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# Choreography of silk spinning by webspinners (Insecta: Embioptera) reflects lifestyle and hints at phylogeny

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Silk spinning defines the morphologically constrained embiopterans. All individuals spin for protection, including immatures, adult males and the wingless females. Enlarged front tarsi are packed with silk glands and clothed with ejectors. They spin by stepping with their front feet and releasing silk against substrates and onto pre-existing silk, often cloth-like. Spinning is stereotypical and appears to differ between species in frequency and probability of transition between two spin-step positions. This spinning choreography was assessed using thousands of spin-steps scored in the laboratory for 22 species to test: (1) the body size hypothesis predicting that spinning would be more complex for larger species; and (2) the phylogeny hypothesis which predicted that spinning would display phylogenetic signal. Tests relied on published phylogenies for the order Embioptera. Independent contrast analysis revealed relationships between five spin characteristics and body size, whereby, for example, larger webspinners invested in relatively larger prothoracic tarsi used for spinning and in spin-steps that would yield expansive silk coverings. Spin-step dynamics displayed a phylogenetic signal for the frequency of six spin-steps and for 16 spin-step transitions. Discussion focuses on patterns revealed by analysis of phylogenetic signal and the relationship to life style and to recently discovered chemical characteristics of silk. © 2016 The Authors. *Biological Journal of the Linnean Society* published by John Wiley & Sons Ltd on behalf of Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **118**, 430–442.

**KEYWORDS:** Blomberg's  $K$  – Embioptera – independent contrast – Insecta – phylogenetic signal – silk – spinning-webspinner.

## INTRODUCTION

The quantification of silk spinning behaviour has been useful for understanding evolution of insects who spin cases and cocoons, including blackflies (Stuart & Hunter, 1998), caddisflies (Stuart & Currie, 2002a, b), and caterpillars (Bucheli, Landry & Wenzel, 2002). For spiders as well, species diversification seems more closely related to innovations in their use of silk than to variation in life styles (Blackledge *et al.*, 2009). These studies on spinning by spiders and insects inspired us to investigate the evolution of another group of silk spinners, the polyneopteran insect order Embioptera. As with spiders, embiopterans show no trophic-level diversification and little diversification in morphology, both of which are unusual characteristics for an order in the otherwise highly diverse Insecta. Observations in our laboratory have prompted the question as to whether sub-

tle differences in spinning might reflect ancestral relationships in Embioptera. Behaviour can be difficult to quantify and for many researchers has also proven to lack phylogenetic signal. But what about behaviour expressed by animals in a taxon that shows little morphological diversity; might behaviour serve as a clue to evolutionary relationships? Also, the behaviour in question is displayed as sequences of spin-steps, and some slight differences that accumulate over evolutionary time might reflect random changes rather than adaptations. Could such changes provide clues to phylogenetic relationships? A brief introduction to the order Embioptera is warranted given the general lack of knowledge of them and to place their silk spinning into context given the questions posed above.

Embiopterans, sister group of the Phasmida according to recent studies (Miller *et al.*, 2012), are mostly subtropical and tropical with fewer than 500 named species and an estimated 2000 worldwide (Ross, 1991). Living within silk, an order-defining

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characteristic, has profoundly constrained the embiopteran body form and lifestyles. The behaviour of hiding within silk and the fact that most species live in the tropics has also made this one of the least known of all orders of insects. They feed on leaf litter and epiphytic algae and lichens, living beneath the ground in silk-lined burrows, under stones, in leaf litter or on bark wherever it is humid and warm. Furthermore, their body form is remarkably uniform (Fig. 1A), no matter the climate or microhabitat. This uniformity stems from neoteny of adult females. The body is elongated and flexible, and combined with the lack of wings, affords females amazing maneuverability within the tight confines of silk tubes. Males, which die soon after mating, are usually winged but even their bodies are flexible and their wings can crumple as they run backwards and forwards within silk. Although females vary in size and colour, they express very few morphological characters that have been used in phylogenetic analyses (Szumik, Edgerly & Hayashi, 2008; Miller *et al.*, 2012). In contrast, male secondary sexual characteristics and wings (Ross, 1991) and more recently molecular data do provide phylogenetic information (Szumik *et al.*, 2008; Miller *et al.*, 2012).

As the webspinner spin-steps in a dance-like routine around the front of the body and sometimes over the back, silk issues forth from swollen glands in the front feet and adheres to the substrate and to previously spun silk (Fig. 1B–E). Silk production varies among species and may be correlated with a variety of abiotic and biotic variables such as temperature, moisture, and predation. For example, burrowers and litter-dwellers appear to produce little silk while some large arboreal species produce copious amounts. Observations of cultures raised in laboratory conditions revealed that these differences persist even under common garden conditions (personal observation; JSE) suggesting the possibility that silk spinning tendencies have diversified. Previous studies of webspinners have sought to understand subsocial and colonial behaviour (see references in Edgerly (1988)), relationships between embiopterans and their environment (Edgerly & Rooks, 2004; Edgerly, Tadimalla & Dahlhoff, 2005; Edgerly, Shenoy & Werner, 2006), and the function and structure of silk (Okada *et al.*, 2008; Collin *et al.*, 2009a, b; Addison *et al.*, 2014) with progress still being made in these areas. Our current goal is to determine whether silk spinning diversity can be quantified, scored, and used to understand embiopteran evolution. Previous work on a few species revealed what appear to be stereotypical behaviours (Edgerly, Davilla & Schoenfeld, 2002; Edgerly, Büsse & Hörnschemeyer, 2012), providing a foundation for a broader sampling of taxa as we seek to answer our research questions.

Silk spinning is performed by webspinners of all ages and both sexes but adult females with nymphs are the most productive (Edgerly, 1988). The structures include tubular galleries (Fig. 1C, E) and/or extensive cloth-like silk (Fig. 1B, D). Generally, most species construct a domicile of thick silk connected to food by more diffuse galleries – sometimes camouflaged with gathered materials, sometimes constructed with clean silk (Ross, 2000; Edgerly *et al.*, 2002). Particular questions emerged from a recent study that revealed that individual female and male *Aposthonia ceylonica* (Enderlein) (Oligotomidae) exhibited spin-steps in an apparently species-specific manner (Edgerly *et al.*, 2012). An evaluation of individuals in three other species chosen to serve as comparisons for *Ap. ceylonica* also hinted at species specificity. That research focused on comparing silk glands and spinning behaviour of males and females, leaving unanswered the question of a phylogenetic signal in silk-spinning behaviour.

