# Trait Evolution in Spiders: Perspectives on the Evolution of Behavioral Syndromes and Web Structures

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Jennifer Marie Bosco
May 2017

Copyright © 2017 by Jennifer Marie Bosco
All rights reserved.

# DEDICATION

To my mother, father

*Karen Marie Bosco and Robert Richard Bosco*

And brother and sister

*Jeana Elizabeth Bosco and Joseph Richard Bosco*

# ACKNOWLEDGEMENTS

I wish to thank my advisors, Susan Riechert and Brian O'Meara, for providing me with intellectual freedom and steadfast support throughout my time in graduate school. They worked diligently to help me bridge two very different fields and always encouraged me that I could do whatever I set my mind to. I would also like to thank my committee members, Gary McCracken, Todd Freeberg, and Haileab Hilafu. Our interactions in and out of the classroom broadened my understanding of animal behavior, evolutionary biology, and statistics. I would also like to express my appreciation to the Department of Ecology & Evolutionary Biology, which provided me with a teaching assistantship and an intellectually stimulating environment in which to conduct my research.

I would like to acknowledge those individuals who helped me collect spiders for this project: Austin Dillon, Evan Bierschmidtt, Angela Chuang, Christina Moynihan, and Domonique Hatton. I am grateful to Veronica Brown for helping me through DNA extraction protocols, gel electrophoresis, and PCR. Your patience and support helped me immeasurably. My fellow students offered stimulating discussion and support, particularly my fellow Riechert lab members – Angela Chuang, Domonique Hatton, and Evan Bierschmidtt. Last but not least, I am most grateful to Kathryn Massana: my Statistics partner, amazing friend, and constant source of support and encouragement. I never would have finished this program without you.

I would also like to acknowledge funding for this project, which came from the Chancellor's Fund at the University of Tennessee, and the Arachnology Fund to Susan Riechert. Finally, I wish to express deep appreciation and love to my family, mom, dad, Jeana, and Joe, who always supported and encouraged me during my graduate education, even though they were all too scared to even set foot in the spider lab.

# ABSTRACT

Organisms have evolved complex behavioral, morphological and physiological traits in response to various selection pressures. These phenotypes are usually composed of many traits that may or may not be genetically or phenotypically correlated. Correlations of both types can lead to evolutionary trade-offs, which may be broken over long evolutionary time periods through such mechanisms as the decoupling of genetic linkages and the development of phenotypic plasticity. Behavioral traits associated with temperament provide an excellent system in which to evaluate underlying mechanisms of the establishment and decoupling of genetic linkages. Other traits, such as the type of web that a spider builds, may not be so labile since there is greater complexity associated with, for example, web spinning organs and prey specialization. I initiated my investigation into these questions by examining the extent to which behavioral traits and their correlations change over ontogeny and how this varies between males and females of the grass spider *Agelenopsis lisa* (Chapter 1). I then considered how these behavioral traits change over macro-evolutionary time by using a dated phylogeny of 19 spider species of the RT3 spider clade (Chapter 2). Finally, I considered web evolution across all of spiders (Araneae) to examine how web type influences spider diversification (Chapter 3). My results indicate that behavioral traits are highly repeatable at certain life-stages, such as the penultimate stage in males that corresponds with increased prey consumption in preparation for searching for mates as an adult. While there are very few significant behavioral trait correlations that would suggest the presence of a behavioral syndrome, the weak correlations are consistent across ontogeny. Behavioral trait correlations are not conserved across macro-evolutionary time, suggesting that temperament traits are likely free to evolve independently from other behavioral

traits. Several of the traits examined are evolving towards phenotypic optima related to the habitat they reside in. However, some traits are particularly slow to evolve, which may result in maladaptive scenarios where species get "stuck" when the environment changes quickly. Finally, I found that weblessness is associated with higher diversification rates in spiders and reduced rates of diversification in orb weaving spiders.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# INTRODUCTION

Throughout their evolutionary histories, organisms have evolved complex behavioral, morphological and physiological traits to survive and reproduce. Behavioral phenotypes are composed of multiple traits that may or may not be genetically or phenotypically correlated which can lead to evolutionary trade-offs. Behavioral traits underlying animal temperament offer one set of such tradeoffs. Sih et al. (2004) applied the term behavioral syndrome to behavioral trait correlations associated with temperament, reflecting the fact that such correlations may not be adaptive across all contexts. Thus, for example, an aggressive individual will not only be aggressive towards prey but would also be aggressive towards potential mates if there is correlation across all contexts. This 'spillover' from behavioral trait correlations creates a tradeoff, where the optimal amount of aggressiveness is a value that is intermediate between the optimal levels for dealing with prey and for dealing with mates. Note that behavioral syndromes may lead to tradeoffs under this view but not always. For example, the aggressiveness the spider *Agelenopsis aperta* exhibits across contexts is generally adaptive: evidence of spillover has only been observed where gene flow occurs between spiders from arid and riparian habitats (Riechert et al., 2002).

Several syndromes or suites of correlated behaviors have been described including aggressive/fearful (Maynard Smith and Riechert, 1984; Riechert and Maynard Smith, 1989), bold/shy (Fraser et al., 2001; Réale et al., 2000; Wilson and Godin, 2009b), high activity/low activity (Sih, 1992; Sih et al., 2003) and proactive/reactive (Sih et al., 2004). Early work utilizing breeding experiments and estimates of trait heritability, respectively indicated; 1) that behavioral syndromes may be coded for by a few genes of major effect as assortment was noted in

backcrosses and second generation hybrids (Riechert and Maynard Smith, 1989); and 2) that behavioral syndromes are strongly heritable (Maynard Smith and Riechert, 1984; Pruitt et al., 2008; Riechert and Maynard Smith, 1989). Recent studies utilizing gene-mapping methods etc. applied to model species, suggest that multiple genes of small, overlapping effect often underlie behavioral traits (Anholt and Mackay, 2004; Dierick and Greenspan, 2007; Mackay, 2004, 2013). Plasticity has been observed in traits associated with temperament in some systems as well (Dingemanse et al., 2007; Ruiz-Gomez et al., 2008). Finally, work across species phylogenies suggest that behavioral trait correlations having an underlying genetic basis may be easily broken through evolutionary time.

Spiders have emerged as one of the most prominent models of behavioral syndrome research (Sih et al. 2004), and studies from distantly related species have documented similar syndromes. Aggressiveness towards prey, prospective mates, predators, and territorial encounters are correlated in at least three families (*Theridiidae*: Johnson et al. 2010; Pruitt et al. 2008, 2009 *Pisauridae*: Johnson & Sih 2005; 2007, *Agelenidae*: reviewed in Riechert et al. 2001). In light of this fact, I ask how the behavior of spiders might change over ontogeny and over long macro-evolutionary time scales, and whether behavioral syndromes might evolve under adaptive conditions, rather than being constrained by one or a few genes.

## Behavioral Trait Stability

Over the past four decades, much research has centered upon the proposition that individual animals alter their behavior to cope with changing local environmental conditions (Piersma and Drent, 2003; Réale and Dingemanse, 2010). While an individual does not express the full range of behavioral trait values present in its population (Réale and Dingemanse, 2010),

environmental effects have the potential to temporarily (Padilla and Adolph, 1996; Pigliucci, 2005) and permanently affect the phenotype produced by a particular genotype (Lynch and Walsh, 1998). One way to look at behavioral correlations is over deep evolutionary time and the other is to look at these behaviors and correlations across ontogeny and with experience. Behavioral and physiological traits show relatively low heritability, similar to life-history traits, and much lower than morphological traits (Mousseau and Roff, 1987; Stirling et al., 2002). It is worth noting that while low heritability suggests low additive genetic variation, there is a large body of work suggesting that pervasive epistatic interactions underlie many behaviors (Anholt, 2004; Anholt and Mackay, 2004; Mackay, 2001, 2004, 2013).

Behavioral traits, like many other traits, have some degree of temporal and situational plasticity. For example, shifts associated with ontogeny/state within the life cycle is seen in female mice who exhibit increased aggressive responses that are targeted towards male intruders while nursing pups. The aggressive response to males is lost when the pups have been weaned (Yu et al., 2014). Studies have also shown that innate temperament can influence the degree to which temporal and situational plasticity might be exhibited (Koolhaas et al., 1999; Sih and Bell, 2008). Thus, inherently less aggressive mice have been shown to adjust their levels of aggression according to social context, while aggressive individuals do not (Natarajan et al., 2009). Measuring individual variation and plasticity through repeated trials of individuals will provide information about the degree to which particular traits are stable or plastic.

## Behavioral Trait Evolution

Traits operating in the same direction may come to share a common regulatory mechanism (trait canalization) and tend to remain so (Cheverud, 1996; Cheverud, 2001; Tierney,

1986; Wagner et al., 2000). Given sufficient time under different selection pressures, however, decoupling may occur because it is possible for regulatory modulators to evolve (Cheverud, 1996; Cheverud, 2001; Wagner et al., 2000). Within particular species (e.g., *A. aperta),* traits may show positive correlations that underlie behavioral syndromes. The presence of a behavioral syndrome may reflect the fact that behavioral trait correlation is adaptive (aggressiveness being advantageous in multiple contexts) (Huntingford, 1976) or is maladaptive (trait correlations not being under strong negative selection for long enough to escape this constraint) (Henriksson, 1997). We expect in the case of a maladaptive correlation that over enough species with sufficient time, some will have escaped this constraint. This is an advantage of the comparative approach we will use here. Rather than looking at particular traits that may happen to be correlated in a single species, we can determine whether such correlations persist over hundreds of thousands of years and gain insight into how this may occur.

Behavioral traits are notorious for being strongly subject to both immediate environmental effects and those measured may show large seasonal or year-to-year variation in the case of longer-lived species. Behavioral and physiological traits show relatively low heritability, similar to life-history traits, and much lower than morphological traits (Mousseau and Roff, 1987; Stirling et al., 2002). It is worth noting that while low heritability suggests low additive genetic variation, there is a large body of work suggesting that pervasive epistatic interactions underlie many behaviors (Anholt et al., 2003; Anholt and Mackay, 2004; Mackay, 2001, 2004). In principle, low heritability could reduce the susceptibility of trait correlations to change, but the heritability estimates for behavioral traits are still generally high enough to allow rapid response to selection (Blomberg et al., 2003).

Two extreme possibilities have been considered. 1) Behavioral traits may be so labile

(plastic) in some species that even local populations can quickly move to different optima (Blomberg et al., 2003; Urbani, 1989). Knowing what one species does in this case is uninformative for a closely related species. 2) There are sufficiently strong barriers to evolve new behavioral traits, to limit adaptation to conditions that predict different optimal trait values (Gittleman et al., 1996). Our preliminary data suggest the true answer is somewhere in between these extremes, with a bias in the direction of more rapid evolution and thus less predictability (phylogenetic signal) offered across species.

Spider species occupying habitats offering limited food resources and few predators, such as arid regions, will be bold (not fearful of potential predation cues), aggressive in food (attack any potential food item) and social contexts (defend against other spiders moving into a territory where they will compete for what little food there is). Spiders in areas with abundant resources but also high predation risk will tend to be more timid. Here competition between spiders is based on who survives predation longest to achieve more matings (males) or produce more egg clutches (females) rather than who gets the most food. Thus, aggressiveness towards conspecifics should be low (Riechert and Hall, 2000).

## Web Evolution

The evolutionary diversification of spiders is attributed to spectacular innovations in silk use, particularly in the production of the prey capture web. Here, we construct a large molecular phylogeny of spiders to examine the diversification of the spider order Araneae in relation to web architecture and putative hypotheses about the evolutionary transitions between different web types/states. All spiders produce and use silk throughout their lives, making it an integral part of their behavioral repertoire. Spiders are most known, however for their silk webs that aid

in the capture of prey. The most advanced web type is the geometric, araneoid orb web. This web, which provides access to flying prey, exhibits an open structure. (Wind currents at the heights orb weavers encounter prey would tear apart the more dense web structures characteristic of the scattered and sheet webs that are constructed either in close proximity to the ground or in vegetation sheltering them from the wind.) Sticky droplets laid down on the capture spiral of the orb web lessens the probability of insect escape (Agnarsson et al., 2006; Bond and Opell, 1998; Coddington, 1990), as does dry silk that clings to prey through van der Waals interactions and hydroscopic forces (Hawthorn and Opell, 2003).

Both Bond and Opell (1998) and Blackledge et al. (2009) note a trend toward increased diversification in orb-web producing lineages. Bond and Opell (1998) further propose that the orb web represents a key innovation that has led to the diversification of the Order Araneae. In part, this reflects changes in capture thread and web features that allow orb-weavers to shift into new adaptive zones. In order to understand the diversification of this important arthropod order, it is essential to discover the evolutionary pathway of silk utilization in spiders. The evolution of different web types will affect diversification rates across spider lineages differently.

## Goals of the Dissertation Research

My dissertation research aims to characterize the behavioral tendencies of spiders in the family Agelenidae, particularly the genus *Agelenopsis*, with particular regards to how various behavioral traits (e.g., predatory behavior, activity level) vary across ontogeny. I then consider how these traits correlate across phylogeny. If the spiders in this study exhibit consistent behavioral trait correlations across phylogeny, it could potentially lend insight into the underlying evolutionary mechanisms influencing these traits. By understanding these

mechanisms, we can also gain insight into how these traits and their correlations might evolve if environmental conditions or selective agents change. I also aim to understand how different web types influence diversification and trait evolution across the entire spider order Araneae. By applying a very broad set of phylogenetic methods to these questions, we can understand how the processes of species diversification relate to the distribution of web traits during the radiation of spiders and in future studies, how these trends might relate to different personality traits that these spiders exhibit.

Chapter I describes how behavioral traits change over the course of ontogeny in *Agelenopsis lisa* (Araneae: Agelenidae). These data are compared to determine how repeatability, mean trait values, and trait correlations change over the course of the life cycle, and how these differences vary between males and females.

Chapter II compares behavioral trait evolution across 19 spider species (16 from the family Agelenidae, 3 species from the family Lycosidae). If behavioral syndromes are conserved over macro-evolutionary time scales, these traits should remain correlated across the phylogeny. I also compare trait evolution between two different habitat types (desert and riparian) to examine how differences in habitat might influence the evolutionary trajectory of behavioral traits.

Chapter III examines the relationship between rates of diversification and web evolution across ~2,600 species of spiders (Araneae) through the generation of a time-scaled, fossil-calibrated molecular phylogeny. We apply this phylogenetic dataset and a very broad set of current phylogenetic methods to test whether the processes of species diversification relate to the distribution of web traits during the radiation of spiders. I also compare transition rates between different web states and test different hypotheses about how web evolution can occur.

## Works Cited


Agnarsson I, Avilés L, Coddington JA, Maddison WP, 2006. Sociality in theridiid spiders: repeated origins of an evolutionary dead end. Evolution 60:2342-2351.

Anholt RR, 2004. Genetic modules and networks for behavior: lessons from Drosophila. BioEssays 26:1299-1306.

Anholt RR, Mackay TF, 2004. Quantitative genetic analyses of complex behaviours in Drosophila. Nature Reviews Genetics 5:838-849.

Anholt RR, Dilda CL, Chang S, Fanara J-J, Kulkarni NH, Ganguly I, Rollmann SM, Kamdar KP, Mackay TF, 2003. The genetic architecture of odor-guided behavior in Drosophila: epistasis and the transcriptome. Nature genetics 35:180-184.

Blackledge TA, Scharff N, Coddington JA, Szüts T, Wenzel JW, Hayashi CY, Agnarsson I, 2009. Reconstructing web evolution and spider diversification in the molecular era. Proceedings of the National Academy of Sciences 106:5229-5234.

Blomberg SP, Garland T, Ives AR, 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57:717-745.

Bond JE, Opell BD, 1998. Testing adaptive radiation and key innovation hypotheses in spiders. Evolution:403-414.

Cheverud JM, 1996. Developmental integration and the evolution of pleiotropy. American Zoologist 36:44-50.

Cheverud JM, 2001. The genetic architecture of pleiotropic relations and differential epistasis. The character concept in evolutionary biology:411-434.

Coddington JA, 1990. Cladistics and spider classification: araneomorph phylogeny and the monophyly of orbweavers (Araneae: Araneomorphae; Orbiculariae). Acta Zoologica Fennica 190:75-87.

Gittleman JL, Anderson CG, Kot M, Luh H-K, 1996. Phylogenetic lability and rates of evolution: a comparison of behavioral, morphological and life history traits. Phylogenies and the comparative method in animal behavior:166-205.

Hawthorn AC, Opell BD, 2003. van der Waals and hygroscopic forces of adhesion generated by spider capture threads. Journal of Experimental Biology 206:3905-3911.

Henriksson S, 1997. Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. Evolutionary Ecology 11:255-273.

Huntingford FA, 1976. The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback,< i> Gasterosteus Aculeatus</i>. Animal Behaviour 24:245-260.

Koolhaas J, Korte S, De Boer S, Van Der Vegt B, Van Reenen C, Hopster H, De Jong I, Ruis M, Blokhuis H, 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience & Biobehavioral Reviews 23:925-935.

Lynch M, Walsh B, 1998. Sinauer Associates; Sunderland, MA: 1998. Genetics and analysis of quantitative traits.

Mackay TF, 2001. Quantitative trait loci in Drosophila. Nature Reviews Genetics 2:11-20.

Mackay TF, 2004. The genetic architecture of quantitative traits: lessons from< i> Drosophila</i>. Current opinion in genetics & development 14:253-257.

Mackay TF, 2013. Epistasis and quantitative traits: using model organisms to study gene-gene interactions. Nature Reviews Genetics.

Mousseau TA, Roff DA, 1987. Natural selection and the heritability of fitness components. Heredity 59:181-197.

Natarajan D, de Vries H, Saaltink D-J, De Boer SF, Koolhaas JM, 2009. Delineation of violence from functional aggression in mice: an ethological approach. Behavior genetics 39:73-90.

Padilla DK, Adolph SC, 1996. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. Evolutionary Ecology 10:105-117.

Piersma T, Drent J, 2003. Phenotypic flexibility and the evolution of organismal design. Trends in Ecology & Evolution 18:228-233.

Pigliucci M, 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution 20:481-486.

Réale D, Dingemanse NJ, 2010. Personality and individual social specialisation. Social behaviour: genes, ecology and evolution:562.

Riechert SE, Hall RF, 2000. Local population success in heterogeneous habitats: reciprocal transplant experiments completed on a desert spider. Journal of Evolutionary Biology 13:541-550.

Sih A, Bell AM, 2008. Insights for behavioral ecology from behavioral syndromes. Advances in the Study of Behavior 38:227-281.

Stirling D, Réale D, Roff D, 2002. Selection, structure and the heritability of behaviour. Journal of Evolutionary Biology 15:277-289.

Tierney AJ, 1986. The evolution of learned and innate behavior: contributions from genetics and neurobiology to a theory of behavioral evolution. Animal Learning & Behavior 14:339-348.

Urbani CB, 1989. Phytogeny and behavioural evolution in ants, with a discussion of the role of behaviour in evolutionary processes. Ethology Ecology & Evolution 1:137-168.

Wagner GP, Chiu C-H, Laubichler M, 2000. Developmental evolution as a mechanistic science: the inference from developmental mechanisms to evolutionary processes. American Zoologist 40:819-831.

Yu Q, Teixeira C, Mahadevia D, Huang Y, Balsam D, Mann J, Gingrich J, Ansorge M, 2014. Dopamine and serotonin signaling during two sensitive developmental periods differentially impact adult aggressive and affective behaviors in mice. Molecular psychiatry 19:688-698.

# CHAPTER I: THE ONTOGENY OF PERSONALITY TRAITS IN THE DESERT FUNNEL-WEB SPIDER, *AGELENOPSIS LISA*

This chapter is based on an original research article submitted to *Ethology* in September 2016. My primary contributions to this paper include: (i) collection and analysis of the data, (ii) figure development, (iii) completion of a first draft of the manuscript, and (iv) responding to reviewer comments.

**Bosco JM**, Riechert SE, O'Meara BC (2016) The ontogeny of personality traits in the desert funnel-web spider, *Agelenopsis lisa*.

## Abstract

Consistent behavioral differences among individuals, that is, personality, have been described in numerous species. Nevertheless, behavioral consistency over the course of development remains unclear. We investigated the ontogeny of personality in the desert funnel-web spider (*Agelenopsis lisa*) by scoring personality traits at six time points during its life cycle, including the transition to sexual maturity (i.e., respective penultimate and adult stages). We demonstrate that trait values for half of the personality traits examined vary across ontogeny. Further, repeatabilities of behavioral traits are low within a life stage across ontogeny. Exceptions are penultimate males and sexually mature females. We also find no evidence of the existence of behavioral syndromes, as trait correlations vary with life stage. Our results demonstrate that both absolute values and consistency of behavioral traits may change across ontogeny and that increased consistency may coincide with developmentally important changes associated with sexual maturation in spiders. These results have implications for future studies on personality. In particular, the life history of the organism should be considered in determining what life stage(s) are to be examined.

## Background

Behavioral biologists have long been interested in the mechanisms by which consistent individual-level differences in behavior may evolve and be maintained over time or across contexts. In recent years, this consistency in behavior has been documented in a wide range of taxa and has generally been characterized as representing animal personalities (Dall et al., 2004; Réale et al., 2007) or behavioral syndromes (Sih et al., 2004). Numerous conceptual and empirical advances have been made in this area (Favati et al., 2015; Krause et al., 2010; Sweeney et al., 2013; Wilson and Godin, 2009a; Wilson et al., 2010; Wilson and Krause, 2012; Wuerz and Krüger, 2015). However, Stamps and Groothuis (2010a) noted that there remains a need to understand how life history and developmental processes are integrated with personality. To date, individual consistency in behavior typically refers to measures taken over short time periods or within a given life history stage. This approach tends to underestimate the potential consequences of long-term consistency in behavior and fails to recognize the potential importance of seemingly maladaptive behaviors later in life. Additionally, personality traits may also couple with life-history characteristics over ontogeny, with some general combinations of behavioral and developmental traits being superior to other in terms of fitness (Réale and Dingemanse, 2010; Réale et al., 2010; Wolf et al., 2007).

Stamps and Groothuis (2010a) further offered a framework to investigate temporal change and stability in ontogenetic studies of personality, applying terms originally derived from human developmental psychology to target measurements of consistency that are the most relevant to studies of ontogeny of personality in animals (e.g., “mean-level consistency”,

"differential consistency", and "structural consistency"). A brief description of each of these terms follows.

Mean consistency is an estimate of temporal change in behavior. It compares the mean response of a focal group (expressed in a particular context at one time point with that expressed under similar conditions at a later time point). This index reflects the general patterns of consistency in behavior for the focal group as a whole. For example, mean-level consistency may be low in a case where juveniles are more prone to show flight responses than adults in a species where juveniles face a higher risk of predation than adults (Dangles et al., 2007). Mean-level consistency is not a measure of personality on its own but provides a background for studies of development of personality traits against which other indices of temporal variation (e.g. individual stability) can be interpreted (Stamps and Groothuis, 2010a).

Differential consistency is defined as the extent to which scores for a given behavioral trait are consistent across individuals and time for a given context. It is a central concept for describing personality (Stamps and Groothuis, 2010a). Note that some personality traits may be predominantly shaped by factors that do not arise until a certain life stage is reached, for example, hormonal state or profile when an individual has reached sexual maturity. In these cases, we do not expect consistency in behavioral responses to appear until after a life-stage transition has occurred. Tracing the developmental process of various personality traits may therefore suggest the proximate, often unknown mechanism underlying personalities (Groothuis and Trillmich, 2011; Stamps and Groothuis, 2010a; Stamps and Groothuis, 2010b).

