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Neurochemical Levels Correlate with Population Level Differences in Social Structure and Individual Behavior in the Polyphenic Spider, *Anelosimus studiosus.*

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Neurochemical Levels Correlate with Population Level Differences in Social Structure and Individual Behavior in the Polyphenic Spider, *Anelosimus studiosus*

A thesis

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East Tennessee State University

in partial fulfillment

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Master of Science in Biology

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by

Jennifer Bryson Price

December 2010

Thomas C. Jones, Chair

Darrell J. Moore

David S. Roane

Keywords: *Anelosimus studiosus*, Behavioral phenotype, Social structure, Octopamine, Serotonin, Spider behavior

ABSTRACT

Neurochemical Levels Correlate with Population Level Differences in Social Structure and Individual Behavior in the Polyphenic Spider, *Anelosimus studiosus*

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Anelosimus studiosus is a socially polyphenic spider. Individuals can be classified as social/tolerant or solitary/aggressive. These behavioral differences are associated with considerable variation in social structure. Here, we begin to examine the physiological differences that may underlie the behavioral dimorphism in this species and possible implications for the evolution of sociality. Octopamine is a neurotransmitter that has been found to elevate aggression in invertebrates. Serotonin has been shown, in some cases, to interact antagonistically with octopamine. We used High Pressure Liquid Chromatography with Electrochemical Detection to quantify levels of these neurochemicals among adult females from social (multi-female) and solitary (single-female) webs in east Tennessee. A subset of spiders was scored for individual social tendency. We found that higher octopamine levels are associated with a greater degree of aggression and intolerance, both at the individual level and the population level, while higher levels of serotonin are found in multi-female colonies and social individuals.

DEDICATION

I would like to dedicate this work to the memory of my father, who instilled in me a curiosity in all things great and small, and to my daughters, who inspire it still. Also, to my mother for exhibiting extended maternal care and extreme tolerance, to my sister for reminding me that nothing is impossible, to Buddy for the amazing 15-year journey, and to Vegas for coming back from Colorado.

ACKNOWLEDGMENTS

I would like to thank my committee members, Dr. Thomas Jones, Dr. Darrell Moore, and Dr. David Roane, for their advice, wisdom, and insight, particularly in helping me to whittle this project to a manageable size. I would especially like to thank Dr. Jones for taking a nontraditional and unmanageable student into his lab and under his wing and being a true mentor through 2.5 difficult years *and* for allowing me to take part in such an interesting and important project. A special thanks to Angela Shepherd Hanley at Bill Gatton College of Pharmacy for her grace and patience in teaching me the ways of HPLC-ECD. Additional thanks to Jonathan Pruitt of UT Knoxville for his spider expertise, to Dr. Karl Joplin for ideas and input, and to Nathan Weber for lab support and comic relief. I am grateful for the brave assistance of Kathy Bryson, Haley Price, and Hannah Price in the collection of spider samples.

I would like to express my deep appreciation to Dr. Gordon Anderson, Dr. Anant Godbole, Dr. Aimee Govett, the faculty and staff of North Side School of Science, Math, and Technology, and the National Science Foundation GK-12 Fellowship Program for two years of support and invaluable experience without which this venture could not have occurred.

I also want to thank Dr. Michael Zavada, Dr. Cecilia Macintosh, and the School of Graduate Studies for the opportunity to be part of such a vibrant Biological Sciences department and thriving research program.

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CHAPTER 1

INTRODUCTION

Animal Behavior and the Evolution of Cooperation

Interactions among organisms and between organisms and their environments drive the activities of the biosphere that we know as planet Earth. Beginning with plants as primary producers, vital energy works its way through the food chains and food webs around the globe, passing from living thing to living thing and providing the basis for life as we know it. Resources that sustain life are of supreme importance and serve as both currency and loot in the game of survival. Charles Darwin succeeded in explaining the tragedy and reward associated with this truth. The fittest among us acquire the necessary resources to survive and pass our genetic batons to our offspring and their offspring and so on. And so it would seem that it would behoove us to be ever-selfish, fighting to find and dominate the goods, keeping them for ourselves and our mates and our offspring, leaving others to wither away, taking with them the genes that would compete with our own in future generations. For most life on earth, that is exactly what happens. But somehow, and for some reason, some organisms have come to cooperate with, and even depend upon, others. Given what we know about natural selection, how does this happen?

Evolution drives sociality only when it is beneficial to the individuals within the group to do so. The evolution of cooperation (Axelrod and Hamilton 1981) involves the application of Game Theory, which has long been used to analyze phenomena in economics and business, to biology (Smith 1973; Smith 1982) and to animal behavior (Fisher 1930; Edwards 2000). The concept of transitioning from selfish motivation to cooperation-for-self-benefit is effectively illustrated by the Prisoner's Dilemma. The Prisoner's Dilemma is a game in which two players independently and simultaneously choose to either defect or cooperate. Each choice has a payoff (in terms of fitness) determined by the choice of the other player. Whether the other player chooses to cooperate or defect, the payoff is highest for defection. *However,* if *both* players defect, then the payoff for both players is less than if they had both chosen to cooperate. Therein lies the dilemma. How does one decide whether to defect or cooperate? The answer to this question depends on the probability of future interaction between the players.

If the players only interact once and never again, then the best strategy is always to defect, but as the likelihood of future interaction increases, the strategy of cooperation becomes more valuable. The "Tit-for-Tat" game demonstrates that, over time and repeated interactions with the same individual, cooperation based on reciprocity results in the highest overall payoff for the players. If the probability that the players will continue to interact in the future is relatively high, then it is in their best interest to cooperate (Axelrod and Hamilton 1981).

