because it should have caused an undulating movement of the walls, causing convection in the air space. We believe that these pillars serve as spacers to keep the lamellae apart. While the haemolymph pressure would prevent the air spaces from expanding, and might limit them to their minimal volume, there is a priori no reason why the lamellae should maintain more or less the same distance from each other throughout the lung. They might equally well become closely stacked in one place, and fall wide

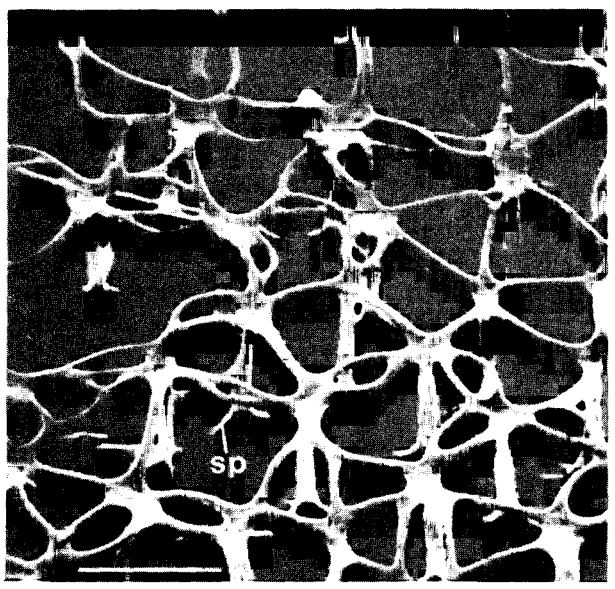

Fig. 8: Oblique view of cuticular struts, showing spurs (sp) at their base and elaborate network of connecting trabeculae radiating from the end plates of the struts. SEM. Scale line = 5 μm.

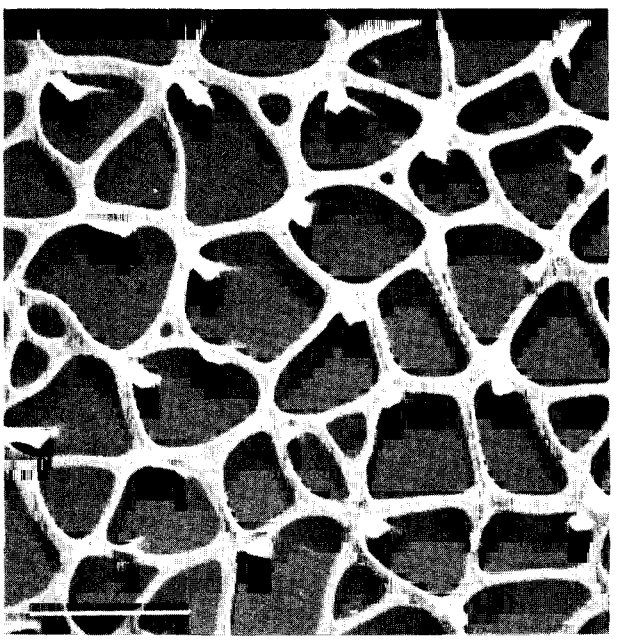

Fig. 9: Top view of trabecular network which supports the dorsal sacculus wall. Area covered is 29% in this sample. SEM. Scale line = 5 μm.

apart in others. The pillar cells may be extended or compressed to a certain extent but definitely serve to keep the lamellae apart and thus ensure a homogeneous perfusion of the lung. The cell bodies may cause some turbulence in the haemolymph flow, which would also be advantageous for gas exchange.

It is probable that the hexagonal fields seen in the lung hypodermis represent the extent of the cytoplasm belonging to each pillar. These fields have already been observed by MacLeod (1884, quoted by Berteaux, 1889: 284) who wrote, ". . . at the surface of the pulmonary lamellae polygonal areas can be seen . . . We consider these areas to be the borders of cells having extremely thin lamelliform attachments to the internal surface of the chitinous cuticle of the pulmonary lamellae. Towards the centre of each of the above-mentioned areas the nucleus of the cell can be found, surrounded by a small amount of protoplasm . . .". Berteaux (1889) argues against this interpretation but is probably misled by his own observation of a network of cuticular reinforcements connecting the bases of the cuticular spines. We regard these hexagonal fields and the cells pertaining to them as being the hypodermal units of the spider lung.

