

AN EXAMINATION OF THE DIFFERENTIAL PRODUCTION OF SILK BY
JUVENILE MALE AND FEMALE *APOSTHONIA CEYLONICA* (ENDERLEIN,
1912) (EMBIIDINA: OLIGOTOMIDAE)

A thesis presented to the faculty of the Graduate School of Western Carolina
University in partial fulfillment of the requirements for the degree of Master of
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ABSTRACT

AN EXAMINATION OF THE DIFFERENTIAL PRODUCTION OF SILK BY JUVENILE MALE AND FEMALE *APOSTHONIA CEYLONICA* (ENDERLEIN, 1912) (EMBIIDINA: OLIGOTOMIDAE)

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Members of the elusive insect order Embiidina are known for their prolific use of silk, and are unusual among insects in that silk is produced by both sexes and all life stages. However, the relative costs of silk spinning might vary considerably between the sexes, as male embiids do not feed as adults and are thus expected to be more resource-limited than females, which feed continuously throughout their lifetimes. Previous research on this group has also shown that the tarsal silk glands of male embiids contain significantly larger silk reservoirs than those of females, and it has been suggested that juvenile males may use these enlarged reservoirs to store silk proteins for use during adulthood, when the amount of energy available for silk production is low. The purpose of this study was to evaluate the plausibility of these conclusions by comparing the silk spinning behaviors of immature male and female embiids in the Indian species *Aposthonia ceylonica* (Enderlein, 1912) (Embiidina: Oligotomidae). Since this species is gregarious, spinning was also examined in a group context in order to test the hypothesis that males can adjust the amount of silk production in response to

group composition, as they may be expected to conserve their own silk in the presence of spinning females. The quantity of silk produced and the amount of time spent spinning were measured over 24 hours for solitary late instar male and female nymphs, as well as for nymphs in single sex and mixed sex groups. The results of this study suggest that juvenile males do produce significantly less silk than juvenile females, but no significant differences were found between groups and solitary individuals or between single sex and mixed sex groups. These findings corroborate the suggestion that males store silk as juveniles, which is likely a strategy to compensate for limited energy availability during adulthood. However, the data do not provide any evidence for social context-dependent silk spinning in the Embiidina. This study is among the first to address these questions in this little-studied insect order, but further research which incorporates more species of embiids as well as field observations is necessary to fully assess sex-related differences in silk production.

INTRODUCTION

The Insect Order Embiidina

The Embiidina (also known as Embioptera, embiids, or colloquially as “webspinners”) comprise a relatively small order of enigmatic insects which contains some 360 currently described species in 8 families, although as many as 2,000 species are estimated to exist worldwide (Ross 2000, 2009; Grimaldi and Engel 2005). The order is distributed across all continents excluding Antarctica, although a vast majority of the known species is restricted to the tropics, with only a small handful of species known to exist in the warmer portions of the temperate zone (Ross 2000, Edgerly et al. 2012).

Morphologically, the different species of embiids are remarkably uniform; they are generally small insects (with most species typically obtaining a length of less than 1cm as adults), with elongated bodies, soft integument, generalized chewing mandibles, and long moniliform antennae (Ross 2000, Grimaldi and Engel 2005). Adult males of many species are winged, but all adult females are neotenous and therefore apterous (Figure 1). They are most commonly encountered living on the surfaces of trees and rocks, or in subterranean retreats in soil and leaf litter. To date, all species examined in the field appear to be generalist herbivores or detritivores, with lichen, tree bark, and algae being the most common food sources for surface-dwelling species and leaf litter being the most common food source for the subterranean species (Edgerly 1997).

The order Embiidina is generally accepted to have strong systematic affinities with the so-called “orthopteroid” orders within the Neoptera, although the exact phylogenetic placement of the group relative to the other orthopteroid orders is still debated (Flook and Rowell 1998, Wheeler et al. 2001, Grimaldi and Engel 2005, Kjer et al. 2006). Systematic analyses variously suggest that they may be closely related to the Zoraptera (Minet and Bourgoïn 1986, Engel and Grimaldi 2000), Plecoptera (Wheeler et al. 2001), or Phasmatodea (Flook and Rowell 1998, Kjer et al. 2006), but none of these studies are conclusive. Although the evolutionary relationships of the order remain uncertain, the monophyly of the Embiidina is very well supported by both molecular and morphological analyses, and the members of the group are united by several notable synapomorphies, including desclerotized longitudinal wing veins, unique hemolymph sinuses at the base of the wing, the absence of ocelli, and three-segmented tarsi (Beutel and Gorb 2001, Wheeler et al. 2001, Grimaldi and Engel 2005, Kjer 2006, Szumik et al. 2008).

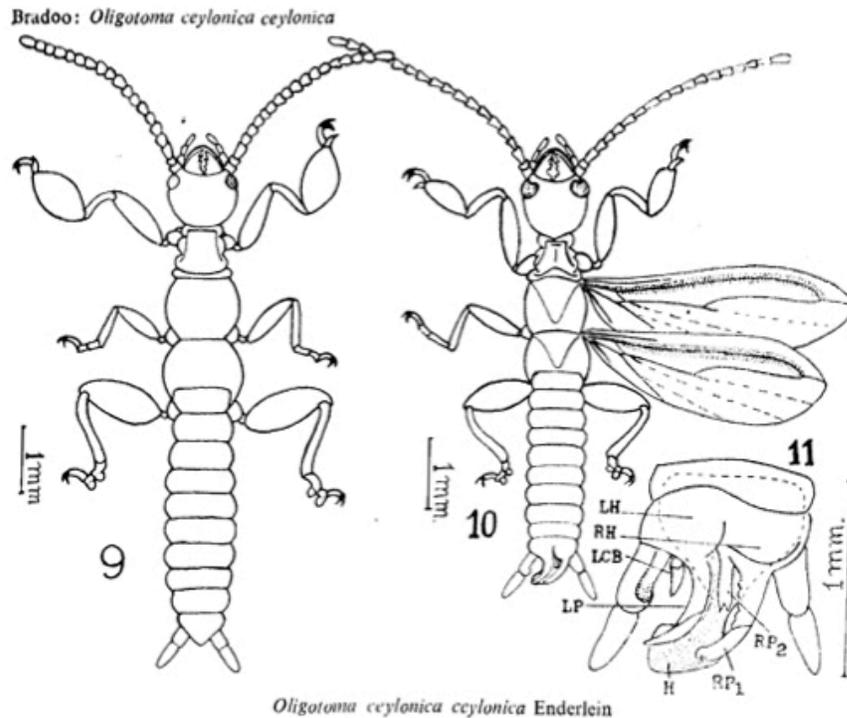


Figure 1. Illustration of an adult female (left) and male (right) embiid of the species *Aposthonia ceylonica*, which highlights many of the morphological characters that unite the Embiidina. Reproduced from Bradoo (1967).

The most salient synapomorphy of the group, however, is the presence of specialized silk glands housed in the enlarged fore basitarsi of both sexes and all life stages (Ross 2000) (Figure 2). Silk is exuded in thick sheets from these glands through a plate of hollow setae that lines the surface of the basitarsus; aside from one tribe of dance flies (Diptera: Empididae: Hilarini) (Young and Merritt 2003), they are the only known insects to spin silk from their tarsi, and it is their prolific production and use of silk that has given the embiids the common name “webspinners” (Edgerly 1997, Ross 2000).