Fig-pollinating wasps often skew their broods toward female-biased ratios when there are many sets of competing foundresses in multiple fruits on the fig tree. Within a single fruit, as the number of foundresses increases, the proportion of male offspring also increases and approaches a Fisherian sex ratio of 50:50 in response to the intensity of mate competition. But because male wasps don't pollinate the figs, it is not in the best interest of the tree or the wasps (because they need fertilized flowers/developing seeds for their own development) to have an even sex ratio. When put in terms of the Prisoner's Dilemma game, we consider the payoffs associated with different possible combinations of sex ratio strategies chosen by two hypothetical foundress wasps. For the fig wasps, there exists a balance between *between-group* and *within-group* selection. Within each deme (in this case, each syconium), the female who uses the even sex ratio strategy (50:50) receives the higher payoff, but across demes (in the situation where there are multiple foundresses and multiple fruits), the highest payoff goes to those *groups* producing the most mated females. The selection acting upon the groups (or across demes) works to overwhelm and override the Fisherian ratios within them. The most productive scenario for the group is the one in which both females "cooperate" by using a female-biased strategy, thereby producing the greatest number of productive (mating *and* pollinating) female offspring (Herre 1999).

Each syconium/fruit on a wasp-pollinated fig tree contains its own local population of interbreeding individuals, and when the mated females leave that fruit as adults, they invade a new syconium to begin a new colony. This situation is analogous to that found in populations of permanent-social spiders that inbreed and, like the fig wasps, have evolved highly female-biased sex ratios (Avilés 1993). Permanent-social spiders demonstrate "intercolony selection leading to female-biased sex ratios" (Avilés 1997).

These models contributes to our understanding of how a strategy of cooperation can invade a strategy of pure defection, how it can take hold and spread throughout the population, and how it can eventually become an evolutionarily stable strategy. It allows us to bridge the theoretical gap between selfish motivations and the good of the whole, and it helps us to understand how individuals who are, by necessity, looking out for themselves have somehow managed to form cooperative relationships and even societies.

If cooperation and social behavior can evolve from a former state of selfishness and aggressive 'survival of the fittest' tendencies, what are the physiological transferrable and transmutable traits that parallel this transformation? What are the internal biological factors that influence these differences in intraspecific social and aggressive behaviors over time? What physical condition causes aggression or lack thereof?

Physiology of Aggression

Octopamine (OA) and serotonin (5-HT) are biogenic amines known to modulate aggression-related behaviors in invertebrates (Kravitz 1988). Octopamine acts as a neurohormone, a neurotransmitter, and a neuromodulator. It has been suggested that octopamine modulates almost every physiological process in invertebrates, and it is considered homologous to the noradrenergic system in vertebrates (Roeder 1999). Octopamine is released when energy-demanding behaviors such as sustained flight (in flying insects), fights, predatory attacks, or anti-predator escape maneuvers are needed (Roeder 1999). OA has a broad range of effects in honeybees, including modulation of dance behavior (Barron et al. 2007), sucrose responsiveness (Scheiner et al. 2002), and age-related division of labor (Schulz and Robinson 2001). In some cases, serotonin has been shown to have an opposite modulatory effect. In crayfish, octopamine enhances an escape response, but serotonin suppresses the response (Glanzman and Krasne 1983).

Both social and non-social invertebrates offer powerful model systems for studying the effects of biogenic amines on aggression (Kravitz and Huber 2003). Octopamine null *Drosophila* mutants showed greatly reduced aggression (Baier et al. 2002), and in the same study, serotonin was found to have no effect on aggression. However, another study found that increased levels of serotonin in the *Drosophila* brain increased aggressive behavior (Dierick and Greenspan 2007). Serotonin is responsible for an extreme behavioral transformation in desert locusts, causing a switch from solitary to group behavior within a matter of hours, with the major behavioral change being loss of aversion to conspecifics (Anstey et al. 2009).

Octopamine and Serotonin in Spiders

Octopamine in spiders acts both in the CNS and peripheral tissues and is found freely circulating in the hemolymph. Its role as a spider neurohormone is indicated by this and the concentration of octopamine immunoreactive neurons found near hemolymph spaces throughout the spider's CNS (Seyfarth et al. 1993). Octopamine also acts to modulate numerous physiological processes including sensitization and desensitization of mechanosensory neurons in spiders (Widmer et al. 2005). Because a spider's relationship with its environment occurs primarily via interpretation of vibratory cues detected by these mechanosensory neurons associated with sensilla (small sensory organs embedded in the exoskeleton) and trichobothria (hairs on the exoskeleton) (Foelix 1996), it follows that increased levels of octopamine could be associated with greater sensitivity to environmental signals, such as air movement (flying predators) and web vibration (conspecifics, prey, or predators).

Information regarding the role of serotonin in spiders is scarce, and as far as we know, only one study involving serotonin and spider behavior has been published. Punzo and Punzo (2001) explored the effects of intraspecific male agonistic interaction on serotonin and octopamine levels in tarantulas, finding that levels of both serotonin and octopamine decreased in both winners and losers following a fight, but they decreased more in the subordinate spiders than in the dominant spiders.

