Influence of Web-Monitoring Tactics on the Density of Mitochondria in Leg Muscles of the Spider Family Uloboridae

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ABSTRACT From electron micrographs we determined the ratio of mitochondrial to myofibril cross sectional area in cells of the first leg anterior depressor muscles of adult females of four spider species, each from a different genus. Species with more active web-monitoring tactics and greater tracheal supplies to their first legs have muscle cells that are better supplied with mitochondria than those with less active tactics and less well-developed tracheal systems. These results demonstrate that, even in homologous tissues of closely related species, mitochondrial supply can change to accommodate changes in metabolic activity. © 1992 Wiley-Liss, Inc.

Mitochondria are the sites of oxidative phosphorylation and are found in greater numbers in more active tissues. For example, in insects mitochondrial density is low in small visceral muscles and inactive fat cells (Dean et al., '85; Smith, '68; Sohal, '73) and high in metabolically active tissues such as flight muscles (Chapman, '54; Edwards and Ruska, '55; Levenbook and Williams, '56; Smith, '62), Malpighian tubules (Smith, '68; Wigglesworth and Salpeter, '62), and anal papillae (Copeland, '64). The purpose of this study is to determine if changes in mitochondrial density accompany diverging activity levels in homologous arthropod muscles. We do this by comparing the mitochondrial supplies of the leg muscles of spiders that have different web-monitoring tactics.

The orb-web is the primitive web form in the family Uloboridae (Coddington, '86, '90; Opell, '79). Members of orb-weaving genera such as Uloborus and Octonoba hang beneath the hubs of their horizontal webs while waiting for prey (Lubin, '86; Cushing and Opell, '90; Peaslee and Peck, '83). In contrast, species that construct reduced webs more actively monitor and manipulate them during prey capture. Members of the genus Hyptiotes spin taut, vertical triangle-webs which they monitor from the anchoring point of the web's apex thread (Lubin, '86; Opell, '82). Members of the genus Miagrammopes construct irregular webs that have one to eight prey capture lines that diverge at varying angles (Lubin, '86; Lubin et al., '78; Opell, '90a). Like Hyptiotes, these spiders use a

single monitoring line and shake the web when a prey strikes it (Opell, '87a). In both orb-web and reduced-web spiders, the first legs play an important role in web monitoring and manipulation.

As summarized in Table 1, previous studies show that these differences in web-monitoring tactics are reflected in both the webmonitoring force expressed by a species and the degree of its tracheal development (Opell, '87a,b). Hyptiotes cavatus has the greatest absolute and relative resting forces (force exerted on a single, resting thread) and also the greatest tracheal supply to its first legs (i.e., the smallest volume of tissue served by each μm^2 of tracheal cross section). Uloborus glomosus has the next greatest resting force and the next greatest first leg tracheal supply, followed by Miagrammopes animotus. The tracheal spiracle of uloborids is near the abdomen's posterior tip and the long abdomen of *M. animotus* reduces the first leg's oxygen supply by increasing tracheal length and, thereby, the distance over which oxygen must diffuse. The resting force expressed by Octonoba sinensis has not been measured and attempts to do so for this study proved unsuccessful, as spiders hung for only a few seconds on resting threads and failed to establish stable resting forces. However, as this species' tracheal system is restricted to its abdomen (Opell, '90b) and its prosomal and leg muscles are supplied solely by hemolymph borne oxygen, it probably exerts less monitoring force than the other three species.

	Hyptiotes cavatus	Uloborus glomosus	Miagrammopes animotus	Octonoba sinensis
Leg I volume \times 10 ⁴ μ m ^{3 1}	27,438	48,822	104,188	
Prosomal and leg weight mg ³	1.49	1.91	2.38	3.43
Resting force N \times 10 ⁻⁵ ²	13.42	10.72	7.09	-
Resting force/leg I volume $\times 10^{-4}$ /mm ³	4.89	2.02	0.68	_
Resting force/prosomal and leg weight	9.01	5.61	2.89	-
Tracheal area serving leg I µm ²	387	289	891	0
Leg I volume/leg I tracheal area/square root distance from spiracle to leg I^1	3,239	8,565	7,180	0

TABLE 1. Comparison of the mean leg volumes, masses, tracheal areas, and resting forces of adult female uloborids

³Opell, '90.

