

## Relating the Cost of Spinning Silk to the Tendency to Share It for Three Embiidids with Different Lifestyles (Order Embiidina: Clothodidae, Notoligotomidae, and Australembiidae)

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**ABSTRACT** Although adult female embiidids (Order Embiidina) superficially lack morphological diversity, their variety of habitats may impose distinct selective pressures on behavior, such as their use of silk and their tendency to aggregate. For example, where silk serves as a primary defense from environmental threats, coloniality might be adaptive. The cost of production or spinning might also prompt them to share silk. These ideas were tested in laboratory trials involving three species of embiidids with different lifestyles: an arboreal species (*Antipaluria urichi* (Saussure) from a neotropical rain forest, a species (*Notoligotoma hardyi* (Frederichs) that dwells on surfaces of granite outcrops in Australia, and another Australian species (*Australembia incompta* Ross) that stitches leaf litter together. The cost of spinning silk was analyzed by recording CO<sub>2</sub> output in the short term during spinning and by measuring performance in long-term trials where embiidids were forced to repeatedly replace their silk. Their subsequent development or reproductive output was scored. Overall, the cost of spinning was relatively low. However, the tendencies to spin and to aggregate varied in a manner related to how silk is used in the field. As such, the more exposed the embiidid is to the elements, as for the two species that spin on surfaces, the more silk they spun and the more likely they settled near a neighbor. In contrast, the embiidid that used dead leaves (not silk) as walls for their abodes produced scant silk and showed little tendency to aggregate.

**KEY WORDS** Embioptera, webspinner, silk, social evolution, metabolic cost

LIVING IN SILKEN ABODES defines lifestyles for many arthropods, especially for masterful weavers such as insects in the order Embiidina. Silk serves as nesting material, retreats, protection for eggs, and coverings over foraging zones. Because silk-using arthropods range from solitary to colonial to communal, researchers have been able to apply comparative methods to determine how silk use relates to social evolution (as in social spiders; see references in Avilés 1997, Uetz and Hieber 1997). An argument with some support is that coloniality may be favored when the costs of silk production are high for any individual and benefits of sharing are great. The purpose of this report is to summarize results of a comparative study studying silk spinning in the context of natural history in the order Embiidina (or Embioidea, see Grimaldi and Engel 2005). Through a series of laboratory experiments, we evaluated possible costs of spinning and explored whether embiidids can reduce costs by sharing silk. Embiidids that construct complex, extensive silk abodes may be more likely to form groups; those relying more on building materials in the environment may be less prone to do so. Before describing methods for this

study, we will briefly review relevant research on arthropod silks.

Generalizations cannot easily be made about the cost of silk production. In some cases, spinning is not costly materially, metabolically, or behaviorally, whereas in others, direct impacts on performance have been shown. For example, caddisfly larvae that spin extra silk lose more weight than starved individuals, suggesting a high cost of spinning for these insects (Dudgeon 1987). Behavioral mechanisms may have evolved in response to costs associated with spinning. For example, Jakob (1991) showed that pholcid spiderlings benefited by sharing silk with older conspecifics because of the relatively high cost of spinning, despite a loss of food to competitors. Tietjen (1986) showed that as colony size increased for the social spider *Mallos gregalis* (Simon) individuals benefited by living in a group because each contributed less silk to the shared nest and spent more time sitting still. In their review of spider evolution, Uetz and Hieber (1997) emphasized that a common element promoting coloniality for some spider species is the three-dimensionality of their aggregations and considerable energetic savings for individuals sharing silk. In contrast, Avilés (1997), in a review of permanent-sociality

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in spiders, did not identify silk as a catalyst for social evolution.

While it seems that costs can promote group-living, alternatively, dependence on silk may lead to aggression. For example, in laboratory experiments, parsnip webworm larvae defended webs from potential usurpers and, when given a choice, adopted empty webs rather than spun new ones (Berenbaum et al. 1993, Green et al. 1998). Comparative work on spider species that range in how much silk they use showed that the more individuals invested in silk for construction, the less likely they were to relocate where they had to spin anew (Tanaka 1989). Apparently, the function of silk can influence dispersion of individuals.

The degree of reliance on silk and the tendency to share it may depend on challenges imposed by environmental factors. In a search for function, Seibt and Wickler (1990) found that silk nests of two social species of *Stegodyphus* spiders in South Africa did not protect occupants from potentially serious threats—wind, hail, direct sun radiation, desiccation, or overheating. The authors concluded that predation on the spiders was more significant in promoting sharing silk and sociality (Seibt and Wickler 1988). Likewise, Edgerly et al. (2005) discovered that embiid silk in Queensland, Australia, did not protect against overheating in colonies exposed to direct sun. The embiids in that study [*Notoligotoma hardyi* (Friederichs): *Notoligotomidae*] relied instead on internal heat-shock proteins and microhabitat choice (see also Edgerly and Rooks 2004). In contrast, silken abodes of caterpillars do play a significant role in behavioral thermoregulation, offering warmth or shade depending on where the larvae sit in their three-dimensional tents (Carlberg 1980, Joos et al. 1988, Fitzgerald and Underwood 2000).

Reliance on silk is fundamental to embiids, an order of  $\approx 2,000$  species, many still undescribed (Ross 2000). Individuals of all ages and both sexes spin with their front tarsi by releasing numerous strands as their silk ejectors touch the substratum (Edgerly et al. 2002). Females are wingless, elongate, flexible, and juvenile in form, traits that are well suited to life in narrow silk passages. The few detailed behavioral studies show that adult females contribute the most silk to the colony, and indeed, spinning silk is a key component of maternal care (Edgerly 1988). Adult males spin little silk—in most species, they fly off to mate and die soon thereafter without feeding or contributing much to the silk covering. The differential contributions to spinning made by males and females still needs study, especially for those species where males are wingless and more likely to spend time in silk galleries, particularly true for embiids in arid environments.