Sociality in Spiders

Nearly 42,000 spider species are currently known (Platnick 2010), and of those, the vast majority display solitary and aggressive behavior. Only 23 spider species are classified as social (Avilés 1997; Agnarsson 2006), and they live, almost without exception, in tropical and subtropical areas. Of those 23 social spider species, 11 to 12 occur within the family Theridiidae (Agnarsson et al. 2006). Theridiids build tangled three-dimensional cobwebs and exhibit extended maternal care of their offspring. It has been suggested that these traits are preadaptations (creating a predisposition) for the evolution of social behavior because all that is further required to become social is a mutual tolerance of conspecifics (Kullmann 1968; Shear 1970; Brach 1977). *Anelosimus studiosus* (Araneae: Theridiidae) is the only species currently known to display cooperatively social behavior in a temperate region (Furey 1998; Jones et al. 2007). Additionally, it is the only species of the described cooperatively social spiders to exhibit a behavioral polyphenism, having both social and solitary phenotypes within the species (Riechert and Jones 2008; Pruitt and Riechert 2009). The habitat range of *A. studiosus* extends from Argentina in South America, all the way through Central America, and into the New England states in North America (Agnarsson 2006). These spiders are small (in this study, mean body mass = 0.00636g), and they typically nest in trees and shrubs along waterways (Brach 1977; Furey 1998). Members of the species residing in the tropical and subtropical latitudes exhibit purely solitary behavior, but with an increase in latitude (moving out of the tropics and into more temperate climes), observations of multi-female webs become more frequent (Jones et al. 2007; Riechert and Jones 2008). This is surprising considering the fact that evolution of spider sociality is thought to be favored by numerous environmental factors, such as greater year-round food supply, larger size of spider prey (profitable sharing), niche exploitation in response to more intense competition, and group-living/extended maternal care in response to higher levels of predation found in tropical regions (Avilés 1997). While most *A. studiosus* webs in east Tennessee are occupied by a single female and her offspring who cooperate in prey capture and web maintenance to their mutual benefit (Jones and Parker 2002), spiders occupying relatively cooler microhabitats may build webs that contain over a hundred females (Furey 1998; Jones et al. 2007). Benefits of this cooperative arrangement, as compared to social structures of tropical spiders, are explained by Jones et al. in a brood-fostering model (2007) and supported by findings of higher reproductive success by the multi-female colonies in cooler microclimates (Jones et al. 2007; Jones and Riechert 2008). There is much variation not only between the multi-female colonies and the single-female clusters but also within them. Some individuals within the multi-female colonies exhibit solitary behavior, maintaining web space adjacent to others while exhibiting aggressive and sometimes even cannibalistic behaviors. Likewise, some females in solitary nests show decreased aggression toward prey and tolerance of intraspecific and interspecific intrusion into their webs (Pruitt et al. 2008). Considering the broad range of behaviors observed in this species, a question arises: What physiological differences might underlie this observed behavioral phenomenon?

Quantifying Behavior

Based on observations of individual behavioral differences within a given social structure, quantification of these differences is necessary to gain deeper ecological understanding of this particular system. Because spiders must both acquire prey *and* avoid predation, they must be capable of performing acts of aggression *and* acts of avoidance. Spiders that are less aggressive toward one another also tend to be generally less aggressive toward prey, and the inverse is also true. Because social tendency and anti-predator response are both considered aspects of an overall behavioral syndrome (Sih et al. 2004; Pruitt et al. 2008), a spider's response to the presence of a predator is related to the spider's level of aggression. Cob-web spiders such as *A. studiosus*, when detecting the presence of a predator via vibratory or convective cues, will crouch, pull in its legs, and remain motionless for a period of time. This is referred to as a 'huddle response.' The duration of the huddle response is typically an accurate predictor of aggressive tendency, with more aggressive spiders huddling for shorter periods of time and more social spiders huddling for longer periods of time (Riechert and Johns 2003; Pruitt et al. 2008). Likewise, self-determined distance between individuals (social tendency) can be used as a measure of tolerance of conspecifics has been shown to be strongly correlated with living strategy (Riechert and Jones 2008; Pruitt et al. 2008).

To explore the neurochemical underpinnings of population- and colony-level differences in social behavior, we investigate the physiological differences underlying the social polyphenism in *A. studiosus* and quantify neurochemical differences between the solitary and social phenotypes within the species. Because aggression is the primary behavior displayed toward conspecifics by the solitary phenotype, we look for correlations in naturally occurring levels of octopamine and serotonin with social and aggression-related behaviors.

Implications for the Evolution of Sociality

Results from a recent phylogenetic study suggest that sociality is evolving locally and independently in populations of *Anelosimus studiosus* in east Tennessee (Weber et al., unpublished data). Modifications in neurochemical levels may be an evolutionary pathway to the development of different social behaviors. It is theoretically possible that individual populations of *A. studiosus* could adopt various neurochemical strategies to address the issue of maximized fitness by cooperation in the cooler microclimates of our study area.

Hypothesis

Given data from other arthropod studies, we hypothesize that octopamine levels should be higher in individuals, and groups of individuals, displaying more aggressive and less social behavior. Based on evidence that serotonin is an antagonist of octopamine (Glanzman and Krasne 1983) and that it may increase tolerance of conspecifics in other invertebrates (Anstey et al. 2009), we predict that serotonin levels will be higher in multi-female colonies and social individuals than in their solitary counterparts.

CHAPTER 2

NEUROCHEMICAL LEVELS CORRELATE WITH POPULATION LEVEL DIFFERENCES IN SOCIAL STRUCTURE AND INDIVIDUAL BEHAVIOR IN THE POLYPHENIC SPIDER, *ANELOSIMUS STUDIOSUS*

Jennifer B. Price [1], Thomas C. Jones [1]*, David S. Roane [2], Jonathan N. Pruitt[3], and Susan E. Riechert[4]

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Abstract: *Anelosimus studiosus* is a socially polyphenic spider exhibiting both social and subsocial behaviors. Individuals can be classified as social/tolerant or solitary/aggressive phenotypes. These behavioral differences are associated with considerable variation in social structure. Populations between 26°N latitude (Florida) and 36°N latitude (Tennessee) exhibit a behavioral cline, with an increasing proportion of social colonies and social individuals occurring as northern latitude increases. In this study, we begin to examine the physiological differences that may underlie social and aggressive behavior in this species. Octopamine (OA) is a neurotransmitter, neuromodulator, and neurohormone that has been found to elevate aggression in several invertebrate species and is commonly thought of as the invertebrate counterpart of norepinephrine. Serotonin (5-HT) has been shown to interact agonistically with OA. We used High Pressure Liquid Chromatography with Electrochemical Detection (HPLC-ECD) to quantify levels of OA and 5-HT among adult females from social (multi-female) and solitary (single-female) webs in east Tennessee. A subset of spiders was scored for individual anti-predator behavior and social tendency. We found that, in general, higher octopamine levels are associated with a greater degree of aggression and intolerance, both at the individual level and the population level, while higher levels of serotonin are found in multi-female colonies and social individuals.

