The Relationship of Book Lung and Tracheal Systems in the Spider
Family Uloboridae

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ABSTRACT The book lung surface areas of representatives of six genera were measured and divided by spider prosomal and leg mass and by the product of prosomal length and width to obtain two indexes of book lung area. Both indexes are greatest in species that have the simplest tracheal systems and smallest in those with the most extensive tracheal systems. When species are ordered phylogenetically and the lung area indexes of their hypothetical ancestors computed, a transformational increase in lung area is indicated. Neither the lung area of a species nor its phylogenetic position is significantly related to the general moisture regime of its habitat. The number of leaves in a book lung is positively correlated with both spider size and total lung area. However, leaf number is not correlated with tracheal development and shows no transformational change.

The Tracheospira (sensu, Platnick, '77) contains spiders that have two respiratory components: a pair of book lungs that oxygenate the hemolymph and a tracheal system that carries oxygen directly to tissues. A variety of spider tracheal patterns have been documented and their adaptiveness discussed (Bromhall, '87; Opell, '87a). However, the relationship between these patterns and book lung development has not been studied.

Spider book lungs reside in the anterior, ventral region of the opisthosoma. Each lung is formed of a stack of elongate, flattened, cuticle-lined leaves, between which hemolymph circulates. The air space between the two lamellae of each leaf is maintained by cuticular struts and communicates with a common atrium that opens to the outside via a spiracular slit (Levi, '67; Moore, '76; Anderson and Prestwich, '80; Hechter, '82). The width of the interlamellar air space within each leaf shows a negative allometric relationship with spider mass, an apparent adaptation to reduce the distance over which oxygen must diffuse to reach the hemolymph (Anderson and Prestwich, '80).

Members of the family Uloboridae exhibit the full range of tracheal development found in spiders (Opell, '79, '87a; Bromhall, '87). The orb-weaving genera Waitkera and Uloborus, the triangle-weaving genus Hyptiotes, and the irregular-web-weaving genus Miagrammopes retain the family's plesiomorphic pattern, characterized by two stout tracheal trunks that extend through the pedicel, into the prosoma, and branch to enter the legs. Tracheae entering the prosomae of Hyptiotes and Miagrammopes have diameters that are, relative to combined prosomal and leg masses, nearly twice those of Waitkera and Uloborus (Table 1). This difference is attributed to the fact that they more actively monitor and manipulate their reduced prey capture webs (Opell '87a,b). Orb-weavers of the genera Octonoba and Philoponella have tracheae that are restricted to the opisthosoma (Opell, '79). Opell ('79) reported that the tracheoles in Octonoba extend into the prosoma, but none appeared in cross sections through the pedicels of North American Octonoba sinensis used in this study (unpublished observations).

