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Molecular evidence suggests multiple evolutionary origins of sociality in the polyphenic spider
Anelosimus studiosus (Araneae: Theridiidae)

A thesis
presented to
the faculty of the Department of Biological Sciences
East Tennessee State University

In partial fulfillment
of the requirements for the degree
Master of Science in Biology

by
Nathaniel O Weber
December 2010

Thomas C Jones, Chair
Foster Levy
Karl Joplin
Thomas Laughlin

Keywords: *Anelosimus studiosus*, local evolution, evolution of sociality, social polyphenism, microsatellites

ABSTRACT

Molecular evidence suggests multiple evolutionary origins of sociality in the polyphenic spider
Anelosimus studiosus (Araneae: Theridiidae)

by

Nathaniel O Weber

Anelosimus studiosus exhibits two behavioral phenotypes: subsocial and social. This is the only documented spider inhabiting a temperate climate exhibiting social behavior. While the subsocial phenotype is most common throughout the range, the social behavior occurs in isolated pockets in northern latitudes. This study examines the origins of the social phenotype within a segment of the spider's range. Two hypotheses are tested: 1) pockets of social behavior represent a single origin or 2) pockets of social behavior represent local evolutions, thus leading to multiple origins of evolution. Microsatellite loci were used to determine genetic structure of the population and to estimate the origins of social behavior. All loci showed lower observed than expected heterozygosities and all populations show indications of high levels of inbreeding. A phylogeny indicates four of the six populations fall out by location, not phenotype. We propose these results reflect multiple local evolutions of the social strategy.

DEDICATION

To TJ, Emily, and David.....thanks!

ACKNOWLEDGMENTS

Great appreciation to my committee Tom Laughlin, Karl Joplin, and Foster Levy for thoughtful and forthright comments. Thank you to D. Kumar for all the help, to A. Bray for answering my numerous questions, and to the Department of Biological Sciences of ETSU and Mikey Z for the all mighty dollar.

And to TJ, with your distractive but ideally attentive personality that somehow perfectly guided me through this process. Your attention was hard to keep, but those times when I needed it, your attention was complete. There were ups, and there were downs, but I would not have changed a thing. Thank you!

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CHAPTER 1
INTRODUCTION

Sociality

Sociality in spiders is rare, as aggressive and solitary behaviors describe the majority of the nearly 42,000 spider species (Platnick 2010). However, spider species exhibit a wide range of behaviors that vary from solitary nests to aggregations of individual webs to communal colonies. Solitary nests consist of an adult female and the current offspring generation. Multiple solitary nests can be found in a common location, but each web is maintained individually without cooperation between spiders. Communal colonies are those in which multiple adult females and offspring cooperate in nest tasks. Varying stages of sociality have been extensively described in the literature but in only 23 species (for review see Avilés 1997; Uetz & Hieber 1997). Species that have evolved sociality; *Anelosimus eximius* (Theridiidae), *Agelena consociata* (Agelenidae), *Stegodyphus mimosarum* (Eresidae), benefit from cooperation but also must deal with potential costs. Selection for sociality may increase the fitness of the individual as a function of group cooperation (Whitehouse & Lubin 2005), but the evolution of sociality requires the benefits of those behaviors to outweigh the costs. An individual can increase its fitness by either reproducing (direct fitness) or providing for an increase in reproduction of siblings/relatives (indirect fitness) (Hamilton 1964). Increased fitness can be achieved by group living or cooperative behaviors as cooperative brood care can increase the indirect fitness of the population. These behaviors include caring for the egg case of or fostering the offspring of a related adult female.

In subsocial species (Wilson 1971; Krafft 1979), also described as non-territorial periodic-social (Avilés 1997), nests consist of a single adult female and her offspring from the most recent generation. Non-territorial periodic-social refers to the time of delayed juvenile dispersal and extended maternal care. These two behaviors typify subsocial species (Wilson 1971) and have been described in the spider families Theridiidae, Eresidae, and Desidae (see Avilés 1997). The juveniles remain in the natal web until they reach maturity when they disperse. Adult females are aggressive towards other conspecific adult females.

Social species, also described as “quasi-social” (Wilson 1971), non-territorial permanent social (Avilés 1997), and cooperative (Riechert 1985), are typified by multiple adult females and various aged offspring maintaining a common nest and cooperatively participating in web maintenance, prey capture, and brood care. Cooperative brood care, where egg sacs and young can be cared for by multiple females, increases the likelihood of survival in the event of the death of a mother (Jones *et al.* 2007). As offspring mature, they increasingly participate in tasks within the colony which is presumed to increase the overall fitness of the group (Jones & Parker 2002). Cooperative behavior can potentially allow for an increase in prey capture as a larger, more robust nest and multiple adult females improve the chance of the capture of larger or more prey (Nentwig & Christenson 1986). This ability to increase prey capture can lead to increased survivorship of a social colony. Most social species of spider are tropical, as optimal year round weather conditions allow for the development and maintenance of social behavior (see Avilés 1997).