Structural consistency is a measure of the degree to which trait correlations across contexts are consistent through time as in over ontogeny. This is the term that underlies the concept of the behavioral syndrome coined in Sih et al. (2004). Because the correlations meeting

the criterion of the term structural consistency may vary depending on the situation (e.g. over developmental transitions (Bell and Stamps, 2004) or degree of predator pressure (Bell and Sih, 2007)), behavioral syndromes are not necessarily stable over time. In fact studies fitting the criteria of tests of structural consistency to date have produced mixed results. Structural consistency has been found to vary between populations or breeding lines (Bell and Stamps, 2004; Carere et al., 2005), to first appear after or during a particular developmental period (Günther et al., 2014; Johnson and Sih, 2007; Sweeney et al., 2013), and to be absent at all investigated age classes (Kralj-Fišer and Schneider, 2012; Sinn et al., 2008) in various studies.

There are few examples of studies of development of spider personality traits. Boldness and aggression have shown to be uncorrelated in field-caught juvenile grass spiders *Agelenopsis pennsylvanica* (Agelenidae), but a boldness-aggression syndrome manifests at the penultimate stage and the two behavioral traits tested exhibit high repeatability of this syndrome at this stage (Sweeney et al., 2013). A study of the bridge *Larinioides sclopetarius* (Araneidae), failed to detect the presence of a behavioral syndrome at any stage of the life cycle (Kralj-Fišer and Schneider, 2012).

In this study, we use a lab-reared population of the desert grass spider (*Agelenopsis lisa*) to quantitatively ascertain whether contextual individual-level differences in personality (i.e. activity, boldness, and aggressiveness) are consistent both within a life-history stage (juvenile, penultimate, and adult) and across ontogeny. This study entails examination of all three measures of behavioral consistency across two different ontogenetic transitions: 'leaving the natal web ($2^{nd}$-$3^{rd}$ instar)' and 'sexual maturation ($8^{th}$-$9^{th}$ instar)'. The first transition, termed 'dispersal' herein, is primarily an alteration of the spider's social environment, as individuals disperse from their siblings on a group web to build individual webs elsewhere. The second

transition, ‘sexual maturity’, is associated with large-scale hormonal changes. In males, there is the abandonment of the web and feeding as they search for potential matings. In females, there is an increase in food demands associated with producing offspring clutch(es). Stamps and Groothuis (2010a) suggest examining trait correlation patterns across such life history transitions as described here for *A. lisa*, can provide valuable insights into factors affecting behavioral stability, for example, when stability of personality traits appear and if consistency varies over different periods in ontogeny.

Using the framework outlined above, we pose the following main questions: 1) How does mean level behavior of spiders change over ontogeny? 2) Is intra-individual consistency of behavioral responses stable over ontogeny? 3) Are correlation structures among behavioral traits stable over ontogeny?

## Methods

### Collection and Laboratory Maintenance

We completed this study on the F1 generation offspring reared in the laboratory from eight families of field collected grass spiders, *Agelenopsis lisa* (Araneae, Agelenidae). Like its close desert relative, *A. aperta*, *A. lisa* occupies arid habitats and builds its sheet web in low vegetation such as in grasses and at the bases of shrubs and cacti. The non-sticky silk sheet is used to sense and locate prey while the spider sits in the protected environment of its funnel retreat. We collected the parental generation of spiders as late instars along a stretch of tall grass habitat bordering State Hwy 17 at Balmorea State Park (Balmorea, TX, 30.944829, -103.785147) during March of 2012. We did not score the behavior of these collected individuals, but merely established each individual in its own container in the laboratory maintained at 22-24°C on a

12:12 h light: dark cycle. We offered the spiders a diet of *ad libitum* crickets and randomly mated individuals as they matured to produce the F1 generation.

We communally reared the offspring produced by each of the eight matings through the 1st two instars. We offered these 'spiderlings' both termite workers (*Reticulitermes flavipes*) and crickets (*Acheta domesticus*) of appropriate size *ad libitum*. We also weekly misted the communal webs. As individuals molted to the third instar, we assigned them unique identities and moved them from the communal container they had been housed in into individual plastic containers, measuring 11 mm in diameter and 2mm in height.

**Test Schedule**

We subjected each of 108 surviving spiders to a battery of 11 behavioral trait tests at three stages in the life cycle: juvenile (3rd-5th instar), penultimate (one molt removed from sexual maturity) and sexually mature. Two weeks separated replicate within stage tests. We completed the first trial within a particular life stage seven days following an individual's molt to a new stage and three days after an *ad libitum* feeding.

**Behavioral Test Trials**

We applied the series of behavioral tests developed for examination of ecotypic variation in the behavior of *A. aperta* in this study of *A. lisa* (See Maupin and Riechert (2001); Riechert and Hedrick (1990, 1993); Riechert and Johns (2003); Riechert and Maupin (1998) for original descriptions of the tests). Table 1.1 shows our assignment of the 11 behavioral traits scored in this study to respective behavioral syndromes – activity (the general level of activity in a novel environment), aggressiveness (an individual's agonistic reaction towards a prey item), and

boldness (an individual's reaction to a novel situation). The behavioral tests are briefly summarized below in the order in which we completed them.

*Boldness and Activity.* Seventy-two hours after a routine feeding, we moved the test subject from its home container to a novel container of the same dimensions. We recorded the distance the spider moved before it had ceased movement for five seconds. We then scored the 'Latency to start Exploring a Novel Environment' as the time elapsed between cessation of movement and its resumption. After the individual began moving, we measured the amount of time an individual spent active over a 10-minute period. Immediately following completion of the activity and boldness assays, we allowed 10 minutes for habituation before commencing the next test. In the prod track tests, we touched the spider at its posterior or anterior end with the eraser end of a pencil (after Riechert and Johns (2003)). We recorded (1) the individual's behavior after the prod (run, walk, attack prod, etc.), (2) the distance travelled around the test arena and (3) the time elapsed between the individual's cessation of movement and when the individual began moving again. We allowed the individual five minutes for habituation between each prod. In each trial, each eraser touched was wiped with a wet paper towel before the next individual's test (to avoid uncontrolled olfactory cues).

Spiders were given twenty-four hours to build a web after completing the boldness trials listed above. After removing the lids from the test arena spiders were allotted as much time as necessary to appear at their funnel entrance (usually 10-15 minutes after removing the lid from the container), following Riechert and Hedrick (1990, 1993). We held a camera-cleaning bulb at a 45° angle to the web, at a height of eight cm and directed a single puff of air at a spot six cm in front of the funnel entrance. We recorded (1) the spider's response to the puff (whether it

displayed an aggressive response such as running out toward the cue or a fearful/non-aggressive response such as retreating into the funnel), (2) the length of time that elapsed between retreat and the reappearance of the spider at the funnel entrance. No retreat or aggressive responses were scored as 0 s.

*Aggressiveness.* After the puff test, spiders were given 10 minutes to habituate before completing the tests of aggression towards prey. We offered the individual a single 2-week old cricket. The cricket was placed on the web approximately 2.0 cm from the test individual and the latency to attack (make contact with the prey) was recorded. The superfluous killing trials followed the protocols developed previously (Maupin and Riechert, 2001; Riechert and Maupin, 1998) to test for general foraging aggressiveness. Crickets were introduced, one at a time, at three minute intervals to the individual's web. Additional time was provided if an individual needed more time to subdue its prey. Trials were terminated when an individual failed to attack two consecutive prey items. At the end of the observation period, all rejected prey items were removed from the web and the individual was given 24 hours to feed on the prey. After this 24 hour period, the captured prey items were assigned to one of the following categories based on the remaining percentage of mass relative to their original body mass: fully consumed (<10%), partially consumed (10-25%) or uneaten (>25%). If an individual was feeding on prey after 24 hours, it was given an additional 24 hours to feed and the prey identified as to feeding classification after that time.

**Statistical Analyses**

*Mean-Level Consistency.* In investigating mean-level consistency, we first tested for life stage differences in each of the behavioral traits, utilizing a general linear mixed model ('lmer') for

traits with a Gaussian distribution and generalized linear mixed models ('glmer') for traits with a Poisson distribution. We specifically utilized the 'lme4' package v1.1-11 (Bates, 2010) with life-stage as a fixed effect and family and individual included as random effects. We fitted separate models for each behavioral variable entered as the response variable.

*Repeatability.* We used the package 'rptR' (Schielzeth and Nakagawa, 2013) to calculate repeatibilities within and between life-stages. This provided a test of the consistency of behavior across ontogeny, which can be defined as differential consistency or broad-sense repeatability.

In completing this analysis, we ran a separate model for each life-stage with the two observations for each behavior as the response variable and individual as the random effects. We included no fixed effects as we wished to provide a conservative estimate of within- and between- individual variation (Dingemanse and Dochtermann, 2013; Schielzeth and Nakagawa, 2013). We also used a Gaussian error distribution for the (ln+1) transformed traits that met the assumptions of normality. Note that two traits ('latency to return from a predatory puff cue' and 'latency to capture prey') did not meet the assumptions of normality after applying various transformations. We used a Poisson distribution on the untransformed count data for them.

To enable comparison across models, we needed to first mean-center and scale the variance components of our Gaussian response variables. This is because variance estimates are inherently tied to the total variation present in the response. We used a square root link identity and added observation number as a random variable for the Poisson response variables in order to capture the residual variance. This approach corresponds to calculating repeatability on the latent scale (Nakagawa and Schielzeth, 2010). We calculated repeatabilities within each life-

stage (comprising two subsequence test rounds ("Within Life Stage Repeatability"), as well as for the complete dataset including all six-test rounds ("Across Life Stage Repeatability").

*Behavioral Syndromes.* Using the data from the first trial, we computed a Spearman rank correlation matrix for each age to test for structural consistency (i.e., whether correlation structures of behavioral responses change over time and developmental stages). We also applied Mantel randomization tests with 10000 permutations to determine whether correlation structures were similar for males and females at each time point. We did not use a Bonferroni correction to adjust the P-values for multiple comparisons as it tends to be overly conservative and can lead to an increase in Type II error. Instead we employed the false recovery estimate of Benjamini and Hochberg (1995). This method controls the expected proportion of false discoveries (rejected hypotheses). The discovery rate is a less-stringent condition than the family-wise error rate employed by methods such as the Bonferroni correction. Thus it is more powerful in minimizing Type I and II errors when many pairwise comparisons are conducted. R version (3.2.4) was used for all statistical analyses (Team, 2014) and the package 'ade4' v1.7-4 was used for the Mantel randomization test (Dray and Dufour, 2007).

# Results

## Repeatability

After correcting for multiple comparisons of the eleven measured behavioral variables we find only the latency to start exploring a novel environment to be significantly repeatable across life stages (Table 1.2). Further, only this boldness trait estimate is significantly greater than zero across the entire life cycle (R = 0.23, 95% CI: 0.12, 0.33). This trait does not show significant within life stage repeatability, however. Though failing to meet significance criteria, several

instars do show relatively high repeatabilities in this boldness trait with penultimate males and mature females exhibiting the highest repeatabilities of all instars measured (R = 0.52, 95% CI: 0, 0.90 and R = 0.36, 95% CI: 0.08, 0.60, respectively).

The only other significantly repeatable boldness trait is the latency to return from a predatory puff cue for mature females (R = 0.52, 95% CI: 0, 0.59). Note, however, that penultimate females also exhibit a high but non-significant repeatability for this trait (R = 0.45, 95% CI: 0, 0.57). Females demonstrate significant repeatability only within the juvenile stage of the life cycle for latency to capture prey but are highly repeatable overall (juvenile female R = 0.82, 95% CI: 0.47, 0.83; penultimate female R = 0.51, 95% CI: 0.02, 0.59; mature female R = 0.33, 95% CI: 0, 0.49). Males exhibit high repeatability only at the penultimate stage (R = 0.82, 95% CI: 0, 0.94). Only penultimate males and mature females exhibit high (but non-significant) repeatability for prey capture and superfluous killing (penultimate male: R = 0.60, 95% CI: 0, 0.92; R = 0. 78, 95% CI: 0.35, 0.96; mature female: R = 0.36, 95% CI: 0.08, 0.58; R = 0.38, 95% CI: 0.10, 0.61, respectively).

**Mean-level consistency**

*Variation in Boldness*. Of the seven boldness traits tested, latency to return from a predatory puff cue, response to front prod, and response to rear prod show significant differences across the life cycle (Tables 1.3 & 1.4). Penultimate males exhibit the shortest latency to return to a foraging position after receiving a predatory puff cue (mean ± SE: 6.3s ± 1.8s). They also take significantly less time to return to foraging mode than juvenile males (36.7s ± 1.8s; $P < 0.0001$), mature males (27.7s ± 1.8s; $P < 0.0001$), and mature females (22.6s ± 1.4s; $P = 0.0034$). Juvenile females exhibit the longest latency to return of all sex and age classes (117.1s ± 1.4s) and take

significantly more time to return that mature females ($P < 0.0001$), penultimate females (38.9s ± 1.40; $P < 0.0001$), and penultimate males (result listed above). Penultimate females exhibit a significantly longer mean latency than juvenile females ($P < 0.0001$) and penultimate males ($P < 0.0001$). Finally juvenile males take about 5x longer than mature males to return to a foraging position ($P < 0.0001$).

Neither response to the front prod nor response to the rear prod show any significant pairwise differences between any life-stage even though the overall effects are significant ($P = 0.01$ and $P = 0.03$, respectively).

*Variation in Activity*. The total amount of activity over 10 minutes does not differ over ontogeny or over time (Table 1.3 & 1.4). However, there are two trends worth noting: (1) Juvenile and penultimate males are equally active but decrease activity by approximately 41% when they mature (juvenile: 73.5s ± 1.2s; penultimate: 75.1s ± 1.3s; mature: 44.2s ± 1.2s), and (2) Juvenile and penultimate females are equally active, less active than their male counterparts, and increase their activity by approximately 20% when they mature (juvenile: 53.1s ± 1.2s; penultimate: 52.8s ± 1.2s; mature: 66.3s ± 1.2s).

*Variation in Aggressive Behaviors.* All aggressiveness traits measured exhibit significant effects of sex and life-stage (Table 1.3 & 1.4). The prey capture estimate is the only aggressiveness trait that does not change significantly over time. Penultimate males and mature females exhibit the lowest latencies to attack prey (1.7s ± 1.5s and 5.6s ± 1.3s, respectively), while mature males exhibit the longest latencies of attack (50.4s ± 1.5s). Penultimate males attack prey significantly faster than juvenile females ($P < 0.0001$), penultimate females ($P < 0.0001$), juvenile males ($P < 0.0001$), and mature males ($P < 0.0001$). Mature females attack prey significantly faster than

juvenile females ($P < 0.0001$), penultimate females ($P < 0.0001$), juvenile males ($P < 0.0001$), and mature males ($P < 0.0001$). Juvenile males attack prey significantly faster than mature males ($P < 0.0001$). Penultimate males exhibit the highest estimates of prey capture ($3.0 \pm 1.1$), which drop drastically once they mature ($2.0 \pm 1.1$; $P < 0.0001$). Mature males exhibit the lowest estimates of prey capture and capture significantly fewer prey items than juvenile females ($2.7 \pm 1.1$; $P = 0.0016$), penultimate females ($2.6 \pm 1.0$; $P = 0.0009$), mature females ($2.5 \pm 1.0$; $P = 0.0049$), and juvenile males ($2.6 \pm 1.1$; $P = 0.0007$). Penultimate males exhibit the highest estimate of wasteful killing ($1.9 \pm 1.1$), which is significantly higher than the waste estimate for mature females ($1.3 \pm 1.1$; $P = 0.0001$). Juvenile females exhibit the highest levels of superfluous killing of any female stage ($1.9 \pm 1.06$), higher than both penultimate females ($1.6 \pm 1.1$) and mature females ($1.3 \pm 1.1$; $P = 0.0074$ and $P < 0.0001$, respectively). Penultimate females also waste more than mature females ($P = 0.0008$) (see Table 1.4 for all mean differences).

**Structural consistency**

All of the pairwise Mantel's tests between the correlation matrices are significant (Table 1.5) except for the Mantel's test between juvenile males and juvenile females ($n_{males} = 22$, $n_{females} = 54$: $r_s = 0.234$, $P = 0.051$). This result indicates that the correlational structure of the measured variables is similar across life-stages. A comparison of correlation matrices between subsequent time points reveal that the relationship between behavioral traits does not change during development from juvenile to adult in both sexes (Table 1.5).

The only consistently strong correlations are: 1) a positive correlation between prey capture and superfluous killing, which is present at every life stage ($\rho = 0.54 – 0.87$), 2) a positive correlation between distance traveled after a front prod and the distance traveled after a

rear prod present only in females at all life stages ($\rho = 0.53 – 0.55$), and 3) a positive correlation between the latency to start searching after a front prod and latency to start searching after a rear prod. This last relationship is present only in females at all life stages and mature males ($\rho = 0.36 – 0.59$) (Table 1.5). The similarity among the correlation matrices found, thus, seem to be based on the absence of strong correlations except the positive ones listed above. All pairwise Spearman rank correlations can be found in Figure 1.1.

## Discussion

In our investigation of the consistency of individual behavior (personality) across ontogeny in A. *lisa*, we find mean expression of behavioral traits to vary across different developmental stages. Further, many traits do not stabilize until later in ontogeny, if they stabilize at all. We also show that repeatability (differential consistency) of particular behavioral traits appear at different ages in ontogeny, and that individual consistencies are, in general, low across major events in ontogeny. Thus, behavioral syndromes are largely absent. This could be an artifact of having small sample sizes for males because our sample turned out to be strongly (3:1) female biased. To our knowledge, our study is the first to both characterize this many personality traits across spider development, as well as to provide measures of consistency of those traits across such a large portion (~75%) of the life-cycle in any invertebrate species. We discuss additional results in turn below as well as suggestions for future studies of this type.

Stamps and Groothuis (2010a) explanations for instability of personality traits across ontogeny include, in part, the possibility that personality traits are linked to physiological factors such as rate of growth and hormonal profile changes through development. The authors also recognize that personality traits may be strongly influenced by external factors such as changes in group structure or feeding levels associated with weather patterns. Because we controlled for

external factors in our study, we can conclude that physiological changes must play a significant role in personality shifts observed. One would thus, expect personality changes to coincide with the occurrence of physiological reorganizations such as those that occur at sexual maturation. Our results, suggest that a shift occurs prior to undergoing the final molt in males: penultimate males show markedly higher levels of aggressiveness towards prey that would provide increases in the rate of growth prior to abandonment of the web and feeding that mature males exhibit as they search for potential mating opportunities. Females exhibit a similar shift in aggressiveness, but it occurs following sexual maturation and is associated with accumulating the resources needed for the production of eggs. An example of an external factor that might influence personality shifts in *A. lisa* and other spiders is the departure of juveniles from the natal web they occupy for some period following emergence from the egg case. As individuals increase in size and undergo 1-2 molts, they become independent and disperse to build their own webs. While we did not test for behavioral consistency in the gregarious phase in this study, ongoing work (Riechert, in prep), indicates that this shift from the gregarious web to independent webs is mediated by competitive interactions that occur during feeding bouts.

Certain behavioral types and processes are also likely influenced by state-dependent factors (e.g., hunger level, parasite load) and asset protection principles would suggest that individuals modify their behavior based on current status and future needs (Wolf et al., 2007). The prevalence of change and consistency are discussed below in relation to both physiological and external events. Because the social and physiological changes occur at different time points in the life cycle of spiders, we are able to speculate on their different roles in generating stability in behavior.

**Mean-level consistency**

Behavioral responses to the simulated predator attacks increased in males at the penultimate stage and remained high after maturation. Female flight responses to simulated predator attacks also consistently increased across each life stage, reaching a maximum after maturation. Adults thus seem to be risk averse compared to juveniles who are more risk prone and have a less active flight response. This pattern observed in *A. lisa* has been observed in other species as well (Dangles et al., 2007; Favati et al., 2015; Gyuris et al., 2012; Hedrick and Kortet, 2012). Various explanations have been suggested for this change in behavior, such as that higher juvenile growth rates call for a more risk-prone feeding behavior (Gyuris et al., 2012) or that juveniles and adults experience different predatory challenges (Hedrick and Kortet, 2012). In *A. lisa*, several explanations might influence these changes in behavior. Perhaps the most compelling argument is related to the fact that birds have been shown to be major predators on *Agelenopsis aperta*, a close relative of *A. lisa* that occupies similar habitats in the desert southwest (Riechert and Hedrick, 1993). Birds are more likely to focus on larger instar individuals rather than juveniles, which would not offer much of a meal to adults or the fledglings they are feeding in the nest. Penultimate males and mature females experience increased energy expenditures and thus must maximize insect intake at a time when predation risk is greatest on them. The exhibition of more risk-averse behavior at this stage would an important balancing strategy: more active foraging but with greater vigilance. Male spiders likewise experience greater predation risk during the wandering period when they are searching for females. They lack the protection the web retreat offers them as well as the sensory information the web sheet and scaffolding afford.

Latencies to attack prey also varied predictably between penultimate males (short latencies to attack) that are maximizing growth, and mature males (long latencies to attack), that are about to abandon the web in search of matings and will have fewer foraging opportunities. Prey attack latencies consistently decreased over ontogeny in females, reaching a minimum at the adult stage when females are investing energy in the production of one to three clutches of eggs. Estimates of the number of prey captured and superfluous killing exhibit a similar trend, peaking at the penultimate stage in males and the adult stage in females.

The mean level of activity and level of responses in several boldness/exploration tests remained constant during ontogeny. This is in congruence with the general pattern of activity and boldness/explorations across species (Wexler et al., 2016; Wilson and Krause, 2012), though there are species where these traits increase (Mazué et al., 2015) or decrease over ontogeny (Bajer et al., 2015). Overall, natural selection may not favor behavioral trait consistency throughout the life span because selection pressures vary predictably over the lifespan and the life history of a particular species likely drives observed levels of consistency.

**Differential consistency (repeatability)**

We found in our study, that individual consistencies were generally quite low across the ontogenetic development of this species, which is one of very few to investigate ontogeny of personality in spiders (but see Kralj-Fišer and Schneider (2012); Sweeney et al. (2013)). Our repeatability estimates are lower than Kralj-Fišer and Schneider (2012), who measured similar traits to our study, but only applied the estimates to mature males and females (N=31 and N=30, respectively), using a seven-day repeat interval. They further used a different measure of repeatability than mixed modeling approach we applied in our study (Dingemanse and

Dochtermann, 2013; Nakagawa and Schielzeth, 2010). Our repeatability estimates also are on par with Sweeney et al. (2013), who measured only latency to move and latency to attack prey in penultimate spiders with a three-day compared to our 14-day repeat interval.

Reports to date on personality shifts across ontogeny and over longer periods of the life history of a species indicate that some aspects of personality are set early in life (Gyuris et al., 2012; Mazué et al., 2015; Wilson and Krause, 2012), whereas other aspects of personality are unstable across ontogeny (Bell and Stamps, 2004; Hedrick and Kortet, 2012; Johnson and Sih, 2007; Niemelä et al., 2012; Petelle et al., 2013; Sinn et al., 2008; Wuerz and Krüger, 2015). Boldness/exploration (latency to explore a novel environment) was the only trait that showed significant repeatability across the life cycle, in most studies. In our study, this was likely driven by the high repeatability of this trait in penultimate males and mature females. While none of the other traits exhibited significant repeatability across the life-cycle, the aggressiveness traits and the predator response to a puff cue exhibited the same pattern of high repeatability before sexual maturation in males with a sharp decline in repeatability after sexual maturation, and high repeatability in females only after sexual maturation (with the exception of latency to capture prey). The important change at this age in penultimate males involves increased energy expenditure on growth in preparation for sexual maturation, and on allocation of energy to egg development in mature females.

