

# Coadaptive changes in physiological and biophysical traits related to thermal stress in web spiders

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**Abstract** Web spiders are considered to have expanded their habitats from dim to bright environments during the evolutionary history. Because they are sedentary predators exposed to the sun, they may have developed a suite of adaptive traits to cope with thermal stress. We examined the critical thermal maximum, spectral reflectance of solar energy by the body surface, and surface–volume ratio (SVR) for 11 spider species. Analysis of the four genera having a pair of species inhabiting both bright and dim environments showed that species in bright environments exhibited higher lethal temperatures, but spectral reflectance and SVR did not differ. Independent contrasts using the 11 species indicated that critical thermal maximum was positively correlated with spectral reflectance and spectral reflectance was negatively correlated with SVR. These results suggest that physiological tolerance to high temperatures and a biophysical mechanism to reduce heat gain evolved jointly during the history of habitat expansion in araneoid spiders.

**Keywords** Lethal temperature · Spectral reflectance · Thermal adaptation · Comparative method

## Introduction

Arthropods living in open environments may be exposed to extreme thermal stress from full sunlight. To cope with this stress, arthropods must develop biophysical mechanisms to decrease radiative heat gain by reflecting solar radiation

(e.g., Pereboom and Biesmeijer 2003) and/or physiological adaptations by increasing lethal temperatures (e.g., Starrett and Waters 2007). Arthropods that experience severe heat stress in open environments may have jointly evolved increases in both biophysical and physiological traits (i.e., the abilities to reduce heat gain and increase lethal temperatures), although this is not necessarily the case in less severe conditions. An important factor that could affect the evolution of such traits is body size because surface–volume ratio (SVR) greatly affects heat loss, i.e., convective heat loss is higher in smaller insects due to a larger SVR, leading to reduced overall heat gain and lower body temperature (e.g., Willmer and Unwin 1981; Pereboom and Biesmeijer 2003). Accordingly, thermal adaptive traits are likely correlated with body size in environments with heat stress. However, few studies have examined the correlated evolution of traits related to thermal adaptation.

Orb-web spiders inhabit various light environments ranging from dim forest floors to full-sun grasslands. The ancestral habitat of orb-weavers is thought to be dark or dim environments, and several derived species have shifted their habitats to bright environments (Craig et al. 1994). This habitat expansion may have contributed in part to the adaptive radiation of araneoid spiders (Bond and Opell 1998) and allowed them to exploit novel prey (Miyashita and Shimazaki 2006). Because most diurnal orb-weavers sit motionless at the center of the web, those inhabiting bright environments with full sunlight may experience severe heat stress in the daytime. Some web spiders are known to reduce heat stress by orienting the long axis of their abdomen to the sun or by making stabilimenta as parasols (Humphreys 1992), but these behavioral changes appear to be insufficient to cope with severe heat stress (Pulz 1987). Therefore, we expect that orb-weavers might have increased their critical thermal maximum and/or increased

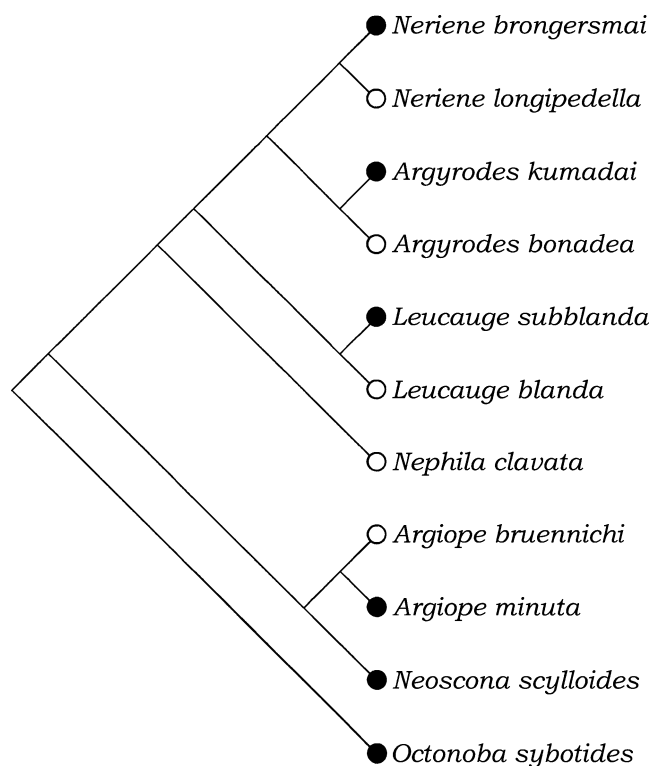
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their ability to reduce radiative heat gain during their evolutionary history of habitat expansion. To explore this possibility, we examined critical thermal maximum, visible and near infrared spectral reflectances of body surfaces, and SVR for ten araneoid and one deinopoid spider species. Because the spectral energy of sunlight is derived primarily from visible and near infrared spectra (Campbell and Norman 1998), this spectral reflectance represents an ability to reduce heat gain in full sunlight. We first analyzed the relationship between environment (dim vs. bright) and the traits of spiders to determine if spiders in bright habitats exhibit adaptive traits for heat stress. We further analyzed the evolutionary association between lethal temperature, spectral reflectance, and SVR in the 11 spider species. We expected that (1) spiders with a small SVR would have higher lethal temperatures and/or spectral reflection to cope with increased heat gain and (2) lethal temperature would exhibit a positive or no association with spectral reflectance, depending on the strength of the selective pressure of the thermal environment.