Keywords: *Anelosimus studiosus*, behavioral phenotype, social structure, octopamine, serotonin

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Methods

Collection and Rearing

Adult female *A. studiosus* were collected from both multi-female and single-female webs along waterways in east Tennessee (Boone Lake [*36°26'51.02" N , 82°25'41.14" W*], near Warrior's Path State Park [*36°29'43.26" N , 82°28'21.96" W*], Melton Hill Lake [*35°59'29.76"*

N, 84°11’44.55” W], and Kingsport [*36°32’40.83” N, 82°33’16.00” W*]) in the months of April through July, 2009, and May, 2010. In the laboratory, all spiders were housed in individual plastic containers (2 oz.). The spiders were maintained in stable laboratory conditions, with ad libitum food and water for a minimum of two weeks to ensure that behavior was not influenced by hunger.

Correlation of Biogenic Amines with Colony Social Structure

Octopamine and serotonin levels of individual spiders from both social and solitary webs from three populations (Boone Lake, Melton Hill, and Warriors Path) were determined using the extraction and HPLC techniques described below. A two-way ANOVA was used to identify differences in neurochemical levels (designated as responses) between ‘populations,’ ‘colony strategies,’ and ‘interactions between population and colony strategy’ (designated as factors).

Correlation of Biogenic Amines with Individual Behavior

Huddle Response

Behavioral assays were conducted in order to quantify social and aggressive tendencies of individuals. Huddle response duration (discussed above) was used as a measure of anti-predator response. Each spider was removed from her container and lowered into the center of a circular glass dish (10 cm diameter) that had been cleaned thoroughly with ethanol and allowed to dry. A puff of air from a rubber squeeze bulb was directed at the spider, and if a huddle response was induced, a stopwatch was used to record its duration (modeled after Riechert and Johns 2003; Pruitt et al. 2008). If a huddle response did not occur, another attempt was made 60 seconds later. After two attempts, if no response was elicited, the spider was put back in her container and tested 24 hours later. If, after two additional trials, the spider still did not huddle, and if she appeared otherwise normal, healthy, and active, she received a huddle response score of zero seconds. If, however, a spider did not huddle and appeared lethargic or otherwise unhealthy, she was removed from the study.

Interindividual Distance

Following Riechert and Jones (2008) and Pruitt and Riechert (2009), two randomly selected females were individually marked with colored paint and placed in the center of a square transparent plastic container (16cm x 16cm). After 24 hours, distance between the individuals (henceforth referred to as ‘interindividual distance’) was measured to the nearest 0.1 cm using a ruler and, in cases of close proximity, a caliper. Spatial orientation was also noted. If both females were occupying the same corner of the container, they were classified as ‘social’ and were subsequently used as ‘testers.’ All spiders found occupying adjacent or opposite corners were eventually tested against a known social tester. If, after 24 hours, a test subject (‘testee’) was found to occupy the same corner as the known social tester, then the testee was also labeled as social. If, however, the test subject positioned herself in an adjacent or opposite corner in relation to the tester, she was labeled as ‘solitary’. Spiders exhibiting cannibalistic behavior (as well as the victims of the cannibalistic behavior) were removed from the study. All behavioral assays were performed in the laboratory.

Extraction of Biogenic Amines

Individual body mass was recorded to the nearest 0.00001 g (Sartorius CP225D analytical balance), and each spider was placed in a 2.0 mL screw-cap vial. The vials were subsequently placed in a freezer (-20°C) for 30 to 60 minutes until spiders had expired. 1 mL of chilled (4°C) 0.2M perchlorate buffer containing internal standards (10 µg/mL syneprhine for octopamine, 30µg/mL α-methylserotonin for serotonin), along with a ¼” ceramic bead, was added to each vial. Spiders were homogenized with a MP Fast-prep 24 sample preparation system (tissue grinder), each with two runs at 4.0 m/s for 40 seconds. The vials were centrifuged at 4°C at 13,000 RPM for 10 minutes. 500 µL of supernatant from each vial was transferred to a 2.0 mL filter spin tube (Costar Spin-X, 0.22µm cellulose acetate filter) and centrifuged for 6 minutes at 13,000 RPM at 4°C to pass all supernatant through the filters. Samples were frozen at -80°C until HPLC analysis could be performed.

Analysis of Biogenic Amines

HPLC-ECD analysis was performed using an ESA Coulochem III with autoinjector and autodetector. We used an MD150 column and MD-TM mobile phase (ESA, Inc. Chelmsford, MA). Cell potential settings were -150 mV for Channel 1, +650 mV for Channel 2, and +700 mV for the guard/conditioning cell. Sensitivity was set at 50 µA. 100 µL of each thawed sample was transferred into a .25 mL crimp-top vial with polytetrafluoroethylene crimp seal and loaded into 5°C storage tray of the autosampler. Controls of pure HPLC-grade water, 0.2M perchlorate, and buffer with internal standards were analyzed at the beginning, in the middle, and at the end of every sample batch to check for contamination and establish a baseline. External standards (octopamine and serotonin) were also analyzed in every run to verify retention times of target amines. 40 µL of each sample was injected for analysis, and each sample was analyzed for 40 minutes.

Statistical analysis

Minitab 16 was used for statistical analysis. Neurochemical levels were measured using methods outlined above. Because our goal was to conduct a relative comparison between groups, we used absolute areas provided by HPLC, rather than estimated concentrations, as indicators of neurochemical content. Octopamine levels were normalized by dividing the absolute area under the curve for octopamine by that of synephrine (internal standard for octopamine) within each sample, and serotonin levels were normalized by dividing serotonin's absolute area by that of α-methylserotonin (internal standard for serotonin) within each sample. Body mass was not used to normalize data because in adult spiders (all spiders used in this study were adult females) the CNS comprises less than 2% of total body mass in some spiders and less than 1% in most (Meyer et al. 1984), and neurophysiology and neurochemistry do not change as a function of size/mass (Foelix 1996). In adult female spiders, mass is affected by diet (controlled in this study) and by reproductive condition (Foelix 1996), and we felt that the use of body mass in data normalization would be a source of introduced error.

Comparisons of means of neurochemical levels (designated as responses) between 'populations,' 'test strategies,' and 'interactions between population and test strategy' (designated as factors) were conducted by performing a 2-way ANOVA. Significance of relationships between neurochemical levels and interindividual distance, neurochemical levels and huddle response durations, and interindividual distance and huddle response durations were analyzed using regression analysis.