Embiids display remarkable morphological uniformity despite their occurrence in a wide range of habitats: tropical to subtropical, arid to humid, arboreal to subterranean. Whereas most insects rely on tough exoskeletons and waxy cuticles for protection, embiids seem to rely on silk instead. When threatened, they run rapidly backward or forward and negotiate tight turns within narrow silken tunnels that connect rest-

ing sites (domiciles) to foraging zones. Generally, embiids live hidden within silk in mother-offspring associations. In the humid tropics, large-bodied embiids can form conspicuous and extensive colonies that cover entire tree trunks where they graze on algae and lichens (see references in Edgerly 1997, Ross 2000). In arid zones, they retreat underground in crevices, emerging (especially after rain) at the surface, particularly at rock-soil boundaries, to feed on vegetation or detritus.

To test our ideas, we selected three species that vary in their use of silk in the field. The focal species were as follows: the relatively large arboreal embiid *Antipaluria urichi* (Saussure) (Clothodidae) of the Trinidad rain forest, a facultatively colonial species that often forms large colonies (Edgerly 1987b, 1994); *N. hardyi* of subtropical Queensland, Australia, which lives mostly on granite outcrops, but also on bark, where they spin dense silk and feed on lichens; and *Austrolembia incompta* Ross (Austrolembiidae), also of Queensland, which stitches dead leaves together and feeds on detritus on the forest floor (Edgerly and Rooks 2004). We evaluated short- and long-term costs of spinning and tested whether sharing silk may function to reduce such costs. We also tested how readily adult females spun silk and whether they tended to aggregate. We related these behaviors to their apparent reliance on silk in the field. Our prediction was that embiids living exposed on rock-faces or trees are more dependent on silk, which serves as primary protection against abiotic and biotic threats. These species should spin more and aggregate more than the leaf-litter dweller, which should be less reliant on silk which functions more as stitching than as primary cover.

## Materials and Methods

**Laboratory Cultures and General Methods.** Embiids were reared at Santa Clara University at 27°C with 12 h of overhead fluorescent lighting each day. *A. urichi* and *A. incompta* fed on romaine lettuce and lived in a mulch of dead oak leaves, typical rearing medium for embiids (Ross 2000), whereas *N. hardyi* would only feed on lichens scraped from oak bark. All lichens and leaves were from local California Live Oak (*Quercus agrifolia*). Adult females were used in all experiments unless noted. Their mean weights ranged from 0.042 g for *A. urichi* ( $n = 20$ ) to 0.021 g for *A. incompta* ( $n = 14$ ), and 0.012 g for *N. hardyi* ( $n = 20$ ). Body length follows the same relationship: *A. urichi* at  $1.60 \pm 0.02$  cm (Edgerly 1987a), *A. incompta* at  $1.18 \pm 0.36$  cm ( $n = 155$  from laboratory cultures) and *N. hardyi* at  $1.04 \pm 0.23$  cm ( $n = 307$  from laboratory cultures). A spot of Testor's enamel paint was applied when individual identification was required. In trials when silk was removed, silk was torn and removed with forceps during a period of time specified below. The control groups in these experiments were provided with forceps to generate a similar level of disturbance for treatment and control replicates.

**Evaluating Species-specific Tendency to Spin Silk.** We used quantitative biochemical techniques rather than a top-loading scale to determine amount spun because silk samples were too small to weigh accurately. The first step in estimating silk production was determining the amino acid composition of silk for each species and identifying which amino acids would be useful for quantitative analysis. Amino acid composition of the embiid silks (unpublished data), determined for us by Dr. Kathy Schegg of University of Nevada, is predominately serine (30%), glycine (between 40 and 50%), and alanine (10%). Also present in small amounts is tyrosine, which because of its aromatic ring can be quantified with UV spectrometry. For *N. hardyi* silk, the mean proportion of tyrosine is 0.40 Mole% ( $n = 3$  samples) and for *A. urichi* silk is 1.63 Mole% ( $n = 3$  samples). Surprisingly, *A. incompta* would not spin in clean petri dishes, and their silk spinning could not be analyzed in this manner. Absorbance at 280 nm caused by tyrosine in silk, hydrolyzed in 1N HCl at 37°C, can be quantified as micrograms per milliliter of sample. By comparing absorbance to a standard curve, the amount of tyrosine per sample was determined; to calculate total amount of silk, we extrapolated from the estimated amount of tyrosine. To determine how much an embiid spins, adult females ( $n = 20$ ) of each species were placed individually in petri dishes (100 mm diameter, 15 mm high) for 3 d. Total output of silk was expressed as microgram of silk per gram of female ( $n = 20$ /species).

In a second experiment, dead leaves were added to petri dishes to trigger spinning in *A. incompta*. *N. hardyi* and *A. urichi* were tested for comparison. The dishes contained four 1-cm<sup>2</sup> cut pieces of leaves or held no additional substrate (control). Ten individuals per species were tested in each treatment group. Amino acid analysis was not used because the leaves contaminated the silk. Instead, the length and width of each silk patch was measured after 3 d and multiplied as an estimate of the area covered by silk. Area of silk spun per gram of female was analyzed as a function of two main factors (SPECIES and SUBSTRATE) and their interaction with a two-way analysis of variance (ANOVA) using JMP IN Statistics (Sall et al. 2001, SAS Institute 2001).

**Exploring Species-specific Tendency to Congregate.** An index of aggregation (A) based on the Clark and Evans nearest neighbor method (cited in Brower et al. 1998) was calculated to document how females settled relative to one another. If  $A = 1$ , dispersion is random, if  $A < 1$ , distribution is contagious, and if  $A > 1$ , the embiids are uniformly dispersed. A *t*-test was used to test the null hypothesis that dispersion is random (Brower et al. 1998). The critical value is 1.96 at 0.05 significance level. Our method differed slightly from Clark and Evans in that all embiids used in the laboratory trials were included in the statistical test rather than a random sample, as is typical of field studies where random sampling is a necessity.

