

Adaptation to thermal stress in lichen-eating webspinners (Embioptera): habitat choice, domicile construction and the potential role of heat shock proteins

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Summary

1. Embiids are a unique group of web-spinning insects, which construct silk domiciles. In sunny habitats, silk may provide a shady refuge, or exacerbate exposure to high temperatures. Here we present the first description of the thermal biology of embiids, focusing on *Notoligotoma hardyi* from Magnetic Island, Australia.
2. We recorded natural colony positions and temperature of silk domiciles relative to incident radiation, and manipulated locations of domiciles on sunny (north-facing) and shady (south-facing) rock faces to test whether silk dampens thermal exposure. We also compared responses to laboratory heat stress in *N. hardyi* with two other embiids: *Austrelembia incompta* (Magnetic Island) and *Antipaluria urichi* (rainforest).
3. Temperatures of air (T_A), rocks (T_R) and domiciles (T_D) are significantly hotter for north- than south-facing colonies. North-facing colonies have less extensive silk coverings and are found in crevices. For natural and experimentally manipulated north-facing colonies, T_D was cooler than T_R but hotter than T_A , whereas T_D and T_A were similar for south-facing colonies. Individuals living in north-facing colonies adjusted position to remain in the shade. Ability to recover from heat exposure was greatest in Australian embiids and was linked to expression of heat shock proteins.
4. In sunny microhabitats, silk reduces but does not prevent exposure to physiologically stressful temperatures. *N. hardyi* tolerates thermal extremes using a combination of behavioural thermal regulation, microhabitat selection and a robust heat shock response.

Key-words: Behavioural thermoregulation, Embiidina, heat shock protein

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Introduction

Ectotherms, whose body temperatures are determined by the environment, are vulnerable to fluctuations in environmental temperature owing to effects of temperature on the rates of biological processes and the structural integrity of biomolecules (Hazel 1995; Somero 1995; Willmer, Stone & Johnston 2000). Many ectotherms possess a repertoire of thermal regulatory behaviours, which allows them to reduce variation in body temperature in the face of changing environmental temperatures (Van Dyck & Matthysen 1998; Forsman 2000; Berwaerts *et al.* 2001; Forsman *et al.* 2002). However, few species of ectotherms are able to use behavioural thermal regulation to maintain a completely constant body temperature, and therefore also possess a suite of

physiological strategies for dealing with effects of variable body temperature on metabolic processes (Willmer *et al.* 2000). Unfortunately, there are few examples of studies that integrate investigation of biochemical, physiological and behavioural responses to temperature, in part because of difficulties of measuring the thermal environment at the scale of the organism of interest (Kingsolver 2000; Bryant, Thomas & Bale 2002; Helmuth 2002).

Here we describe behavioural and physiological adaptations to thermal variation in an unusual group of insects – the embiids, or webspinners. Webspinners dwell within silk structures, which they build by methodically touching the substratum with their front feet, ejecting silk threads from tarsal glands with each step (Edgerly 1997; Ross 2000; Edgerly, Davilla & Schoenfeld 2002). Webspinners live in a diverse array of habitats and use their silk accordingly. Some species line subterranean crevices with silk to create deep retreats, others stitch leaf litter together where they feed on detritus, and others form colonies on exposed

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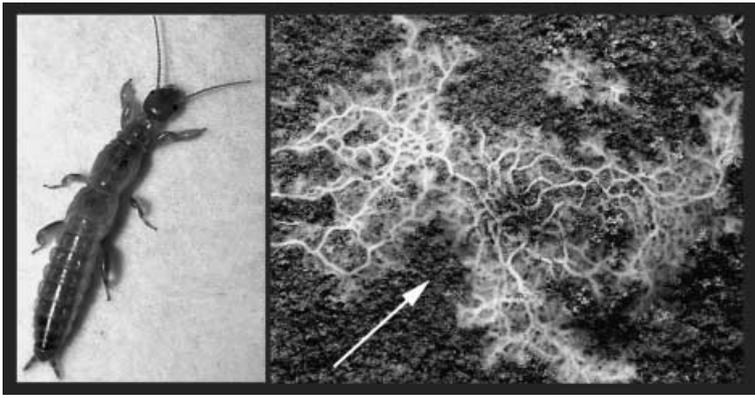


