

EVIDENCE OF KIN-SPECIFIC COMMUNICATION IN A TEMPERATE,
SUBSOCIAL SPIDER *ANELOSIMUS STUDIOUSUS* (ARANEAE, THERIDIIDAE)

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ABSTRACT

EVIDENCE OF KIN-SPECIFIC COMMUNICATION IN A TEMPERATE, SUBSOCIAL SPIDER *ANELOSIMUS STUDIOUS* (ARANEAE, THERIDIIDAE)

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Sociality in spiders is extremely rare but has evolved repeatedly and is found across multiple families, including Theridiidae. Potential benefits of sociality for spiders include the ability to capture larger prey, reduced predation on individuals, and reduced individual silk costs. However, there are also potential costs and risks, including reduced individual fecundity in larger colonies and inbreeding depression. In subsocial spider species, such as *Anelosimus studiosus*, females typically establish nests as solitary individuals and raise offspring without the aid of others. This experiment was designed to test for evidence of kin-specific communication between mother and offspring by inducing choice through a Y-branch experiment. Additionally, the mother's silk production was inhibited to determine if communication could occur through pheromones deposited with silk draglines. The results showed significant differences in the way spiderlings made choices after being exposed to silk draglines from their mother or from an unrelated mother ($p=0.016$). The spiderlings also chose differently when exposed to a mother leaving a silk dragline as opposed to following a mother incapable of producing silk ($p=0.045$). These results suggest a degree of kin-specific communication between

mother and offspring and that the communication may be mediated through silk draglines. Such kin recognition could be important in understanding group cohesion and how kin selection might work to create social networks within typically asocial taxa such as spiders.

INTRODUCTION

Social behavior has evolved in many animals and across many groups of taxa. This begs the question why sociality has evolved in some groups and not others, and why sociality appears more often in some groups of taxa than others. A traditional ecological tool used to explain the ultimate (evolutionary) reasons behind the development of a behavior is the cost-benefit analysis. Behavioral ecology theory predicts that sociality in organisms can and will evolve only when the net benefits of close association with conspecifics exceed the costs (Lin and Michener 1972, Silk 2007). There are many potential benefits to sociality as well as many potential costs, and these benefits and costs vary amongst taxa and habitats. The three overarching categories of benefits of sociality are: increased foraging abilities, protection from predation, and increased reproductive success (Bourke and Franks 1995, Costa 2006, Janson 1998, Lin and Michener 1972, Silk 2007, Uetz et al. 2002). All provide short-term benefits for individuals as well as longer-scale, larger benefits for societies (Avilés and Tufiño 1998, Silk 2007). As such, many evolutionary processes are invoked to explain the evolution of sociality: selection on individuals, kin selection, and even possibly group selection (Avilés 1986, Silk 2007). Kin selection can act on a society of related individuals if their inclusive fitness is higher when living as a group than as individuals (Hamilton 1964). Kin selection is often invoked to explain behaviors that could otherwise seem contradictory because of costs to direct fitness. These costs to direct fitness, however, can be compensated for by gains in indirect fitness. This is referred to as inclusive fitness in the theory of kin selection.

In addition to the benefits, however, there are potential costs to sociality as well. Large societies may be more conspicuous to possible predators, leading to an increased predation risk (Bonds et al. 2005, Uetz et al. 2002, Silk 2007). Group members may also engage in intragroup competition which can affect the individual fitness of individuals within the society (Avilés and Tufiño 1998, Bonds et al. 2005, Bourke and Franks 1995). Group living can increase the occurrence of parasites or diseases as a result of living in such close contact with other individuals (Bonds et al. 2005, Velthuis 1987). Because different taxa have different requirements and priorities, these costs and benefits have different weights and thus sociality is more likely to evolve in certain groups than in others.

Eusociality is an example of a high level of sociality that can be found in Hymenoptera (wasps, bees, and ants) and Isoptera (termites), as well as in a few other taxa including some thrips, aphids, and naked mole rats (Pamilo 1984, Jarvis 1981, Nowak et al. 2010). Eusociality is characterized by overlapping generations, a division of labor in reproduction, and cooperative care of offspring (Danforth 2002, Fewell 2003). These eusocial taxa can serve as interesting study groups for sociality in general because of the high level of social interactions within colonies. Before Hamilton's concept of inclusive fitness, altruism, a key element of eusocial taxa, was considered to be an evolutionary paradox because it seems to conflict with the core Darwinian concept of reproductive self-interest (Thorne et al. 2003). The explanation for the evolution of eusociality rests on two major premises: kin selection and inclusive fitness (Evans 1977, Hamilton 1964a, b). Genetic processes such as a haplo/diploid sex determination system or high levels of inbreeding can influence the degree of relationship between members of

the colony, because females in each family-based colony in a haplo/diploid system are more closely related to one another than in traditional family groups with a diploid system of genetic determination (Evans 1977). In short, having this higher coefficient of relatedness amplifies the effect of kin selection, which relies on increased individual fitness due to increased reproductive success by their relatives (Hamilton 1964). Therefore, eusociality could have evolved in these taxa based on the chance of nest inheritance, cooperative breeding, and kin selection (Thorne et al. 2003, Lin and Michener 1972).

Not all Hymenoptera exhibit eusocial behavior, however. In fact, there can be a wide variety in the amount of sociality seen in these taxa. In halictid bees (Halictidae), for example, there is intraspecific variation in the level of sociality. Some of this variation appears to be associated with changes in altitude or latitude (Danforth 2002). In general, tropical habitats appear to support more social insects, with all groups of social insects best represented in the tropics and subtropics (Evans 1977). Additionally, as in many areas of biogeography, elevation parallels latitude, and social species are more common at lower elevations in the tropics than at higher altitudes at the same latitude (Avilés et al. 2007). One explanation for these patterns is that tropical climates more readily permit the overlap of generations necessary in eusocial societies, because of a more stable climate. There tend to be high levels of competition and predation in warmer climates which could support higher levels of sociality in organisms (Evans 1977).

