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Kin Recognition at the Spiderling Level in the Common House Spider, Parasteatoda Tepidariorum (Araneae, Theridiidae)

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KIN RECOGNITION AT THE SPIDERLING LEVEL IN THE COMMON HOUSE SPIDER, *PARASTEATODA TEPIDARIORUM* (ARANEAE, THERIDIIDAE)

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Title: Kin Recognition at the Spiderling Level in the Common House Spider, Parasteatoda Tepidariorum (Araneae, Theridiidae)

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The theory of kin selection, formalized by W.D. Hamilton in 1963, provides an explanation for the occurrence of altruistic behaviors within a population and has since been a subject of intense study and investigation. Using kin recognition, the differential treatment of conspecifics with regard to relatedness, scientists have begun probing populations for the presence of kin selection.

In this study, I investigated kin recognition in *Parasteatoda tepidariorum* (C.L. Koch). By pairing related and non-related individuals, and measuring the frequency of cannibalism between treatments, I have found that this population does not exhibit behaviors associated with kin recognition; cannibalism is equally frequent between siblings and non-siblings. While kin recognition does not appear to be present, an alternative avoidance strategy is; *P. tepidariorum* spiderlings appear to delay aggression and disperse before predatory instincts initiate. In this way, siblings avoid cannibalizing each other by removing themselves from the natal web.

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CHAPTER I: INTRODUCTION

Evolution describes how an organism enhances its ability to survive and reproduce in a given environment. To accomplish this task, an organism must change or adapt to its environment. Adaptation is by no means an accident, rather it is a process driven by a natural selection. This process of selection has many forms, one of which is kin selection, the focus of this particular study. Kin selection can be described as the evolutionary process through which organisms acquire altruistic and or nepotistic adaptations (Agrawal 2001). This theory of kin selection is heavily reliant on inclusive fitness, that is, the sum of indirect and direct fitness. Together these concepts can be utilized to explain how altruism and or nepotism may evolve within a population.

There have been many studies that have employed the theory of kin selection to explain altruistic interactions within a population. These studies have utilized kin recognition, i.e. the differential treatment of individuals of the same species (conspecifics) with respect to kinship (Sherman and Holmes 1985), to identify kin selection in a population. A number of different model systems have been used to study kin selection: mammals, birds, amphibians, insects, arachnids, etc. For this particular study, I investigated kin selection in the common house spider, *Parasteatoda tepidariorum* (C.L. Koch). Previous studies, using other species of spiders as the model system, have shown that individuals are able to identify and differentiate siblings from non-siblings (Bilde and Lubin 2001, Evans 1999, Roberts et al. 2003). However, many of these studies utilized spiders known for their social behaviors and or terrestrial dispersal. Unlike those species, *P. tepidariorum* has been described as both a solitary and subsocial spider (Rypstra 1986, Common…c2009). Additionally, *P. tepidariorum* solely

disperses aerially, via ballooning (Suter 1999). A review of the literature failed to reveal any previous studies that have investigated kin selection in a spider species exhibiting these behaviors.

The primary objective of this study was to determine if *P. tepidariorum* spiderlings exhibit behaviors associated with kin recognition; i.e. do they discriminate siblings from non-siblings. More specifically, I have tested the hypothesis that sibling pairs exhibit a lower frequency of cannibalism than non-sibling pairs. Also, I have investigated how the amount of time raised apart, i.e. reduced association, affects the rate of cannibalism in this species. I hypothesized that kin recognition might be mediated by association rather than genetic markers. To test this hypothesis I isolated spiderlings for varying lengths of time prior to exposure. Lastly, I have investigated the rate of aerial dispersal for this species. The literature suggests that spiderlings disperse by the 10^{th} day (Valerio 1975), however this literature is dated. As this was a test of previous research, I expected similar results as those previously observed.

CHAPTER II: LITERATURE REVIEW

Evolution & Natural Selection

As a population changes and adapts to an environment, it acquires traits that enable it to survive and grow. Those populations that fail to grow or expand are less suited to the environment and will become extinct as time progresses. This natural interaction between organisms and the environment facilitates the evolution of populations. It transforms and shapes an organisms form and behavior, and creates the buds from which new species bloom in the tree of life.

While the process of evolution has been stirring since life itself began, the actual scientific study of evolution began only in the mid-nineteenth century (Gould 2002). A growing fossil record and observations of the diversity of living species provided the soil from which the theory of evolution grew. Charles Darwin, and the less accredited Alfred Wallace, both independently developed the process that explains evolution (Darwin and Wallace 1858). Charles Darwin, in his book, *On the Origin of Species*, coined the original term that describes this process. Darwin writes, "This preservation of favourable variation and the rejection of injurious variation, I call Natural Selection" (1859). This selective process drives evolution and shapes populations into those that are highly fit. The process of natural selection has many forms: direct selection, indirect selection, group selection, kin selection, runaway selection, and sexual selection are among these. Guided by these avenues of selection, populations are modified and adaptations arise. Of those named, this particular project focuses on kin selection, a form of natural selection acting on relatedness within a population (Hamilton 1963).

Kin Selection

The term kin selection was coined by John Maynard Smith in 1964. Maynard Smith wrote, "By kin selection I mean the evolution of characteristics which favour the survival of close relatives of the affected individual…" (Maynard Smith 1964). More generally, kin selection can be described as an evolutionary process through which organisms acquire altruistic and/or nepotistic adaptations (Agrawal 2001). While Maynard Smith is credited for providing the term that describes this process, W.D. Hamilton first formalized the concept of kin selection. In 1963, Hamilton used mathematics to describe the evolution of altruistic behaviors. Hamilton describes how gene G, a gene that causes some altruistic behavior, increases in a population, compared to gene g, which is null. The frequency of G in the gene-pool will increase if the advantage of G is greater than the personal disadvantage of carrying, possessing, and expressing G. Individuals affected by G must also be a relative of the acting individual, that is, having an increased chance of carrying G, so as to benefit gene G itself (Hamilton 1964). This inequality, formally known as Hamilton's Rule, is expressed as $rB - C > 0$ or $rB > C$, where "r" is the coefficient of relatedness, "B" the reproductive benefit gained by the recipient and "C" the reproductive cost to the individual possessing the particular gene (Hamilton 1964). When $rB < C$, the gene of concern will be selected against. However, if $rB > C$, then the particular gene will be selected for (Pfennig 1997).

Inclusive Fitness

To study kin selection, and its presence in a population, one must first be familiar with the concept of fitness and more specifically, inclusive fitness. Fitness itself is simply an individual's reproductive success; the ability to pass one's genes on to the next generation (Roff 2008). Inclusive fitness however describes an individual's overall

reproductive success and can be broken down into two components, direct fitness and indirect fitness. Direct fitness refers to the fitness that a particular individual gains by reproducing, i.e. through personal reproduction. Indirect fitness refers to the fitness an individual gains by helping non-descendant kin (relatives other than offspring) survive and reproduce (Brown 1987, Roff 2008). In studying kin selection, it is necessary to consider all aspects of an individual's fitness, i.e. their inclusive fitness. Together, fitness, along with kin selection can be utilized to explain how behaviors of altruism and or nepotism may evolve in a population.

Kin Recognition

There have been many studies that have employed the theory of kin selection to explain social interaction, and or nepotism in a population (Salzen and Cornell 1968, Ross and Gamboa 1981, Holmes and Sherman, 1982, Walls and Roudebush 1991, Pfennig 1992, Wagner 1995, Evans 1998, Mateo and Johnston 2000, Bilde and Lubin 2001, Opell 2001, Giraud et al. 2002, Japyassú et al. 2003, Roberts et al. 2003, Smukalla et al. 2008). Much of this research has focused on the mechanisms of kin recognition. Kin recognition occurs when individuals, within a randomly mating population, interact with one another in a non-random way, with respect to kinship (Wade 1980). More generally, kin recognition may be defined as the differential treatment of conspecifics, depending on their degree of genetic relatedness (Sherman and Holmes 1985). This behavior requires that an individual be able to recognize and discriminate kin from non-kin. The literature has identified at least four proximate mechanisms that facilitate kin recognition. These are: (1) spatial distribution (Ross and Gamboa 1981), (2) association (Holmes and Sherman 1982), (3) phenotype matching (Salzen and Cornell 1968, Holmes and Sherman 1982, Mateo and Johnston 2000), and (4) "recognition alleles" (Smukalla et

al. 2008). I will discuss each of these four mechanisms below, using examples from the literature as illustrations. However, while these experiments (often) identify one recognition mechanism in use by the study organism, it is likely that the study organism is actually using a combination of all four recognition mechanisms (as well as other mechanisms still unknown) to identify and discriminate relatives and nonrelatives.

SPATIAL DISTRIBUTION

Spatial distribution, in the context of kin recognition, refers to the location of an individual in a particular space with respect to the location of other individuals sharing that same space. Evolutionarily speaking, these nonrandom interactions may work to reduce inbreeding (relative avoidance), or conversely, offer protection in numbers (relative gathering) (Holmes and Sherman 1982). Ross and Gamboa (1981) investigated the role that kin recognition plays in the spatial distribution of paper wasps (*Polistes metricus*). Female foundresses of this species are known to construct new nests within a few meters of their natal nests. Often, two or more cooperating co-foundresses may found these nests together. On a closer examination of the relationship amongst co-foundresses, it was observed that these individuals were often siblings. The question however remained, were these individuals recognizing siblings (phenotype matching) or was this the result of spatial distribution? Adult females, and their nests, were collected in the field and isolated; females and nests were isolated from other females and other nests. Nests and females were then placed in separate cardboard boxes where they overwintered. Meanwhile, identification and relationship status of females and nests were recorded. Additionally, all overwintering females were reared on the same diet, under the same conditions, and provided the same nest constructing materials. Thus,

phenotypic cues that arise from diet and nest-building materials were variables removed from the experiment. As females came out of their overwintering state, they were released into a controlled laboratory setting. In this setting, females had access to their original nests. Once co-foundresses began to create new nests, observers recorded who was pairing, or co-founding, with whom. Of the 44 associations observed, 41 consisted of related individuals. Therefore, siblings were able to associate with relatives without the phenotypic cues that are derived from diet and nesting materials. These data suggest that the selective pressures of kin selection direct how these wasps distribute themselves in an environment.

ASSOCIATION

Kin recognition through association is the result of direct interaction (Holmes and Sherman 1982). This form of recognition has been uncovered in two species of ground squirrels, the Arctic ground squirrel (*Spermophilus parryii)* and the Belding's ground squirrel (*Spermophilus beldingi).* Holmes and Sherman (1982) used these organisms in their study of kin recognition because females are known to mate with multiple males, which leads to the production of offspring that vary in their degree of relation; pups that inhabit the same nest may be born from the same mother and yet vary in their relation to each other.

In their experiments, pups that were reared together (nestmates) were found to be equally aggressive to each other (only in male-male and male-female pairings). Regardless of relation, full sibling, half siblings or no relation, the aggressive behaviors exhibited by the subjects were equal. However, individuals that were not nestmates, exhibited aggressive behaviors toward one another. Again, this occurred regardless of relation to each other and only in male-male and male-female pairings. This suggests

that pups reared together, regardless of relation, identify and recognize each other as relatives.

PHENOTYPE MATCHING

Phenotype matching is a process that relies on identifying (learning) a particular trait, be it one of your own or one from a relative, and using that trait as the cue to identify unrelated individuals (Holmes and Sherman 1982). Often these traits, or signals, are chemical (Carlin and Hölldobler 1986, Pfennig 1997, Giraud et al. 2002) however there are cases of acoustic or auditory phenotype matching (Waldman 1988) and visual phenotype matching (Salzen and Cornell 1968). In essence, phenotype matching is only limited by the sensory systems used by organisms in their social encounters.

Phenotype matching in which an individual uses itself to create the template used for relative identification, self referent phenotype matching, also goes by another name, 'The Armpit Effect' (Dawkins 1982). Mateo and Johnston (2000) tested 'the armpit effect' in their work with golden hamsters (*Mesocricetus auratus*). In their experiments, researchers reared *M. auratus* individuals with non-relatives (exposure began at birth) and assessed their response to the odors of non-relatives and unfamiliar relatives. A glass plate was rubbed on the flank glands of donors in order to impregnate the glass plate with the donors' odor. Odor impregnated plates were then presented to female *M. auratus* which responded differently to the odors of non-relatives and unfamiliar relatives; females spent more time investigating the odors of unfamiliar relatives compared to the odors of non-relatives. As the subjects had received no postnatal exposure to relatives, it would seem that they were able to use their own scent for phenotypic matching.

Just as organisms use themselves to create a phenotype matching template, organisms may also use the phenotypic traits of known relatives to identify and

discriminate related individuals from non-related individuals (Holmes and Sherman 1982). As previously discussed, Holmes and Sherman (1982), in their work with ground squirrels, were able to show that male-male and male-female nestmate pairings do not discriminate amongst unrelated, partially related, or fully related individuals. Female-female pairings however had different results. In their tests, sister exposures had fewer aggressive interactions than non-sister (nonrelated female) exposures. This suggests that female ground squirrels are able to identify some phenotypic cue exhibited by their female siblings. Therefore, it appears that the ground squirrels use both association and phenotypic matching to identify and distinguish related individuals from nonrelated individuals.

Visual cues are also a means through which organisms identify and distinguish a related individual from a non-related individual. Statements like, "he has his mothers' eyes," or "she has her fathers' smile," are often made by humans verbalizing a visible similarity (relation) between parent and offspring. And while these statements are merely that, they shed light on a real means and mechanism through which people identify and discriminate relatives from non-relatives. This concept was investigated by Salzen and Cornell (1968) in their work with White Leghorn chicks. In their experiments, these scientists reared chicks in environments lacking another individual (isolates) and environments with other chicks dyed a particular color such as red and green (socials). Individual chicks were then placed in an experimental chamber in which they where exposed to three goal boxes containing: 1) three green chicks, 2) three red chicks, and 3) no chicks. Isolate chicks showed no preference in either of the three goal options. Social chicks however showed a strong preference for the goal box that housed individuals dyed

the same color that the test subject was socialized with. To expand on this experiment, two groups of isolated and dyed individuals received one of two additional treatments: they were provided a dish with water or they were not provided a dish with water. The dish with water allowed individuals to see their own reflection. These groups were then tested under conditions similar to those already described. As with the previous experiment, dyed individuals, having access to their own reflection, approached conspecifics that were dyed similarly. Those individuals lacking the water dish showed no specific preference. These results demonstrate how visual cues are used to identify and distinguish familiar or similar looking individuals from unfamiliar, non-similar individuals.