Fig. 1. An adult female webspinner (length = 1.0 cm) of *Notoligotoma hardyi* of Magnetic Island, Queensland, Australia, and silk of a colony spun onto granite. The central area of the colony (indicated by arrow) is thick and sprinkled with faecal pellets (black dots) that have been pushed out by the occupants. The silken tubes, visible as dense white lines in the photograph, form passageways to and from lichens, food for *N. hardyi*. The passageways are approximately the width of the adult female; colony-mates travel through them, running backwards and forwards. Adult females of all species in the Order Embioptera resemble this individual in shape (wingless, elongate and nymphoid).

vertical surfaces, a lifestyle typical of embiid in tropical regions (Edgerly 1987; Edgerly 1994; Ross 2000). A standard silk dwelling includes thick, reinforced areas (domiciles) where females lay eggs and reside during the day, as well as passageways to where they feed at night (Edgerly 1987; Edgerly *et al.* 2002). The diversity of habitats in which webspinners are found means that different species may experience unique environmental conditions.

Embiids from diverse habitats have surprisingly similar body plans, probably because of constraints imposed by living within silk (Ross 2000). Adults are elongate and flexible. Females are wingless and nymph-like in form; males are winged. Having a soft, unarmoured body may leave embiid especially vulnerable to predation. Silk domiciles and other constructions appear to protect embiid from ants, which generally walk over silk without responding to it (Edgerly 1994). Silk domiciles may also provide protection from heavy tropical rains, which would knock webspinners from their perches. It has been proposed that silk provides a shield against solar radiation or elevated temperature. However, being constrained inside silk may expose embiid to stressfully high temperatures in dry, sunny habitats. Trade-offs between predation avoidance and thermal stress exposure may drive behavioural ecology of these insects. Unfortunately, we know nothing of the thermal biology of webspinners, or of the physiological problems associated with being constrained inside a silk domicile.

One physiological strategy many organisms use to cope with thermal extremes in their environment is the up-regulation of heat shock proteins (Hsps), which minimize stress-induced protein aggregation and facilitate removal of damaged proteins (Krebs & Feder 1997; Feder & Hofmann 1999; Feder *et al.* 2002;

Frydenberg, Hoffmann & Loeschcke 2003; Garbuz *et al.* 2003; Norry & Loeschcke 2003). Hsp expression enhances survival and increases thermal tolerance, but over-expression of Hsps may negatively influence growth and reproduction or disrupt cellular metabolism (Krebs & Feder 1998; Feder & Hofmann 1999; Krebs & Holbrook 2001; Feder *et al.* 2002; Hamdoun, Cheney & Cherr 2003). Consequently, Hsp production may enhance survival at the cost of reduced fitness. The importance of the heat shock response for animals in nature has been well established (Roberts, Hofmann & Somero 1997; Tomanek & Somero 1999; Dahlhoff & Rank 2000; Buckley, Owen & Hofmann 2001; Michaelak *et al.* 2001). Thus, production of Hsps is a probable physiological solution for embiid living in hot climates. To date no investigation of Hsps has been conducted on a silk-dwelling organism.

Our studies focus on Magnetic Island (Queensland, Australia: 19°25' S, 146°77' E) populations of *Notoligotoma hardyi* (Notoligotomidae; Fig. 1), a lichen-eating embiid that constructs domiciles on granite boulders (Edgerly & Rooks 2004). Because they are sedentary once they settle and lay eggs, *N. hardyi* colonies are susceptible to thermal fluctuations that occur within their domiciles. Thus, choice of substratum seems imperative in regions of high sun exposure. In an earlier study, Edgerly & Rooks (2004) found that differences in sun exposure affected microhabitat selection by *N. hardyi*. Those findings led to experiments reported here, designed to describe the thermal biology of *N. hardyi*. First, we made temperature measurements of naturally occurring *N. hardyi* colonies, and second, we experimentally manipulated the amount of sun exposure received by colonies to help us understand how silk and microhabitat selection aids in thermal regulation. Third, to assess the importance of physiological response to heat stress, we compared behavioural and physiological responses to laboratory heat stress for *N. hardyi* with two other embiid from different thermal habitats: a leaf-litter detritivore from Magnetic Island (*Austrelembia incompta* (Austrelembiidae)) and a rainforest species from Trinidad, West Indies (*Antipaluria urichi* (Clothodidae)). The rainforest embiid *A. urichi* experiences habitat temperatures between 18 and 30 °C (Asa Wright Nature Center; www.AsaWright.org), whereas Australian embiid routinely experience ambient temperatures between 30 and 45 °C during austral summer (Bureau of Meteorology; www.bom.gov.au).