In contrast with previous studies of silk in arthropods (such as spider orb webs) the architecture of webspinner silk cannot be sufficiently characterized because, to a large extent, its form varies in subtle ways from tubular galleries to sheet-like coverings. Instead, subtle movements during spinning may provide more useful characters for phylogenetic analysis than the end products of spinning, as has been shown for blackfly larvae (Stuart & Hunter, 1998). Our alternative hypotheses are spinning behaviour style: (1) is related to body size because larger webspinners, often arboreal, may require more complex spin-steps to create a protective silk covering (the body size hypothesis); and (2) reflects phylogenetic relationships as differences accumulated in lineages over evolutionary time (the phylogeny hypothesis).

To test these hypotheses, behaviours of adult females from 22 species representing ten families were scored. Their body lengths ranged from ~ 0.8–2.0 cm, thus providing variability needed to test the body size hypothesis. Because larger species tend to live in the open on tree bark, we predicted that they would differ from smaller, more hidden, species in spinning choreographies. Smaller species were predicted to spin in a manner that would result in a lining for a burrow (similar to ‘wall-papering’ seen in some spiders and that shown beneath the female in Fig. 1D). In contrast, larger species were predicted to spin in a more complex way because they can make tubes that extend up the surface of bark providing a barrier to the elements in this exposed microhabitat. To test if these relationships hold, an independent contrast analysis was performed. Of note, a recent study of a set of closely related species did not reveal such a relationship between spinning behaviours and body size (Büsse *et al.*, 2015).





**Figure 1.** Diversity of adult female embiopterans and their silk. (A) From left to right: *Metoligotoma pentanesiana* (Austrolembiidae), *Archembia* n. sp. (Archembiidae), *Haploembia tarsalis* (Oligotomidae), *Macrembia* sp. ('Embiidae'), and *Antipaluria urichi* (Clothodidae). Females range in length from ~1.2–2.0 cm. (B) Silk of *Gibocercus* sp. in the field in Ecuador showing the sheet-like covering over tubular route-ways typical for tropical rainforest species. Grazing on epiphytic algae and mosses is evidenced by the scraped surface of the bark, (C) Silk of *Pararhagadochir trinitatis* (Scelembiidae) in a laboratory container showing typical tubular galleries, (D) *Aposthonia borneensis* (Oligotomidae) guarding her eggs hidden by tissue-like silk covering, in the laboratory, E. Silk of *Embia nuragica* ('Embiidae')(top) displaying openings at the top of the culture and at bottom, similarly at the top of a leaf-litter colony of *Metoligotoma incompta* in the laboratory.

If spinning behaviour is related strongly to the resultant domicile structure, which ultimately relates to demands of the microhabitat, then the link between phylogeny and spinning will be tenuous. Detection of subtle differences in silk spinning choreography in a previous study, mentioned above (Edgerly *et al.*, 2012), suggested that spin-step dynamics might actually reveal phylogenetic signal. A recently published phylogeny of the order (Miller *et al.*, 2012) and our decade-long intensive collecting of these hard-to-find insects and subsequent filming efforts provided the specimens and data necessary to test for such a signal.

## MATERIAL AND METHODS

### CULTURES

Embipterans collected in the field were maintained in laboratory colonies prior to the beginning of spinning behaviour trials (see Table S1, Miller *et al.*, 2012 for collection locations). All colonies were maintained at room temperature (~24°C) in plastic or glass containers filled with dry leaf litter (Coastal Live Oak, *Quercus agrifolia*) forming a matrix where individuals could spin and feed. Romaine lettuce and locally collected lichens served as food. Lighting was set on a diurnal cycle (14 h light: 10 h dark) and water was provided every 3–4 days. Collecting, rearing, filming, and scoring behaviours took place over a 10-year period (2001–2011).

### RECORDING AND SCORING SPINNING BEHAVIOUR

Spinning behaviour was evaluated only in adult females to minimize variability due to a sex effect. To induce spinning, individuals were placed in a narrow burrow (0.3 cm wide × 0.5 cm deep × 5.8 cm long) carved into a plywood block, covered with a transparent plastic lid, and/or in an open chamber with a bark-covered piece of wood within a plexiglass box (6 cm long by 6 cm wide by 4 cm deep) leaving a 1.5 cm air space above the bark). The bark served as a tree model. Because different microhabitats might trigger different types of or different investment in spinning, these two arenas were employed with the goal of capturing the fullest expression of spinning from a species. In the snug burrow, spinning might be reduced for arboreal webspinners because the tight space simulates a silk gallery and suppresses spinning. Alternatively, specialists that naturally live in litter or burrows might be triggered to line the burrow with silk as they would in nature. On average, five females per species were filmed in each arena but the replicate number varied depending on the availability of test subjects (details in Supporting