Sociality in spiders has repeated origins across phylogenetically distant groups with 18 or 19 independent evolutions among only 23 species (Agnarsson *et al.* 2007). This indicates the transition to sociality, though rare, has occurred multiple times and at different locations. Most social species are phylogenetically adjacent to species that exhibit subsocial behavior, suggesting that subsociality is antecedent to permanent sociality (Agnarsson *et al.* 2007). Congruent to the evolution of sociality is a transition from outbred to inbred mating systems.

Routes to Sociality

Sociality in spiders can be reached by two routes: parasocial and subsocial. The parasocial route to sociality can be characterized by aggregations of individual spiders in a common area. These aggregations of typically aggressive solitary spiders can form colonies. In some species, these aggregations can be temporary, lasting for only part of or for one generation or, in other species, can be permanent, lasting successive generations. The individuals share supporting web structure; however, they forage on individual orbs (Uetz & Hieber 1997) and exhibit no maternal care beyond egg laying (see

Whitehouse & Lubin 2005). The subsocial pathway leads to sociality through extended maternal care and delayed offspring dispersal. This leads to varying degrees of family interactions on a single web.

Species can be pre-adapted for sociality (Krafft 1979) due to delayed juvenile dispersal, extended maternal care and the ability to use a common web. This predisposition may have arisen from prolonged interaction with conspecifics and reduced pre-mating dispersal (Kullmann 1972; Avilés 1997).

One critical question is the mechanism by which the alternative social behavior is evolving. Because sociality in spiders is so rare and phylogenetically scattered, the commonly accepted hypothesis is that the solitary phenotype is the ancestral state (Avilés 1997; Agnarsson *et al.* 2006). Species that exhibit both subsocial and social phenotypes in the same area can provide insight into the evolution of sociality.

Microsatellites

Microsatellites are simple sequence tandem repeats of usually 1-6 nucleotides occurring in the nuclear genomes of most taxa. Microsatellites are in non-coding regions, either intergenic regions or introns, and presumably mutate neutrally (Ellegren 2004), but at high rates, in a stepwise fashion (for review see Selkoe & Toonen 2006). Their location and mutation patterns create high allelic diversity in individuals through successive generations, making them appropriate for population-level studies. These characteristics have positioned microsatellites as powerful genetic markers. The analysis of microsatellite variation has allowed estimations of relatedness in many species including ants (Goropashnaya *et al.* 2001), mites (Carbonnelle *et al.* 2007), bees (Paxton *et al.* 1996), crab spiders (Evans & Goodisman 2002) and sheep (Mukesh *et al.* 2006) and have been used in phylogenetic estimates in animals: *Drosophila* (Orsini *et al.* 2004) and bettongs (Pope *et al.* 2000) as well as plants: *Cicer reticulatum* (Sethy *et al.* 2006) and *Sinojackia* (Yao *et al.* 2008). Microsatellites allow for the estimation of evolutionary history of a given population over time, which can be combined with the geography of the organism to develop a phylogeography of the species as it evolves. The validity of using microsatellites markers in *Anelosimus studiosus* was established by Duncan *et al.* (2010).

Anelosimus studiosus

The comb-footed spider, *Anelosimus studiosus* (Hentz) (Theridiidae), has a range from Argentina in South America to New England in North America. It has been described as a subsocial species (Brach 1977) as throughout most of its range are webs containing single adult females that exhibit extended maternal care and delayed juvenile dispersal. A subsocial nest consists of a single adult females and the most recent offspring generation. After dispersal of the current generation a second brood, though uncommon, can be produced (Jones & Parker 2002). Juveniles are fed regurgitated food until odd enough to take prey without assistance. Interspersed within the larger and most common subsocial nest populations in northern latitudes are isolated pockets of social colonies. Though *A. studiosus* was previously documented in eastern Tennessee, Furey (1998) was first to describe the social colony structure of the species as far north as 36°N, a temperate climate. Furey also described evidence for social behavior (e.g. cooperative feeding, brood care, and web maintenance) and noted that subsocial nests are generally annual while social colonies are perennial. The presence of social colonies in northern temperate climates is contrary to the notion that year- round activity in the tropics allows for social behavior, whereas shorter optimum seasons at higher latitudes better suit solitary colonies (Lin & Michener 1972). All other described social spider species are only found in tropical climates, which highlights the uniqueness of this system. Social colonies of *A. studiosus* are most commonly found along waterways, apparently having some level of dependency on water bodies. Social colonies in the southeastern United States are found in correlation with increasing latitude; from 1% at 26°N to 33% at 36°N (Riechert & Jones 2008). A model suggest that the transition to sociality in *A. studiosus* is a “bet-hedging” strategy (Jones *et al.* 2007), predicting mothers will forgo their potential maximum relative fitness for a more stable, but less-than-maximal relative fitness, giving the best average chance of success.