Materials and methods

DETERMINATION OF PREFERRED HABITAT OF *N. HARDYI*

To quantify the thermal environment, a peak along Forts Trail was selected where *N. hardyi* was common. To evaluate the thermal environment, temperatures and compass directions of available substratum (rocks) and embiid microhabitat (within silk domiciles)

were quantified and compared. All temperatures were recorded on a single day (12 June 2001) for randomly selected rocks on north- and south-facing slopes. Temperatures of rock faces (T_R), colony surroundings (T_C) and domiciles (T_D) were recorded with an infrared thermometer (Oakton TempTestr®, Omni Controls Co., Tampa, FL). Ambient air temperature (T_A) was recorded continually with a datalogger (HOBO® H8 Pro, Onset Computer Co., Pocasset, MA), which measured T_A s identical to those measured by the infrared thermometer. Two active colonies from each rock were randomly selected for the survey. If a randomly selected rock did not support a colony, the closest colonized rock was used to complete the set. The length and width of each colony's silk were used to estimate colony area for each exposure. Temperature at four points around the silk was measured and the highest temperature of the four was used to represent T_C . For comparison purposes, T_A was recorded before each colony was measured. The position of each domicile was characterized as being spun on a flat surface, within a crevice (the depth of which was measured), or in the shade of a piece of exfoliating rock (a common feature for granite exposed to strong sun). To measure T_D , the domicile silk was lifted and the temperature of the rock surface beneath was immediately recorded with the IR thermometer. Temperature data were analysed with *t*-tests or matched pair *t*-tests. These and all subsequent statistical analyses were conducted using JMP-In (Version 4.0, SAS Institute Inc., Cary, NC).

TEMPERATURE PROFILES OF EXPERIMENTAL COLONIES

To focus specifically on the effect of silk on microhabitat temperature, experimental field colonies ($n = 20$) were established on small rocks set in either shade or sun. Continuously recording temperature dataloggers (HOBO® H8 Pro) were used to measure T_A and T_D . The objective was to compare temperatures experienced by colonies in direct sun (north-facing) with those exposed to shadier conditions (south-facing). Two trials were conducted in Austral Fall of 2001 (Trial 1: 10 March to 7 April; Trial 2: 11–29 April). Ten lichen-covered rocks (mean size: $12\,528\text{ cm}^3 \pm 2415$ (SEM)) were collected from a shaded hillside where *N. hardyi* colonies were abundant. Rocks were placed on a wooden bench in two rows, one facing south, one facing north, with a Masonite board propped between the two rows to shade backs of south-facing rocks. Each rock was equipped with a temperature logger shielded by a plastic solar radiation guard. A 5-mm diameter external temperature probe was affixed to each rock with tape or a clay-like adhesive (Blu-Tack Re-usable Adhesive, Bostick®). Temperature loggers recorded the ambient temperature next to each rock and under silk every 15 min for 9 days. A silk patch for each replicate was created by carefully excising from a rock a natural domicile filled with embiid nymphs collected

from a nearby field site. These patches averaged $6.6 \times 2.75\text{ cm}^2$ in size. Each patch was affixed with small pieces of labelling tape to the temperature probe on its experimental rock. Cloth mesh was taped over the patch to protect it during the first days after transfer and to trap the nymphs inside, forcing them to live and spin directly on the probe.

Experimental colonies were protected from exposure to elements and predators with Masonite boards and chicken wire until most of the domiciles were adhering to probes and rocks, and embiids were feeding on lichens. This required 19 days for Trial 1 and 8 days for Trial 2. During Trial 1, temperature loggers were hung onto the sides of the rocks with wire, but in Trial 2 they were hung from Masonite board behind each rock. Seven replicates were excluded overall, because the silk formed an inadequate seal over the probes ($n = 6$) or the probe was faulty ($n = 1$). Thirteen replicates, six facing north and seven facing south, were included in the statistical analysis. Excess temperature experienced beneath the silk patch was calculated as T_D minus T_A and plotted as a function of T_A . Slopes of the lines were calculated and compared using heterogeneity of slopes analysis in JMP.