Boldness (responses to predatory cues and exploration in the prod tests) was inconsistent across the life cycle and failed to stabilize in the mature spiders. Inconsistency in behavior across sexual maturation has also been demonstrated for boldness in dumpling squid (Sinn et al., 2008), zebra finches (Wuerz and Krüger, 2015), and crickets (Hedrick and Kortet, 2012; Niemelä et al., 2012), and superfluous killing and courtship in bridge spiders (Kralj-Fišer and Schneider, 2012).

There are studies, however, that suggest individual behavior becomes more consistent during adulthood (Favati et al., 2015; Gyuris et al., 2012; Roberts et al., 2001; Sinn et al., 2008). Consistent with our study, Wuerz and Krüger (2015) noted that traits can be repeatable across the life cycle (i.e. fearlessness/ boldness) or only consistent within certain life stages between sexes (aggression, exploration, and activity), which is within the range of repeatabilities described in general (Bell et al., 2009; Wolak et al., 2012). These results encourage further studies investigating the role of hormonal reorganization during sexual maturation versus the role of diverging natural and sexual selection pressures between sexes in generating inconsistency of these behaviors.

It is important that such studies should focus on multiple assessments of personality traits over the course of development on a number of different personality traits. Preliminary analyses should be completed prior to data collection to determine stage interval lengths for different periods of the life cycle. Combined, the approach will permit estimation not only of the consistency of single personality traits, but also their functional coupling and how this changes over time.

**Behavioral syndromes**

Correlation structure among traits did not vary across ontogeny in both sexes. This means that juveniles do not exhibit behavioral syndromes and no clear behavioral syndromes appeared, which is inconsistent with a previous study in penultimate field-collected *Agelenopsis pennsylvanica* (Sweeney et al., 2013), where a boldness-aggression behavioral syndrome was present in the penultimate stage. Our study is consistent with studies of domestic red jungle fowl and yellow-bellied mamots in that behavioral syndromes did not appear across major life

transitions (Favati et al., 2015; Petelle et al., 2013), even though previous studies noted that correlations among traits changed over ontogeny in red jungle fowl (Favati et al., 2014a; Favati et al., 2014b). Studies in convict cichlids (Mazué et al., 2015) and firebugs (Gyuris et al., 2012) also show that the correlations among behavioral traits are consistent over ontogeny, with behavioral syndromes present from juveniles through adulthood. While behavioral syndromes were not present in our system, this absence was consistent across ontogeny, suggesting that behavioral syndromes may not be adaptive at any time point in some systems, similar to results found in the bridge spider, *Larinioides sclopetarius* (Kralj-Fišer and Schneider, 2012). The spiders we used were reared in the laboratory, as well as in groups until the 3$^{rd}$ instar, and we cannot rule out that this might have affected our findings regarding behavioral syndrome structure over time or other aspects of behavioral consistency. Nevertheless, the finding that behavioral trait correlations were consistently absent supports the perspective of behavioral syndromes as plastic and not mainly due to genetic constraints (Dingemanse et al., 2007; Johnson and Sih, 2007). Given the inconsistent results among studies of behavioral syndromes across ontogeny, why and when traits are expected to correlate warrant both further theoretical work and empirical studies. Furthermore, the lack of correlation between most of the traits suggests that these are separate responses that may play different roles in an individual's behavioral repertoire, or be explained by different underlying mechanisms.

In conclusion, we found that in contrast to the idea of behavioral syndromes, there was little consistency in behavior across life stages in the desert grass spider *Agelenopsis lisa*. These correlations are not manifest through development. The process of sexual maturation appears as an event reducing consistency in personality during ontogeny. In addition to that the influence of these events differed among the behavioral traits studied here. The underlying mechanisms of the

physiologically, environmentally and socially generated consistency and change remain unclear. Our results emphasize the need for future studies that are designed to disentangle internal (e.g., physiology/ hormones, molt cycle variation) and external (e.g., social reorganization, environmental) factors that operate during ontogenetic development to better understand the underlying processes responsible for the observed organization and stability of personality traits in individuals collected from the field.

## Works Cited


Bajer K, Horváth G, Molnár O, Török J, Garamszegi LZ, Herczeg G, 2015. European green lizard (Lacerta viridis) personalities: Linking behavioural types to ecologically relevant traits at different ontogenetic stages. Behavioural processes 111:67-74.

Bates DM, 2010. lme4: Mixed-effects modeling with R. URL http://lme4 r-forge r-project org/book.

Bell AM, Stamps JA, 2004. Development of behavioural differences between individuals and populations of sticklebacks, Gasterosteus aculeatus. Animal Behaviour 68:1339-1348.

Bell AM, Sih A, 2007. Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). Ecology Letters 10:828-834.

Bell AM, Hankison SJ, Laskowski KL, 2009. The repeatability of behaviour: a meta-analysis. Animal Behaviour 77:771-783.

Benjamini Y, Hochberg Y, 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological):289-300.

Carere C, Drent PJ, Privitera L, Koolhaas JM, Groothuis TG, 2005. Personalities in great tits, Parus major: stability and consistency. Animal Behaviour 70:795-805.

Core R, 2012. Team. R: A language and environment for statistical computing.

Dall SR, Houston AI, McNamara JM, 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecology letters 7:734-739.

Dangles O, Pierre D, Christides J, Casas J, 2007. Escape performance decreases during ontogeny in wild crickets. Journal of Experimental Biology 210:3165-3170.

Dingemanse NJ, Dochtermann NA, 2013. Quantifying individual variation in behaviour: mixed-effect modelling approaches. Journal of Animal Ecology 82:39-54.

Dingemanse NJ, Wright J, Kazem AJN, Thomas DK, Hickling R, Dawnay N, 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. Journal of Animal Ecology 76:1128-1138.

Dray S, Dufour A-B, 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of statistical software 22:1-20.

Favati A, Leimar O, Løvlie H, 2014a. Personality predicts social dominance in male domestic fowl. PloS one 9:e103535.

Favati A, Leimar O, Radesäter T, Løvlie H, 2014b. Social status and personality: stability in social state can promote consistency of behavioural responses. Proceedings of the Royal Society of London B: Biological Sciences 281:20132531.

Favati A, Zidar J, Thorpe H, Jensen P, Løvlie H, 2015. The ontogeny of personality traits in the red junglefowl, Gallus gallus. Behavioral Ecology:arv177.

Groothuis TG, Trillmich F, 2011. Unfolding personalities: the importance of studying ontogeny. Developmental psychobiology 53:641-655.

Günther A, Finkemeier M-A, Trillmich F, 2014. The ontogeny of personality in the wild guinea pig. Animal Behaviour 90:131-139.

Gyuris E, Feró O, Barta Z, 2012. Personality traits across ontogeny in firebugs, Pyrrhocoris apterus. Animal Behaviour 84:103-109.

Hedrick AV, Kortet R, 2012. Sex differences in the repeatability of boldness over metamorphosis. Behavioral Ecology and Sociobiology 66:407-412.

Johnson JC, Sih A, 2007. Fear, food, sex and parental care: a syndrome of boldness in the fishing spider, Dolomedes triton. Animal Behaviour 74:1131-1138.

Kralj-Fišer S, Schneider JM, 2012. Individual behavioural consistency and plasticity in an urban spider. Animal Behaviour 84:197-204.

Krause J, James R, Croft D, 2010. Personality in the context of social networks. Philosophical Transactions of the Royal Society of London B: Biological Sciences 365:4099-4106.

Maupin JL, Riechert SE, 2001. Superfluous killing in spiders: a consequence of adaptation to food-limited environments? Behavioral Ecology 12:569-576.

Mazué GP, Dechaume-Moncharmont F-X, Godin J-GJ, 2015. Boldness–exploration behavioral syndrome: interfamily variability and repeatability of personality traits in the young of the convict cichlid (Amatitlania siquia). Behavioral Ecology:arv030.

Niemelä PT, Vainikka A, Hedrick AV, Kortet R, 2012. Integrating behaviour with life history: boldness of the field cricket, Gryllus integer, during ontogeny. Functional Ecology 26:450-456.

Petelle MB, McCoy DE, Alejandro V, Martin JG, Blumstein DT, 2013. Development of boldness and docility in yellow-bellied marmots. Animal Behaviour 86:1147-1154.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ, 2007. Integrating animal temperament within ecology and evolution. Biological reviews 82:291-318.

Réale D, Dingemanse NJ, Kazem AJ, Wright J, 2010. Evolutionary and ecological approaches to the study of personality. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3937-3946.

Réale D, Dingemanse NJ, 2010. Personality and individual social specialisation. Social behaviour: genes, ecology and evolution:562.

Riechert SE, Hedrick AV, 1990. Levels of predation and genetically based anti-predator behaviour in the spider, *Agelenopsis aperta*. Animal Behaviour 40:679-687.

Riechert SE, Hedrick AV, 1993. A test for correlations among fitness-linked behavioural traits in the spider *Agelenopsis aperta* (Araneae, Agelenidae). Animal Behaviour 46:669-675.

Riechert SE, Johns PM, 2003. Do female spiders select heavier males for the genes for behavioral aggressiveness they offer their offspring? Evolution 57:1367-1373.

Riechert SE, Maupin J, Spider effects on prey: Tests for superfluous killing in five web builders. Proc 17th European Colloquium Arachnol(PA Selden, ed) Bull British Arachnol Soc1998. p. 203-210.

Roberts BW, Caspi A, Moffitt TE, 2001. The kids are alright: growth and stability in personality development from adolescence to adulthood. Journal of personality and social psychology 81:670.

Schielzeth H, Nakagawa S, 2013. rptR: Repeatability for Gaussian and non-Gaussian data. R package version 0.6. 405/r52.

Sih A, Bell A, Johnson JC, 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19:372-378.

Sinn DL, Gosling SD, Moltschaniwskyj NA, 2008. Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. Animal Behaviour 75:433-442.

Stamps J, Groothuis TG, 2010a. The development of animal personality: relevance, concepts and perspectives. Biological Reviews 85:301-325.

Stamps JA, Groothuis TG, 2010b. Developmental perspectives on personality: implications for ecological and evolutionary studies of individual differences. Philosophical Transactions of the Royal Society of London B: Biological Sciences 365:4029-4041.

Sweeney K, Gadd RD, Hess ZL, McDermott DR, MacDonald L, Cotter P, Armagost F, Chen JZ, Berning AW, DiRienzo N, 2013. Assessing the effects of rearing environment, natural selection, and developmental stage on the emergence of a behavioral syndrome. Ethology 119:436-447.

Team RC, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013. ISBN 3-900051-07-0.

Wexler Y, Subach A, Pruitt JN, Scharf I, 2016. Behavioral repeatability of flour beetles before and after metamorphosis and throughout aging. Behavioral Ecology and Sociobiology 70:745-753.

Wilson AD, Godin J-GJ, 2009a. Boldness and behavioral syndromes in the bluegill sunfish, Lepomis macrochirus. Behavioral Ecology:arp018.

Wilson ADM, Godin J-GJ, 2009b. Boldness and behavioral syndromes in the bluegill sunfish, Lepomis macrochirus. Behavioral ecology 20:231-237.

Wilson AD, Godin JGJ, Ward AJ, 2010. Boldness and reproductive fitness correlates in the eastern mosquitofish, Gambusia holbrooki. Ethology 116:96-104.

Wilson AD, Krause J, 2012. Personality and metamorphosis: is behavioral variation consistent across ontogenetic niche shifts? Behavioral Ecology 23:1316-1323.

Wolak ME, Fairbairn DJ, Paulsen YR, 2012. Guidelines for estimating repeatability. Methods in Ecology and Evolution 3:129-137.

Wolf M, Van Doorn GS, Leimar O, Weissing FJ, 2007. Life-history trade-offs favour the evolution of animal personalities. Nature 447:581-584.

Wuerz Y, Krüger O, 2015. Personality over ontogeny in zebra finches: long-term repeatable traits but unstable behavioural syndromes. Frontiers in Zoology 12:1-14.

# APPENDIX A

**Table 1.1.** Assignment of 11 behavioral traits examined to behavioral syndrome classifications.

| Activity | Aggressiveness | Boldness |
|---|---|---|
| Activity over 10 mins | Latency to attack prey | Latency: Explore novel environment |
| | Capture estimate | Distance moved upon introduction |
| | Wasteful killing estimate | Front prod: Retreat distance |
| | | Latency: Explore following front prod |
| | | Rear Prod: Retreat distance |
| | | Puff: Behavioral response |
| | | Puff: Latency to return |

**Table 1.2.** Repeatability estimates, P-values (produced by likelihood ratio tests), and 95% confidence limits within and across life-stages for 11 behavioral traits.

| Trait | Life stage | R | CI (95%) | P |
|---|---|---|---|---|
| **BOLDNESS** | | | | |
| Latency to explore novel environment | **All** | **0.23** | **(0.12, 0.33)** | **<0.0001** |
| | Juvenile Male | 0 | (0, 0.65) | > 0.05 |
| | Juvenile Female | 0.16 | (0, 0.43) | > 0.05 |
| | Penultimate Male | 0.52 | (0, 0.91) | > 0.05 |
| | Penultimate Female | 0.05 | (0, 0.34) | > 0.05 |
| | Mature Male | 0.16 | (0, 0.62) | > 0.05 |
| | Mature Female | 0.36 | (0.08, 0.60) | > 0.05 |
| Distance upon introduction | All | 0.10 | (0.01, 0.19) | > 0.05 |
| | Juvenile Male | 0.27 | (0, 0.76) | > 0.05 |
| | Juvenile Female | 0.22 | (0, 0.49) | > 0.05 |
| | Penultimate Male | 0.17 | (0, 0.83) | > 0.05 |
| | Penultimate Female | 0.22 | (0, 0.48) | > 0.05 |
| | Mature Male | 0 | (0, 0.50) | > 0.05 |
| | Mature Female | 0 | (0, 0.30) | > 0.05 |
| Front prod distance | All | 0.03 | (0, 0.12) | > 0.05 |
| | Juvenile Male | 0 | (0, 0.68) | > 0.05 |
| | Juvenile Female | 0.19 | (0, 0.47) | > 0.05 |
| | Penultimate Male | 0.25 | (0, 0.82) | > 0.05 |
| | Penultimate Female | 0 | (0, 0.29) | > 0.05 |
| | Mature Male | 0.16 | (0, 0.65) | > 0.05 |
| | Mature Female | 0.12 | (0, 0.41) | > 0.05 |
| Latency to explore after front prod | All | 0.03 | (0, 0.12) | > 0.05 |
| | Juvenile Male | 0.46 | (0, 0.83) | > 0.05 |
| | Juvenile Female | 0 | (0, 0.31) | > 0.05 |
| | Penultimate Male | 0.18 | (0, 0.83) | > 0.05 |
| | Penultimate Female | 0.10 | (0, 0.38) | > 0.05 |
| | Mature Male | 0 | (0, 0.49) | > 0.05 |
| | Mature Female | 0.11 | (0, 0.38) | > 0.05 |
| Rear prod distance | All | 0.08 | (0, 0.17) | > 0.05 |
| | Juvenile Male | 0.21 | (0, 0.73) | > 0.05 |
| | Juvenile Female | 0.04 | (0, 0.31) | > 0.05 |
| | Penultimate Male | 0 | (0, 0.77) | > 0.05 |
| | Penultimate Female | 0.03 | (0, 0.31) | > 0.05 |
| | Mature Male | 0.35 | (0, 0.72) | > 0.05 |
| | Mature Female | 0.03 | (0, 0.33) | > 0.05 |
| Latency to explore after rear prod | All | 0.02 | (0, 0.07) | > 0.05 |
| | Juvenile Male | 0 | (0, 0.62) | > 0.05 |

**Table 1.2.** Continued.

| Trait | Life stage | R | CI (95%) | P |
|---|---|---|---|---|
| | Juvenile Female | 0 | (0, 0.31) | > 0.05 |
| | Penultimate Male | 0.40 | (0, 0.88) | > 0.05 |
| | Penultimate Female | 0 | (0, 0.26) | > 0.05 |
| | Mature Male | 0 | (0, 0.52) | > 0.05 |
| | Mature Female | 0.15 | (0, 0.42) | > 0.05 |
| Latency to return after puff | All | 0.01 | (0, 0.08) | > 0.05 |
| | Juvenile Male | 0 | (0, 0.66) | > 0.05 |
| | Juvenile Female | 0 | (0, 0.29) | > 0.05 |
| | Penultimate Male | 0 | (0, 0.80) | > 0.05 |
| | Penultimate Female | 0.45 | (0, 0.57) | > 0.05 |
| | Mature Male | 0 | (0, 0.54) | > 0.05 |
| | **Mature Female** | **0.54** | **(0, 0.59)** | **= 0.04** |
| **ACTIVITY** | | | | |
| Activity over 10 minutes | All | 0.11 | (0.02, 0.21) | > 0.05 |
| | Juvenile Male | 0.09 | (0, 0.70) | > 0.05 |
| | Juvenile Female | 0.01 | (0, 0.32) | > 0.05 |
| | Penultimate Male | 0 | (0, 0.78) | > 0.05 |
| | Penultimate Female | 0.15 | (0, 0.41) | > 0.05 |
| | Mature Male | 0.36 | (0, 0.73) | > 0.05 |
| | Mature Female | 0.10 | (0, 0.39) | > 0.05 |
| **AGGRESSIVENESS** | | | | |
| Latency to capture prey | All | 0.15 | (0, 0.15) | > 0.05 |
| | Juvenile Male | 0 | (0, 0.67) | > 0.05 |
| | **Juvenile Female** | **0.82** | **(0.47, 0.83)** | **= 0.002** |
| | Penultimate Male | 0.82 | (0, 0.94) | > 0.05 |
| | Penultimate Female | 0.51 | (0.02, 0.59) | > 0.05 |
| | Mature Male | 0 | (0, 0.51) | > 0.05 |
| | Mature Female | 0.33 | (0, 0.49) | > 0.05 |
| Capture estimate | All | 0.02 | (0, 0.10) | > 0.05 |
| | Juvenile Male | 0 | (0, 0.70) | > 0.05 |
| | Juvenile Female | 0 | (0, 0.32) | > 0.05 |
| | Penultimate Male | 0.60 | (0, 0.92) | > 0.05 |
| | Penultimate Female | 0 | (0, 0.30) | > 0.05 |
| | Mature Male | 0 | (0, 0.50) | > 0.05 |
| | Mature Female | 0.36 | (0.08, 0.58) | > 0.05 |
| Wasteful killing | All | 0.02 | (0, 0.08) | > 0.05 |
| | Juvenile Male | 0.19 | (0, 0.73) | > 0.05 |
| | Juvenile Female | 0 | (0, 0.29) | > 0.05 |
| | Penultimate Male | 0.78 | (0.35, 0.96) | > 0.05 |
| | Penultimate Female | 0.18 | (0, 0.45) | > 0.05 |
| | Mature Male | 0 | (0, 0.50) | > 0.05 |
| | Mature Female | 0.38 | (0.10, 0.61) | > 0.05 |

P-values presented are corrected for multiple comparisons. Repeatabilities that had a P-value < 0.05 before adjustment of P values are bolded.

**Table 1.3.** F-statistics and P-values for the fixed effects from the linear mixed models across all behavioral traits measured. Significant results (alpha = 0.05) are highlighted in bold.

| Trait | Developmental Stage |
|---|---|
| **BOLDNESS** | |
| Latency to explore novel environment | $F_{5,388} = 2.24$, P = 0.05 |
| Distance upon introduction | $F_{5,388} = 2.10$, P = 0.07 |
| Front prod distance | **$F_{5,388} = 2.95$, P = 0.01** |
| Latency to explore after front prod | $F_{5,388} = 1.08$, P = 0.38 |
| Rear prod distance | **$F_{5,388} = 2.47$, P = 0.03** |
| Latency to explore after rear prod | $F_{5,388} = 0.81$, P = 0.54 |
| Latency to return after puff | **$F_{5,388} = 6925.8$, P < 0.0001** |
| **ACTIVITY** | |
| Activity over 10 minutes | $F_{5,388} = 2.02$, P = 0.08 |
| **AGGRESSIVENESS** | |
| Latency to capture prey | **$F_{5,388} = 1554.7$, P < 0.0001** |
| Capture estimate | **$F_{5,388} = 9.45$, P < 0.0001** |
| Wasteful killing | **$F_{5,388} = 12.18$, P < 0.0001** |

**Table 1.4.** Mean estimates of each behavioral trait with 95% confidence limits (in parentheses) for each developmental stage.

| Trait | JM | JF | PM | PF | MM | MF |
|---|---|---|---|---|---|---|
| **BOLDNESS** | | | | | | |
| Latency to explore novel environment (s) | 275.5 (152.5, 497.6) | 385.2 (181.3, 818.2) | 696.8 (330.7, 1468.1) | 363.2 (204.7, 644.7) | 659.3 (323.3, 1344.5) | 389.3 (219.1, 691.6) |
| Distance upon introduction (cm) | 8.4 (5.2, 13.6) | 16.5 (11.9, 23.0) | 12.9 (8.0, 20.7) | 14.5 (10.7, 19.7) | 17.6 (11.4, 27.3) | 13.4 (9.9, 18.3) |
| Front prod distance (cm) | 8.9 (5.9, 14.7) | 14.7 (9.4, 23.0) | 8.8 (5.9, 13.1) | 13.0 (8.9, 19.1) | 11.5 (8.0, 16.5) | 9.5 (6.8, 13.1) |
| Latency to explore after front prod (cm) | 584.6 (313.8, 1088.8) | 646.0 (409.1, 1020.2) | 711.1 (386.0, 1310.0) | 751.2 (500.8, 1126.7) | 781.0 (448.8, 1359.1) | 490.8 (329.4, 731.3) |
| Rear prod distance (cm) | 15.5 (10.7, 22.5) | 16.7 (11.1, 25.1) | 12.5 (8.7, 17.9) | 19.4 (13.5, 27.9) | 14.6 (10.5, 20.3) | 13.4 (9.9, 18.1) |
| Latency to explore after rear prod (s) | 505.3 (186.6, 1019.9) | 638.9 (392.8, 1039.3) | 662.9 (331.8, 1324.5) | 814.9 (527.7, 1258.5) | 826.4 (442.3, 1544.3) | 562.3 (364.7, 867.0) |
| Latency to return after puff (s) | 36.7 (11.8, 114.1) | 117.1 (60.6, 226.3) | 6.3 (2.0, 19.6) | 38.9 (20.1, 75.2) | 27.7 (8.9, 86.1) | 22.6 (11.7, 43.7) |
| **ACTIVITY** | | | | | | |
| Activity over 10 minutes (s) | 73.5 (31.0, 115.6) | 53.1 (39.2, 72.0) | 75.1 (48.1, 117.1) | 52.8 (39.9, 69.9) | 44.2 (29.3, 66.8) | 66.3 (50.0, 87.9) |
| **AGGRESSIVENESS** | | | | | | |
| Latency to capture prey (s) | 17.4 (8.0, 37.8) | 20.3 (12.8, 32.2) | 1.7 (0.8, 3.7) | 16.9 (10.7, 26.7) | 50.4 (23.2, 109.7) | 5.6 (3.6, 8.9) |
| Capture estimate | 2.6 (2.3, 2.9) | 2.7 (2.4, 3.1) | 3.0 (2.7, 3.4) | 2.6 (2.4, 2.9) | 2.0 (1.8, 2.2) | 2.5 (2.3, 2.8) |
| Wasteful killing | 1.6 (1.3, 1.9) | 1.9 (1.7, 2.1) | 1.9 (1.6, 2.2) | 1.6 (1.4, 1.7) | 1.5 (1.3, 1.7) | 1.3 (1.1, 1.4) |

**Table 1.5.** Structural consistency of the relations among behavioral traits in *Agelenopsis lisa*.

| Distance matrices | $r_s$ | P |
|---|---|---|
| Juvenile male vs. juvenile female | 0.234 | 0.051 |
| Penultimate male vs. penultimate female | 0.320 | 0.0068 |
| Mature male vs. mature female | 0.500 | 0.001 |
| Juvenile male vs. penultimate male | 0.398 | 0.0042 |
| Juvenile male vs. mature male | 0.413 | 0.0033 |
| Penultimate male vs. mature male | 0.333 | 0.016 |
| Juvenile female vs. penultimate female | 0.533 | 0.0003 |
| Juvenile female vs. mature female | 0.619 | 0.0001 |
| Penultimate female vs. mature female | 0.583 | 0.0003 |

Pairwise Mantel tests between rank correlation matrices ($r_s$) of behavioral scores and their significance (P-value) at different life stages. All significant P-values remained significant after Benjamini & Hochberg (1995) correction.