Comparisons of the relative book lung surface areas in these taxa make it possible to determine how tracheal development, activity patterns, and phylogenetic position influence book lung development. If spiders with more extensive tracheal systems have greater book lung surface areas than those with more weakly developed tracheae, this would suggest that both systems act in consort to supply a spider's increased oxygen demands. If more extensive tracheal systems are associated with smaller book lung surface areas, this would indicate that the tracheae meet more acute or more specific oxygen demands (such as those imposed by web-monitoring behaviors) and, therefore have assumed a greater role in meeting a spider's total oxygen demands. Any transformational changes in book lungs of these species should be apparent when their relative surface areas are ordered phylogenetically.
<table>
<thead>
<tr>
<th>Tracheae, web type, species</th>
<th>Do not enter prosoma</th>
<th>Enter prosoma</th>
<th>Reduced-web</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. tingeri</td>
<td>O. sinensis</td>
<td>W. waitakeriensis</td>
</tr>
<tr>
<td>Pedicle tracheal area²/ prosomal and leg mass</td>
<td>0</td>
<td>0</td>
<td>410</td>
</tr>
<tr>
<td>Carapace length (µm)</td>
<td>1,460 ± 158 (10)</td>
<td>1,484 ± 126 (10)</td>
<td>1,252 ± 88 (5)</td>
</tr>
<tr>
<td>Carapace width (µm)</td>
<td>1,358 ± 145 (10)</td>
<td>1,384 ± 134 (10)</td>
<td>1,168 ± 70 (5)</td>
</tr>
<tr>
<td>Prosomal and leg mass (mg)</td>
<td>2.84 ± 0.95 (10)</td>
<td>3.43 ± 1.25 (10)</td>
<td>2.17 ± 0.26 (7)</td>
</tr>
<tr>
<td>Book lung leaves</td>
<td>29.4 ± 6.3 (10)</td>
<td>30.9 ± 6.7 (9)</td>
<td>17.2 ± 2.8 (5)</td>
</tr>
<tr>
<td>Number of lung leaves/ prosomal and leg mass (mg)</td>
<td>10.4 ± 2.4 (10)</td>
<td>9.2 ± 2.2 (9)</td>
<td>8.2 ± 2.5 (5)</td>
</tr>
<tr>
<td>Book lung area (mm²)</td>
<td>6.97 ± 2.55 (10)</td>
<td>7.47 ± 2.76 (9)</td>
<td>2.53 ± 0.47 (7)</td>
</tr>
<tr>
<td>Lung area (mm²)/ prosomal and leg mass (mg)</td>
<td>2.35 ± 0.51 (10)</td>
<td>2.13 ± 0.33 (9)</td>
<td>1.19 ± 0.31 (5)</td>
</tr>
<tr>
<td>Lung area (µm³)/ carapace length × width</td>
<td>0.83 ± 0.20 (10)</td>
<td>0.87 ± 0.17 (9)</td>
<td>0.44 ± 0.08 (5)</td>
</tr>
<tr>
<td>Live spider mass (mg)</td>
<td>—</td>
<td>14.52 ± 3.63 (20)</td>
<td>—</td>
</tr>
<tr>
<td>Predicted interlamellar air space width (µm)</td>
<td>—</td>
<td>1.6</td>
<td>—</td>
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<tr>
<td>Measured interlamellar air space width (µm)</td>
<td>1.5 ± 0.1 (5)</td>
<td>1.3 ± 0.1 (5)</td>
<td>1.2 ± 0.1 (5)</td>
</tr>
</tbody>
</table>

¹Mean values from Opell, '87a.
²Mean values from Opell, '87b.
METHODS AND MATERIALS

Adult females, representing 6 of the 18 described uloborid genera studied: the orb-weavers Waitkera waitakeriensis (Chamberlain, '46) from northern New Zealand, Uloborus glomosus (Walckenaer, 1841) from Virginia, Octonoba sinensis (Simon, 1880) an introduced Asian species collected in Virginia, and Philoponella tingena (Chamberlin and Ivie, '36) from Panama and Colombia; the irregular-web weaver Miagrammopes animotus (Chickering, '68) from Puerto Rico; and the triangle-web weaver Hyptiotes cavatus (Hentz, 1847) from Virginia. These species were identified using the following revisions (Muma and Gertsch, '64; Chickering, '68; Opell, '79; and Yoshida, '80).

The anterior region of the opisthosoma containing the book lungs was removed, cleared in hot 10% (weight/volume) NaOH to remove non-chitinous tissue, rinsed in water, and stained to make the thin book lung leaves visible. One book lung from each specimen was dissected in a drop of mounting medium and its isolated leaves flattened under a coverslip.

The surface areas of the leaves of a book lung were measured with a digitizing tablet, summed, and this value multiplied by four to obtain a spider's total book lung surface area. The width of the air space separating a leaf's lamellae was measured from compound microscope photographs of a folded leaf (Anderson and Prestwick, '80). The shape of a species' book lung leaves was reconstructed from outline drawings of one of the three largest leaves of five specimens of each species. A midline was drawn from a leaf's tip to the center of a line connecting the corners of its base. The perpendicular distance from this midline to the leaf's left and right margins was measured at 15 equidistant points (Fig. 2). Mean values were used to reconstruct leaf shapes.