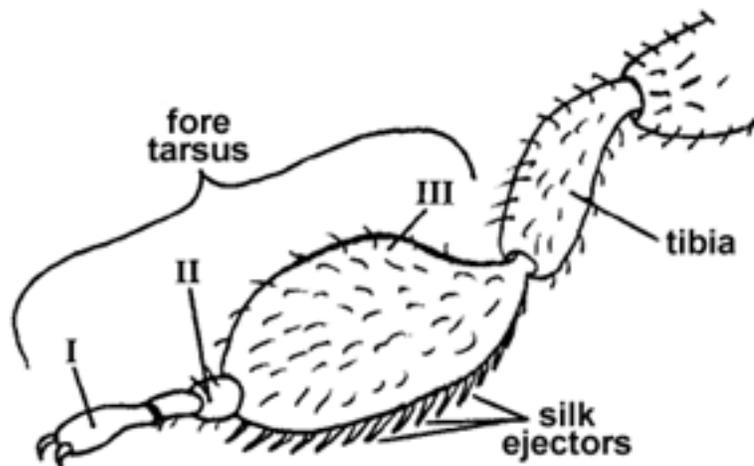


Figure 2. Illustration of the enlarged fore basitarsus of the Embiidina, which houses specialized silk glands characteristic of the order. Reproduced from Stoffolano (1997).

Indeed, silk appears to be so vital to the embiids that nearly all aspects of their lives are intimately tied to its use, and many of the morphological and behavioral characters which are so constant across all of the species in the order are specializations for a life spent almost exclusively beneath silk walls (Edgerly 1997). Although silk use varies among embiid species, most spin a thick sheet of silk over the substrate, sometimes incorporating elements of the substrate itself into the silk. Distinctive tubular galleries are then produced under the silk via repeated, circular motions of the front legs over their bodies (Edgerly et al. 2002). These silken domiciles are typically constructed over a food source, such as a patch of lichens on the side of a tree, and most species feed from under the silk, subsequently expanding it over new food sources as the old ones are depleted (Edgerly 1997, Ross 2000). By doing so, members of most species spend nearly their entire lives covered by a protective layer of silk, leaving it only to disperse or

mate; however, there is limited evidence that a some species may occasionally leave the domicile to forage (J.S. Edgerly, pers. comm.).

Subsocial Behavior in the Embiidina

Embiids are of potential interest to behavioral entomologists because they exhibit several behavioral characteristics that may promote the evolution of complex social behavior, including parental care of eggs (and in many cases newly-hatched young) and the construction of nests in which related conspecifics may interact (Edgerly 1986, Edgerly 1997, Costa 2006). Indeed, accounts by biologists who have observed or collected embiids in the field suggest that most species are gregarious (Edgerly 1997, Costa 2006). Despite this, there have been relatively few published studies of social behavior in the Embiidina. Prolonged mother-offspring interactions have been briefly described for several species of *Aposthonia* and *Oligotoma* (Bradoo 1967, Bradoo and Joseph 1970), *Anisembia texana* (Choe 1994), *Embia ramburi* (Edgerly 1997), and several others (Edgerly 1997, Miller and Edgerly 2008). However, to date, the only species to be rigorously tested using a combination of lab and field experiments is the Trinidadian species *Antipaluria urichi* in the family Clothodidae (Edgerly 1997). Since *A. urichi* is the only species of embiid whose behavior has been thoroughly investigated, findings related its behavior are often extrapolated and considered to be typical of all subsocial embiids. However, until more species are thoroughly examined, this cannot be said with certainty.

Aggregations of *A. urichi* in the field are variable in size, ranging from a single adult female and her offspring to larger networks of multiple mother-offspring family units. In these large colonies, galleries of multiple adult females may fuse, but interactions between adults are uncommon (Edgerly 1987a). Similar patterns have been noted for other species in the field and in the laboratory (J.S. Edgerly, pers. comm.). The evolution of social behavior in *A. urichi* appears to have been driven largely by heavy selective pressures imposed by predators and parasitoids (Edgerly 1987b, Edgerly 1994, Edgerly 1997). Since they are adapted for high maneuverability within their silken galleries, embiids are by necessity relatively soft-bodied insects that can easily be attacked by parasitic Hymenoptera and Diptera. Members of the widely distributed wasp family Sclerogibbidae are specialist ectoparasitoids on embiid nymphs, and members of several other families (including Braconidae and Scelionidae within Hymenoptera and Tachinidae within Diptera) attack clothodids of all life stages in South America (Edgerly 1997). Presumably, similar parasitic relationships exist in other embiid lineages from other parts of the world as well, as has been shown to be the case in India (Bradoo 1967).

In response to a large parasite load, *A. urichi* mothers will spin a thick protective layer of silk over their egg masses and actively guard the eggs from parasitoids through aggressive lunging displays (Edgerly 1987b). The thick silken barrier is often sufficient to deter natural enemies from attempting to enter the domicile (Edgerly 1994), and lunging displays have been shown to repel both scelionid egg parasitoids and opportunistic predators such as ploikiophilid bugs

(Edgerly 1987b, 1988). The maternal care exhibited by this species appears to strongly increase the hatching success of the young embiids; Edgerly (1987b) found that approximately 71% of eggs hatched when actively guarded by adult females while only 12% hatched when the female was experimentally removed. Guarded nymphs also appear to develop at an accelerated rate when compared to unguarded nymphs, possibly because of reduced energy expenditure due to shared silk production with their mother (Edgerly 1988). The results of these early studies by Edgerly as well as similar observations from Choe (1994) on the species *Anisembia texana* suggest that maternal protection from natural enemies increases the probability that young nymphs will survive to a reproductive age, and this is likely a major benefit driving the evolution of sociality in the order.

Silk Use and Production

These studies on group-living embiids provide evidence that silk serves a vital protective function for embiids by sometimes drastically decreasing the rates of egg parasitism and predation in the field. However, the function of embiid silk is likely multifaceted (Edgerly 1997). Embiid silk is waterproof, and thus the thick sheet of silk typically spun over the individual galleries may aid in shielding the embiids from heavy rains and monsoons that are common in the tropical habitats of most species; this hypothesis is supported by several observations of *A. urichi* silk in Trinidad (Edgerly 1986, 1997). Although silks of several other arthropod groups have thermoregulatory properties, this does not seem to be the case for embiid silk; instead, embiids appear to regulate their body temperatures via a

combination of behavioral and physiological adaptations (Edgerly et al. 2005, Costa 2006). Nevertheless, it is apparent that silk plays a vital role in the lives of embiids, an idea that is corroborated by fact that no species of embiid is known that does not spin silk (Edgerly 1997).