## Materials and methods

### Spiders

We examined ten araneoid species belonging to five families (Araneidae [*Argiope*, *Neoscona*], Tetragnathidae [*Leucauge*], Nephilidae [*Nephila*], Theridiidae [*Argyrodus*], and Linyphiidae [*Neriene*]) and one deinopoid species (Uloboridae [*Octonoba*]; Fig. 1). The phylogeny was constructed after Scharff and Coddington (1997) and Kuntner (2007). In each of four genera (*Neriene*, *Argyrodus*, *Leucauge*, and *Argiope*), the members of one pair of species, respectively, inhabit dim and bright environments. We defined bright environments as grasslands and forest edges in which spiders are exposed to the sun during the daytime and dim environments as the forest floor and understory in which spiders seldom receive full sunlight. All species are widely distributed in temperate areas in Japan with adult stages occurring in early to late summer. Experimental spiders were collected from Chiba and Saitama Prefectures in eastern Japan from June to August. The maximum temperature in summer occasionally reaches 35°C with even higher temperatures at exposed sites. Experimental spiders were primarily adult females with the exceptions of juveniles for *Nephila* and *Argiope* due to their extremely large adult body sizes. These differences in stages did not appear to affect the traits measured in this study because spiders do not exhibit metamorphosis (ametabola), and the species examined do not change their microhabitats with development.



**Fig. 1** Phylogeny of spider species used in this study with habitat type indicated by circles (white bright environment, black dim environments). All species other than *Octonoba* belong to the Araneoidea

### Trait measurements

**Critical thermal maximum** Although several indices have been used to measure physiological tolerance to high temperatures, we used the critical thermal maximum ( $CT_{max}$ ) because we were primarily interested in the ability of spiders to tolerate immediate increases in heat stress under full sunlight. Each individual collected from the field was confined in a 20-mL plastic cup with a small piece of gauze as a scaffold. Prior to measurements, individuals were held for 5 days at 25°C under long-day conditions (14 h light, 10 h dark) for acclimation and were fed artificial food containing sucrose and amino acids. A 5-day acclimation period could be justified in comparison with 2–7 days of other studies (Hoffmann and Watson 1993; Klock and Chown 2003). At the time of measurements, the cup containing a spider was placed into an incubator. An additional cup with an electric thermometer probe (Type T thermocouple, Model BAT-12, 0.1°C resolution, Physitemp Instruments) was also introduced into the same incubator to measure the temperature inside the cups. The temperature in the incubator was then raised at a rate of 0.1°C min<sup>-1</sup>, and the behavior of the spiders was observed; preliminary experiments revealed that body and air temperatures became nearly identical at this increasing rate. We defined