**Figure 1.1.** Spearman rank correlations for 11 behavioral traits across life-stages.

A)

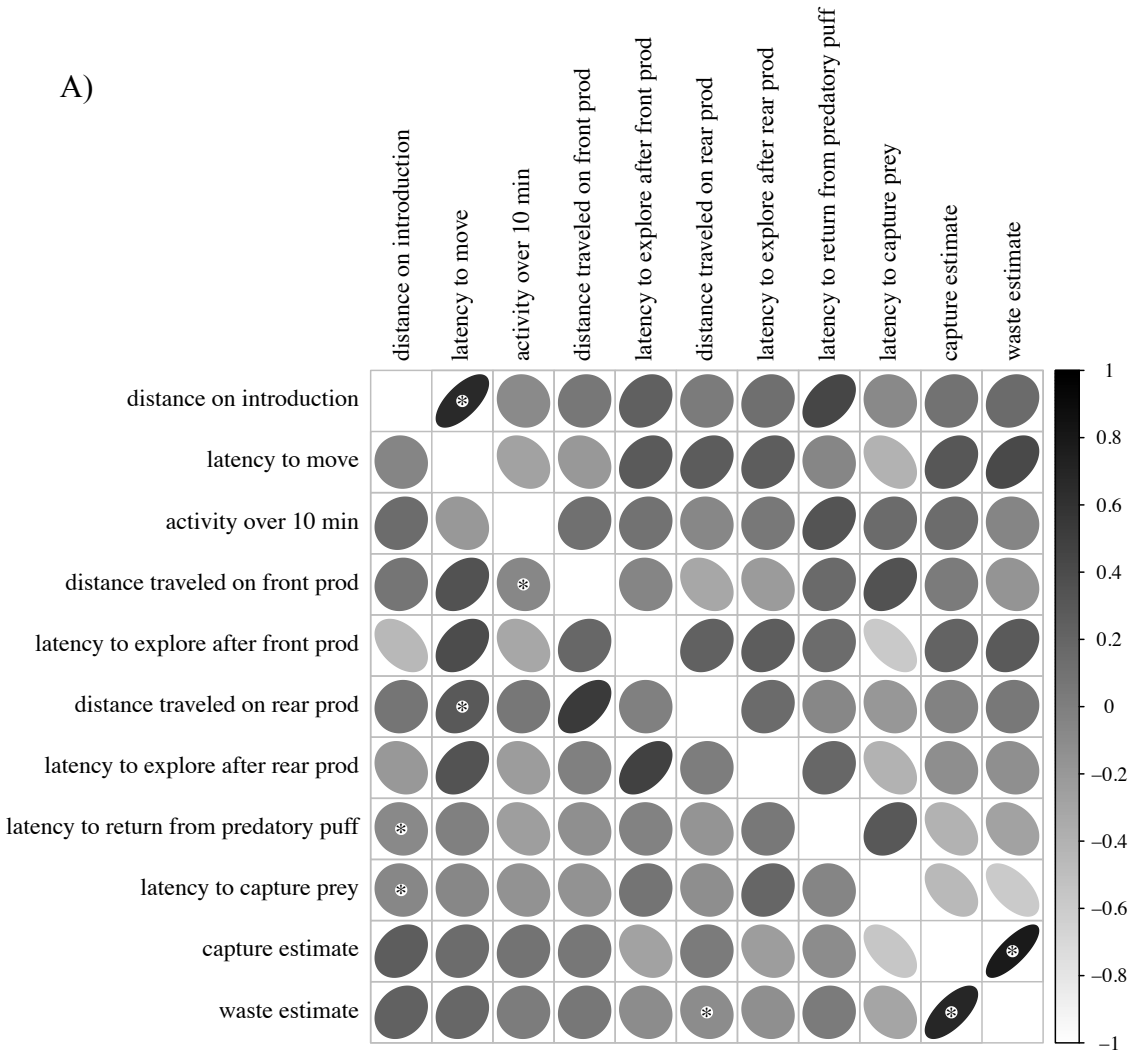


**Figure 1.1.** Continued.

B)

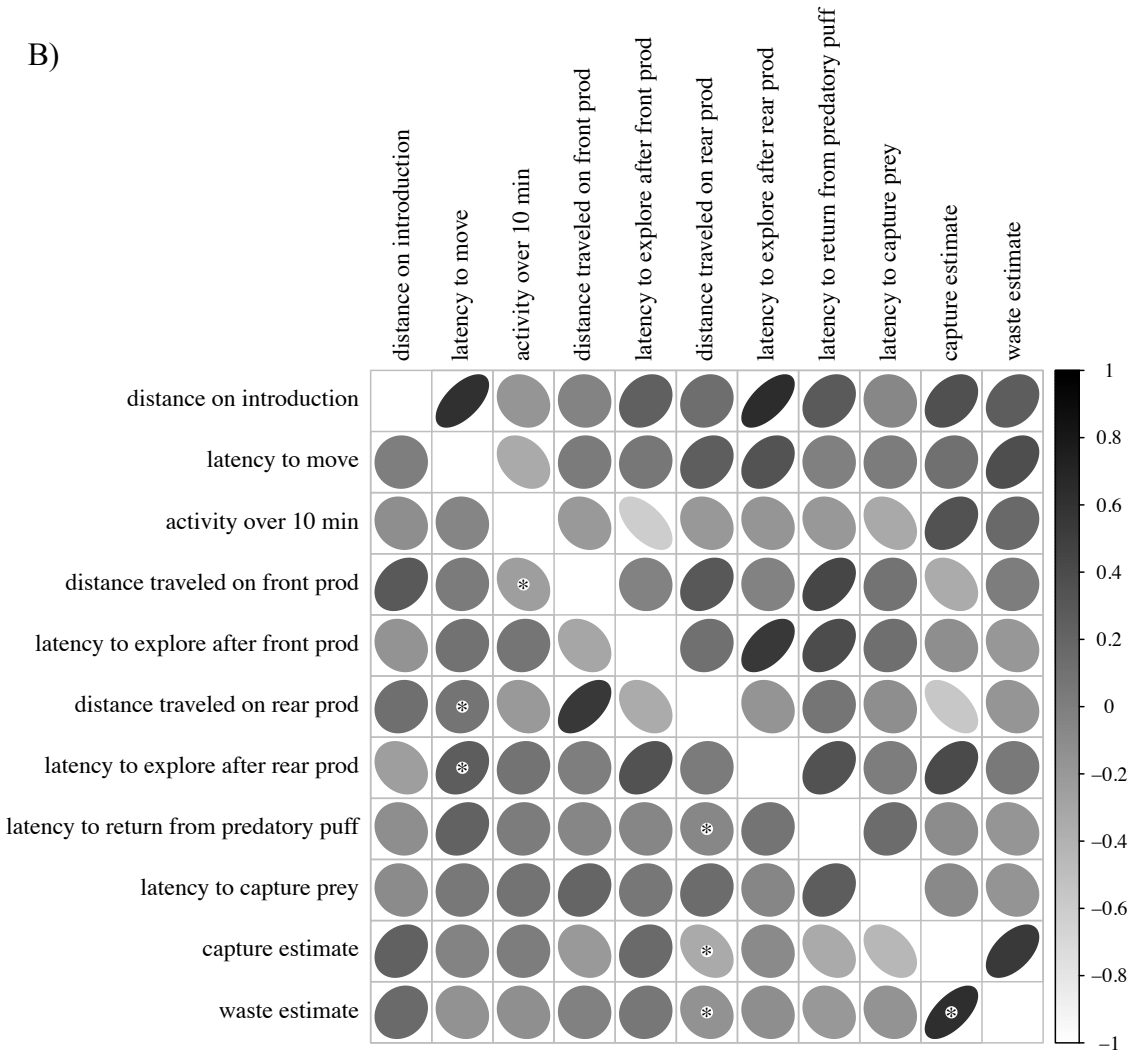


**Figure 1.1.** Continued.

C)

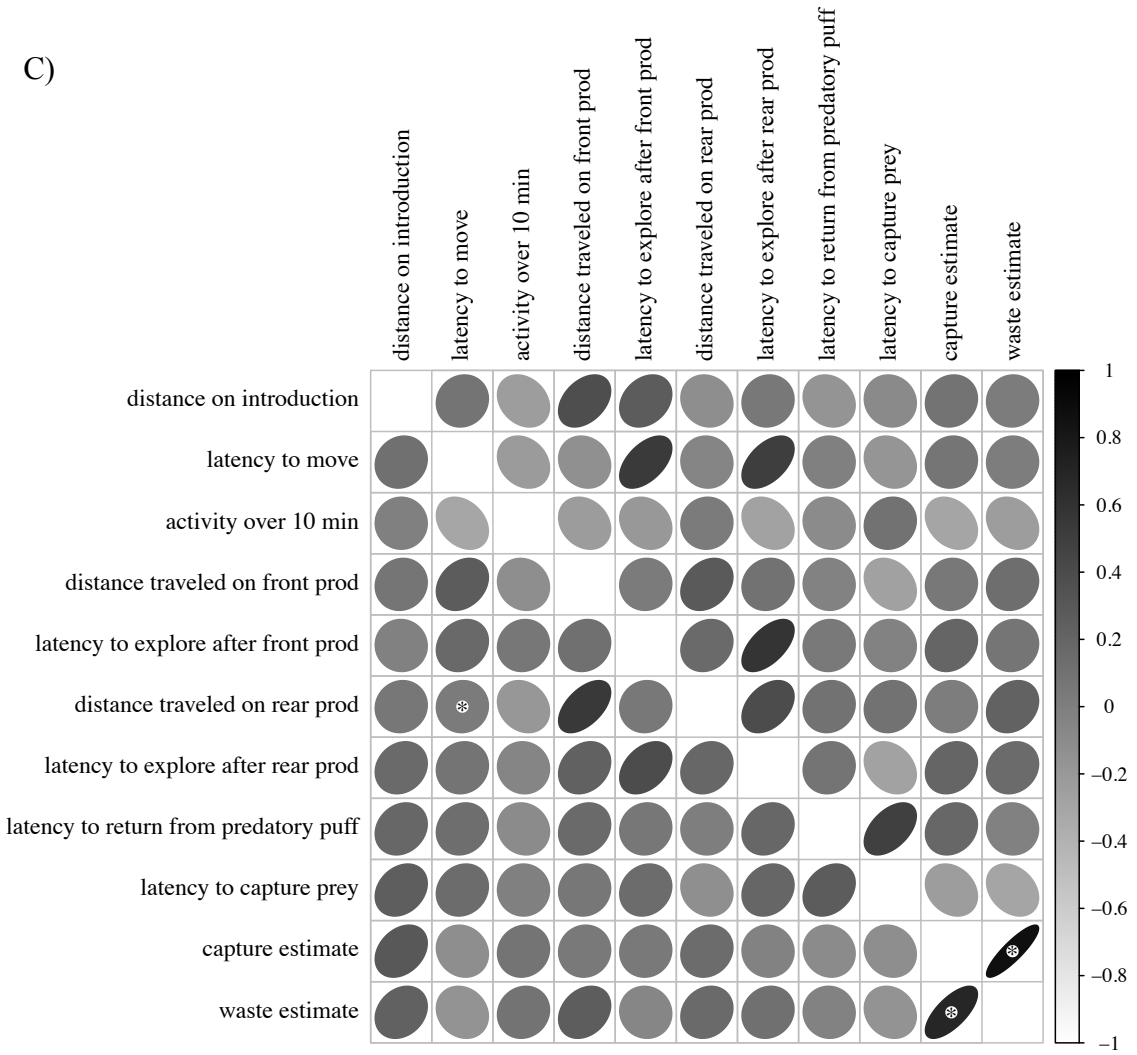


**Figure 1.1.** Continued.

Spearman rank correlation coefficients are given for females (below diagonal) and males (above diagonal), (A) Juveniles: $n_{female} = 54$, $n_{male} = 19$ (B) Penultimate: $n_{female} = 67$, $n_{male} = 22$, and (C) Mature: $n_{female} = 54$, $n_{male} = 22$. Correlations that had a P-value < 0.05 before adjustment of P values are marked with (*).
Significance at the *P < 0.05 after adjusting P-values for multiple comparisons.

# CHAPTER II: HABITAT USE DRIVES BEHAVIORAL SYNDROME EVOLUTION IN AMERICAN FUNNEL-WEB SPIDERS (AGELENIDAE)

This chapter is based on an original research article submitted to *American Naturalist* in January 2017. My primary contributions to this paper include: (i) collection and analysis of the data, (ii) table development, (iii) completion of a first draft of the manuscript.

**Bosco JM**, Riechert SE, O'Meara BC. Habitat use drives behavioral syndrome evolution in American funnel-web spiders (Agelenidae).

## Abstract

Although many descriptive studies on behavioral syndromes have been performed, the factors that underlie the evolution of behavioral syndromes remain poorly understood. To test the hypothesis that behavioral syndrome evolution is affected by habitat type, we completed a phylogenetic comparative analysis of habitat association and behavioral trait data sets for 19 North American representative species of the RTA spider clade: 16 species of grass spiders (Agelenidae), and three species of wolf spiders (Lycosidae). We find that these species show substantial variation in behavioral trait correlations in general and in behavioral aggressiveness and boldness in particular. We find no evidence of the conservation of behavioral trait correlations across phylogeny. Rather, behavioral trait correlations in these spider species appear to be evolutionarily labile and are correlated with species habitat associations. The specific environmental factors driving trait correlation divergence remain to be delineated.

## Background

Behavioral syndromes are known to vary widely within and between populations (Huntingford, 1976; Riechert and Hedrick, 1993; Sih et al., 2004). Understanding the origin and maintenance of this phenotypic variation is of current interest to both behavioral and evolutionary ecologists. A behavioral syndrome consists of a suite of behaviors that are correlated through time within a population along two (or more) behavioral axes (Huntingford, 1976; Réale et al., 2007; Sih et al., 2004; Sih and Bell, 2008). The most commonly measured behaviors are activity, aggression, boldness and sociability (Réale et al., 2007).

There are two competing hypotheses pertaining to the ecological and evolutionary formation and maintenance of behavioral syndromes, 'constraint' and 'adaptive'. The 'constraint' hypothesis suggests that syndromes are the result of internal or external constraining force(s). Examples of internal constraining forces include gene pleiotropy, physiological constraints or hormonal influences (Maynard Smith and Riechert, 1984; Stamps, 1991). External constraints include environmental factors such as predation pressure (Riechert and Hall, 2000) and latitude (Pruitt et al., 2008). The 'adaptive' hypothesis describes individuals as altering their behavior, depending on their situation (Bell, 2005; Pruitt et al., 2011b). Because considerable supporting evidence exists for both 'constraint' and 'adaptive' hypotheses, one or both effects may be operating in any given system. The true proximate mechanism may well be the blending of both constraining and adaptive forces.

Many examples of behavioral syndromes have been documented across different species (Brown et al., 2005; Carter et al., 2010; Dingemanse et al., 2007; Huntingford, 1976; Kortet and Hedrick, 2007; Moretz et al., 2007; Pruitt et al., 2008; Riechert and Hedrick, 1993) and

behavioral syndromes in spiders have been particularly well studied (Johnson and Sih, 2005, 2007; Maynard Smith and Riechert, 1984; Pruitt et al., 2008; Riechert and Hall, 2000; Riechert and Hedrick, 1993; Riechert and Maynard Smith, 1989). As compared to other taxa, behavioral syndromes are believed to be relatively stable in spiders (Pruitt and Riechert, 2012), because distantly related species (Johnson and Sih, 2005; Kralj-Fišer and Schneider, 2012; Pruitt et al., 2011a; Sweeney et al., 2013) and populations from ecologically distinct habitats (Riechert, 1993; Riechert and Hedrick, 1993) often exhibit similar syndromes. The most pervasive syndrome observed in spiders is a positive correlation between individual boldness and their aggressiveness towards prey, competitors, and mates (Johnson and Sih, 2005; Pruitt et al., 2008; Riechert et al., 2002).

We test the adaptive model of behavioral syndrome evolution by combining comprehensive behavioral analyses with a robust, species-level phylogenetic framework. By characterizing the behavioral correlates of animal personality and testing their functional consequences, we hope to begin to elucidate the general selective pressures favoring the coupling or decoupling of behavioral trait correlations. This is the first study of its kind to tie behavioral trait evolution to the concept of diversification on a macro-evolutionary adaptive landscape, a multivariate phenotype surface where it is possible for species to evolve up local adaptive peaks (Karr and James, 1975; Simpson, 1953). In this case, it is possible that the habitat that a species occupies shapes its temperament and behavioral trait correlations more so than genetic constraints. Therefore, rather than closely related species exhibiting more similar behaviors, it appears that species inhabiting particular habitats have converged on more similar behaviors that allow them to succeed in a particular environment. Convergent evolution is among the most powerful lines of evidence for the power of natural selection to shape organisms to their

environment, implying a deterministic aspect of phenotypic evolution over macro-evolutionary time scales (Losos, 2010; Simpson, 1953). Habitat specialization is thought to be one of the main forces driving the evolution of morphological traits (lizards: Goodman et al., 2008; Harrison et al., 2015, snails: Hirano et al., 2015, snakes: Fabre et al., 2016, fish: Davis et al., 2014) as well as behavioral traits such as social behavior (Legendre et al., 2014; Pruitt et al., 2012), alarm call behavior (García-Navas and Blumstein, 2016), territorial behavior (Johnson et al., 2010), and mating behavior (York et al., 2015), but such a hypothesis has yet to be tested in a comparative framework for personality traits associated with behavioral syndromes. New comparative frameworks that model multiple phenotypic trait optima (Beaulieu et al., 2012; Beaulieu and O'Meara, 2012; Butler and King, 2004) facilitate mechanistic questions on the nature of the phenotypic evolution, particularly regarding the scale of macro-evolutionary regimes.

Examinations of behavioral syndrome evolution have to date only been addressed in individual species (Brown et al., 2005; Carter et al., 2010; Dingemanse et al., 2007; Huntingford, 1976; Kortet and Hedrick, 2007; Moretz et al., 2007; Pruitt et al., 2008; Riechert and Hedrick, 1993, but see Bell et al., 2009). The implications of a phylogenetic study for the evolution of behavioral syndromes have yet to be explored. Using a data set comprising 19 species of a spider clade from the continental United States, we test herein the following hypotheses: (1) Are behavioral traits characterized by high phylogenetic signal?; (2) Have differences in traits associated with behavioral syndromes evolved among species occupying particular habitats?; and (3) Have correlations between behavioral traits remained consistent both across phylogeny and among members of the different habitat classes?

# Methods

## Taxon Sampling and Phylogeny Reconstruction

For all analyses, we use an ultrametric, maximum likelihood phylogenetic tree pruned from a larger phylogeny encompassing the entire spider order, Araneae (Bosco & O'Meara unpublished). Prior to completing tree pruning and phylogenetic analyses, we resolved polytomies randomly with the 'multi2di' function in the 'ape' package (Paradis et al., 2004). Our tree contains 19 species of the RTA spider clade. Our focus is on this group because it is best known behaviorally and one that we have had considerable experience with in delineating behavioral trait correlations. Sixteen species represent the family Agelenidae (i.e.,12 of the 14 American *Agelenopsis* species, two of the four *Barronopsis* species, one *Hololena* species and one *Novalena* species). The three outgroup species are from the family Lycosidae (two of 19 American *Hogna* species, and one *Schizocosa* species).

## Specimen Collection and Laboratory Maintenance

We collected spiders of the American *Agelenopsis* group and close relatives for behavioral quantification from across the United States between 2012 and 2014.

*Agelenopsis* Giebel 1869 (Chamberlin and Ivie, 1941) terminals include 19-126 individuals representing 12 of the 14 *Agelenopsis* species described to date (see Table 2.1 for species list). For the molecular data we include all described species for which we obtained fresh material. Outgroups within the family Agelenidae include the closely related *Hololena* Chamberlin and Gertsch and *Novalena* Chamberlin and Ivie 1942 (Chamberlin and Ivie, 1942). To root the phylogeny, we also include more distant outgroups from the family Lycosidae (*Hogna carolinensis*, *Hogna helluo*, and *Schizocosa avida*).

Our maintenance protocol includes individual housing under 22-24°C on a 12:12 h light: dark cycle with a weekly diet of *ad libitum* termite workers for the younger instars. Domestic crickets are offered to the larger instar spiders and all spiders are misted at the time of feeding.

**Behavioral Measures**

We score individual spiders for the following behaviors once they reach the penultimate stage ($8^{th}$-$9^{th}$ molt). Each individual is released into an unfamiliar container (10.5 cm diameter x 5 cm height), three days after a routine feeding. We then record (1) the distance the spider moves on the track on introduction before pausing, (2) the time elapsed between the individual's cessation of movement and the first movement as measured by stopwatch, and (3) the amount of time that the individual spends moving during the entire ten minute test period. Note that movements are scored as independent if they are separated by three seconds or more of quiescence and a stopwatch is used in timing all time periods in these trials.

After completion of the exploratory and boldness assays, individuals that have been settled for 5 minutes are touched at the rear with a prod (the eraser end of a pencil after Riechert and Johns (2003)) and we record the distance it travels. We allow the individual to settle for 5 minutes before prodding it from the front with the probe. Rear prods versus front prods are administered first versus second in alternate trials.

One day after completion of the trials listed above, we subject each test spider to a second anti-predator test following Riechert and Hedrick (1990). In this trial we direct a single puff of air towards a test subject sitting face out at its funnel entrance. The puff of air is produced by a camera bulb held at a 45° angle to the web and at a height of 8 cm above the web. It is directed at a spot 6 cm in front of the funnel entrance. We record (1) the spider's response to the puff (e.g.,

whether it displays an aggressive response such as running out toward the cue or a fearful/non-aggressive response such as retreating into the funnel), (2) the length of time that elapses between retreat and the reappearance of the spider at the funnel entrance. 'No retreat' or aggressive responses directed at the bulb are scored as 0 s.

One day after completion of the exploration, boldness and anti-predator tests, an individual is offered a single 2-week old cricket, placed on the web 2.0 cm from the test individual. We record the spider's latency to attack (make contact with the prey). The superfluous killing trials follow the protocols used to test for general foraging aggressiveness in other studies of spider behavior by our lab (Maupin and Riechert, 2001; Riechert and Maupin, 1998). We introduce a size appropriate domestic cricket at three-minute intervals to the test subject's web. Additional time is provided if an individual needs more time to subdue a particular prey item. Trials are terminated when an individual fails to attack two consecutive prey items. At the end of the observation period, all rejected prey items are removed from the web and the individual is given 24h to feed on the prey. After this 24h period, the captured prey items are examined and assigned to one of the following categories based on the remaining percentage of mass relative to their original body mass: fully consumed (<10%), partially consumed (10-25%) or uneaten (>25%). If an individual is still feeding on prey after 24h, it is given an additional 24h to feed and the prey identified as to feeding classification after that time.