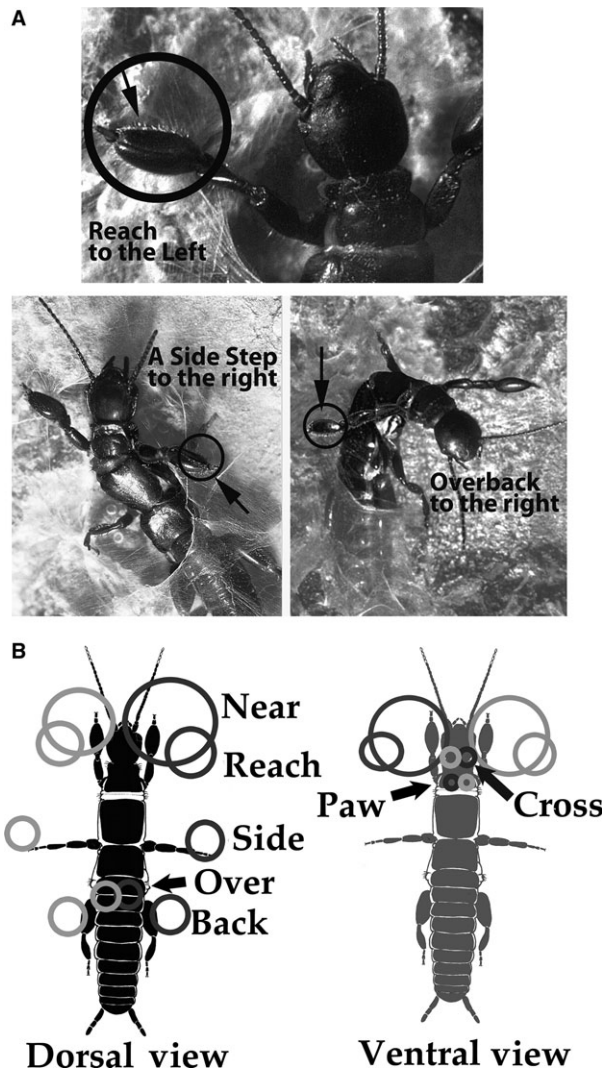
Information, Table S1) and three arboreal species were only tested in the chamber apparatus. We had not decided during the time these three species were available to run a comparison. Each trial consisted of the burrow or chamber being mounted approximately vertical but tilted slightly relative to the camera. Each hour-long session was recorded using a solid-state camera (Javelin Electronics, Torrance, CA, USA) with a zoom lens (18–108 mm, F 2.5) and digital videodiscs. Fibre optic lamps mounted on either side of the camera provided additional lighting needed to highlight the subtle actions of spinning. Behavioural acts, such as sit still, travel, and spin, were scored during playback using Observer software (version 5, Noldus Information Technology, Wageningen, the Netherlands). The resulting records showed time spent spinning and provided time stamps directing us to spinning bouts scored during subsequent slow-motion playbacks (see below). The same observer called out the spin-steps to an assistant who typed the actions into the Observer program running simultaneously with the video playback. The Observer program computed the order of spin-steps and transition probabilities for each replicate.

### ANALYSIS OF SPINNING CHOREOGRAPHY

Choreography of spinning was quantified based on methods described previously in Edgerly *et al.* (2012). Each spin-step was named based on the position of the front foot during spinning. Examples of spin-steps called *reach*, *side*, and *over the back* are shown, as example, in Fig. 2 for *Antipaluria urichi* (Saussure) (Clothodidae). Spin-step dynamics were quantified as probability of transition from one spin-step to another (or others), proportion of spin-steps in the different foot positions, spin-step diversity (see details below), and proportion of spin-steps to the *side*, *back* and *over*. The latter combination captures investment in spinning that yields more extensive silk covering over the body. Preliminary statistical analyses helped identify the more informative spin-step transitions allowing deletion of rare or uninformative spin-step transitions and a reduction in the number of possible spin-step transitions from 83 to 32 (see details in Edgerly *et al.*, 2012).

Of note, *Haploembia tarsalis* (Ross) (Oligotomidae) would not spin silk during the 1 h bouts, an unusual result given that webspinners tend to naturally spin when placed in an open container (JSE personal observation). After trying and failing to trigger spinning in dozens of individuals a revised protocol was developed. To induce spinning, individual *H. tarsalis* were placed in a burrow-like arena with another female; three females spun under these conditions and the spin-step details were scored. It is unknown





**Figure 2.** (A) Adult female *Antipaluria urichi* (Clothodidae) spinning silk and demonstrating three different spin-step positions (*reach*, *side*, and *over*). The emerging silk covering, thin and tissue-like, is visible covering her abdomen in the lower photographs. Opercula of eggs are visible (as white circles) beneath her head in the top and beneath her middle leg in the lower left photograph. Females typically guard their eggs and the silk covering is a component of maternal investment. (B) Kinematic diagram of spinning behaviour showing typical spin-steps for this species. Proportion of spin-steps in a position are relative to the size of the circle, using data from spinning in the chamber arena as an example. The insect spins by stepping with the front legs around the body, releasing silk from glands in the swollen basal tarsal segment. Spin-steps are designated by the foot positions as *near* the head, *reach* out from there, next to the *side* of the body, *back* along the abdomen, and *over* the dorsum. *Cross* also occurs when the webspinner has her venter against the substrate and entails bringing a front leg underneath the prosternum and across from one side to the other. ‘Dorsal view’ means that the venter of the body is against the substrate. In this position, silk emerges along the length of the body, around the head and over the back. During spinning, they flip over such that their venter is pressed against the emerging silk structure in the orientation dubbed ‘Ventral view’. Typically she spins around the anterior of her body with *near*, *reach*, and *cross*, but also *paw*, when she flaps her prothoracic tarsi toward her sternum.

why these females were reluctant to spin, but their behaviour reflects a general feature in that they seem to produce little silk in culture or in the field. Four species were filmed elsewhere and/or in different arenas but their spin-step dynamics were easily scored using videodisc recordings and the Observer program in our lab. These were two Ecuadorian species *Clothoda* nr. *longicauda* (Ross) (Clothodidae), and *Gibocercus napoa* Ross (Scelembiidae) and two Thai species *Oedembia* sp. Ross (Oligotomidae) and *Ptilocerembia catherinae* (Poolprasert & Edgerly) (Ptilocerembiidae).

#### INVESTMENT IN SILK GLANDS

The relative size of the front basal tarsomere, housing the silk glands, was used as a stand-in for potential investment in silk. The assumption was that the bigger the foot, the greater the potential for silk

production and storage. The front tarsi of four to six adult females per species, anesthetized with CO<sub>2</sub> gas, were photographed from the side and top aspect through an ocular lens of a dissecting scope (Olympus) with a Nikon Coolpix 990 camera. Digital images were measured with calibration to scale using ImageJ free-ware (<http://imagej.nih.gov/ij/>). A composite tarsal size score was based on circumference of the side view added to the circumference of the top view divided by head width (measured as the widest point from outer edge of eye to the other eye). The fairly consistent shape of females, as displayed in Figure 1A, is due to their nymph-like bodies and constraints imposed by the tight confines of silk tubes; hence, body length and head width are correlated and therefore, head width is a good measure of size of an adult female (Independent Contrast Analysis for head width and body length, Pearson Product-Moment Correlation Coefficient = 0.78; see Supporting Information, Table S1 for size data and below for statistical methods used).