**Testing the Arboreal Embiids.** Four rectangular, flat wooden panels (15 by 8 cm) were constructed by strapping small diameter branches together with plas-

tic ties. Each panel provided lichens on wood, on two flat sides, emulating natural habitat for *A. urichi* and *N. hardyi*. Each panel was propped up against an inner wall of a translucent plastic box (16 by 24 by 46 cm). Large females (who neither had eggs nor appeared gravid) were selected from cultures in an attempt to use females that might be prone to disperse. This selection protocol derives from unpublished field observations (J.S.E.) where such females have been seen wandering outside their silk or living within isolated, fresh patches of silk (evidence of recent dispersal). Embiids were marked and allowed to spin for a few hours in a petri dish with leaves. To begin the trial, an open dish containing three individuals was placed opposite the upright panel and left for 3 d, during which time the females could leave their silk and move onto the wooden panel, as they would onto bark or rock-faces in the field. This process was repeated three times, allowing the females to disperse onto the panel, where they could find others to join or choose empty regions where each could spin alone. In this manner, the embiids accumulated on the wooden panel and we could evaluate their tendency to cluster. Nearest neighbor distances were measured for all females that had settled onto the wood. Different sample sizes of females (12 and 15) were used in the trials (two per species) because of limited numbers of adults available during the trials.

**Testing the Detritivore.** Dead oak leaves arranged in a pattern were provided as habitat to test the aggregation tendency of *A. incompta*. In the first trial, adults ( $n = 7$ ) were placed in the petri dish with 12 1.5-cm leaf discs arranged in a circle; the position of each female's silk domicile was recorded 2 wk later. A second trial was conducted that allowed females to walk from a dish to a new habitat (resembling more closely the experimental design used for the other two species). In this test, 6-cm<sup>2</sup> pieces of leaves ( $n = 16$ ) were hot-glued onto a wooden board to form a rectangular patch of leaves. Marked females ( $n = 9$ ) were added every 3 d in groups of three (as described above); however, this time, the apparatus was placed on the bottom of the container.

**Determining the Short-term Metabolic Cost of Spinning Silk.** A behavioral repertoire for *A. urichi* and *N. hardyi* was recorded for 20 min while each individual was in a chamber (6 cm<sup>2</sup> by 0.7 cm deep) connected to a CO<sub>2</sub> analyzer (Qubit Systems, Kingston, Canada). *A. incompta* tends to stay still for long periods after handling and therefore were not used in this study. Metabolic rate was scored as CO<sub>2</sub> output as a function of time spent in each behavioral act. Behavioral acts were spinning, pushing up against the silk, antennation, traveling around the chamber (included climbing up and walking), and sitting still. Preliminary data analysis revealed that *A. urichi* females fit into distinct categories of activity, especially related to the behavior of spinning: some rarely spun, whereas others spun most of the time. We sorted the females based on their behavior using the multivariate ordination technique of discriminant analysis and cluster analysis using JMP IN. Both discriminant anal-