Hypotheses

Locations with both subsocial and social phenotypes may provide insight into the evolutionary question of how the social strategy is evolving. Presuming that the social behavior has evolved from the ancestral subsocial phenotype, this study examines two possible hypotheses for evolution of the social strategy.

First, the number of origins of evolution of social behavior can be few, potentially only one, with the split in behaviors occurring further back in evolutionary history. Each phenotype would belong to a separate lineage and the social strategy would spread simply by range expansion of the strategy or of the species as a whole. In this case, individuals of the same phenotype would form a monophyletic group regardless of location. The limited evolution hypothesis, hypothesis 1 (Figure 1A), predicts the number of origins of sociality are few, grouping individuals by phenotype not location. Evidence for limited evolution can be seen in *Halictus rubicundus* (Soucy & Danforth 2002). They found a limited number of origins of alternate behavior with individuals expressing different phenotypes belonging to phylogenetically separate lineages. Alternatively, the social strategy could be evolving multiple times from the ancestral phenotype locally across the population. For this, individuals would group together according to location not phenotype. The local evolution hypothesis, hypothesis 2 (Figure 1B), predicts many local origins of social behavior. Evidence of local evolution can be seen in a *Tetramorium* ant (Schlick-Steiner *et al.* 2007). The ancestral phenotype in *Tetramorium moravicum* is a strategy of a single large queen while the alternate strategy is multiple small queens in the same nest. This species shows two distinct geographical lineages with the alternate strategy evolving separately in both. In our study, two mutually exclusive hypotheses (Figure 1) are tested to gauge the evolutionary relationship of subsocial and social populations of *A. studiosus*.

We examined populations in a 600 km range in east Tennessee along the Tennessee River watershed. Six microsatellite loci were used to test for genetic differentiation and estimate the number of origins of evolution of social behavior in the study population.

CHAPTER 2

ARTICLE FOR SUBMISSION TO *MOLECULAR ECOLOGY*

Molecular evidence suggests multiple evolutionary origins of sociality in the polyphenic spider

Anelosimus studiosus (Araneae: Theridiidae)

Nathaniel O Weber¹ and Thomas C Jones¹

Department of Biological Sciences, East Tennessee State University, Johnson City, TN 37614 USA

Corresponding author: Thomas C Jones - email: jonestc@etsu.edu tel: 4234396930

Keywords: *Anelosimus studiosus*, local evolution, evolution of sociality, social polyphenism, microsatellites

Abstract

The northern social spider, *Anelosimus studiosus*, exhibits two behavioral phenotypes: subsocial and social. This is the only documented spider species inhabiting a temperate climate exhibiting social behavior. While the subsocial and aggressive, phenotype is most common and ubiquitous throughout the range, the alternate social behavioral phenotype occurs in small isolated pockets in northern latitudes. This study examines the evolution of the social phenotype within a segment of the spider's range. Two mutually exclusive hypotheses are tested: 1) the pockets of social behavior represent a single origin of evolution that is spreading through the population; possibly in concert with range expansion or 2) the pockets of social behavior represent local evolutions within a locale, thus leading to multiple origins of social behavior. In six locations where both phenotypes are observed, six microsatellite loci were used to determine genetic structure of the population and to estimate the evolution of social behavior. All loci showed lower observed than expected heterozygosities and all populations showed indications of high levels of inbreeding. This suggests the demography of subsocial populations could purge deleterious alleles, facilitating the transition to sociality. An UPGMA analysis indicates four of the six populations group by location, not phenotype. We propose these results reflect multiple, and possibly recent, local evolutions of the social behavior.