#### STATISTICAL METHODS

Spinning choreography details were used to generate a matrix of the probability of transition between each

spin-step type (using Observer's statistics module). A female was excluded from the final analysis if she generated fewer than 20% of the maximum spin-steps for individuals of her species filmed for this study. This criterion was established because those that spun a limited amount of time during the session did not produce a silk structure visible to our eyes and as such did not express the fullness of their spinning potential. Supporting Information, Table S1 shows numbers of females ultimately analyzed for spin-step dynamics for each species.

To compare spin-step diversity among species, Simpson's Diversity Index, usually applied to describe richness and evenness of ecological communities, was used to represent spinning complexity (Brower, Zar & von Ende, 1998). Each spin-step position was treated as a 'species' and an index was computed to reflect the diversity of spin-steps taken by each individual overall: the higher the value of the index, the higher the diversity of spin-steps. A mean value was attributed to each species.

A test of independent contrasts was used to assess whether characteristics of spinning behaviour and the differences in morphological investment in silk glands are related to body size. This test corrects for the phylogenetic relationships among species. The test requires accurate topology of a phylogeny containing the species of interest as well as the assumption that the character traits of interest express Brownian motion (Felsenstein 1985). We adapted a phylogeny of the focal species ( $n = 22$ ) from the known phylogeny of the order Embioptera (Miller *et al.*, 2012). The Miller *et al.*, 2012 phylogeny was developed based on 82 species using molecular data from five genes (16S rRNA, 18S rRNA, 28S rRNA, cytochrome oxidase I and histone III; 6844 bp) and 95 morphological traits. The adapted phylogeny was used to perform a test of independent contrasts in the program Mesquite (Maddison & Maddison, 2015) and a test to detect phylogenetic signal (described below). Branch lengths on the tree were set to unity. Means were used for all multiple replicates to represent a species' scores. We performed linear regressions on all morphological and behavioural traits (using the 'Y contrast vs. X contrast' tool in PDAP) to test which traits were dependent or independent of the species' average adult female body length.

To estimate phylogenetic signal in spinning behaviour and related measurements, we computed Blomberg's  $K$  analyzed using R (R Development Core Team 2008) by implementing the *phylosignal* function of the *picante* package (Kembel *et al.*, 2010). Blomberg's  $K$  varies from zero (the null expectation) indicating no phylogenetic signal to  $K = 1$  which indicates phylogenetic signal to  $K > 1$  suggesting

traits are more similar (reflecting convergence in close relatives) than expected based on a Brownian Motion scenario (see Vanhooydonck *et al.* (2010) for similar methods for interpreting values of  $K$ ). Other measurements exist for detecting phylogenetic signal, such as Pagel's Lambda, but according to a recent review of methods by Kamilar & Cooper (2013), a sample size  $> 30$  taxa is required; our sampling effort did not meet this criterion. In addition, phylogenetic PCA was performed using the function in R of *phyl.pca* (Revell, 2012) to generate graphics that display characters that showed phylogenetic signal. We display the Bayes tree with Burrow data and the Maximum Likelihood Tree with Chamber data because those analyses had some of the higher  $K$ -values detected, although results were very similar irrespective of the phylogenetic trees of Miller *et al.* (2012) used in the analysis (Supporting Information, Table S2).

## RESULTS AND DISCUSSION

### GENERAL BEHAVIOUR

The two arenas elicited different spinning responses somewhat in line with the differences in microhabitats (Supporting Information, Fig. 1): arboreals tended to spin more in the bark Chamber and leaf litter species showed more action in the Burrow. We did not conduct tests to determine if the arenas triggered statistically different behavioural responses because not all species were tested in both arenas and insufficient replicates were available. A close inspection of the spinning details showed that different species tended to express more or fewer spin-steps or different types in the different arenas. Because spinning tended to vary, we evaluated three different datasets of spin-step dynamics: Burrow trials, Chamber trials and trials where spinning was expressed fully, dubbed Best spinning. Best spinning replicates were in the arenas where the individuals of a species spun the most and with the most complex styles. Of particular importance was to include trials where the females expressed the *side*, *back* and *over* spin-steps because these occur when building more complex silk structures. Of note, some leaf litter individuals, such as *Metoligotoma pentanesiana* Davis (Australambiidae), would not spin in the Chamber and many feigned death while in this setting. When placed in the Burrow, these would spin for many minutes at a time. The records for each species were scrutinized for spinning complexity and the final selections for inclusion in the analysis are indicated in bold-faced font in Supporting Information, Table S1.

## ANALYSIS OF SPIN-STEP DYNAMICS: INDEPENDENT CONTRASTS AND PHYLOGENETIC SIGNAL

*Testing the body size hypothesis*

Independent contrast analysis showed that five traits (four spinning and one morphological) varied as a function of body size (Fig. 3). For example, the circumference of the spinning tarsus relative to head width increased with body length, suggesting that larger webspinners are investing more in silk glands than the smaller (Fig. 3D). This finding aligns with a recent analysis of silk glands for six oligotomids, which showed that tarsi of the larger insects housed glands with greater reservoir volumes (Büsse *et al.*, 2015). Dorsal spin-steps, predicted to be displayed by species with elaborate silk coverings in the field, were expressed at a higher rate by larger species (Fig. 3B). Samples of kinematic diagrams of spinning and close-up photographs of field silk have been added to the graph to show the variety of silk potentially related to the variation in dorsal spin-steps (Fig. 3B). For example, *Notoligotoma hardyi* Friedrichs (Notoligotomidae) creates layers of tight tubes by spinning over the back and to the side in a complex pattern. *Clothoda nr longicauda* mostly produces dorsal spin-steps, with very few ventral, and their silk is sheet-like (shown with scraps and moss pieces stuck to the surface). *Metoligotoma incompta* (formerly known as *Austrolembia incompta*) lives in leaf litter and uses silk to stitch leaves together; they rarely displayed the full range of spin-steps to the side, back and over. Instead, they spin mostly around their head, flipping over repeatedly (a behaviour reflected in almost equal investment in spinning with the dorsum or venter toward the camera lens).