Additionally, a hypothesis posited by E.O. Wilson suggests that the continuous colony function seen in tropical areas allows for cooperative behavior to evolve with fewer adaptations (Wilson 1975 in Jones et al. 2007). In temperate areas, where prey

items are scarcer, it may be difficult to support the large population sizes typical of the social species. Cooperative prey capture by social societies allows a colony to obtain a larger food source than would be possible to obtain by individuals (Jones et al. 2007, Evans 1977, Guevara and Avilés 2007, Powers and Avilés 2007, Purcell and Avilés 2008). This is also consistent with Wilson's hypothesis that sociality in arthropods evolved first in the tropics, and then evolved in more temperate areas secondarily (Wilson 1975). Like most things in ecology, however, it is a delicate balance of costs and benefits in sociality, which might explain why there is such great variation both between and within species.

Sociality in Spiders

The science behind the evolution of sociality in non-eusocial social arthropods such as spiders raises many questions. How do different degrees of sociality arise, and why do some species show social behavior while congeners may not? Sociality in spiders, for example, is extremely rare, occurring in only approximately 23 of over 39,000 described spider species, but it has evolved repeatedly and is found across multiple families (Agnarsson et al. 2006, Avilés et al. 2007, Avilés and Harwood 2012). Within the spider family Theridiidae, social behavior is known from 11-12 species (Agnarsson et al. 2006). Based on current phylogenetic hypotheses, it appears that sociality has evolved approximately six times within the genus *Anelosimus* (Theridiidae) alone but only two pairs of sister species (*A. lorenzo* and *A. rupununi*; *A. domingo* and *A. dubiosus*) share social behavior (Agnarsson et al. 2006). There are potential benefits of sociality for spiders, including the ability to capture larger prey, reduced predation on individuals, and reduced individual silk costs (Agnarsson et al. 2006, Gonzaga and

Vasconcellos-Neto 2002, Guevara et al. 2011). However, there are also potential costs and risks inherent in social spider groups, including reduced individual fecundity in larger colonies and inbreeding depression (Jones and Parker 2000, Tietjen 1986, Agnarsson et al. 2013).

To be considered “social” under the definition provided by Agnarsson et al. (2006), colony members must remain in the natal web past reproductive maturity. The nests of social spiders usually contain multiple adult females and the progeny of multiple adult females (Avilés and Harwood 2012, Furey 1998). Typically, dispersal in social societies occurs only after a colony has reached a relatively large size (Powers and Avilés 2003, 2007). As a result of multiple generations breeding and remaining in the web, social spider populations tend to be strongly inbred (Agnarsson et al. 2006). Also, social spiders tend to show highly skewed sex ratios, with sometimes 10 times more females than males in the webs (Agnarsson et al. 2006, Avilés and Gelsey 1998). Social spiders are found in diverse habitats, but tend to be concentrated in tropical regions of the world (Agnarsson et al. 2006, Avilés et al. 2007).

Sociality is, however, a continuum, ranging from eusocial to social to subsocial to asocial (Avilés and Harwood 2012). As such, the term “subsocial” is an abstract word without a clear definition, though it generally refers to any form of parental care (Costa 2006). Powers and Avilés have suggested that subsocial can refer to non-territorial periodic-social colonies (2007). In subsocial species of spiders, females typically establish their nests as solitary individuals and raise their offspring without the aid of others. This differs from social species in which females typically establish their nests with other female individuals and may participate in some level of cooperative brood care

(Powers and Avilés 2007). Although it can be difficult to define the terms “social” and “subsocial,” considering it as a gradient or a continuum can alleviate some of the need for discrete categorical descriptions.

Kin-Recognition and Communication in Spiders

Communication between members is important in social societies, so we would predict some level of communication to occur within social and subsocial spider colonies. Such communication may be the result of vibrations, pheromones, or other behaviors. Somehow, the spiders have to be able to establish themselves as family members so as to show “peer tolerance” among siblings and “offspring tolerance” between mother and offspring (Agnarsson 2002). This is especially important in these generalist predators, to reduce uncertainty about others’ intentions. Early research showed that the web is the key to communication, and specific vibrations transmitted through the web give spiders very specific information regarding intraspecific recognition and mating possibilities (West 1979). Currently, relatively little is known about spider pheromones and their possible role in social communication (Agnarsson 2002, Roland 1983). However, studies have shown that spiders will often cue on the movements of other spiders, appearing to follow drag lines deposited on a substrate (Furey 1998). It is unclear if this behavior is the result of a response to silk that could then lead to aggregation, or if the silk mediates a more complex system of communication (Jeanson et al. 2004, Mailleux et al. 2008).

It is possible that collective-decision making is simply an amplified aggregation process as a result of multiple silk trails deposited in the same direction. Previous studies have shown there to be chemical signals in silk. At the very least sex pheromones used in communication between males and females have been isolated from silk (Roland 1983,

Tietjen 1978). Spider silk also appears to have some function that contributes to group cohesion (Roland 1983, Tietjen 1978, Evans 1999). The further question is, then, is this communication kin-specific? Natural selection predicts it would be advantageous for some degree of kin-specific recognition within families if kin selection is at work within a colony. However, some social spiders, including *A. eximius*, show a tendency to accept unrelated individuals into their family-groups without any form of discrimination (Pasquet et al. 1997, Evans 1999). This could be due to contribution of unrelated spiders to web building increasing the fitness of the family, so there could be a benefit to accepting unrelated immigrants into the nest (Evans 1999). Further, there is evidence of some degree of kin recognition among siblings, especially while they are young and the risk of being separated from a social unit would be high (Evans 1999).