LABORATORY HEAT SHOCK

Study organisms

Specimens were selected from laboratory-reared colonies of two Australian embiids (*N. hardyi* and *A. incompta*) and the rainforest species (*A. urichi*). All three species of laboratory-reared embiids were maintained for 2 years in an environmental chamber at 25 °C. Embiids were held in small glass containers filled with fresh, lichen-covered bark and leaves obtained from local (Northern California) oak woodlands.

Thermal tolerance

Thermal tolerance experiments were conducted following methods modified from Neargarder, Dahlhoff & Rank (2003). Individuals ($n = 5$ per species per treatment temperature) were transferred to microfuge tubes filled with small glass beads to a level even with the top of a digital hot-cold block incubator (Tropicooler, Boekel Scientific, Feasterville, PA, USA). Each tube was moistened with a small amount of water to limit desiccation. To monitor treatment temperature accurately, tubes were equipped with a T-type 36-gauge fine wire thermocouple attached to a hand-held 2-channel thermometer (HH-82, Omega Engineering Inc., Stamford, CT). Individuals were exposed to one of the following treatment temperatures for 4 h: 25, 30, 33, 36, 39 or 42 °C, and allowed to recover for 1 h at 25 °C. For temperatures above 25 °C, animals were placed in tubes at ambient temperature and Tropicooler block temperature was increased 1 °C every 2 min until experimental temperature was reached.

After heat treatment and recovery, web-spinners were scored: active (4), active when prodded only (somewhat active, 3), involuntary twitching, or sluggish response to stimuli (barely active, 2), not responding to repeated stimuli (dead, 1), following published methods (Nearing *et al.* 2003). This score was weighted by number of individuals receiving it, and the mean of resulting values was reported as the recovery value for each species at each temperature. Recovery values were log-transformed and analysed by ANOVA, with species and treatment temperature as main effects. Immediately after assessing recovery status, individuals were flash-frozen on dry ice and stored at -70°C until Hsp analysis.

We also evaluated responses to stress for *N. hardyi* in laboratory-constructed domiciles. Individuals from the laboratory colony were provided with lichen-covered rocks and allowed to spin domiciles for several weeks. Once domiciles were mature, colonies were heat-shocked by exposure to a 275-watt full-spectrum solar lamp (T-Rex reptile lamp, Dolphin Pet Co., Campbell, CA). The lamp was placed about 46 cm away from domicile, to bring temperatures near those typical of the hottest field temperatures ($T_R = 37^{\circ}\text{C}$, $T_D = 35^{\circ}\text{C}$). Rock temperature (for light and shaded side of the rock) was monitored throughout experiment using an infrared thermometer. Temperature inside the domicile was monitored using a hand-held digital thermometer equipped with a T-type 36-gauge thermocouple (HH-82, Omega Engineering Inc.).

ANALYSIS OF HSP70 EXPRESSION LEVEL

Expression of Hsp70-class stress proteins was quantified by Western blot analysis following published methods (Rank & Dahloff 2002; Nearing *et al.* 2003). Expression of Hsp70 was resolved using a mouse monoclonal anti-Hsp70 (SPA-822, StressGen Inc., Vancouver, BC). After electrophoresis, blotting and treatment with peroxidase-linked antimouse IgG and signal-enhancing chemicals (ECL Plus, Amersham Biosciences, UK), blots were scanned and analysed using a Storm80 Imaging System (Amersham Biosciences, UK). Hsp70 expression levels were analysed using ANCOVA, with species and treatment temperature as main effects and body mass as a covariate.