Surprisingly, spin-step diversity decreased with body length. Closer inspection of this trend indicates that species that spin-step around the front of their bodies more than over the dorsum produce a variety of dorsal and ventral spin-steps, such as *cross* and *paw*, thereby contributing to high spin-step diversity scores. Arboreal species (black circles in the graph) displayed higher spin-step diversity whereas leaf litter, subterranean and mixed lifestyle species were lower (Fig 3C). Therefore, even if small, arboreal webspinners showed higher spin-step diversity than similarly sized leaf litter or subterranean species. Proportion *dorsal cross* (Fig. 3A) decreased whereas repeatedly spin-stepping to the *side* (Fig. 3E) increased with body length. Repeat spin-steps in one position reinforces the emerging silk. Lifestyles seem to partially explain the distribution of points relative to body length because webspinners that live in leaf litter, even part of the time, were less likely to repeat the *side* spin-step (Fig 3E). For embiopterans, we suspect that the larger bodies need more complex

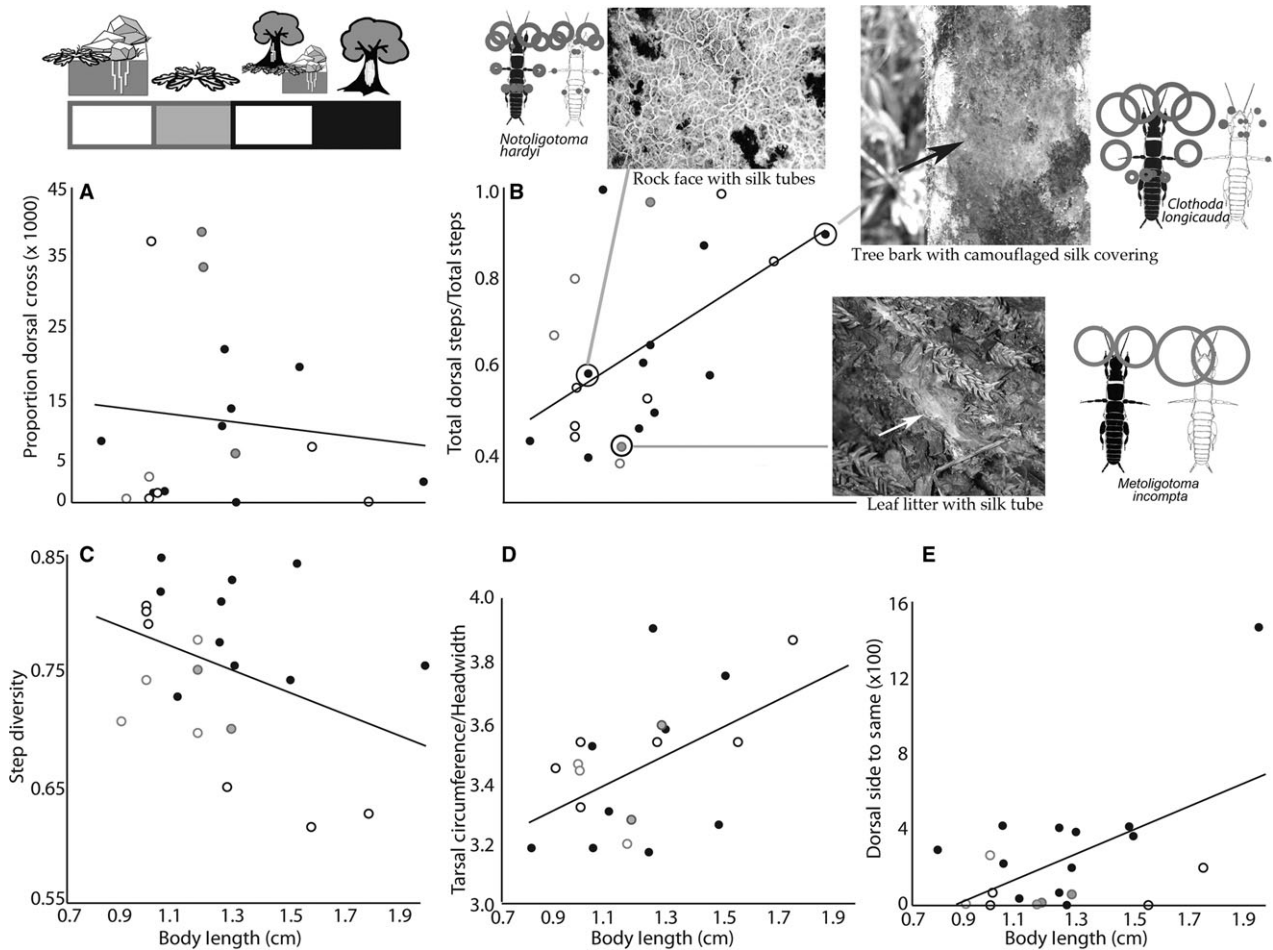
coverings and therefore, silk spinning methods relate to some degree with external demands that seem to vary with the size of the animal.

