Evolution

Silk Spinning Behavior Varies from Species-Specific to Individualistic in Embioptera: Do Environmental Correlates Account for this Diversity?

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Abstract

String sequence analysis revealed that silk spinning behavior of adult female Embioptera varies from species-specific to individualistic. This analysis included 26 species from ten taxonomic families with a total of 115 individuals. Spin-steps, 28 possible positions of the front feet during spinning, were scored from hour-long DVD recordings produced in the laboratory. Entire transcripts of hundreds to thousands of spin-steps per individual were compared by computing Levenshtein edit distances between all possible pairs of subsequences, with lengths ranging from 5 to 25—intraspecific similarity scores were then computed. Silk gallery characteristics and architecture, body size, climatic variables, and phylogenetic relationships were tested as possible drivers of intraspecific similarity in spinning behavior. Significant differences in intraspecific similarity aligned most strongly with climatic variables such that those species living in regions with high temperature seasonality, low annual precipitation, and high annual temperatures displayed more species-stereotypical spinning sequences than those from other regions, such as tropical forests. Phylogenetic signal was significant but weakly so, suggesting that environmental drivers play a stronger role in shaping the evolution of silk spinning. Body size also appears to play a role in that those of similar size are more like each other, even if not related.

Key words: embioptera, silk spinning, string sequence analysis, environmental correlates

Complex patterns and sequences characterize communication signals, construction routines, and dances performed by a variety of animals. Bird song (Alger et al. 2016), courtship displays (Ligon et al. 2018), orb weaving (Benjamin and Zschokke 2003), spider predatory behavior (Tourinho et al. 2016), dolphin social interactions (Dans et al. 2008), and the subjects of this study—silk spinning in embiopterans (McMillan et al. 2016)—are among the many examples of behaviors expressed as a series of acts, often complex and species-specific, and sometimes with a stamp of individual personality. Variability in how individuals display sequences of behavioral acts, such as singing styles in birds, might indicate quality, how they learned, or if the song reflects particular information (Alger et al. 2016). Variation in behavior may also arise due to stochastic variation generated by the central nervous system of individuals (and, therefore, unrelated to the environment), a hypothesis termed 'imprecision' and proposed to describe features of silk spinning in a fly larva (Eberhard 1990). For embiopterans, analyzing behavioral sequences to address evolutionary and ecological questions can be challenging especially because the behavioral act, in this case silk spinning, is highly variable and expressed in long series. Their absolute reliance on silk for protection makes silk spinning behavior a key adaptation promoting

their survival and reproductive success (reviewed in Edgerly 2018). Thus, behavioral sequences expressed while spinning may reflect biologically significant attributes under selection, reflecting the interplay of the organism's phenotype and environmental characteristics. Variation in sequences might also shed light on phylogenetic relationships among species. The purpose of this study was to apply new methods for analyzing behavioral sequences to test hypotheses concerning the evolution and diversification of silk spinning behavior in Embioptera, also called webspinners. Our approach is to search for similarity and differences in sequences by comparing individuals sampled from across the phylogeny of the group and from a broad range of climates and microhabitats.

Embiopterans, including an estimated 1,500 species (Ross 2000), are outliers compared to species in other insect taxa because their body form is uniform, an unusual characteristic for the otherwise diverse Class Insecta. Although webspinners vary in size and color, they do not vary in overall body shape (for typical diversity of adult females, see Fig. 1A–D). Uniformity so characterizes webspinners that for many species adult females are described as displaying no distinguishing traits, even at the taxonomic family level, and they are often left without any description at all. For

example, for Clothodidae, Ross (1987) states after a detailed paragraph on male traits: 'Females: Without unique family-level characteristics.' In fact, species are generally described based only on male sexual characteristics and wings. The embiopteran lifestyle of stepping with their front feet to create silk tubes for travel and protection apparently imposed a homogenizing constraint on female body form (Ross 2000).

Adult females are neotenous, never winged, long, narrow, soft-bodied, and flexible (Fig. 1A–D, see Supp Video 1 [online only] for demonstration of typical flexibility). Although all individuals spin silk, adult females appear responsible for most of the silk for a colony (e.g., for *Antipaluria urichi* (Saussure) (Clothodidae) see Edgerly 1988). Mature males are also flexible and typically sport wings (Fig. 1D) that crumple easily while they traverse the silk tubes
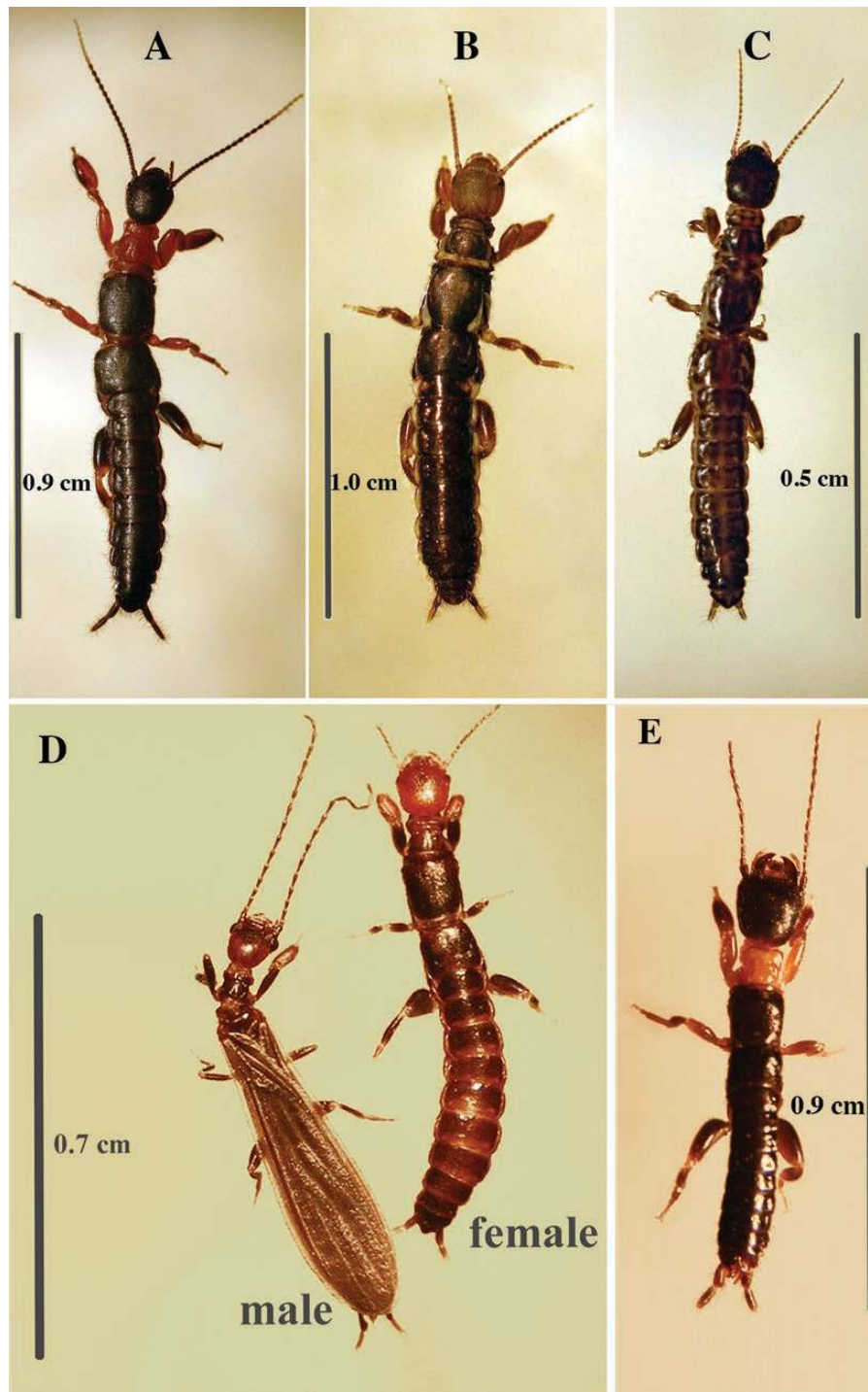

Fig. 1. Adult Embioptera illustrating morphological uniformity of females and typical male traits. (A) Female *Eosembia auripecta* Ross (Oligotomidae), (B) Female *Antipaluria urichi* (Saussure) (Clothodidae), (C) Female *Metoligotoma pentanesiana* Davis (Australembiidae), (D) Male and female *Aposthonia ceylonica* (Enderlein) (Oligotomidae), (E) *Haploembia solieri* (Rambur) (Oligotomidae) male.