RECOGNITION ALLELES

Kin recognition via "recognition alleles" is a concept that was first discussed by Hamilton in 1964. Richard Dawkins however popularized this concept in 1976, referring to it as the Green Beard Altruism Effect, or simply the Green Beard Effect. The Green Beard Effect demonstrates how a particular gene, when expressed, produces some trait or cue in the carrier, a green beard for example. Other individuals who carry and express the associated gene, recognize this trait, or cue. Individuals expressing this gene are therefore able to identify each other as relatives, i.e. carriers of the green beard gene (Dawkins 1976). While this appears very similar to phenotype matching, there exists a very important and defining difference, that is, the recognition cue is not learned; rather it is (hypothetically) innate (Sherman and Holmes 1982). In the scope of kin recognition, The Green Beard Effect illustrates a special and likely rare case where the phenotypic recognition signal is unlearned. While this particular behavior may be rare, there is an organism that exhibits a 'green beard'. *Saccharomyces cerevisiae* is a budding yeast

which exhibits aggregation phenotypes, one example being flocculation. Flocculation, a self-adherence phenotype, is a behavior that few laboratory *S. cerevisiaecan* strains display. However, activation of the FLO1 gene in laboratory strains can restore the cells' flocculation capabilities. Two strains of *S. cerevisiaecan* were used in an experiment performed by Smukalla et al. (2008), a non-flocculent lab strain (S288C) and its flocculating feral ancestors. FLO1 genes present in S288C were activated and these cells were exposed to the flocculating feral ancestor strain. Upon exposure, these two strains formed a floc, a concentrated aggregation of cells. Prior exposures, where the FLO1 gene was inactivated, resulted in an oil and water reaction; flocculating cells preferentially aggregated together forming a floc, while nonflocculating cells exhibited no aggregation. Therefore, it appears that cells expressing this gene (FLO1) preferentially stick to one another regardless of genetic relatedness. Thus FLO1 is a true 'green beard' gene directing cooperation toward other cells expressing this gene.

Kin Recognition Research

There has been a great deal of research on kin recognition and its role in kin selection. In one particular study, Walls and Roudebush (1991) used larval salamanders, *Ambystoma opacum*, to investigate kin recognition. After mating, female *A. opacum* will produce numerous eggs that are held together in a gelatinous egg sac. This egg sac usually resides in water or in a terrestrial area that will become aquatic sometime during the year. Once hatched, the larval salamanders seek refuge in the aquatic environment (Kaplan and Salthe 1979). During this point in their life cycle, and under certain selective pressures, *A. opacum* larvae are known to be cannibalistic, feeding on other *A. opacum* larvae (Walls and Roudebush 1991). Walls and Roudebush investigated the

cannibalistic tendencies of the larvae questioning who they were feeding on, kin, non-kin, or both? *Ambystoma opacum* larvae were paired based on relatedness (siblings and non-siblings) and familiarity, that is, were the larvae reared in the same aquarium (neighboring or non-neighboring). In the pairings, Walls and Roudebush found that the larval salamanders showed significantly less aggression towards siblings than towards non-siblings. As for unfamiliar larval salamander pairings (non-neighboring; larvae did not share the same aquarium), less aggressive behaviors were observed between siblings than between non-siblings. These results suggest that the salamander larvae can recognize and discriminate kin from non-kin, regardless of familiarity.

As with *A. opacum*, similar behaviors have been associated with the desert dwelling amphibian, the spadefoot toad (Pfennig 1992). Spadefoot toads, genus *Spea*, lay their eggs in clutches. Upon hatching, the tadpoles are by default non-cannibalistic omnivores, feeding on detritus and plankton. Occasionally, an individual, by chance, may eat another tadpole or a freshwater shrimp. This action triggers changes in the individual's musculature, physical morphology, dietary preference, and behavior. Tadpoles that have eaten another tadpole, or freshwater shrimp, will from that point become primarily carnivorous. These carnivorous or cannibalistic individuals forgo schooling with siblings and preferentially school with non-siblings. When a carnivorous tadpole strikes another tadpole, via a nipping behavior, that tadpole will, by taste, determine whether it's attacking a sibling. If the attacked individual happens to be an unrelated conspecific, the tadpole will eat that individual. However, if the attacked individual tastes like a "sibling", the attacking tadpole will halt its assault. A behavior

such as this suggests that the carnivorous tadpoles use phenotype matching to identify and discriminate related individuals from non-related individuals.

Dobler and Kölliker (2009) investigated kin recognition and siblicide in the European earwig, *Forficula auricularia*. In this species, nestmates are often sired by multiple males and therefore vary in their relation to one another. Additionally, nestmates tend to stay together during their first juvenile instar, approximately 10 days. During this time period, killing of nestmates (siblicide) is not uncommon. With this in mind, researchers paired siblings and non-siblings together with the hypothesis that *F. auricularia* juveniles kill and cannibalize unrelated nestmates earlier and more often than related nestmates. This was exactly what was observed ($p = 0.027$). The results of this experiment suggest that *F. auricularia* juveniles can identify and distinguish siblings from non-siblings.

In another experiment, researchers investigated the recognition ability of 33 different colonies of Argentine ants, *Linepithema humile,* an invasive species throughout Europe (Giraud et al. 2002). This particular species exhibits a social system in which individuals may safely interact with conspecifics from other colonies, multicoloniality. In this way, an individual from colony A may traverse, unharmed, through colony B to colony C. This interaction is referred to as unicoloniality and allows for an increase in the density of worker ants. Aggression tests were conducted between individuals of each of the 33 colonies. The results of these exposures identified two distinct supercolony groups, a Catalonian supercolony and the main super colony. The Catalonia supercolony consisted only of three colonies, while the remaining colonies (30) made up the main supercolony. When individuals from each supercolony were exposed to one another, the

exposure always resulted in severe aggression and the death of one of the two individuals. However, aggression never occurred between individuals from different colonies within the same supercolony. That is, two individuals within the same supercolony, but from two separate colonies 6,000 km apart, did not exhibit aggression toward one another. These data suggest that *L. humile* nestmate recognition is genetically driven.

Related Research Involving Spiders

A number of studies have utilized spiders to examine questions surrounding kin selection. The focus of most of these experiments has been on the parent to egg sac relationship (Opell 2001, Japyassú et al. 2003), the parent to offspring relationship (Bilde and Lubin 2001, Evans 1998, Wagner 1995), and the spiderling to spiderling relationship (Roberts et al. 2003, Bilde and Lubin 2001). Such experiments have also employed species of spiders differing in their social state (social/sub-social and solitary) while developing questions concerning kin selection (Evans 1998, Bilde and Lubin 2001, Opell 2001, Roberts et al. 2003).

Evans (1998) sought to investigate the offspring recognition abilities of maternal spiders. *Diaea ergandros*, a species of crab spiders known to display extreme sacrificial maternal care (nest fortification, guarding and matriphagy), was exposed to either her own offspring or "adopted" spiderlings. Although both groups of spiderlings had equal survival rates, there was a difference in spiderling growth rates between the two treatments. Spiderlings that were exposed to their maternal caregiver (related) were significantly heavier than those spiderlings unrelated to their maternal caregiver; $p < 0.01$. These results suggest that mother *D. ergandros* provided more care, i.e. caught larger prey, for related offspring compared to "adopted" or non-related offspring.

Additionally, these results suggest that maternal spiders are able to identify and discriminate kin from non-kin via some unidentified cue. Furthermore, it demonstrates how extensive maternal care can provide the selective pressures necessary for the development of nepotistic kin recognition.

Bilde and Lubin (2001) also investigated kin recognition abilities in spiders, however, their focus was on the spiderling to spiderling relationship. Their study organism was *Stegodphus lineatus*, a species that exhibits a subsocial lifestyle. Females of this species, similar to *D. ergandros*, also display extensive maternal care, feeding spiderlings actively for approximately two weeks. Following the two week feeding period and two molts, *S. lineatus* spiderlings will begin consuming their mother; matriphagy. Shortly after matriphagy, spiderlings mature to the dispersing stage. During this stage, spiderling siblings will form small groups, often remaining together to hunt and feed. Considering this lifestyle, it would seem highly advantageous for related individuals to recognize one another so as to avoid cannibalizing a sibling. In their experiment, Bilde and Lubin paired *S. lineatus* juveniles with either a sibling, or a conspecific (non-related individual of the same species), of the approximate same age. These pairs were confined to plastic containers. Exposure lasted up to three weeks during which containers were checked for mortalities (acts of cannibalism). Bilde and Lubin found that sibling pairs had a lower frequency of cannibalism than non-sibling pairs; $p < 0.048$. These findings suggest that juvenile *S. lineatus* can recognize and discriminate kin from non-kin and furthermore, that kin selection has a hand in shaping cannibalistic behaviors.

Roberts et al. (2003) investigated kin recognition in the solitary spider species, *Hogna helluo,* a large North American wolf spider. *Hogna helluo* adults were collected and reared under laboratory conditions for the purpose of producing egg sacs. As spiderlings began to emerge from the egg sacs, they were collected. Juveniles, of the approximate same age, were paired following two different procedures: related individual pair or non-related individual pair. Spiderling pairs were held in plastic containers that were checked each day until a mortality was discovered. When one of the two individuals was found dead, the dead individual was removed and examined to determine a cause of death. Overall, the occurrence of cannibalism of *H. helluo* juvenile spiderlings was more frequent in non-sibling pairs than in sibling pairs; $p < 0.02$. These data suggest that the solitary spider, *H. helluo,* is capable of kin recognition and that it displays behaviors resulting from kin selection.

Experimental Summary

For this particular study, I too have focused on kin selection at the spiderling level, with particular attention paid to kin recognition. The spider species I used was *Parasteatoda tepidariorum* (C.L. Koch), generally referred to as the common house spider. This species was formerly identified as *Achaearanea tepidariorumm,* but was transferred to *Parasteatoda tepidariorum* by Yoshida (2008). *Parasteatoda tepidariorum* is a common synanthropic spider, that is, it is highly prevalent in and around human dwellings (Ross 2008). *Parasteatoda tepidariorum* is a web building spider species that produces a tangle web, a loosely constructed web lacking any real pattern. The web itself is primarily utilized for prey capture, conducting vibrations as an organism becomes ensnared in it (Common . . . c2009). This species, whose size ranges

from 4-8 mm in length (Common . . . c2009), is found worldwide and has been reported in North America, South America, Central America, Germany (Common . . . c2009) and Japan (Miyashita 1987b). In southern regions of the United States, such as Florida, this species remains active throughout the year (Common . . . c2009). In those regions where temperatures dramatically decrease during the winter, this species retreats to a safe location where, in diapause, it awaits warm weather (Tanaka 1989). The social status of this organism is still a topic under investigation. The literature has described it as both a solitary species, which does not cohabitate with conspecifics (Rypstra 1986), and also as a sub-social species, whose webs may interweave with other conspecifics (Common . . . c2009). The social interaction of males and females is generally restricted to times of mating. Males may be found at the outskirts of a female's web (Lubin 1986) and will move into the webs interior for mating. Such movements often occur when the female is preoccupied with food, or has just finished molting (Lubin 1986). Once successfully mated, a female in a temperate region can produce on average 4 to 7 egg sacs (Miyashita 1987a), each of which can contain between 140 to 380 eggs (Commonly . . . c2006). When the eggs begin to hatch, the nymphal spiderlings remain within the egg sac until their second instar. During this time, spiderlings are known to feed on non-viable eggs, a behavior exhibited by many spider species (Valerio 1974). As the spiderlings begin to emerge from the egg sac, approximately 15 days later (Valerio 1974), they aggregate into a dense cluster near the egg sac. The spiderlings then begin to construct a communal web within the natal web (Valerio 1975). By day ten, the spiderlings have all dispersed from the web by ballooning (Valerio 1975). As spiderlings prepare for dispersal, they position themselves at a high point in or around their natal web and release a length of silk from

their spinnerets. This silk is also referred to as gossamer (Ubick et al. 2005). Moving air catches the gossamer generating the lift necessary for dispersal. Much like a kite rises in the wind so does the spiderling, traveling with the currents through the atmosphere (Suter 1999).

Choosing *P. tepidariorum* as the spider species for this study was an easy decision. While *P. tepidariorum* is not active year-round in western Pennsylvania, it is quite common and numerous during the spring, summer and fall months. This makes acquiring a large healthy population easy and cost efficient. Also, *P. tepidariorum* is fairly passive as well as harmless. While it is within the same family (Theridiidae) as the venomous Black Widow, a bite from *P. tepidariorum* will do minimal damage (Commonly . . . c2006). Another positive feature that this organism displays is its simple lifestyle. *Parasteatoda tepidariorum* is a fairly stationary species and will construct a web in nearly any container, given a reasonable amount of space. It suspends itself from its web, which it uses to acquire food, produce egg sacs, and mate. Also, since this organism is quite small, a large population can be kept in a small laboratory setting. As for care and maintenance, this organism requires a fairly limited amount. Small insects, juvenile crickets or houseflies for example, provide excellent meals and are easy to acquire or rear. Insects, and a daily mist of water, are really all this species requires. Additionally, *P. tepidariorum* females yield large amounts of offspring throughout their lifetime. This permits an experiment with a large sample size and numerous replicates.

While those features, previously mentioned, were all considered in choosing *P. tepidariorum* as my study organism, there was one trait that carried a lot of weight in the final decision. As noted, the focus of this study is kin recognition at the spiderling level.

Previous studies, with goals and questions similar to mine, utilized species of spiders that primarily disperse terrestrially (Bilde and Lubin 2001, Evans 1999, Roberts et al. 2003). However, in a review of the literature, I was unable to find any similar studies that employed a spider species that disperses primarily through the air (aerially). Ballooning, which is the means of dispersal for *P. tepidariorum,* enables this species to cover large distances in a relatively short period of time. So incredible is this feat that ballooning spiders have even been observed out at sea (Darwin 1909). However, this technique can be fairly unpredictable as wind patterns change in direction and velocity (Suter 1999). With that in mind, the potential of two individuals from the same egg sac dispersing to the same location is probably low (a personal hypothesis). Thus, this dispersal behavior adds an interesting element to the kin recognition question.

Experimental Aims

Much like Roberts et al. (2003) and Dobler and Kölliker (2009), I have exposed my study organisms, *P. tepidariorum*, to siblings and non-siblings, recording the frequency of cannibalistic acts. If kin recognition were present in this species, I would expect to see more cannibalistic occurrences between non-siblings pairs than sibling pairs. Such observations have been recorded with other spider species (Bilde and Lubin 2001, Roberts et al. 2003), thus I expected to see similar results with *P. tepidariorum*.

In addition to exploring the cannibalistic tendencies of *P. tepidariorum*, I have also investigated how association affects the rate of cannibalism in sibling and non-sibling pairs. As previously mentioned, Holmes and Sherman (1982) identified kin recognition through association in ground squirrels; related and non-related pups reared together from birth displayed fewer aggressive behaviors compared to related and non-

related pups reared apart. Therefore, were kin recognition through association present in *P. tepidariorum*, I expected to observe similar responses; as the level of association decreases the incidence of cannibalism in spiderling pairs will increase.

Lastly, while the literature suggests that *P. tepidariorum* disperses by the tenth day, following emergence from the egg sac (Valerio 1975), this information is dated and not specific. Therefore, as a means of testing the literature, I have explored the rate of dispersal of *P. tepidariorum.* As this is a test of previous research, I expect similar findings as those observed by Valerio (1975).