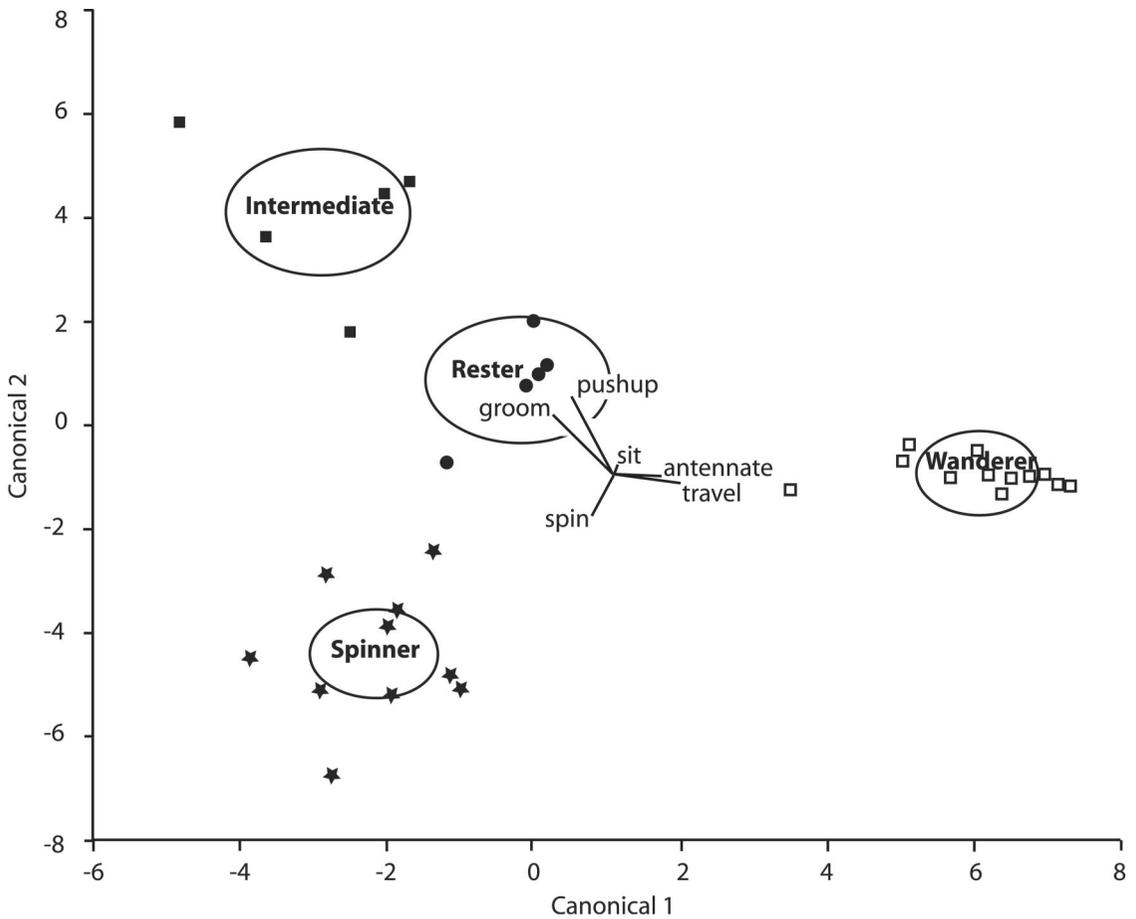


Fig. 1. Canonical plot of 32 *A. urichi* females based on discriminant analysis of time spent in behaviors scored using an event recorder during 20-m trials in a chamber connected to a CO<sub>2</sub> detector. The plot shows the two dimensions that best separate the groups. The biplot rays show the directions of the original variables (behavioral acts) in the canonical space. Each ellipse is based on 95% confidence. All behavioral acts shown in the graph are self-explanatory except *pushup*, which refers to the behavior of pushing up against the silk with the front part of the body, apparently stretching the silk. ■, Intermediate (*n* = 5), showing a range of activities; ●, Resters (*n* = 5), which tended to sit still; □, Wanderers (*n* = 12), which tended to travel more than the other embiids; ★, Spinners (*n* = 10), which spun silk more than the other embiids. See Fig. 2 for details on time spent in behavioral acts.

ysis and clustering were 100% successful in sorting individuals into the same four categories; because the analyses were completely consistent, only results of the discriminant analysis are shown here (Fig. 1). We used a post hoc criterion, based on the ordination, to separate *A. urichi* into the categories named wanderer, spinner, and rester (Fig. 1). Because we were interested in the cost of spinning compared with other distinct behaviors such as resting, intermediate females were ignored in a subsequent Kruskal-Wallis Test (JMP IN) comparing metabolic rates of females engaged in different activities. We used a nonparametric test because females were not assigned randomly to categories as required by parametric tests. The average time spent by *A. urichi* in the most common activities for Spinners (*n* = 10), Wanderers (*n* = 12), and Resters (*n* = 5) is shown in Fig. 2A. When *N. hardyi* females were placed into the chamber, they spun silk throughout all trials. We generated a set of

nonspinners by allowing each female to spin in the chamber before testing. After these females had stopped spinning, the trial started. Comparison groups for *N. hardyi* were Spinners (*n* = 12) and Resters (*n* = 12; Fig. 2B).

During the trials, air from a compressed air tank was sent through a flow rate regulator positioned before the air stream enters the chamber. The flow rate (0.42 ml/s) was slow so that CO<sub>2</sub> produced by the insect could accumulate before the air moved onto the CO<sub>2</sub> detector. A computer software program, Logger Pro Version 2.1 (Vernier Software and Technology 2000), receiving information from the detector, recorded CO<sub>2</sub> output. Air flowed through the empty chamber (control) for 10 min before the female was added and for 10 min after the female was removed to allow us to calculate the CO<sub>2</sub> in control air. One observer recorded embiid behavior using an event recorder programmed with The Observer software by Noldus In-

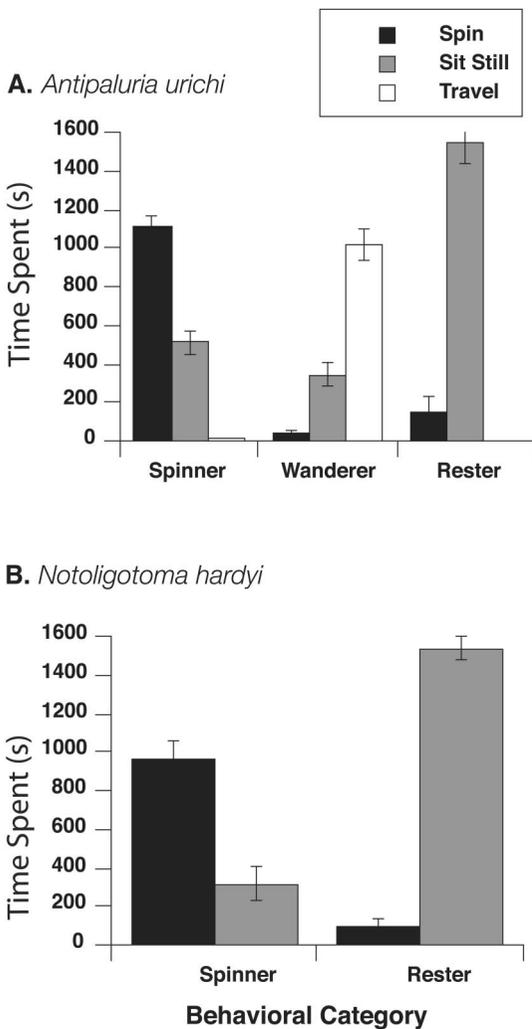


Fig. 2. Behavioral categories of adult females scored for  $\text{CO}_2$  output during various activities. The average ( $\pm$ SE) time spent in each of three main behaviors (spin, sit still, or travel) for (A) *A. urichi* and (B) *N. hardyi*. Sample sizes are indicated in Fig. 4.

formation Technology (1995). Control  $\text{CO}_2$  was subtracted from total  $\text{CO}_2$  output to find the amount of  $\text{CO}_2$  (converted to  $\mu\text{mole/s/g}$  female) produced only by the female. To achieve a normal distribution,  $\text{CO}_2$  output for *N. hardyi* was transformed by the square root before statistical testing with a Student's *t*-test.

**Studying Reproductive and Developmental Trade-offs.** We evaluated the relationship between spinning and development or reproduction in three short-term studies (one per species) and two additional long-term studies for *A. urichi*. In a short-term study, *A. incompta* and *N. hardyi* adult females were reared in separate containers with leaves for the detritivore or lichens for the lichenivore. Individuals ( $n = 6/\text{species}$ ) were exposed to a silk removal treatment (1-min session, twice per week for 2 wk) or prodding in the

control group ( $n = 6/\text{species}$ ). Embiids in the treatment group always replaced the torn silk. Trials stopped when nymphs hatched in some containers. Eggs were counted 5 wk after the start for *A. incompta* and after 2 mo for *N. hardyi*. Reproductive output for the silk treatment was compared with controls with a Student's *t*-test.