so that the wings do not tear or catch (Ross 2000). A few species, including *Haploembia solieri* (Rambur) (Oligotomidae), have wingless males (Fig. 1E). Embiopterans execute U-turns and run backwards and forwards with agility in the tight confines of their silken homes where they graze on lichens, epiphytic algae or leaf litter. Even though webspinners are constrained to live in tubes, they do vary in where they construct those tubes (reviewed in Ross 2000 and Edgerly 2018). Some are exposed on tree bark or rock outcrops (Fig. 2A–D), often living in expansive colonies within thick silk coverings as in Fig. 2A. Others stay hidden under bark or in crevices (Fig. 2E and F), stitch dead leaves together (Fig. 2G and H) or live under rocks and underground (Fig. 2I and J). In tropical rainforests

where temperature and humidity are conducive to insect life, embiopterans spin their silk out in the open usually on tree bark or other upright substrates that support their food (epiphytic algae and lichens). In dry and hot climes, they burrow under rocks, lining crevices with silk. Previous experiments demonstrated that a leaf-litter dwelling species produced much less silk and did not form social groups (Edgerly et al. 2006). Two arboreal species in the same study produced significantly more silk and tended toward gregariousness, allowing individuals an opportunity to share the silk that serves to protect them in their more exposed perches. These results suggest environmental drivers as selective pressures on silk spinning that might differ depending on habitat characteristics of a species.
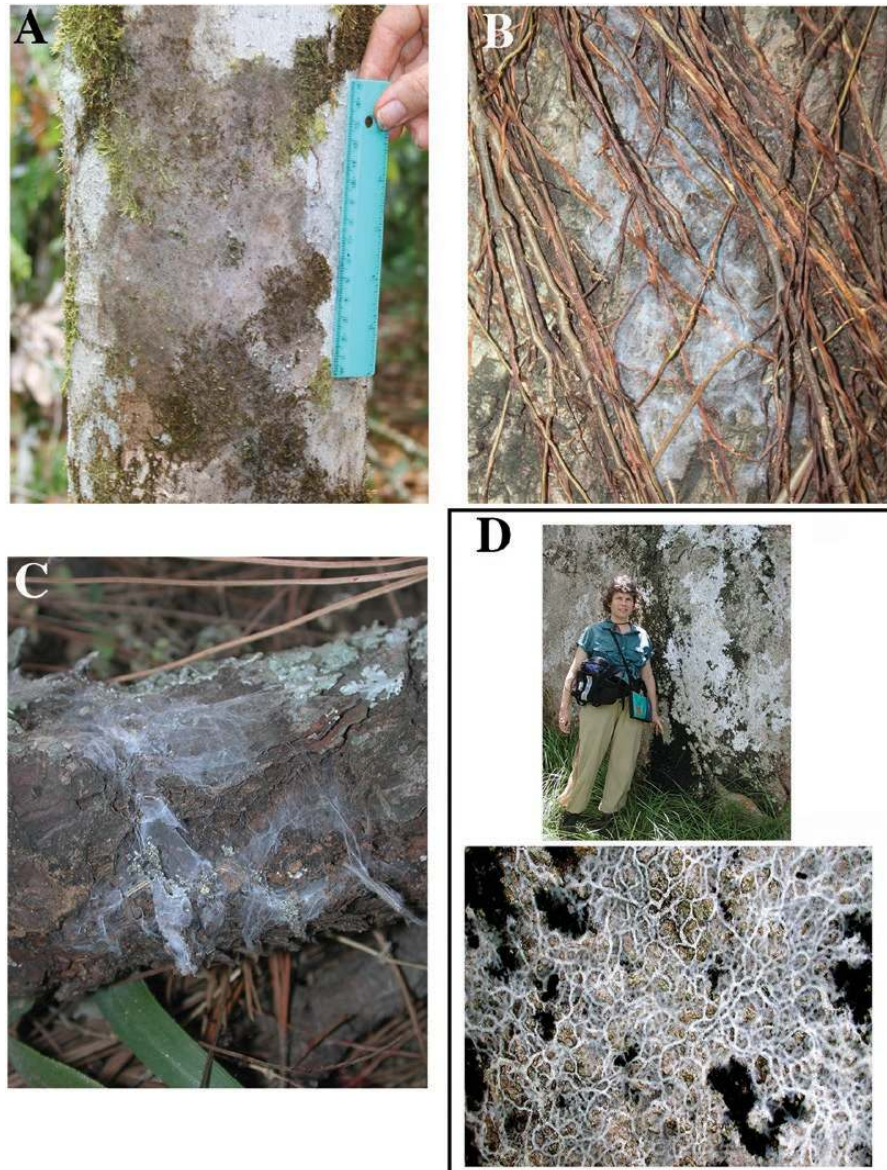

Fig. 2. Examples of silk spun by embiopterans in their natural habitats. Domicile and gallery structure scores used in the statistical analyses are reported in SuppTable 2 [online only]. (A) *Clothoda nr. longicauda* Ross (Clothodidae) with exposed silk typified by a thick outer coating of silk, in this case reinforced even further with gathered materials, (B) *Pararhagadochir trinitatis* (Saussure) (Scelembiidae) with galleries exposed to the elements but quite thin, (C) *Conicercembia septentrionalis* (Mariño & Márquez) (Scelembiidae) with very thin exposed silk over the bark, (D) *Notoligotoma hardyi* (Friederichs) (Notoligotomidae) showing a typical colony on lichen-covered granite with a close-up of the exposed thick tubes of silk, (E) *Neorhagadochir moreliensis* (Ross) (Scelembiidae) with thick silk tubes found under bark flaps, (F) *Ptilocerembia thaidina* Poolprasert & Edgerly (Ptilocerembiidae) with very thin silk, also found mostly living under bark and inside crevices, (G and H) *Metoligotoma* species Davis (Australembiidae) all of which live in leaf litter; (I) *Haploembia tarsalis* (Ross) (Oligotomidae) with thin silk lining underground crevices, (J) *Haploembia solieri* with thin silk under rocks and in leaf litter.
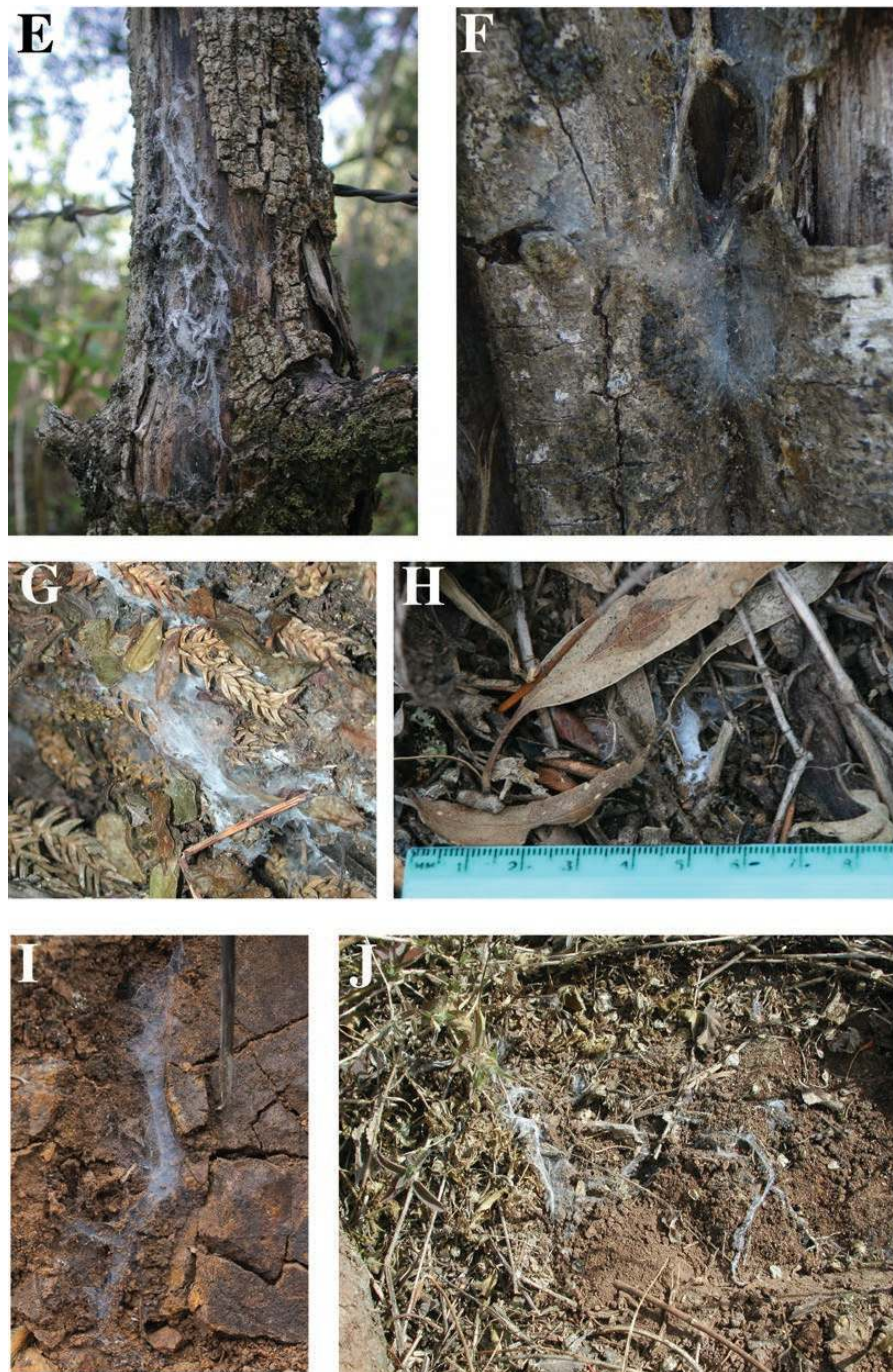
Fig. 2. Continued.