CHAPTER III: MATERIALS AND METHODS

Equipment

Holding Chamber: These chambers were made from colorless 3-liter soda bottles and consisted of two sections, a base and living area (Figure 1). The base consisted of the bottom portion of a 3-liter soda bottle cut in half. Fish tank gravel was placed into the bottom to provide stability. The living area was made by cutting off the bottom portion of a second soda bottle and inverting the larger of the two remaining portions, and placing it into the base. The smaller of the two remaining portions was then used as a lid for the holding chamber. A small circle, with a diameter of 4 cm, was cut into the living area portion of the holding chamber and covered with a patch of window screening or white synthetic chiffon fabric, glued down at its edges. This provided air circulation for each specimen. A cotton ball was placed in the inverted mouth of the living area to prevent specimens from falling into the base. Additionally, the inside of the living area was scored with 3M sand paper, to provide a surface on which spiders could crawl and secure their webs. Holding chambers were also washed with hot soapy water and given unique identifying numbers.

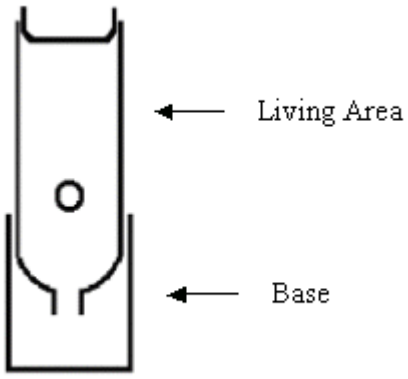

Figure 1 Spider holding chamber: These chambers were made from colorless 3-liter soda bottles and consisted of two sections, a base and living area. This device was used to house adult female spiders. In total, 40 of these chambers were constructed for this experiment.

Experimental Chamber: Fisherbrand, 60 mm x 15mm, sterile plastic petri plates were used to create the experimental chamber (Figure 2). Each petri plate had a 3 mm circular hole punched into its bottom with a heated nail. The hole was positioned in the center of the bottom portion of the petri plate. Each hole was plugged with a 40 mm x 3 mm strand of felt. Each felt strand was threaded through the hole, from outside to inside, with a sterile dissecting pin. The felt "head" rested 5 to 10 mm above the petri plate base while the "legs", laid flat against the outside surface of the bottom of the petri plate. All experimental chambers were placed on the experimental platform (pages 25 and 26), which was draped with felt that was soaked with water. Each of the felt wicks, of the experimental chambers, were primed with water; the "legs" were dipped in water. Having been primed, the wick provided the spiderlings ad libitum access to water throughout the duration of the experiment. Each experimental chamber was given a unique number, 1 – 389, which was written on the lid of the petri plate.

Temporary Experimental Chamber: Temporary experimental chambers were identical to the experimental chambers in every way except that they were labeled differently. The purpose of these labels was to identify the specimens within. In this way, a spiderling arising from the first egg sac of the Armstrong spider, A-04, would be given the label A-04-E-01. Translated, this means that the spiderling emerged from the first egg sac of the Armstrong spider, A-04. Also, these chambers only housed one spiderling at a time, i.e. these chambers housed isolated spiderlings.

Figure 2 Experimental chamber and temporary holding chamber**:** These devices served as the holding apparatus for spiderlings during an experiment or prior to spiderling pairings.

Experimental Platform: The experimental platform consisted of five pieces: a frame, a platform, plastic sheeting, felt strips and twelve plastic containers (Figure 3). The frame was constructed of wood and measured 1.45 x 0.43 x 0.19 meters. The platform, which rested on the frame, was cut from plywood sheeting to measure 1.84 x 0.67 meters. A layer of plastic sheeting was then draped over the platform followed by a layer of felt. The felt was cut into strips that measured approximately 1.05 x 0.3 meters. Prior to the experiment, these strips were soaked with water and draped over the platform so that approximately 0.67 meters hung off both the left and right side of the platform. Twelve plastic dishes, six per side, were then positioned under the left and right sides of the platform. These dishes, which measured 0.32 x 0.18 x 0.09 meters, were filled with tap water. The ends of the felt strips, hanging off the left and right side of the platform, were then placed in the water filled containers. Two experimental platforms were made following these specifications. One was strictly used for the actual experiment, and the other was used to hold the temporary experimental chambers.

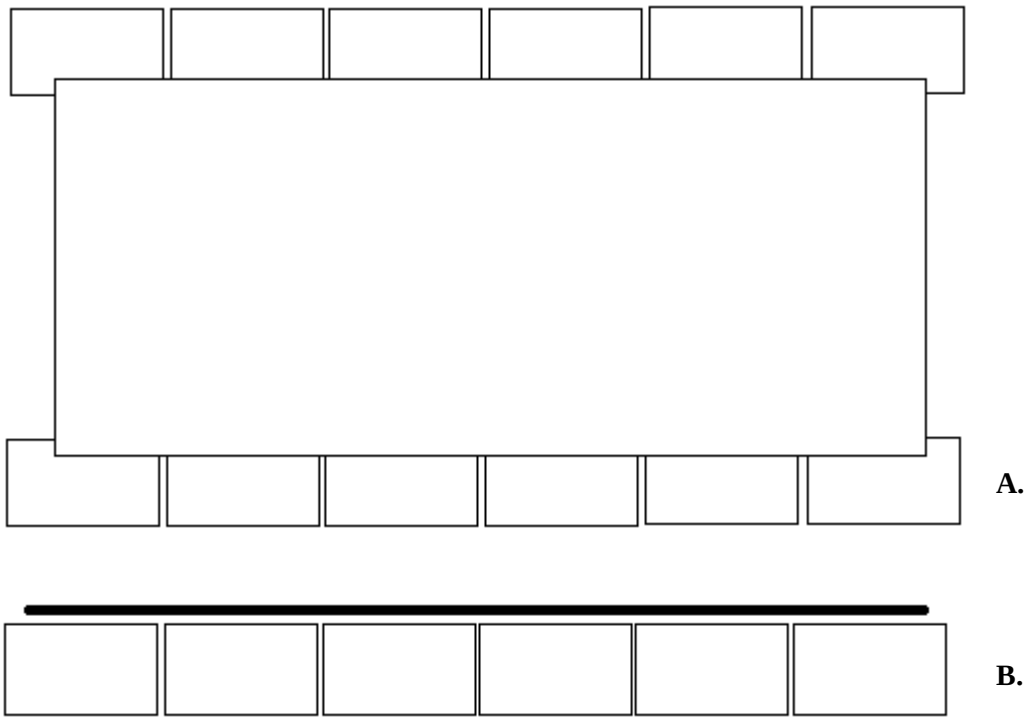

Figure 3 Experimental platform: The experimental platform consisted of five pieces: a frame, a platform, plastic sheeting, felt strips and twelve plastic containers. This structure held the spiderling pairs prior to and during an experiment; the experimental and temporary experimental chambers were placed on this structure (A. overhead view, B. side view).

Thermometer: The EasyLog EL-USB-2 - Humidity, Temperature and Dew Point Data Logger (with LCD) was the instrument that recorded laboratory conditions throughout the duration of this experiment. It was set to automatically take a reading every five minutes. This information was then loaded into an Excel data sheet for analysis and interpretation.

Spider Populations

Adult Spiders: Mature female spiders were collected in June of 2011 from Armstrong and Indiana County (Pennsylvania). Two populations were required for the non-sibling pair treatments; it was assumed that spiderlings emmerging from Armstrong and Indiana egg sacs were not related. A Magellan Meridian Gold Water Resistant Hiking GPS device was used to record all collection locations (Table 1). Up to 20 individuals were collected from each county for a total of 40 mature females. Spiders taken from collection locations were held in the laboratory. In the lab, data were recorded on each individual: date captured, capture location, weight, presence of egg sac(s) and any additional comments. Individuals were then separated and placed into holding chambers (Figure 1). All 40 holding chambers were grouped together in a large terrarium where they were exposed to a specific lighting regime, 14hr light/10hr dark. Three times a week, specimens were fed crickets, purchased from a local pet store. To simplify feedings, crickets were placed in the freezer for two to three minutes and then placed into the specimens' webs. Lightweight forceps were used for this procedure. As crickets began to warm up and move around, their movements would attract the spider. One cricket was provided to each individual during a feeding. Additionally, mature females were provided water, via a spray bottle; one spray per day, seven days a week.

Table 1 GPS spider collection locations: A Magellan Meridian Gold Water Resistant Hiking GPS device was used to record all collection locations of adult spiders.

Spider	Latitude	Longitude
A-01	40:45:2.1 N	79:27:46.44 W
A-02	40:45:2.0 N	79:27:46.44 W
A-03	40:45:2.0 N	79:27:46.44 W
A-04	40:45:2.0 N	79:27:46.44 W
A-05	40:45:2.0 N	79:27:46.44 W
A-06	40:45:1.8 N	79:27:49.1 W
A-07	40:45:1.8 N	79:27:49.1 W
A-08	40:44:49.3 N	79:27:56.9 W
A-09	40:44:49.3 N	79:27:56.9 W
A-10	40:42:58.3 N	79:30:18.6 W
A-11	40:42:58.3 N	79:30:18.6 W
A-12	40:42:58.3 N	79:30:18.6 W
A-13	40:42:58.3 N	79:30:18.6 W
A-14	40:42:58.3 N	79:30:18.6 W
A-15	40:42:58.3 N	79:30:18.6 W
A-17	40:42:58.3 N	79:30:18.6 W
A-18	40:42:58.3 N	79:30:18.6 W
A-19	40:42:58.3 N	79:30:18.6 W
A-20	40:42:58.3 N	79:30:18.6 W
I-01	40:41:19.9 N	79:09:42.0 W
I-02	40:41:19.9 N	79:09:42.0 W
I-03	40:41:19.9 N	79:09:42.0 W
I-04	40:41:19.9 N	79:09:42.0 W
I-05	40:41:23.4 N	79:09:40.1 W
I-06	40:41:23.4 N	79:09:40.1 W
I-07	40:41:23.4 N	79:09:40.1 W
I-08	40:41:22.0 N	79:09:38.2 W
I-09	40:41:22.0 N	79:09:38.2 W
I-10	40:41:22.0 N	79:09:38.2 W
I-11	40:41:22.0 N	79:09:38.2 W
I-12	40:41:22.8 N	79:09”35.3 W
I-13	40:41:22.8 N	79:09”35.3 W
I-14	40:41:22.8 N	79:09”35.3 W
I-15	40:41:22.8 N	79:09:37.6 W
I-16	40:41:22.8 N	79:09:37.6 W
I-17	40:41:23.4 N	79:09:40.1 W
I-18	40:41:23.4 N	79:09:40.1 W
I-19	40:41:23.4 N	79:09:40.1 W
I-20	40:41:23.4 N	79:09:40.1 W

Egg Sacs: Female spiders were observed daily for the production of egg sacs. Two days after an egg sac was produced, it was placed in its own petri dish and provided a unique label indicating its relation with other spiders and spiderlings. For example, the third egg sac taken from the spider I-09 was labeled I-09-E-03. Sterilized dissecting scissors and lightweight forceps were used to carefully free egg sacs from webs. Data on each egg sac were also recorded: parent, date produced, date of first spiderling emergence, days between egg sac production and first spiderling emergence, estimated total number of spiderlings and any additional comments.

Paired Siblings & Paired Non-Siblings Experiment

Experimental Design and Methods: As spiderlings emerged from their egg sacs, 40 individuals were haphazardly collected and isolated from one another in 60 x 15 mm petri plates (temporary holding chambers). Once all spiderlings were collected, they were paired according to one of two treatments, paired siblings or paired non-siblings (see experimental controls below for the spiderling selection procedure). For the paired sibling's treatment, two spiderlings, which emerged from the same egg sac, were haphazardly selected and placed in an experimental chamber. For the paired non-sibling treatment, two spiderlings, one having emerged from an Armstrong egg sac and the other an Indiana egg sac (non-siblings), were haphazardly selected and placed in an experimental chamber. Each experimental chamber was labeled 1 – 389 and was haphazardly selected for each pairing. A synthetic fiber paintbrush, rinsed with alcohol and dried, was carefully used to collect and transport spiderlings to the experimental chamber. In addition to the sibling and non-sibling pairing treatments, spiderlings were also assigned to one of five different age regime treatments: a) 0 days following

emergence from the egg sac (DFE), b) 5 DFE c) 7 DFE d) 10 DFE and e) 20 DFE. In these experiments, spiderlings having previously been assigned to either the sibling treatment or the non-sibling treatment, where then exposed to each other at 0, 5, 7, 10 or 20 DFE. The 0 DFE pairings were placed in the experimental chambers within 24 hours of emergence. Spiderlings in the 5, 7, 10 and 20 DFE treatments were isolated from each other in the temporary experimental chambers until the day of exposure. Following exposure, "spiderling pairs" were checked daily for mortalities. This continued until one individual from the spiderling pair died. When a mortality was discovered, the dead spiderling was inspected with a dissecting microscope to determine the cause of death.

Sample Size: In total, 880 spiderlings were collected from 33 different egg sacs; 17 egg sacs were from Armstrong County and 16 were from Indiana County. See table 2 for a summary of the number of spiderlings collected and paired (sibling vs. non-sibling). In a perfect world, sample sizes would be equal between sibling pairs and the non-sibling pairs, however spiderling mortalities occurring prior to a pairing greatly impacted sample size (table 2, see 20 DFE). Of the total spiderlings collected (880) in this experiment, 352 were either not used in an experimental pair (but served as a control) or died prior to a pairing.

Table 2 Spiderling pairing summary**:** In total, 880 spiderlings were collected from 33 different egg sacs; 17 egg sacs were from Armstrong County and 16 were from Indiana County. Of those collected, only 528 individuals, 264 pairs, were used in this experiment.

	Spiderlings Collected	**N (Siblings vs. Non-Siblings)**
0 DFE	212	52 pairs vs. 54 pairs
5 DFE	60	15 pairs vs. 15 pairs
7 DFE	58	14 pairs vs. 15 pairs
10 DFE	156	38 pairs vs. 40 pairs
20 DFE	42	20 pairs vs. 1 pair
All Levels (Sum)	528	139 pairs vs. 125 pairs

EXPERIMENTAL CONTROLS

Spiderlings: For each egg sac pairing, 40 spiderlings were haphazardly selected and isolated; when an egg sac hatched, 40 spiderlings were collected. Of those 40 spiderlings that were isolated, only 30 were used in the experiments. Those that were not paired remained isolated until they died. These individuals served as controls, or a comparison group, to those spiders (0, 5, 7, 10, and 20 DFE) that were not cannibalized, that is, those that died from starvation or some means other than being cannibalized. In this way, I was able to discriminate a cannibalized spiderling from a non-cannibalized spiderling.

Spiderling Age: Spiderlings that were paired under any pairing treatment, sibling or non-siblings, 0, 5, 7, 10 or 20 DFE, were the same age; where age is defined as the number of days following emergence from an egg sac. This meant that a non-sibling pairing could only take place if the spiderlings being paired emerged from their egg sac on the same day. Although this limited sample size, it ensured that one spiderling would not have a size, weight, and age advantage.