**Table 1** Traits of spiders related to thermal tolerance in eight species in four genera (mean±SE (*n*))

Spider genus	Environment (species)	CT <sub>max</sub> (°C)	Spectral reflectance (%)	SVR (m <sup>2</sup> /m <sup>3</sup> )
<i>Neriene</i>	Bright ( <i>longipedella</i> )	44.2±0.1 (10)	19.9±1.5	3.38±0.12 (5)
	Dim ( <i>brongersmai</i> )	41.0±0.1 (10)	14.7±1.1	3.65±0.43 (5)
<i>Argyrodes</i>	Bright ( <i>bonadea</i> )	46.9±0.1 (2)	22.2±1.7	3.64±0.20 (5)
	Dim ( <i>kumadai</i> )	44.4±0.0 (8)	22.0±1.7	3.13±0.34 (5)
<i>Leucauge</i>	Bright ( <i>blanda</i> )	44.0±0.0 (6)	24.0±1.8	1.99±0.19 (5)
	Dim ( <i>subblanda</i> )	42.4±0.1(6)	24.8±1.9	1.52±0.03 (5)
<i>Argiope</i>	Bright ( <i>bruennichii</i> )	51.7±0.2 (18)	25.1±1.9	0.74±0.03 (5)
	Dim ( <i>minuta</i> )	47.8±0.2 (12)	23.0±1.7	1.72±0.17(5)

CT<sub>max</sub> as the temperature at which spiders began to draw in their legs and were paralyzed. To maintain a high humidity, wet cotton was placed in the cups.

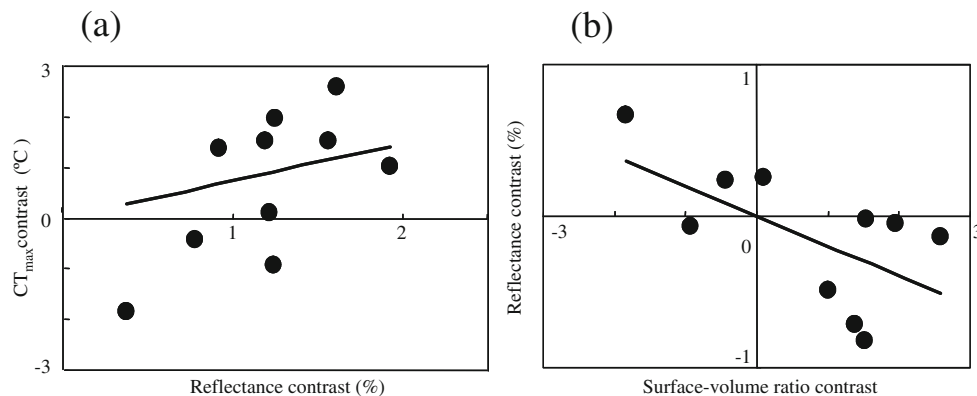
**Spectral reflectance of body surface** A spectrophotometer (JASCO V570) was used to measure the spectral reflectance of spider body surfaces at ranges from 400 to 2,200 nm, at intervals of 1 nm. Individuals killed with ethyl acetate were immediately mounted in a cell consisting of transparent quartz plates on two opposing sides (1 × 1 cm). Prior to mounting, spider legs had been cut off. To ensure that at least 95% area of the transparent plates was covered by the spider body surface, three to 40 individuals were mounted; all individuals directed the same side (dorsal/ventral) to one side of the cell. Spectral reflectance was measured from both sides of the cell (i.e., from dorsal and ventral sides). We observed that, in the wild, all species other than *Argyrodes* oriented the long axis (tip) of their abdomen to the sun, whereas *Argyrodes* exposed their ventral side to the sun. Thus, ventral-side reflectance was used for *Argyrodes* in subsequent calculations, while the mean reflectance of the two sides was used for the other species. The fraction of solar energy reflected by the spider body surface was then calculated using this value and the spectral distribution of standard sunlight (Japanese Industrial Standards Committee 1989). To estimate measurement

error caused by light diffusion, the spectral reflectance of rubber tubes of different sizes (large tube=8 mm diameter, 10 mm length; small tube=2 mm diameter, 3 mm length) were measured as references. The reflectance of each tube was measured five times, and their coefficients of variation (CV) were calculated. The CVs of large and small tubes were used for estimating measurement error for larger (*Argiope bruennichi*, *A. minuta*, *Leucauge blanda*, *L. subblanda*, and *Nephila clavata*) and all other (smaller) spider species, respectively, which were multiplied by the reflectance of each species to yield SE.

**Surface–volume ratio** As variation in spider body shape is mostly due to the difference in abdomen shape, we measured length (*l*) and width (*w*) of abdomen for five individuals of each species, and defined SVR as 2(*l*+*w*)/(*lw*), assuming abdominal shape to be a cylinder.