Introduction

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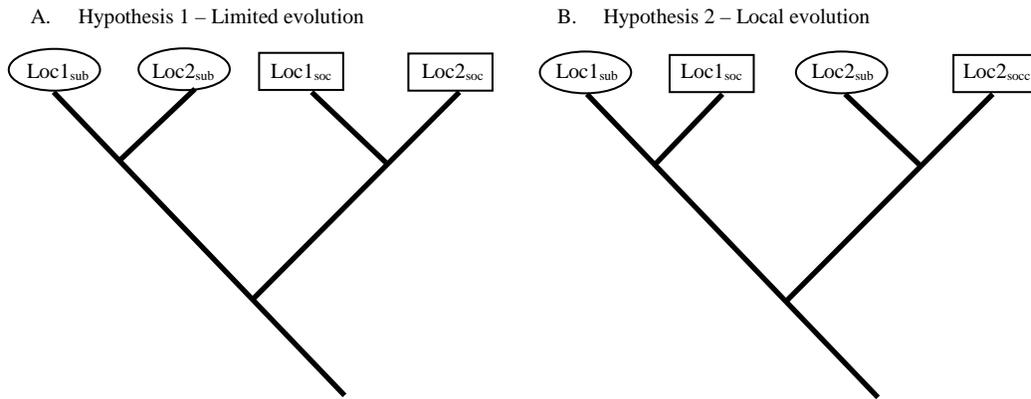


Figure 2.1 Graphical representation of hypotheses. Hypothetical populations are indicated by collection site (Loc1 or Loc2) and phenotype (sub = subsocial, soc = social) with tree A indicating limited origins and tree B local (multiple) origins of evolution of social behavior

We examined populations in a 600 km range in east Tennessee along the Tennessee River watershed. Six microsatellite loci were used to test for genetic differentiation and estimate the number of origins of evolution of social behavior in the study population.

Materials and Methods

Sampling

A study area was defined along the Tennessee River watershed in northern Alabama and eastern Tennessee. The total distance by waterway of the study was 600 km from Guntersville Lake, AL (34°N) to Steele Creek Lake, TN (36°N). Collections were completed between June and October 2008 and in August 2009. Locations were selected where known *A. studiosus* populations existed and where both phenotypes were present and interspersed. Individuals were taken from multiple colonies of each

phenotype per site to decrease the chance of sampling siblings. Sampling in this manner was an attempt to reduce clustering based on relatedness. A total of 113 individuals were sampled from 6 localities (Table 1, Figure 2) with sample sizes as follows: $BL_{soc} = 10$, $BL_{sub} = 9$; $WP_{soc} = 10$, $WP_{sub} = 10$; $SC_{soc} = 8$, $SC_{sub} = 10$; $MH_{soc} = 9$, $MH_{sub} = 9$; $KN_{soc} = 9$, $KN_{sub} = 9$; $GL_{soc} = 10$, $GL_{sub} = 10$. Specimens were collected in the field and transported to the laboratory for preparation. Individuals not immediately prepared were preserved in 70% ethanol prior to DNA extraction.

Table 2.1 Collection site information: site abbreviation, GPS coordinates, elevation, sample size of social, subsocial, and total individuals

Location	GPS Coordinates	Elevation (ft)	N_{soc}	N_{sub}	N_{tot}
Boone Lake (BL)	36°26'51.02" N 82°25'41.14" W	1385	10	9	19
Warriors Path (WP)	36°29'43.26" N 82°28'21.96" W	1264	10	10	20
Steele Creek (SC)	36°34'16.55" N 82°13'58.75" W	1581	8	10	18
Melton Hill (MH)	35°59'29.76" N 84°11'44.55" W	795	9	9	18
Kingston (KN)	35°51'53.41" N 84°32'37.04" W	743	9	9	18
Guntersville, AL (GL)	34°22'19.85" N 86°14'55.58" W	601	10	10	20