Two indexes of spider size were used for comparisons: 1) the product of prosomal length and width and 2) combined prosomal and leg masses. The former index accounts for differences in uloborid prosomal shape (Opell, '84) and the latter for differences in a spider's feeding and the mass of forming eggs in its opisthosoma. For example, in Octonoba sinensis, prosomal length is poorly correlated with live weight (r = 0.60, P > 0.05, N = 9) but more strongly correlated with combined prosomal and leg mass (r = 0.94, P = 0.0005, N = 9).

Specimens of Philoponella tingena were stored in ethanol and transferred to buffer one day before being weighed. All others were fixed in 3% glutaraldehyde/3% formaldehyde and stored in sodium cacodylate buffer. This treatment leaches little material from tissue, has little effect on mass, and coagulates hemolymph, preventing its loss when a specimen's pedicel is severed. Prior to weighing, specimens were blotted and air dried for 30 seconds. The masses of eight Miagrammopes animotus so treated averaged 103% (range 95–105%) of their live weights.

Species were assigned to one of three groups of increasing tracheal development, one of four phylogenetic groups, and one of three habitat groups of increasing moisture availability (Fig. 1). Within the limited context of this study, Hyptiotes and Miagrammopes and Philoponella and Octonoba are sister taxa, making it inappropriate to assign one member of the pair a more advanced status than the other. One factor analysis of variance (ANOVA) and Kruskal-Wallis K-sample ranking (K-W) tests were used to determine if tracheal type and habitat explained the differences in relative book lung surface area.

Phylogenetic tracts were evaluated using ANOVA tests, K-W tests, and transformational analysis. The latter approach infers from terminal (living) taxa the states of hypothetical ancestral taxa and determines if these states exhibit directional changes indicative of an evolutionary trend (Lauder, '81; Huey, '87; Huey and Bennett, '87). Hypothetical ancestors are used in an attempt to neutralize the unique selective forces that have operated on each terminal taxon since its origin. I used the following scheme of iterative averaging to determine the states of hypothetical ancestors H1-H4.

\[
\begin{align*}
H_1 &= \text{Hyptiotes + Miagrammopes/2} \\
H_2 &= (H_1 + \text{Waitkera}) + (H_1 + \text{Uloborus}) \\
&\quad + (\text{Waitkera + Uloborus})/6 \\
H_3 &= (H_1 + \text{Uloborus}) + (\text{Uloborus + Octonoba}) \\
&\quad + (H_1 + \text{Octonoba})/6 \\
H_4 &= (\text{Uloborus + Octonoba}) + (\text{Octonoba + Philop.}) \\
&\quad + (\text{Uloborus + Philop.})/6.
\end{align*}
\]

RESULTS

Table 1 presents mean values for the six species studied. Habitat differences do not confound the interpretation of these results. Neither the tracheal patterns of the six study species nor the two indexes of relative book lung surface area are affected by habitat (ANOVA P > 0.18). Likewise, phylogenetic position has no effect on the habitat of a species (ANOVA P < 0.35). The three tracheal groups of increasing tracheal development are characterized by decreasing book lung surface area. When book lung surface area is divided by combined prosomal and leg mass, the mean ratios for tracheal groups 1–3 are 2.25, 1.64, and 1.33 mm²/mg, respec-
Fig. 1. Phylogenetic, tracheal, and habitat categories of the genera studied. To the right of H₁–H₄, estimated values are given for: 1) book lung area relative to prosomal and leg mass (upper), 2) book lung area relative to the product of prosoma length and width (middle), and 3) the number of book lung leaves relative to prosomal and leg mass (lower). Tracheal categories: 1) tracheae restricted to the opisthosoma, 2) smaller tracheae enter the prosoma and legs, 3) stout tracheae enter the prosoma and legs (Opell '79, '87b). Habitat categories: 1) dry temperate shrubbery and forest underbrush, 2) moist temperate forests and buildings, and 3) wet tropical forests (Comstock '40; Forster '67; Opell '79, '82, '87a,b; Peaslee and Peck '83; David Court and Denis Gibbs, personal communication).