An abundance of literature suggests that silk threads play a major role in communication between individual spiders (Evans 1999, West 1979, Roland 1983, Saffre et al. 1999, Saffre and Deneubourg 2000). There is evidence that signals that help individuals recognize kin groups may include pheromones incorporated into the silk of the mother that are recognizable by the offspring. Previous experiments have found that the presence of dragline silk can influence collective decision making in colony spiders, and a binary choice experiment showed that groups of spiders generally selected the same nest site with 80% making the same choice (Saffre et al. 1999). Jeanson et al. (2004) found that in a collective displacement study, spiders would either travel up a cotton thread or a silk shortcut laid down by a previous spider, but they almost all ended up on the same side of a binary choice experiment. In social spiders, attraction of kin to their natal web is often compared to pheromone trails laid by ant colonies (Trabalon and Assi-

Bessekon 2008). This evidence suggests that spiders may use pheromones or some other aspect of silk production as a mode of communication and to aid in aggregation and further that this communication may be kin-specific (Furey 1998).

Anelosimus eximius

A well-studied member of the genus *Anelosimus* is *Anelosimus eximius*, which exhibits more characteristics of social behavior than *A. studiosus*, and is therefore classified as a social theridiid (Agnarsson et al. 2006). *A. eximius* also displays the skewed sex ratio associated with sociality, showing a sex ratio of 10 females to 1 male. This skewed sex ratio is thought to possibly be the result of group selection due to the increased success of a colony's survival due to a preponderance of females (Avilés 1986). This relies on the fact that females tend to be the "limiting sex" to reproduction so having more females in a colony will contribute more individuals to the colony than having an equal number of females and males. *A. eximius* is a neotropical species, living in colonies often in excess of a thousand individuals in a nest (Vollrath 1986, Agnarsson 2012).

As previously stated, sociality is more commonly seen in tropical or warm climate species. Multiple studies have found that insects in the lowland rainforests where social species within *Anelosimus* occur are significantly larger than insect species at higher elevations where subsocial congeners occur (Avilés et al. 2007, Guevara 2007, Yip et al. 2008, Purcell and Avilés 2008). This suggests that sociality in *Anelosimus* could confer an evolutionary advantage to social species because cooperative prey capture allows access to prey items otherwise inaccessible due to their size (Avilés et al. 2007, Guevara and Avilés 2007, Powers and Avilés 2007, Yip et al. 2008, Jones and Parker 2008). All the members of the *A. eximius* colony, from the third instar onward, contribute to the

maintenance and construction of the web, prey capture, and colony maintenance (Vollrath 1986).

Another hypothesis explaining why *A. eximius* is more social than *A. studiosus* is that *A. eximius* and other social species of *Anelosimus* may already exhibit high levels of inbreeding. If so, the presence of multiple generations and siblings could help buffer the possible negative effects of inbreeding depression (Agnarsson et al. 2006). There are thought to be two alternative pathways to permanent sociality in spiders: from a subsocial precursor or from a parasocial precursor. A subsocial precursor involves group living due to extended maternal care, and a parasocial precursor results in social living derived from aggregations around a resource (Whitehouse and Lubin 2005, Avilés and Gelsey 1998). Evidence from other *Anelosimus* species suggests that evolution of sociality in the genus has evolved as a gradual transition over time from short-term maternal care (subsocial) to permanent sociality, so it is likely that *A. eximius* also evolved from a subsocial precursor (Agnarsson 2012).

Anelosimus studiosus

Anelosimus studiosus Hentz, the study species in this investigation, is considered a subsocial species because they generally are found with a natal web containing a colony of up to 50 spiders (Brach 1977, Agnarsson et al. 2006). Unlike a social species, however, the progeny of an *A. studiosus* female generally disperse prior to mating. Although the spiderlings disperse before maturity, they do contribute some amount to the natal web (Agnarsson et al. 2006). That contribution can be through silk production, web maintenance, and food capture. Additionally, *A. studiosus* has been shown to produce an even number of males and females, not exhibiting the skewed sex ratio of the more social

congeners (Avilés and Maddison 1991). There is a degree of variation in the level of sociality in *Anelosimus studiosus*, however, and occasionally colonies are found with multiple mature females within one extended web structure (Agnarsson et al. 2006, Duncan et al. 2010). Additionally, such variation in sociality appears to follow a latitudinal gradient (Riechert and Jones 2008, Pruitt et al. 2008, Duncan et al. 2010). A study by Pruitt et al. (2008) showed that latitudinal behavioral polymorphisms are apparent, with more “asocial” behaviors present at higher latitudes than at lower latitudes in North America. Since sociality can be an evolutionary way to colonize a particular ecological niche that would otherwise be inaccessible, social spiders are able, through cooperative prey capture, to obtain prey items otherwise inaccessible to solitary individuals (Yip et al. 2008).

This experiment was designed to examine some unanswered questions specific to *Anelosimus studiosus*. For example, assuming an evolutionary advantage through kin selection keeping mother and offspring together in the natal web, is there evidence of communication between adult and spiderlings that could function to keep kin groups together? If evidence of communication is found between adults and spiderlings, is it kin specific? And, does silk appear to play a role as the media of this communication?

Hypotheses

1. Kin spiderlings choosing the same refuge as their mother in an induced choice experiment (cf non-kin spiderling groups) provides evidence of kin-specific communication.

2. Changes in the choice patterns of spiderlings following inhibition of the mothers' spinnerets may provide evidence that pheromones transmitted via silk are the mode of communication.

METHODS AND MATERIALS

Study Taxon and Maintenance of the Study Populations

Anelosimus studiosus is a temperate colony web-building theridiid. A relatively common spider with a distribution throughout the southeastern United States (Brach 1977), it is found in relatively high abundance on and near the campus of Western Carolina University (Jackson County, North Carolina). Colonies are often found near water, although on the campus of Western Carolina University the colonies seem to occur most commonly on low landscape bushes and do not seem localized to available water. Colonies occur in webs approximately 60x60 mm and contain up to 50 spiders. Generally, these webs consist of a mother and her offspring, although there is the possibility of unrelated immigrants existing within the web (Brach 1977). Generally the spiders found in a single web are a mother and her offspring from one reproductive event (Levi 1963). For use in this study, 20 colonies of *A studiosus* were collected from the campus of Western Carolina University. Ten of these colonies were collected from a cluster of landscape box bushes (*Buxus* sp.) from one site, and 10 were collected from a row of yew bushes (*Taxus* sp.) at a second, nearby site. Colonies were collected between 28 June and 1 July 2011. In order to maximize the number of spiderlings collected from the web, a Ziploc bag was first placed around the site of the web and then the branch was clipped from behind, thereby enclosing the entire web and all of the spiders inside (based on Pruitt et al. 2008). Some webs were collected that contained only a mother and egg sac that was allowed to hatch in the lab. All egg sacs collected hatched within five days of being in the lab.