Results

Octopamine Among Populations and Social Strategies

A total of 160 adult female spiders from three populations in east Tennessee (Boone Lake, Warriors Path, and Melton Hill) were used to test the hypothesis that octopamine levels vary between populations and colony types (Fig. 1). Octopamine levels were significantly different between populations (two-way ANOVA: F=6.11, df=2, p=0.003), with the Boone Lake population having the lowest mean and the least variation, while Melton Hill and Warriors Path exhibited a wider range of octopamine levels. Single-female colonies had significantly higher levels of octopamine than multi-female colonies in all three populations (F=5.89, df=1, p=0.016), but there was not a significant interaction between population and colony strategy (F=0.76, df=2, p=0.469) (multi N=111, single N=49).

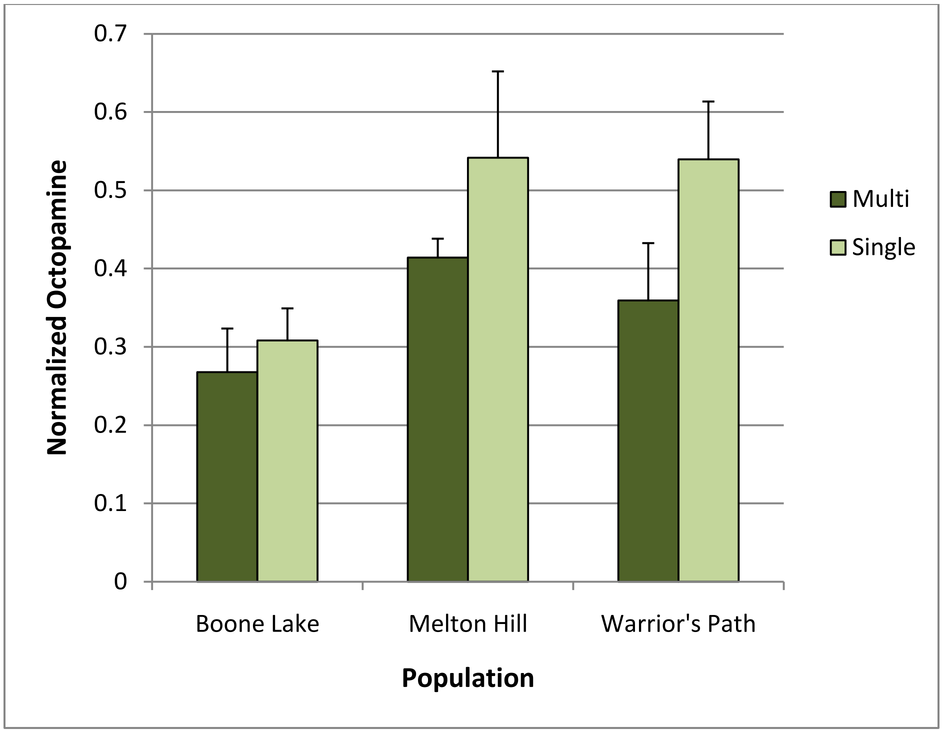

Fig. 1 Octopamine levels associated with different colony strategies. Light-colored bars indicate mean normalized octopamine levels of single-female colonies, and the dark bars indicate mean normalized octopamine levels of multi-female colonies and from three populations of *Anelosimus studiosus* in east Tennessee. Multi-female colonies consist of several to a hundred adult females and their offspring, while single-female colonies consist of one adult female and her sub-adult offspring.

Serotonin Among Populations and Social Strategies

Serotonin levels of 144 spiders from Boone Lake, Melton Hill, and Warriors Path were analyzed to determine differences between populations and between strategies within those populations. Results (Fig. 2) revealed that, like octopamine, serotonin levels were found to differ significantly between populations (F=10.71, df=2, p=<0.000). In this case however, serotonin levels were higher in colonies displaying the multi-female strategy and lower in the single-female colonies (F=15.21, df=1, p=<0.000). This trend was only found in two of the populations, resulting in a significant interaction between population and colony strategy (F=3.44, df=2, p=0.035) (multi N=88, single N=46).

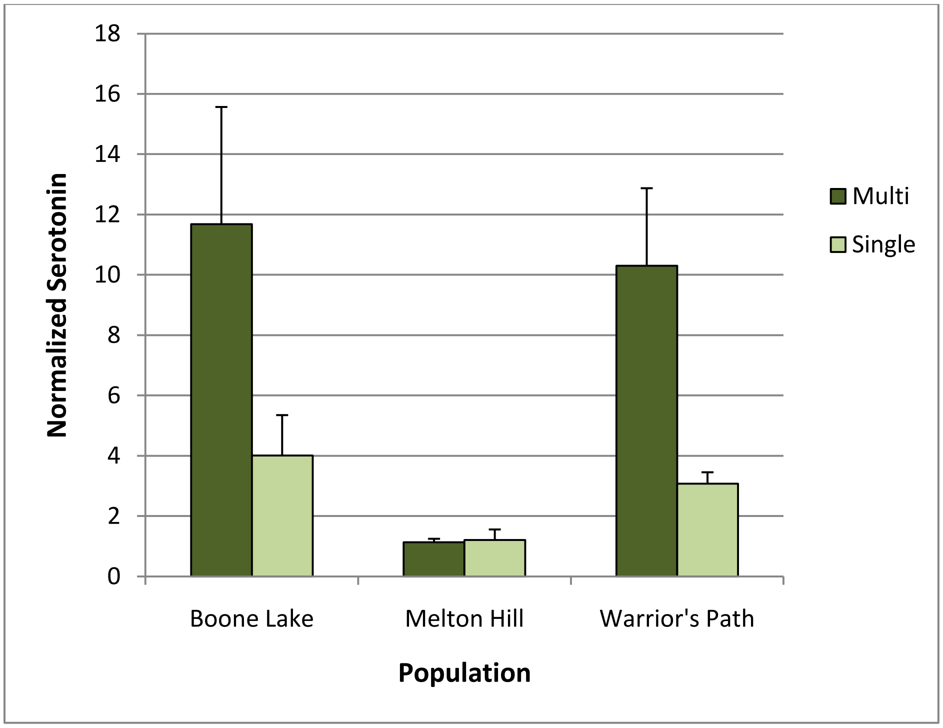

Fig. 2 Serotonin levels associated with different *Anelosimus studiosus* colony strategies in east Tennessee. Light-colored bars indicate mean serotonin levels of spiders from single-female colonies, and the dark bars indicate mean serotonin levels of spiders from multi-female colonies.