Spiderling Selection: As it was impossible to predict when a particular egg sac would hatch, it was impractical to preselect egg sac pairings. Therefore, egg sac pairings were decided the day a hatch or hatches occurred. Thus, on the occasion that an egg sac, or egg sacs, from only one county hatched, the resulting pairing was a sibling pairing (or pairings). On the occasion that one egg sac from each county hatched, the result was a non-sibling pairing. In the event that multiple Armstrong and Indiana egg sacs hatched, egg sacs were haphazardly assigned to a pairing regime (sibling or non-sibling). In this way, the pairing regime was left to the randomness of egg sac hatching.

Data Collection: As spiderlings were paired according to a particular pairing regime, they were placed into an experimental chamber, which was labeled with a particular number, 1-389. This number was recorded in an Excel spreadsheet (A) along with the exposure type, DFE treatment, the identification of the individual spiderlings involved, and the exposure start date. Later, when a mortality was discovered in an experimental chamber, that experimental chamber was removed from the experimental platform. The entire experimental chamber was then placed under a dissecting microscope for analysis. Prior to cause of death investigation, the date and the experimental chambers' identification number were recorded by hand. The dissecting scope was then utilized to determine the cause of death. Once this was determined, it was also recorded. This data was later entered into an additional Excel spreadsheet (B). Data recorded in this spreadsheet included: the date a mortality was discovered, the experimental chambers identification number, the initial start date, the treatment level, the number of days following emergence from the egg sac, the number of days till a mortality, and the cause of death. As the mortality date and the experimental chambers identification number were inserted into B, the remaining data, excluding cause of death, were pulled from A, and inserted into B. In this way I was unaware of, or blind, to the exposure that the experimental chambers received (paired siblings, paired non-siblings).

Measurement(s): Cause of death was the dependent variable in this experiment. Spiderlings that were alive were very easy to distinguish from those that were not alive, based on their mobility and physique (Figures 4a and 5a). However, when spiderling mortality was observed, there were two possible explanations; the spiderling died "naturally" (1), that is by any means other than being cannibalized, or the spiderling was

cannibalized (2) by the spiderling it was paired with. Differentiating between these two causes of death was imperative for this study and was accomplished using a few key characteristics. A spiderling that died of "natural" causes generally lacked silk fibers and bodily damage, and also had legs that appeared to be stiffened and collapsed in toward the body (Figure 4). Cannibalism was evident from the presence of feeding damage on the body. This often included a deflated abdomen and mangled legs and cephalothorax. Silk fibers were also usually present (Figure 5). I was not able to control for (spiderling) body damages received post mortality; non-cannibalistic mortalities may have been identified as cannibalistic mortalities due to post mortality feeding.

Statistical analysis: Chi-Square tests (Sokal and Rohlf 1995) were performed to determine if the frequency of cannibalism was significantly different between siblings and non-siblings. This was done separately for each DFE level (0,5,7,10 and 20 DFE) and for the entire data set. Chi-Square values were calculated with the critical value set at 3.841 (n = 1, alpha = 0.05). In addition to Chi-Square, the statistics program SPSS, version 19, was used to conduct an analysis of variance (ANOVA) for independence and a Pairwise Comparisons test on the days until the first mortality (DTFM) data sets. Two tailed T-tests for independence were also used to analyze (DTFM) data sets.

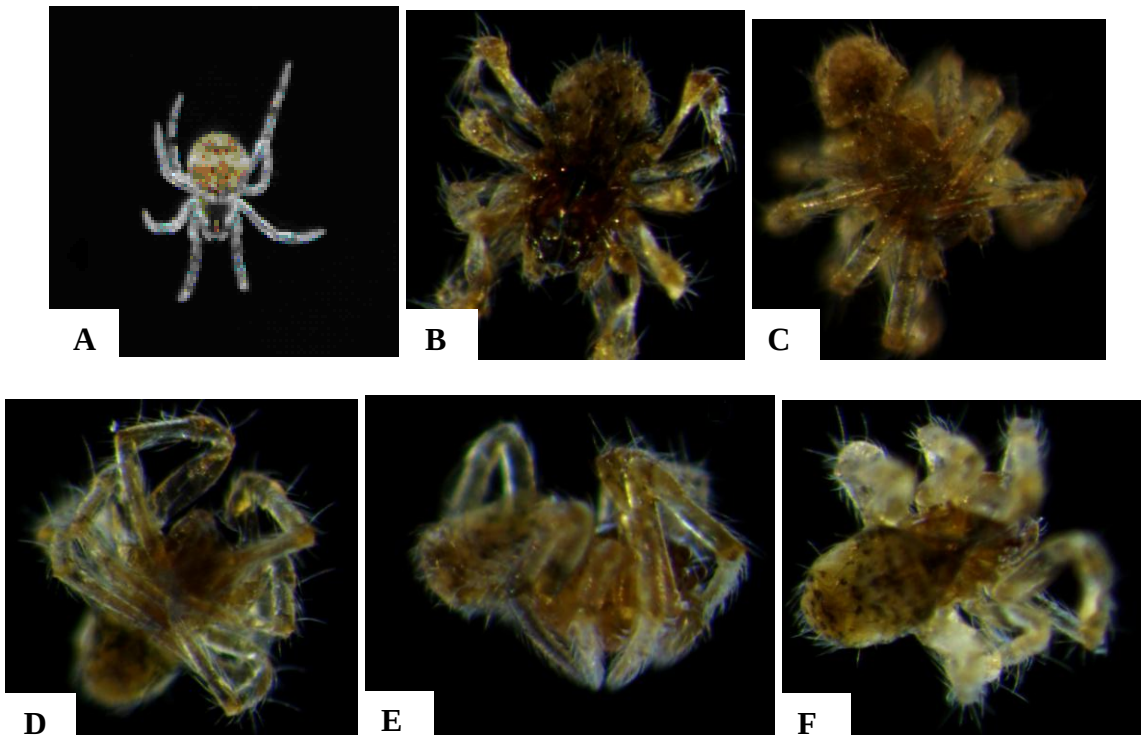

Figure 4 Non-cannibalized spiderling images**:** An Olympus stereo microscope SZX10 paired with an Olympus DP72 high-resolution digital camera was used to capture these images. All images are post mortality with the exception of A, a live specimen (10x). Images B, C, and D, *P. tepidariorum* ventral views (63x). Image E, *P. tepidariorum* lateral view (63x). Image F *P. tepidariorum* dorsal view (63x). Note how the limbs appear folded and collapsed (B, C, D). Additionally, legs and body parts are intact and full.

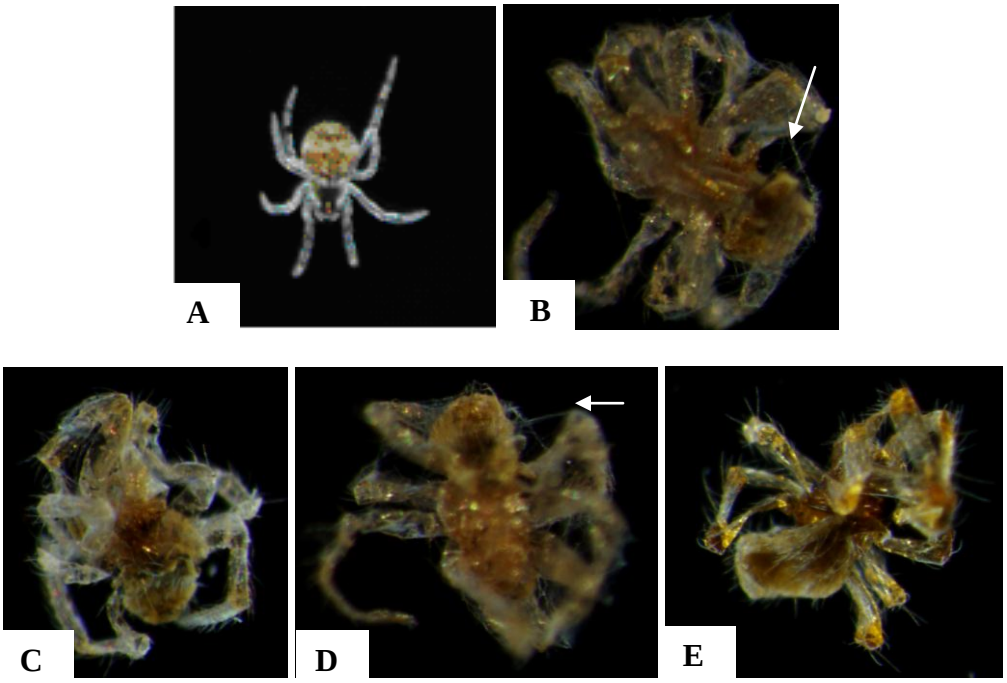

Figure 5 Cannibalized spiderling images**:** An Olympus stereo microscope SZX10 paired with an Olympus DP72 high-resolution digital camera was used to capture these images. All images are post mortality with the exception of A, a live specimen (10x). All views are dorsa (63x)l. Note the appearance of the limbs, mangled, broken, deflated (B, C, D, E). The arrows in B and D identify the presence of silk fibers. Additionally, note the deflated appearance of the abdomen (E).

Spiderling Dispersal Experiment

Experimental Design and Methods: When an egg sac was discovered to have produced spiderlings, the date was recorded and the egg sac, contained within a 60 mm x 15mm plastic petri plate, was placed onto the experimental platform. The lid of the petri plate was then permanently removed and an image was taken of the petri plate and spiderlings within. An additional photograph was taken daily until all the spiderlings were gone (dispersed). A sample of these images may be viewed in Figure 6. A Canon PowerShot A550, 71 megapixels camera, was used to take all pictures. Each image was labeled with the appropriate date. In total, 13 egg sacs were utilized for this experiment.

Measurements: Images were used to estimate the total number of individual spiderlings present at a particular point in time, 0 hours, 24 hours, 48 hours, etc. Each image was copied into the computer program "Paint", which was used to mark each individual spiderling. Each mark was then counted and a total number of spiderlings, for a particular point in time, was determined. Percentages were calculated for each day's total, for each individual egg sac. Additionally, the average daily dispersal was determined and the standard error was calculated.

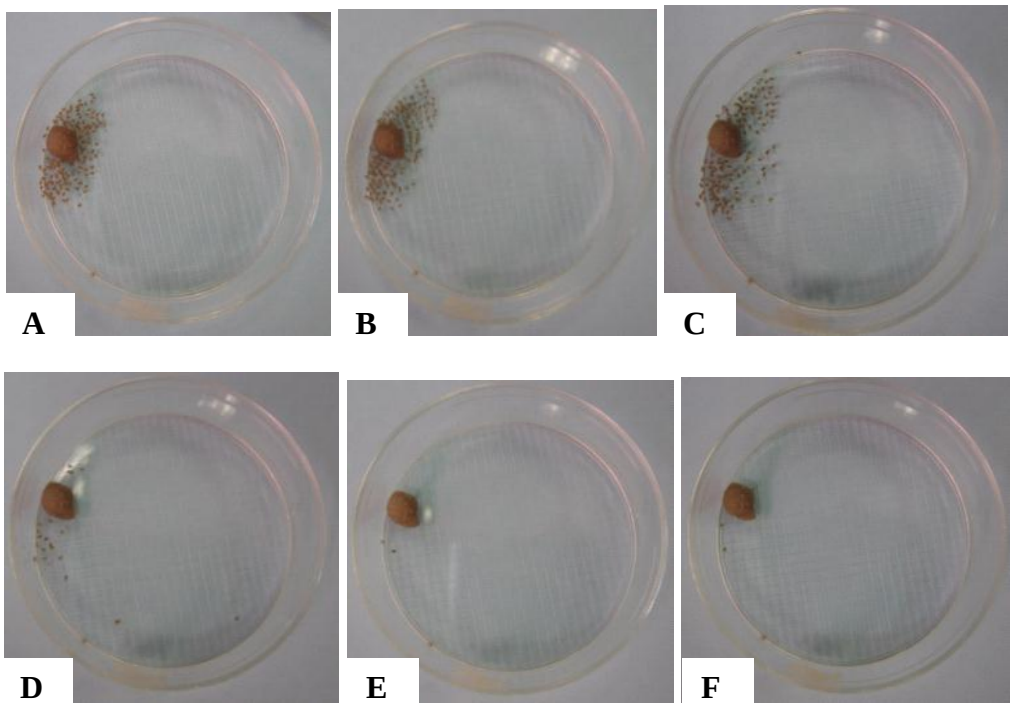

Figure 6 Spiderling dispersal images, I-01-E-05: A Canon PowerShot A550, 71-megapixel camera, was used to take daily images of spiderling clutches. (A) Day one, 131 spiderlings present (97% of the spiderlings have not dispersed). (B) Day 2, 132 spiderlings present (97.8%). (C) Day 3, 135 spiderlings present (100%). (D) Day 4, 19 spiderlings present (14.1%). (E) Day 5, 2 spiderlings present (1.5%). (F) Day 6, 2 spiderlings present (1.5%). The spiderlings within this particular egg sac were completely dispersed by the 7^{th} day, 0 spiderlings present (0%) (Last image not shown). There were no spiderling mortalities observed during this experiment.

CHAPTER IV: RESULTS

Paired Siblings & Paired Non-Siblings Experiment

Cannibalism was the primary cause of mortality in this experiment, accounting for over 80% of all deaths (Figure 7). However, contrary to my hypothesis, the overall occurrence of cannibalism in *P. tepidariorum* spiderlings was the same for sibling and non-sibling pairs, regardless of DFE treatments (All Data, $\chi^2 = 0.379$, $p = 0.153$; Figure 8, Table 3).

In order to identify if the rate of cannibalism changed over time, I determined the daily percentage of cannibalistic mortalities for sibling and non-sibling pairs. At 0 DFE in the sibling pairs, cannibalism first occurred on day 2. For non-sibling pairs, cannibalistic mortalities first appear on the 3rd day. Overall, the distribution of cannibalistic mortalities was similar between sibling and non-sibling pairs (Figure 9). I also identified the cumulative percentage of cannibalistic mortalities between sibling and non-siblings pairs for 0 DFE (Figure 10). The number of cannibalistic mortalities per day appears to increase at the same rate for both sibling and non-sibling pairs. However, between days 6 and 7, this increase was more noticeable.

In addition to 0 DFE, the cumulative percentage of cannibalistic mortalities was also determined for 5, 7 and 10 DFE for both sibling and non-sibling pairs. The 20 DFE data were excluded from this analysis because no (0) cannibalistic mortalities were observed between non-sibling pairs. For the spiderlings paired immediately after emergence from the egg sac (0 DFE, in both siblings and non-siblings) the cumulative cannibalistic mortalities (expressed as a percentage, Figures 11 and 12) initially rose slowly. By day 5, the mortality rate began to increase.