The colonies were placed into individual 10 x 10 x 7.5 cm Tupperware containers with screening on top secured by two large rubber bands. The foliage was clipped down to approximately the last 6 cm to serve as substrate upon which the spiders could be maintained. Colonies were offered food twice weekly, either pinhead crickets or fruit flies. The amount of food offered increased as the spiders grew, but was kept consistent between colonies to maintain uniformity. Once a week, the area of the laboratory where the spiders were kept was lightly misted with distilled water to provide some water but not enough to encourage mold growth. The spiders, even as adults, grow only to a maximum size of 5mm, so they are very difficult to handle, especially as spiderlings. As a result, great care was taken to handle both mother and offspring with extreme care to prevent accidentally injuring or killing individual spiders. Spiders were handled as little as possible and usually moved by means of a fine paintbrush when necessary.

Binary Choice Experimental Design

Y-branch experiments are a traditional binary-choice experimental design (Saffre et al. 1999). In this case, the idea was to determine if there is evidence of a chemical communication system that might be kin specific. In this experiment, the Y-branch was made out of 10-ply white cotton string. From the bottom, the structure was 10 cm to the branch and then 15 cm to the refuge, making a total direct travel distance of 25 cm. The Y-branch was set-up with the bottom of the branch emerging from a plastic platform surrounded by water. The water is used to discourage the spiders from attempting escape off the platform and to force them to travel up the apparatus. At the end of each branch (referred to as Left and Right throughout the experiment) was a refuge. The refuge consisted of an opaque plastic cup that was easily sterilized and yet provided an attractive

shelter for the spiders. The entire Y-branch was suspended via an apparatus constructed with PVC piping. This binary choice design was used in both experiment one and experiment two.

Experiment One

Experiment one was designed to test for a difference in choice-making behaviors between kin groups and non-kin groups. Colonies were chosen randomly for the tests, within the constraints of whether it was a kin or a non-kin test. When kin tests were performed, only one colony was used with mother and offspring coming from the same colony. For a non-kin test two colonies were chosen; the adult female from the first colony was used with the offspring from the second colony. In both cases, the experiment was conducted in the same way. First, the adult female was carefully placed, with the assistance of a paintbrush, on the plastic platform and allowed to travel up one of the arms of the Y-branch experimental set-up, choosing one of the refuges (scored as Left or Right). Then, 5 offspring (either kin or non-kin) were carefully placed directly on the string very close to the plastic platform and allowed to travel up the same Y-branch. Each individual was recorded (in order) as choosing Left or Right. Spiderlings were placed on the string instead of the platform was so that they would immediately come in contact with the dragline left behind by the adult. Also, the very small spiderlings were likely to get lost or wander around on the plastic platform and perhaps never find their way to the Y-branch, so placing them directly on the string increased the possibility that they would complete the binary choice. Both adult and offspring were scored as choosing either left or right, thus providing data that could be recorded as the number or proportion of

spiderlings that chose the same side as the adult, regardless of which side was actually chosen.

Experiment Two

Experiment two was designed to test for a difference in the choice-making behavior of spiderlings when silk was not available as a possible cue. It began when the spiderlings began to molt into the fourth instar. At this point the following modifications were made: five mothers out of the ten collected from each site were randomly selected and their spinnerets were cauterized to inhibit their ability to produce silk. The cauterization was performed by first sedating the mother spider by placing it in a closed vial in a 4 °C refrigerator for 1 minute. This was found to slow the spiders down sufficiently but was not enough to kill them. The anesthetized spider was removed and placed in a small petri dish under a dissecting microscope. A small pin was held in the flame of a Bunsen burner for 20 seconds and then carefully touched against the spinnerets of the mother spider. When the spider began moving again, it was allowed to move around the petri dish for approximately 5 minutes, until its behavior seemed normal. It was then removed and the petri dish was misted with water to improve visibility of silk and examined for evidence of silk trails. If no silk trails were found, the spinnerets were assumed to be non-functional. The 10 remaining mothers remained unaltered to serve as controls.

After 10 of the mothers were cauterized, the experiment was repeated as before. However, this time mothers were only tested against their own kin. Therefore, the two groups tested consisted only of kin groups in which the mother was unable to produce

silk and kin groups in which the mother had intact spinnerets. The other procedures remained the same.

The data were analyzed using the software “R.” A Fisher’s exact test for independence with a 2x2 contingency table was used to test for a difference in the observed and expected values for how the first spiderlings in each replicate chose their refuges. Single-sample t-tests were used to see if spiderling decision making was significantly different from random assortment for each treatment group. Independent sample t-tests allowed to test for a significant difference in the proportion of spiderlings choosing the same side as the adult between treatment groups.

RESULTS

Experiment One

Each adult spider and each spiderling were scored as choosing either the left or right side, or as making no choice. The order in which choices were made was also recorded. Spiders designated as “no choice” were those that had not chosen a refuge at the end of a thirty minute period. This was a more common event when the spiderlings were younger. In the first two instars, the spiderlings were very tiny and on occasion would not move at all from where they were placed on the Y-branch, or would only move a short distance up and down. As the spiderlings molted and matured they became more decisive and it was a rare event for a spider not to have chosen a side by the end of the thirty-minute time period.