Although the functions of embiid silk and the relative fitness benefits afforded by this silken microenvironment have been investigated in several different embiid lineages, the energetic costs associated with silk production in this group are less well understood. In addition to embiids, numerous other arthropod groups spin silk during at least one life stage, the most well studied being the spiders (Araneae), larval amphisomenopteran (Trichoptera and Lepidoptera), and larval hymenopteran. In all of these groups, silk is a highly expressed protein, and since it is continuously being synthesized within the silk glands, silk production must require significant amounts cellular energy to produce (Craig et al. 1999). The relative metabolic cost of silk production in relation to other sources of energy expenditure and gain, however, is still debated. An analysis conducted by Craig et al. (1999) demonstrated that the silk proteins produced by different arthropod taxa vary considerably in amino acid composition, and since each amino acid requires a different amount of cellular energy to synthesize, this leads to different energetic costs associated with each of these different types of silk. While this study did not focus on embiid silk, it did indicate that lepidopteran silk, which is rich in the amino acids glycine, alanine, and serine, requires less ATP to produce than the more heterogeneous silk proteins of araneomorph spiders. Data from Edgerly et al. (2006) indicate that

embiid silk, like lepidopteran silk, is primarily composed of glycine (40-50%), serine (30%), and alanine (10%), and so it is expected to be relatively inexpensive to produce as well. This expectation is consistent with other data which show that, at least in three species of embiids, the energetic costs of silk spinning (measured via the amount of CO₂ released during the activity) did not significantly differ from the energetic costs of wandering along the substrate (Edgerly et al. 2006). This same study also demonstrated that the induction of additional silk production, via the removal of silk from an adult female's domicile, did not lead to a reduction in fecundity. This indicates that silk production does not appear to divert energy away from egg production and further suggests that the costs associated with silk spinning in embiids is relatively low.

The primary focus of such studies has been on silk spinning in adult female embiids, but there is evidence to suggest that silk spinning behaviors and the relative costs associated with silk production may vary with the age and sex of the individual. Earlier experiments on *Anisembia texana* showed that nymphs reared in the laboratory without a mother spun relatively little silk and matured more slowly than nymphs reared in the presence of maternal silk, which may indicate that silk production is costly enough for juveniles to divert resources away from nymphal development (Choe 1994). Similar findings were reported for nymphal *Antipaluria urichi* in the field and in the laboratory (Edgerly 1988, Edgerly et al. 2006). Silk production may also be more metabolically expensive for male embiids than it is for females due to fundamental differences in the biology of the two sexes. Whereas females continue to feed into adulthood, the

males of most species do not feed after the final molt (Ross 2000). Therefore, males may experience greater physiological constraints on silk production than do females, since they do not have the capacity to obtain energy after the final molt for use in silk production.

The Biology of *Aposthonia ceylonica*

To date, sex-related differences in silk production have only been studied in one species of embiid, *Aposthonia ceylonica* (Embioptera: Oligotomidae) from India. Like many of the other embiid species previously described, *A. ceylonica* is subsocial and exhibits strong sexual dimorphism between the neotenous females and the smaller alate males (Edgerly et al. 2012). The social behavior of *A. ceylonica* was first described by Bradoo (1967), and subsequent observations of the species in the laboratory have shown that its behavior does not drastically differ from that known for *Antipaluria urichi* (Edgerly et al. 2012). As is typical for embiid societies, *A. ceylonica* groups are composed of a single mother and her offspring, although it is not uncommon for multiple mother-offspring groups to coalesce and form much more extensive aggregations (Bradoo 1967, Edgerly et al. 2012). Typically, these groups are found on the surfaces of trees, particularly those with multiple crevices in which the embiids may hide (Edgerly et al. 2012), although one curious subspecies (*A. ceylonica ceylonica*) is an inquiline of subsocial *Stegodyphus* spiders and constructs silken galleries within the spiders' silken sheet webs (Bradoo 1967). In this species, the mother conceals her egg mass with feces and other debris and subsequently guards them against

parasitic scelionid wasps; after hatching, the nymphs remain in the colony for several instars, often following the mother closely early during their development (Bradoo 1967, Edgerly et al. 2012). During this time, the adult female embiid contributes the most silk to domicile extension and repair, although the nymphs are capable of spinning silk and do so in smaller quantities. It is probable, based on comparisons with *A. urichi*, that the mother dies before nymphal development is complete, at which point the nymphs will begin to lay down silk and extend the natal domicile (Edgerly et al. 2012). The young typically do not disperse away from the natal domicile to form new colonies until they are fifth instar subadults (Bradoo 1967).

As appears to be the case for all embiid species examined to date, male *A. ceylonica* do not feed as adults, despite possessing fully formed mandibles (Edgerly et al. 2012). Based on this observation, Edgerly et al. (2012) hypothesized that adult males of *A. ceylonica* would spin less silk than adult females; however, this hypothesis was not supported when the silk spinning behavior of the two sexes was compared. Once the silk measurements were adjusted to account for the smaller body sizes of the males, it was found that members of both sexes spun statistically equivalent amounts of silk (Edgerly et al. 2012). The authors of the study speculated that *A. ceylonica* males might be able to spin copious amounts of silk as adults by producing the necessary silk proteins when they feed as juveniles and subsequently storing these silk proteins in tarsal reservoirs until adulthood. This notion is consistent with morphological data which show that silk gland reservoirs are both larger and more densely

packed in the tarsi of male embiids than in female tarsi, but the hypothesis that males store silk produced during nymphal development has thus far not been tested.

Goals of the Present Study

The goals of the present study are to corroborate the conclusions made by Edgerly et al. (2012) and to examine the possibility that male embiids can conserve silk as nymphs by comparing the silk spinning biology of male and female *A. ceylonica* juveniles in the lab. It is hypothesized that juvenile males will spin smaller quantities of silk compared to comparably-aged juvenile females, as they are expected to withhold a significant proportion of their synthesized silk proteins for later use during adulthood. Since *A. ceylonica* is a social species, sex-related differences in silk spinning in a group context were also examined, by comparing the total amount of silk produced by nymphs in all-male groups vs. all-female groups vs. mixed-sex groups. It is hypothesized that juvenile males will produce less silk in mixed-sex groups than in all-male groups, as the presence of the silk spun by females will negate the requirement for males to contribute as much silk to domicile construction. Female nymphs are hypothesized to show no significant differences in silk production between the different treatments, as they are not subject to the same physiological constraints as males and presumably do not need to store as much silk as juvenile males.

If it can be shown that juvenile males alter the amount of silk deposited in response to the presence or absence of females in the group, it will provide

compelling evidence of complex social context-dependent decision behaviors which are currently unknown for any embiid species. Behavioral plasticity, or the ability to alter certain aspects of a behavior in response different environmental conditions, is well known from many arthropod taxa (Nylin and Gotthard 1998). With respect to silk spinning behaviors, this phenomenon has been especially well documented in spiders, which are able to adjust the structure and composition of prey-capture webs depending on local prey densities (Herberstein et al. 2000), the presence of predators (Li and Lee 2004) and the spiders' own body condition (Zevenbergen et al. 2008). However, the importance of social context (i.e. the presence or absence of conspecifics) in shaping silk spinning behaviors has not yet been thoroughly investigated in arthropods.

Social context-dependent behaviors of any kind have been rarely documented in insects. Such behaviors have been demonstrated in *Drosophila* fruit flies, the females of which appear to change the frequency at which they mate depending upon the relative abundance of males from different strains in the group (Billeter et al. 2012). Social context has also been shown to influence chemical signaling in *Drosophila*, with males emitting structurally different pheromones depending upon the number of different genotypes represented by the female flies in the group (Kent et al. 2008).