Because no significant effect of silk removal occurred (see Results), the regimen was modified in an attempt to impose a higher cost of spinning in the larger embiid, *A. urichi*. Three experiments were conducted: the impact of spinning on development, the effect of sharing silk by adult females on egg production, and the impact of spinning by nymphs on egg production later on. To study development in nymphs, *A. urichi* were raised in groups of four ( $n = 10$  replicates) or alone ( $n = 20$  replicates) in an attempt to manipulate how much silk an individual spins. Silk in one half the replicates was removed for 45 s twice per week for 15 wk; the remainder served as controls. Adult head width (distance between outer edges of the eyes) was used as a measure of developmental consequences of silk replacement and/or group size. Male and female head widths did not differ significantly ( $t = 0.02$ ;  $df = 37$ ;  $P = 0.51$ ), and therefore, grand means were computed for each replicate to use in the analysis. A two-way ANOVA was used to test mean head width as a function of SILK and GROUP SIZE and the interaction between these two factors.

*Antipaluria urichi* adult females were tested to determine if reproduction is enhanced when individuals share silk and for a possible cost of spinning that might accrue during development. Because colonial females in the field are known to lay more eggs than solitary females (Edgerly 1987b), the hypothesis is reasonable that sharing silk is a benefit. The protocol controlled all variables (except living together) that might affect reproductive output, such as food availability, humidity, and lighting. Predators and parasitoids, another source of differences between colonial and solitary females in the field, were absent. To test for a benefit of sharing silk, females placed in quart glass jars, either in groups of three or singly, were allowed to spin silk on upright sticks bearing lichens, which were replenished whenever 75% of the lichens were covered with silk. Adults in the field are often in groups of three (e.g., median and mode = 3 for 19 colonies with colonial females) (Edgerly 1987b). Males were added periodically as mates. Replicates were set up 8 June 2002; eggs were counted on 18 September 2002 when nymphs started to hatch in some jars. Egg count for each colonial replicate was computed as the mean number of eggs per egg mass and for each solitary female as the total number of eggs. Egg counts were compared with a Student's *t*-test.

Nymphs that hatched from eggs in the experiment just described were used in a follow-up experiment on the reproductive cost of silk. In the treatment group ( $n = 9$ ), silk was removed during a 3-min trial once a week for 17 wk (24 October 2002 through 1 February 2003). Nymphs in the control group ( $n = 9$ ) were prodded for 3 m each time. When mature embiids

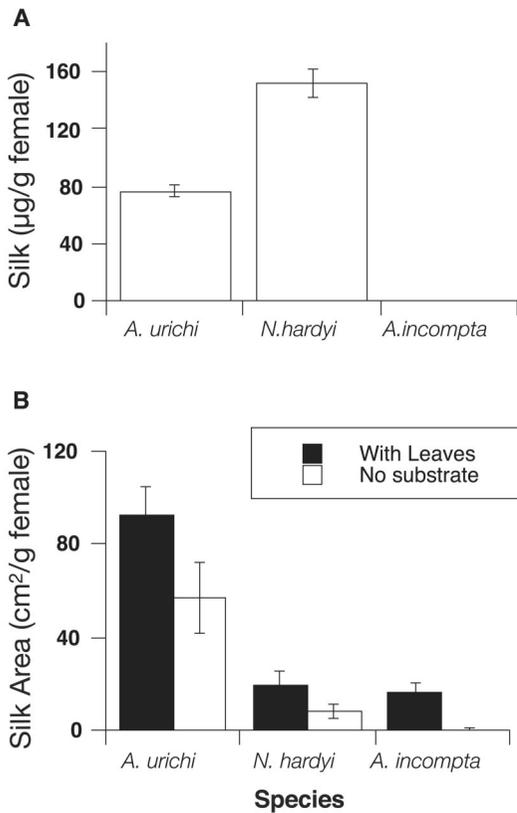


Fig. 3. Amount of silk spun (mean  $\pm$  SE) by adult females in three species of embiids when placed in petri dishes in the laboratory, measured either as (A) microgram silk per gram female ( $n = 20$ /species) or (B) mean area of silk (square centimeter per gram female) laid down in petri dishes stocked with dead leaves (10 replicates/species) or without the extra substrate (10 replicates/species).

appeared in one of the jars, all embiids were removed from the sticks, and each replicate group was placed into containers filled with leaf mulch where it was easier to watch for egg laying. Containers were checked for completed egg masses every week from

May through July (for description of egg mass, see Edgerly, 1987a). The mean number of eggs per egg mass for each replicate for treatment and control groups were compared with a Student's  $t$ -test.

**Results**

**Tendency to Spin Silk.** When placed into empty dishes, females in the three species produced different amounts of silk, measured as mass of silk ( $\mu\text{g/g}$  female) or as area of silk expanse ( $\text{cm}^2/\text{g}$  female). In the experiment employing UV spectrometry to quantify silk samples, embiids affixed silk to their dishes. *N. hardyi* produced significantly heavier silk ( $\mu\text{g}$ ) than *A. urichi* per gram female ( $t = 6.9$ ,  $df = 38$ ,  $P < 0.001$ ), and both spun more than *A. incompta*, who did not spin (Fig. 3A). In the second experiment, embiids housed with leaves spun significantly more than those without leaves (Fig. 3B). SPECIES ( $F = 9.397$ ;  $df = 2,59$ ;  $P = 0.003$ ) and SUBSTRATE ( $F = 38.6$ ;  $df = 1,59$ ;  $P < 0.001$ ) were significant factors, but the two-way interaction was not significant ( $F = 1.2$ ;  $df = 2,59$ ;  $P = 0.305$ ). In empty dishes, silk domiciles of *A. urichi* were the most expansive, *N. hardyi* were second, and *A. incompta* produced either nothing ( $n = 8$ ) or scant silk ( $n = 2$ ). Leaves stimulated more spinning in all species: *A. urichi* increased their output by 1.6, *N. hardyi* by 2.3, and *A. incompta* by 49.1 times.