Because the first legs are important in web monitoring, the activity level of their muscles, as predicted by a species' resting force and tracheal supply, should be reflected in their mitochondrial supply. This study will test the hypothesis that the density of mitochondria in first leg muscles is ordered, from greatest to least, as follows: *Hyptiotes cavatus, Uloborus glomosus, Miagrammopes animotus, Octonoba sinensis.*

MATERIALS AND METHODS

Adult females of the following species were used in this investigation: the introduced Asian orb-weaver, Octonoba sinensis (Simon), collected from free ranging populations in greenhouses at Virginia Polytechnic Institute and State University (VPISU); the eastern North American orb-weaver, Uloborus glomosus (Walckenaer), collected from shrubbery on the VPISU campus; the eastern North American triangle-web-weaver, Hyptiotes cavatus (Hentz), collected in the forests of Montgomery and Craig Counties of Virginia; and the Puerto Rican "single-lineweaver," Miagrammopes animotus Chickering, '68, collected at the Center for Energy and Environment Research's El Verde field station in the Luquillo National Forest. These species were identified by reference to the taxonomic revisions of Chickering ('68), Muma and Gertsch ('64), Opell ('79), and Yoshida ('80).

Specimens were anesthetized with carbon dioxide, fixed at $20-26^{\circ}$ C for 12-18 hr in 3%glutaraldehyde/3% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3), and rinsed in 0.1 M sodium cacodylate buffer. First legs were removed at the trochanter and their retrolateral and prolateral anterior dorsal depressor muscles (ADDM; Whitehead and Rempel, '59) were exposed. We chose these muscles because their large size and position make them easy to identify, isolate, and handle and because their role as flexors of the patella is important for the leg movements associated with web monitoring and manipulation.

The ADDM were post-fixed for 30 min in 1% osmium tetroxide, washed with sodium cacodylate buffer for 5 min, and dehydrated through a series of ethanol dilutions. Muscles were then infiltrated with Spurr's epoxy resin (hard formula) and polymerized for 24 hr at 65°C. Thin cross and longitudinal sections were stained with lead citrate and uranyl acetate and examined and photographed at $\times 1,600-3,300$ with a transmission electron microscope.

Enlarged cross sectional photographs of individual muscle cells (Fig. 1) were measured with a digitizing tablet connected to a computer. We first determined the cell's total surface area (TA), the combined surface areas of its mitochondria (MA), the surface area of its central clear region where a nucleus was often found (CA), and the combined surface areas of the mitochondria found within the central clear region (CMA). We used the following formula to determine the area occupied by a cell's contractile elements (CEA):

CEA = TA - CA - (MA - CMA)

However, in addition to myofibril bundles, this cortex region (= CEA) also contains sarcoplasmic reticula, T-system tubules, and intracellular space (Figs. 1, 2). We determined the density of myofibrils within the CEA (MYD) by dividing the total area of a representative cortex region into the total area occupied by this region's myofibril bundles. We then multiplied each cell's CEA by its MYD to determine its corrected total myofibril surface area (CMYA). When CMYA is divided by MA it yields an index of how densely a cell's

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¹Opell, '87b. ²Opell, '87a.



Fig. 1. Cross section of the first leg anterior dorsal depressor muscle of *Uloborus glomosus*. CA, central clear area; M, mitochondria; MYO, myofibrils; N, nucleus; T, tracheole.

Fig. 2. Longitudinal section of the first leg anterior dorsal depressor muscle of *Uloborus glomosus*. M, mitochondria; MYO, myofibrils.

myofibrils are supplied by mitochondria. The larger this index, the greater the cell's mitochondrial supply.

This method assumes that the mitochondria of all species have similar shapes. If a species' mitochondria are greatly elongated in the longitudinal dimension (Fig. 2) this method might underestimate that species' mitochondrial surface area. We evaluated each species' mitochondrial shape by measuring the maximum and minimum dimensions of mitochondria in cross and longitudinal sections of muscle cells (Figs. 1, 2). We measured either all of the mitochondria in a cell (or, in the case of longitudinal sections, all of the cell shown in a photograph) or a subset of mitochondria chosen by placing a numbered grid over a photograph and using a random number table to select the mitochondria to be measured.

We determined the general shape of a species' mitochondria by comparing the ratio of its maximum to minimum mitochondrial dimensions in cross section with that measured in longitudinal section. If mitochondria are spherical then these ratios will be equal. If mitochondria are elongated along the muscle's length then the ratio from longitudinal sections will exceed that from cross sections.

The phylogenetic relationship of the genera represented in this study is shown in Figure 3. *Hyptiotes* and *Miagrammopes* form a monophyletic group and *Uloborus* and *Octonoba* form a paraphyletic group (Coddington, '90). We performed a transformational analysis of phylogenetic changes in the mitochondrial supply of the ADDM by using the scheme of iterative averaging (Huey and Bennett, '87) shown below, where H = Hyptiotes,



Fig. 3. Phylogenetic relationship of the four genera represented in this study, with their mean mitochondrial area to corrected myofibril area ratios and those for their hypothetical ancestors H_1-H_3 .