Variation in spinning behavior by adult female embiopterans appears to hold clues about phylogenetic relationships or adaptations related to lifestyle that are not easily observed (McMillan et al. 2016). Spinning routines have proven to be very long; individual females display many hundreds, even thousands, of spin-steps executed during hour-long filming sessions (McMillan et al. 2016). When spinning, they step with their front feet, swollen with silk glands, and release silk by pressing hair-like ejectors against the substrate (Ross 2000; Fig. 20 in Büsse et al. 2015, Osborn Popp et al. 2016, Büsse et al. 2019). An adhesion disc forms to stick down the secreted liquid silk (Büsse et al. 2019). When they pull their foot back, the silk protein is sheared into nano-scale fibers (Okada et al. 2008, Addison et al. 2014, Stokes et al. 2018), dozens produced with each footfall. The spinning routine includes up to 28 different spin-steps with the right and left front leg placed one step at a time around the body (Edgerly et al. 2002; McMillan et al. 2016). They spin over and around their dorsum (dubbed 'dorsal spinning') and then turn to face the emerging structure to thicken that with more silk (dubbed 'ventral spinning'). Thus, an embiopteran typically displays subroutines to first create, and then to reinforce, a framework. The spin-steps are named for their positions: near (the head), reach (out from the head), side, back (along the side of the thorax and abdomen), and overback (above the dorsum of the abdomen), cross (from one side to the other near

the head), and paw (reach forward down in front of the venter) (Fig. 3). Their stepping is symmetrical around the body as shown in the two examples in Fig. 3, without handedness, unless they are up against the side of the arena as displayed by a female of *H. solieri* in Supp Video 2 [online only].

McMillan et al. (2016) found that 16 different spin-step transitions and frequencies revealed phylogenetic signal for 22 species of webspinners examined. For that investigation, choreographies were quantified as frequencies of the different spin-steps and transitions from one to another. Such an approach is typical for studying behavioral sequences (e.g., Martin and Bateson 1993, Dans et al. 2008, Legendre et al. 2008, Lunardi and Ferreira 2014) and the discovery of phylogenetic signal in spinning behavior was significant (McMillan et al. 2016). However, the method employed resulted in a potential loss of meaningful information because exact sequences are not intact or represented when the spin-step routines were transformed into frequencies and probabilities of transitions. Our agenda

herein was to develop a measure of similarity among individuals exhibiting long, complex behavioral sequences. An example of the differences between the summary data analyzed in McMillan et al. (2016) and a complete spin-step dataset for one individual we analyzed herein is displayed in Supp Table 1 [online only].

We further explored phylogenetic signal and environmental drivers of spinning behavior that had not been tested previously. In addition, we developed a scoring mechanism to define different silk architectures and tested how spin-step sequences might relate to the end-products of spinning as has been done for spiders (e.g., Blackledge et al. 2009) and black flies (Diptera: Simuliidae) (e.g., Stuart and Hunter 1998). The varying silk architectures might reflect similarities along phylogenetic lines or, alternatively, products shaped by the environmental drivers of heat, humidity, and rainfall patterns that might impact ground-dwellers and arboreal species differently. Despite the lack of morphological variability in the order, we speculated that behavioral variability might provide clues about
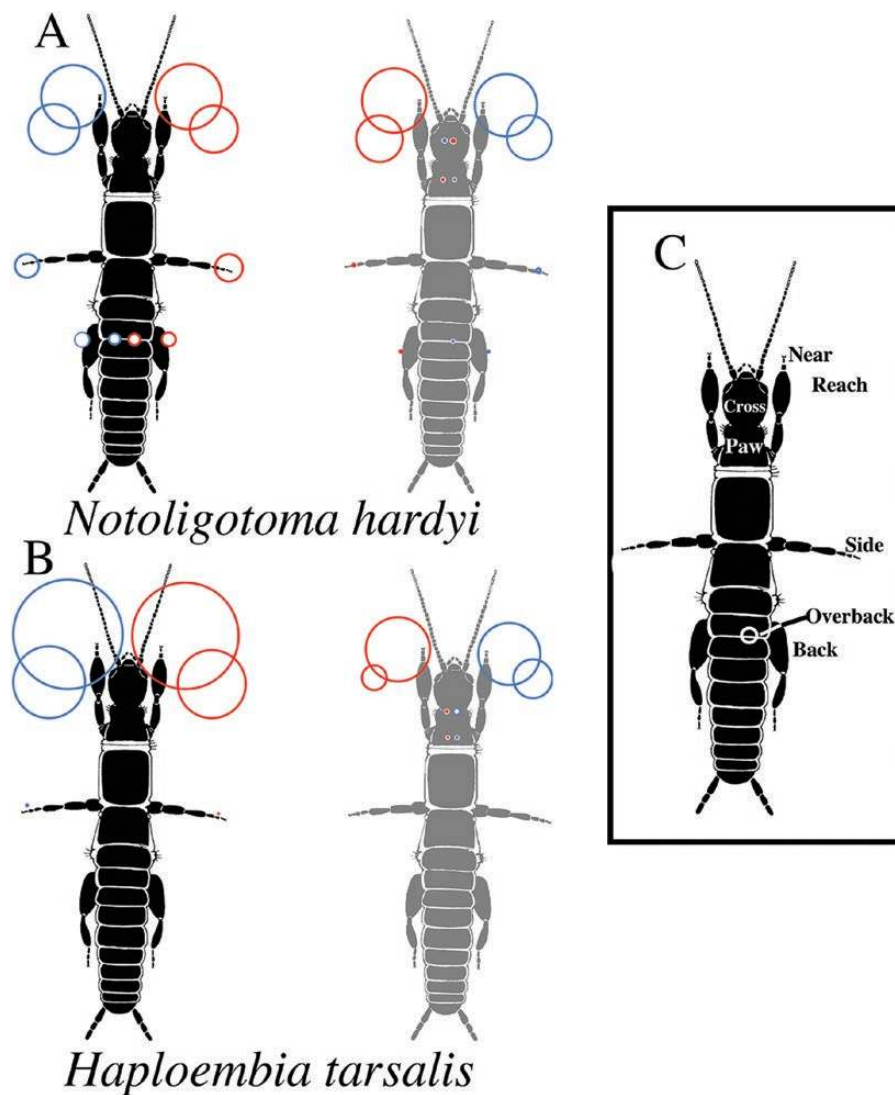

Fig. 3. Kinematic diagrams displaying relative proportion of spin-steps in each position as relative size of the circles. Saturated black color of the body represents dorsal spinning; dark gray represents kinematics when the embiopteran faces the framework silk and spins with her ventral surface facing the camera and the emerging silk structure. Spinning was recorded during hour-long filming sessions in the laboratory in an apparatus as shown in Supp Fig. 1 [online only]. (A) *Notoligotoma hardyi* average spin dynamics, (B) *Haploembia tarsalis* average spin dynamics, (C) Diagram shows the positions of the different possible spin-steps whereby the words are placed in the position of the front foot as the embiopteran steps around her body to release silk with each foot fall. The same steps are taken on the left as well during spinning. See Supp Video 2 [online only] for examples of spinning behavior exhibited by individual females.

how or whether embiopterans have diversified and which selective forces might be acting.