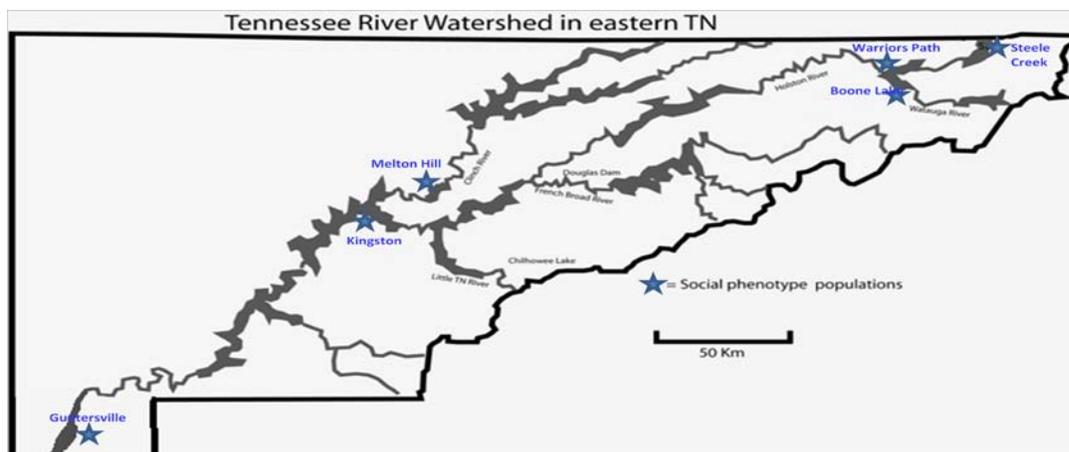


Figure 2.2 Locations of collection sites

DNA extraction and amplification

DNA from living samples was extracted following the Qiagen DNeasy July 2006 protocol for Animal Tissue. Non-living, preserved samples were air dried for 15 minutes prior to DNA extraction as residual ethanol can interfere with DNA extraction. Adult female *A. studiosus* were ground in 180 μ L lysis Buffer

ATL followed by 20 μ L proteinase K. The samples were incubated at 57°C for 4 hours then vortexed. Next, 200 μ L of both Buffer AL and ethanol were combined and added to the sample. The tube was then centrifuged thrice with corresponding applications of 500 μ L Buffer AW1 and AW2 and a final application of 200 μ L Buffer AE. The resulting DNA material was refrigerated until used.

Six microsatellite loci we used were amplified using a modified version of the protocol in Duncan *et al.* (2010). PCR reactions were run with a final volume of 20 μ L, consisting of 10 μ L Sigma Aldrich ReadyMix, 6.4 μ L biology grade water, 0.8 μ L forward and reverse primers and 2 μ L template DNA. All amplifications were performed using a Bio Rad MyCycler thermocycler with general parameters as follows: an initial denaturation of 94°C for 3 min, 35 iterations of 94°C for 40s, annealing at 55.7 – 57.6°C (optimized by locus – Table 2) for 45s, and extension at 72°C for 35s. That was followed by 72°C for 20 min and a final hold at 10°C.

Table 2.2 Six polymorphic loci information: forward (F) and reverse (R) primer sequence, optimal annealing temperature, allele range, number of alleles per locus, dilution concentration, repeat motif and HWE deviation

Locus	Primer Sequence	T _{opt} (°C)	Allele range	Allele per locus	Dilution conc.	Repeat motif	HWE deviation p-value
D5	F – AGAGCCACTAAAGCAAGCA R – TAAGGGCATTTTTGTAGCG	55.7	105 – 153	47	1:10	TAGA	0.1522
D225	F – AATTCGACTGTCGTATCC R – TCAGGGGCATTTTAGATTC	55.6	223 – 265	9	1:10	TAGA	< 0.0001
C106	F – AAGCAAAATGCCTCCTT R – GCTCAGAAGACGAGTGATTC	55.7	139 – 182	10	1:50	ATG	< 0.0001
B112	F – CGTCATCTAAACGTGGTTC R – TAGCTTGATGTTGTCCAGTTT	56.8	142 – 309	29	1:25	AAC	< 0.0001
D103	F – TCCAACGGCTGTCATTTTC R – GGGGCACCTGGTAACATT	56.8	99 – 187	12	1:25	TATC	< 0.0001
D110	F – GGAGAAATTCTGTCAAATCTGG R – GGCGATGTTACCTTTATTAACG	57.6	218 – 271	16	1:75	TAGA	< 0.0001

Agarose gel electrophoresis was used to ensure adequate product was being obtained. For analysis, loci were multiplexed by individual, diluted (dilution concentrations in Table 2) and combined with formamide to a final volume of 10 μ L. GeneScan 600 LIZ Standard, a size standard used with fragments

between 20 and 600 bp, was added and analysis was completed at Yale DNA Facility on an Applied Biosystems 3730xl 96-Capillary Genetic Analyzer.

Data Analysis

Microsatellite fragments for each individual locus pair were visualized and scored with Softgenetics® GeneMarker® (www.softgenetics.com) with each specimen assigned a genotype for each locus.

Genepop 4.0.10 (Raymond & Rousset 1995) was used to calculate alleles per locus, allele frequencies, heterozygosities, F statistics, and deviations from Hardy-Weinberg equilibrium (HWE).

Analyses of Molecular Variance (AMOVA) were carried out using Arlequin (Excoffier *et al.* 2005) to examine potential population differentiation. This program partitions the sum of the squared deviations into hierarchical variance components. In Duncan *et al.* (2010), AMOVA was used to determine if the phenotypes represent demographically separated populations, focusing on a single population covering 200 m (two-100 m transects), and found no detectable genetic differentiation between subsocial (subsocal referred to as solitary in Duncan *et al.* (2010)) and social phenotypes. We expanded this to test for population differentiation throughout our study range, roughly 600 km, running two AMOVAs to gauge the levels of differentiation throughout the study area. Two levels of AMOVA were used. The first level had two groups with each phenotype making a group. The second level had six groups with each collection site making a group. These levels provide insight to the cause of variation in the population.