**Analysis of Phylogenetic Signal**

Phylogenetic signal is the tendency of related species to resemble each other more than species drawn at random from the same tree (Blomberg and Garland, 2002). It is a measure of the statistical non-independence among species trait values because of their phylogenetic

relatedness (Felsenstein, 1985; Münkemüller et al., 2012; Revell et al., 2008). The phylogenetic signal of each behavior is determined for each species using Blomberg's K, as it allows for comparison among different phylogenies for continuous traits, across traits, and tree types (Blomberg et al., 2003; Münkemüller et al., 2012). Blomberg's K expresses the strength of phylogenetic signal as the ratio of the mean squared error of the tip data measured from the phylogenetic corrected mean and the mean squared error based on the variance-covariance matrix derived from the given phylogeny under the assumption of Brownian motion (Blomberg et al., 2003; Münkemüller et al., 2012). If the resemblance of species in the trait of interest is due to the degree of shared evolutionary history, K should be 1. On the other hand, a small K value (close to zero) implies phylogenetic independence. Overdispersion (high levels of trait value variation) in trait expression may be due to adaptive evolution or to high measurement errors in the trait or in the construction of the tree (Blomberg et al., 2003). Blomberg's K statistic and associated P-values are calculated for each behavioral trait using the phylosignal function of the 'picante' package in R (Kembel and Kembel, 2014).

**Testing Adaptive Models for Spider Behavioral Evolution**

Our assessment of what evolutionary model best fits the evolution of behavioral personality in spiders entails stochastic character-mapped reconstructions of habitat (SIMMAP; Bollback, 2006; Nielsen, 2002), which we apply in producing a maximum-likelihood tree. Our two main habitat types are 'desert' (species inhabiting arid desert habitats), and 'riparian' (species inhabiting the more mesic riparian habitats). Using this simple character coding, the distribution of species habitats conforms well to a two-state framework (Table 2.1).

We examine several evolutionary models to find the best fit in explaining the evolution of behavioral traits associated with personality in spiders. First, we fit single-rate Brownian motion (BM1) to each log-transformed behavioral trait, a time-homogenous (random walk) process in which behavioral disparity varies at random and increases uniformly as a function of time. We then test a two-rate BM model (BMS; O'Meara et al., 2006), in which riparian and desert species exhibit different rates of BM evolution for its application to the two habitat use patterns. Secondly, we fit a single-optimum Ornstein-Uhlenbeck (OU) adaptive model (Hansen 1997; Butler and King, 2004) with one parameter for the variance of random walk ($\sigma^2$) and strength of selection ($\alpha$) towards a global optimum for all species (OU1). The OU process is another variation of BM that models phenotypic variation oscillating around one or more phenotypic optima ($\theta$), as well as a so-called rubber band parameter ($\alpha$) that determines whether the trait is drawn back to its optimal value as it evolves away under stabilizing selection. The deviation of the trait value from the optimum ($\sigma^2$) is interpreted as the rate of stochastic motion or, more simply, the rate of evolution (Beaulieu and O'Meara, 2012; Butler and King, 2004; Hansen, 1997). Thirdly, we assessed the fit of OU models with separate optima for each habitat, but global $\sigma^2$ and $\alpha$ parameters for the different riparian ($\theta_R$), and desert ($\theta_D$) selective regimes (OUM; Butler and King, 2004). Finally, we measured phylogenetic half-life ($t_{1/2}$) (Hansen et al., 2008) for each trait to assess the length of time it takes a trait to move half the distance from the ancestral state to the optimum. If the half-life is short, it means that adaptation to the primary optimum is rapid, and if the half-life is long, it means that species are likely poorly adapted to the primary niche due to influence of the ancestral state.

We explored applying more complex OU models such as scenarios with separate trait optima, variances, and attractors for each selective regime (Beaulieu and O'Meara, 2012).

However, analyses utilizing these more complex models produce frequent optimization failures, likely due to limited sample sizes. Thus, we have limited multi-peak model fitting to the simpler OUM approach described in the above paragraph. Our model-averaging approach entails the calculation of Akaike weights for each model (i.e. the relative likelihood of each model) by means of the second-order Akaike information criterion ($AIC_c$), which includes a correction for smaller sample sizes.

We utilize functions from 'R' packages in conducting all data manipulations: 'phytools', 'ape', and 'geiger' (Harmon et al., 2008; Paradis et al., 2004; Revell, 2012). We also utilize 'R' version 3.2.4 (Core, 2012) in performing all analyses and fit adaptive models with the 'R' package 'OUwie' (Beaulieu and O'Meara, 2012).

### Phylogenetic Independent Contrasts for Behavioral Trait Coevolution

We calculate phylogenetic independent contrasts using the 'pic' function in the 'ape' package (Paradis et al., 2004). Branch lengths are obtained from the ultrametric tree and standardized independent contrasts are verified by plotting absolute values of standardized independent contrasts versus their standard deviation (Garland et al., 1992). Sets of independent contrasts with values for log of each behavioral trait "positivized" have been regressed through the origin.

## Results

### Phylogenetic Signal

Analyses using the K statistic reveal variation in levels of phylogenetic signal across traits. The strength of the phylogenetic signal ranges from 0.18 to 0.34 (mean ± SD: 0.24 ± 0.05). Five traits of the 10 measured exhibit phylogenetic signal significantly different from zero (Table

2.2). The phylogenetic signals of these traits range from 0.27 to 0.34 (0.29 ± 0.03). These traits are best modeled by OUM. The traits that do not exhibit phylogenetic signal are also the traits that are best modeled using an Ornstein-Uhlenbeck process rather than Brownian motion.

**Evolutionary Model Fitting**

In our analyses, both the single- and multi-rate BM models receive less support than any of the OU models for all behavioral axes (Table 2.3). This suggests that the evolution of all behavioral traits oscillate, at least in part, around one or more phenotypic optima. There is substantial support for the OUM models of evolution ($AIC_w$ = 0.53-0.98; Table 2.3) for the puff score, latency to return from a predatory puff cue, latency to capture prey, the prey capture estimate, and the waste estimate with half-lives ranging from 0.35 to 60.4 million year. With the exception of OU1, all alternative models receive low support ($AIC_w < 0.05$). The model-averaged parameter estimates from these two OU models suggest that the adaptive optima differ among ecotypes (see mean phenotypic optimum scores in Table 2.3). Model fitting for the remaining behavioral traits suggest OU1 is the best model with half-lives ranging from 3.37 to 17.2 million years, although OUM receives some support as well.

**Phylogenetic Independent Contrasts**

Of the 45 possible trait correlations, only one is significant at the Bonferroni adjusted $\alpha$ = 0.0011. Latency to return from a predatory puff cue is highly correlated with the behavior exhibited following the presentation of the cue ($r$ = -0.85, P < 0.0001). While the distance traveled after presentation of a front prod is highly correlated with the distance traveled after the presentation of a rear prod, it does not meet the threshold for significance ($r$ = 0.70, P = 0.0013). In this instance, the correlation between latency to start exploring a novel environment and

latency to capture prey, as would be expected in a boldness-aggression syndrome across the phylogeny is weak and non-significant ($r$ = 0.10, P = 0.70). Nor is there a significant correlation between latency to start exploring a novel environment and activity, as would be expected in a boldness-activity syndrome ($r$ = -0.46, P = 0.053). All pairwise correlations are available in Table 2.4.

## Discussion

The evolutionary transition to living in another habitat may require a shift in behavioral tendencies. Identifying the environmental correlates of personality traits within and across species can help elucidate the environments that favor, for example, increased aggression or sociality. This may also provide evidence as to why certain species exhibit such divergent behavioral tendencies. A multitude of investigations have tested for associations between habitat parameters and behavioral traits using either intraspecific trait variation (Bókony et al., 2012; Dingemanse et al., 2007; Jones et al., 2007; Pruitt et al., 2011a; Pruitt et al., 2011b; Pruitt et al., 2012; Pruitt and Riechert, 2009; Pruitt et al., 2008; Riechert and Hall, 2000; Royauté et al., 2014; Wilson and Godin, 2009a) or small-scale comparative studies (Bierbach et al., 2013; Guevara and Aviles, 2011; Samuk et al., 2011; Thierry, 2013). However, to date, studies considering enough species to use comparative analyses to test for associations and changes of different behavioral traits and habitats are rare (but see Pruitt et al. (2012)).

Our analysis suggests that behavioral traits associated with personality are not conserved deep within evolutionary history but are rather determined by ecological factors associated with habitat, such as predation pressure, availability of prey and competition for favorable sites. There are, thus, distinct optima associated on the one hand with desert habitats and on the other

with riparian habitats for the aggressiveness traits and traits associated with the predatory puff cue (boldness). Desert *Agelenopsis* species tend to exhibit higher frequencies of aggressive behaviors and shorter latencies to return from predator puff cues, shorter latencies to attack prey, as well as higher estimates of prey capture and superfluous killing relative to riparian species. Thus, in agelenids, desert species are more aggressive toward prey and less fearful towards predators than riparian species. These results are consistent with a previous assessment of the behavioral trait differences between desert and riparian ecotypes of *Agelenopsis aperta* (Riechert & Hall 2000). These authors argue that the selective advantage of higher-level aggressive/bold responses is driven simultaneously by a lack of bird predation and scarcity of prey in desert habitats. Conversely, in riparian habitats where bird predation is common and prey abundance high, this spider exhibits longer latencies to return from predator cues and longer latencies to attack prey. Thus, we find strong support for an adaptive macro-evolutionary landscape in agelenids with different phenotypic optima shaped (constrained) by habitat.

While several traits show phylogenetic signal (i.e. more closely related species have more similar trait values), there is strong evidence that these traits can adapt quickly to different habitat optima, particularly behavioral responses to predatory puff cues, latency to return from a predatory puff cue and the estimate of prey capture ($t_{1/2}$ = 0.35, 0.76, and 0.79 Ma, respectively). However, there is a single trait (latency to capture prey) that exhibits high phylogenetic signal (K = 0.34) and a large half-life ($t_{1/2}$ = 60.40 Ma). This suggests that if new optima were to arise for the different habitats, these species would likely have a difficult time adjusting and could go extinct as a result. In addition, if other behaviors are significantly correlated with this trait, it seems likely that these traits would be constrained in their evolution as well, forming a newly maladaptive behavioral syndrome that could result in reduced fitness or even extinction (Sih et

al., 2004). Note, however, that work in desert and riparian populations of *Agelenopsis aperta* show that this behavioral shift does occur (Riechert and Hall, 2000), possible as a result of an over-dominance relationship between autosomal and sex chromosome gene complexes (Maynard Smith and Riechert, 1984; Riechert and Maynard Smith, 1989; Riechert et al., 2002).

Although other behavioral syndrome studies have reported correlation values (*r)* ranging from 0.4 to 0.8 (Bell, 2005; Bell and Stamps, 2004; Hedrick and Kortet, 2012; Moretz et al., 2007; Sih et al., 2004; Sih et al., 2003), such strong correlations may have only a short-term effect on behavioral evolution. Over time, such strong phenotypic or genetic correlations decrease differences among individuals in a population. Thus, unless correlational selection is acting on the two traits or the animals exist in environments that fluctuate over time (Roff, 1996), the originally strong phenotypic or genetic correlations are likely to disappear as the two traits reach fixation. Alternatively, weak but persistent genetic correlations may have a more profound evolutionary impact when considered over long periods of time, especially for behavioral traits that are influenced by tens or hundreds of genes (e.g., Anholt et al., 2003; Flint, 2003; Mackay, 2004). In these cases, strong genetic linkages between subsets of these genes may produce only weak phenotypic correlations, which over long periods of time have profound effects on the direction of evolutionary change. Comparative studies that infer selection from interspecific variation (e.g., Hansen, 1997; Prum, 1997) are needed to determine whether behavioral syndromes measured in a single generation are sufficiently large to lead to long-term behavioral evolution.

Comparative datasets on behavioral syndromes are rare, and the existing data have revealed remarkable inter-population variation in syndromes (Bell, 2005; Bell and Sih, 2007; Dingemanse et al., 2007; Herczeg et al., 2009; Huntingford, 1976; Riechert and Hall, 2000;

Snyder and Dingle, 1989; Wilson and Godin, 2009a). These population comparisons have been interpreted as evidence that syndromes are readily generated and dissolved over evolutionary time, and that their importance in shaping evolutionary trends is trivial. However, this is not the case in *Anelosimus studiosus*, which exhibits the opposite trend (i.e. remarkable consistency in syndromes across populations), even though the data come from a large number of source populations and habitat types (Pruitt et al., 2010).

Taken together, it appears that minute changes in selective regimes (e.g. habitat type, level of predation pressure, climate) can alter behavioral syndromes (Bell, 2007; Dingemanse et al., 2007; Pruitt et al., 2008; Sih and Bell, 2008; Wolf et al., 2007; Wolf et al., 2008) suggesting that behavioral syndromes are hardly "rigid" against selection because the observed behavioral correlations might represent a complex, adaptive plastic response to environment. However, due to the fact that other studies (Pruitt et al., 2010) find that behavioral syndromes are consistent across habitats and situations, there may be genetic "constraints" on the evolution of behavioral syndromes. These "constraints" act as quantitative resistance to change in certain directions, and can influence the direction of evolution and lead to a population becoming trapped on a local, but globally suboptimal peak on an adaptive landscape (Schluter, 1996; Wright, 1932). For example, in *Anelosimus studiosus* it is clear that the lack of plasticity is maladaptive. However, social colonies contain a mix of behavioral phenotypes and produce offspring of varying phenotypes, which can adjust colony composition to specific selection pressures. It could be possible that social living evolved as a way for this species to escape the maladaptive consequences associated with the lack of behavioral plasticity. Behavioral syndrome evolution is likely a combination of the "adaptive" and "constraint" hypotheses and future work should endeavor to explain how genetic constraints impact the evolution of behavioral syndromes as selective regimes change.

# Works Cited


Anholt RR, Dilda CL, Chang S, Fanara J-J, Kulkarni NH, Ganguly I, Rollmann SM, Kamdar KP, Mackay TF, 2003. The genetic architecture of odor-guided behavior in Drosophila: epistasis and the transcriptome. Nature genetics 35:180-184.

Beaulieu JM, Jhwueng DC, Boettiger C, O'Meara BC, 2012. Modeling stabilizing selection: expanding the Ornstein–Uhlenbeck model of adaptive evolution. Evolution 66:2369-2383.

Beaulieu JM, O'Meara B, 2012. OUwie: analysis of evolutionary rates in an OU framework. R package version 1.

Bell A, 2005. Behavioural differences between individuals and two populations of stickleback (Gasterosteus aculeatus). Journal of evolutionary biology 18:464-473.

Bell AM, Hankison SJ, Laskowski KL, 2009. The repeatability of behaviour: a meta-analysis. Animal Behaviour 77:771-783.

Bell AM, Sih A, 2007. Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). Ecology Letters 10:828-834.

Bell AM, Stamps JA, 2004. Development of behavioural differences between individuals and populations of sticklebacks, Gasterosteus aculeatus. Animal Behaviour 68:1339-1348.

Bierbach D, Makowicz AM, Schlupp I, Geupel H, Streit B, Plath M, 2013. Casanovas are liars: behavioral syndromes, sperm competition risk, and the evolution of deceptive male mating behavior in live-bearing fishes. F1000Research 2.

Blomberg SP, Garland T, 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. Journal of Evolutionary Biology 15:899-910.

Blomberg SP, Garland T, Ives AR, 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57:717-745.

Bókony V, Kulcsár A, Tóth Z, Liker A, 2012. Personality traits and behavioral syndromes in differently urbanized populations of house sparrows (Passer domesticus). PLoS One 7:e36639.

Bollback JP, 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. BMC bioinformatics 7:1.

Brown C, Jones F, Braithwaite V, 2005. In situ examination of boldness–shyness traits in the tropical poeciliid,< i> Brachyraphis episcopi</i>. Animal Behaviour 70:1003-1009.

Butler MA, King AA, 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. The American Naturalist 164:683-695.

Carter AJ, Goldizen AW, Tromp SA, 2010. Agamas exhibit behavioral syndromes: bolder males bask and feed more but may suffer higher predation. Behavioral Ecology:arq036.

Chamberlin RV, Ivie W, 1941. North American Agelenidae of the genera Agelenopsis, Calilena, Ritalena and Tortolena. Annals of the Entomological Society of America 34:585-628.

Chamberlin RV, Ivie W, 1942. Agelenidae of the genera Hololena, Novalena, Rualena, and Melpomene. Annals of the Entomological Society of America 35:203-241.

Core R, 2012. Team. R: A language and environment for statistical computing.

Davis AM, Unmack PJ, Pusey BJ, Pearson RG, Morgan DL, 2014. Evidence for a multi-peak adaptive landscape in the evolution of trophic morphology in terapontid fishes. Biological Journal of the Linnean Society 113:623-634.

Dingemanse NJ, Wright J, Kazem AJN, Thomas DK, Hickling R, Dawnay N, 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. Journal of Animal Ecology 76:1128-1138.

Fabre AC, Bickford D, Segall M, Herrel A, 2016. The impact of diet, habitat use, and behaviour on head shape evolution in homalopsid snakes. Biological Journal of the Linnean Society.

Felsenstein J, 1985. Phylogenies and the comparative method. American Naturalist:1-15.

Flint J, 2003. Analysis of quantitative trait loci that influence animal behavior. Journal of neurobiology 54:46-77.

García-Navas V, Blumstein DT, 2016. The effect of body size and habitat on the evolution of alarm vocalizations in rodents. Biological Journal of the Linnean Society.

Garland T, Harvey PH, Ives AR, 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Systematic biology 41:18-32.

Goodman BA, Miles DB, Schwarzkopf L, 2008. Life on the rocks: habitat use drives morphological and performance evolution in lizards. Ecology 89:3462-3471.

Guevara J, Aviles L, 2011. Influence of body size and level of cooperation on the prey capture efficiency of two sympatric social spiders exhibiting an included niche pattern. Functional Ecology 25:859-867.

Hansen TF, 1997. Stabilizing selection and the comparative analysis of adaptation. Evolution:1341-1351.

Hansen TF, Pienaar J, Orzack SH, 2008. A comparative method for studying adaptation to a randomly evolving environment. Evolution 62:1965-1977.

Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W, 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129-131.

Harrison AS, Revell LJ, Losos JB, 2015. Correlated evolution of microhabitat, morphology, and behavior in West Indian Anolislizards: a test of the habitat matrix model. Behaviour 152:1187-1207.

Hedrick AV, Kortet R, 2012. Sex differences in the repeatability of boldness over metamorphosis. Behavioral Ecology and Sociobiology 66:407-412.

Herczeg G, Gonda A, Merilä J, 2009. Predation mediated population divergence in complex behaviour of nine-spined stickleback (Pungitius pungitius). Journal of evolutionary biology 22:544-552.

Hirano T, Kameda Y, Kimura K, Chiba S, 2015. Divergence in the shell morphology of the land snail genus Aegista (Pulmonata: Bradybaenidae) under phylogenetic constraints. Biological Journal of the Linnean Society 114:229-241.

Huntingford FA, 1976. The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback,< i> Gasterosteus Aculeatus</i>. Animal Behaviour 24:245-260.

Johnson JC, Sih A, 2005. Precopulatory sexual cannibalism in fishing spiders (Dolomedes triton): a role for behavioral syndromes. Behavioral Ecology and Sociobiology 58:390-396.

Johnson JC, Sih A, 2007. Fear, food, sex and parental care: a syndrome of boldness in the fishing spider, Dolomedes triton. Animal Behaviour 74:1131-1138.

Johnson MA, Revell LJ, Losos JB, 2010. Behavioral convergence and adaptive radiation: effects of habitat use on territorial behavior in Anolis lizards. Evolution 64:1151-1159.

Jones TC, Riechert SE, Dalrymple SE, Parker PG, 2007. Fostering model explains variation in levels of sociality in a spider system. Animal Behaviour 73:195-204.

Karr JR, James FC, 1975. Eco-morphological configurations and convergent evolution in species and communities. Ecology and evolution of communities:258-291.

Kembel SW, Kembel MSW, 2014. Package 'picante'.

Kralj-Fišer S, Schneider JM, 2012. Individual behavioural consistency and plasticity in an urban spider. Animal Behaviour 84:197-204.

Kortet R, Hedrick A, 2007. A behavioural syndrome in the field cricket Gryllus integer: intrasexual aggression is correlated with activity in a novel environment. Biological Journal of the Linnean Society 91:475-482.

Legendre F, D'Haese CA, Deleporte P, Pellens R, Whiting MF, Schliep K, Grandcolas P, 2014. The evolution of social behaviour in Blaberid cockroaches with diverse habitats and social systems: phylogenetic analysis of behavioural sequences. Biological Journal of the Linnean Society 111:58-77.

Losos JB, 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. The American Naturalist 175:623-639.

Mackay TF, 2004. The genetic architecture of quantitative traits: lessons from< i> Drosophila</i>. Current opinion in genetics & development 14:253-257.

Maupin JL, Riechert SE, 2001. Superfluous killing in spiders: a consequence of adaptation to food-limited environments? Behavioral Ecology 12:569-576.

Maynard Smith J, Riechert SE, 1984. A conflicting-tendency model of spider agonistic behaviour: Hybrid-pure population line comparisons. Animal behaviour 32:564-578.

Moretz JA, Martins EP, Robison BD, 2007. Behavioral syndromes and the evolution of correlated behavior in zebrafish. Behavioral ecology 18:556-562.

Münkemüller T, Lavergne S, Bzeznik B, Dray S, Jombart T, Schiffers K, Thuiller W, 2012. How to measure and test phylogenetic signal. Methods in Ecology and Evolution 3:743-756.

Nielsen R, 2002. Mapping mutations on phylogenies. Systematic biology 51:729-739.

O'Meara BC, Ané C, Sanderson MJ, Wainwright PC, 2006. Testing for different rates of continuous trait evolution using likelihood. Evolution 60:922-933.

Paradis E, Claude J, Strimmer K, 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289-290.

Pruitt JN, DiRienzo N, Kralj-Fišer S, Johnson JC, Sih A, 2011a. Individual-and condition-dependent effects on habitat choice and choosiness. Behavioral Ecology and Sociobiology 65:1987-1995.

Pruitt JN, Iturralde G, Avilés L, Riechert SE, 2011b. Amazonian social spiders share similar within-colony behavioural variation and behavioural syndromes. Animal Behaviour 82:1449-1455.

Pruitt JN, Oufiero CE, Avilés L, Riechert SE, 2012. Iterative evolution of increased behavioral variation characterizes the transition to sociality in spiders and proves advantageous. The American Naturalist 180:496-510.

Pruitt JN, Riechert SE, Jones TC, 2008. Behavioural syndromes and their fitness consequences in a socially polymorphic spider,< i> Anelosimus studiosus</i>. Animal Behaviour 76:871-879.

Pruitt JN, Riechert SE, Iturralde G, Vega M, Fitzpatrick BM, Aviles L, 2010. Population differences in behaviour are explained by shared within-population trait correlations. Journal of evolutionary biology 23:748-756.

Pruitt JN, Riechert SE, 2009. Sex matters: sexually dimorphic fitness consequences of a behavioural syndrome. Animal Behaviour 78:175-181.

Pruitt JN, Riechert SE, 2012. The ecological consequences of temperament in spiders. Current Zoology 58:588-595.

Prum RO, 1997. Phylogenetic tests of alternative intersexual selection mechanisms: trait macroevolution in a polygynous clade (Aves: Pipridae). American Naturalist:668-692.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ, 2007. Integrating animal temperament within ecology and evolution. Biological reviews 82:291-318.