First, the data were analyzed by looking at the choice made by the first spiderling in each trial. The first spider to travel up the apparatus would receive less of an effect from the behavior or silk output of its fellow spiderlings. Of 80 trials for each treatment group, 63 of the first spiderlings in the kin treatment group chose the same refuge as their mother. In the non-kin treatment group, only 48 of the first spiderlings chose the same refuge as the adult spider (Figure 1). These data were analyzed using a Fisher’s Exact Test and the difference is significant ($p=0.016$) suggesting that, using this criterion, a kin effect does exist and provides evidence that supports hypothesis one.

To analyze the choices of all the spiderlings involved in the trials, the data were blocked by colony to account for any genetic difference in the behavior of individual families. The results of each trial were combined by family and transformed into a

proportion. After performing an arcsine transformation on the proportion to account for non-normalcy of proportions, a 1-sided independent samples t-test was run on the data. This test also showed a significant difference between the kin and non-kin treatment groups ($p < 0.01$). The back-transformed mean proportion of spiderlings choosing the same side as the adult for the kin treatment group was 0.79 and for the non-kin treatment group it was 0.49.

In addition, one sample t-tests were performed on the data to test if the true mean of the proportion of spiderlings choosing the same side as the adult was significantly different than 0.5, which would be expected by random assortment. Random assortment would be likely to occur if the spiderlings were not receiving or attending to any communicative signals from their mother but were, instead, choosing a refuge randomly. The results fail to reject the null hypothesis for the non-kin groups with a p-value of 0.98, but reject the null hypothesis for the kin groups with a p-value < 0.01 , thereby accepting the alternative hypothesis that the true mean is not equal to 0.5. This suggests that the spiders in the mixed groups were randomly selecting refuges without making their choice based on any communication. The spiders in the kin-groups, however, do not appear to be assorting randomly but are aggregating at a level that suggests they are acting on a signal of some sort.

Table 1. Results from two one-sample t-tests for experiment one. The null hypothesis was set as the true mean being equal to 0.5, as would be expected by random assortment. Kin groups behaved significantly different than expected by random assortment but non-kin groups did not.

Treatment Groups	t-value	Df	p-value
Kin	6.14	19	<0.01
Non-kin	-0.016	19	0.98

Experiment Two

Experiment two was designed to explore the way spiderlings followed a mother with her spinnerets inhibited and whether that was different from the way spiderlings followed a mother with normally functioning spinnerets. The purpose was to determine if spider assortment or aggregation is influenced by a component found in the silk of the mother. If there is some influence of silk on the decision making of the spiderlings, we would expect to see a significant difference between the aggregation behavior of spiderlings following a mother with intact spinnerets and the behavior of spiderlings following a mother with spinnerets inhibited.

Looking at the way the first spiderling chose, there is a significant difference when exposed to a mother with intact spinnerets and a mother with inhibited spinnerets. Of the 25 trials where the mother had intact spinnerets, the first spider chose the same refuge as the mother 18 times. In the 25 trials involving mothers with inhibited spinnerets the first spider choose the same side as the adult only 10 times (Figure 2). In the Fisher's exact test this is a significant difference between altered and unaltered treatment groups ($p=0.045$).

As in experiment one, the choice data for all the spiderlings were combined and blocked by groups, then transformed so a t-test could be run. This two sample t-test did not show significance ($p\text{-value}=0.1796$), so there does not appear to be a difference in the proportion of spiderlings that chose the same side as the silk producing adult than those that chose the same side as the adult with spinnerets inhibited. The back-transformed mean proportion of spiderlings choosing the adult in the silk treatment group was 0.68 and in the no silk treatment group was 0.56.

Again, a 1-sample t-test was performed on the data for both treatment groups to test for significant differences from random assortment. The results fail to reject the null hypothesis for the no silk treatment groups ($p=0.334$), but rejects the null hypothesis for the silk treatment group with a p-value of 0.033, thereby accepting the alternative hypothesis that the true mean is not equal to 0.5. This suggests that the spiders in the no silk treatment group could be randomly selecting refuges without basing their choice on any communication.

Table 2. Results from two one-sample t-tests for experiment two. The null hypothesis was set as the true mean being equal to 0.5, as would be expected by random assortment. Silk groups behaved significantly different than expected by random assortment but the no silk groups did not.

Treatment Group	t-value	Df	p-value
Silk	2.574	8	0.033
No Silk	1.0205	9	0.334

In both experiments there was a chance that the order spiderlings traveled up the apparatus had an effect on their decision making. Saffre et al. (2000) found that collective decision making in young *Anelosimus eximius* was very effective, with over 80% of spiderlings generally making the same choice. This was without the benefit of a signal from a mother's silk but simply offspring aggregating with one another, with some effect of silk of fellow spiderlings. Saffre et al. (1999) also discussed the process of "dragline mediated amplification" in decision making. Like ants on a trail, draglines of silk left by each individual as they travel up the apparatus could amplify the effect so that the last spider to make a choice has evidence of the choices of each spider that went before them to influence their choice. Since only 5 spiderlings were used in each test, any amplified dragline effect would be relatively small, but it was still necessary to test for a detectable effect.

To do this, differences in the average choice of spiders at each position were recorded (1-5, depending on how they made their choice). The data were examined to see if spiderlings later in the order were more likely to choose the same side as the spider in front of them. If there was an amplification effect, we would expect to see that the later spiders would be more likely to choose the same side as the spider in front of them because of multiple silk trails for them to follow. There appears to be no difference in how the spiders chose based on their position (Figure 3), which suggests that spiderling choice was independent of their order in the experimental protocol. Either the spiderlings were not cuing on the silk strands of their siblings while making a decision or the effect was not strong enough with only five spiderlings to be an issue.