Octopamine and Individual Behavior

We scored 138 adult females from two populations (Melton Hill and Kingsport) in the laboratory behavioral assays. Of these, 117 scored as solitary, and 21 scored as social. Octopamine levels were significantly higher in spiders exhibiting the solitary behavioral phenotypes than in spiders with the social phenotype (Fig. 3) (two-way ANOVA: F=6.59, df=2, p=0.012), but there was no significant difference between the two populations (F=0.45, df=1, p=0.503). There was also not a significant interaction between population and test strategy (F=0.01, df=2, p=0.930). The mean interindividual distance between social individuals was 6.28 cm, and the mean distance between solitary individuals and their social testers was 17.25 cm.

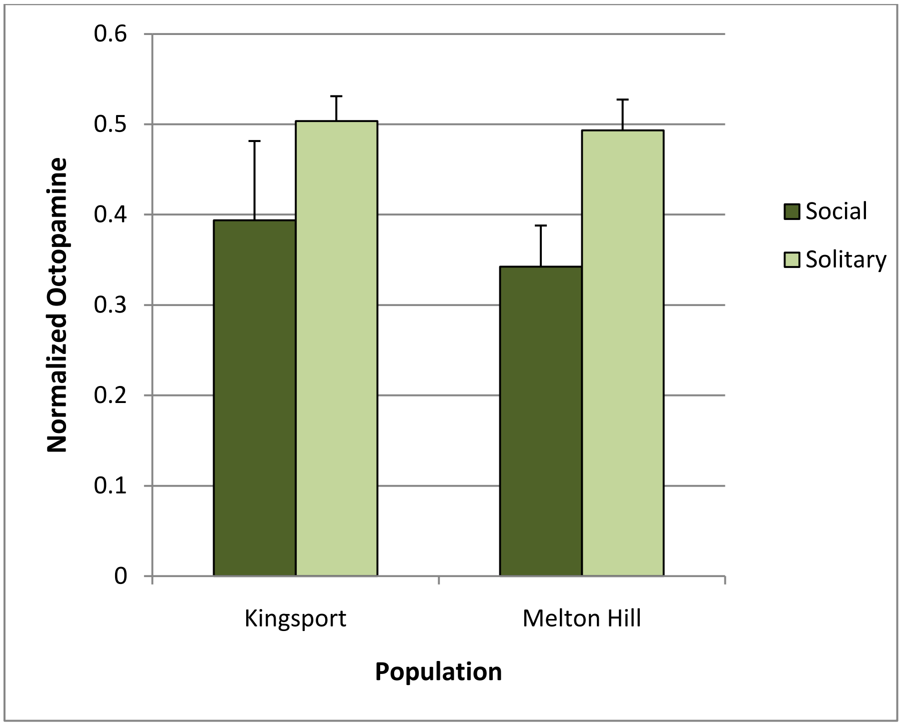

Fig. 3 Octopamine levels associated with individual spider behavior. The bars indicate normalized octopamine levels of behaviorally scored social (dark bars) and solitary (light bars) *Anelosimus studiosus* individuals from two populations.

Huddle response duration was positively correlated with octopamine levels (Fig. 4), indicating that as octopamine levels increase, huddle duration times lengthen.

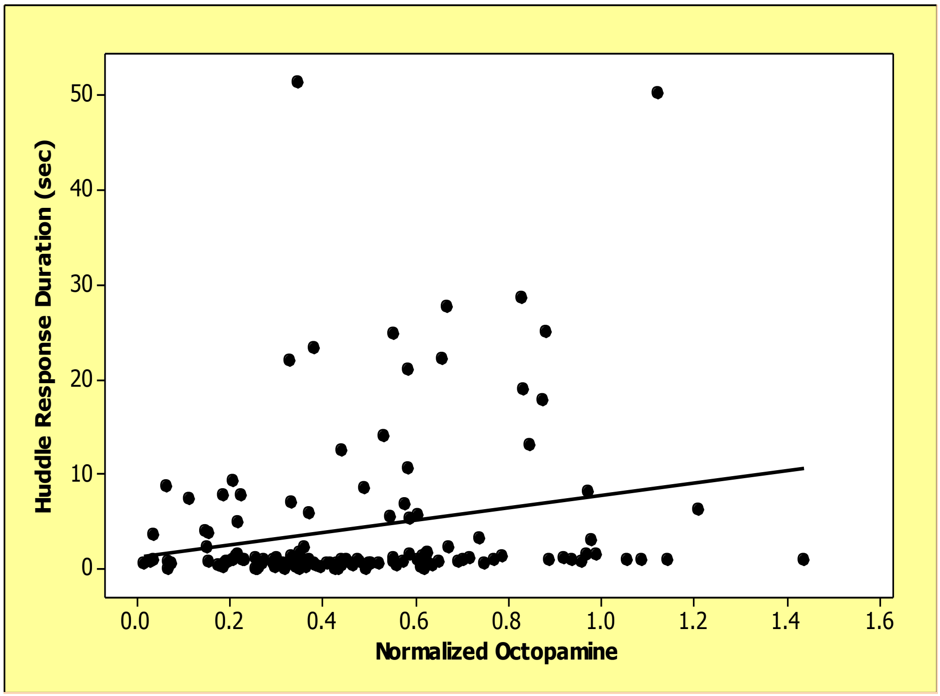

Fig. 4 Octopamine and huddle response. A scatterplot with regression fit line compares octopamine levels of individual spiders with their huddle response duration times. Huddle response duration was positively correlated with octopamine levels, indicating that as octopamine levels increase, huddle duration times lengthen. The relationship was significant (regression analysis: p=0.012, R^2=0.039)

Serotonin and Individual Behavior

Of the 138 spiders from Kingsport and Melton Hill populations scored in the laboratory behavioral assays, we could quantify serotonin levels for 115 of them. Of these, 95 scored as solitary, and 20 scored as social. There was no difference in serotonin levels between the two populations (F=0.71, df=2, p=0.400), but within them, serotonin levels were significantly higher in the social phenotype than in the solitary phenotype (F=10.41, df=1, p=0.002). There was no significant interaction between population and test strategy (F=0.14, df=2, p=0.711) (Fig. 5).