Revell LJ, 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3:217-223.

Revell LJ, Harmon LJ, Collar DC, 2008. Phylogenetic signal, evolutionary process, and rate. Systematic Biology 57:591-601.

Riechert SE, 1993. Investigation of potential gene flow limitation of behavioral adaptation in an aridlands spider. Behavioral Ecology and Sociobiology 32:355-363.

Riechert SE, Hall RF, 2000. Local population success in heterogeneous habitats: reciprocal transplant experiments completed on a desert spider. Journal of Evolutionary Biology 13:541-550.

Riechert SE, Hedrick AV, 1990. Levels of predation and genetically based anti-predator behaviour in the spider, *Agelenopsis aperta*. Animal Behaviour 40:679-687.

Riechert SE, Hedrick AV, 1993. A test for correlations among fitness-linked behavioural traits in the spider *Agelenopsis aperta* (Araneae, Agelenidae). Animal Behaviour 46:669-675.

Riechert SE, Johns PM, 2003. Do female spiders select heavier males for the genes for behavioral aggressiveness they offer their offspring? Evolution 57:1367-1373.

Riechert SE, Maupin J, Spider effects on prey: Tests for superfluous killing in five web builders. Proc 17th European Colloquium Arachnol(PA Selden, ed) Bull British Arachnol Soc1998. p. 203-210.

Riechert SE, Maynard Smith J, 1989. Genetic analyses of two behavioural traits linked to individual fitness in the desert spider< i> Agelenopsis aperta</i>. Animal Behaviour 37:624-637.

Roff DA, 1996. The evolution of genetic correlations: an analysis of patterns. Evolution:1392-1403.

Royauté R, Buddle CM, Vincent C, 2014. Interpopulation variations in behavioral syndromes of a jumping spider from insecticide-treated and insecticide-free orchards. Ethology 120:127-139.

Samuk KM, LeDue EE, Avilés L, 2011. Sister clade comparisons reveal reduced maternal care behavior in social cobweb spiders (Anelosimus spp.). Behavioral Ecology:arr146.

Schluter D, 1996. Adaptive radiation along genetic lines of least resistance. Evolution:1766-1774.

Sih A, Bell A, Johnson JC, 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19:372-378.

Sih A, Kats LB, Maurer EF, 2003. Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish–salamander system. Animal Behaviour 65:29-44.

Simpson GG, 1953. The major features of evolution. 1953. and G Simpson, Tempo and Mode in:143.

Sih A, Bell AM, 2008. Insights for behavioral ecology from behavioral syndromes. Advances in the Study of Behavior 38:227-281.

Snyder RJ, Dingle H, 1989. Adaptive, genetically based differences in life history between estuary and freshwater threespine sticklebacks (Gasterosteus aculeatus L.). Canadian Journal of Zoology 67:2448-2454.

Stamps JA, 1991. Why evolutionary issues are reviving interest in proximate behavioral mechanisms. American Zoologist 31:338-348.

Sweeney K, Gadd RD, Hess ZL, McDermott DR, MacDonald L, Cotter P, Armagost F, Chen JZ, Berning AW, DiRienzo N, 2013. Assessing the effects of rearing environment, natural selection, and developmental stage on the emergence of a behavioral syndrome. Ethology 119:436-447.

Team RC, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013. ISBN 3-900051-07-0.

Thierry B, 2013. Identifying constraints in the evolution of primate societies. Philosophical Transactions of the Royal Society of London B: Biological Sciences 368:20120342.

Wilson AD, Godin J-GJ, 2009a. Boldness and behavioral syndromes in the bluegill sunfish, Lepomis macrochirus. Behavioral Ecology:arp018.

Wright S, 1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution: na.

York RA, Patil C, Hulsey CD, Anoruo O, Streelman JT, Fernald RD, 2015. Evolution of bower building in Lake Malawi cichlid fish: phylogeny, morphology, and behavior. Frontiers in Ecology and Evolution 3:18.

# APPENDIX B

**Table 2.1.** Habitat information for species tested in this study.

| Species | Family | N | Habitat |
|---|---|---|---|
| *Agelenopsis aleenae* | Agelenidae | 48 | Desert |
| *Agelenopsis aperta* | Agelenidae | 63 | Desert |
| *Agelenopsis emertoni* | Agelenidae | 26 | Riparian |
| *Agelenopsis kastoni* | Agelenidae | 62 | Riparian |
| *Agelenopsis lisa* | Agelenidae | 60 | Desert |
| *Agelenopsis naevia* | Agelenidae | 20 | Riparian |
| *Agelenopsis oklahoma* | Agelenidae | 49 | Desert |
| *Agelenopsis oregonensis* | Agelenidae | 126 | Riparian |
| *Agelenopsis pennsylvanica* | Agelenidae | 75 | Riparian |
| *Agelenopsis potteri* | Agelenidae | 61 | Riparian |
| *Agelenopsis spatula* | Agelenidae | 86 | Desert |
| *Agelenopsis utahana* | Agelenidae | 19 | Riparian |
| *Barronopsis floridensis* | Agelenidae | 88 | Riparian |
| *Barronopsis texana* | Agelenidae | 48 | Riparian |
| *Hogna carolinensis* | Lycosidae | 26 | Riparian |
| *Hogna helluo* | Lycosidae | 26 | Riparian |
| *Hololena sula* | Agelenidae | 83 | Riparian |
| *Novalena intermedia* | Agelenidae | 26 | Riparian |
| *Schizocosa avida* | Lycosidae | 18 | Riparian |

N signifies specimen numbers used in behavioral quantification.

**Table 2.2.** Blomberg's K estimates for the 10 behavioral traits measured. Traits are ordered by the values of Blomberg's K from largest to smallest.

| Trait | Blomberg's K | P-value |
|---|---|---|
| Latency to capture prey | 0.34 | 0.002 |
| Distance traveled upon introduction to novel environment | 0.30 | 0.026 |
| Wasteful killing estimate | 0.29 | 0.016 |
| Distance traveled after front prod | 0.27 | 0.032 |
| Distance traveled after rear prod | 0.26 | 0.032 |
| Latency to start exploring novel environment | 0.23 | 0.13 |
| Activity over five minutes | 0.21 | 0.171 |
| Latency to return from puff | 0.19 | 0.115 |
| Capture estimate | 0.19 | 0.166 |
| Behavior score in reaction to puff | 0.18 | 0.142 |

**Table 2.3.** Average AIC weights ($AIC_w$) representing the relative likelihood or each of the four evolutionary models investigated to fit data for 10 behavioral traits, computed after fitting all evolutionary models.

| | **Model** | **ΔAIC** | **Average AIC weights ($AIC_w$)** | **$\alpha_1$** | **$\alpha_2$** | **$\sigma^2_1$** | **$\sigma^2_2$** | **Phenotypic Optima 1 (Riparian; $\theta_R$ )** | **Phenotypic Optima 2 (Desert; $\theta_{D)}$** | **Half-life1** | **Half-life2** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1ST move Distance | BM1 | 1.87 | 0.15 | - | - | 0.03 | 0.03 | 9.04 | 9.04 | - | - |
| | BMS | 4.72 | 0.06 | - | - | 0.03 | 0.03 | 9.04 | 9.04 | - | - |
| | OU1 | 0 | 0.58 | 0.05 | 0.05 | 0.06 | 0.06 | 10.69 | 10.69 | 13.90 | 13.90 |
| | OUM | 3.23 | 0.22 | 0.05 | 0.05 | 0.06 | 0.06 | 10.62 | 13.35 | 13.24 | 13.24 |
| | AVG | - | - | 0.04 | 0.04 | 0.05 | 0.05 | 10.32 | 9.44 | 17.22 | 17.22 |
| Latency to move | BM1 | 4.58 | 0.05 | - | - | 0.07 | 0.07 | 225.11 | 225.11 | - | - |
| | BMS | 6.62 | 0.03 | - | - | 0.08 | 0.04 | 222.18 | 222.18 | - | - |
| | OU1 | 0 | 0.68 | 0.12 | 0.12 | 0.21 | 0.21 | 310.06 | 310.06 | 5.58 | 5.58 |
| | OUM | 3.25 | 0.25 | 0.11 | 0.11 | 0.19 | 0.19 | 305.43 | 269.16 | 6.52 | 6.52 |
| | AVG | - | - | 0.11 | 0.11 | 0.20 | 0.19 | 302.08 | 255.47 | 6.25 | 6.25 |
| Activity over five minutes | BM1 | 1.67 | 0.16 | - | - | 0.01 | 0.01 | 31.10 | 31.10 | - | - |
| | BMS | 4.51 | 0.06 | - | - | 0.01 | 0.01 | 31.10 | 31.10 | - | - |
| | OU1 | 0 | 0.56 | 0.27 | 0.27 | 0.06 | 0.06 | 38.02 | 38.02 | 2.54 | 2.54 |
| | OUM | 3.05 | 0.23 | 0.24 | 0.24 | 0.05 | 0.05 | 36.79 | 41.14 | 2.92 | 2.92 |
| | AVG | - | - | 0.21 | 0.21 | 0.05 | 0.05 | 36.11 | 30.09 | 3.37 | 3.37 |

**Table 2.3.** Continued.

| | **Model** | **ΔAIC** | **Average AIC weights ($AIC_w$)** | **$\alpha_1$** | **$\alpha_2$** | **$\sigma^2_1$** | **$\sigma^2_2$** | **Phenotypic Optima 1 (Riparian; $\theta_R$)** | **Phenotypic Optima 2 (Desert; $\theta_D$)** | **Half-life1** | **Half-life 2** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Distance traveled after front prod | BM1 | 2.80 | 0.09 | - | - | 0.03 | 0.03 | 3.90 | 3.90 | - | - |
| | BMS | 5.30 | 0.04 | - | - | 0.04 | 0.02 | 3.91 | 3.91 | - | - |
| | OU1 | 0 | 0.57 | 0.07 | 0.07 | 0.07 | 0.07 | 4.74 | 4.74 | | |
| | OUM | 2.54 | 0.30 | 0.09 | 0.09 | 0.09 | 0.09 | 4.51 | 10.91 | | |
| | AVG | - | - | 0.07 | 0.07 | 0.07 | 0.07 | 4.56 | 5.56 | 10.54 | 10.54 |
| Distance traveled after rear prod | BM1 | 2.78 | 0.09 | - | - | 0.03 | 0.03 | 5.58 | 5.58 | - | - |
| | BMS | 5.20 | 0.04 | - | - | 0.02 | 0.04 | 5.56 | 5.56 | - | - |
| | OU1 | 0 | 0.58 | 0.07 | 0.07 | 0.06 | 0.06 | 6.50 | 6.50 | 9.48 | 9.48 |
| | OUM | 2.66 | 0.29 | 0.09 | 0.09 | 0.07 | 0.07 | 6.19 | 12.08 | 0.09 | 0.09 |
| | AVG | - | - | 0.07 | 0.07 | 0.06 | 0.06 | 6.28 | 6.96 | 10.35 | 10.35 |
| Behavior score in reaction to puff | BM1 | 7.79 | 0.0059 | - | - | 8.21e-4 | 8.21e-4 | 0.45 | 0.45 | - | - |
| | BMS | 10.33 | 0.0025 | - | - | 6.94e-4 | 1.31e-3 | 0.45 | 0.45 | - | - |
| | OU1 | 1.99 | 0.16 | 0.15 | 0.15 | 2.81e-3 | 2.81e-3 | 0.43 | 0.43 | 4.70 | 4.70 |
| | OUM | 0 | 0.83 | 2.34 | 2.34 | 0.03 | 0.03 | 0.39 | 0.57 | 0.30 | 0.30 |
| | AVG | - | - | 1.97 | 1.97 | 0.03 | 0.03 | 0.40 | 0.54 | 0.35 | 0.35 |

**Table 2.3.** Continued.

| | **Model** | **ΔAIC** | **Average AIC weights ($AIC_w$)** | **$\alpha_1$** | **$\alpha_2$** | **$\sigma^2_1$** | **$\sigma^2_2$** | **Phenotypic Optima 1 (Riparian; $\theta_R$)** | **Phenotypic Optima 2 (Desert; $\theta_D$)** | **Half-life1** | **Half-life 2** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Latency to return from puff | BM1 | 5.83 | 0.01 | - | - | 0.13 | 0.13 | 21.92 | 21.92 | - | - |
| | BMS | 7.94 | 0.01 | - | - | 0.17 | 0.04 | 20.61 | 20.61 | - | - |
| | OU1 | 0.51 | 0.29 | 0.16 | 0.16 | 0.49 | 0.49 | 27.70 | 27.70 | 4.39 | 4.39 |
| | OUM | 0 | 0.69 | 1.25 | 1.25 | 2.79 | 2.79 | 40.43 | 9.90 | 0.55 | 0.55 |
| | AVG | - | - | 0.91 | 0.91 | 2.08 | 2.08 | 35.82 | 13.30 | 0.76 | 0.76 |
| Latency to capture prey | BM1 | 8.58 | 0.005 | - | - | 0.03 | 0.03 | 7.43 | 7.43 | - | - |
| | BMS | 10.31 | 0.003 | - | - | 0.05 | 9.22e-05 | 6.92 | 5.92 | - | - |
| | OU1 | 9.07 | 0.01 | 0.03 | 0.03 | 0.05 | 0.05 | 8.14 | 8.14 | 20.31 | 20.31 |
| | OUM | 0 | 0.99 | 0.01 | 0.01 | 0.01 | 0.01 | 8.96 | 5.63e-4 | 60.60 | 60.60 |
| | AVG | - | - | 0.01 | 0.01 | 0.01 | 0.01 | 8.94 | 6.33e-4 | 60.40 | 60.40 |
| Capture estimate | BM1 | 12.44 | 0.001 | - | - | 8.79e-4 | 8.79e-4 | 1.63 | 1.63 | - | - |
| | BMS | 14.71 | 0.0003 | - | - | 1.11e-3 | 4.13e-4 | 1.64 | 1.64 | - | - |
| | OU1 | 6.42 | 0.02 | 0.11 | 0.11 | 2.56e-3 | 2.56e-3 | 1.69 | 1.69 | 6.10 | 6.10 |
| | OUM | 0 | 0.98 | 0.89 | 0.89 | 9.58e-3 | 9.58e-3 | 1.62 | 2.09 | 0.78 | 0.78 |
| | AVG | - | - | 0.88 | 0.88 | 9.42e-3 | 9.42e-3 | 1.62 | 2.08 | 0.79 | 0.79 |

**Table 2.3.** Continued.

| | **Model** | **ΔAIC** | **Average AIC weights ($AIC_w$)** | **$\alpha_1$** | **$\alpha_2$** | **$\sigma^2_1$** | **$\sigma^2_2$** | **Phenotypic Optima 1 (Riparian; $\theta_R$)** | **Phenotypic Optima 2 (Desert; $\theta_D$)** | **Half-life1** | **Half-life 2** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wasteful killing estimate | BM1 | 3.94 | 0.04 | - | - | 4.32e-3 | 4.32e-3 | 0.47 | 0.47 | - | - |
| | BMS | 6.67 | 0.01 | - | - | 4.00e-3 | 5.41e-3 | 0.47 | 0.47 | - | - |
| | OU1 | 0 | 0.41 | 0.10 | 0.10 | 0.01 | 0.01 | 0.52 | 0.52 | 7.17 | 7.17 |
| | OUM | 0.68 | 0.54 | 0.22 | 0.22 | 0.02 | 0.02 | 0.48 | 0.92 | 3.13 | 3.13 |
| | AVG | - | - | 0.16 | 0.16 | 0.02 | 0.02 | 0.50 | 0.71 | 4.36 | 4.36 |

$AIC_w$ values of the best-fit models are in bold. Fitted evolutionary models include BM1, single-rate Brownian motion; BMS, two-rate BM model; OU1, a single-optimum Ornstein-Uhlenbeck (OU) adaptive model with one parameter for the variance of random walk ($\sigma^2$) and strength of selection ($\alpha$) towards a global optimum for all species; and OUM, an OU model with separate behavioral optima for each habitat, but global $\sigma^2$ and $\alpha$ parameters for the different desert and riparian selective regimes.

**Table 2.4.** Correlations between traits across the phylogeny with correlations ranked from largest to smallest. Significant correlations after Bonferroni correction (alpha = 0.0011) are bolded.

| Trait Pairings | Correlation | P-value |
|---|---|---|
| Latency to return from puff + Behavior score in reaction to puff | -0.85 | 6.44E-06 |
| Distance traveled after rear prod + Distance traveled after front prod | 0.70 | 0.0013 |
| Distance traveled after rear prod + Distance traveled upon introduction to novel environment | 0.63 | 0.005 |
| Capture estimate + Latency to capture prey | -0.58 | 0.01 |
| Capture estimate + Distance traveled upon introduction to novel environment | -0.54 | 0.02 |
| Activity over five minutes + Latency to start exploring novel environment | -0.46 | 0.05 |
| Latency to return from puff + Distance traveled after rear prod | -0.46 | 0.05 |
| Latency to capture prey + Distance traveled upon introduction to novel environment | 0.46 | 0.06 |
| Wasteful killing estimate + Distance traveled upon introduction to novel environment | -0.45 | 0.06 |
| Latency to return from puff + Distance traveled after front prod | -0.41 | 0.09 |
| Distance traveled after front prod + Distance traveled upon introduction to novel environment | 0.41 | 0.09 |
| Wasteful killing estimate + Distance traveled after front prod | -0.40 | 0.10 |
| Capture estimate + Latency to start exploring novel environment | -0.40 | 0.10 |
| Behavior score in reaction to puff + Latency to start exploring novel environment | -0.32 | 0.19 |
| Behavior score in reaction to puff + Distance traveled after rear prod | 0.32 | 0.20 |
| Latency to capture prey + Behavior score in reaction to puff | -0.31 | 0.21 |
| Latency to capture prey + Distance traveled after rear prod | 0.29 | 0.25 |
| Distance traveled after front prod + Latency to start exploring novel environment | -0.28 | 0.26 |
| Latency to capture prey + Distance traveled after front prod | 0.28 | 0.27 |
| Wasteful killing estimate + Distance traveled after rear prod | -0.28 | 0.27 |
| Capture estimate + Behavior score in reaction to puff | 0.26 | 0.29 |
| Distance traveled after rear prod + Activity over five minutes | -0.26 | 0.30 |
| Behavior score in reaction to puff + Distance traveled after front prod | 0.26 | 0.30 |
| Wasteful killing estimate + Capture estimate | 0.24 | 0.33 |
| Latency to capture prey + Latency to return from puff | 0.23 | 0.35 |
| Capture estimate + Latency to return from puff | -0.23 | 0.36 |

**Table 2.4.** Continued.

| Trait Pairings | Correlation | P-value |
|---|---|---|
| Distance traveled after rear prod + Latency to start exploring novel environment | -0.23 | 0.36 |
| Wasteful killing estimate + Activity over five minutes | 0.20 | 0.42 |
| Latency to return from puff + Latency to start exploring novel environment | 0.19 | 0.44 |
| Distance traveled after front prod + Activity over five minutes | -0.19 | 0.44 |
| Behavior score in reaction to puff + Distance traveled upon introduction to novel environment | 0.16 | 0.52 |
| Latency to return from puff + Distance traveled upon introduction to novel environment | -0.16 | 0.52 |
| Capture estimate + Distance traveled after front prod | -0.15 | 0.55 |
| Behavior score in reaction to puff + Activity over five minutes | 0.13 | 0.61 |
| Capture estimate + Distance traveled after rear prod | -0.12 | 0.63 |
| Latency to capture prey + Latency to start exploring novel environment | 0.10 | 0.70 |
| Capture estimate + Activity over five minutes | 0.09 | 0.73 |
| Activity over five minutes + Distance traveled upon introduction to novel environment | -0.09 | 0.73 |
| Latency to capture prey + Activity over five minutes | -0.08 | 0.74 |
| Latency to start exploring novel environment + Distance traveled upon introduction to novel environment | 0.08 | 0.75 |
| Wasteful killing estimate + Latency to capture prey | 0.06 | 0.81 |
| Wasteful killing estimate + Behavior score in reaction to puff | -0.05 | 0.84 |
| Latency to return from puff + Activity over five minutes | 0.04 | 0.88 |
| Wasteful killing estimate + Latency to start exploring novel environment | 0.04 | 0.89 |
| Wasteful killing estimate + Latency to return from puff | 0.02 | 0.92 |

# CHAPTER III: STUCK IN THE WEB: SPIDER DIVERSIFICATION ACCELERATES WHEN THEY LEAVE WEBS BEHIND

This chapter is based on an original research article to be submitted to *Science* in February 2017. My primary contributions to this paper include: (i) collection and analysis of the data, (ii) development of the phylogeny, (iii) figure and table development, (iv) completion of a first draft of the manuscript.

**Bosco JM**, O'Meara BC, Riechert SE. Stuck in the web: Spider diversification accelerates when they leave webs behind.

## Abstract

The evolutionary diversification of spiders is attributed to spectacular innovations in silk use, particularly in the production of the prey capture web. Here, we construct a large molecular phylogeny of spiders to examine the diversification of the spider order Araneae in relation to web architecture and putative hypotheses about the evolutionary transitions between different web types/states. Contrary to long held beliefs, our analyses indicate that the aerial orb web is not the key innovation that leads to increased diversification in the Araneae. Rather it was abandonment of the web altogether in taking on a more cursorial lifestyle. This occurred through the lineages of scattered and sheet web types constructed in close proximity to the ground. In the cursorial spider groups, silk use is limited to the production of a retreat and drag line(s) that prevent injury during wandering as well as being used in dispersal through aerial ballooning. Molecular dating estimates indicate the branching event from scattered line and sheet webs to cursorial foraging strategies occurs during the Cretaceous Terrestrial revolution 125-190 MYA. The diversification of cursorial spider types at this time may coincide with a major increase in biomass of non-flying insects. This behavioral shift in spider use of silk likely played a role in the dramatic evolutionary success and ecological dominance of spiders as predators of insects.

## Background

Spiders (Order: Araneae) prey on arthropods, and are the dominant consumers at intermediate trophic levels (Foelix, 2010; Wise, 1993). They are also exceptionally diverse and abundant in terrestrial ecosystems, numbering ~45,500 described species. In contrast to the mega-diverse orders of their arthropod relatives, insects, the evolutionary diversification of spiders has not been linked to major trophic shifts. Rather, diversification in the Araneae is linked to innovations in silk use (Blackledge et al., 2003; Blackledge et al., 2009; Bond and Opell, 1998).

All spiders produce and use silk throughout their lives, making it an integral part of their behavioral repertoire. Spiders are most known, however for their silk webs that aid in the capture of prey. The most advanced web type is the geometric, araneoid orb web. This web, which provides access to flying prey, exhibits an open structure. (Wind currents at the heights orb weavers encounter prey would tear apart the more dense web structures characteristic of the scattered and sheet webs that are constructed either in close proximity to the ground or in vegetation sheltering them from the wind). Sticky droplets laid down on the capture spiral of the orb web lessens the probability of insect escape (Bond and Opell, 1998; Coddington, 1990), as does dry silk that clings to prey through van der Waals interactions and hydroscopic forces (Hawthorn and Opell, 2003).