The problem of identifying similarity among sequences of arbitrary length and complexity has arisen in a variety of fields, including text analysis (Gomaa and Fahmy 2013), bioinformatics (e.g., Abouelhoda and Ghanem 2009), and musicology (Clifford and Iliopoulos 2004, Lartillot 2005). Our data have several particular features that led us to develop a new approach that builds on previous work. First, we expect that spin-step sequences might be organized into motifs—relatively short sequences that might be repeated in different orders among individuals. Thus, a simple measure of overall string similarity such as the Levenshtein edit distance does not suit our purposes very well. As an illustration, consider two different motifs, A and B, with very different step sequences. Individual 1 might spin with a sequence A, A, B, B, while individual two might spin B, B, A, A. We would like to score these as very similar sequences, but a simple overall string similarity measure would not do this. It is also true that transition matrices previously analyzed by McMillan et al. (2016) collapsed the information possibly embedded in spin-step motifs, as mentioned earlier (Supp Table 1 [online only]).

At the same time, these motifs are only hypothesized; we have no a priori knowledge of what they might be, or how long. Thus, it was not possible to define a particular behavioral pattern of interest and search for that pattern (or variants of it) in the sequence. We needed to allow these motifs to arise naturally from the analysis itself.

A number of approaches for analyzing similarity and finding motifs in behavioral sequences already exist. These rely mostly on transition probabilities (e.g., for embiopterans see McMillan et al. 2016, for dolphins see Dans et al. 2008, for spiders see Japyassú and Caires 2008, for cockroaches see Legendre et al. 2008) or on Markov Chain analyses (for bird song see Alger et al. 2016). Analyses typically focus on more easily categorized behavioral acts, such as wrapping and biting prey for the spiders or whistles, clicks and trills for bird song. In contrast, the granular nature of our data and the very long sequences during one bout of spinning meant that employing probability of transitioning from one act to another resulted in potential loss of information. The structure of our data is a series of 28 possible spin-steps, that repeat again and again in obvious subroutines, interspersed with bouts of unpredictable spin-steps, reminded us very much of the structure of musical compositions. Upon exploring the musicology literature (e.g., Bartsch and Wakefield 2001, Cooper and Foote 2003), we discovered useful methods: the sliding-window method of comparing long sequences of data points (musical notes for musicologists and spin-steps for us) and heat maps to display the comparisons among data sets (musical scores vs webspinner species).

In summary, we compared silk-spinning sequences of individuals to determine how similar they are to each other. We tested three main noncompeting hypotheses about diversification of embiopteran spinning behavior: 1) The Environmental Driver Hypothesis whereby environmental correlates, such as seasonality, temperature, and precipitation, underlie similarities and differences in spin-step dynamics between individuals which dwell in different microhabitats in response to climate, 2) The Phylogenetic Lineage Hypothesis whereby spin-step sequences expressed as interspecific similarity scores correlate with phylogenetic distances, as predicted by the McMillan et al. (2016) analysis showing phylogenetic signal in several spin-step transition probabilities and frequencies, and 3) The Body Size Hypothesis whereby larger webspinners typically live within complex silk galleries exposed on surfaces and therefore their spinning behavior might be more complex than expressed by smaller individuals, which can hide in cervices and gain protection more from substrate than from silk. For both Hypotheses 1 and 3, we do not know if species exposed on surfaces would be predisposed to have higher intraspecific similarity scores or lower than leaf-litter or underground dwellers. Because arboreal species rely on thicker silk for protection, they might express species-specific routines or alternatively, they might be responsive as individuals to the vines, cracks, and other irregularities that tree bark presents. According to Eberhard (2000), imprecision in how the central nervous system controls expression of web construction behavior, as seen in spiders of the surprisingly variable spinners in genus *Wendilgarda*, suggests a reduction in the intensity of selection for stereotypy combined with an increase in behavioral variability. If embiopterans display similar degrees of variability across and within species, we would predict that the crevice dwellers with more constrained spinning substrates might be more stereotypical than the arboreals who contend with variable silk attachment sites. Our methods are designed to probe these questions.

Materials and Methods

Behavioral Observations

The individuals and recording methods used in this study were previously described in McMillan et al. (2016) but will be briefly summarized herein. Laboratory culturing information is also described in McMillan et al. (2016). Adult males were not used as subjects because they do not feed after reaching maturity and die soon after mating. Individual adult females (typically five for each species), selected from laboratory cultures held at Santa Clara University (SCU), were videotaped for 1 h each and placed in either a bark-lined chamber or into a burrow carved into a wooden block (Supp Fig. 1 [online only]). The only exceptions were *Gibocercus napoe* Ross (Scelembiidae) and *Clothoda nr. longicauda* Ross (Clothodidae), which were videotaped in Ecuador. These videos were analyzed at SCU. Preliminary trials showed that an hour-long session triggered spinning and lasted long enough that individuals stopped spinning before the end of the hour. These apparatuses resembled tree bark and crevices, respectively, reflecting microhabitats of the different species. A leaf litter dweller was much more likely to spin in a burrow whereas the bark-lined chamber elicited more spinning from an arboreal, tropical species (Büsse et al. 2015, McMillan et al. 2016). Spin-steps, as described earlier, produced in whichever situation generated the most spinning from an individual, were selected for data analysis to ensure the insect was producing the most complete record possible. Playback of the recording on DVDs was run at half- or quarter-speed depending on how quickly the webspinner moved. A single observer (Edgerly) voiced the spin-steps being displayed while an assistant simultaneously recorded these steps on a laptop running the Observer software (version 5, Noldus Information Technology, Wageningen, the Netherlands). An example of a kinematic diagram summarizing the average spinning for two species is shown in Fig. 3. A selection of kinematic diagrams for four different embiopteran species is also in Edgerly et al. (2012).

The data set generated by this procedure is a complete sequence of all spin-steps for each individual with the maximum being approximately 9,000 spin-steps for an individual of *H. solieri*. The average number of steps overall was 2,500. The final set of individuals (*n* = 115) who spun enough to be included in the analysis of spin-step sequences was 26 species in eight of the 13 possible taxonomic families plus the unnamed clades 'Embiidae 1 & 2' and 'Embiidae 3' (Miller et al. 2012). Individuals were excluded if they did not spin as much as one quarter of the spin-steps displayed by the top spinner in its species, because we found that stingy spinners

did not express the diversity of steps seen in the top spinner. The total evaluated also included four species not previously tested by McMillan et al. (2016): *Pararhagadochir trinitatis* (Saussure) and *Rhagadochir virgo* (Ross) (Sclembiidae), *Parthenembia reclusa* Ross ('Embiidae'), and *Saussurembia davisi* Ross (Anisembiidae). Body length and head widths of adult females, number of females and mean number of spin-steps per species, and taxonomic classification are described in Supp Table 2 [online only].

Spin Sequence Analysis

Pairwise Similarity

Given the spin-step sequences of two individuals (1 and 2, Fig. 4A and B), our goal was to compute a similarity score. Here, we define the similarity of 1 to 2 ($S_{1,2}$) as the proportion of all possible subsequences of length N in sequence 1 that have a close analog in some subsequence of 2 (Supp Fig. 2A [online only]). To obtain this value, we computed the Levenshtein edit distances (Supp Fig. 2B [online only]) between all possible pairs of N-length subsequences between 1 and 2 (Fig. 4C). For each possible N-length subsequence of 1, we then asked whether a close analog occurred in 2 by computing the minimum edit distance between that substring of 1 and all substrings of 2 (marginal plots in Fig. 4C). Finally, we computed $S_{1,2}$ as the proportion of all possible substrings of 1 whose minimum edit distance to any substring of 2 fell below a particular threshold (T, horizontal or vertical lines in marginal plots of Fig. 4C).

Because we are interested in spinning motifs that might be expressed over different numbers of steps, we considered a range of values for N, and adjusted T accordingly. N was 5, 10, 15, 20, or 25, and T was N/5 (i.e., 1, 2, 3, 4, 5).

This $S_{1,2}$ value provides a measure of how similar sequence 1 is to 2. It is asymmetrical, since for example, 1 might be entirely contained within 2, but not the reverse. As a result, shorter 1 and longer 2 will tend to have the highest $S_{1,2}$ values. To limit this effect, we only analyzed a maximum of 4,000 spin steps from each sequence, though some sequences were shorter.

Computation and Analysis of Similarity Among Individuals

We used the above approach to compute the similarity between all individual spin sequences, for values of N in {5, 10, 15, 20, 25}. This produced five 115 by 115 similarity matrices describing the similarity of all pairs of spinning sequences. We computed intraspecific similarity scores as the means of the $S_{i,j}$ values for all pairwise comparisons within each species (where i and j are individuals of the same species).