*Testing for phylogenetic signal*

Spin-step dynamics that showed significant phylogenetic signal included the proportional representation of six spin-steps in particular positions (such as *dorsal cross*, *dorsal side*, and *ventral side*), nine transitions of spin-stepping around the whole body (such as *dorsal side to back* and *dorsal reach to side*) and seven spin-step transitions involving spin-steps just around the front of the body (such as *dorsal near to adjacent near*) (Fig. 4, Supporting Information, Table S2). Spin-step dynamics scored in the Burrow revealed strong phylogenetic signal, with 16 characters being significant (Figs 4, 5A). Trials in the Chamber revealed seven of the same spin-steps detected in the Burrow that showed phylogenetic signal and six others that were not detected in the Burrow (Figs 4, 5B). Four of the spin-step transitions were to the front of the body such as *ventral paw to reach*. For the analysis using Best trials, 11 characters showed phylogenetic signal, with only two being unique: *dorsal near to adjacent near* and spin-step diversity. Chamber and Burrow trials enhanced the differences between species because their response to microhabitat to some degree appears to modify their expression of spinning. Furthermore, the Burrow dataset revealed four spin characters with  $K > 1$  indicating convergence between closely related species. Analyzing Best spinning data for each species is a more conservative approach to detect phylogenetic signal because the data selected were the most complex for each species. Even with this collapsing of potential variability, phylogenetic signal in 11 characters of spin-step dynamics, plus spin-step diversity, was detected in the Best data set.

Close inspection of phylogenetic signal based on spinning in the Burrow, when phylogeny is based on the Bayes tree, revealed spin-step dynamics that might be shared because of evolutionary history especially in the families Oligotomidae and Austrolembiidae. The other species, especially the larger arboreal webspinners, did not spin much in the Burrow. Of interest, the two *Haploembia* species seem to have diverged from others in the oligotomid lineage—*Oligotoma*, *Aposthonia*, and *Lobosembia* species, which in turn displayed similar spin-step dynamics to each other perhaps contributing to the higher  $K$ -values. In the laboratory and field, *Haploembia*, especially *H. tarsalis*, tend to spin little silk and exhibit less complex spinning, a tendency that may be related to living in underground burrows. They appear to rely more on substrate materials and

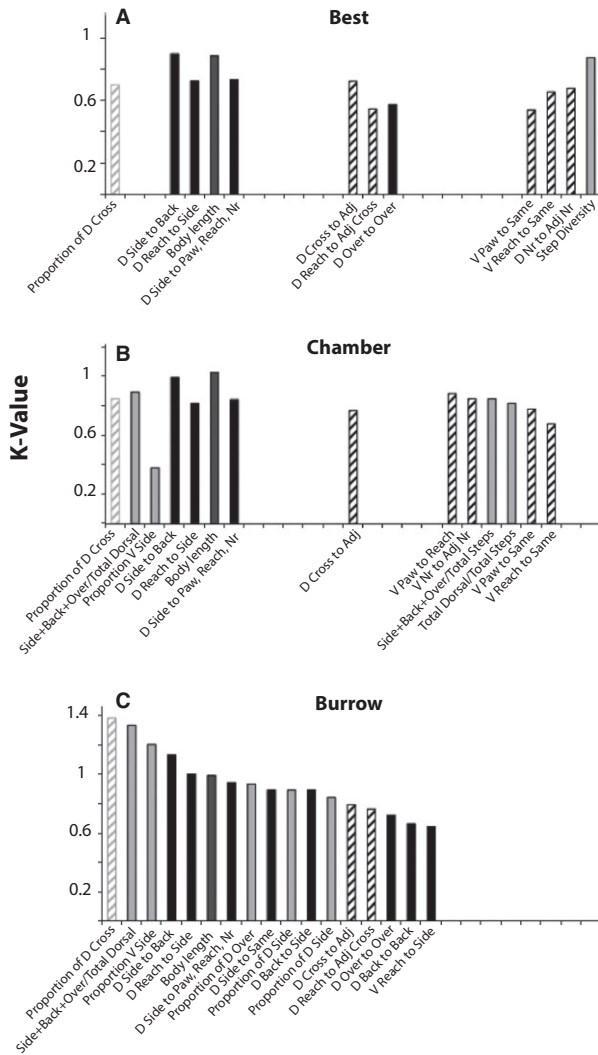




**Figure 3.** Significant relationships between spinning behaviours and body length, as indicated by independent contrast analysis. As shown in the graphic at top left, grey symbols indicate subterranean and leaf litter (open circles) or leaf-litter (solid circles) dwellers while black indicate mixed habitat (open circles) or arboreal (solid circles) species. (A) Proportion of spin-steps in the *dorsal cross* position. (B) Total Dorsal spin-steps divided by total number of spin-steps. Samples of kinematic diagrams (as described in Fig. 2) illustrate some of the variation displayed by small (*Notoligotoma hardyi* and *Metoligotoma incompta*) and large (*Clothoda* nr. *longicauda*) species. Photographs of silk in the field display their silk structures ranging from tubes (*N. hardyi*) to diffuse stitching and patches in leaf litter (*M. incompta*) to sheet-like coverings over bark (*C. nr. longicauda*). (C) Spin-step diversity, based on Simpson's Diversity Index, as a function of body length. (D) Tarsal circumference relative to headwidth as a stand-in for investment in silk glands and (E) *Dorsal side to same*, which is when the female spin-steps to the side and repeats that spin-step again.

cracks within the soil, which they line with silk using simple spin-steps, than they do on silk. *Haploembia solieri* Ramb. display more complex spinning, and their aggregations, in California at least, produce more silk than their parthenogenetic congener *H. tarsalis*. Recent studies of silk proteins revealed that *H. tarsalis* (referred to as the asexual race of *H. solieri* in that paper) produced silk unlike the five other species analyzed (Collin, Edgerly & Hayashi, 2011). Their lack of cysteine in the fibroin carboxyl-terminal region might play a role in fibre formation. The authors suggested that *H. tarsalis* might be less effective at silk production. Their silk

is wispy, perhaps also related to their apparent reliance on substrate materials for their domiciles. In a separate study, Collin *et al.* (2009b) found their silk proteins differed from that of six other species in having the highest percentage of *Beta*-sheet in the proteins and the least extensibility of the fibres. Their silk has increased rigidity and decreased ability to stretch. In many ways, this species differs from all others tested and the problems we had trying to get them to spin silk might be related to how selection has acted to reduce their reliance on silk spinning and production. Their silk is different and their spinning is different.