Embiid societies have been traditionally described as relatively simple mother-offspring associations, but anecdotal reports of communal foraging, nest provisioning, and perhaps even a guarding caste in several species all provide tantalizing hints that social behavior in this group may be more complex than

previously thought (Edgerly 1997, Costa 2006). Evidence of social context-dependent silk spinning behaviors in *Aposthonia ceylonica* nymphs would corroborate this argument and provide a compelling reason for entomologists to study this secretive and often-neglected group of insects more thoroughly.

METHODS

Study Organisms

The focal taxon of this study is *Aposthonia ceylonica*. Large juveniles (4th and 5th instars) of both sexes were obtained from breeding cultures reared by Dr. Janice Edgerly-Rooks at Santa Clara University in California; these individuals are descendants of specimens originally collected in India. Prior to the experiment, the embiids were housed in tall glass jars on a substrate of dry oak leaves and fed on foliose lichens and organic Romaine lettuce twice per week. All lichens used in this study were collected from the surfaces of trees and fallen logs located on the Western Carolina University campus and the North Carolina Center for the Advancement of Teaching (NCCAT), both in Jackson County, North Carolina. A pair of 50-watt infrared heat lamps were used to keep the embiid colonies at a temperature of 22-25 °C and all colonies were misted with water every other day to maintain proper humidity levels. The light regimen was set to alternate between 12 hours of light and 12 hours of darkness in an effort to better mimic the conditions of *A. ceylonica*'s natural habitat.

Individuals chosen for experimentation were sexed under a dissecting microscope. Late-instar embiids are sexually dimorphic, with males displaying conspicuous wing pads in addition to having somewhat smaller and more slender bodies than the females.

Assessing Silk Production by Solitary Juveniles

To determine whether there is a difference in overall silk production by male and female nymphs, experimental arenas were constructed by cutting a single groove measuring 6.35cm long by 0.32cm wide by 0.64cm deep in each of 12 plywood blocks measuring 6.35cm long by 2.54cm wide by 1.27cm deep (Figure 3). This setup is based on the design used by Edgerly et al. (2012) and is intended to mimic the bark crevices that *A. ceylonica* naturally occupies in the field. At the start of each trial, a single late-instar nymph was selected from the source colony, sexed as described above, and weighed to the nearest ten-thousandth of a gram using a Mettler AJ100 electronic scale. The embiid was then placed in the center of the groove of one of the blocks using a camelhair paintbrush. To prevent escapes, the block was taped to the underside of an 8cm diameter petri dish lid, which acted as a covering. The embiid was subsequently filmed for 24 hours (12 hours in the light followed by 12 hours in darkness) using a JVC EverioS GZ-MS240 digital camera set to record 5 second intervals every minute. Infrared lamps were used to illuminate the arenas during the 12 hours of darkness. At the end of the recording period, the embiids were removed to a distinct colony jar (to prevent accidental resampling from the original source colony) and the wooden block with all of the silk that had been spun with on it was photographed using a tripod-mounted Pentax K-X dSLR camera equipped with a Sigma 17-70mm f/2.8-4 DC Macro OS HSM lens. This image was then imported into the computer program ImageJ64 (<http://rsbweb.nih.gov/ij>), and the total area covered by silk was selected and measured using the program's

“Analyze” menu. This measurement was then divided by the mass of the individual embiid to obtain a measure of silk production per unit of body weight. The videos were then reviewed and the amount of time each individual spent spinning silk (evident from the characteristic movement of the forelegs displayed by spinning embiids) was also calculated, and any other behaviors relevant to silk spinning were noted. All blocks were thoroughly cleaned and freed of any residual silk before being reused in subsequent trials. Trials in which individuals which did not spin any silk during the allotted time were discarded. A total of $n=8$ males and $n=8$ females were used for this portion of the study.

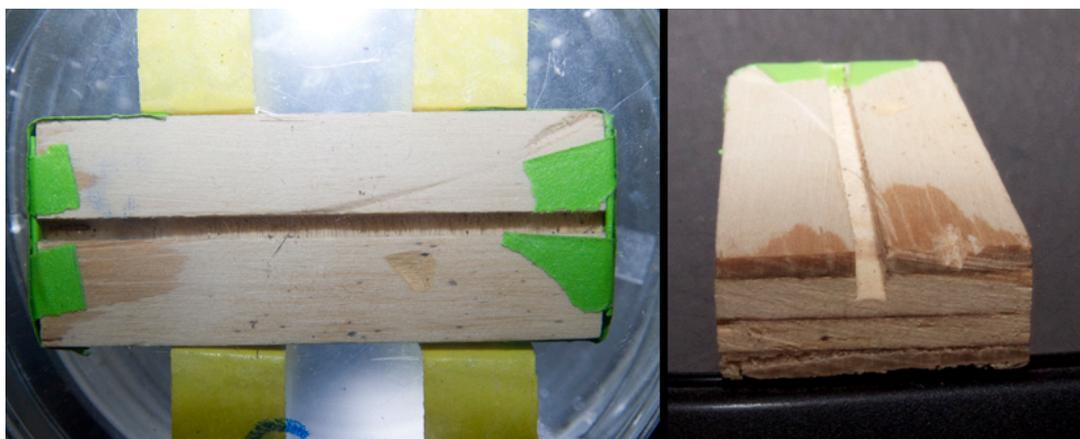


Figure 3. A depiction of an experimental arena constructed for this study, as seen from above (left) and from the side (right).

Assessing Silk Production in a Group Context

In order to ascertain the relative contributions of male and female nymphs to the construction of silken domiciles in groups, three additional treatment groups were established: one consisting of a group of 4 males, one consisting of a group of 4 females, and one mixed group consisting of 2 males and 2 females.

The individuals that comprised these groups were both selected from the original source colony and, due to limitations on the availability of appropriately aged nymphs, randomly resampled from the colony containing individuals previously used in the individual trials. The combined mass of each group was measured to the nearest ten-thousandth of a gram using the Mettler AJ100 electronic scale and then the embiids were placed into a groove of one of the plywood arenas described above. The arenas were then covered and recorded exactly as previously described, and the total amount of silk spun by the end of the recording period was again calculated using ImageJ64 and expressed as a function of the total mass of the embiids in the group. At the end of the experiment, the video recordings were again analyzed, but since the individuals used in this study were too small to safely mark with identifying paint, individuals within a group were tracked by eye. The amount of time spent spinning for each individual in the each of the three treatments was then recorded, and individuals which did not spin at all were excluded from the statistical analyses. Due to a limited number of available individuals during this portion of the study, total of $n=3$ replicates were created for the all-male treatment and a total of $n=4$ replicates were made for each of the remaining two treatment groups.

Statistical Analyses

After all of the measurements were recorded, the data were imported into *R* 2.14.2 for Mac OSX (<http://www.R-project.org/>). The Mann-Whitney U test was used to compare median values for the solitary spinning trials, as the small

sample sizes obtained prohibited the use of an independent-samples Student's t test (Wheater and Cook 2000). The specific comparisons that were made are as follows: (1) the area of silk deposited (per milligram bodyweight) by individual males vs. individual females and (2) the amount of time spent spinning silk by individual males vs. individual females.