Results

NATURAL FIELD COLONIES AND THEIR THERMAL ENVIRONMENTS

Habitat locality of naturally occurring colonies of *N. hardyi* was highly dependent on their position relative to the sun (Fig. 2). In particular, north-facing domiciles tended to be tucked into crevices or under exfoliating sheets of rock and rarely on the flat surface (6%), whereas those facing south were typically spun on flat, exposed surfaces of the rocks (56%). In addition, the mean aspect of 16 north-facing colonies was 215° ,

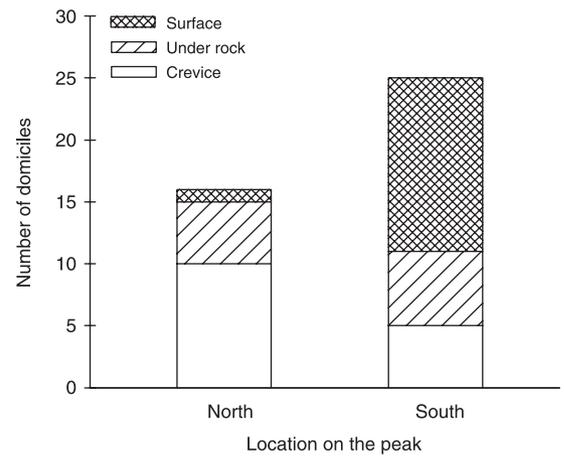


Fig. 2. Microhabitat selection of natural colonies of the Australian embiid *Notoligotoma hardyi*. Data shown are total number of domiciles located on north- or south-facing slopes in each of three distinct microhabitats: on exposed, flat rock surface (surface), under exfoliating sheets of rock (under rock), or in rock crevices (crevice). Statistical analyses shown in text.

those on south-facing slopes was 180° (Rayleigh's Circular Statistic, north: NS; south: $w = 11.3$, significant at $P < 0.05$). Three south-facing colonies included multiple domiciles connected by foraging trails that had coalesced, whereas all colonies on the north-facing slope had single domiciles. The expansiveness and microhabitats of silk differed between the two sites as well. The south-facing colonies averaged $29.1\text{ cm}^2 \pm 5.2$ (one south-facing colony of 4100 cm^2 , composed of nine domiciles fused together, was an outlier and eliminated from analysis) and ranged in size from 4 to 69 cm^2 . Average area of north-facing colonies ($16.3\text{ cm}^2 \pm 2.5$) was significantly smaller than south-facing colonies ($t = 2.21$, $df = 29$; $P = 0.035$). North-facing domiciles averaged $1.0\text{ cm} \pm 0.2$ below the surface of the rock, significantly deeper than those to the south ($0.4\text{ cm} \pm 0.2$; $t = 2.06$; $df = 30$; $P = 0.048$).

Thermal experience was distinct for north- and south-facing colonies (Fig. 3). For north-facing rocks, T_R was significantly hotter than T_A , whereas T_R for south-facing rocks was indistinguishable from T_A (north: $t = 6.06$, $df = 37$, $P < 0.001$; south: $t = 0.86$, $df = 38$, $P = 0.39$). Rocks facing NW were on average 20°C hotter than T_A . T_D for colonies on north-facing rocks was significantly higher than T_A ($t = 2.19$, $df = 15$, $P = 0.04$), but 4°C lower than T_C (matched pairs t -test: $t = 6.07$, $df = 15$, $P < 0.001$). In contrast, T_D for colonies on south-facing rocks was actually lower than T_R (matched pairs t -test: $t = 2.02$, $df = 15$, $P = 0.03$).

THERMAL PROFILES OF EXPERIMENTAL COLONIES

For the comparisons of experimentally manipulated north- and south-facing colonies, mean ($26.8^{\circ}\text{C} \pm 0.09$), minimum (average 22.1°C) and maximum (average

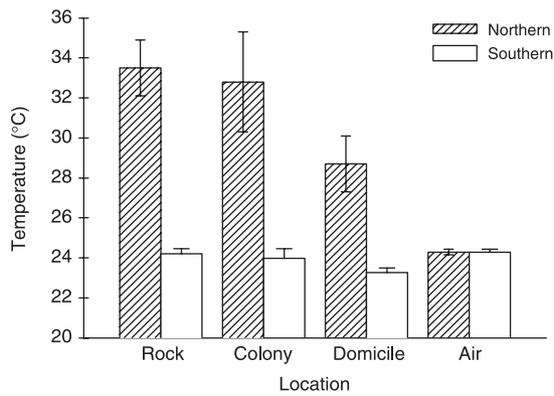


Fig. 3. Temperatures in and near natural embiid domiciles in north- and south-facing colonies measured on 12 June 2001. Data shown are least-squares means (\pm SEM) of temperatures collected for $n = 9$ time points (between 13.00 and 17.00 h) per randomly selected locality of bare rock (T_R), colony surroundings (T_C) or domiciles (T_D). Air temperature (T_A) was also measured. Additional statistical analyses described in text.