M = Miagrammopes, U = Uloborus, and O = Octonoba:

$$\begin{split} H_1 &= (H + M) + (H + (U + O/2)) + (M + (U + O/2))/6 \\ H_2 &= (U + O) + (U + (H + M/2)) + (O + (H + M/2))/6 \\ H_3 &= H_1 + H_2/2 \end{split}$$

We inferred the resting force and tracheal supply of the common ancestor of *Hyptiotes* and *Miagrammopes* from the mean values of Waitkera waitakerensis, an orb-weaver that is a primitive member of the outgroup of the four species included in this study, and Uloborus glomosus, the most primitive member of the outgroup of the *Hyptiotes-Miagram-*mopes clade (Coddington, '90). The values of U. glomosus resting forces and first leg tracheal supply are given in Table 1. Those for W. waitakerensis are: relative resting forces, $1.93~\times~10^{-4}~N/mm^3$ of leg I volume and 5.58×10^{-5} N/mg of prosomal and leg weight; relative tracheal supply, $10,519 \times 10^4 \,\mu m^3$ of leg I volume/ μ m² of tracheal cross section/ square root of distance from tracheal spiracle to leg I base in µm (Opell, '87b, '90b, '92).

RESULTS

Results are presented in Table 2. Because data were not normally distributed for all species, we used a Kruskal-Wallis k-sample test to determine if there were interspecific differences in these values. There were no significant differences (P > 0.145) in either the number of mitochondria per cell or the density of myofibrils in the cells' cortex. Mean mitochondrial area differed significantly among species, although one-tailed Wilcoxon two-sample tests showed that the values of Hyptiotes cavatus, Uloborus glomosus, and Octonoba sinensis did not differ significantly (P > 0.333) but that Miagrammopes animotus had a smaller value than each of the other species (P < 0.033).

Both total cell area and corrected myofibril area per cell differed significantly between species, although there was no interspecific difference in corrected myofibril area per cell. As hypothesized, the ratio of mitochondrial area to corrected myofibril area differed significantly between species and differs in the direction predicted by the hypothesis. Onetailed Wilcoxon two-sample tests show that this ratio is significantly greater in *H. cavatus* than in both *U. glomosus* (P = 0.013) and *M. animotus* (P = 0.007) and in *U. glomosus* than in *O. sinensis* (P = 0.015). However, the ratios do not differ significantly between ei-

Variable	Hyptiotes cavatus	Uloborus glomosus	Miagrammopes animotus	Octonoba sinensis	P value
No. specimens	6	4	5	4	
No. cells per specimen	4.8 ± 0.4	3.8 ± 1.0	5.2 ± 1.6	4.0 ± 2.5	_
No. mitochondria per cell	48.9 ± 23.1	37.3 ± 16.0	34.2 ± 17.6	19.1 ± 5.4	0.145
Area per mitochondria µm ²	0.90 ± 0.22	1.00 ± 0.51	0.40 ± 0.17	1.11 ± 0.42	0.022
Total cell area µm ²	287.6 ± 126.8	460.8 ± 132.2	367.4 ± 79.4	634.3 ± 149.6	0.013
Myofibril density in cortex	0.60 ± 0.13	0.64 ± 0.06	0.69 ± 0.12	0.65 ± 0.06	0.545
Corrected myofibril area µm ²	133.9 ± 69.2	242.6 ± 62.8	236.8 ± 86.4	350.6 ± 85.9	0.015
Corrected myofibril area/cell area	0.45 ± 0.12	0.54 ± 0.09	0.62 ± 0.15	0.55 ± 0.07	0.253
Mitochondrial area/corrected myo-	0.35 ± 0.10	0.15 ± 0.08	0.10 ± 0.11	0.06 ± 0.02	0.006
fibril area					
Max./min. mitochondria diameter					
In cross section	1.28 ± 0.09	1.42 ± 0.16	1.21 ± 0.05	1.24 ± 0.10	0.087
No. specimens	4	4	5	4	_
No. mitochondria per cell	20.0 ± 9.4	24.0 ± 10.6	10.0 ± 0.0	30.8 ± 27.2	_
In longitudinal section	1.50 ± 0.16	1.34 ± 0.12	1.40 ± 0.19	1.32 ± 0.04	0.398
No. specimens	4	2	4	4	
No. mitochondria per cell	26.3 ± 2.2	50.0 ± 0.0	12.5 ± 5.6	52.5 ± 22.3	-

TABLE 2. Comparison of mitochondrial densities in the first leg anterior dorsal depressor muscles of adult female Uloboridae¹

¹Mean values are followed by standard deviations. P values are for Kruskal-Wallis k-sample tests of species effects on the variables.

ther U. glomosus and M. animotus (P = 0.196) or M. animotus and O. sinensis (P = 0.357).