For comparison with previous results (McMillan et al. 2016), we also computed a step transition matrix for each individual. For one individual, we considered all step transitions displayed during its spinning behavior and calculated the probabilities of moving from each possible step to each other possible step. To obtain a score that describes the dissimilarity between two individuals, we then computed the Euclidean distance between the transition matrices between those individuals.

Predictors of Similarity

To provide some insight into possible drivers of variation in intraspecific similarity and between-species similarity, we compiled a range of variables describing the silk characteristics, latitude and longitude coordinates, size and phylogenetic relationships among species (Supp Table 2 [online only]). These included a classification into degree of exposure to the elements of their silk domiciles and the form of their silk galleries, based on observations in the field,

photographs, and behavior in laboratory cultures at SCU. Degree of exposure of their silk domiciles ranged from completely underground to hidden under bark flaps to completely exposed. Structure of their silk galleries ranged from silk acting as a lining of crevices in the substrate to silk tubes covered by a thick cloth of silk (see Fig. 2 and Supp Table 2 [online only] for details). As a measure of habitat characteristics, we scored mean annual temperature, annual precipitation and temperature seasonality based on the collection location (or, if not available, at the holotype locality). Using the geographic coordinates, we extracted climate data from CHELSA (Karger et al. 2017). We used average body length and head width divided by body length as measures of size.

We used the Bayesian phylogeny of Miller et al. (2012) to define the topology of the relationships among all species in this study, adding species at the family level if that species was missing from the species-level phylogeny. To obtain an ultra-metric tree, we used the penalized likelihood method of Sanderson (2002), implemented in the R function chronopl (ape package Paradis and Schliep 2018).

Statistical Analysis

We began by assessing the drivers of variation in intraspecific similarity. To do this, we used phylogenetic linear models (implemented in the R package phylolm, Ho and Ane 2014) to predict intraspecific similarity scores from the three climate variables, two size variables, two habitat variables and three variables describing silk and domicile structure. We used Brownian motion to model the covariance. Because there was a relatively large number of potential predictor variables relative to the sample size, we performed a stepwise model reduction based on AIC to obtain a simpler model.

To assess the drivers of similarity among all individuals, we used Mantel tests on pairs of distance matrices. We first converted our sequence similarity matrix into a distance matrix, then we related that to three distance matrices computed from 1) the phylogeny, 2) the Euclidean distance in the three-dimensional z-scaled climate space, and 3) the Euclidean distance in the two-dimensional z-scaled size space.

Results

In total, we computed the pairwise sequence similarities between 115 individuals, in subsequence lengths (N) of 5, 10, 15, 20, and 25. Across all comparisons, mean sequence similarity declined as N increased, from 0.77 for $n = 5$ to 0.10 for $n = 25$. The upper triangle of the pairwise distance matrices (Fig. 5) is not identical to the lower triangle because one sequence could be nested entirely within another, but not vice versa. Nevertheless, the two halves of the matrix were fairly well correlated for large N ($r > 0.5$ for N ≥ 15, $r = 0.36$ for $n = 10$, $r = 0.11$ for $n = 5$).

The pairwise similarity values were weakly negatively related to phylogenetic distances for N values of 5, 10, and 15 (Mantel Test, $P = 0.01$, $P = 0.02$, and $P = 0.04$). For larger N, the relationship was not significant ($P = 0.09$, $P = 0.11$). Dissimilarities in climate were unrelated to sequence similarity, but there was a trend towards a negative relationship between body size differences and sequence similarity for large N (20 and 25, $P = 0.066$, $P = 0.049$).

One matrix of similarity values can be converted into a distance matrix and visualized in two dimensions using multidimensional scaling (MDS). The resulting plot represents the distances between each pair of individuals as accurately as possible, given the inevitable distortion that occurs during compression into a two-dimensional space. Nearby points in the MDS plot have similar spin sequences
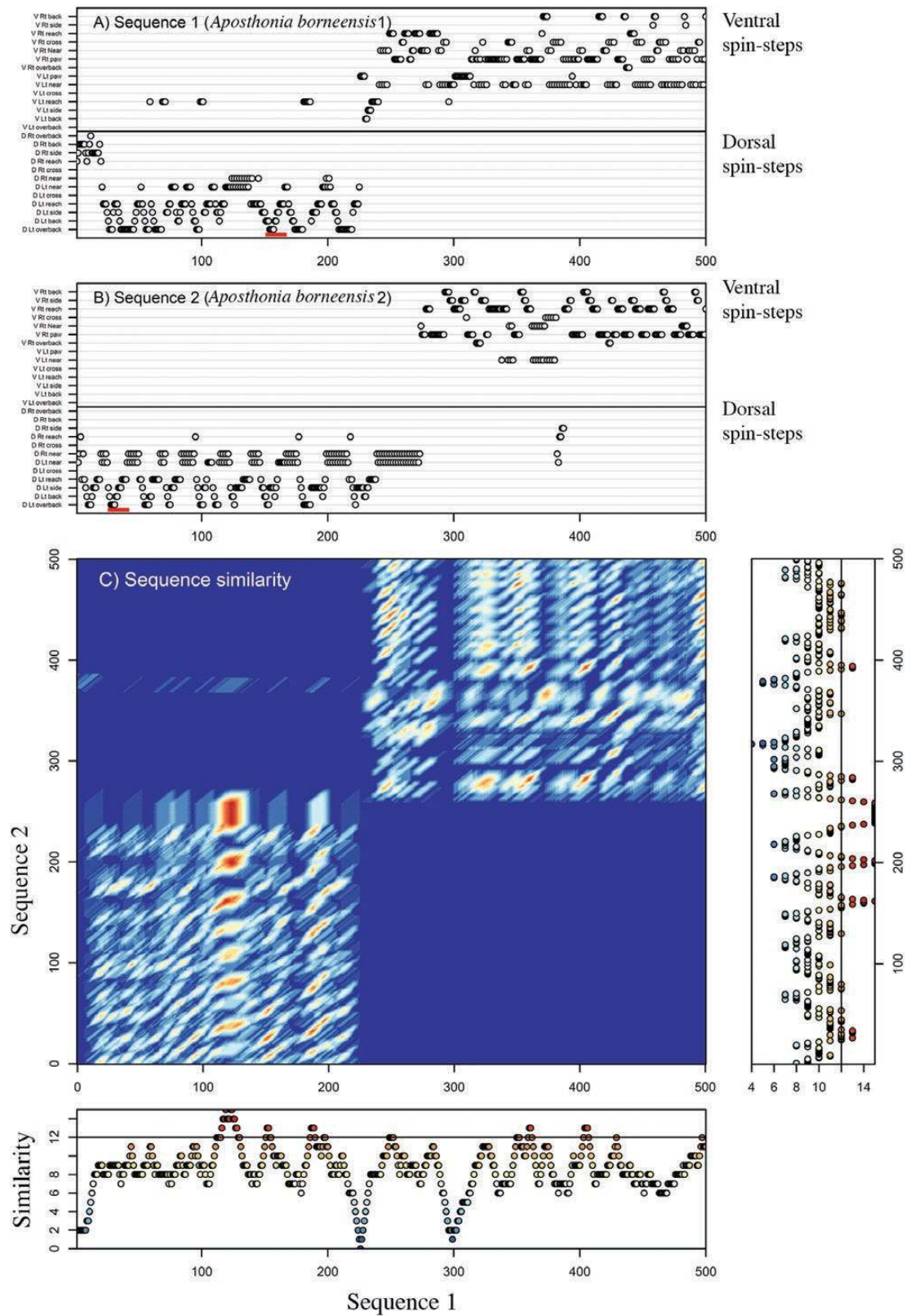
Fig. 4. Computation of similarity between two sequences. For two individuals of *Aposthonia borneensis* (Hagen) (Oligotomidae), the first 500 steps of their spin sequences are shown (A and B). The red bar underlining a short sequence indicates one 15-step subsequence that is highly similar between these two individuals. For all possible pairs of 15 step subsequences, the heatmap displays the sequence similarity (C), with red areas indicating regions of the spin sequence that are highly similar. The profiles on the margins of the heat map indicate the marginal maxima—that is, for each 15-step subsequence, what is the similarity to the most similar subsequence in the other individual. Portions of the sequence with similarities about 12 were deemed sufficiently similar to the other sequence (vertical or horizontal lines), amounting to about 5% of individual 1's sequence and 10% of individual 2's sequence.

(Fig. 6). Some species, such as *Neorhagadochir moreliensis* (Ross) (Scelembiidae) (Nm in Fig. 6A), *Metoligotoma pentanesiana* Davis (Australembiidae) (Mp in Fig. 6B) and *Ptilocerembia catherinae* Poolprasert & Edgerly (Ptilocerembiidae) (Pc in Fig. 6B), are highly clustered, indicating high intra-specific similarity. On the other hand, others including *A. urichi*, *P. reclusa* (Au and Pr in Fig. 5A) and *Embia nuragica* Stefani ('Embiidae 1&2,' Miller at al. 2012) (En in Fig. 5C) are widely scattered. Individuals of these species were no more similar to one another than to individuals of other species.