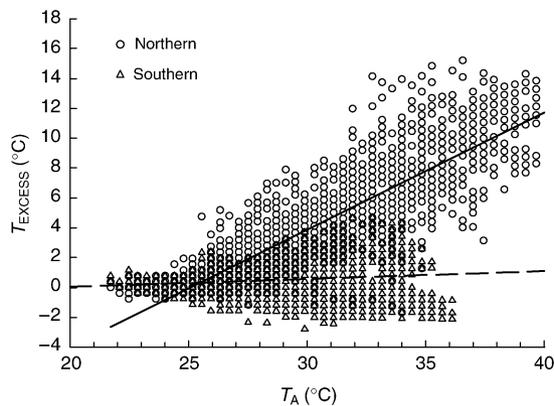


Fig. 4. Thermal profile of silk microhabitat for experimental embiid colonies on north- and south-facing rocks. Each data point shows T_A and the difference between T_A and T_D ($T_D - T_A = T_{EXCESS}$). Data from two different time trials (lasting 19 and 8 days, respectively) are combined. North-facing colony temperatures increased with increasing T_A ($y = -20 + 0.79x$; $R^2 = 0.80$; $F_{1,2864} = 11\,166$; $P < 0.001$), whereas south-facing colony temperatures tracked T_A ($y = -1.0 + 0.05x$; $R^2 = 0.03$; $F_{1,4192} = 113$; $P < 0.001$). Additional statistical analyses described in text.

35.7 °C) T_A did not vary between trials. The typical range of T_A was about 22 °C at night and between 35 and 41 °C during the day. Thermal excess ($T_D - T_A$) of experimentally manipulated colonies was significantly higher for colonies facing north (0.79 ± 0.07) than those facing south (0.05 ± 0.07 ; heterogeneity of slopes; $F_{1,7056} = 6785$; $P < 0.0001$; Fig. 4). During Trial 1, all north-facing colonies shifted their silk spinning and feeding activities to the backs or sides of the rocks, or under temperature loggers suspended from the rocks. In Trial 2, loggers were moved off the rocks and were hung from the back wall to prevent their shade from attracting the embiids. Nymphs in three of five north-