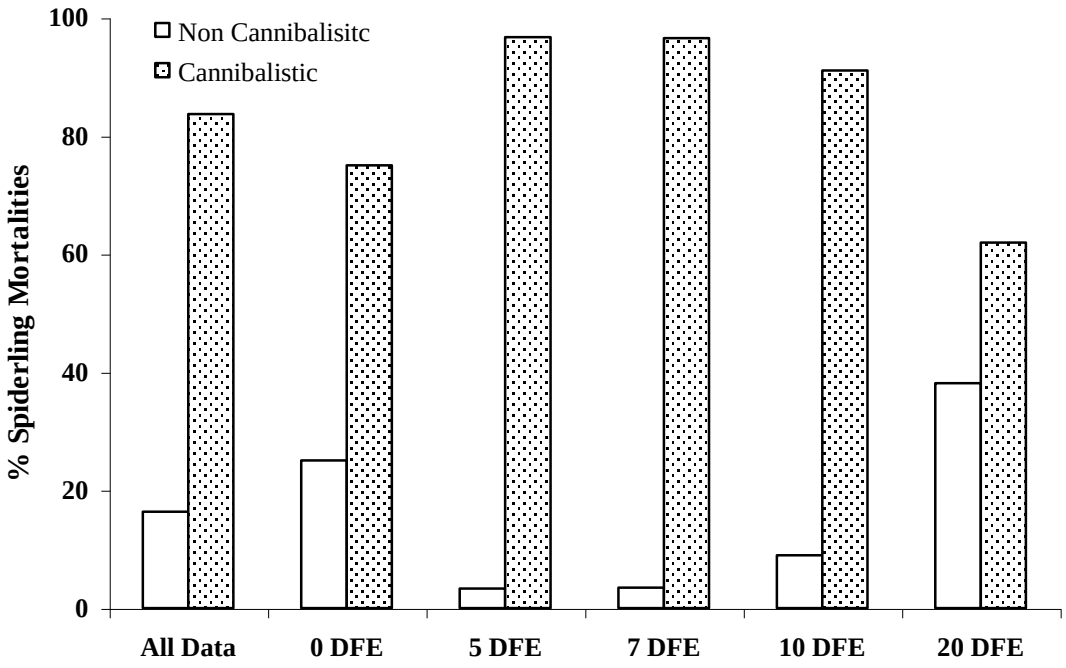

Figure 7 Overall percentages of cannibalistic and non-cannibalistic spiderling mortalities: The open bars represent non-cannibalistic mortalities while the stippled bars represent cannibalistic mortalities. There were more cannibalistic mortalities than non-cannibalistic mortalities for all DFE levels.

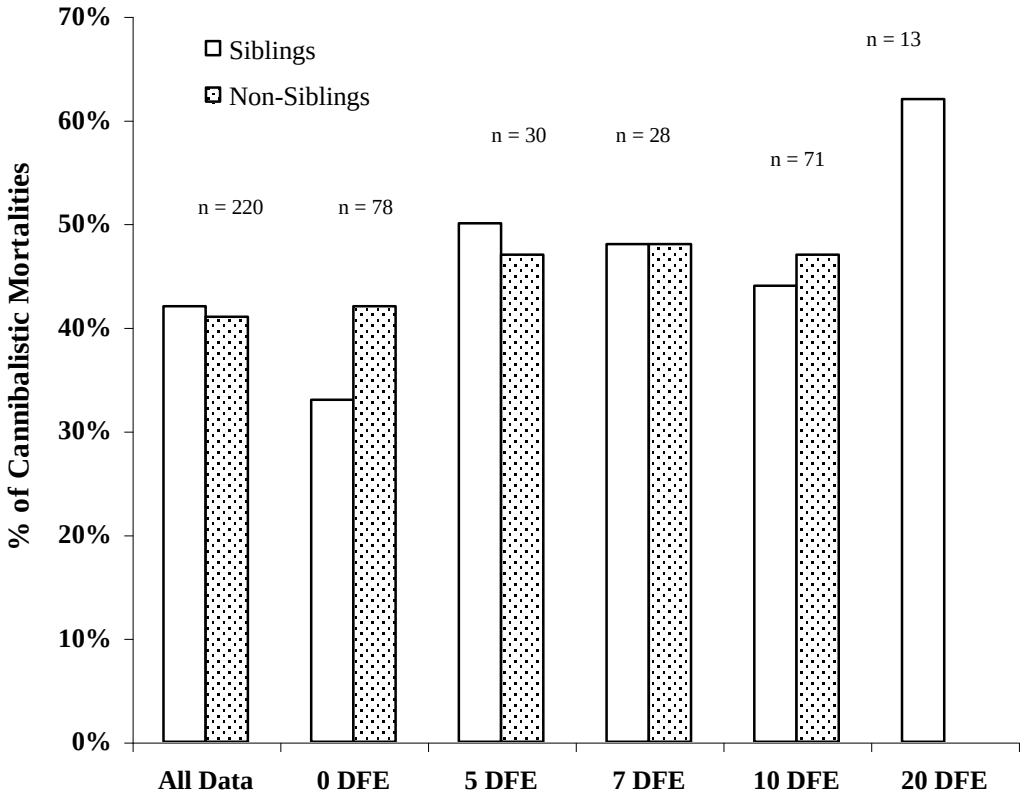

Figure 8 Percentage of cannibalistic mortalities in sibling and non-sibling pairs: The open bars represent sibling pairs while the stippled bars represent non-sibling pairs. Note that no (0) cannibalistic mortalities were observed between non-sibling pairs for the 20 DFE level. A Chi-Square statistic was calculated for each DFE level (Table 3). There were no significant differences between sibling and non-sibling pairs; the occurrence of cannibalism was the same for sibling and non-sibling pairs.

Table 3 The frequency of cannibalistic mortalities for sibling pairs and non-sibling pairs: Pairs were formed at different ages, measured here as days following emergence from the egg sac (DFE). Chi-Square values were calculated with the critical value set at 3.841 (n = 1, alpha = 0.05). P values were also calculated. The occurrence of cannibalism was the same for sibling and non-sibling pairs.

	Sibling Pair Cannibalisms	**Non-Sibling Pair Cannibalisms**	**Chi-Square**	**df**	**P value**
0 DFE	34 (n = 50)	44 (n = 54)	0.628	1	0.143
5 DFE	15 (n = 15)	14 (n = 15)	0.052	1	0.31
7 DFE	14 (n = 14)	14 (n = 15)	0.034	1	0.326
10 DFE	34 (n = 38)	37 (n = 40)	0.0133	1	0.640
20 DFE	13 (n = 20)	0 (n = 1)	1.031	1	0.191
All Data	111 (n =138)	109 (n = 125)	0.379	1	0.153

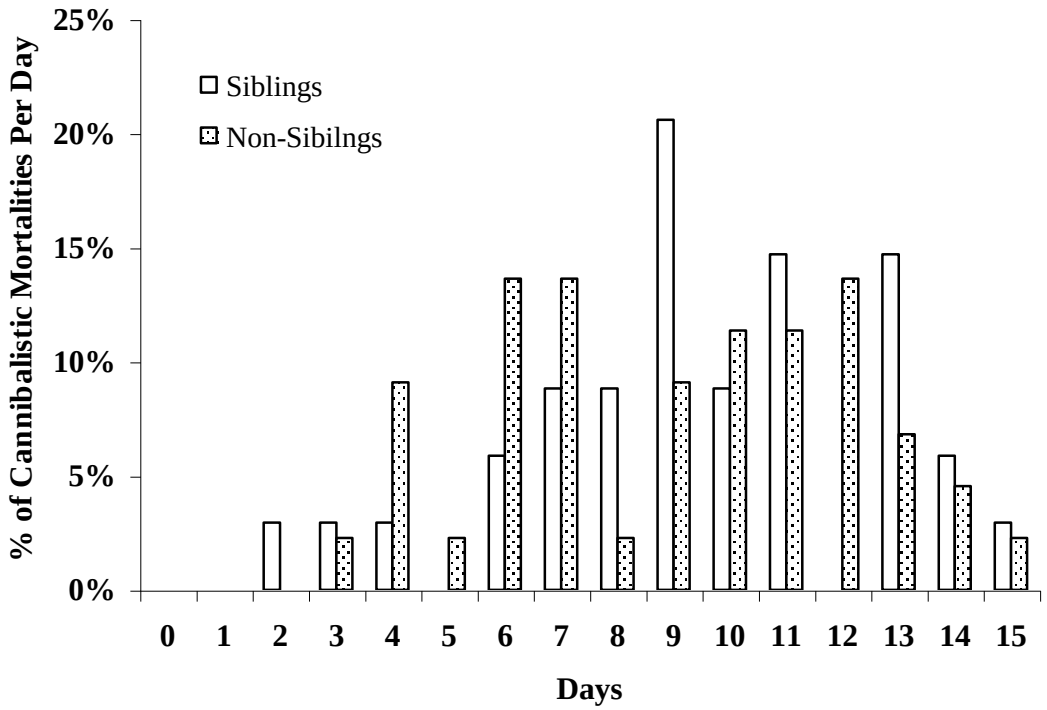

Figure 9 Percentage of cannibalistic mortalities per day between sibling and non-sibling pairs (0 DFE): The open bars represent sibling pairs while the stippled bars represent non-sibling pairs. In sibling pairs, cannibalistic mortalities are not observed until the 2nd day. As for non-sibling pairs, cannibalistic mortalities appear on the 3rd day. The overall distribution of cannibalistic mortalities appears to be the same between sibling and non-sibling pairs.

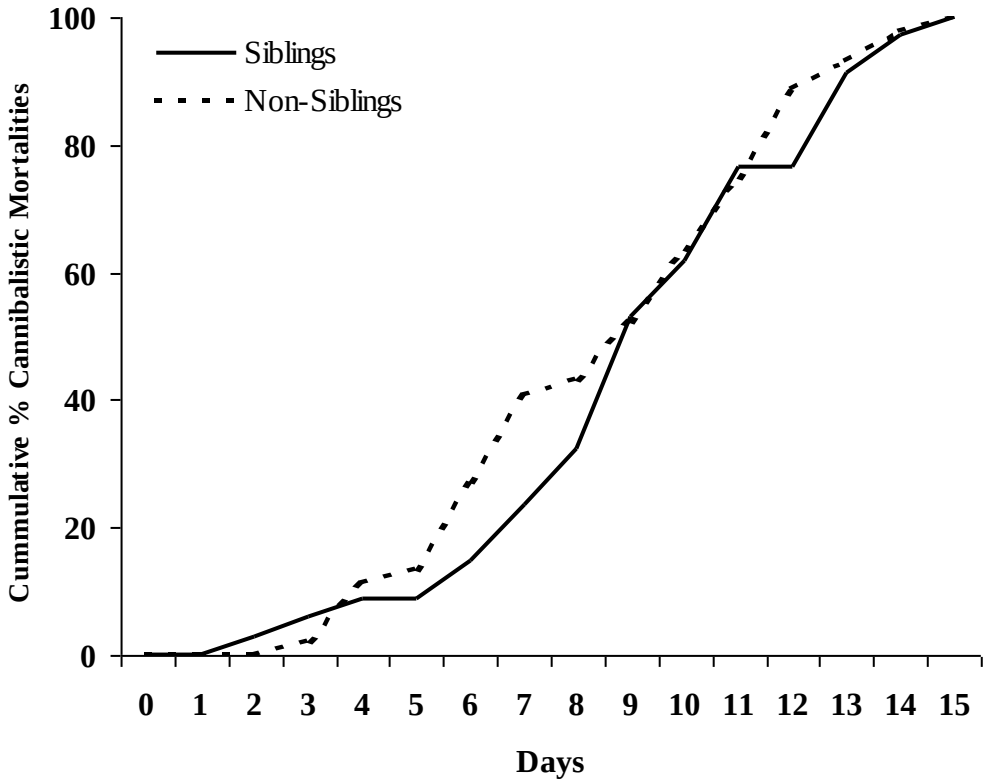

Figure 10 Cumulative percent of cannibalistic mortalities per day between sibling and non-sibling pairs (0 DFE): The solid lines represent sibling pairs while the dashed lines represent non-sibling pairs. The number of cannibalistic mortalities per day appears to increase at the same rate for both sibling and non-sibling pairs. Although non-sibling pairs exhibit a higher percentage of cannibalistic mortalities on days 4, 5, 6, and 7, sibling pairs appear to begin cannibalizing sooner, and at a greater percentage (days 2 and 3). Overall, the rate of cannibalistic mortalities for both treatments appears to gradually increase. However, between days 6 and 7, that increase is more noticeable.

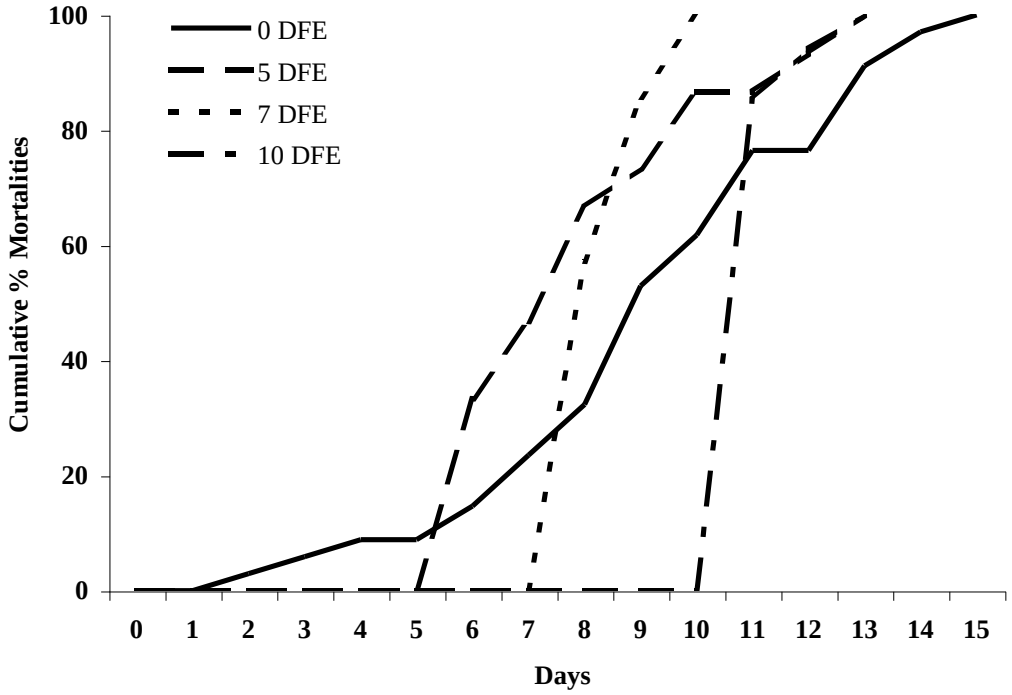

Figure 11 Cumulative percent of cannibalistic mortalities per day (sibling pairs): The cumulative percentage of cannibalistic mortalities per day, for sibling pairs, was calculated for each DFE level. The 20 DFE level was excluded from this figure because all cannibalistic mortalities occurred one day after exposure (day 21). At 0 DFE, the percentage of mortalities initially rises slowly. However at day 5, the mortality rate begins to increase. Spiderlings paired at days 5, 7, and 10 DFE show no such delay. Thus, there appears to be an association effect; siblings that are isolated from one another (5, 7, and 10 DFE) fail to recognize each other as relatives and feed.