The strong variation among species in their intraspecific similarity was related to characteristics of the species and their environments (Tables 1 and 2). For example, for *n* = 15, intraspecific similarity increased with the mean annual temperature (Fig. 7A) and temperature seasonality of species' environment (Fig. 7B), and was higher for species that produce more structured silk galleries (Fig. 7C; Supp Fig. 3 [online only]). Across the different values of N, intraspecific similarity tended to be highest in warm, seasonal and dry environments and for species with the most structured galleries (see Supp Video 2 [online only] for a sample of diversity in spin-steps).

Across all values of N, sequence similarity was positively correlated with transition matrix similarity, though this correlation declined with increasing N (from $r = 0.462$ for $n = 5$ to $r = 0.204$ for $n = 25$, Supp Fig. 4 [online only]). Predictors of intraspecific similarity of the transition matrices were generally similar to those for sequence similarity, especially for $n = 5$ (Table 2).

Discussion

One of our goals (noted in McMillan et al. 2016) was to develop a method for scoring silk gallery structure for embiopterans, and as has been shown for spiders (e.g., Eberhard et al. 2007, Vollrath and Selden 2007, Blackledge et al. 2009), we detected an association between behavior and structure. Similarly, Blackledge and Gillespie (2004) found that 11 species of *Tetragnatha* spiders on Hawaiian island displayed ecological differentiation in their web construction; species on different islands appear to have converged with one another suggesting common selective pressures yielding what the authors dubbed 'ethotypes.' We also sought to include ecological attributes as predictors to test for a link between spinning and ecology and found evidence for such a link. Furthermore, an intriguing new line of research focuses on extended spider cognition (reviewed in Japyassú and Laland 2017). Evidence of lower species-specificity and greater individuality in silk spinning by some embiopterans suggests that they as well might be capable of 'cognitive connectivity to variable habitat features' as described by Japyassú and Laland. Below we will address this novel idea as well as each of the three
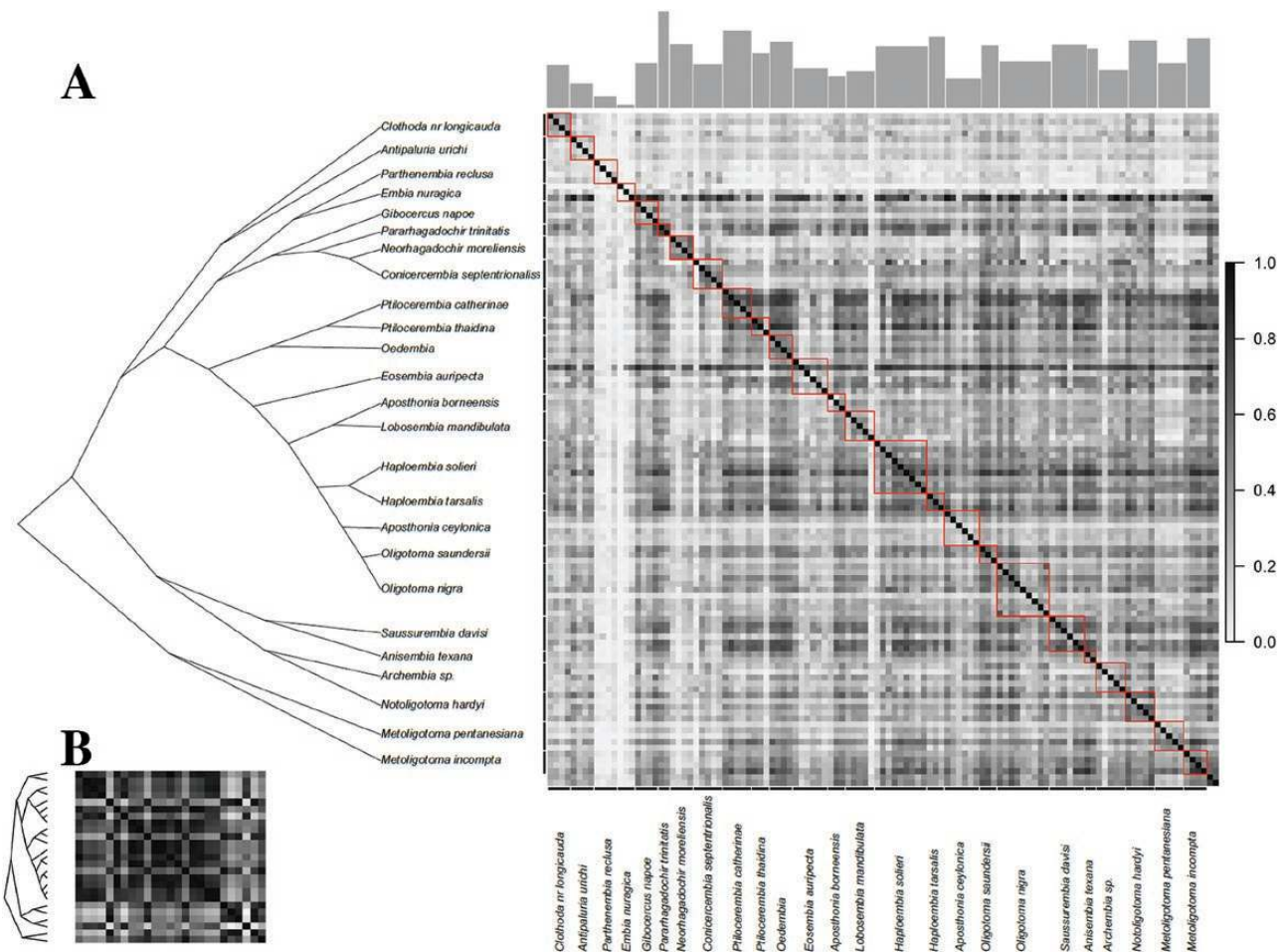

Fig. 5. Phylogenetic relationships and sequence similarities (*n* = 15) for all individuals in this study. (A) Sequence similarities are depicted as a heat map, with the diagonal representing self-similarities. The small outlined boxes along the diagonal indicate all intraspecific comparisons, and the mean intraspecific similarity for each species is depicted above the heat map. For comparison, the inset graph, (B) shows the similarity among pairs of species for a trait that is evolving according to the Ornstein-Uhlenbeck model. The large clade constituting the top 19 species shows high similarity among species (mostly dark colors in the upper left), but low similarity to the two outgroups to this clade (bottom six species, shows as lighter gray colors). The phylogenetic tree is based on Miller et al. 2012.
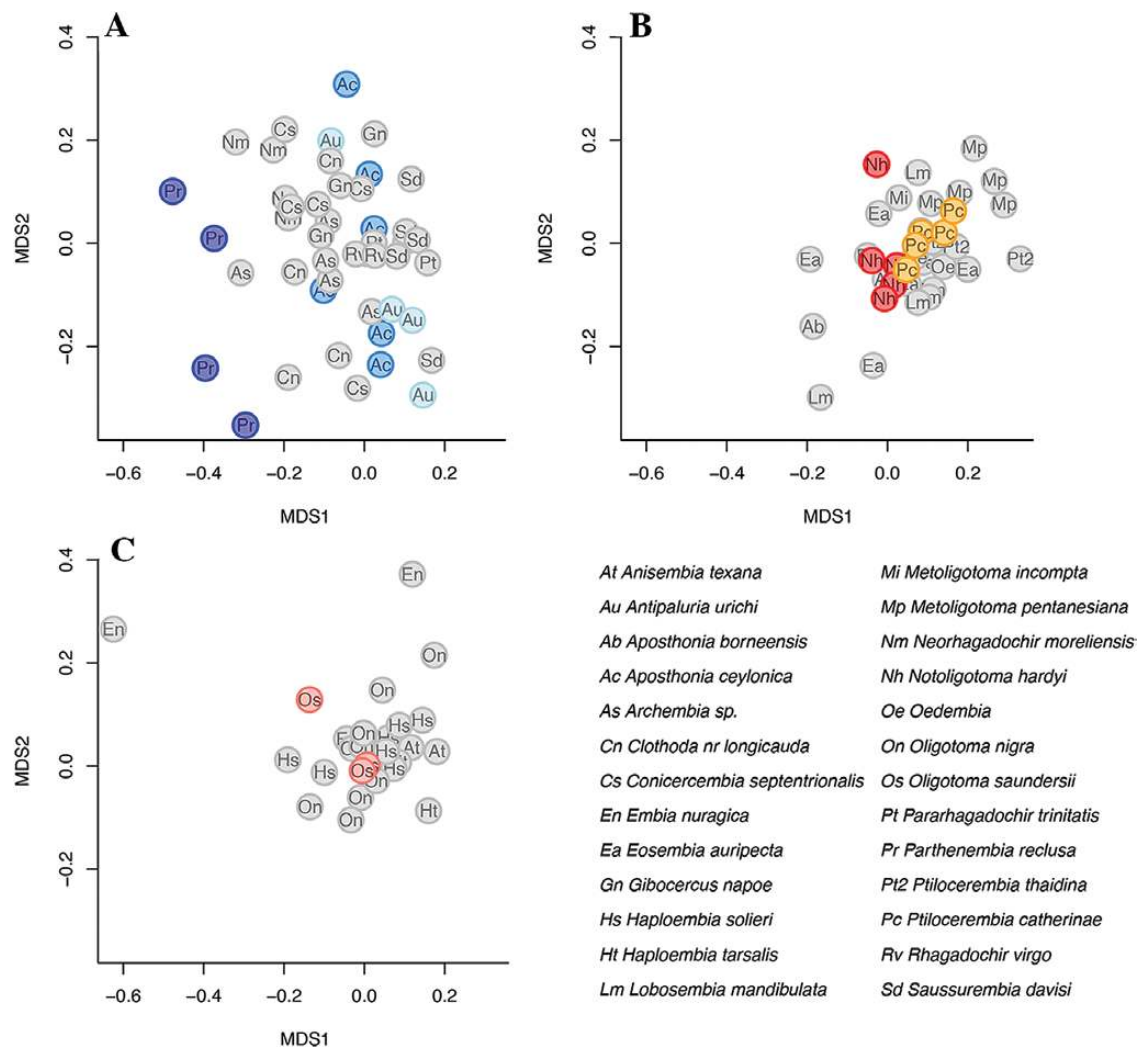
Fig. 6. Multidimensional scaling plot of sequence similarity ($n = 15$). Each individual is plotted with its species code, and six species are highlighted in color. Those in shades of blue showed particularly low intraspecific similarity, while those in warm shades showed high intraspecific similarity. (A) Low temperature seasonality (<2000), (B) Moderate temperature seasonality (2000–4000), (C) High temperature seasonality (>4000). Legend shows species names and abbreviations.