**Figure 4.** Blomberg's  $K$ -values, a measure of phylogenetic signal, for different spinning traits in three data-sets: (A) Best spinning, where only the most complete records for a species were used; (B) Chamber, where only spinning in the Chamber apparatus was analyzed; and (C) Burrow, where only spinning in the Burrow apparatus was analyzed. Black bars: spin-step transitions involving *side*, *back* and *over* spin-steps, which contribute more to sheet-like coverings, Striped Black: spin-step transitions between spin-steps around the anterior of the body, Grey bars: proportion of spin-steps in side, back or over, Striped Grey: proportion of spin-steps around the anterior of the body. Spin-step Diversity showed significant phylogenetic signal only in the Best spinning data-set, and that score includes all spin-steps.

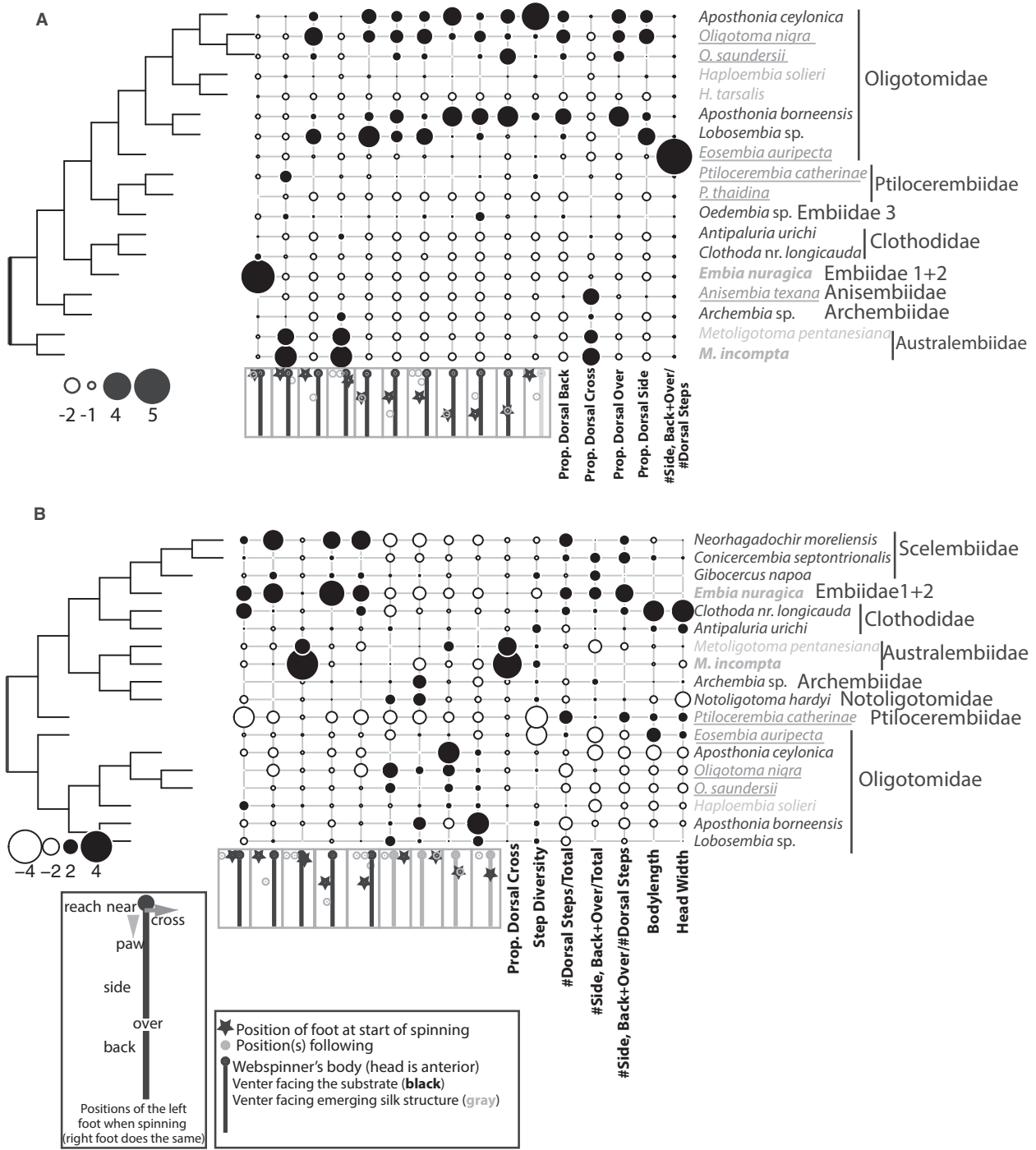
*Metoligotoma incompta* (Ross) and *M. pentaneisiana* displayed the *dorsal cross* spin-step regularly, something not seen much in other species scored by us. Spin-step dynamics that include *cross* appear to

separate these australembiids from the species in the other families examined.

In the Chamber, which emulated bark on a tree, arboreal species tended to spin more than when in the Burrow. Some of leaf litter species shut down completely, either sitting still or wandering around as if in search of acceptable habitat. Many of the same spin-step dynamics as in the Burrow trials appear to have phylogenetic signal for the Chamber trials. The pPCA graph based on the Maximum Likelihood Tree and the Chamber data (Fig. 5B) shows again a split between the Oligotomidae and the remaining families. Scelembiids, all arboreals, along with *Embia nuragica* Stefani, appear very similar corroborating the placement of *E. nuragica* near Scelembiidae as proposed by Miller *et al.* (2012). As in the Burrow samples, the australembiids displayed *cross* spin-steps while in the Chamber, and resembled each other more than they do any other species in their spin styles. Another pair of closely related species from two different families with similar spin-step dynamics in the Chamber, as much as they did in the Burrow. However, in this case (Fig. 5B), *Ap. borneensis* (Hagen) in some aspects resembles *Lobosembia mandibulata* Ross, a finding that aligns with the results of the Miller *et al.* (2012) phylogenetic analysis.

Spin-step dynamics that show phylogenetic signal are quirky. Some spin choreographies appear related to the end products of spinning (for example, thick sheets of silk which are more typical of arboreal species), whereas other styles are not apparently so. This latter finding is expected if slight differences in choreography accumulated in lineages over time. For example, the sequence of spin-stepping from *near* to *reach* might involve multiple repeats in one position before a switch to another position or might involve alternations back and forth.