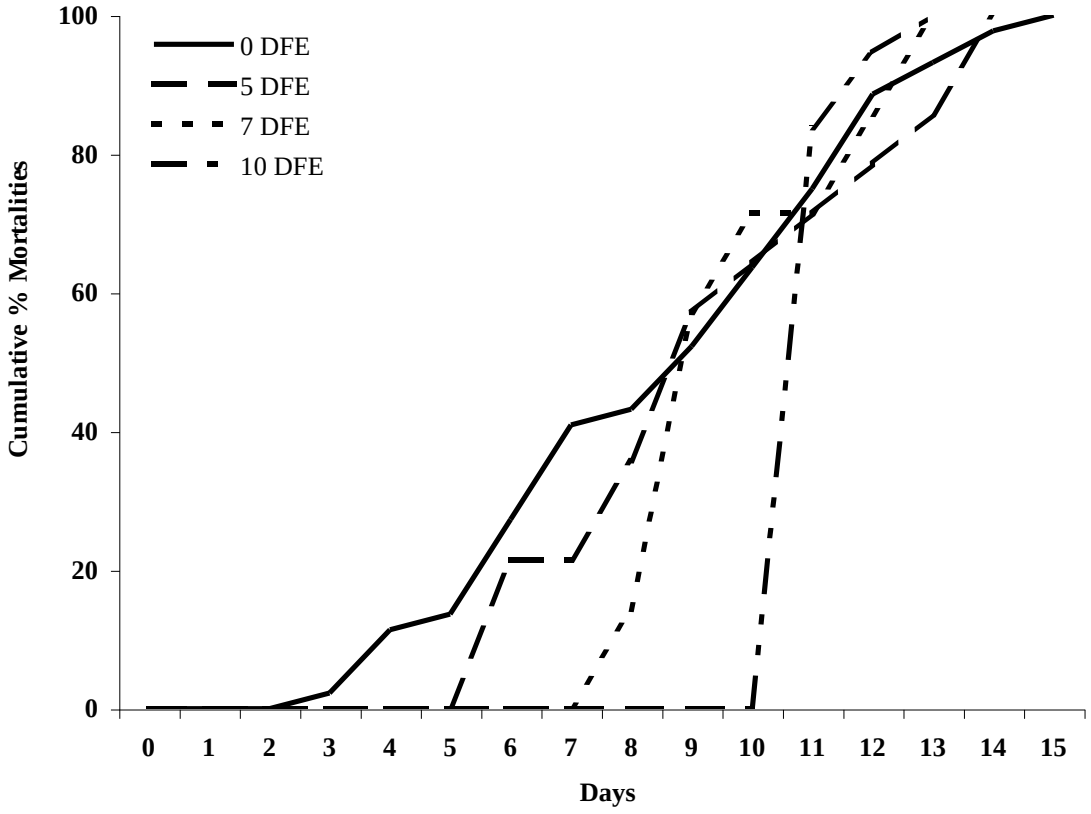

Figure 12 Cumulative percent of cannibalistic mortalities per day (non-sibling pairs): The percentage of mortalities per day, for non-sibling pairs, was calculated for each DFE level. The 20 DFE level was excluded from this figure because there were no (0) cannibalistic mortalities observed between non-sibling pairs at this level. At 0 DFE, the percentage of mortalities initially rises slowly. Spiderlings paired at days 5, 7, and 10 DFE show no such delay. Lines appear to converge around day 11. Following the convergence, all lines appear to increase at the same rate. There does not appear to be an association effect.

Spiderlings paired on days 5, 7, and 10 show no such delay. The frequency of cannibalism appears to increase with spiderling age with little delay at 7 and 10 DFE. As spiderling increase in age, the incidence of cannibalism appears to also increase.

On average, the first mortality was observed 11.1 days (S.E. = 0.20 days) following the formation of spiderling pairs (Table 4). Average DTFM values for 0, 5, 7, 10 and 20 DFE appear in Table 4 and are illustrated in Figure 13. A one-way ANOVA and Pairwise Comparison of 0, 5, 7, 10 and 20 DFE DTFM values revealed similar ($p \geq 0.05$) DTFM values amongst 0, 5, and 7 DFE. However, 10 and 20 DFE were significantly different from all other average DTFM's (Table 5). This difference was probably largely due to the fact that the 10 and 20 DFE spiderlings were isolated and thus had no opportunity to feed until 10 or 20 days following emergence from the egg sac. Two tailed T-Tests for independence were used to identify significant differences between the average DTFM of cannibalistic mortalities (siblings and non-siblings) and non-cannibalistic mortalities (siblings and non-siblings). Non-cannibalistic mortalities (combined for all DFE's) occurred significantly later than cannibalistic mortalities ($p = 6.82*10^{-07}$; Figure 14). Looking specifically at cannibalistic mortalities between sibling pairs and non-sibling pairs (all DFE treatments combined), siblings displayed a higher average DTFM than non-siblings (siblings 11.1 +/- 0.41; non-siblings 9.8 +/- 0.25) ($p = 0.008$; Table 7, Figure 15). In other words, siblings appeared to delay cannibalistic predation longer than non-siblings. However when each DFE group was examined separately this pattern disappeared. Contrary to the overall DTFM, at 7 DFE, non-sibling pairs displayed a higher average DTFM than sibling pairs (siblings 8.5 +/- 0.20; non-siblings 10.0 +/- 0.47); non-siblings delay cannibalistic predation longer than siblings.

Table 4 Average number of days until the first mortality: Pairs were formed at different ages, measured here as days following emergence from the egg sac (DFE). The average number of days until the first mortality (DTFM) was calculated for each DFE from the emergence day and the exposure day. Sibling and non-sibling data, and cannibalistic and non-cannibalistic mortality data were combined. Standard error was calculated for average DTFM values.

	n	**Average DTFM After Emergence (Days)**	**Average DTFM After Exposure (Days)**	**S.E. (+/- Days)**
0 DFE	106	10.0	10.0	0.3
5 DFE	30	9.0	4.0	0.5
7 DFE	29	9.5	2.5	0.3
10 DFE	78	11.4	1.4	0.1
20 DFE	21	21.2	1.2	0.2
All Data	264	11.1	11.1	0.2

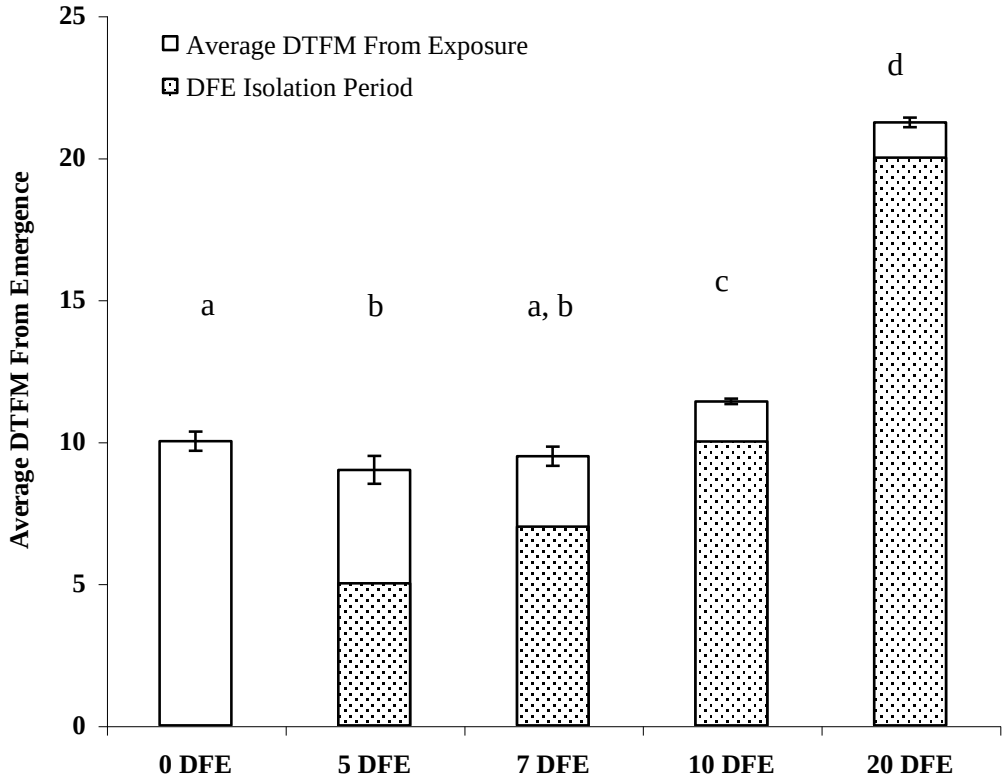

Figure 13 Average number of days until the first mortality (DTFM): Pairs were formed at different ages, measured here as days following emergence from the egg sac (DFE). The average number of days until the first mortality (DTFM) was calculated for each DFE. For most treatments spiderlings were kept in isolation for a period of time (stippled portion of each bar) before being paired. The 0 DFE level has no stippled section because exposure began within 24 hours of spiderlings emerging from the egg sac. Standard error was calculated for each DFE level. Bars with the same letter are not significantly different based on Pairwise Comparisons summarized in table 5.

Table 5 P values computed from a one-way ANOVA Pairwise Comparison test of the DTFM data set: Pairs were formed at different ages, measured here as days following emergence from the egg sac (DFE). The average number of days until the first mortality (DTFM) was calculated for each DFE. For this particular study, the values of interest are those that are not significant ($p > 0.05$), in the bold print. Non-significant values suggest that the DTFM between DFE levels are the same; spiderlings appear to delay their aggression until the ~9th day. Ten DFE was likely similar to 0, 5, and 7 DFE, however the experimental procedures exaggerated 10 DFE DTFM values. Spiderlings under this treatment could not cannibalize until the 11th day, raising the average to 11.4 days.

	0 DFE	**5 DFE**	**7 DFE**	**10 DFE**	**20 DFE**
0 DFE	---------	.010	**.207**	.000	.000
5 DFE	.010	---------	**.299**	.000	.000
7 DFE	**.207**	**.299**	---------	.000	.000
10 DFE	.000	.000	.000	---------	.000
20 DFE	.000	.000	.000	.000	---------

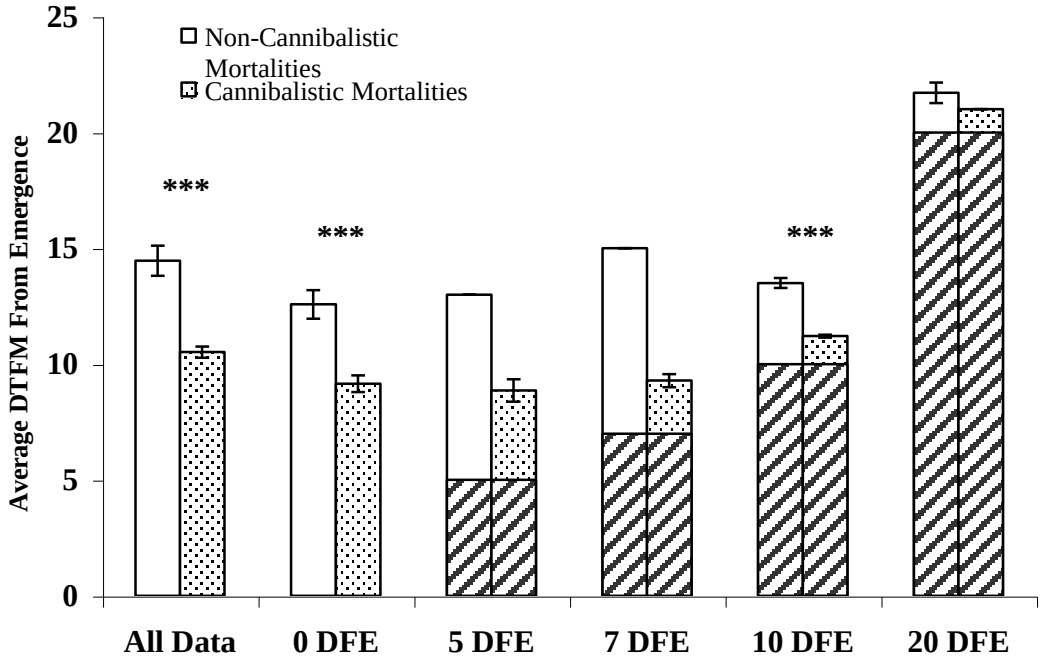

Figure 14 Average number of days until the first mortality (DTFM, non-cannibalistic vs. cannibalistic): The open bars represent non-cannibalistic mortalities while the stippled bars represent cannibalistic mortalities. Pairs were formed at different ages, measured here as days following emergence from the egg sac (DFE). The hatched region of the bars represents the period of time that spiderlings were isolated prior to exposure. The 0 DFE level has no hatched section because emergence began within 24 hours of spiderlings emerging from the egg sac. The average number of days until the first mortality (DTFM) was calculated for each DFE. Standard error was calculated for each DFE level. Note that standard error could not be calculated for non-cannibalistic mortalities at the 5 DFE and 7 DFE due to sample size (n = 1 for both treatments). At 20 DFE, non-siblings standard error was 0; n = 13, all DTFM = 1. Asterisks note significant p values (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

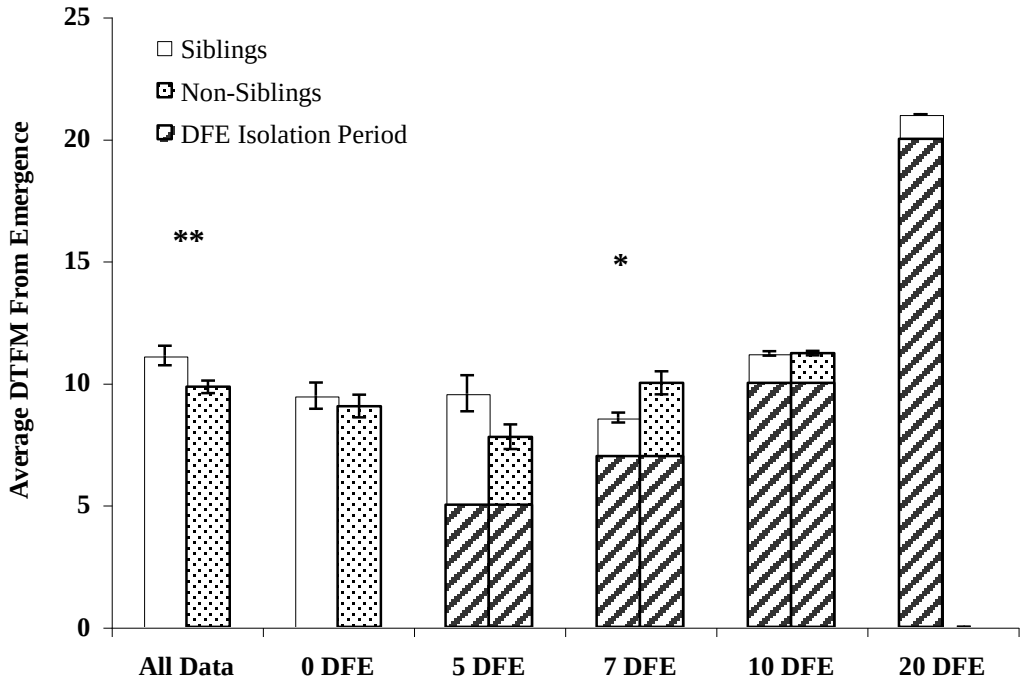

Figure 15 Average number of days until the first mortality (DTFM, cannibalistic mortalities): The open bars represent sibling pairs while the stippled bars represent non-siblings pairs. Pairs were formed at different ages, measured here as days following emergence from the egg sac (DFE). The hatched region of the bars represents the period of time that spiderlings were isolated prior to exposure. The 0 DFE level has no hatched section because emergence began within 24 hours of spiderlings emerging from the egg sac. The average number of days until the first mortality (DTFM) was calculated for each DFE. Standard error was calculated for each DFE. At 20 DFE, for sibling pairs, the standard error was 0; n = 13, all DTFM = 1. At All Data, significantly more mortalities (cannibalistic) where observed between sibling pairs than between non-sibling pairs. However this likely an artifact stemming from 20 DFE; no cannibalistic mortalities were observed between non-sibling pairs at 20 DFE. At 7 DFE, the opposite was observed; there were significantly more mortalities (cannibalistic) observed between non-sibling pairs than between sibling pairs. Asterisks note significant p values (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Not surprisingly, looking at non-cannibalistic mortalities (overall), no significant differences were observed between sibling pairs and non-sibling pairs ($p = 0.754$; Figure 16). Results for figures 13, 14 and 15 are summarized in Table 6.