For group spinning trials, a Kruskal–Wallis one-way analysis of variance was used to compare the median area of silk deposited (per milligram bodyweight) by male groups vs. female groups vs. mixed groups in place of an ANOVA, again due to small sample sizes. A Nemenyi-Damico-Wolfe-Dunn test was then performed to calculate pairwise differences in the event that the Kruskal-Wallis test yielded significant results (Wheater and Cook 2000). In addition, Mann-Whitney U tests were performed to make the following comparisons: (1) the amount of time spent spinning silk by males in all-male groups vs. solitary males, (2) the amount of time spent spinning silk by females in all-female groups vs. solitary females, (3) the amount of time spent spinning by males in male groups vs. mixed-sex groups and (4) the amount of time spent spinning silk by females in female groups vs. mixed groups.

RESULTS

Silk Production by Solitary Males and Females

In all trials, the amount of silk produced by members of both sexes was very scant and was typically spun in the form of thin, nearly translucent sheets over the tops of the crevices. Nevertheless, solitary male nymphs were found to spin significantly less silk per milligram of body mass (median=0.0681 cm²/mg, n=8) than did females (median=0.0784 cm²/mg, n=8) (Mann-Whitney U test U=51, p=0.04988, n=16). The medians and range of values for each of the two treatment groups are displayed in Figure 4 below.

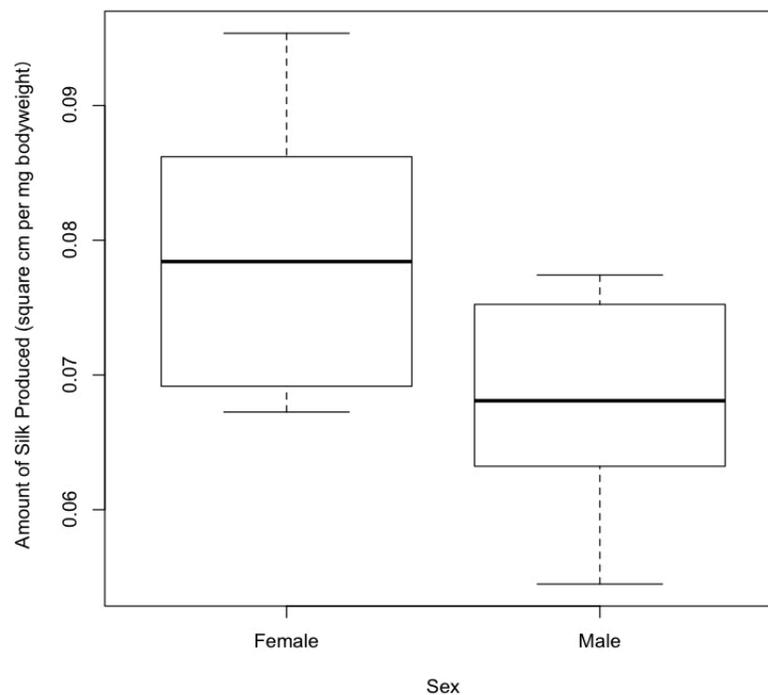


Figure 4. Median area of silk deposition (cm²) as a function of body mass (mg) for solitary female vs. male *Aposthonia ceylonica* juveniles.

Analysis of the video recordings revealed that embiids of both sexes spent relatively little time spinning silk during the trials. In a majority of the observations, an individual embiid actively wandered along the length of the arena whilst touching its antennae to the sides and floor of the crevice during the first several minutes of the trial. Most individuals then laid down silk relatively quickly during the first hour of the trial and subsequently remained nearly motionless beneath the silken sheet for the remainder of the experiment, though some individuals were more active and wandered underneath the silken domicile later in the trial. This pattern of activity was very similar for both males and females, and males were not found to significantly differ from females in the amount of time spent spinning ($U=44$, $p=0.2268$, $n=16$). The median time spent spinning was 9.9 minutes for males ($n=8$) and 11.3 minutes ($n=8$) for females out of the 24 hour recording period (Figure 5).

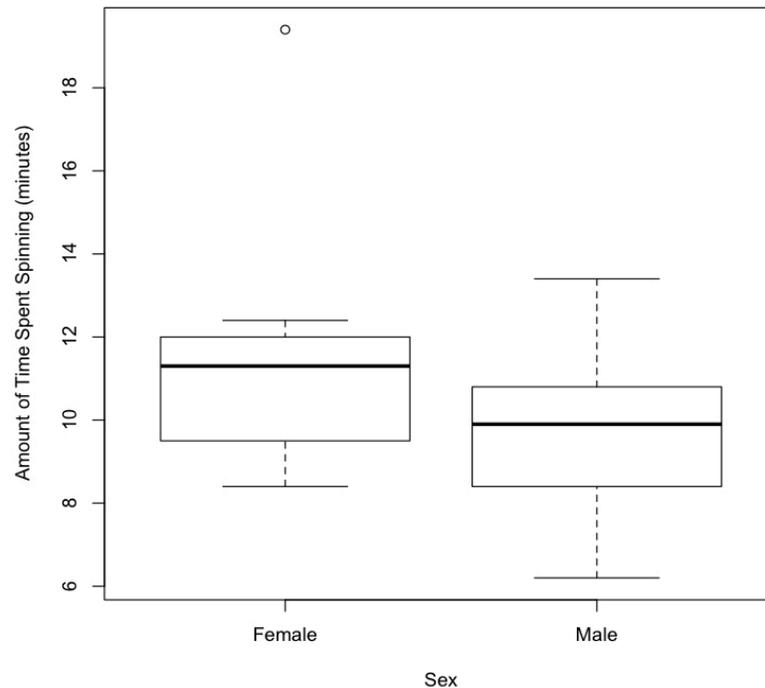


Figure 5. Median time (min) spent engaging in silk spinning behaviors for solitary female vs. male *Aposthonia ceylonica* juveniles during the 24-hour observation period.

Silk Production by Males and Females in a Group Context

Just as was observed in the solitary trials, the amount of silk spun in the group trials was again very scant and limited to thin sheets or tubes along the top surface of the arenas' crevices. The median amount silk spun per milligram of body mass was significantly different across the three treatment groups (Kruskal-Wallis $\chi^2=6.1818$, $df=2$, $p=0.04546$, $n=11$). The all-male groups spun the smallest amounts of silk (median=0.0220 cm^2/mg , $n=3$), followed by the mixed sex groups (median=0.0299 cm^2/mg , $n=4$), while the all-female groups spun the largest amounts of silk (median=0.0307 cm^2/mg , $n=4$) (Figure 6). However, the Nemenyi-Damico-Wolfe-Dunn test revealed that the only significant pairwise

difference was between the all-female groups and the all-male groups ($p=0.033$, $n=7$, $\alpha=0.05$); there was no significant difference in the medians between all-male groups and mixed sex groups ($p=0.135$, $n=7$) or between all-female groups and mixed sex groups ($p=0.921$, $n=8$).