**Exploring Species-specific Tendency to Congregate.** Depending on species, embiids expressed different tendencies to aggregate as reflected in the mean number of body lengths between nearest neighbors, and in the index of aggregation,  $A$  (Table 1). ANOVA revealed significant differences between nearest neighbor distances as a function of species ( $F = 4.205$ ;  $df = 2,56$ ;  $P = 0.02$ ). *A. urichi* displayed the lowest distance between nearest neighbors (Table 1). They were not tightly clumped as reflected in the intermediate values of  $A$ —in one case, statistically clumped; in the other, borderline random. Individuals spun their own domiciles but linked them with silk to others on the bark. One trial of *N. hardyi* yielded the most aggregated pattern of all trials, but in the other trial, dispersion was not significantly different from ran-

Table 1. Index of aggregation and nearest neighbor distances for three species of embiids tested in the laboratory

Species and trial ( $n$ )	Percent settling in arena	Nearest neighbor distance (body lengths) (mean $\pm$ SE)	Index of aggregation ( $A$ )	Dispersion pattern	Test statistic ( $t$ ) <sup>a</sup>
<i>A. urichi</i>		1.009 $\pm$ 0.16 <sup>b</sup>			
Trial 1 (15)	93		0.70	Aggregated	2.17
Trial 2 (12)	92		0.77	Random	1.46
<i>N. hardyi</i>		1.53 $\pm$ 0.19 <sup>c</sup>			
Trial 1 (15)	66.7		0.41	Aggregated	3.57
Trial 2 (12)	70		0.81	Random	1.11
<i>A. incompta</i>		1.71 $\pm$ 0.21 <sup>c</sup>			
Trial 1 (9)	100		1.18	Random	1.01
Trial 2 (7)	100		1.26	Random	1.33

<sup>a</sup> Critical value at significance level 0.05 is 1.96. When animals are randomly dispersed,  $A = 1.0$ ,  $A < 1.0$  denotes contagious dispersion, and for  $A > 1.0$ , dispersion tends toward uniformity. Nearest neighbor distance was computed for each species by determining the distance between nearest neighbors for all individuals and then dividing by avg length of females. Means with different superscripts are statistically different (Tukey-Kramer HSD). Sample size ( $n$ ) is the no. of embiids allowed into an arena.

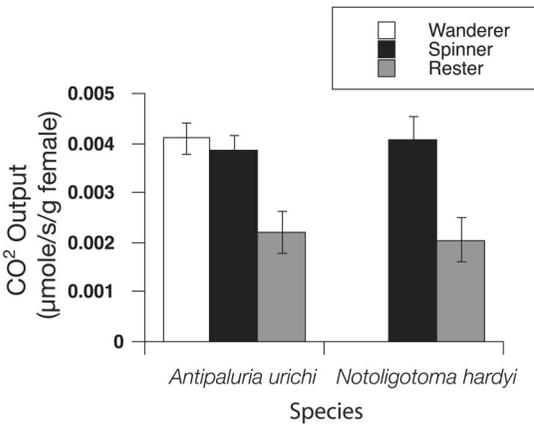


Fig. 4. CO<sub>2</sub> output (mean ± SE) produced during various activities (wandering, spinning, or resting) for two species of embiids, *A. urichi* and *N. hardyi*. Numbers of females in each category are shown above the bars.

dom. Inspection of nearest neighbor distances in this latter trial revealed that two individuals were far apart at 4.5 cm, whereas all other nearest neighbors were <1.8 cm apart. If the outliers are dropped, the A of 0.48 indicates a significantly clumped distribution ( $t = 2.65$ ), more consistent with the other trial of this species. *N. hardyi* was the most reluctant to disperse onto the apparatus from the petri dishes, with ≈30% staying behind in each trial. Dispersal rates of the other species were between 92 and 100%. Finally, *A. incompta* showed a random dispersion and the nearest neighbor distances were the greatest of the three species (Table

1). In all cases, *A. incompta* individuals created separate domiciles under the leaf pieces.

**Cost of Spinning Silk.** CO<sub>2</sub> output was significantly higher for active embiids (Spinners and Wanderers for *A. urichi* and Spinners for *N. hardyi*) than for Resters (Fig. 4; for *A. urichi*: Krusal-Wallis Test  $\chi^2$  [K-W] = 8.678, df = 2,  $P = 0.013$ ; for *N. hardyi*:  $t = 2.288$ , df = 18,  $P = 0.017$  for a one-tailed test). Females in both species produced statistically equivalent amounts of CO<sub>2</sub> when resting (Fig. 4; K-W = 0.044, df = 1,  $P = 0.83$ ) and when spinning (K-W = 0.024, df = 1,  $P = 0.62$ ). CO<sub>2</sub> output was 1.7 (*A. urichi*) and 2.0 (*N. hardyi*) times higher for Spinners than for Resters.