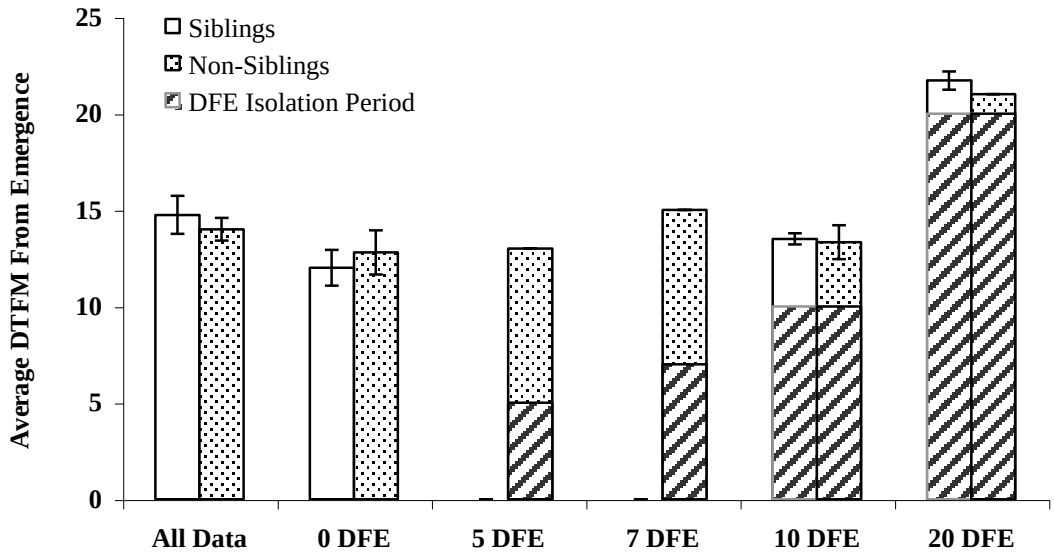

Figure 16 Average number of days until the first mortality (DTFM, non-cannibalistic mortalities): The open bars represent sibling pairs while the stippled bars represent non-siblings pairs. Pairs were formed at different ages, measured here as days following emergence from the egg sac (DFE). The hatched region of the bars represents the period of time that spiderlings were isolated prior to exposure. The 0 DFE assignment has no hatched section because emergence began within 24 hours of spiderlings emerging from the egg sac; no isolation period. The average number of days until the first mortality (DTFM) was calculated for each DFE. No (0) non-cannibalistic mortalities were observed between sibling pairs at 5 and 7 DFE. Standard error was calculated for each DFE. Note that standard error could not be calculated for non-siblings at 5, 7 and 20 DFE due to sample size (n = 1 for all treatments). There were no significant differences between sibling and non-sibling pairs.

Table 6 Two tailed T-Tests of DTFM values, cannibalistic mortalities vs. non-cannibalistic mortalities, cannibalistic mortalities (siblings vs. non-siblings), and non-cannibalistic mortalities (siblings vs. non-siblings): Pairs were formed at different ages, measured here as days following emergence from the egg sac (DFE). N/A identifies areas where a T-Test was not possible due to sample size ($n \leq 1$). Asterisks note significant p values (*$p<0.05$, **$p<0.01$, ***$p<0.001$). In regards to cannibalistic mortalities (siblings vs. non-siblings), at All Data, sibling pair mortalities occurred significantly later than non-sibling pairs. However at 7 DFE, the opposite was observed; non-sibling pair mortalities occurred significantly later than sibling pairs.

	Cannibalistic vs. Non-Cannibalistic	**Cannibalistic** (Siblings vs. Non-Siblings)	**Non-Cannibalistic** (Siblings vs. Non-Siblings
All Data	*** 6.82 E -07	** 0.008	0.754
0 DFE	*** 1.84 E -05	0.436	0.172
5 DFE	**N/A**	0.220	**N/A**
7 DFE	**N/A**	* 0.012	**N/A**
10 DFE	*** 0.00083	0.935	0.118
20 DFE	0.18	**N/A**	**N/A**

Spiderling Dispersal Experiment

Thirteen egg sacs were used in this experiment. Those egg sacs produced on average 120 spiderlings per egg sac (S.E. = 12.0 spiderlings). On average, spiderlings emerged 11.15 days (S.E. = 0.10 days) following the formation of the egg sac. Upon emergence from the egg sac, spiderlings lingered within the vicinity of the egg sac for an average of 8.61 days (S.E. = 0.95); on average all spiderlings were dispersed from the egg sac after 8.61 days. However, between days 2 and 3, and 3 and 4, there was a significant decrease in the spiderling population (Figures 17 and 18).

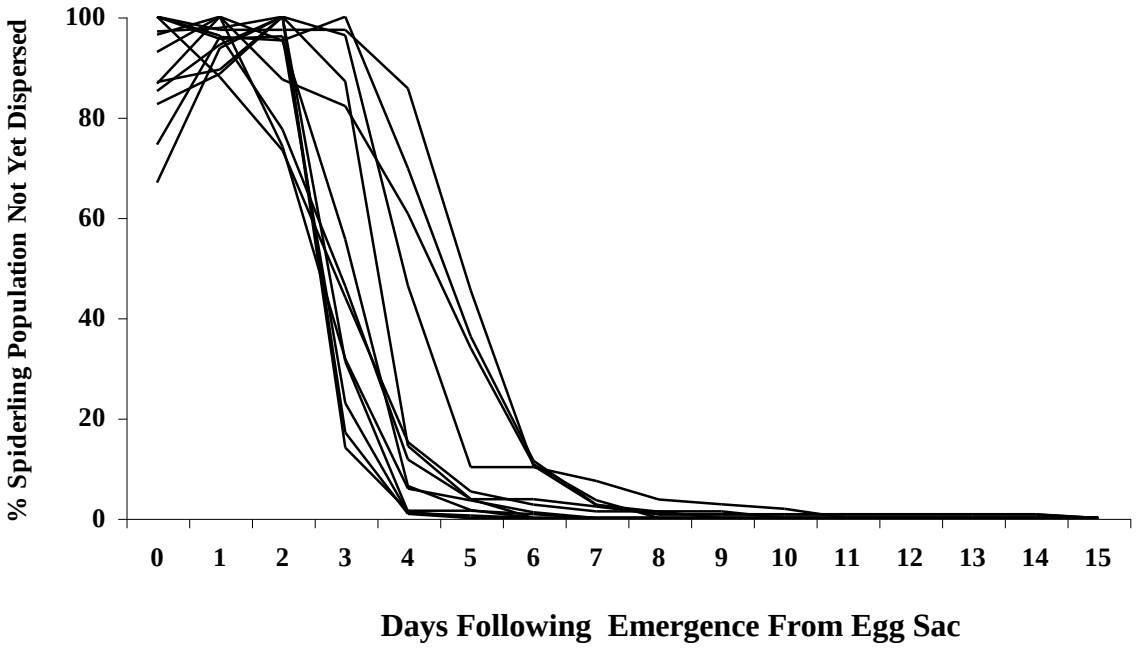

Figure 17 Rate of spiderling dispersal: As time progresses, the number of spiderlings within these 13 egg sacs exhibit a dramatic decrease in size; spiderlings leave the egg sac dispersing to another location. This decrease is most noticeable between days 2 and 3, and 3 and 4. Spiderlings however do appear to stay within the vicinity of the egg sac until the second day.

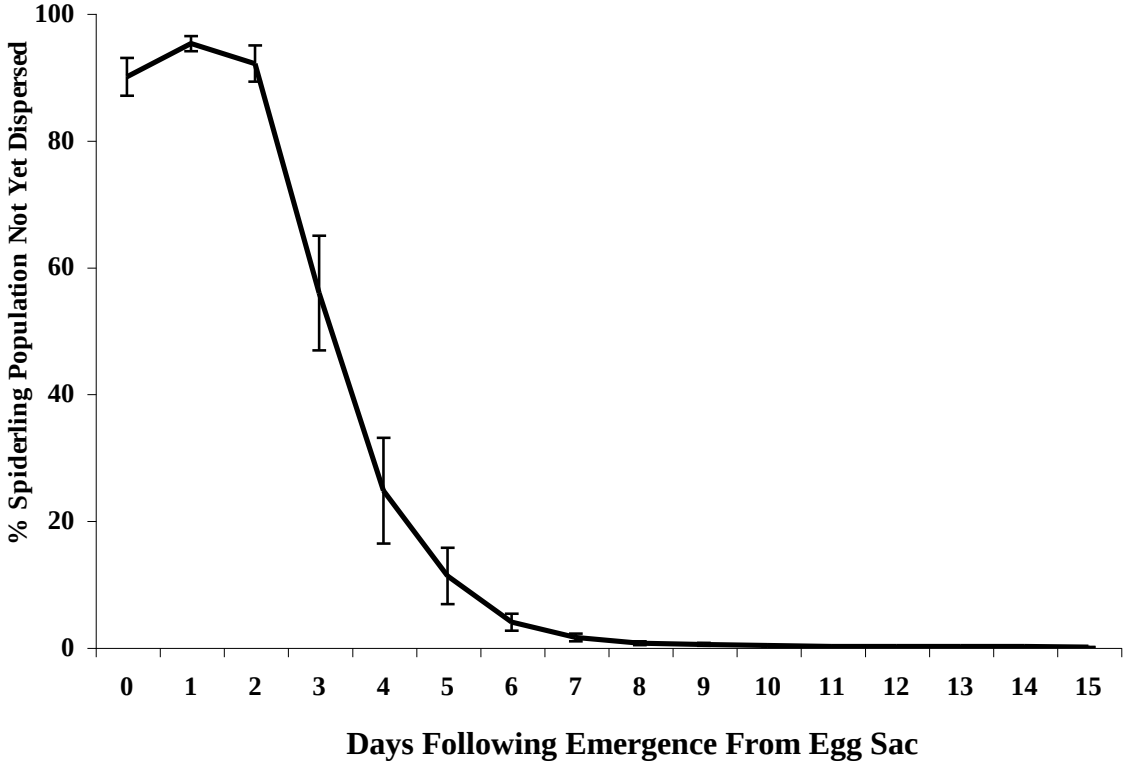

Figure 18 Average spiderling dispersal rate: The average was calculated from 13 spiderling cohorts (13 egg sacs). As time progresses, this average dramatically decreases in size; spiderlings leave the egg sac dispersing to another location. This decrease is most noticeable between days 2 and 3, and 3 and 4. Spiderlings however do appear to stay within the vicinity of the egg sac until the second day. Standard error was calculated for each day.

Spider & Egg Sac Data

Throughout this experiment, 230 egg sacs were produced and collected, with females producing an average of 5 egg sacs (S.E. = 0.72). Females produced a new egg sac approximately every 5^{th} day (mean = 5.31, S.E.= 0.84). Of the 230 egg sacs produced, 132 (57.4%) hatched or produced spiderlings, 44 (24%) did not hatch or produce spiderlings, and 54 (23.5%) were lost, damaged or used in other experiments. (Appendix A)

CHAPTER V: DISCUSSION

"It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change" (Megginson 1963, paraphrasing from Charles Darwin's *On the Origin of Species)*.

Parasteatoda tepidariorum, the common house spider, is a remarkable organism. It is a skilled web builder, and a patient and efficient predator. Clearly this species has acquired a number of adaptations that have allowed it to be successful. This study aimed to address whether or not kin recognition was one of those adaptations. Kin recognition can easily be assessed, as was done in this study, by the presence or absence of cannibalism. In this experiment, I had expected to observe fewer cannibalistic mortalities between siblings than between non-siblings. However, this was not the case; cannibalistic mortalities were equally frequent between sibling pairs and non-sibling pairs. This suggests that *P. tepidariorum* spiderlings do not discriminate siblings from non-siblings.

In addition to the frequency of cannibalism between sibling and non-sibling pairs, the rate of cannibalism between sibling and non-sibling pairs also appears to be the same (Figure 10). Thus, kin recognition is not supported by these data either. However, the rate of cannibalism does appear to increase the longer spiderlings are isolated from one another. In this way, as a spiderlings association with another spiderling decreases, the rate of cannibalism between spiderlings will increase. This is exactly what was observed between sibling pairs (Figure 11). These data therefore suggest the presence of kin recognition by association. However, the results from the non-sibling pairs do not support this hypothesis; the rate of cannibalism appears to be the same for each DFE

level (Figure 12). These data are therefore too inconclusive to suggest the presence or absence of kin recognition, through association, in these *P. tepidariorum* populations.

Although the results of this experiment suggests that kin recognition is not present in either of these *P. tepidariorum* populations, I would be remiss if I did not discuss the bit of data that counters this argument. Referring back to the pooled data in Figure 15, sibling pairs (overall) display a significantly higher average DTFM than non-sibling pairs ($p < 0.01$). That is, sibling pairs appear to delay predation (cannibalism) longer than non-sibling pairs. This behavior is suggestive of kin recognition, however it is likely an artifact stemming from the 20 DFE level; recall that there were no cannibalistic mortalities observed between non-sibling pairs (Figure 15). Additionally, this observation is not consistent at all DFE levels. For example, in that same figure (15), at 7 DFE, non-sibling pairs appear to delay predation (cannibalism) longer than sibling pairs ($p < 0.01$); this is contrary to the overall data. Grubbs (1969) test for outliers was employed to determine if any outliers might account for this, but no outliers were identified (All data, 0, 5, 7, 10 and 20 DFE). As these data are not unanimous in predicting the spiderlings behavior, I argue that there is not enough evidence for the presence of kin recognition in these *P. tepidariorum* populations. Certainly retesting, paired with a larger sample size, would help to clarify these conflicting arguments.

While it appears that kin recognition is not present in these populations, the results of this experiment suggest that an alternative adaptation, dispersal, may make kin recognition unnecessary. On average, mortalities between spiderling pairs (both sibling and non-sibling) occurred after the 9^{th} day of exposure (+/- 0.21 days). This behavior was observed at each DFE with the exception of the 10 and 20 DFE. However, this

simply reflects the experiments design, since in these cases spiderlings were unable to feed until the 11th or 21st day, respectively. Regardless, this suggests that *P. tepidariorum* spiderlings delay predation for a period of 9 days (approximately), by which time they would have normally dispersed.