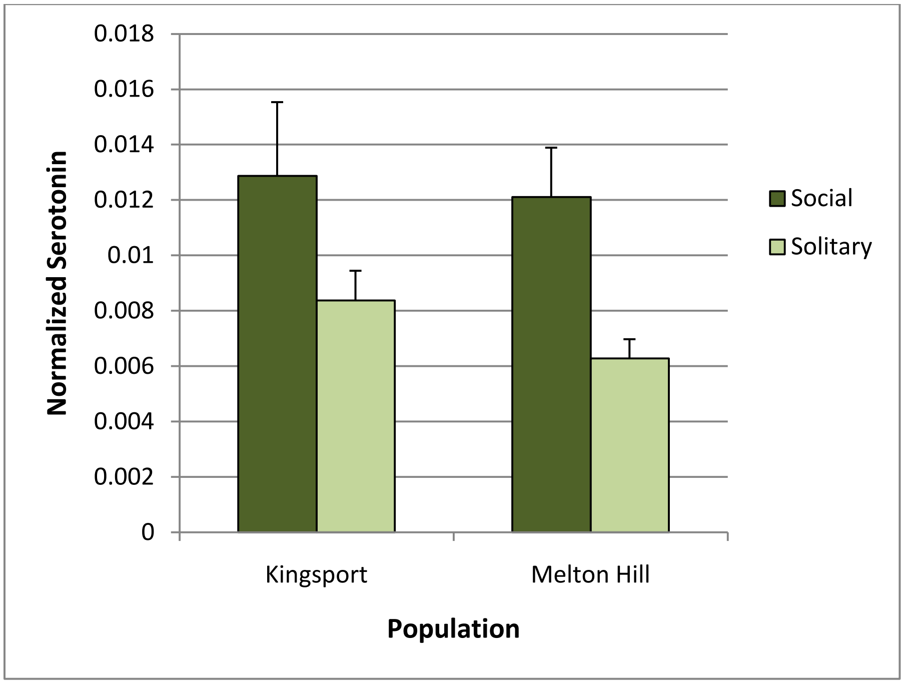

Fig. 5 Serotonin levels associated with individual spider behavior. The bars indicate serotonin levels of social (light bars) and solitary (dark bars) individual spiders from two populations. Spiders (total N=115, 95 solitary, 20 social) from Kingsport and Melton Hill populations were scored in the laboratory behavioral assays to determine social tendency based on interindividual distance. Serotonin levels were significantly higher in the social phenotype than in the solitary phenotype (F=10.41, df=1, p=0.002). There was no difference in serotonin levels between the two populations (F=0.71, df=2, p=0.400), and there was no significant interaction between population and test strategy (F=0.14, df=2, p=0.711)

Regression analysis showed that there was no relationship between huddle response duration and serotonin levels (p=0.479, R-square=0.00) (Fig. 6).

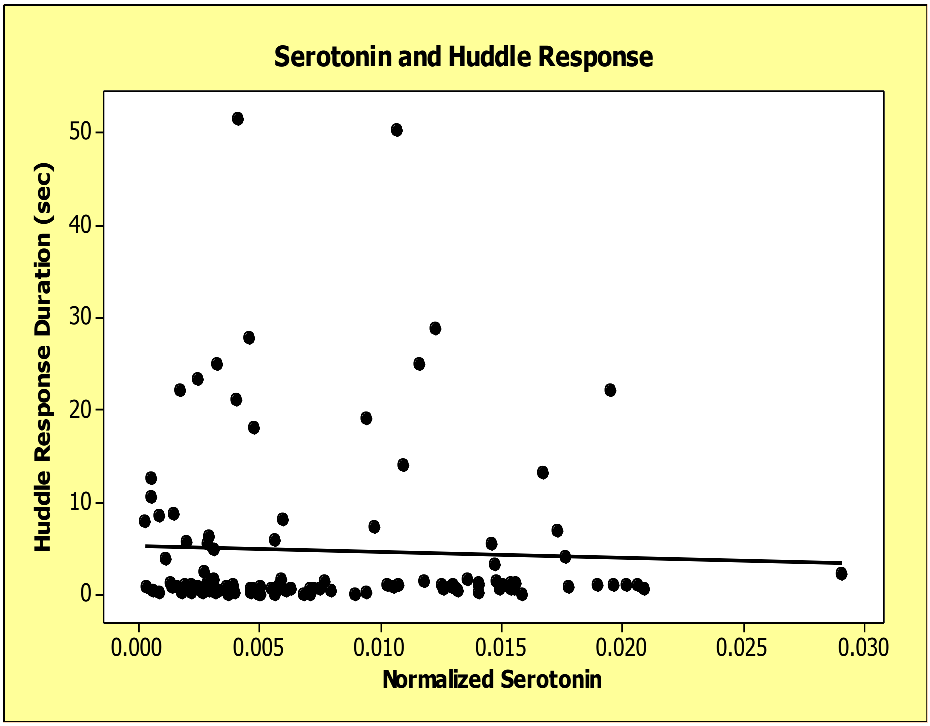

Fig. 6 Regression plot of serotonin and huddle response (p=0.479, R^2=0.00)

Discussion

The population-level study showed, as predicted, that overall octopamine levels within populations were lower in multi-female colonies than in single-female nests. We also found that serotonin levels follow the opposite trend, occurring in higher levels in the multi-female colonies than in the single-female colonies. Also, based on scores from individual behavioral assays, we determined that spiders exhibiting the ‘solitary’ phenotype generally have higher levels of octopamine and lower levels of serotonin than ‘social’ spiders. These findings support our hypothesis that greater social tendencies in spiders are associated with higher levels of serotonin, and that higher levels of octopamine may increase aggressive behavior.