A preponderance of spin-steps to the *side*, *back* and *over the back* add silk along the length of the body and result in tubular or sheet-like coverings. But the spin-step pattern, not just the frequency, showed phylogenetic signal. For example, *Eosembia auripecta* Ross displayed a high proportion of spin-steps to the *side*, *back* and *over the back*, but did not tend to repeat any one of these spin-steps before moving on (Fig. 5A). Their unique style of spin-steps from one position to the next without many repeats may relate to the resulting wispy 'swiss-cheese' appearance of their silk (Fig. 1E). Two other spin-steps that showed phylogenetic signal were *paw* and *cross*. *Paw* appeared most commonly in some oligotomid species, such as *Ap. ceylonica* and *Oligotoma nigra* (Hagen), who typically extend tightly



**Figure 5.** Phylogenetic principal component analysis (pPCA) of silk spinning behaviour data aligned with (A) the phylogeny based on Bayes analysis from Miller *et al.* (2012) and spinning data collected from the Burrow observations; and (B) the phylogeny based on Maximum Likelihood analysis from Miller *et al.* (2012) and spinning data collected from webspinners placed in the Chamber apparatus. Positive and negative values of traits and PCs are indicated by black and white circles, respectively (see, Jombart *et al.*, 2010). Symbols, resembling domino tiles, along the horizontal axis show the spin-step transitions as indicated in the legend. Species' names are coded in colours of the font to indicate habitats as follows: Black is arboreal, Grey underlined is mixed habitat, Grey is leaf litter, and Light Grey is underground as well as in leaf litter.



spun silk tubes up the sides of their laboratory containers. *Paw* appears to reinforce the tube that develops anteriorly around the body as the insect spins around the head and thorax.

## CONCLUSION

Embiopteran silks tested thus far have not diverged much in structural profiles (Addison *et al.*, 2014), tensile strengths, or amino acid sequences (Collin *et al.*, 2009b, 2011). *Haploembia tarsalis* is the exception discovered to date; Collin *et al.* suggested that selection on their silk may be relaxed because of their reliance on substrate materials, as mentioned above. Webspinner spinning routines, however, did vary in a range of details. Together these findings align well with work on spiders by Sensenig *et al.* (2010) who proposed that the intrinsic properties of biomaterials appear relatively fixed suggesting strong selection on silk proteins. They also proposed that shifts might have occurred in web architecture, behaviour and quantity of silk produced. These last two aspects appear to have diversified in the Embioptera as well. For spiders, diversification relates to the stopping potential of the webs, and larger spiders appear to need better performing webs because of the need for more food (Sensenig *et al.*, 2010). For embiopterans, some spin characters also varied with respect to body size, and to some extent with lifestyle as reflected in the higher spin-step diversity scores for arboreals irrespective of size of individual. We also found support for the hypothesis that more effective coverings are needed to camouflage and protect the larger webspinners. They also protect their eggs by covering them with silk, a behaviour shared with spiders. In fact, for spiders, protecting eggs and their own bodies seems to have preceded the evolution of the use of silk as traps (Vollrath & Selden, 2007).

Embiopteran body shape varies little compared with spiders. Vollrath & Selden (2007) reviewed the role of behaviour in the evolution of spiders, silks and webs, and discovered a clear link between variable body structures and behaviour because their spinning actions require specific adaptations in body shape and leg dimensions, spinning glands and spigots. They also reported that deconstruction of a spider's web provides a continuous record of the spin-steps taken by the spider. Embiopteran silk products are not so informative, perhaps because they use their front legs (which house the silk glands) to walk and run, a function that would limit variability. We have seen similarly shaped silk tubes produced by almost every species collected in the field by us reflecting the reach of the front legs as they

spin-step around their bodies while releasing silk. We have not succeeded in designing a method for capturing the slight microscopic differences in the layering of silk fibres.

Early-on in our filming trials we detected similarities in spinning routines in related species, and this sparked our interest, because before this we had not seen any hint of phylogenetic signal in silk tubes or body morphology of adult females. The end result of scoring thousands of spin-steps showed that routines display phylogenetic signal and, therefore, their silk spinning reveals hints of relationships, although not as easily detectable as in spiders for which behaviour and obvious web architectures align. Future studies might include evaluating the sequencing of spin-step, which may hold more information than the probability of switching from one position to the next, the variable analyzed in this study. A suitable method might resemble that developed for analyzing sequences displayed by interacting cockroaches (Legendre *et al.*, 2008) or for even more complex sequences exhibited by cats grooming or spiders foraging (see references in Japyassu *et al.*, 2006). Finally, it is worth noting that webspinners produce many spin-step sub-routines, repeating them over and over, until switching to another sub-routine, as they create their silken tubes. Discovering a suitable method for capturing and coding such complex sub-routines is the next spin-step in seeking phylogenetic signal in the complex behaviour of silk spinning in this order of insect.

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### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Figure S1.** Time spent spinning in the two different arenas (Burrow or Chamber) used to record silk spinning behaviour. The graphics across the top indicate the lifestyle of the species named along the horizontal axis in order from left to right. Means are typically based on five or fewer females (see Supporting Information, Table S2 for sample sizes per species). Some species were recorded in only one arena type, as discussed in Methods. Those species that tend toward arboreal lifestyles were prone to spend more time spinning in the bark-lined chamber in contrast with leaf litter or subterranean species, which tended to spend more time spinning when in the burrow arena.

**Table S1.** Habitat information, sizes, and details of spinning behaviours for embiopteran species videotaped for 1 h sessions in artificial arenas in the laboratory.

**Table S2.** Results of tests for phylogenetic signal and independent contrast values for behavioural data related to silk spinning behaviour in embiopterans. Bold-faced font = Blomberg's  $K > 0.7$ . \* = Based on Figure 4 in Miller *et al.*, 2012. \*\* = Based on figure 3 in Miller *et al.* (2012).