major hypotheses posed earlier: Phylogenetic Lineage, Body Size, and Environmental Driver.

Phylogenetic Lineage and Body Size Hypotheses

Analysis of a number of broad characteristics of embiopteran silk spinning revealed phylogenetic signal and relationships between spinning and body size in an earlier study (McMillan et al. 2016), features also known for spiders (e.g., Vollrath and Selden 2007, Blackledge et al. 2009). That 2016 report on Embioptera combined spin-steps into batches such as the frequency of the spin-steps *side-back-overback*, rather than the sequences of all the steps. Comparing the complete sequences in the current study revealed weak but significant support for the Phylogenetic Lineage Hypothesis. When analyzing spin-step sequences, individual variability is intact and, therefore, if individuals of a species are more unpredictable, phylogenetic signal is less likely to emerge from our data than when spin-steps are counted as overall frequencies. We believe evaluating complete sequences is a more accurate picture of silk spinning evolution in webspinners and, therefore, the apparently stronger phylogenetic signal reported in McMillan et al.

(2016) overemphasized a fairly weak signal. The diffuse pattern shown in the heat map (Fig. 5) also reflects a weak link between sequence similarity and phylogeny. As evidenced by the variability in intraspecific similarity scores, some species, like *P. trinitatis*, displayed a species-specific style while others, like *A. urichi*, were more individualistic. On the other hand, analysis of size (relative head width) as a predictor of spin-step sequence similarity between individuals irrespective of species or phylogenetic relationships revealed that for sequence lengths of $n = 25$, the more alike in body size the individuals are, the more similar their spin-step sequences are. This finding was one of the predictions of the Body Size Hypothesis. This result aligns with the findings in McMillan et al. (2016), whereby larger embiopterans emphasized and repeated dorsal *side* spin-steps and showed fewer dorsal *cross* spin-steps that characterized smaller species. Repeating dorsal *side* spin-steps typify large species, such as the large clothodids, that invest in creating complex gallery coverings. *Cross* spin-steps, placed around the front of the head, were more common for the smaller australembiid species, for example, which use silk for stitching leaf litter pieces together.

Table 1. Intraspecific sequence similarity scores and habitat information for the species of Embioptera sampled for their spinning behavior

Family	Species	Habitat	Microhabitat	Intraspecific Similarity Scores[a]					
				5	10	15	20	25	Transition
Anisembiidae	*Anisembia texana* (Melander)	Mixed	Crevice	0.90	0.67	0.45	0.28	0.18	0.48
	Saussurembia davisi Ross	Arboreal	Crevice	0.89	0.67	0.48	0.34	0.24	0.40
Archembiidae	*Archembia sp* Ross	Arboreal	Exposed	0.80	0.57	0.29	0.13	0.06	0.36
Australembiidae	*Metoligotoma incompta* (Ross)	Leaf litter	Crevice	0.87	0.69	0.53	0.39	0.27	0.41
	Metoligotoma pentanesiana Davis	Underground + Leaf litter	Crevice	0.87	0.59	0.34	0.18	0.11	0.40
Clothodidae	*Antipaluria urichi* (Saussure)	Arboreal	Exposed	0.91	0.51	0.19	0.07	0.03	0.45
	Clothoda nr longicauda Ross	Arboreal	Exposed	0.87	0.62	0.33	0.14	0.06	0.38
Embiidae 2 and 1	*Embia nuragica* Stefani	Leaf litter	Crevice	0.32	0.09	0.03	0.02	0.01	0.15
	Parthenembia reclusa Rosss	Arboreal	Intermediate	0.87	0.38	0.09	0.02	0.01	0.41
Embiidae 3	*Oedembia sp.* Ross	Arboreal	Exposed	0.93	0.73	0.50	0.31	0.17	0.46
Notoligotomidae	*Notoligotoma hardyi* (Friederichs)	Arboreal	Exposed	0.93	0.77	0.51	0.32	0.21	0.44
Oligotomidae	*Aposthonia borneensis* (Hagen)	Arboreal	Crevice	0.72	0.46	0.24	0.11	0.06	0.43
	Aposthonia ceylonica (Enderlein)	Arboreal	Crevice	0.84	0.48	0.22	0.11	0.06	0.40
	Eosembia auripecta Ross	Arboreal	Exposed	0.74	0.50	0.30	0.17	0.11	0.26
	Haploembia tarsalis (Ross)	Underground + Leaf litter	Crevice	0.94	0.77	0.54	0.35	0.20	0.44
	Haploembia solieri (Rambur)	Underground + Leaf litter	Crevice	0.83	0.63	0.47	0.35	0.27	0.39
	Lobosembia mandibulata Ross	Arboreal	Exposed	0.84	0.54	0.28	0.14	0.07	0.39
	Oligotoma nigra (Hagen)	Leaf litter	Crevice	0.89	0.62	0.35	0.19	0.11	0.41
	Oligotoma saundersii (Westwood)	Arboreal	Crevice	0.94	0.74	0.48	0.29	0.18	0.44
Ptilocerembiidae	*Ptilocerembia thaidina* Poolprasert & Edgerly	Arboreal	Intermediate	0.94	0.68	0.42	0.27	0.17	0.40
	Ptilocerembia catherinae Poolprasert & Edgerly	Arboreal	Exposed	0.92	0.76	0.58	0.42	0.28	0.41
Scelembiidae	*Neorhagadochir moreliensis* (Ross)	Arboreal	Intermediate	0.97	0.79	0.48	0.25	0.11	0.45
	Conicercembia septentrionalis (Mariño & Márquez)	Arboreal	Intermediate	0.71	0.54	0.33	0.21	0.12	0.31
	Gibocercus napoe Ross	Arboreal	Exposed	0.87	0.60	0.34	0.19	0.11	0.40
	Pararhagadochir trinitatis (Saussure)	Arboreal	Exposed	0.93	0.85	0.73	0.60	0.45	0.45
	Rhagadochir virgo (Ross)	Arboreal	Intermediate	0.95	0.83	0.70	0.52	0.36	0.44

[a]The values 5–25 indicate the length of the spin-step sequences being compared using the sliding window method described in the Methods. 'Transition' refers to the probabilities of moving from one spin-step to another, a common data point used for analyzing simple behavioral transitions.