A potential drawback when using microsatellites for phylogenetics is the presence of null alleles. Any allele that continually fails to amplify can be defined as a null allele and is usually caused by poor primer annealing caused by point mutations. It is important to accurately accommodate for null alleles as failure to do so may lead to over-estimates of homozygosity and false deviations from Hardy-Weinberg Equilibrium. To account for null alleles, the software package ML-Relate (Kalinowski *et al.* 2006) was used and the data set was analyzed with and without assumptions of null alleles. Finding no differences between the two analyses, we report the data assuming no null alleles. Dendrograms were created with POPTREE2 (Takezaki *et al.* 2010) by UPGMA using Nei's D_A distance (Nei *et al.* 1983). Unweighted

pair group method with arithmetic mean (UPGMA) is a method of hierarchical clustering commonly used in reconstruction of phylogenetic trees from diploid markers (i.e. Petren *et al.* 1999; Sethy *et al.* 2006; Yao *et al.* 2008;).

Results

Phylogenetic analysis

The clustering of the study populations was evaluated by construction of a UPGMA-based tree (Figure 3). In four of the six population pairs (Kingston, Warriors Path, Melton Hill, Guntersville) the tree significantly (by bootstrap values indicated on tree) grouped the phenotypes within each location; indicating the phenotypes are more similar within location rather than over the whole study area.

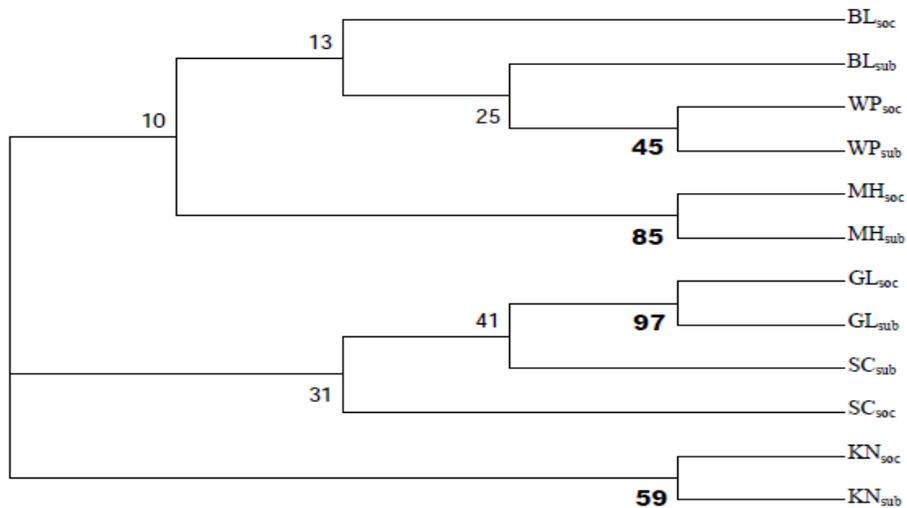


Figure 2.3 Dendrogram of collection sites: using UPGMA with significant bootstrap values indicated in bold. Labels indicate location and phenotype (subscript: soc = social, sub = subsocial)

Not as strong support exists for the Kingston population forming its own group. Steele Creek populations do not group together and cluster with populations from Guntersville Lake, AL. Though support is not as strong, populations from Boone Lake and Warriors Path tend to group together, potentially representing a lack of differentiation due to geographical proximity (11 km).

Genetic structure

Genetic data for populations and loci are summarized in Table 3. All populations showed significantly lower observed heterozygosities (mean $H_{OBS} = 0.39$) compared to expected (mean $H_{EXP} = 0.65$) by Hardy-Weinberg supporting the position that social spider species exhibit inbreeding. Five of six loci showed deviations from Hardy-Weinberg, with marker D5 being within parameters of equilibrium (Table 3).