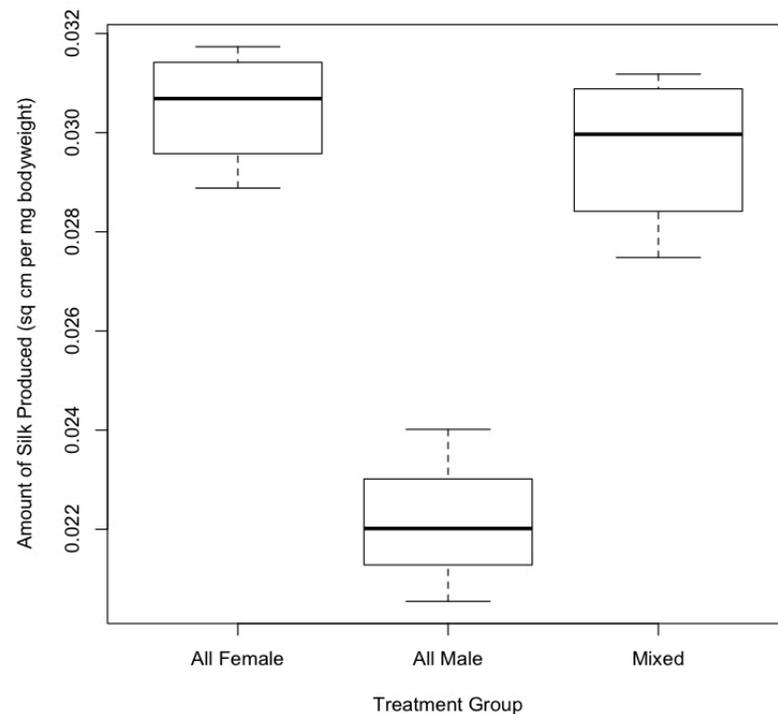


Figure 6. Median area of silk deposition (cm^2) as a function of body mass (mg) for all male vs. all female vs. mixed sex groups of *Aposthonia ceylonica* juveniles.

As was observed during the solitary trials, the amount of time spent spinning silk in all groups was relatively brief and generally limited to the first hour of the trials. Analysis of the video recordings also revealed that not all individuals within each group actually spun silk during the entire 24-hour observation period. In three of the four all-female groups observed in this study,

only one individual female exhibited spinning behavior, while in one group two different individuals were observed to spin silk. In the majority of these observations, the individuals that did not spin eventually took refuge under the silken walls laid down by the other group member(s). This same general pattern was observed for the other treatments. Of the three all-male groups constructed, spinning was performed by just one individual in two groups and by two different embiids in just one group. In the case of the mixed sex groups, spinning was performed by one male and one female in two of the groups, by two females and one male in one group, and by two females but no males in one group.

The amount of time spent spinning by individual male embiids in the all-male groups was not found to significantly differ from the amount of time spent spinning by males in the solitary treatment ($U=15$, $p=0.9333$, $n=12$). The median amount of time for males in a group was 9.5 minutes ($n=4$), compared to a median of 9.9 minutes ($n=8$) for solitary males. Similar patterns were found with respect to females; the amount of time spent spinning by females in an all-female group was not significantly different from that of the solitary females ($U=16$, $p=0.6064$, $n=13$). The median amount of time spent spinning by females in groups was 10.2 minutes ($n=5$), compared to the median of 11.3 minutes ($n=8$) for solitary males. These comparisons are shown in Figure 7.

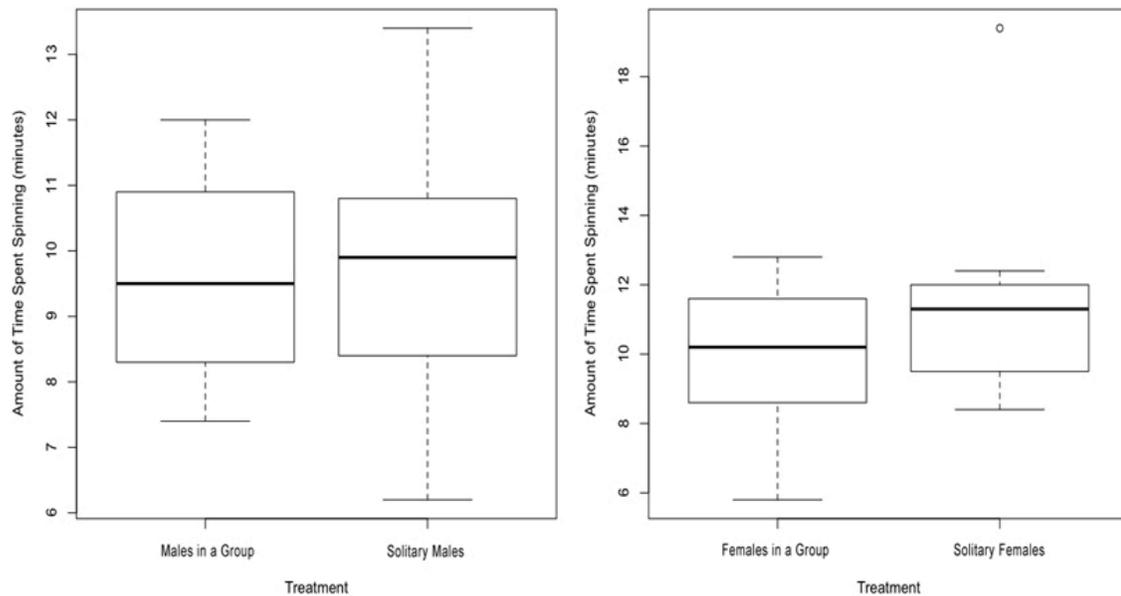


Figure 7. Median time (min) spent engaging in silk spinning behaviors for males in all-male groups vs. solitary males (left) and for females in all-female groups vs. solitary females (right) during the 24-hour observation period.

The median amount of time spent spinning by males in the mixed-sex groups was 5.4 minutes ($n=3$). This median did not significantly differ from the median amount of time spent spinning by males in the all-male groups ($U=11$, $p=0.1143$, $n=7$). However, despite the lack of significance, there was a noticeable trend which showed males in the mixed sex groups spun silk for slightly less time than those in the all-male groups (Figure 8). Females in the all-female groups were not found to spend a significantly different amount of time spinning than the females in the mixed sex treatment ($U=16$, $p=0.9307$, $n=11$). The median amount of time spent spinning by females in mixed sex groups was 10.1 minutes (Figure 8).

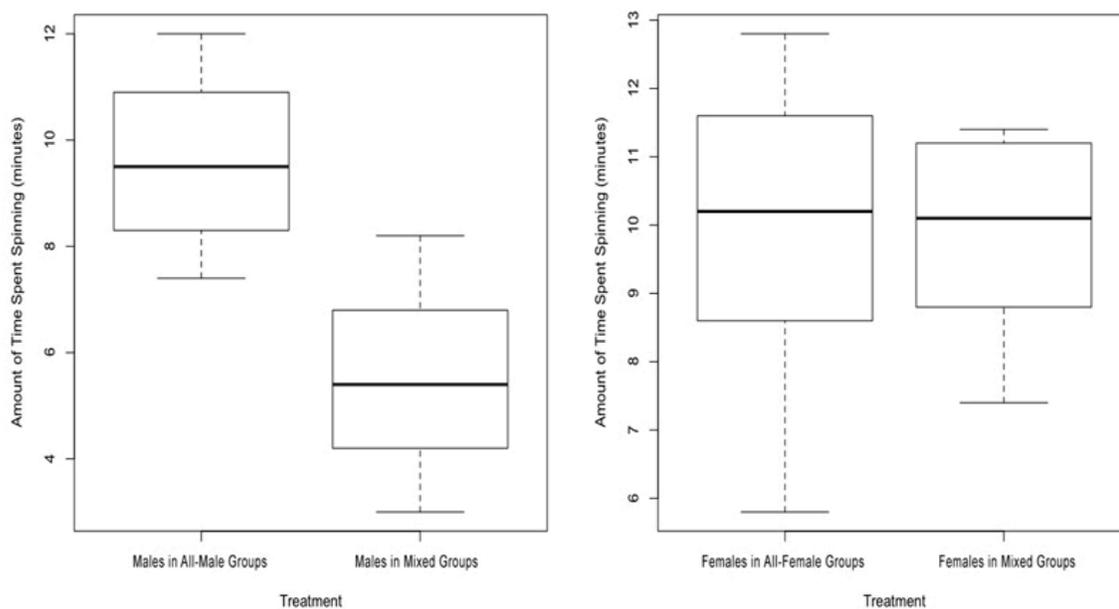


Figure 8. Median time (min) spent engaging in silk spinning behaviors for males in all-male groups vs. mixed-sex groups (left) and for females in all-female groups vs. mixed-sex groups (right) during the 24-hour observation period.