Silk removal and/or varying group size affected the ultimate size of adults in *A. urichi* but not reproductive output for the three species tested (Table 2). *A. urichi* reared as nymphs from replicates with torn silk were smaller than those in the control group, and those reared in groups were smaller than those raised alone. The mean number of eggs produced by *A. urichi* was similar whether they were reared as adults alone or in groups of three. The number of eggs produced by *A. urichi* started as nymphs and exposed to 17 wk of having their silk torn also did not differ significantly from those in the control group (Table 2). In this latter experiment, one replicate in the torn silk treatment produced extraordinarily large egg masses, averaging 103 eggs per mass, which is >2 SD above the mean for the treatment group overall. If this outlier is removed, the  $P$  value increased from 0.06 to 0.19. For each of the other two species, *N. hardyi* and *A. incompta*, adult females exposed to silk tearing or to disturbance but

Table 2. Comparison of performance for embiids exposed to treatments designed to affect investment in silk spinning

Species (time exposed to treatment)	Measure of performance per treatment group		Test statistic ( $P$ )
	least square mean head width (mm ± SE)		
<i>Antipaluria urichi</i> Tested as nymphs (15 wk)	<b>Solitary:</b> 1.86 ± 0.015 $n = 19$	<b>Group of 4:</b> 1.76 ± 0.016 $n = 18$	$F = 23.28$ $P < 0.0001^b$ $df = 1$
	<b>Control Silk:</b> 1.83 ± 0.016 $n = 19$	<b>Torn Silk:</b> 1.79 ± 0.015 $n = 18$	$F = 4.565$ $P = 0.04^a$ $df = 1$
<i>A. urichi</i> Started as adults (13 wk)	<b># Eggs per Batch (Mean ± SE)</b>		
	<b>Solitary:</b> 32.5 ± 5.6 $n = 18$	<b>Groups of 3:</b> 32.4 ± 4.4 $n = 20$	$t = 0.52$ $P = 0.61$ NS
<i>A. urichi</i> Started as nymphs (17 wk)	<b>Control Silk:</b> 51.5 ± 7.2 $n = 6$	<b>Torn Silk:</b> 71.9 ± 6.65 $n = 7$	$t = 2.09$ $P = 0.06$ NS
	<b>Control Silk:</b> 6.2 ± 1.51 $n = 6$	<b>Torn Silk:</b> 10.17 ± 2.59 $n = 6$	$t = 1.33$ $P = 0.21$ NS
<i>Australembia incompta</i> Tested as adults (5 wk)	6.2 ± 1.51 $n = 6$	10.17 ± 2.59 $n = 6$	$t = 1.33$ $P = 0.21$ NS
<i>Notoligotoma hardyi</i> Tested as adults (8 wk)	16.2 ± 1.49 $n = 6$	13.4 ± 1.63 $n = 5$	$t = 1.29$ $P = 0.23$ NS

Statistical results are shown for the ANOVA ( $F$ ) or Student's  $t$ -test. The statistical interaction between group size and silk treatment was not significant ( $P = 0.39$ ) for the exp on *A. urichi* nymphal development. See text for details of how silk was torn. Statistical significance at  $^aP < 0.05$ ;  $^bP < 0.001$ ; NS, not significant

no silk removal (controls) did not differ significantly in the average number of eggs produced (Table 2).

### Discussion

Two interrelated themes guided our study on silk spinning and its relationship to lifestyle. First, a high cost of spinning might dictate how embiids use silk and whether they will share the task. To study this theme, we took a multifaceted approach to quantify short-term costs of spinning and to manipulate possible trade-offs in spinning and development or reproduction. Second, we explored how embiids with different lifestyles use silk when placed under controlled conditions and whether their tendency to spin silk relates to their tendency to share it. In the following sections, we discuss our findings in relationship to our predictions and speculate about environmental contexts for the differences between the three species.

**Costs of Spinning—a Question of Trade-offs.** Forcing *A. urichi* nymphs to replace silk was the only manipulation of spinning that yielded a significant effect—experimental nymphs developed into smaller adults. In a previous field experiment (Edgerly 1988), *A. urichi* nymphs reared without their mothers grew more slowly than controls. Other field observations showed that once eggs hatch, an increase in maternal spinning yields an exponential increase in the silk covering for the family (Edgerly 1988). Maternal silk may provide a developmental advantage to nymphs. Choe (1994) similarly found that nymphs of *Anisembia texana* (Anisembiiidae), reared without their mothers in the laboratory, spun thin silk and grew more slowly than controls. In contrast, in this study, when adult females, in the three species, were forced to replace silk, they produced the same number of eggs as controls. This was true even if silk was stolen 17 times as they developed from the juvenile stage to adulthood in *A. urichi*. Furthermore, adult *A. urichi* living in small groups did not produce more eggs than did singletons, a finding that suggests that a potential reduction in physiological costs by reducing spinning, by sharing silk in this case, did not occur for adults. Therefore, overall laboratory evidence supports the contention that extra investment in silk spinning does not affect fitness for adult webspinners. Colonial female *A. urichi*, which lay more eggs than solitary females in the field (Edgerly 1987b), may do so for reasons other than physiological benefits gained by sharing silk. It is not known if silk spinning is costly for food-limited embiids, a condition that remains to be studied.

**Metabolic Cost of Spinning.** The similarity in metabolic rates of wanderers and spinners is not surprising because both activities require stepping; wanderers use all six legs, spinners mostly move their front legs. Spinners step rapidly as they create scaffolding for their silk domiciles (Edgerly et al. 2002). Our method does not allow us to separate the cost of producing silk proteins from the cost of stepping, referred to as step energy by Peakall and Witt (1975) in their study of an orb web-building spider. The increase in respiration

during spinning in *A. urichi* and *N. hardyi* was significant and very similar for the two species, but did not translate into reduced egg production in our long-term trials. It is possible that our silk-stealing regimen was not severe enough to force females to shunt energy and materials away from ovaries and into stepping and production of the silk protein. Dudgeon (1987), in contrast, found that stealing silk every day from caddisfly larvae imposed a cost because of extra silk synthesis. Starved caddisflies built smaller nets and those forced to spin every day lost weight. Another silk spinner, a leaf-rolling gelechiid caterpillar, displayed the opposite response to stolen silk. Loeffler (1996) discovered that larvae forced to spin new refuges actually grew faster than controls. Larvae displaced from their refuges suffered lower survival rates because they lost contact with the host plant, but if they did survive, their ability to select fresh leaves serving as new refuges and as food compensated for costs incurred during spinning. Loeffler suggested that, under normal conditions, the caterpillars balanced the risks of predation and loss of contact with the host plant against the problem of staying safely inside leaves that lose nutritional value as they age. Apparently, the cost of spinning did not figure strongly as a selective pressure in this species. Our laboratory results lead us to similarly propose that the functional aspects of silk, rather than the cost of spinning, may select for group living in some embiids.