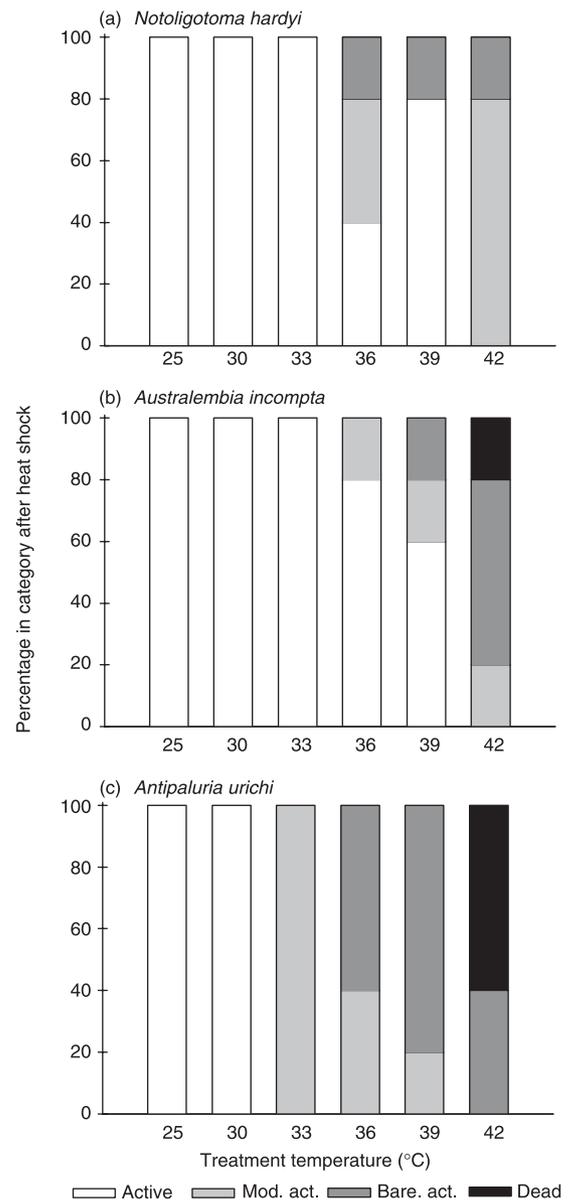


Fig. 5. Differences in recovery from heat shock among three species of embiid. Recovery status was rated according to methods described in text: active, somewhat active, barely active or dead. Data shown are percentage of individuals ($n = 5$ individuals per species for each treatment temperature) grouped in each recovery category after 4-h heat shock. Statistical analyses described in text.

facing colonies shifted away from the sun and spun their silk on the back of the rocks during the 9-day exposure to the sun. The other two domiciles were abandoned. The south-facing colonies, in contrast, did not shift substantially from their initial positions.

BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES TO THERMAL STRESS IN THE LABORATORY

Recovery from exposure to laboratory thermal stress was correlated with the typical thermal experience of each species in nature (Fig. 5). The Australian embiids

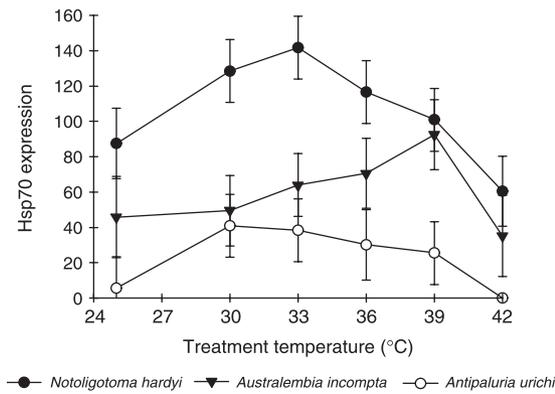


Fig. 6. Differences in Hsp70 induction profiles among three species of embiid. Hsp70 expression was induced at different temperatures in the laboratory and is reported as ng Hsp70 per mg total body wall muscle protein. Data shown are least-squares means (\pm SEM) of $n = 5$ individuals per species for each treatment temperature, except for 42 °C, where $n = 3$ for *N. hardyi*, 2 for *A. incompta* and 1 for *A. urichi*. Additional statistical analyses described in text.

recovered activity after thermal stress much more readily (mean scaled log score *N. hardyi*: 1.26 ± 0.02 , *A. incompta*: 1.23 ± 0.02) than the rainforest species (*A. urichi*: 1.12 ± 0.02 ; ANOVA: species–treatment temperature interaction: $F_{2,17} = 3.7$, $P = 0.048$). Expression levels of Hsp70-class stress proteins were significantly different among webspinner species as well (Fig. 6). Hsp70 expression levels for *N. hardyi* were higher than for *A. incompta*, which were in turn higher than for *A. urichi* (ANOVA; species effect: $F_{2,67} = 31.3$; $P < 0.0001$). Temperature of maximal Hsp70 expression tended to be higher for the Australian species (33 °C for *N. hardyi*, 39 °C for *A. incompta*) than the rainforest species *A. urichi* (30 °C), though the effect of temperature on Hsp expression did not vary between species (ANOVA; species–temperature interaction: $F_{2,8} = 0.8$; $P > 0.5$). When *N. hardyi* were exposed to thermal stress (35–37 °C) in more natural conditions in the laboratory (female-constructed domiciles irradiated by a heat lamp), five of the six individuals from the first colony escaped to a cooler (22–23 °C) side of the rock within 5 min.