Table 2.3 Location and phenotype data: expected and observed heterozygosities and HWE deviation

Location	Phenotype	H_{EXP}	H_{OBS}	HWE deviation p-value
Boone Lake (BL)	Subsocial	0.72	0.62	p = 0.0016
	Social	0.79	0.44	p < 0.0001
Warriors Path (WP)	Subsocial	0.69	0.40	p < 0.0001
	Social	0.72	0.43	p < 0.0001
Steele Creek (SC)	Subsocial	0.67	0.33	p = 0.0002
	Social	0.65	0.34	p < 0.0001
Melton Hill (MH)	Subsocial	0.57	0.49	p = 0.0049
	Social	0.59	0.37	p < 0.0001
Kingston (KN)	Subsocial	0.39	0.10	p = 0.0009
	Social	0.61	0.32	p < 0.0001
Guntersville, AL (GL)	Subsocial	0.74	0.45	p < 0.0001
	Social	0.64	0.39	p < 0.0001

Two models of AMOVA (Table 4) were used to estimate variation between phenotypes (A) and among locations (B). The largest amount of variation was explained by differences among individuals within populations (A: 31.54%, B: 31.04) and within individuals (A: 49.13%, B: 48.36) in both models. Both phenotypes show high levels of inbreeding, subsocial $F_{IS} = 0.39$ (p < 0.0001); social $F_{IS} = 0.52$ (p < 0.0001). Differences between phenotypes (Table 4a) account for no (~0.0%) variation, while differences between locations (Table 4b) contribute to the variation (~8%). F_{CT} (among group index) values are low among phenotypes (A: $F_{CT} = -0.02$, p = 0.9045) indicating little contribution to shaping the population.

Table 2.4 AMOVA results from Arlequin: source of variation, percent of variation from source and fixation indices. Part A: data in two groups, by phenotype. Part B: data in six groups, by location

A.				B.			
Cause of variation	Percent variation	Fixation index	p-value	Cause of variation	Percent variation	Fixation index	p-value
Among phenotypes	0.0	$F_{CT} = -0.017$	0.9045	Among locations	7.67	$F_{CT} = 0.08$	< 0.0001
Among populations within phenotypes	21.03	$F_{SC} = 0.21$	< 0.0001	Among populations within locations	2.92	$F_{SC} = 0.14$	< 0.0001
Among individuals within populations	31.54	$F_{IS} = 0.39$	< 0.0001	Among individuals within populations	31.04	$F_{IS} = 0.39$	< 0.0001
Within individuals	47.43	$F_{IT} = 0.51$	< 0.0001	Within individuals	48.37	$F_{IT} = 0.52$	< 0.0001

For variation among locations a small but significant effect is indicated (B: $F_{CT} = 0.08$, $p < 0.00010$). A regression of F_{ST} by geographic distance (Figure 4) indicates a weak but significant correlation between the two ($R^2 = 0.012$, $p = 0.03$).

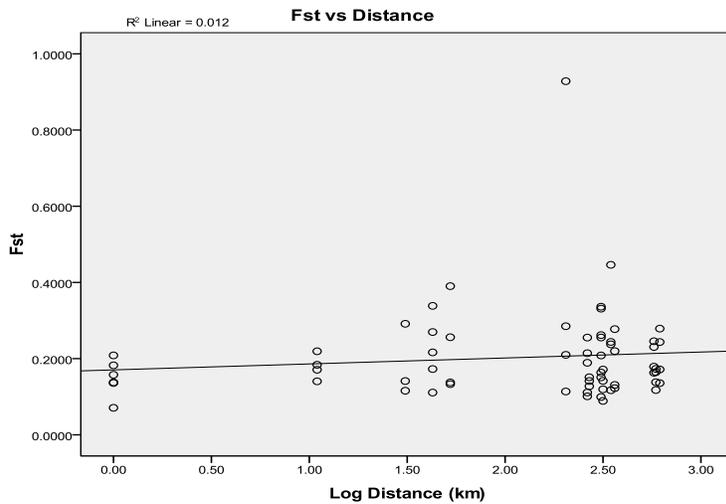


Figure 2.4 F_{ST} / Distance regression: F_{ST} regressed with log transformed distance by waterway.

Discussion

Anelosimus studiosus provides a distinctive system in which to investigate the transition to social behavior. With the species exhibiting both subsocial and social behavioral phenotypes sympatrically, insight is provided into many of the questions surrounding the evolution and maintenance of sociality (Jones *et al.* 2007; Jones & Riechert 2008). The clustering seen on the dendrogram suggests phenotypes are more similar genetically within locations than across the study area. These results support hypothesis 2 of multiple local evolutions of social behavior. It is generally accepted the subsocial phenotype is ancestral to the social strategy (Avilés 1997; Agnarsson *et al.* 2006) and given the prominence of subsocial nests across the study range, we presume this is also the case in *A. studiosus*. Considering this and the cluster strength in Warriors Path, Melton Hill, Guntersville Lake, and Kingston (Figure 3), we propose the social phenotype has evolved, or is currently evolving, from the subsocial phenotype. The strongest support is for the clusters of Guntersville Lake and Melton Hill with values of 96 and 85 respectively. Strong support is presented for Kingston populations grouping by phenotype but also separating from the other populations. The reasons behind this separation remain unknown. Marginal but convincing support is shown for Warriors Path with a bootstrap value of 44. The Steele Creek population is unusual as it groups, though weakly, with the geographically distant Guntersville Lake population. This could reflect the horticultural history of Steele Creek. Many of the trees were planted from nursery stock 20 – 25 years ago (Laughlin, personal communication) potentially introducing a more distant founder population. The group containing Boone Lake and Warriors Path, though not strongly supported, could reflect effects of geography. The two sites represent the shortest distance between any two study locations, 11 kilometers. This short distance could allow for enough gene flow to prevent further differentiation of these two sites. A regression of F_{ST} by geographic distance shows weak but significant support for isolation by distance.