DISCUSSION

Differential Silk Production by Male and Female Nymphs

The examination of the silk-spinning behaviors of solitary embiids revealed that male nymphs produced significantly less silk than comparably aged female nymphs. This lends support to the first main hypothesis of this study, which posited that male juveniles would spin less silk (potentially due to selective pressures on male nymphs to conserve silk proteins until adulthood). Edgerly et al. (2012) found that adult male and female *A. ceylonica* spin comparable amounts of silk, despite the fact that adult males do not feed unlike females and thus must have smaller energy budgets that may quickly be depleted with frequent silk production. In response to these observations, the authors of the study hypothesized that males would store silk proteins in enlarged tarsal silk reservoirs as juveniles for use during adulthood (Edgerly et al. 2012). The finding that juvenile males spin less silk than juvenile females is consistent with this interpretation, as it is expected that males would conserve as much silk as possible during later immature instars. This would result in the overall smaller quantities of silk that were observed with respect to the solitary male embiids. Dissections of nymphal male tarsal glands and subsequent protein analyses could also help to confirm the presence of significant quantities of stored silk proteins; however, this has yet to be performed, as it was outside the scope of the current study.

This is the first study to present evidence for sex-related differences in silk production for juvenile embiids and is one of only two studies to address embiid silk spinning behaviors in the context of sexual dimorphism. Differential silk production between the two sexes has been rarely documented for juvenile insects in general. Underwood and Shapiro (1999) examined the relative silk contributions of male and female *Eucheira socialis westwoodi* caterpillars (Lepidoptera: Pieridae) to communal nest construction and discovered that male caterpillars both produced more silk and spent more time spinning than did females. This finding, which presents a pattern directly opposite to the results of the present study, is likely the result of selective pressures related to lepidopteran biology that are not applicable to embiids. The authors hypothesized that female larvae may conserve silk because they are under strong pressure to allocate food energy into obtaining large adult body weights, as body mass is linked to fecundity in these moths (Underwood and Shapiro 1999). This is typical of many lepidopterans, which either feed little or do not feed at all as adults. Female embiids, in contrast, feed both as immatures and as adults, and therefore are not subject to the same selective pressures. A second study, conducted by Wilson and Hölldobler (1980), found that female larvae of the ant species *Oecophylla longinoda* (Hymenoptera: Formicidae: Formicinae) produce more silk in relation to male larvae, a trend similar to that found for *A. ceylonica*. However, Wilson and Hölldobler (1980) proposed that their observations could best be interpreted as a byproduct of strong kin selection, which is plausible in the case of haplodiploid insects such as ants, since males are less related (on average) to

the rest of the colony than are females and thus have more incentive to cheat. However, this is not applicable to embiids, in which there is no discrepancy in relatedness between males and females. Instead, the patterns observed for *A. ceylonica* are likely best explained as the result of the physiological constraints associated with silk production in adult males as described previously.

Despite the fact that the amount of silk deposited was found to significantly differ between the two sexes, the amount of time spent engaging in spinning behaviors was not significantly different. This curious finding could suggest that the males in this study produced less silk not by regulating how long they spun, but rather by regulating the volume of silk that they secreted from their silk glands during each period of spinning. The particulars of embiid silk spinning biology are only beginning to be thoroughly studied (Edgerly et al. 2005, 2006, 2012), and much is still unknown about the differences between the two sexes in this regard. The assessment of adult *A. ceylonica* by Edgerly et al. (2012) demonstrated that the spinning choreographies of adult males and females were statistically indistinguishable, but it is unknown if there are any differences between the two sexes in the amount of silk deposited per “spin step” or the degree to which this can be regulated and adjusted by the embiids. It is also possible that this finding is an artifact of the small sample sizes used in this portion of the study; the addition of further replicates into the analysis could potentially produce a significant difference in spinning times that would be more consistent with the observed significant difference in silk quantity.

Silk Production in a Group Context

The adjusted amount of silk deposited was found to be significantly different across the three treatment groups tested. Although there is an apparent trend in the data which suggests that all-male groups produce less silk than both all-female and mixed sex groups, the Nemenyi-Damico-Wolfe-Dunn post hoc test revealed that the only significant contrast was between the all-male and all-female groups. Since the video analyses suggest that a majority of the silk production within any given group is typically performed by one individual embiid, this particular difference is likely due to the differences in silk deposition between male and female juveniles that were observed in the solitary embiid trials. Thus, these results corroborate the conclusion that male *A. ceylonica* nymphs spin significantly less silk than female nymphs and therefore provide further support for the hypothesis that male nymphs withhold silk proteins until adulthood.

Since the amount of silk spun in the mixed-sex groups was found to be indistinguishable from the amounts produced in the other two treatment groups, the second main hypothesis of this study, which predicted that males in mixed-sex groups would produce less silk than males in all-male groups, must be rejected. This suggests that juvenile male *A. ceylonica* do not adjust the amount of silk they produce in response to the composition of the spinning group, but rather tend to spin relatively comparable amounts of silk both in the presence and absence of females, which were predicted to produce silk more readily due to more relaxed physiological constraints during adulthood. The observations made in the present study therefore provide no direct evidence for the presence

of any social context-dependent allocation of resources in the Embiidina. However, further studies that focus on different behaviors in a larger array of embiid species need to be performed before the existence of such context-dependent behaviors in this order is completely dismissed.

The various comparisons concerning the amount of time spent spinning within groups (as revealed from the analysis of the video recordings) yielded few significant patterns. The amount of time that males spent spinning silk in all-male groups was not found to be significantly different than the amount of time spent spinning by males in the mixed sex groups, which further suggests that juvenile male embiids do not adjust their silk spinning behaviors based on group composition. This is consistent with the patterns observed in relation to silk quantity. The same is true for females, as no significant difference in spinning time was detected between females in all-female groups and those in the mixed sex groups; however, this was an expected result. But again, due to the small sample sizes used in this experiment and especially in relation to the group context portion of the study, caution is warranted in completely discounting the possibility that silk spinning in *A. ceylonica* juveniles is context-dependent.