<table>
<thead>
<tr>
<th>TRACHEAL PATTERN</th>
<th>HABITAT</th>
<th>PHYSOGENETIC POSITION</th>
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<tbody>
<tr>
<td>2</td>
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</table>

When all six species are considered, the number of leaves per book lung correlates positively with spider prosomal and leg mass (r = 0.76, P < 0.0001, N = 47) and with total book lung area (r = 0.91, P < 0.0001, N = 47). Therefore, mass-specific indexes (Table 1) were used in comparisons of leaf number. A transformational analysis of the number of leaves per prosomal and leg mass shows an average change of only 3% (Fig. 1). Likewise, ANOVA tests show an insignificant effect (P > 0.51) of the three tracheal and four phylogenetic categories on the ratio of book lung leaves to prosomal and leg mass.

The shape of the six species' book lung leaves is similar, although those with greater book lung areas and more leaves have narrower leaves (Fig. 2). When the mean value of a leaf's 16 width measurements is divided by its length, the resulting index is inversely related to both the number of a specimen's book lung leaves (r = -0.55, P < 0.002, N = 30) and to its book lung area (r = -0.41, P < 0.024, N = 30). However, this index of leaf width is not correlated with a specimen's combined prosomal and leg mass (P < 0.11).

**DISCUSSION**

In the Uloboridae, increased book lung area is achieved by increases in leaf number and area. Tracheae and book lungs are complementary...
respiratory mechanisms; when one system is better developed the other provides less of a spider's total oxygen. The inverse relationship between these two systems suggests that their development is governed not only by a spider's total respiratory demands, but also by the specificity of these demands.

The tracheae of *Hyptiotes cauatus* and *Mia grammopes aninxotus* extend into the prosoma and legs (Opell, '87a), where they can directly meet the oxygen demands imposed by these spiders' active web monitoring and manipulation tactics (Lubin et al., '78; Lubin, '86; Opell, '87b). These species' smaller relative book lung areas probably supply their non-locomotor respiratory demands. In contrast, the tracheae of *Octonoba sinensis* and *Philoponella tingena* are confined to the opisthosoma, requiring all locomotor oxygen demands to be met by hemolymph-borne oxygen. These spiders' book lungs must meet both locomotor and non-locomotor respiratory demands. Not surprisingly, these orb-weavers have the largest relative book lung areas of the six species studied.

Within the Uloboridae, there is a transformational increase in book lung area. This is seen despite the fact that book lung area is directly related to metabolic rate (Anderson, '70; Anderson and Prestwich, '82). However, this measurement does not include the area of fine tracheoles across which oxygen diffuses directly into tissues and, therefore, underestimates total respiratory surface area, particularly of spiders with well developed tracheal systems. Therefore, it is inappropriate to conclude that ul oborids with the greatest book lung areas also have the highest metabolic rates.

Across spiders of different sizes, book lung area is directly related to metabolic rate (Anderson, '70; Anderson and Prestwich, '82). However, this measurement does not include the area of fine tracheoles across which oxygen diffuses directly into tissues and, therefore, underestimates total respiratory surface area, particularly of spiders with well developed tracheal systems. Therefore, it is inappropriate to conclude that ul oborids with the greatest book lung areas also have the highest metabolic rates.

On average, Anderson and Prestwich's ('80) formula: width of interlamellar air space = 0.90 body mass0.621 predicts to within 10% the mean interlamellar separation of the four species whose adult masses are known (Table 1). Considering that the standard deviations of the six species' measured interlamellar separations average 12% of their mean values, the generality of Anderson and Prestwich's model is supported by this study.

Orb-weavers of the families Uloboridae and Araneidae have similar relative book lung surface areas. The four large araneids studied by Anderson and Prestwich ('82) [Acanthepeira stellata, Argiope aurantia, Mecynogea lemniscata, and Metazygia wittfeldae; mean mass of about 382 mg] had a mean value of 0.54 mm² of book lung area per milligram of live mass. The
uloborid *Octonoba sinensis*, with a poorly developed tracheal system similar to those found in the four araneid species, has a value of 0.51 mm²/mg. *Uloborus glomosus*, with a more extensive tracheal system, has a value of 0.38 mm²/mg. However, before it can be concluded with certainty that the metabolic costs of operating an orb-web is similar in both families, the lung areas of smaller araneid species must also be measured.

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**LITERATURE CITED**