Table 2. Standardized regression coefficients for stepwise phylogenetic regressions predicting intraspecific behavioral similarity from species characteristics. If no coefficient is given for a variable in a particular model, that variable was not selected in that model (*P*-values: * = 0.05, ** = 0.001)

| Predictors	Length of Spin-step Sequences					
	n = 5	*n* = 10	*n* = 15	*n* = 20	*n* = 25	Transition
Mean annual temperature	0.626**	0.715**	0.632**	0.586**	0.600*	0.827*
Temperature seasonality		0.534	0.712*	0.882**	0.772*	
Annual precipitation	-0.705**	–0.534*	-0.343			-0.653**
Body length						
Head width			-0.258	-0.245	-0.291	
Domicile Score	-0.548*	–0.317	-0.335			-0.560*
Silk Galleries	0.882**	0.807**	0.833**	0.489	0.401	0.592*
Pseudo-R^2	0.291	0.295	0.302	0.245	0.279	0.358
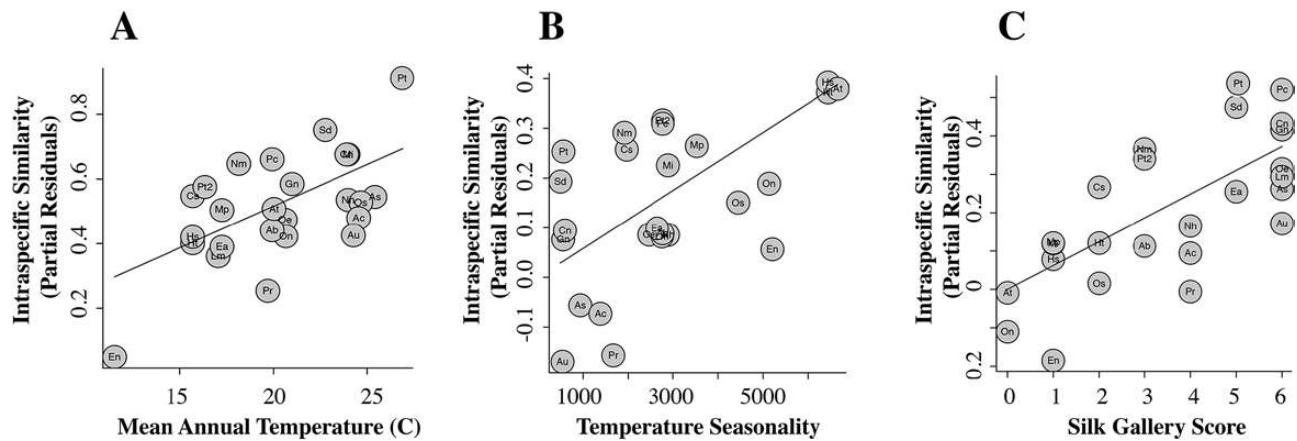
Fig. 7. Predictors of intraspecific similarity scores. The three panels show the partial residual plots for the three variables selected in the final model for a subsequence length of 15. Intraspecific similarity as a function of: (A) mean annual temperature, (B) temperature seasonality, and (C) silk gallery structure. Two-letter codes indicate the species as in Fig. 6.

Environmental Drivers of Silk Spinning Styles

Because dry habitats force embiopterans underground or into leaf litter and more humid environments allow them to live more exposed on tree bark, we predicted that there might be a difference in how silk-spinning and silk architecture would be expressed. We were not sure in which direction intraspecific similarity scores would go—higher for one lifestyle and lower for the other? Analysis of climatic variables as predictors of intraspecific similarity in spinning did reveal an interesting pattern and possible ecological context for behavioral evolution in Embioptera. Those species living in regions with higher annual temperatures, greater seasonality, and lower annual precipitation tended to display higher intraspecific similarity scores for spinning behavior. Even for sequences as long as $n = 25$ spin-steps, temperature seasonality was a significant predictor in the model. The MDS plot of species based on spinning behaviors in seasonally variable sites (Fig. 6C) shows that the individuals even in different species are clustered together whereas those with low temperature variability are spread out in the plot indicating higher variability within a species in spinning overall (Fig. 6A). The fairly low R^2 values (Table 2) reflect the high variability in the behavioral data set. Despite this, however, the fit between climatic predictors and similarity scores suggests that species from subtropical and Mediterranean-like climates are more species-stereotypical in their spinning compared to tropical rainforest species which showed higher intraspecific variability.

After accounting for the climatic variables, the analysis also showed that species with greater complexity in their gallery structures had higher intraspecific similarity scores. As such, for those species that live in similar regions, species with more complex gallery structures had higher intraspecific similarity scores. An example would be *N. hardyi* compared to *M. incompta*; populations overlap on Magnetic Island in Queensland but *N. hardyi* lives in more shaded areas and spins their silk as complex tubes exposed on granite outcrops where they feed on lichens (Fig. 2D). An experimental field and laboratory study demonstrated that their silk did not protect them from thermal stress— their domiciles were about the same temperature as the substratum. In fact, when forced to dwell facing the sun, colonies would move to shaded sites. Instead of using silk to thermoregulate, they rely on microhabitat selection and a robust heat-shock response (Edgerly et al. 2005). In contrast, *M. incompta* lives in leaf litter (Fig. 2G) where they inhabit drier, hotter conditions even on the same island (Edgerly and Rooks 2004). They also have a strong

heat shock response but cope with extreme heat, particularly in the warmest locations on the island, by sheltering close to the ground in their silk-leaf domiciles. In this example, the lichenivore had higher intraspecific similarity scores than the detritivore for spin-step sequence lengths of $n = 5$ of 0.93 versus 0.87 and $n = 10$ of 0.77 versus 0.69, respectively.

Overall the relationships between the predictor variables and silk spinning are complex as illustrated in the contour plots (Supp Fig. 3 [online only]). Despite high variability between individuals within a species, we can claim that the evidence points to environmental drivers as a stronger force than phylogenetic inertia as an explanation of intraspecific similarity scores.

Our approach to computing sequence similarity was intended to resolve weaknesses in traditional approaches relying on behavioral transition matrices. However, it comes at a massive computational cost. The comparison of all possible pairs of substrings of a given length is a time-consuming process, often requiring millions of string comparisons for a pair of individuals. Thus, it is useful to ask whether this computational investment was worth it—did this analysis reveal something more than can be observed from the more traditional transition matrix? First, while sequence similarities and transition matrix similarities were positively correlated, these correlations were moderate to weak, especially for larger subsequence lengths. Thus, the sequence similarity approach appears to capture patterns that the transition matrix alone misses. This was confirmed by the analysis of drivers of intraspecific similarity. Some predictors, such as the degree of structure in silk galleries, were consistently related to intraspecific similarity for the transition matrix and for various subsequence lengths. However, others were not. Most notably, temperature seasonality is a strong positive predictor of sequence similarity, but was not a good predictor of transition matrix similarity. Furthermore, the sequence similarity approach has great potential for detecting other patterns. For example, it is possible to identify the subsequences that are most characteristic of any particular species or larger group. Our next goal is to work more on identifying these behavioral 'motifs.'

Conclusion

If we focus on a few extreme cases, a pattern emerges that might hold as a prediction for how an embiopteran spins silk: large tropical rainforest embiopterans are more likely to be individualistic in their silk spinning routines than smaller species from habitats with less rainfall and more variability in temperature. Embedded in our

recordings of silk spinning may be clues about how the large tropical embiopterans are behaving in a less robotic style. As they spin, they stop periodically and display 'push-ups,' actions that appear to stretch the emerging silk roof of their domicile. Push-ups appear to give information to the insect about what they need to do next to reinforce the new domicile. They also move along the length of the gallery, and if their antennae poke through a hole, they display spin-steps that resemble darning (as depicted in Fig. 5 in Edgerly et al. 2002). The intraspecific variability of some species we have seen after scoring dozens of videos contrasted with the more robotic behaviors of other species, like *N. hardyi*, *P. trinitatis*, and *N. moreliensis*. In the current study, we detected interesting links between environment and silk-spinning variability. The underlying cause of intraspecific variability might be 'imprecision' in control by the central nervous system, as proposed by Eberhard (1990, 2000) for other variable silk spinners. What remains as another intriguing question is whether the 'extended cognition' posed as an explanation for the abilities of some orb weaving spiders (Japyassú and Laland 2017) is also true for the large-bodied tropical rainforest embiopterans, for whom life involves numerous challenges such as heavy rainfall requiring thick reinforced layers of silk, attacks by predators, including birds (e.g., Edgerly 1994) and social behavior (reviewed in Edgerly 1997).

Supplementary Data

Acknowledgments

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