Discussion

The importance of silk for insect thermal regulation has been well described, most notably for tent-building caterpillars (Carlberg 1980; Joos *et al.* 1988; Fitzgerald 1995; Fitzgerald & Underwood 2000; Ruf & Fiedler 2000). Caterpillar tents encompass many layers of silk, are replete with air pockets and are thermally heterogeneous, and caterpillars use this heterogeneity to thermoregulate. When the air is cool, they huddle in the middle of the tent, which elevates their body temperature to the point where they are able to feed; when it is warm, caterpillars remain near the edge, or even dangle outside in the shade provided by the tent, to avoid overheating (Joos *et al.* 1988). By contrast, while

the presence of silk is clearly important for webspinner survival (adult females migrate onto rocks that support lichens and spin silk as quickly as possible), it does not appear to be the primary means of thermoregulation. This may be because embiid domiciles, consisting of layers of air-filled silk tubes lined with faecal pellets, are spun flat against the substratum and are thus subject to rapid heating by the underlying granite. Although the tubes are a potential source of insulation, we found that colonies facing the sun (or a heat lamp) heated up dramatically despite the silk covering, rapidly reaching temperatures higher than ambient air temperature. Instead of using silk, webspinners appear to rely primarily upon behavioural (microhabitat selection) and physiological (heat shock response) adaptations to mitigate potential effects of stress from exposure to thermal extremes.

Temperature is clearly an important factor in determining habitat selection for *N. hardyi*. Thermal experience of an individual is highly dependent on substratum aspect, with webspinners facing sunny, northern slopes experiencing significantly higher temperatures than those facing cooler, southern slopes. Colonies on north-facing slopes prefer shady crevices to sunny, flat rock surfaces (Fig. 2). The fact that colony temperatures were cooler than rock temperatures on north-facing slopes (Fig. 3) was probably a result of this preference for crevices, though we did not directly compare crevice to surface temperatures in the absence of webspinner colonies to confirm this hypothesis. Webspinners experimentally forced to live in sun-exposed microhabitats attempted to adjust their position to utilize any available shade, or abandoned these sites altogether. Silk did provide some cooling in very sunny habitats ($T_D < T_C$ on north-facing slopes, Fig. 3), because of either thermal resistance of air pockets in silk-filled crevices or higher microhabitat humidity. Taken together, these results suggest that the main function of silk in embiids is not thermoregulation. Instead, silk may act to camouflage these soft-bodied insects from predators, or to protect them from heavy rainfall (J. Edgerly, unpublished observations). Alternatively, silk may inhibit desiccation or trap moisture from humid night air, reducing desiccation stress experienced on sun-exposed surfaces. These alternative functions for silk have not yet been evaluated experimentally.

Field studies of a number of ectotherm species implicate the energetic cost of routinely up-regulating Hsps as one of the major causes for lower growth rates, activities and reproductive outputs in high thermal stress microhabitats (Roberts *et al.* 1997; Hofmann 1999; Tomanek & Somero 1999; Dahlhoff, Buckley & Menge 2001). We found that webspinner domiciles on sunny, north-facing slopes had less extensive silk coverings and were smaller than their south-facing counterparts. Differences in colony size between sun exposures may be the direct result of metabolic costs of maintaining a high standing stock of heat shock proteins. For all laboratory-reared webspinners, Hsp70 was present and expression peaked at a moderately high

temperature, above which Hsp70 expression decreased, probably the result of a decline in the efficacy of protein synthesis; however, the two Australian species tended to up-regulate Hsp70 at higher temperatures (33, 39 °C) than the rainforest species (30 °C), implying adaptation to higher habitat temperatures in the Australian species.

We measured total expression of Hsp70 by Western blot analysis, which quantifies the total pool of Hsps present at the time the animal was assayed. Thus, observed Hsp70 expression levels (Fig. 6) may include both cognate (expressed at all times) and inducible (expressed only after stress exposure) forms. Australian species, and especially *N. hardyi*, appear to have higher cognate Hsp70 expression than the rainforest species, as evidenced by higher standing stock Hsp70 expression after 2 years of acclimation to common-garden, laboratory conditions (Fig. 6, 25 °C comparison). Australian webspinners living in sunny microhabitats may use Hsps to tolerate less optimal habitats for short or extended periods of time, while they seek out more hospitable microclimates. In areas where desirable microhabitats are limited, Hsp expression may allow individuals to survive extreme conditions, though growth and reproductive rates may be reduced. Future studies of Hsp expression in a natural context for these unusual insects will allow us to test this hypothesis.

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