Kruskal-Wallis tests show no interspecific differences between the ratio of maximum-tominimum mitochondrial dimensions in either cross or longitudinal sections (Table 2). When one-tailed Wilcoxon two-sample tests are used to compare the cross and longitudinal section ratios of each species, significant differences are found in *H. cavatus* (P = 0.030) and M. animotus (P = 0.019), but not in U. glomosus (P = 0.409) or O. sinensis (P = 0.156).In *H. cavatus* the longitudinal section ratio is 1.17 times greater than the cross section ratio and in M. animotus it is 1.16 times greater. This indicates that in these species the mitochondria are not spherical but are elongated along the myofibril axes and that mitochondrial cross sectional area underestimates the total mitochondrial supply of their muscle cells.

To account for this difference, we recalculated mean total mitochondrial surface areas for *H. cavatus* and *M. animotus* muscle cells. We did this by treating each cell's total cross sectional mitochondrial surface area as that of a circle, determining its diameter, increasing this diameter by 1.17 or 1.16, respectively, determining the surface area of this larger circle, and then dividing this increased mitochondrial surface area by the cell's corrected total myofibril area.

As a result of these recalculations, the mean mitochondrial area to corrected myofibril area for *H. cavatus* increased to 0.47 ± 0.15 and

for *M. animotus* to 0.11 ± 0.13 . However, these corrected values do not change the study's outcome. A Kurskal-Wallis test still showed significant interspecific differences (P = 0.006); *H. cavatus* still had a greater ratio than either *U. glomosus* (one-tailed Wilcoxon test, P = 0.007) or *M. animotus* (P = 0.007); the ratios of *U. glomosus* and *M. animotus* do not differ significantly (P = 0.270); and the ratios of *M. animotus* and *O. sinensis* do not differ significantly (P = 0.357).

The transformational analysis presented in Figure 3 shows that the mitochondrial supply of the hypothetical ancestor of the four species included in this study was most similar to that of Uloborus. It also shows that this supply has increased in Hyptiotes and decreased independently in *Miagrammopes* and Octonoba. Resting force and tracheal supply values for the hypothetical ancestor of Hyptiotes and Miagrammopes are: relative resting forces, $1.98 \times 10^{-4} \text{ N/mm}^3$ of leg volume and 5.60 \times 10⁻⁵ N/mg of prosomal and leg weight; relative tracheal supply, $9.545 \times 10^4 \ \mu m^3/\mu m^2/\mu m$. Like the mitochondrial density of this hypothetical ancestor (Fig. 3), these values are intermediate between those of Hyptiotes and Miagrammopes.

DISCUSSION

The results of this study support the hypothesis that the mitochondrial supply of uloborid first leg muscles complements the activity levels of this tissue. The density of mitochondria is greatest in the first leg muscles of species that exert the most resting force and have the best developed tracheal supplies to their first legs and is least in species that exert the least force and have the poorest tracheal development. These conclusions are not altered when the shape of each species' mitochondria is taken into account.

These differences in mitochondrial density may have been driven by changes in activity levels and tracheal patterns or existing differences in mitochondrial densities and tracheal supplies may have permitted only certain species to achieve greater levels of activity. Support for the former hypothesis comes from the contrasting mitochondrial densities, resting forces, and tracheal supplies of the sister genera Hyptiotes and Miagrammopes (Tables 1, 2) and from the fact that the hypothetical ancestor of these genera appears to have had mitochondrial and tracheal supplies that were intermediate for these genera. Such a condition could not preadapt this ancestor to the condition found in either extant genus. Instead, mitochondrial and tracheal supplies had to increase in *Hyptiotes* and decrease in Miagrammopes to accommodate the diverging web-monitoring forces associated with changes in the web architecture of these two genera.

Mitochondrial supply is known to increase during development to meet the increased metabolic demands of insect flight muscles (Brosemer et al., '63) and fat bodies (Sohal, '73). However, to our knowledge, this is the first study to demonstrate a phylogenetic change in the mitochondrial density of homologous arthropod muscles to accommodate the changing activity patterns of adults. A limited phylogenetic analysis (Fig. 3) suggests that mitochondrial density may be quite plastic. In the four genera of Uloboridae that we studied, one showed an increase and two showed independent decreases in mitochondrial density from the group's hypothetical ancestor.

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