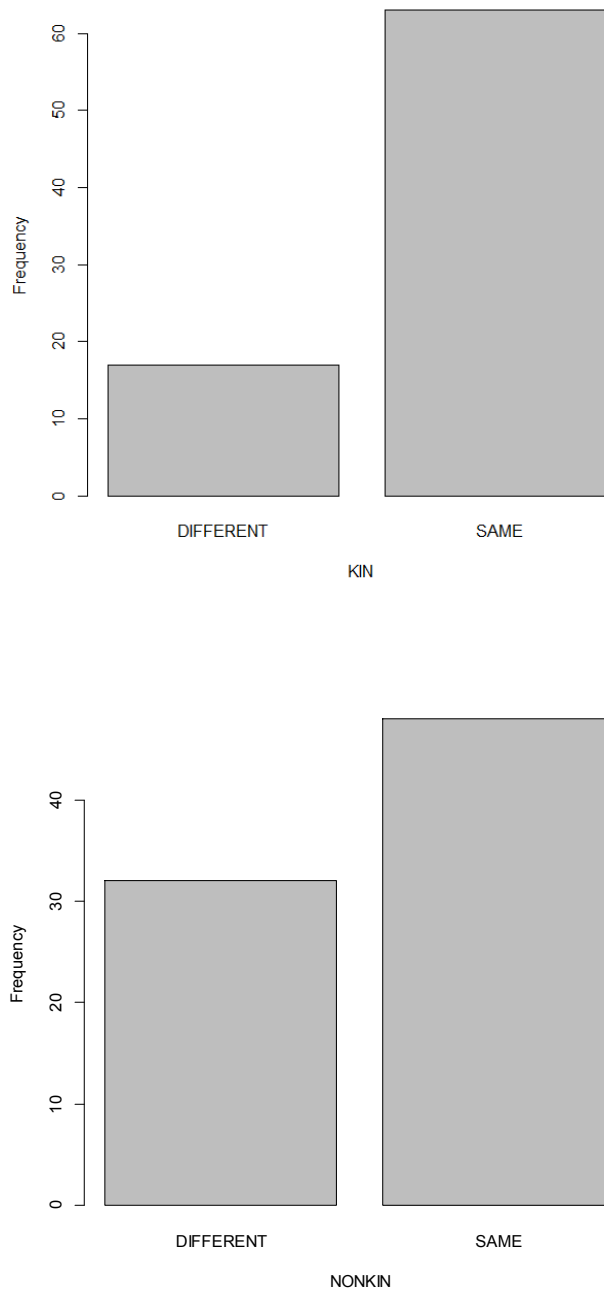


Figure 1. Choice of the first spiderling following the adult in the kin vs. non-kin treatment groups. There was a significant difference between these two treatment groups ($p=0.016$). “Same” represents the first spiderlings that chose the same refuge as the adult (Kin =63, Non-kin= 48), and “Different” represents the first spiderlings that chose the different refuge as the adult (Kin =17, Non-kin=32).

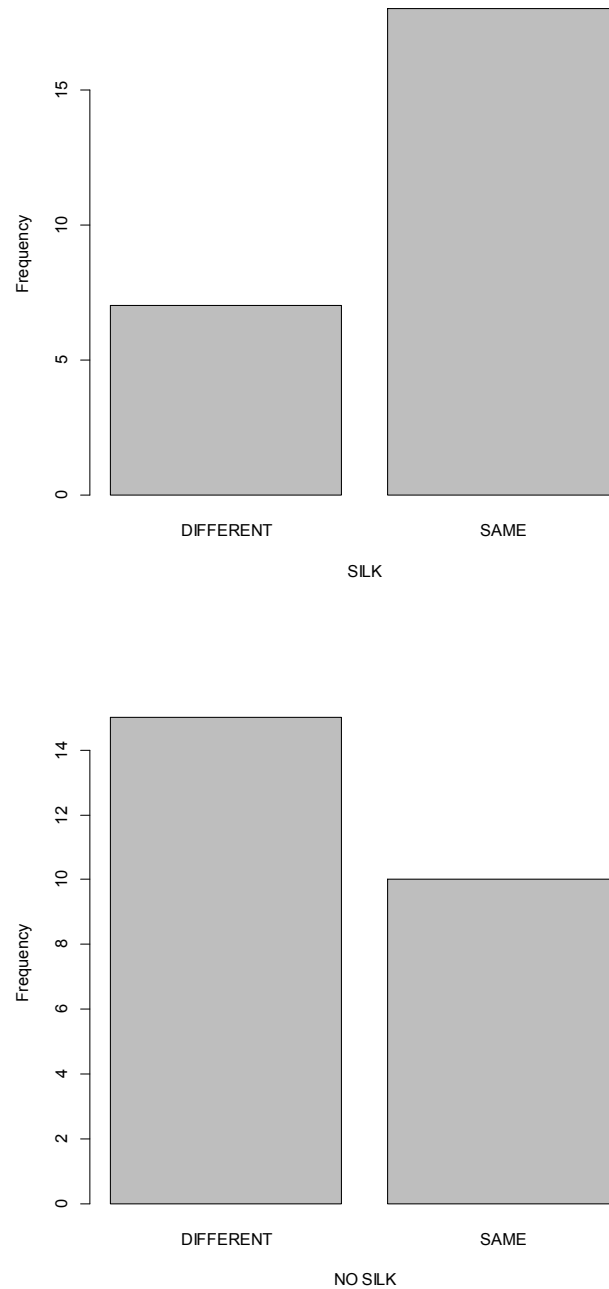


Figure 2. Choice of the first spiderling following the adult in the silk vs. no silk treatment groups. There was a significant difference between these two treatment groups ($p=0.045$). “Same” represents the first spiderlings choosing the same refuge as the adult (Silk = 18, No Silk = 10), and “Different” represents the first spiderlings that chose the different refuge as the adult (Silk = 7, No Silk = 15).