The cuticular struts (or spines) projecting into the air space from the bottom of each sacculus, are connected at their distal ends by a network of trabeculae. At the posterior edge of the sacculus, where it opens into the atrium, this branching and anastomosing seems to be typical in all spiders examined so far. However, its occurrence over the greater part if not all of the sacculus area may be a characteristic of the Theraphosidae (although only a few species have been examined). As a support to the upper sacculus wall this network would make sense only if a corresponding network existed at the base of the cuticular struts, since the haemolymph pressure is identical from both sides. Such

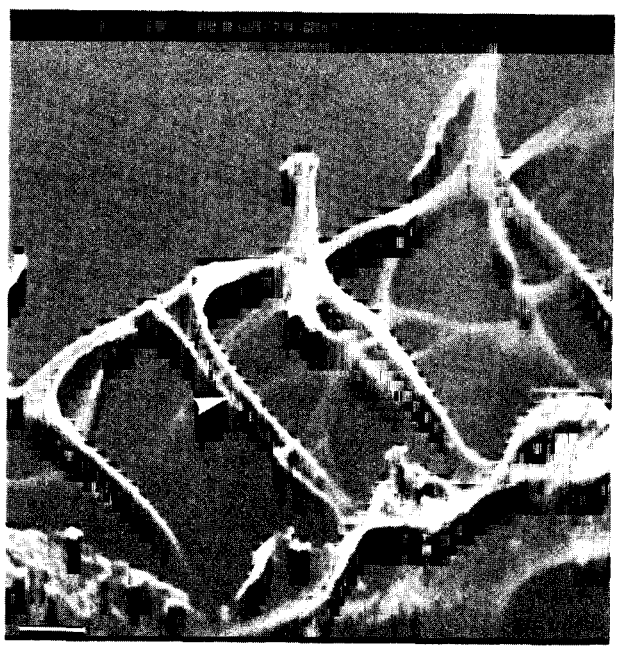

Fig. 10: Sacculus wall cut with a razor blade. One of the cuticular struts torn, revealing hollow structure (arrow). Note surface of the haemolymph space forming funnels into the bases of the struts. SEM. Scale line = 1 μm.

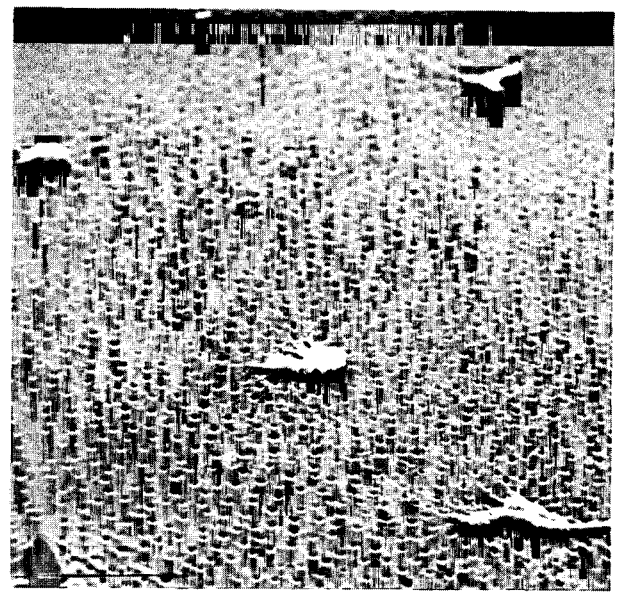

Fig. 11: Haemolymph face of the ventral sacculus wall (i.e. ventral surface of lamella), showing remnants of pillar cells and funnel-shaped depressions leading into the (bases of) the struts. SEM. Scale line = 20 μm.

a basal network indeed exists, as indicated by the spurs radiating from the cuticular struts in Figs. 8, 9. This basal network is certainly formed by bundles of microfibrils as described by Peters (1969). Near the edges and towards the tips of the sacculi (i.e. away from the atrium), the cuticular struts are unbranched or branch very little.

The cuticular struts are hollow (Fig. 10), a fact already seen by Berteaux (1889) but which has received no attention since. Berteaux maintains that this lumen is separated from the haemolymph space by a thin membrane. This might possibly be an optical artifact.