Using character optimization and sister clade comparisons, both Bond and Opell (1998) and Blackledge et al. (2009) note a trend toward increased diversification in orb-web producing lineages. Bond and Opell (1998) further propose that the orb web represents a key innovation that has led to the diversification of the Order Araneae. In part, this reflects changes in capture

thread and web features that allow orb-weavers to shift into new adaptive zones. The question we address here is whether the key innovation that has led to the diversity of extant spider species is indeed the development of the advanced orb web structure or whether some other pathway contributes more to the 37,500 described spider species populating the world today. In order to understand the diversification of this important arthropod order, it is essential to discover the evolutionary pathway of silk utilization in spiders. The evolution of different web types will affect diversification rates across spider lineages differently. The impact of putative key innovations on the diversification of the Order Araneae has not been thoroughly investigated in a statistical framework (but see Blackledge et al., 2009; Garrison et al., 2016). Blackledge et al. (2009), for instance noted that the "RTA clade" (the sister group to orb-weaving spiders) contains half of all spider diversity and exhibits a trend toward increased diversification associated with the abandonment of prey capture webs and the loss of cribellate (mechanically sticky) silk. Most recently, Garrison et al. (2016), using a phylogeny of 70 species suggest that while the evolution of the orb web and adhesive sticky threads has led to elevated rates of diversification among the Araneoidea, the highest rates of diversification likely has occurred among species in the RTA clade.

Here we examine the relationship between rates of diversification and web evolution across spiders (Araneae) through the generation of a time-scaled molecular phylogeny of spider species. We apply this phylogenetic dataset and a very broad set of current phylogenetic methods to test whether the processes of species diversification relate to the distribution of web traits during the radiation of spiders.

# Methods

## Molecular data

The molecular data set we use to reconstruct the phylogenetic relationships in Araneae consists of 2,257 spider species. Taxa in the sequence matrix meet the criterion of having at least half the number of base pairs as the longest sequence for each gene. Further, we apply the program Mesquite (Maddison and Maddison, 2001) to remove those taxa that have long branches due to bad sequence data pulled from GenBank. While this is a very conservative approach, our analyses focus on defined species to which we can assign characters for web characteristics.

Reconstructing the phylogeny consists of the following steps: 1) examine each gene separately using maximum likelihood; 2) visually assess the resulting data sets for conflicts; and 3) construct a concatenated sequence matrix for the eight genes (6621 bp), using the procedures described in Smith et al. (2009) and implemented in the program PHLAWD.

Studies of higher-level phylogeny and divergence times of spiders (e.g. Agnarsson et al., 2007; Ayoub et al., 2005; Blackledge et al., 2009; Hedin and Bond, 2006; Vink et al., 2008; Wood et al., 2012) use the mitochondrial genes (16S, COI, and NADH1) and the nuclear genes (18S, 28S, wingless, histone 3A, and actin) extensively. We use these eight genes in estimating the phylogeny and divergence times for the Order Araneae here.

## Estimation of Phylogeny and Divergence Times

The phylogeny is obtained using maximum likelihood (i.e., RAxML version 7.0.4 and the recommended GTR + Γ model (Stamatakis, 2006). Our analyses of divergence entail examining the resulting concatenated dataset with independent partitions for each gene in identifying the optimal likelihood (penalized) tree. In the event that a genus includes only unknown species, the taxon with the longest concatenated gene sequence is used to represent that genus in the tree.

We utilize Sanderson (2002) penalized likelihood approach for large phylogenies (i.e., TreePL, after Smith and O'Meara, 2012) to estimate divergence times in our reconstructed phylogeny. Cross-validated assessment is used in selecting the best-fitting smoothing parameter to estimate the ages of clades. This entails ten replicate optimizations. Penalized likelihood analyses requires 22 calibration points, 21 of which serve as minimum age estimates, and one as a fixed root age. All calibration points (fossil and BEAST estimates) come from Wood et al. (2012)(See Table 3.1). In general, the calibration points correspond to the oldest fossil taxon that can be confidently assigned to a given clade, and the minimum age of a fossil is based on the end of the time period that the fossil taxon came from). In many cases, a fossil assigned to a given higher taxon (e.g. family) cannot be confidently assigned to that taxon's crown group. Instead, we use it to estimate the age of the stem group (i.e., the age of the most recent common ancestor of that family and its sister group).

**Modeling Phenotypic Evolutionary Dynamics with MEDUSA**

We use the stepwise approach, MEDUSA (Modeling Evolutionary Diversification Using Stepwise Akaike Information Criterion), to detect multiple shifts in birth and death rates in our phylogeny (Alfaro et al., 2009). This method detects intrinsic changes in diversification rates within our data, without relying on trait-based models. Briefly, it finds the likelihood of obtaining the particular combination of phylogenetic relationships and taxonomic data given particular values of birth (b) and death (d). MEDUSA starts by finding the maximum-likelihood values for b and d, and the stepwise AIC algorithm uses this model as the starting point. In the analysis, we use the percentage of taxa sampled per genus of 5% (World Spider Catalog, 2015) as an estimate of the global frequency. This accounts for non-random missing speciation events.

**Estimating Patterns of Trait Evolution**

Binary and hidden-state speciation-extinction models require trait information to be coded in a binary format (Beaulieu and O'Meara, 2016; Maddison et al., 2007). Each of the 2,597 taxa included in our analyses is assigned a score for the presence (state 0) or absence (state 1) of a web, and for the absence (state 0) or presence (state 1) of an orb web. This allows us to explicitly test the hypothesis that the orb web has an effect on diversification. It also tests a second hypothesis, (i.e., that losing the prey-capture web influences diversification). We apply the following analyses to the time-calibrated trees from TreePL in examining the two patterns: orb web evolution specifically and web evolution in general.

We apply HiSSE (Beaulieu and O'Meara, 2016) to investigate trait correlations associated with diversification rate. Unlike BiSSE (Maddison et al., 2007), this allows for hidden traits to affect diversification, so that rate heterogeneity is not necessarily ascribed to the state of the observed trait. (Maddison and FitzJohn, 2015) discuss the issues of phylogenetic methods that fail to incorporate information about the number of times a trait evolves. HiSSE is an analysis that does not incorporate this information. Because state changes occur frequently in spider evolution, this issue is not likely to be problematic.

In this analysis, we compare the relative fit of 36 different models for both web vs. no web and orb web vs. no orb web states, in which we allow web type to vary in order to account for the potential effects of the different states on diversification rates for all models. We also compare models in which we allow rates of diversification associated with each character state to differ, (i.e. all rates held constant, rates between 0 and 1 held constant, and rates between A and B held constant). This permits us to evaluate whether there is any evidence that a trait influences

diversification or if there is a hidden state associated with a particular trait that influences diversification.

One issue to note is that the sample of spider species we use in our analysis is incomplete (2,597 of > 40,000). This could result in an upward bias in estimates of character-state associated diversification rates (FitzJohn et al., 2009) as well as other analyses. However, these potential biases are minimal in this case as all major clades are represented in our sample and we take sampling frequency into account when running these models.

We use the R-package corHMM (Beaulieu et al., 2013) to generate hidden rates models (HRMs) for each of our web datasets and the associated spider phylogeny. For a given number of rate classes, this package infers transition rates between the various states in the HRM. Classical models of binary character state evolution can be problematic at this phylogenetic scale, because they assume a single rate of evolution and loss of the focal trait. It is instead more realistic to assume that some spider clades have a higher transition rate to and from a specific state (for example, having an orb web) than other clades. To create our HRMs, we, thus, constrain the state of the root node of the phylogeny to have a web (in the case of web vs. no web) and to be orb-less web (in the case of orb web vs. no orb web) as the respective ancestral states of spiders. We do not constrain the transition rates in any way, meaning that they are free to be estimated at any value, including the zero bound. To sample the full parameter space, we use 100 random restarts and generate HRMs assuming one to six rate classes. This permits exploration of a wide range of evolutionary scenarios.

We use AIC weights to determine which HRM best describes our character state distribution data (Table 3.5 and 3.6). We also use the rayDISC command in the corHMM package (Beaulieu et al., 2013) to reconstruct character states of four different web types (1 =

webless, 2 = orb, 3 = ground web, 4= non orb aerial web). Our web groupings follow the characterizations given by Blackledge et al. (2009) and Garrison et al. (2016). However, we group their "brushed sheet", "terminal line", "irregular ground sheet", and "trapdoor/ burrow" categories into our "ground web" type. We also group "cob web" and "stereotypical aerial sheet" categories, labeling them as the "aerial web" category. This method allows for multistate characters, unresolved nodes, and ambiguities (polymorphic or missing data).

We evaluate three models of character evolution under the ML method: equal rates (ER), symmetrical (SYM), and all rates different (ARD). We also restrict various transitions between different states to address more specific questions about web evolution using the ARD transition matrix. These models consist of: (1) restricting the re-evolution of a web from the webless state, (2) restricting the evolution of the webless state by only being able to go through the ground web state, (3) restricting the evolution of the orb web by only being able to go through the aerial web state, (4) combining restrictions from (1) and (2), and (5) combining restrictions from (1), (2), and (3). We fix the root web state as the ground web state, which includes the subterranean burrow and trapdoor burrows. Finally, we use AIC weights in selecting among these varying models of character evolution.

After obtaining the rayDISC matrix, we apply the sim.history function in the phytools package (Revell, 2012) to reconstruct 1000 histories based on the transition rate matrix given from the best rayDISC model and our phylogeny. This provides the median number of state changes across the phylogeny with 95% confidence limits.

### Timing of Web Evolution

In order to gauge when specific groups evolved, we use getMRCA command in the ape package (Paradis et al., 2004) to obtain the node number and height. Estimates of divergence times (in millions of years) are obtained from subtracting the node height from the root age of the tree (Table 3.1**)**.

## Results

### Web data, spider phylogeny, and trait reconstruction

From our comprehensive list of web characteristics for 2,597 spider species, we obtain the simplest model of web evolution given the following three assumptions: (i) there is one rate of acquiring webs across the entire tree and one rate of losing them; (ii) the same as (i), but for orb webs; and (iii) the possibility of the existence of rate heterogeneity, which could include hidden traits that affect the gain or loss rate. We use AIC (Akaike Information Criterion) to determine which model best describes the character state distribution data.

### Time-varying speciation rates

There appears to be a trend towards a speed-up in the rates of net diversification in the RTA clade (Retrolateral Tibial Apophysis), with the highest net diversification rates in the Lycosidae. We also observe high rates of net diversification within the Araneoidea (specifically in the Theriididae, Linyphiidae, and other groups of spiders that build various aerial webs such as cobwebs) as well as increased net diversification in some families of the Haplogynae, such as the Sicariidae and Pholcidae.

**Web-State Evolution**

A heterogeneous rate model with two rate classes best explains the history of spider evolution and distribution of webs (Akaike weight = 79%) (Table 3.2). From this model, we can see that highest transition rate is from the web state to the web-less state both with hidden state B ($q_{0B\text{-}1B}$ = 9.36 transitions / MY). This rate is 4.5 greater than the transition rate from the webless state to the web state within hidden state B ($q_{0B\text{-}1B}$ = 2.08 transitions / MY) (Table 3.3). Our model identifies several other important characteristics of web evolution. First, the transition rates from the web-less state to the web state are 4.5 times smaller than the rates from the web state to the web-less state for state B and over 40,000 times smaller for state A ($q_{1A\text{-}0A}$= 2.06e-09, $q_{g1B\text{-}0B}$ = 2.08, $q_{0A\text{-}1A}$= 0.000085, and $q_{0B\text{-}1B}$= 9.36 transitions / MY, respectively). The rates from hidden state B to hidden state A are 43,622,204 times greater than the rates from hidden state A to hidden state B for the web state and over seven times smaller for the web-less state ($q_{0A\text{-}0B}$= 2.06e-09, $q_{g0B\text{-}0A}$ =0.090, $q_{1A\text{-}1B}$= 0.00073, and $q_{1B\text{-}1A}$= 0.0057 transitions / MY, respectively). This suggests that web-less spiders in state B transition to the web state much less often than web-less spiders in state A and that while transition rates from hidden state A to hidden state B are low, web spiders have made this transition far less often than web-less spiders.

A heterogeneous rate model with two rate classes best explains the history of spider evolution and distribution of orb webs (Akaike weight = 96%) (Table 3.4). From this model, we can see that highest transition rate is from hidden state B to hidden state A within the orbless state ($q_{0B\text{-}0A}$ = 0.039 transitions / MY) (Table 3.5). This rate is 19,043,341 times greater than the transition rate from hidden state A to hidden state B within the orbless state ($q_{0A\text{-}0B}$ = 2.06e-09 transitions / MY). Our model identifies several other important characteristics of orb web evolution. First, the transition rate from the orbless state to the orb state is approximately as the

rate from the orb state to the orbless state for state B and over 14 times smaller for state A ($q_{1A-0A}$= 0.00034, $q_{g1B-0B}$ = 0.073, $q_{0A-1A}$= 0.000023, and $q_{0B-1B}$= 0.075 transitions / MY, respectively). The rates from hidden state B to hidden state A are five to six orders of magnitude greater than the rates from hidden state A to hidden state B for both states ($q_{0A-0B}$= 2.06e-09, $q_{g0B-0A}$ = 0.039, $q_{1A-1B}$= 2.06e-09, and $q_{1B-1A}$= 0.0058 transitions / MY, respectively). This suggests that orbless spiders in state A transition to the orb state less often than orbless spiders in state B (where rates between the two web states are essentially equal) and transition rates from hidden state B to hidden state A are very high and of similar magnitude for spiders with and without orb webs.

**Higher diversification is associated with weblessness but a hidden factor matters more**

The best-fit model for the web/web-less scoring scheme is the full, unconstrained (transition rates and all states unconstrained) HiSSE model with extinction fractions differing in web and web-less lineages fit , and different transition rates between the web state and the web-less state (Akaike weight = 100%) (Table 3.6). The ΔAIC of a model with different diversification rates between web and web-less lineages is 1371.4 (Akaike weight = 1.6E-296%), while the equivalent BiSSE model has a ΔAIC of 1491.73 (Akaike weight = 0%). The full, unconstrained model results in net diversification rates for webless spiders in which hidden trait A are approximately 11x greater than web-less spiders with hidden state B and approximately two times greater than webbed spiders with hidden state A (Table 3.7). These spiders also exhibit the highest turnover and smallest extinction fraction of all trait combinations. Webless spiders with hidden state B have 10.5x greater net diversification rates than web-building spiders with hidden state B. It appears that web spiders with hidden state B also have the lowest turnover and highest extinction fraction of all trait combinations. These results suggest that although

weblessness influences diversification, other biological traits or processes substantially contribute to the observed diversification patterns.

**The orb web is not a key innovation**

The best-fit model for orb/orb-less scoring scheme is the model with unconstrained transition rates, and turnover and extinction fraction constrained to be equal in the hidden states (0A = 1A and 0B = 1B) (Akaike weight = 99.99%) (Table 3.8). The ΔAIC of a model with unequal diversification rates between the orb state and the orb-less state is 1762.1 (Akaike weight = 0%), while the BiSSE equivalent has a ΔAIC of 1773.9 (Akaike weight = 0%). The selected model shows that the spiders with hidden state B (regardless of whether or not they build an orb web) exhibit a net diversification rate over 17 times greater than spiders with hidden state A, over four times greater turnover, and an extinction fraction that is 75% of that of spiders with hidden state A (Table 3.9). These results suggest that whether a species produces an orb or not, does not influence diversification whatsoever. Rather, the observed differences in diversification are driven entirely by the hidden state.

**The ground web is an important precursor**

The all rates different model was the best-fit trait only model for our multi-state data (AIC weight = 85%) (Table 3.10). From this model, we can see that the highest transition rate is from the ground web state to the web-less state ($q_{ground\text{-}web\text{-}less}$ = 0.00075 transitions / MY). This rate is 2.5 to 5 times greater than the transition rates to web-lessness from the aerial web state and orb web state, respectively. The transition rate from the ground web state to the aerial web state is the second highest rate ($q_{ground\text{-}aerial}$ = 0.00047 transitions / MY).

Our model identified several other important characteristics of web evolution. First, transition rates to the orb web state are low. Transition rates to the orb web state from the web-less and ground web states are an order of magnitude smaller than the transition rate to the orb web state from the aerial web state ($q_{web\text{-}less\text{-}orb} = 0.000037$, $q_{ground\text{-}orb} = 0.000081$, $q_{aerial\text{-}orb} = 0.00022$ transitions / MY, respectively). Second, the transition rate from the orb web state to the ground web state is 0. Finally, it is possible to revert to a web-making state from the web-less state, suggesting that weblessness is not an irreversible condition (Table 3.11). The resulting simulated state changes that correspond with the above transition rates are summarized in Table 3.12.

## Discussion

The orb web has been considered the "crowning achievement of aerial spiders" (Gertsch, 1979), because the evolution of adhesive threads and the vertical orientation of the orb web, positioned to intercept and retain flying insects, has long been considered a key innovation that allowed spiders to inhabit a new adaptive zone (Bond and Opell, 1998). Numerous authors have speculated about the adaptive value of the orb web, including Bond and Opell (1998), Coddington (1986), Levi (1980), and Olive (1980).

However, our reconstruction of the evolution of web traits based on a phylogeny of the Araneae does not support the idea that the orb web is a key innovation. This result is not unexpected. (Griswold et al., 1998) noted that over 50% of Araneoidea no longer build recognizable orb webs and suggested that "the orb web has been an evolutionary base camp rather than a summit". Our study is the first to explicitly test this hypothesis. While this does not

discount an orb web as being highly adaptive in terms of individual level selection, the orb web, itself, apparently has no net effect on speciation or extinction rates in the spider order Araneae.

We find that only weblessness correlates with increased net diversification within spiders. The loss of the web altogether, is associated with a 2 to 10-fold increase of the speciation rate in the Araneae. Weblessness paired with some unknown trait or process has increased net diversification the most. Importantly, while the hidden traits could be a single character, like good vision, there may be other factors that vary over the tree (presence of some other group of organisms, continent, time period, etc.). Griswold et al. (1998) suggest that the evolution of weblessness and its hidden trait(s) may allow spiders to exploit resources in their environment that they might not be able to access otherwise. It is important to note that net diversification in the case of orb webs, themselves, is influenced by some unknown trait unassociated with web structure. Our analysis indicates that this 'hidden' trait accounts for a 17-fold increase in the diversification of orb weaving spider species.

While May and Moore (2016) criticized MEDUSA's performance in rejecting trivial null hypotheses and in estimating rates, our analysis here does not try to reject a model of constant diversification rate through time but rather uses MEDUSA's estimates as heuristics for rates across branches. We detected the highest rates of diversification among members of the RTA clade, in particular the family Lycosidae (wolf spiders). We also detected increased diversification in the non-orb spinning groups such as the Linyphiidae and Theridiidae, similar to a previous study of diversification in spiders (Garrison et al., 2016), suggesting that a web modified from the true orb may confer an evolutionary advantage. We did not find the same pattern of increased diversification in the Mygalomorphae (Garrison et al. 2016). These results

imply that other foraging strategies (e.g. cursorial hunting, irregular sheets, and modified orb webs) are more successful than the true orb.

It appears that the point estimate for the RTA node occurs during the early Cretaceous (123 Ma), which is slightly younger than the age (139 Ma) identified by (Garrison et al., 2016) and precedes the subsequent diversification of the RTA clade at 108.6 – 60.1 Ma, which coincides with the Cretaceous Terrestrial Revolution (KTR). This result is interesting because angiosperms radiated extensively around 125-90 Ma (Friis, 1987; Hickey and Doyle, 1977), as did various insect lineages that fed on and inhabited these plants, including ants (Moreau et al., 2006), beetles (McKenna et al., 2015; McNamara et al., 2009; Wang et al., 2013), lepidopterans (Wahlberg et al., 2013), and other holometabolous insects (Misof et al., 2014). Note, however, that this is not the case for all insect lineages (e.g. darkling beetles; Kergoat et al., 2014). Spiders, as important insect predators, likely diversified along with their prey (e.g. Peñalver et al., 2006; Penney et al., 2003; Selden and Penney, 2010). The fossil and phylogenetic data presented here show that most spider lineages predate the KTR (Bond et al., 2014; Garrison et al., 2016; Selden and Penney, 2010), except for the RTA clade, which diversified very soon after the beginning of the KTR. However, it is likely that aerial web building clades, such as the araneoids, which predate the KTR diversified in response to the KTR insect pulse. In the same vein, if forest litter habitats became more complex and spurred ground insect diversification (Moreau et al., 2006), diversification in ground-dwelling spiders likely increased as a result. A major increase in these insect groups may have favored spiders that feed on cursorial prey and thus could help explain the concurrent increase in diversification in the RTA clade and non-orb weaving araneoids such as cobweb weavers (Dziki et al., 2015).

The results of the above analyses suggest the orb web may be a less successful or more limited foraging strategy compared to other foraging strategies (e.g. cursorial hunting). Orb webs are more limited in that they only encounter aerial prey whereas, in most cases, ground webs are able to capture aerial prey as well as prey on the ground. They not only refute the hypothesis proposed by Bond and Opell (1998), but shed light on potential new mechanisms behind the success of the major groups that abandoned the orb web. After all, over half of the extant araneoid spiders do not build an orb web (Fernández et al., 2014; Griswold et al., 1998).

## Works Cited

Agnarsson I, Maddison WP, Avilés L, 2007. The phylogeny of the social< i> Anelosimus</i> spiders (Araneae: Theridiidae) inferred from six molecular loci and morphology. Molecular phylogenetics and evolution 43:833-851.

Alfaro ME, Santini F, Brock C, Alamillo H, Dornburg A, Rabosky DL, Carnevale G, Harmon LJ, 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proceedings of the National Academy of Sciences 106:13410-13414.

Ayoub NA, Riechert SE, Small RL, 2005. Speciation history of the North American funnel web spiders,< i> Agelenopsis</i>(Araneae: Agelenidae): Phylogenetic inferences at the population–species interface. Molecular phylogenetics and evolution 36:42-57.

Beaulieu JM, O'Meara BC, Donoghue MJ, 2013. Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid angiosperms. Systematic biology 62:725-737.

Beaulieu JM, O'Meara BC, 2016. Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. Systematic biology:syw022.

Blackledge TA, Coddington JA, Gillespie RG, 2003. Are three-dimensional spider webs defensive adaptations? Ecology Letters 6:13-18.

Blackledge TA, Scharff N, Coddington JA, Szüts T, Wenzel JW, Hayashi CY, Agnarsson I, 2009. Reconstructing web evolution and spider diversification in the molecular era. Proceedings of the National Academy of Sciences 106:5229-5234.

Bond JE, Garrison NL, Hamilton CA, Godwin RL, Hedin M, Agnarsson I, 2014. Phylogenomics resolves a spider backbone phylogeny and rejects a prevailing paradigm for orb web evolution. Current Biology 24:1765-1771.

Bond JE, Opell BD, 1998. Testing adaptive radiation and key innovation hypotheses in spiders. Evolution:403-414.

Butler MA, King AA, 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. The American Naturalist 164:683-695.

Coddington JA, 1986. The monophyletic origin of the orb web. Spiders: Webs, Behavior, and Evolution(WA Shear, ed) Stanford University Press, Stanford, California:319-363.

Coddington JA, 1990. Cladistics and spider classification: araneomorph phylogeny and the monophyly of orbweavers (Araneae: Araneomorphae; Orbiculariae). Acta Zoologica Fennica 190:75-87.

Core R, 2012. Team. R: A language and environment for statistical computing.

Dziki A, Binford GJ, Coddington JA, Agnarsson I, 2015. Spintharus flavidus in the Caribbean—a 30 million year biogeographical history and radiation of a 'widespread species'. PeerJ 3:e1422.

Fernández R, Hormiga G, Giribet G, 2014. Phylogenomic analysis of spiders reveals nonmonophyly of orb weavers. Current Biology 24:1772-1777.

FitzJohn RG, Maddison WP, Otto SP, 2009. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. Systematic biology 58:595-611.

Foelix R, 2010. Biology of spiders: Oxford University Press.

Friis EM, 1987. The origins of angiosperms and their biological consequences: CUP Archive.