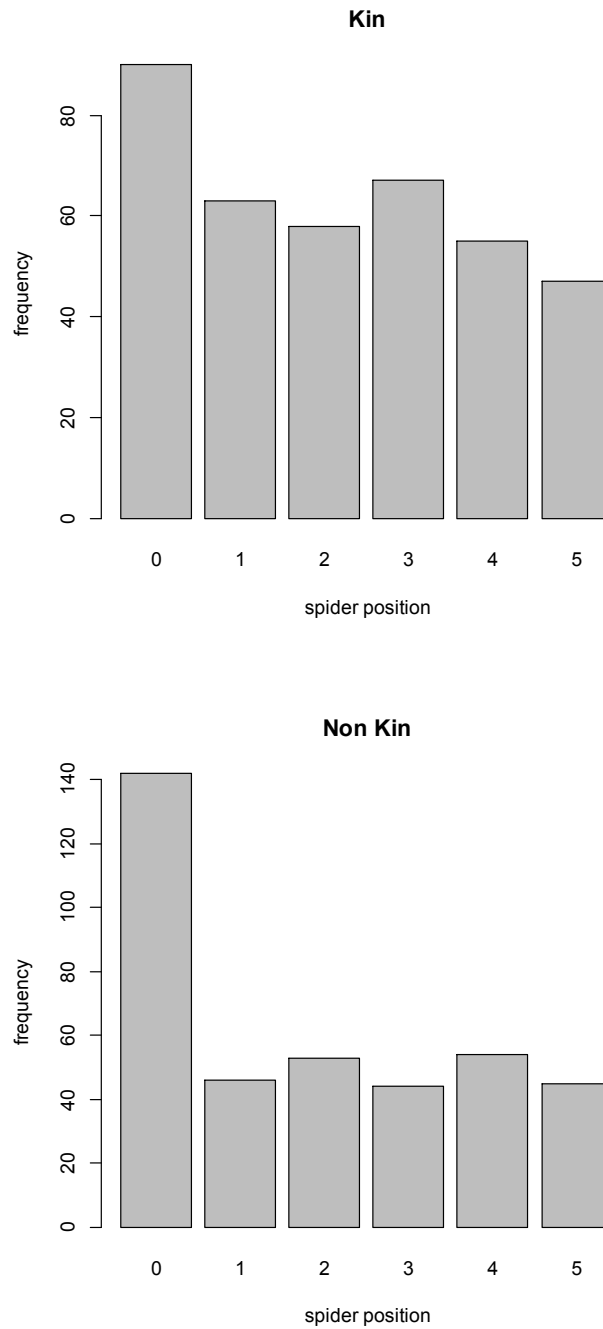


Figure 3. A graphical representation of the test to detect an effect of spider position (order) on behavior. The frequency represents the number of spiders in each position (1-5) that chose the same side as the spider directly before them. There is no noticeable difference in the results between spider position, suggesting there is no amplification or order effect. The “0” bar represents the spiders that chose a side different than the spider in front of them.

DISCUSSION

Sociality in spiders is rare, as might be predicted in consummate sit and wait predators, yet it has evolved independently multiple times (Agnarsson et al. 2006). As a result, understanding the processes that produce social behaviors, such as kin-specific communication and family-based aggregation, can aid in understanding how social spider species have evolved in clades of traditionally asocial taxa. The aim of this study was to provide evidence of kin-specific recognition and communication in the sub-social spider *A. studiosus* using a binary choice behavioral test. Additionally, this study was designed to determine if the silk plays a role in communication between mother and offspring. The results were largely successful in lending support to the hypothesis that there is a communicative mechanism in the silk of the mother to which spiderlings are able to recognize and respond, and moreover that this communication is kin-specific.

Experiment one investigated if spiderlings would choose the same refuge as their mother more often than unrelated spiderlings, thus providing evidence, or not, of kin recognition. The results of the first experiment show that spiderlings follow their mother more often than they follow an unrelated spider, thus supporting the first hypothesis. This suggests some degree of kin recognition, such that spiderlings are able to distinguish between their mother and an unrelated spider. However, such kin recognition was imperfect with spiderlings often choosing a different refuge than their mother.

Multiple studies have shown that spiders use silk draglines to assist in aggregation (Saffre et al. 1999, Saffre et al. 2000). This experiment adds another level, however. These results suggest that following silk is not indiscriminate but instead relies on some

communicative signal that offspring receive from their mother. However, the “random choice” seen when spiders followed an unrelated adult female is, in retrospect, a puzzling result. Roland (1983) found that spiders will almost always follow threads, no matter who laid down the dragline. However, when presented with multiple options spiders tended to follow the thread deposited by the most closely related spider. For example, if presented with a thread deposited by a conspecific and a thread deposited by a non-conspecific, they were more likely to follow the thread of the conspecific. If only presented with a thread of a non-conspecific, however, they were more likely to follow that than not follow a thread at all. Based on these results, the “random choice” seen when spiderlings made a choice when following a non-kin mother is perplexing because it seems they should be more likely to follow a dragline than not. The possibility exists, however, that in that non-kin trials the dragline of the fellow spiderlings provided additional dragline options for subsequent spiderlings to follow.

The results of experiment two lend support to the second hypothesis, that the communication is mediated by silk. When the mother’s silk was inhibited, the spiderlings no longer seemed to receive the signal. The first spiderlings following these altered mothers appeared to choose their refuge randomly. However, with multiple spiderlings in each trial there was not a significant difference between the treatment groups. This may be because the spiderlings, in the absence of silk from an adult, are responding more to silk draglines left by their fellow spiderlings.

Experiment two would have had less meaning in isolation, because all it truly tells us is that the spiders tend to follow silk draglines, a behavior that has been shown many times and one not unique to social spiders. However, when looked at in context of

experiment one, there was a significant difference in the way kin groups made a choice after the silk was removed. In fact, kin groups appeared to act like non-kin groups when silk was removed. It is likely that it is not simply the silk dragline that is cuing movement of the spiderlings but instead that there is a pheromone associated with the silk the mother is producing.

Previous studies have shown that spiders are capable of incorporating pheromones into their silk for reproductive purposes and to aid in aggregation. For example, Roland (1983) found that the silk of adult females of the spider *Stegodyphus sarasinorum* Karsch (1891), a social species, provides a signal that stimulates the aggregation of conspecifics of the same sex, and that this aggregation behavior is not observed when the silk is removed. Further, a bioassay of the dragline of female lycosid spiders indicated the presence of a sex pheromone that was associated with the dragline and not released from the integument (Tietjen 1978). Thus, it is not unreasonable to assume the possibility of a pheromone associated with the silk dragline of adult female *Anelosimus studiosus* that would be recognized by their offspring and assist in keeping family units together.