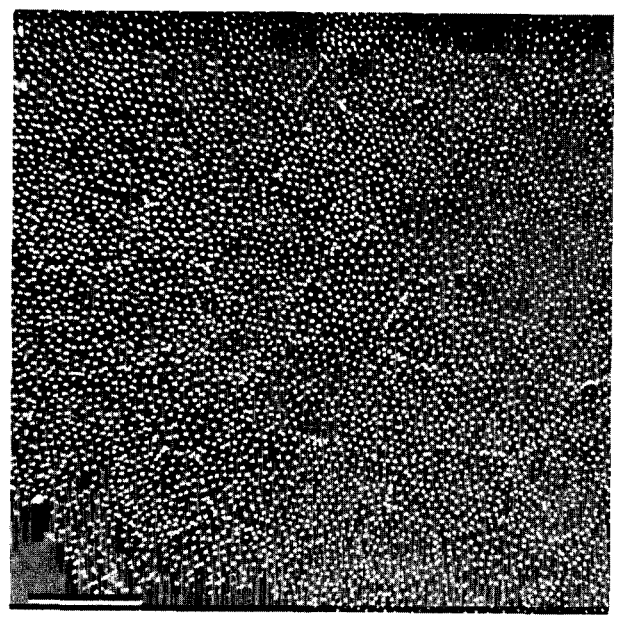

Fig. 12: Ventral sacculus wall (i.e. dorsal surface of lamella), out of focus to show polygonal fields subdividing the hypodermis. These fields are thought to represent the hypodermal cytoplasm belonging to a pillar in the haemolymph space. The white dots correspond to cuticular struts. Phase contrast. Scale line = 50 μm.

The funnel-like openings from the haemolymph space into the bases of the struts suggest that either cytoplasm and/or haemolymph may enter the interior of the struts.

Lung morphometry

Anderson & Prestwich (1980), from a comparison of 32 specimens belonging to 11 species, found that the height of the air space, as determined by cuticular strut length, scaled to the one-fifth power of body mass, while length and width of the sacculi scaled to the one-third power, i.e. isometrically. They concluded that respiration occurred by a combined ventilation-diffusion mechanism.

The chitinous lining of the sacculi is the greatest diffusion barrier between the outside air and the haemolymph. It is of no consequence then if the height of the sacculi (= strut length) is increased as long as diffusion dominates also within the sacculi. In a diffusion lung, selection pressure is on respiratory surface area, i.e. on the number of sacculi per mm. This value was almost constant in the specimens examined by us. It may be determined by the flow properties of the haemolymph, including haemocyte dimensions.

The relation between respiratory surface area (RSA) and body weight followed the equation RSA (mm^2) = $1.00 \cdot W\ (mg)^{0.83}$ (all spiders examined) in Anderson & Prestwich's (1982) work. Our value for the exponent is lower, 0.65, in accordance with the value (0.67) determined by Strazny & Perry (1984) and the value of 0.71 obtained by Greenstone & Bennett (1980). Unfortunately, the body weights of our animals are not well spaced, falling into two groups around 8.5 and 16.8 g (in Strazny & Perry's work, however, sample size is only three). The specific RSA (area per unit body weight) in tarantulas decreases with size, being $430\,mm^2\ g^{-1}$ in an 8.6 g animal and $330\,mm^2\ g^{-1}$ in a 16.8 g animal. These values are higher than those reported by Anderson (1970) for two-lunged spiders, but may be typical for the four-lunged species which are devoid of tracheae.

The minimal air space (= residual lung volume) was calculated from 0.5 RSA times cuticular strut length as predicted from Anderson & Prestwich's (1980) formula (since we did not have measurements from all our specimens), and ranged between 9 μl in an 8 g tarantula and *c.* 22 μl in an animal weighing 17 g. Thus, residual lung volume scales in almost direct proportion to body weight.

Conclusions

The present results support Anderson & Prestwich's (1980) conclusions, but it must be emphasised that for *Eurypelma californicum* at least, diffusion as the major respiratory mechanism has now been experimentally proven (Paul, Fincke & Linzen, 1987). Under resting conditions only a small fraction of the gas exchange capacity of the tarantula lung is utilised. The spiracles remain (almost) closed for most of the time and the arterial P_{O_2} is around 28 Torr (Angersbach, 1978). The spiracle width is the limiting diffusion barrier. The lung characteristics become critical only during, or following activity, when the spiracles are fully opened. Pa_{O_2} then rises to *c.* 75 Torr. The limiting diffusion barrier is now represented by the cuticle. Diffusion through the air space of the sacculi never becomes a critical factor. The calculations by Paul, Fincke & Linzen (1987) were based on an assumed cuticle thickness of 0.9 μm and other conservative estimates. Before a true quantitative description can be given one must be able to measure the thickness of the cuticle with confidence.

Acknowledgements

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