In the case of both sexes, individuals in groups did not spend significantly more or less time spinning silk than did solitary individuals. Unfortunately, since it is impossible to determine the exact proportion of silk laid down by each individual in a group when more than one embiid participates in spinning, comparisons could not be made regarding the amount of silk produced between group-living and solitary individuals in this study. However, if it is assumed that

the amount of time spent spinning silk is proportional to the amount of silk produced, the data collected herein suggest that individuals in groups do not produce significantly different quantities of silk compared to solitary individuals. This is a surprising discovery, as it might be expected that individuals in a group would decrease the amount of silk they produce in response to the presence of silk from other individuals. That such behavior was not found is consistent with the claim that a reduction in energy expenditure via the shared production of silk is not a primary factor that promoted the evolution of group living in the Embiidina (Edgerly 1997). It is worth noting, however, that careful analysis of the recordings reveals that in a majority of the group trials, silk spinning was performed primarily by one individual. Under these circumstances, it should be expected that the patterns observed in the group trials would not differ from those seen in the solitary trials, as most group trials involved a single, solitary spinning embiid.

Although no significant differences in the amount of time spent spinning were found, the observation that most individuals within a given group did not spin any silk is nevertheless intriguing. These individuals did, in many cases, eventually occupy the silken structures produced by other individuals. This, along with the generally scant amount of silk produced in these trials and the surprisingly small amount of time spent spinning by all of the embiids observed, could suggest that *A. ceylonica* nymphs spin relatively little silk of their own under natural conditions and instead preferentially join the silken domiciles constructed by others (perhaps adults). Given that this species frequently occupies the surfaces of trees which have many crevices (Edgerly et al. 2012), it is also

possible that nymphs rely primarily on the cover of the bark itself for protection and this produce little silk of their own until adulthood. There is currently much unknown about the behavior of *A. ceylonica* in the field, with Bradoo (1967)'s observations constituting the only published study on the subject, and to date no study has extensively investigated silk production and use by the species under natural conditions. Thus, it is imperative that such field studies be performed, as it is only by knowing the ecology and life history of this species that we can make full sense of laboratory findings such as those put forth in this study.

Limitations of the Present Study and Areas for Future Research

Although significant differences in silk quantities were found between the sexes, the small sample sizes used in this experiment make it necessary to interpret these data with particular caution. Only eight individuals were chosen from each sex for the solitary trials, due in part to the limited availability of appropriately aged nymphs and partly to time constraints relating to the nymphs' final molt to adulthood. While this sample size appears to have been sufficient to produce a noticeable trend in silk production by males vs. females, the probability value associated with this particular statistical comparison is only marginally smaller than the significance value (α) of 0.05. Therefore, it would be beneficial to repeat the present study with additional replicates, in order to further support that the results presented herein are due to differences in the silk spinning biology of male and female nymphs and not simply artifacts of the small sample sizes that were utilized.

Due to the same constraints of time and specimen availability, no more than four replicates could be constructed for each treatment class in the group trials; thus, it is important to consider those results in light of small sample sizes as well. The need for additional replicates in this portion of the study is perhaps highlighted by Figure 8. Although there was a pronounced tendency for males in mixed sex groups to spin for less time than males in all-male groups, this comparison was not found to be significant. It is possible, however, that the addition of further replicates could reveal a significant difference in this case. Should this be demonstrated, it would suggest that nymphal males do alter their silk spinning behaviors in the presence of females (by spending less time spinning silk), which would support rather than refute the hypothesis that social context-dependent silk spinning does occur in the Embiidina. Therefore, although the current results do not provide any evidence for such behaviors, further research is required to ascertain the validity of this conclusion.

It is also imperative that more detailed field observations are made of *Aposthonia ceylonica*, with particular emphasis on the ways in which juveniles use silk under natural conditions. The only published account of this species in the field focused on a single subspecies, *A. ceylonica ceylonica*, which is an inquiline in social spider webs (Bradoo 1967); little is known about the behavior of other subspecies in the field, including the bark-dwelling populations used in this study. Most of the information about the ecology and life history of these other subspecies is derived from anecdotal observations made by collectors or is inferred based on field observations of another embiid species, *Antipaluria urichi*.

Although *A. urichi* and *A. ceylonica* behave similarly in lab cultures (Edgerly et al. 2012), it may be erroneous to assume that the ecology and life histories of the two species are identical, as they are not only phylogenetically distantly related (Szumik et al. 2008), but are also found on different continents and thus potentially interact with very different sets of biotic and abiotic factors. Therefore, in order to fully understand the silk spinning behavior of this species, laboratory experiments such as the present study must be performed in conjunction with studies that examine silk spinning behaviors in the field.

The observation that only one individual performed the majority of the silk spinning in most of the group trials certainly warrants further attention. In particular, it would be of great interest to determine whether certain individuals in a group are more likely to consistently spin silk than others. If this could be demonstrated, it may suggest the development of a limited form of intra-colony division of labor, with a certain fraction of individuals performing a disproportionately large amount of the domicile construction. This type of behavior is currently unknown in the Embiidina, but given the general paucity of species which have been thoroughly examined, the possibility of such a system should not be immediately discounted.

The results reported herein suggest that late-instar male nymphs of *Aposthonia ceylonica* produce less silk than late-instar female nymphs, but it would be interesting to expand the scope of this study to examine whether these trends are pronounced in earlier nymphal instars as well. It is unknown whether spinning behaviors change significantly during nymphal development and, if so,

in which respects (Edgerly et al. 2012). It is possible that male embiids only begin to sequester silk proteins during the penultimate instar, in which case it would be expected that the trends observed in the present study would not be as readily apparent during earlier instars; however, studies which track silk production throughout the entire life cycle will need to be performed in order to test this hypothesis.

Finally, it would be well worthwhile to examine these questions of sex-based differences in silk spinning behaviors and context dependent silk spinning behaviors in other embiid species. This study is one of the first to address these questions in this group, but both this study and the pioneering study conducted by Edgerly et al. (2012) focused on a single embiid species (*Aposthonia ceylonica*). Given that embiid species with different life histories appear to use silk in different ways (Edgerly et al. 2006) and perhaps produce it differently as well (Edgerly et al. 2012), it would be of great interest to determine whether the patterns observed for *A. ceylonica* can be extended to other species as well, including non-arboreal species which may behave very differently.

Conclusions

The observation that male nymphs of the embiid species *Aposthonia ceylonica* spin smaller quantities of silk relative to female nymphs, when taken together with previous observations regarding sexual dimorphism in silk gland structure and the spinning behaviors of adults, suggests that juvenile male embiids likely store excess silk proteins during nymphal development for use

during adulthood. This is likely an adaptation to compensate for limited energy availability during adulthood, as male embiids do not feed as adults whereas female embiids do. Although such a difference has only been observed for *A. ceylonica*, it is likely that the results of this study can be extended to other species as well, since no species of embiid is known in which males feed past the final molt. Comparative experiments which draw upon a wider range of study species are needed to confirm the validity of this claim.

Although the data available from this study do not provide any evidence that nymphal male embiids can alter their spinning behaviors in response to social context, there are compelling reasons to investigate this area further. Noticeable trends in the data, although not found to be statistically significant, could indicate that such context-dependent behaviors exist and that they were simply undetectable due to low sample sizes. Although several taxa of arthropods have been demonstrated to exhibit complex context-dependent behaviors, including in relation to silk spinning, no study has thus far provided any evidence (in embiids or in other taxa) that silk spinning behavior can be altered depending on social context. Given that so much remains unknown about embiid biology and that many poorly studied species show tantalizing hints of relatively complex social behaviors, these questions and many others relating to these enigmatic insects are certainly worth exploring.

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