Regarding the larger picture, this study provides some insight into the evolution of aspects of behavior of a sub-social spider. As was discussed in the introduction, evolutionary theory predicts sociality should appear in societies where the benefit of living amongst others outweighs the individual fitness costs. In this particular subsocial species, there is little evidence of the necessity to live in natal webs for increased prey capture. Typically the habitat in which *A. studiosus* is found provides sufficient amounts of small prey and there is little evidence that the young contribute very much to prey capture and web maintenance, at least in their first few instars. Instead, it is likely that

what most reinforces social behavior is increased survivorship of the young due to defense from predation by a group.

Jones and Parker (2002) found that the delayed dispersal in *A. studiosus* benefits juveniles while they are in the natal web. They also found that having juveniles in the web also benefits the mother's future reproductive success, due to juveniles helping some with prey capture and web maintenance as they reach maturity (Jones and Parker 2000). This means that there would be an evolutionary advantage not only to group living but to maintaining the group as a family unit, requiring communicative signals that would allow spiders to recognize their kin group and reject unrelated spiders.

Establishing a method of communication between mother and offspring is very important to the maintenance of a cohesive family unit. Without a method of kin-specific communication, maintaining a cohesive family unit in areas with a high density of conspecific webs would be extremely difficult. Insofar as kin selection may promote the evolution of sociality in spiders, it is important that individuals be able to distinguish kin from non-kin in order to determine how to treat fellow spiders in their habitat. Responding to pheromones in the mother's silk and being able to distinguish between their own mother and an unrelated mother would provide family group cohesion even in areas where there are multiple natal family webs within a small area.

Interestingly, however, studies have shown that *Anelosimus* species and other social spider species do not always reject unrelated spiders in their webs. Perkins et al. (2007) found that foreign spider species were frequently observed in or near the nests of *A. studiosus*. These spiders were identified to be either kleptoparasites that feed on prey items in the webs of *A. studiosus* or commensals that build their webs within the same

space (Perkins et al. 2007). Due to the “ricochet effect,” where prey may ricochet off of one web and into a nearby web of a different spider, there could be increased fitness for spiders living near one another even if their neighbors are unrelated spiders. This phenomenon is often referred to as the “selfish herd,” where individuals benefit from living in a group even amongst unrelated neighbors (Agnarsson 2002, Perkins et al. 2007, Uetz 1989). Still, kin selection could be at work and present an additional fitness benefit to group-living spiders, and kin selection requires kin-specific communication or recognition.

There are a couple puzzling results and perhaps some errors in experimental design that could have affected the results of the experiment. For example, in the presence of silk, even if not from a related spider, one would expect to see aggregation rates greater than 50% (Figure 1). In a similar Y-branch experiment with *Anelosimus eximius*, the more social congener of *A. studiosus*, Saffre et al. (2000) found the collective decision of spiderlings to be very easily reached, with over 80% of spiders selecting the same nest site. This was without any input from a mother spider but simply spiderlings choosing refuges on their own within a group. Since spiders can use draglines as cues for aggregation, if each spiderling is laying a dragline as it makes a decision, we would still expect to see higher levels of aggregation in the groups of spiders even if they were following a non-kin mother. One possible explanation is that only five spiderlings were used in each test. The experiment may yield different results if more than 5 spiderlings are used in each trial. This was a conscious choice to run the experiment in this way, in order to keep the trials consistent (some colonies only had 15 spiderlings) and because there was a relatively high chance of mortality in spiderlings during handling for trials.

However, future experiments would be well served to attempt to use more spiderlings in each trial. Another option would be to run more tests using only one adult and one spiderling in each test.

Another unanswered question is whether the behavior of the spiders changes over time. Since sub-social spiders are only periodically social, the spiderlings leave the web around or before the time they reach reproductive maturity (Jones et al. 2007). As a result, there may be a difference in their level of sociality as they mature and approach the time at which they would be leaving the natal web. Even among non-social spiders, there is generally some amount of “peer tolerance” among recently eclosed juvenile spiders and “offspring tolerance” which reduces predation on offspring or the egg sac by adult spiders (Agnarsson 2002). In non-social spiders this peer tolerance lasts only for a brief period of time while the young disperse; in sub-social spider systems peer tolerance lasts for an extended period of time until the spiderlings begin to approach reproductive maturity (Agnarsson 2002). Over the course of this experiment, the first part (kin vs. non kin) took place while the spiderlings were in the first three instars. The second part (altered mothers vs. non-altered mothers) took place when the spiderlings were in the fourth and fifth instars. Because of the way the spinnerets were inhibited (cauterization), there was no reversal and the mothers were permanently altered. As a result, the two parts of the experiment could not be run simultaneously on the same spider colonies. However, it would be interesting to see if there is a change in spiderling sociality or antagonism as the spiders approach reproductive maturity.

This investigation, though performed at a small scale on one sub-social study species, has broader implications for helping to understand how social behavior has

evolved in spiders. *A. studiosus* has been considered an important example of a possible intermediate stage of social development in spiders, and as a good example of a broad range of sociality (Brach 1977). Determining the ability of spiders to distinguish their kin from unrelated spiders could help understand how important kin selection might be in the evolution of social behavior in spiders vs. how important group selection (increased individual fitness as a result of living in a group of spiders, unaffected by relatedness) is in the evolution of these behaviors. If kin selection is more important, then selecting for a method of kin recognition and discrimination would be critical from an evolutionary standpoint. The evidence provided from this research suggests that since spiderlings have a mechanism for recognizing their mother due to a component in the silk, that evolution favors the ability to stay together as a family unit and that kin selection is important in the evolution of sociality in spiders.

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