Analyses

First, to explore the hypothesis that environment type affects traits related to reducing thermal stress, we analyzed the difference in each trait between the congeneric species inhabiting different environments (dim vs. bright) using a randomized block-design ANOVA with “genus” as a



**Fig. 2** Relationship between **a** increment of spectral reflectance and that of CT<sub>max</sub> and **b** increment of SVR and that of spectral reflectance in spiders. Details are described in the text. Lines indicate regressions through the origin

random factor and “environment type” as a fixed factor. Second, we performed a comparative analysis by independent contrasts (CAIC; Purvis and Rambaut 1995) to detect evolutionary associations among traits using 11 species. This analysis uses the difference in a given trait between two pairs of species or higher nodes to control for the statistical problems arising from species relatedness. Using independent contrasts of trait variables, we conducted a regression through the origin (Purvis and Rambaut 1995).

## Results

Table 1 shows the traits of the spiders of the four genera that have a pair of species inhabiting dim and bright environments. The ANOVA indicated that  $CT_{max}$  was significantly different between species inhabiting dim and bright environments ( $F_{1,3}=30.41$ ,  $p=0.012$ ) with spiders from bright environments exhibiting a higher  $CT_{max}$  (about 1.5–4.0°C) (Table 1). However, both reflectance and SVR did not differ significantly between environments (reflectance:  $F_{1,3}=1.53$ ,  $p=0.305$ ; SVR:  $F_{1,3}=0.01$ ,  $p=0.860$ ; Table 1).

Comparative analysis by independent contrasts using 11 spider species gave ten contrasts for each trait. Regression analysis revealed that  $CT_{max}$  was positively associated with reflectance ( $t_8=2.41$ ,  $p=0.039$ ,  $r^2=0.300$ ), but not with SVR ( $t_8=1.49$ ,  $p=0.170$ ,  $r^2=0.141$ ). However, SVR exhibited a negative association with reflectance ( $t_8=-2.63$ ,  $p=0.027$ ,  $r^2=0.395$ ; Fig. 2).

## Discussion

The physiological tolerance to high temperatures as measured by  $CT_{max}$  was higher for spider species inhabiting bright compared to dim environments, and this pattern was consistent across the four araneoid genera studied. Because the ancestral state of this trait is assumed to be a lower  $CT_{max}$ , araneoid spiders appeared to have acquired an ability to tolerate heat stress throughout their evolutionary history of habitat expansion. Contrary to our expectations, spectral reflectance of solar energy by body surfaces as well as SVR did not differ between species inhabiting bright and dim environments. This result might have been caused by the type 2 error derived from the small sample size. This issue is resolved partly by the independent contrasts using more species (see next paragraph).

In terms of the correlated change of traits using 11 species of spiders,  $CT_{max}$  was positively associated with spectral reflectance by the body surface, indicating that these two traits have jointly evolved in spiders. This result suggests that selection pressure by heat stress in open

environments was so strong that spiders evolved both physiological thermal tolerance (higher  $CT_{max}$ ) and a biophysical method of reducing heat gain (by reflecting solar energy), rather than just one of the two mechanisms. De Jong et al. (1996) showed that 2.5% differences in energy transmittance through the body surface altered the body temperature of a ladybird beetle by 1–2°C. Accordingly, increasing reflectance of solar energy by a small percentage appears to be effective for ameliorating heat stress. Reflectance also increased with decreasing SVR, suggesting that larger species with lower SVRs required higher reflectances of solar energy by the body surface to compensate for the higher heat gain when exposed to full sunlight. Increased spectral reflectance might serve functions other than thermal adaptation, such as prey attraction by UV reflection (e.g., Tso et al. 2006). However, we examined wavelengths that were longer than UV radiation; thus, spectral reflection, as studied here, was likely irrelevant to prey attraction.

There remain unmeasured traits in our study that may affect thermal stress. First, higher SVR is likely to increase cuticular water loss, which might cause osmotic problems (Pulz 1987). However, because independent contrast revealed a negative association between SVR and reflectance of solar energy, larger SVR appears to have a positive effect on thermoregulation, suggesting that the advantage of heat loss outweighs the cost of water loss. Second, stabilimenta made by *Argiope* species may function to ameliorate heat stress. This effect, even if present, does not alter our conclusion because  $CT_{max}$ , spectral reflectance, and SVR shown in *Argiope* were more adaptive for ameliorating thermal stress than those in other spider genera (Table 1).

We presented empirical evidence that araneoid spiders have acquired physiological tolerance to high temperatures during their history of habitat expansion, which in turn may be coupled with biophysical adaptations to increase the reflectance of solar energy. Future explorations of the correlated evolution of multiple traits linked to thermal adaptation would further elucidate the mechanisms of adaptive radiation of insects and spiders.

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