If our original prediction had been correct that sharing silk is beneficial, grouped *A. urichi* nymphs should not have attained smaller adult sizes than solitary nymphs. Although we provided abundant lettuce, it is possible that the grouped nymphs were competing for food. Field observations (J.S.E., unpublished data) also provide insight as to a second explanation. As nymphs develop in field colonies, they spin tight open-ended silken tubes around themselves. These tubes are stitched beneath maternal silk and may form a barrier between a nymph and its colony-mates. The purpose of nymphal tubes is not known, but one hypothesis is that nymphs require added protection during ecdysis. In addition, in field trials where adult females were allowed to enter existing colonies, when the occupant of the silk was a nymph, the intruders were attacked, and in some cases, repelled completely (Edgerly 1986). These observations suggest that nymphs are aggressive and therefore living in a group may trigger some form of stress. Green et al. (1998) obtained similar results working on parsnip webworms that fought more when their webs were experimentally removed. As with *A. urichi* nymphs in our experiment, webworms reared alone attained larger sizes than grouped larvae. Therefore, it is possible that the smaller adults emerging from groups in our study were the result of behaviors not related to silk spinning.

**Interface Between Lifestyle and Silk Spinning Behavior.** The responses to tasks that embiids confronted in our experiments lend support to the idea that different lifestyles drive diversity in silk spinning behavior within the order. The tendency to aggregate (albeit

not strong) related positively to the tendency to spin and to the presumed reliance on silk: *A. urichi* constructed extensive silk coverings and settled relatively close to their neighbors, *N. hardyi* was intermediate in aggregating and in spinning, producing relatively heavy but small patches of silk, whereas *A. incompta* produced scant silk and settled randomly with respect to each other into a matrix of leaves. We will briefly review the natural history of the focal species to establish a context for these results. *A. urichi* live in groups of various sizes that form when dispersers establish new colonies or join existing ones (Edgerly 1987b, 1994). During a recent ecological survey on Magnetic Island, Edgerly and Rooks (2004) noted that the dispersion of two sympatric Australian embiidids differed from *A. urichi*. *A. incompta*, the detritivore, relies on a leaf-silk matrix for cover, whereas *N. hardyi*, the lichenivore, lives in thick silk domiciles on vertical surfaces of boulders produced by distinct mother-offspring groups that sometimes coalesce into large aggregations (see also Edgerly et al. 2005). These observations prompted us to propose the hypothesis that females relying to a greater extent on silk for protection tend to form groups. Consistent with this proposal, *A. urichi*, living in humid rain forests, share extensive silk produced by large colonies and reduce risks of predation through the avoidance and dilution effects (Edgerly 1994). They also may benefit by joining others in preexisting silk rather than risk predation while dispersing, a high risk indeed (Edgerly 1986).

Because the detritivore relies less on silk and more on leaves for construction, we expected that they would not spin as much silk as the other two species. Furthermore, if reliance on silk promotes coloniality then *A. incompta* would not be as likely as the two silk-dependent embiidids to seek other individuals when dispersing into fresh habitat. When tested for their tendency to spin, most *A. incompta* did not spin unless given a leaf. The reluctance of *A. incompta* to spin was not because of an inability to produce a silk gallery in empty dishes, but rather they were not stimulated to do so. As a case in point, the few females that laid eggs during the experiment readily spun silk along the edges of the dishes. Such reluctance to spin stands in contrast to behavior of *N. hardyi*, which spin immediately when placed in an empty arena. Exploring the differences between microhabitats might reveal the key to underlying causes for these distinctive behaviors. Unquantified observations (J.S.E.) suggest that differential predation pressures exist for the two embiidids. On the rocks, weaver ants are a pervasive threat; although these ants do not tear into silk, they quickly grab any insect sitting in the open. When we pulled *N. hardyi* out of their silk in the field, they quickly retreated into crevices where they would rapidly spin. In the litter, many species of skinks were seen hunting, scanning the ground for movements of potential prey. When we pulled *A. incompta* out of their silk in the field, they usually stayed motionless for long periods of time and often appeared dead. Because spinning requires movement that might attract predators, the

embiidids' reluctance to spin may be an antipredator behavior.

In conclusion, we propose that benefits of sharing silk by adult embiidids seem related to function rather than to physiological cost-sharing, with the caveat that we have not tested embiidids under the stress of food deprivation. Our results support the hypothesis that the tendency to aggregate in embiidids correlates with their reliance on silk. Our findings are based on small samples—three species in three families, two arboreal and one leaf-litter dweller. Research currently underway in our laboratory has revealed a morphological trait that correlates with the behavioral results in this report. For the three species, the relative sizes of the front basitarsi to head-width scores are 0.247 *A. urichi*, 0.218 *N. hardyi*, and 0.205 *A. incompta*. Hence, morphological evidence suggests that females that invest more in silk also invest more in silk glands, which are housed in their foretarsi. Whether the loss of the reliance on copious silk occurs within the Family Austrelembiidae and/or is associated with the habit of stitching leaf-litter remains an interesting question. The significance of abiotic factors also remains to be tested. Possible water-proofing qualities of embiid silk (J.S.E., unpublished observations) suggest a critical role of silk in protecting embiidids from flooding in leaf litter or heavy stem-flow during rain storms. Further research should be conducted on embiidids from a range of habitats to determine if natural history, spinning behavior, and social tendencies follow the trend detected here. To adequately address these questions will require a broader sweep of the order and an investment in fieldwork to locate more populations of these elusive insects.

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