Individuals of true social phenotype in *Anelosimus studiosus* are the minority. Frequency of social phenotype among spiders tested in laboratory behavior trials was 15% (21 social of 138 total), supporting findings by Riechert and Jones (2008) in which the maximum frequency of social phenotype at 36° N latitude was 14%.

Huddle response duration positively correlated with octopamine levels. This is the opposite of what we expected and could be due to differences in technique by the tester because the huddle response durations from this study were remarkably lower than measured durations from previous studies (Pruitt and Jones, unpublished data). Regression analysis showed that the relationship was significant (p=0.012, R-square=0.039). It should be noted that the scatter of the points around the regression fit line is loose, and the value of the fit line is not predictive.

Our focus on octopamine and serotonin is not to ignore the possible effects of other neurochemicals in the social and aggression-related behaviors of spiders. Future research in this area should focus on behavioral effects of additional neurotransmitters and their interactions, effects of environmental factors on neurochemical levels, and assessing the neurochemical differences between and within additional populations of *A. studiosus*.

Conclusions

Anelosimus studiosus offers a unique opportunity to study the neurochemical underpinnings of social behavior. The results of this study support the hypothesis that octopamine and serotonin levels are related to social behavior in *A. studiosus*. Between and within populations, variations in social and aggressive behavioral tendencies correlate with physiological levels of OA and 5-HT. As far as we know, this is the first description of the neurochemical underpinnings of population-level differences in social structure. Additional studies are needed to further explore the effects of neurochemicals on behavior, social structure, and their potential correlation with the evolution of sociality in spiders.

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All experiments described here within comply with the current laws of the United States. The authors declare that they have no conflict of interest.

CHAPTER 3

CULMINATION

Discussion

Animal behavior is the result of a complex set of interactions between multiple factors. Externally, we can observe the actions of an animal in relationship to its environment and attempt to make connections and assumptions regarding which stimuli induce which responses. However, a true understanding of behavioral mechanisms cannot occur without an investigation of internal processes.

Genetics, epigenetics, nutrition, metabolism, conditioning (experience), neuroanatomy, and neurochemistry all play a role in dictating the relationship between an animal's internal and external worlds. Narrowing the study focus to interactions between animals of the same species provides an excellent opportunity to consider the combined effects of internal and external causes and effects of the evolution of social behavior.

We believe, based on the absence of written and oral indications of the presence of the large multi-female colonies in this area prior to 1998 (Furey 1998), that there has been an increase in the proportion of social individuals and conspicuous multi-female colonies since the introduction of artificial impoundments along the waterways of the Tennessee Valley watershed by the Tennessee Valley Authority beginning in 1933 (Ezzell 2010), which has resulted in cooling of areas immediately below dams where cold water from deep lake bottoms is released.

Given that (1) the natural variation in individual behaviors observed in *Anelosimus studiosus* is heritable (Jones and Riechert 2008; Riechert and Jones 2008; Pruitt and Riechert

2009), and (2) considering the fact that they have a large range that spans from tropical to subtropical to temperate climates, and (3) the spiders living in multi-female colonies in cooler microclimates experience greater relative fitness than those living in single-female webs (Jones et al. 2007), we are seeing natural selection in action. The result of this natural selection is that we are witnessing the local evolution of populations of *A. studiosus* as they adapt to their local environment.

Our goal with this study was to investigate and correlate the neurophysiological differences that accompany this behavioral adaptation. The population-level study showed, as predicted, that overall octopamine levels within populations were lower in multi-female colonies and higher in single-female nests. We also found that serotonin levels follow the opposite trend, occurring in higher levels in the multi-female colonies and lower levels in the single-female colonies. Also, based on scores from individual behavioral assays, we determined that spiders exhibiting the ‘solitary’ phenotype generally have higher levels of octopamine and lower levels of serotonin than ‘social’ spiders. These findings support our hypothesis that greater social tendencies in spiders are associated with higher levels of serotonin, and higher levels of octopamine correlate with decreased tolerance of conspecifics.

Individuals of true social phenotype in *A. studiosus* are certainly the minority. Frequency of social phenotype among spiders tested in laboratory behavior trials was 15% (21 social of 138 total), supporting findings by Riechert and Jones (2008) in which the maximum frequency of social phenotype at 36° N latitude was 14%.

Our focus on octopamine and serotonin is not to ignore the possible effects of other neurochemicals in the social and aggression-related behaviors of spiders.

Conclusions

Anelosimus studiosus offers a unique opportunity to study the neurochemical underpinnings of social behavior. The results of this study support the hypothesis that octopamine and serotonin levels are related to social behavior in *A. studiosus*. Between and within populations, variations in social and aggressive behavioral tendencies appear to correlate with physiological levels of OA and 5-HT.

As far as we know, this is the first description of the neurochemical underpinnings of population-level differences in social structure. Based on the correlations we see here, and based on Weber's recent phylogenetic findings (unpublished data), it is possible that geographically separated populations of *Anelosimus studiosus* are finding different pathways to evolve sociality.

Additional studies are needed to further explore the effects of neurochemicals on behavior, social structure, and their potential correlation with the evolution of sociality in spiders.

Future Directions of This Research

The next important step in the process of investigating this phenomenon will involve an ontogenetic study of the neurochemical changes that occur throughout the development and life stages of *Anelosimus studiosus*. Future research in this area should focus on behavioral effects of additional neurotransmitters, and their interactions, via experimental administration of exogenous monoamines. Effects of environmental factors on neurochemical levels including the effects of varied food intake (scarce versus abundant prey), varied climate (hot versus cold, arid versus humid), and varied proximity to conspecifics should be investigated.

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APPENDIX

“Behavioral Ecology and Sociobiology” Instructions for Authors

- Types of papers
- "Behavioral Ecology and Sociobiology" accepts Reviews, Original Papers, Methods papers and Commentaries:
    - Reviews should cover a topic of current interest. There is no limit to the length of a review.
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