This behavior does not appear to be unique to *P. tepidariorum.* A review of the literature reveals that many social and subsocial spider species exhibit delayed aggression (i.e. increased aggression with age) towards siblings, offspring and conspecifics (Riechert and Roeloffs 1993, Bessekon and Horel 1996, Trabalon et al. 1996, Bessekon 1997, Aviles 1997, Pourie and Trabalon 1999). For example, in *Tegenaria atrica,* siblings appear to show increased aggression toward one another as they approach the dispersal age. Additionally, adult females of this species exhibit a similar behavior, tolerating spiderling presence during the pre-dispersal stage. Following the pre-dispersal stage, adult females, and likely spiderlings, appear to switch their behavior from tolerance to aggression (cannibalism) (Trabalon et al. 1996, Pourie and Trabalon 1999). *Delena cancerides,* a social huntsman spider, also displays increasing levels of aggression correlated with age. As juveniles, this species exhibits low aggression levels toward conspecifics. For example, in sibling and non-sibling pairs, juveniles starved rather than cannibalizing conspecifics. However, as an adult, this species is highly aggressive and intolerant of conspecifics. Thus it would seem that with age, there is a decrease in tolerance and increase in aggression toward conspecifics. However, when cannibalism was observed between *D. cancerides* juveniles, it was always between non-sibling pairs; *D. cancerides* juveniles preferentially cannibalize non-siblings over siblings (Beavis 2007).

Contrary to those examples previously mentioned (Trabalon et al. 1996, Pourie and Trabalon 1999, Beavis 2007), I have found no evidence that *P. tepidariorum* spiderlings recognize and discriminate related individuals from non-related individuals. However, they do delay their cannibalistic tendencies. Why? One explanation for this behavior may come from the dispersal timing of this species. The results of the dispersal experiment demonstrated that most spiderlings dispersed (via ballooning) by the 5^{th} day, following emergence from their egg sac. Pairing that information with the apparent 9 day delayed aggression data set, it would appear that in nature, *P. tepidariorum* disperse before their predatory tendencies manifest. In this way, siblings avoid cannibalizing each other by removing themselves from the natal web. Therefore, I propose that this species' need for kin recognition is greatly reduced, if not eliminated. Figure 19 models this hypothesis I am suggesting.

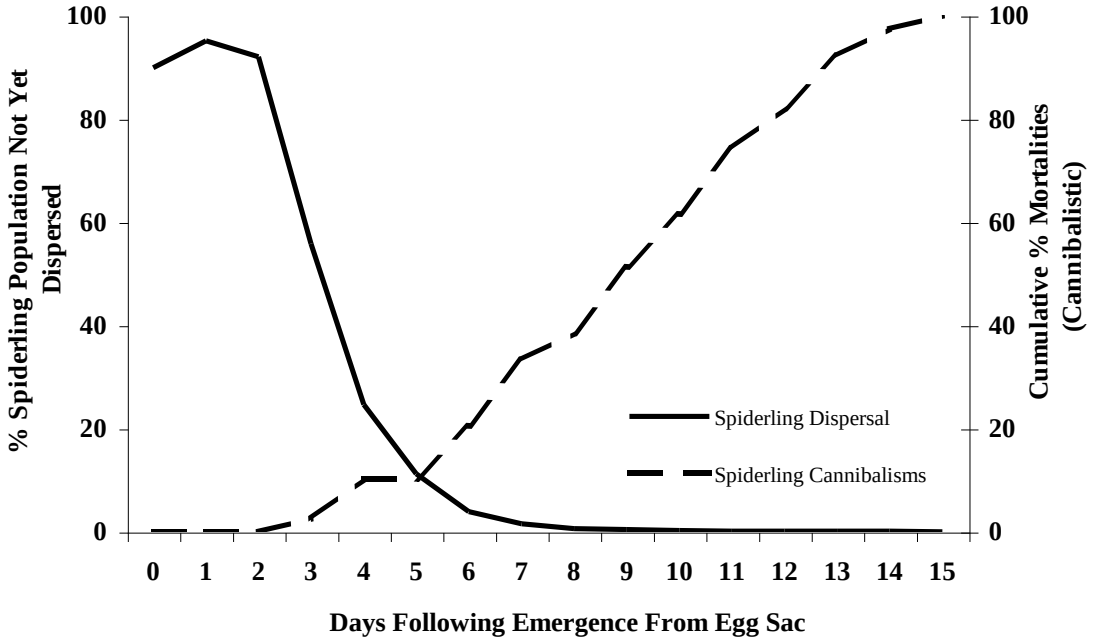

Figure 19 Early dispersal and delayed aggression model: The average spiderling dispersal data was paired with an average (sibling pairs and non-sibling pairs) of the observed cumulative cannibalistic mortalities, both displayed here as percentages. The majority of spiderlings disperse before cannibalistic predation severely reduces spiderling numbers.

In *P. tepidariorum*, it appears that early dispersal enables this species to avoid being cannibalized by conspecifics. As with *P. tepidariorum*, the same can be said for *Zophobas atratus*, a tenebrionid beetle (Tschinkel 1981). In its larval form, *Z. atratus* larvae are known to cannibalize pre-pharate pupae during their vulnerable pupal stage. However, as the larvae increase in age and size, approaching the pre-pharate pupal stage, they begin to disperse, often over-dispersing. These pre-pharate pupae likely disperse, away from larvae conspecifics, to reduce their risk of being cannibalized. Thus it would seem that dispersal reduces the probability of cannibalism in *Z. atratus* too. I was unable to locate any studies that, to date, have identified kin recognition in this species (Rudolf et al. 2010)

As previously mentioned, *P. tepidariorum* disperse by ballooning. This method provides a quick and efficient means of dispersal for spiderlings. Additionally, this technique results in vast separation distances for spiderlings from their natal webs and each other (Suter 1999, Ubick et al. 2005, Szymkowiak et al. 2007). In this way, the likelihood of siblings meeting after dispersal is probably very low. This means of dispersal (ballooning) is very different from those spider species (*Diaea ergandros, Stegodphus lineatus*, and *Hogna helluo)* known to exhibit kin recognition (Evans 1998, Bilde and Lubin 2001, Roberts et al. 2003). The later species disperse by terrestrial movements. Presumably, compared to *P. tepidariorum*, those spiders that disperse terrestrially have a much higher probability of encountering siblings following dispersal. Thus, spiders that disperse terrestrially would experience greater selection for kin recognition, as the ability to avoid eating relatives would increase a spiderling's inclusive fitness (Roberts et al. 2003). For some species, this recognition ability lasts late in life

(Trabalon et al. 1996, Pourie and Trabalon 1999), but because *P. tepidariorum* have a lower probability of reuniting with siblings following dispersal, the evolutionary impetus for kin recognition is likely greatly reduced. *Parasteatoda tepidariorum* spiderlings therefore likely prevent cannibalism, and prevent inclusive fitness losses, by aerial dispersal. This same behavior has been observed in the crab spider, *Misumena vatia*, a species that also disperses aerially. *Misumena vatia* spiderlings, which are also incapable of kin recognition, appear to avoid cannibalism by dispersing within a day of the final emergence from the egg sac (Morse 2011a).

In this experiment, my main objective was to address the question of kin recognition in two *P. tepidariorum* populations. In reviewing the results, I feel that this objective was not only met, but also exceeded. The results of this experiment have shed light onto the behaviors of *P. tepidariorum*, and have laid a foundation from which a number of new and interesting questions can arise.

One such question focuses on whether genetic variation might explain differences in the cannibalistic tendencies of *P. tepidariorum*. Regardless of relationship, cannibalism was certainly more common than non-cannibalism in these populations (Figure 7). With that in mind, one might assume that, per egg sac, there were a higher proportion of cannibalistic individuals than non-cannibalistic individuals. Considering the non-cannibalistic phenotype could only be observed when two non-cannibals were paired together, there were likely more non-cannibalistic individuals than were observed; much like a recessive trait is hidden by the dominant trait, non-cannibals are hidden (from the observer) by the cannibals that eat them. The opposite has been observed in *Misumena vatia*, a species of crab spider. In this species, Morse (2011b) found that 1%

of *M. vatia* nests contained cannibalistic individuals. Of the 1% observed, 8 % of the individuals within each nest were cannibalistic. Additionally, those individuals that were cannibalistic remained in the nest three times longer than average, and were two times heavier than the non-cannibalistic individuals. Hvam et al. (2005) and Mayntz and Toft (2006) report similar findings in their work with wolf spiders. While the inverse was observed in this study with *P. tepidariorum* (there were more cannibals than non-cannibals), the same basic question remains, why does this phenotypes exist within these populations? Is it a genetic or learned characteristic? Do the non-cannibals disperse early compared to the cannibals, or vice versa? The dispersal experiment did reveal that (on average) 4% of the spiderlings from an egg sac remain in the natal web after the fifth day. This percentage decreases over time to 0% by day 15. Whether these late dispersers are cannibalistic or non-cannibalistic is unknown, and enticing.

Another interesting question is, what sex are the cannibals and the non-cannibals? Female spiders are renown for their aggressive and cannibalistic behaviors, often exhibited toward male spiders (Roberts et al. 2003, Ubick et al. 2005). Thus, it would not be all that shocking if a higher proportion of the cannibalistic individuals were identified as females. This could be accomplished by rearing all cannibalistic spiderlings to the earliest sexing stage. Provided adequate feeding, this could be completed within two months.

At the core of every trait exists a population of expressed genes. With that in mind, another question for investigation is the aggression "switch" that appears to be present in these *P. tepidariorum* populations. At approximately 9 days, upon emergence from the egg sac, spiderlings appear to switch their behavior from tolerant to cannibalistic

(Figure 13). Presumably, it is at this time that their aggression toward one another overcomes a threshold, and spiderlings begin to cannibalize one another. This observation begs the question, is the increase in aggression genetic? While other studies have confirmed the occurrence of this behavior in other spider species (Trabalon et al. 1996, Pourie and Trabalon 1999), there appear to be none that have investigated the role genetics play in the "switch". Those studies that do investigate aggression in invertebrates, suggest that the up-regulation of specific hormones, serotonin and octopomine (in crustaceans, *Homarus americanus, Munida quadrispina*), and down-regulation of dopamine (fruit flies, *Drosophila melanogaster*), contributes to aggression (Kravitz and Huber 2003).

As with any experiment, steps were taken to reduce experimental error. All organisms were treated similarly in regards to housing, handling, lighting and feeding. Temperature was also observed throughout this experiment and proved to be relatively stable (Figure 20). Also, data collection was done in a way so that the recorder was blind to the treatment.

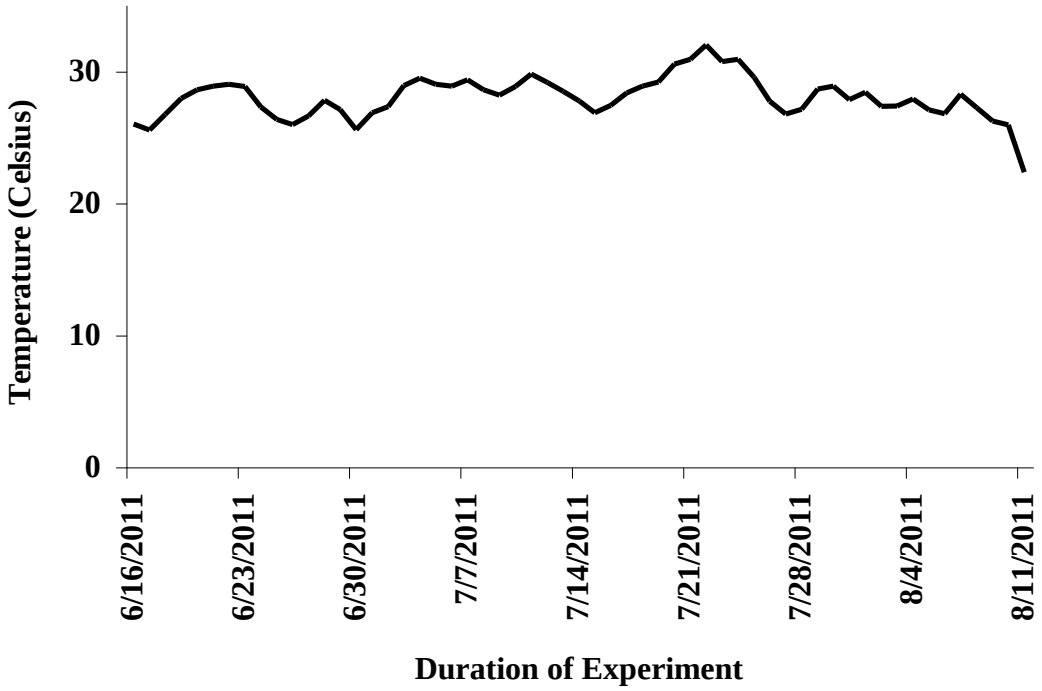

Figure 19 Daily temperature reading of laboratory conditions: An EasyLog EL-USB-2 - Humidity, Temperature and Dew Point Data Logger (with LCD) was the instrument that recorded laboratory conditions throughout the duration of this experiment. It was set to automatically take a reading every five minutes. This information was then loaded into an Excel data sheet for analysis and interpretation. An average was calculated for each day.

Determining the cause of death was also a possible source of error. While there was no confusing a cannibalized spiderling from one that was not, the statement of cannibalistic mortality, or non-cannibalistic mortality, presumed that post mortality feeding did not occur. In other words, it was assumed that *P. tepidariorum* spiderlings did not feed on deceased (non-cannibalized) spiderlings. While a few opportunities arose whereby spiderling attacks were observed, and cannibalistic mortalities were evident, these observations made up only a small fraction of the total spiderling pairings. The literature does provide accounts of *P. tepidariorum* spiderlings feeding on non-viable eggs while still within the egg sac (Valerio 1974), however little is mentioned about feeding habits upon emergence from the egg sac. In other species, mothers permit spiderlings to feed on her prey and even herself (matriphagy), as the time for dispersal nears (Evans 1998, Bilde and Lubin 2001). Such behaviors provide both direct fitness gains as well as inclusive fitness gains for spiderlings and the mother. As of yet, no such behaviors have been reported for *P. tepidariorum*.

In summary, the data collected in this experiment suggest that *P. tepidariorum* spiderlings prevent cannibalizing siblings by delaying their aggression for a period of 9 days. This delay is paired with an early dispersal, whereby individuals leave their natal web before their aggression toward conspecifics manifests. Although *P. tepidariorum* is incapable of kin recognition (based on this experiment), this species is still able to avoid cannibalizing relatives.

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APPENDIX A

Egg Sac Data:	Total	Percentage	Mean (S.E.)
# Egg Sacs Produced	230	100	5.89 (0.72)
# Egg Sacs Hatched	132	57.39	3.38 (0.53)
# Egg Sacs No Hatch	44	19.13	1.13 (0.24)
# Egg Sacs Lost, Damaged, or Used in an Experiment	54	23.39	0.69 (0.22)