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Individuals of *A. studiosus* can be scored as subsocial or social in behavior with subsocial individuals being less tolerant of adult conspecifics and social individuals being more tolerant. Both subsocial and social scored individuals are found throughout the eastern United States (Riechert & Jones 2008) though

social colonies are only found in northern latitudes. Social colonies are an emergent property of an increasing proportion of social individuals presumably reaching a threshold at which the transition to sociality is made. The variation in social structure naturally exists in the population throughout the range which implies that a transition to sociality would not require novel mutations.

Environmental component

Ecological factors also need to be considered when studying the increased prevalence of the social phenotypes. Though ecological conditions do not directly cause the evolution of sociality, they do provide the appropriate microclimate for social colonies. In *A. studiosus*, subsocial nests are predominant throughout the species' range within North America (26° - 36°N). Social colonies do not first appear until northern Florida (30°N) and increase in proportion into northern Tennessee (36°N) (Jones *et al.* 2007). Social colonies in northern, cooler regions seem to contradict the idea that warm, year-round growing seasons allow for larger colonies to persist (Wilson 1971). The more stable temperatures of the tropics allow spiders to forego over-wintering and colonies to grow larger by continuous activity. For similar continuous activity in cooler regions temperatures must be buffered to prevent colony reduction or extinction during harsh winter months. The advent of impoundments along the Tennessee River and its watershed by the Tennessee Valley Authority (TVA) could provide temperature buffering to support larger colony proliferation. Even though social colonies are conspicuous and should have been easily documented if existing, they were not described until 1998 (Furey 1998). Impoundments, which began in 1933 to control flooding, improve navigation, and provide power, created lakes and reservoirs throughout the region. These bodies of water could buffer ambient temperatures along the edges of water providing thermal stability and allowing for suitable habitat for social colonies to persist. It has been shown that *A. studiosus* can adapt to very specific microclimates (Jones *et al.* 2007)

Conclusion

The system is primed for the transition in behavioral strategy. The social variation already exists in the population with subsocial populations containing individuals that score as social. With evidence indicating populations of both phenotypes are inbred, it can be presumed inbreeding depression would not

hinder the shift in strategy. Not surprising, this evidence supports hypothesis 2, multiple local evolutions of social behavior in *A. studiosus*. A more profound question is why the social strategy is the minority throughout the range. An empirically refined model by Jones and Riechert (2008) predicts smaller social colonies would have an advantage in both warm and cool microclimates in the north. The relatively new introduction of suitable environmental conditions and no documentation of conspicuous social colonies until 1998 is evidence for novelty.

With the evidence presented here and the prediction of the Jones and Riechert (2008) model we suggest we are witnessing the active transition of the subsocial phenotype to the predicatively more advantageous social strategy.

Future work

To provide further support for active evolution of sociality in *A. studiosus*, we are currently continuing a long-term and large-scale study to quantify this transition. We are also expanding our study area to include TVA dam sites in middle and west Tennessee. We are also broadening our study northern, into Virginia and further to better track the prevalence of social behavior in the north.

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CHAPTER 3

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VITA

NATHANIEL O WEBER

Personal Data: Date of Birth: 18 January 1979
 Place of Birth: Kingsport, Tennessee

Education: Department of Defense Education Agency
 Colon, Panama
 Bitburg, Germany
 A.S. Biology, Northeast State Community College, Blountville,
 TN 2006
 B.S. Biology, East Tennessee State University, Johnson
 City, TN 2008
 M.S. Biology, East Tennessee State University, Johnson City, TN
 2010

Professional Experience: Graduate Assistant, East Tennessee State University, Department of
 Biological Sciences, 2008 - 2010

Presentations: American Arachnological Society 2008

Honors and Awards: Student-Faculty Collaborate Grant 2008