Garrison NL, Rodriguez J, Agnarsson I, Coddington JA, Griswold CE, Hamilton

CA, Hedin M, Kocot KM, Ledford JM, Bond JE, 2016. Spider phylogenomics: untangling the Spider Tree of Life. PeerJ 4:e1719.

Gertsch WJ, 1979. American spiders: Van Nostrand Reinhold Co.

Griswold CE, Coddington JA, Hormiga G, Scharff N, 1998. Phylogeny of the orb-web building spiders (Araneae, Orbiculariae: Deinopoidea, Araneoidea). Zoological Journal of the Linnean Society 123:1-99.

Hawthorn AC, Opell BD, 2003. van der Waals and hygroscopic forces of adhesion generated by spider capture threads. Journal of Experimental Biology 206:3905-3911.

Hedin M, Bond JE, 2006. Molecular phylogenetics of the spider infraorder Mygalomorphae using nuclear rRNA genes (18S and 28S): Conflict and agreement with the current system of classification. Molecular phylogenetics and evolution 41:454-471.

Hickey LJ, Doyle JA, 1977. Early Cretaceous fossil evidence for angiosperm evolution. The Botanical Review 43:3-104.

Kergoat GJ, Bouchard P, Clamens A-L, Abbate JL, Jourdan H, Jabbour-Zahab R, Genson G, Soldati L, Condamine FL, 2014. Cretaceous environmental changes led to high extinction rates in a hyperdiverse beetle family. BMC evolutionary biology 14:1.

Levi HW, 1980. The orb-weaver genus Mecynogea, the subfamily Metinae and the genera Pachygnatha, Glenognatha and Azilia of the subfamily Tetragnathinae north of Mexico (Araneae: Araneidae). Harvard University Museum of Comparative Zoology Bulletin (USA).

Maddison WP, FitzJohn RG, 2015. The unsolved challenge to phylogenetic correlation tests for categorical characters. Systematic biology 64:127-136.

Maddison WP, Maddison DR, 2001. Mesquite: a modular system for evolutionary analysis.

Maddison WP, Midford PE, Otto SP, 2007. Estimating a binary character's effect on speciation and extinction. Systematic Biology 56:701-710.

May MR, Moore BR, 2016. How Well Can We Detect Lineage-Specific Diversification-Rate Shifts? A Simulation Study of Sequential AIC Methods. Systematic biology:syw026.

McKenna DD, Wild AL, Kanda K, Bellamy CL, Beutel RG, Caterino MS, Farnum CW, Hawks DC, Ivie MA, Jameson ML, 2015. The beetle tree of life reveals that Coleoptera survived end-Permian mass extinction to diversify during the Cretaceous terrestrial revolution. Systematic Entomology 40:835-880.

McNamara JM, Houston AI, Barta Z, Scheuerlein A, Fromhage L, 2009. Deterioration, death and the evolution of reproductive restraint in late life. Proceedings of the Royal Society of London B: Biological Sciences:rspb20090959.

Misof B, Liu S, Meusemann K, Peters RS, Donath A, Mayer C, Frandsen PB, Ware J, Flouri T, Beutel RG, 2014. Phylogenomics resolves the timing and pattern of insect evolution. Science 346:763-767.

Moreau CS, Bell CD, Vila R, Archibald SB, Pierce NE, 2006. Phylogeny of the ants: diversification in the age of angiosperms. Science 312:101-104.

Olive CW, 1980. Foraging Specialization in Orb-Weaving Spiders. Ecology 61:1133-1144.

Paradis E, Claude J, Strimmer K, 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289-290.

Peñalver E, Grimaldi DA, Delclòs X, 2006. Early Cretaceous spider web with its prey. Science 312:1761-1761.

Penney D, Wheater CP, Selden PA, 2003. Resistance of spiders to Cretaceous-Tertiary extinction events. Evolution 57:2599-2607.

Revell LJ, 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3:217-223.

Sanderson MJ, 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Molecular biology and evolution 19:101-109.

Selden PA, Penney D, 2010. Fossil spiders. Biological Reviews 85:171-206.

Smith S, Beaulieu J, Donoghue M, 2009. Mega-phylogeny approach for comparative biology: an alternative to supertree and supermatrix approaches. BMC evolutionary biology 9:37.

Smith SA, O'Meara BC, 2012. treePL: divergence time estimation using penalized likelihood for large phylogenies. Bioinformatics 28:2689-2690.

Stamatakis A, 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688-2690.

Vink CJ, Hedin M, Bodner MR, Maddison WP, Hayashi CY, Garb JE, 2008. Actin 5C, a promising nuclear gene for spider phylogenetics. Molecular phylogenetics and evolution 48:377-382.

Wahlberg N, Wheat CW, Peña C, 2013. Timing and patterns in the taxonomic diversification of Lepidoptera (butterflies and moths). PLoS One 8:e80875.

Wang B, Zhang H, Jarzembowski E, 2013. Early Cretaceous angiosperms and beetle evolution. Frontiers in plant science 4:360.

Wise D, 1993. Il. 1993. Spiders in ecological webs. Cambridge University Press, Cambridge.

Wood HM, Matzke NJ, Gillespie RG, Griswold CE, 2012. Treating fossils as terminal taxa in divergence time estimation reveals ancient vicariance patterns in the palpimanoid spiders. Systematic Biology:sys092.

World Spider C, 2015. World spider catalog, version 16. Natural History Museum Bern.

# APPENDIX C

**Table 3.1**. Estimates of divergence times in millions of years, mean [95% CI] indicates ages used to date the phylogeny (Wood et al., 2012) using species pairs from the phylogeny created in this study.

| Crown Group | Species Pairing | Mean [95% CI] | Node Height (Age in MY) |
|---|---|---|---|
| Huttonidae | *Huttonia sp.* HMW 2012, *Palpiamus sp.* HMW 2012 | 85 [77, 109] | 109 |
| Oecobiidae | *Uroctea durandi, Stegodyphus mimosarum* | 133 [126, 155] | 155 |
| Lycosidae | *Arctosa littoralis, Pardosa milvina, Gnaphosa_parvula* | 27 [16, 53] | 41 |
| Araneoidea | *Badumna longinqua, Araneus diadematus* | 167 [161, 184] | 184 |
| Araneae | *Ryuthela iheyana, Marpissa pikei* | 392 [270, 392] | 392 |
| Eriauchenidae | *Eriauchenius lavatenda, Eriauchenius jeanneli* | 55 [17, 104] | 88 |
| Madagascan archaeids | *Eriauchenius jeanneli, Eriauchenius legendrei* | 81 [34, 135] | 88 |
| AfraEria | *Afraarchaea woodae, Eriauchenius jeanneli* | 116 [68, 177] | 108 |
| EriabEriaw | *Eriauchenius bourgini, Eriachenius workman* | 61 [18, 118] | 32 |
| Australian archaeids | *Austrarchaea nodosa, Austrarchaea mainae* | 81 [30, 131] | 30 |
| SH archaeids | *Afrarchaea woodae, Eriauchenius bourgini* | 161 [108, 221] | 108 |
| ColoEria | *Colopea sp. HMW 2012, Eriauchenius lavatenda* | 228 [181, 287] | 217 |
| NZ mecymaucheniids | *Aotearoa magna, Zearchaea sp. HMW 2012* | 42 [8, 100] | 62 |
| MecyChila | *Mecysmauchenius segmentatus, Chilarchaea quellon* | 48 [10, 114] | 60 |
| Mecysmaucheniidae | *Mecysmauchenius segmentatus, Aotearoa magna* | 87 [33, 172 | 70 |
| Palpimanoidea | *Chilarchaea quellon, Colopea sp. HMW 2012* | 253 [196, 320] | 240 |

**Table 3.1.** Continued.

| Crown Group | Species Pairing | Mean [95% CI] | Node Height (Age in MY) |
|---|---|---|---|
| True Araneoidea | *Holoarchaea sp. New Zealand, Araneus diadematus* | 143 [121, 165] | 164 |
| RTA-clade | *Ambohima andrefana Schizocosa ocreata* | 82 [32, 150] | 123 |
| Entelegynae | *Badumna longinqua, Uroctea durandi* | 194 [166, 247] | 195 |
| Palpimanoidea + Entelegynae | *Stegodyphus mimosarum, Afrarchaea woodae* | 272 [210, 342] | 249 |
| Hickmaniae | *Hickmania troglodytes, Chilarchaea quellon* | 296 [228, 373] | 234 |
| Kukulcaniae | *Kukulcania hibernalis, Chilarchaea quellon* | 317 [243, 392] | 249 |

**Table 3.2.** The fit of alternative models of different transition rate classes of web evolution across the Order Araneae using corHMM. The best model, based on ΔAIC and Akaike weights ($w_i$) is denoted in bold italics.

| **Number of hidden rates** | **AIC** | **ΔAIC** | ***$w_i$*** |
|---|---|---|---|
| 0 | 401.42 | 29.93 | 2.50E-07 |
| 2 | ***371.49*** | ***0*** | ***0.79*** |
| 3 | 374.12 | 2.63 | 0.211 |
| 4 | 388.02 | 16.53 | 0.00020 |
| 5 | 394.42 | 22.92 | 8.30E-06 |

**Table 3.3.** Transition rates (transitions per million years) for web vs. webless traits using corHMM.

| State | Web, A | Webless, A | Web, B | Webless, B |
|---|---|---|---|---|
| Web, A | 0 | 0 0.000085 | 2.06e-09 | 0 |
| Webless, A | 2.06e-09 | 0 | 0 | 0.00073 |
| Web, B | 0.090 | 0 | 0 | 9.36 |
| Webless, B | 0 | 0.0057 | 2.08 | 0 |

**Table 3.4.** The fit of alternative models of different transition rate classes of orb web evolution across the Order Araneae using corHMM. The best model, based on ΔAIC and Akaike weights ($w_i$) is denoted in bold italics.

| **Number of hidden rates** | **AIC** | **ΔAIC** | ***$w_i$*** |
|---|---|---|---|
| 0 | 180.18 | 6.88 | 0.031 |
| 2 | ***173.30*** | **0** | ***0.96*** |
| 3 | 183.07 | 9.77 | 0.0073 |
| 4 | 193.47 | 20.18 | 4.00E-05 |
| 5 | 205.65 | 32.36 | 9.06E-08 |

**Table 3.5.** Transition rates (transitions per million years) for orb vs. orbless traits using corHMM.

| State | Orbless, A | Orb, A | Orbless, B | Orb, B |
|---|---|---|---|---|
| Orbless, A | 0 | 0.000023 | 2.06e-09 | 0 |
| Orb, A | 0.00034 | 0 | 0 | 2.06e-09 |
| Orbless, B | 0.039 | 0 | 0 | 0.075 |
| Orb, B | 0 | 0.0058 | 0.073 | 0 |

**Table 3.6.** The fit of alternative models of web evolution across the Order Araneae using HiSSE. The best model, based on ΔAIC and Akaike weights ($w_i$) is denoted in bold italics.

| Model | AIC | ΔAIC | $w_i$ |
|---|---|---|---|
| web.bisse | 23911.71 | 1491.73 | 0 |
| web.hisse.full.model | ***22419.98*** | ***0*** | ***1*** |
| web.hisse.AandB.fixed | 22552.97 | 132.99 | 1.32E-29 |
| web.hisse.0and1.fixed | 23791.38 | 1371.4 | 1.60E-298 |
| web.hisse.A.fixed | 22649.26 | 229.28 | 1.63E-50 |
| web.hisse.B.fixed | 22701.92 | 281.94 | 5.99E-62 |
| web.hisse.0.fixed | 23124.49 | 704.51 | 1.04E-153 |
| web.hisse.1.fixed | 23093.55 | 673.57 | 5.45E-147 |
| web.hisse.0fixed.no1B | 23277.25 | 857.27 | 7.02E-187 |
| web.hisse.1fixed.no0B | 23080.1 | 660.12 | 4.54E-144 |
| web.hisse.no0B | 22515.94 | 95.96 | 1.45E-21 |
| web.hisse.no1B | 22567.23 | 147.25 | 1.06E-32 |
| web.bisse.null | 23910.59 | 1490.61 | 0 |
| web.hisse.full.model.null | 23469.3 | 1049.32 | 1.39E-228 |
| web.hisse.AandB.fixed.null | 23183.1 | 763.12 | 1.95E-166 |
| web.hisse.0and1.fixed.null | 23910.59 | 1490.61 | 0 |
| web.hisse.A.fixed.null | 23217.65 | 797.67 | 6.14E-174 |
| web.hisse.B.fixed.null | 23219.75 | 799.77 | 2.15E-174 |
| web.hisse.0.fixed.null | 23472.08 | 1052.1 | 3.46E-229 |
| web.hisse.1.fixed.null | 23425.47 | 1005.49 | 4.58E-219 |
| web.hisse.0fixed.no1B.null | 23743.89 | 1323.91 | 3.29E-288 |
| web.hisse.1fixed.no0B.null | 23794.66 | 1374.68 | 3.10E-299 |
| web.hisse.no0B.null | 23465.3 | 1045.32 | 1.03E-227 |
| web.hisse.no1B.null | 23382.15 | 962.17 | 1.17E-209 |
| web.bisse.ABand01Equal | 23911.71 | 1491.73 | 0 |
| web.hisse.full.model.ABand01Equal | 23036.77 | 616.79 | 1.16E-134 |
| web.hisse.AandB.fixed.ABand01Equal | 23185.21 | 765.23 | 6.80E-167 |
| web.hisse.0and1.fixed.ABand01Equal | 23835.13 | 1415.15 | 5.06E-308 |
| web.hisse.A.fixed.ABand01Equal | 23406.87 | 986.89 | 5.01E-215 |
| web.hisse.B.fixed.ABand01Equal | 22818.35 | 398.37 | 3.13E-87 |
| web.hisse.0.fixed.ABand01Equal | 23148.97 | 728.99 | 5.03E-159 |
| web.hisse.1.fixed.ABand01Equal | 23695.82 | 1275.84 | 9.01E-278 |
| web.hisse.0fixed.no1B.ABand01Equal | 23204.69 | 784.71 | 4.00E-171 |
| web.hisse.1fixed.no0B.ABand01Equal | 23422.48 | 1002.5 | 2.04E-218 |
| web.hisse.no0B.ABand01Equal | 23030.99 | 611.01 | 2.09E-133 |
| web.hisse.no1B.ABand01Equal | 22881.15 | 461.17 | 7.21E-101 |

**Table 3.7.** Speciation (lambda), extinction (mu), net diversification (netdiv), extinction (lambda/mu), and turnover (lambda + mu) rates in spiders as inferred from the best-fit trait-dependent diversification model depending on web type (web vs. webless), and an additional, unmeasured ('hidden state') trait. Units are events per million years.

| Model | lambda | mu | netdiv | extinction fraction | turnover |
|---|---|---|---|---|---|
| Webbed, A | 0.54 | 0.44 | 0.11 | 0.81 | 0.98 |
| Webless, A | 0.87 | 0.64 | 0.22 | 0.74 | 1.51 |
| Webbed, B | 0.11 | 0.11 | -0.002 | 1.02 | 0.21 |
| Webless, B | 0.15 | 0.13 | 0.021 | 0.86 | 0.28 |

**Table 3.8.** The fit of alternative models of orb web evolution across the Order Araneae using HiSSE. The best model, based on ΔAIC and Akaike weights ($w_i$) is denoted in bold italics.

| Model | AIC | ΔAIC | $w_i$ |
|---|---|---|---|
| orb.bisse | 24119.1 | 1773.9 | 0 |
| orb.hisse.full.model | 22428.26 | 83.06 | 9.20E-19 |
| orb.hisse.AandB.fixed | ***22345.2*** | ***0*** | ***0.999999951*** |
| orb.hisse.0and1.fixed | 24107.3 | 1762.1 | 0 |
| orb.hisse.A.fixed | 22590.36 | 245.16 | 5.81E-54 |
| orb.hisse.B.fixed | 22597.99 | 252.79 | 1.28E-55 |
| orb.hisse.0.fixed | 23913.96 | 1568.76 | 0 |
| orb.hisse.1.fixed | 22412.03 | 66.83 | 3.08E-15 |
| orb.hisse.0fixed.no1B | 22980.5 | 635.3 | 1.11E-138 |
| orb.hisse.1fixed.no0B | 23899.03 | 1553.83 | 0 |
| orb.hisse.no0B | 22685.25 | 340.05 | 1.44E-74 |
| orb.hisse.no1B | 22378.85 | 33.65 | 4.93E-08 |
| orb.bisse.null | 24130.73 | 1785.53 | 0 |
| orb.hisse.full.model.null | 22933.68 | 588.48 | 1.63E-128 |
| orb.hisse.AandB.fixed.null | 22952.06 | 606.86 | 1.67E-132 |
| orb.hisse.0and1.fixed.null | 24130.74 | 1785.54 | 0 |
| orb.hisse.A.fixed.null | 23013.29 | 668.09 | 8.44E-146 |
| orb.hisse.B.fixed.null | 23013 | 667.8 | 9.75E-146 |
| orb.hisse.0.fixed.null | 24091.81 | 1746.61 | 0 |
| orb.hisse.1.fixed.null | 22937.35 | 592.15 | 2.61E-129 |
| orb.hisse.0fixed.no1B.null | 24116.49 | 1771.29 | 0 |
| orb.hisse.1fixed.no0B.null | 23953.55 | 1608.35 | 0 |
| orb.hisse.no0B.null | 23686.86 | 1341.66 | 4.59E-292 |
| orb.hisse.no1B.null | 22957.69 | 612.49 | 9.99E-134 |
| orb.bisse.ABand01Equal | 24119.1 | 1773.9 | 0 |
| orb.hisse.full.model.ABand01Equal | 23206.44 | 861.24 | 9.64E-188 |
| orb.hisse.AandB.fixed.ABand01Equal | 22935.23 | 590.03 | 7.53E-129 |
| orb.hisse.0and1.fixed.ABand01Equal | 24072.99 | 1727.79 | 0 |
| orb.hisse.A.fixed.ABand01Equal | 23017.34 | 672.14 | 1.11E-146 |
| orb.hisse.B.fixed.ABand01Equal | 23054.92 | 709.72 | 7.70E-155 |
| orb.hisse.0.fixed.ABand01Equal | 23901.31 | 1556.11 | 0 |
| orb.hisse.1.fixed.ABand01Equal | 22939.38 | 594.18 | 9.45E-130 |
| orb.hisse.0fixed.no1B.ABand01Equal | 23897.31 | 1552.11 | 0 |
| orb.hisse.1fixed.no0B.ABand01Equal | 23500.27 | 1155.07 | 1.51E-251 |
| orb.hisse.no0B.ABand01Equal | 23114.48 | 769.28 | 8.97E-168 |
| orb.hisse.no1B.ABand01Equal | 22801.88 | 456.68 | 6.81E-100 |

**Table 3.9.** Speciation (lambda), extinction (mu), net diversification (netdiv), extinction fraction (lambda/mu), and turnover (lambda + mu) rates in spiders as inferred from the best-fit trait-dependent diversification model depending on web type (orb vs. no orb), and an additional, unmeasured ('hidden state') trait. Units are events per million years.

| Model | lambda | mu | netdiv | extinction fraction | turnover |
|---|---|---|---|---|---|
| No Orb, A | 0.18 | 0.19 | -0.011 | 1.06 | 0.37 |
| Orb, A | 0.18 | 0.18 | -0.011 | 1.06 | 0.37 |
| No Orb, B | 0.97 | 0.77 | 0.20 | 0.80 | 1.74 |
| Orb, B | 0.97 | 0.77 | 0.20 | 0.80 | 1.74 |

**Table 3.10.** The fit of alternative models of web evolution across the Order Araneae using rayDISC. The best model, based on ΔAIC and Akaike weights ($w_i$) is denoted in bold italics.

| Model | AIC | ΔAIC | $w_i$ |
|---|---|---|---|
| rayDISC.orb | 750.1827 | 3.6707 | 0.14 |
| rayDISC.web | 998.1236 | 251.6116 | 1.97E-55 |
| rayDISC.webloss | 981.9495 | 235.4375 | 6.40E-52 |
| rayDISC.webevolve | 780.3207 | 33.8087 | 3.89E-08 |
| All rates different | ***746.512*** | ***0*** | ***0.85*** |
| Equal rates | 755.2735 | 8.7615 | 0.01 |
| Symmetric rates | 1268.317 | 521.805 | 4.19E-114 |
| rayDISC.constraint | 1006.456 | 259.944 | 3.05E-57 |

**Table 3.11.** Transition rates between the four web states produced from the rayDISC 'All rates different model'. State 1 = webless, 2 = orb web, 3 = ground web, and 4 = aerial web other than orb.

| State | Webless | Orb | Ground web | Aerial Web |
|---|---|---|---|---|
| Webless | 0 | 3.680023e-05 | 0.0001511829 | 0.0002533237 |
| Orb | 0.0001566533 | 0 | 0 | 0.0003375286 |
| Ground web | 0.0007516287 | 8.059622e-05 | 0 | 0.0004734558 |
| Aerial web | 0.0002338065 | 2.163746e-04 | 0.0002307247 | 0 |

**Table 3.12.** Average number of state changes with 95% confidence limits in parentheses from doing stochastic character mapping (which ignores effects of states on diversification). State 1 = webless, 2 = orb web, 3 = ground web, and 4 = aerial web other than orb.

| State | Webless | Orb | Ground web | Aerial web |
|---|---|---|---|---|
| Webless | 0 | 0 (0,6) | 1 (0,27) | 0 (0,8.02) |
| Orb | 0 (0,0) | 0 | 0 (0,0) | 0 (0,1) |
| Ground web | 13 (1,21) | 0 (0,0) | 0 | 21 (2,32) |
| Aerial web | 0 (0,16) | 1 (0, 22.02) | 1 (0,31) | 0 |

# CONCLUSIONS AND SYNTHESIS

## Chapter 1

The spider *Agelenopsis lisa* exhibits remarkable behavioral inconsistency across ontogeny and sexes. In this study, I identified that, in general, behavioral traits exhibit very low repeatability within life-stages as well as across ontogeny. However, there are several interesting trends worth noting. While none of the other traits exhibited significant repeatability across the life-cycle, the aggressiveness traits and the predator response to a puff cue exhibited the same pattern of high repeatability before sexual maturation in males with a sharp decline in repeatability after sexual maturation, and high repeatability in females only after sexual maturation (with the exception of latency to capture prey). The important change at this age in penultimate males involves increased energy expenditure on growth in preparation for sexual maturation, and on allocation of energy to egg development in mature females. The finding that behavioral trait correlations were consistently absent supports the perspective of behavioral syndromes as plastic and not mainly due to genetic constraints (Dingemanse et al., 2007; Johnson and Sih, 2007). Given the inconsistent results among studies of behavioral syndromes across ontogeny, why and when traits are expected to correlate warrant both further theoretical work and empirical studies. Furthermore, the lack of correlation between most of the traits suggests that these are separate responses that may play different roles in an individual's behavioral repertoire, or be explained by different underlying mechanisms.

## Chapter 2

Similar to the findings in Chapter 1 that behaviors are not repeatable or correlated across ontogeny, in Chapter 2, I identified that behaviors are also not correlated across macro-

evolutionary time. Our analysis suggests that behavioral trait correlations associated with personality are not conserved deep within evolutionary history but trait evolution is rather determined by ecological factors associated with habitat, such as predation pressure, availability of prey and competition for favorable sites. Taken together, it appears that minute changes in selective regimes (e.g. habitat type, level of predation pressure, climate) can alter behavioral syndromes suggesting that behavioral syndromes are hardly "rigid" against selection because the observed behavioral correlations might represent a complex, adaptive plastic response to environment.

## Chapter 3

In Chapter 3, I identified that diversification rates are higher in webless spiders than in spiders that build webs, while the orb web was found not to influence diversification at all. The results of the above analyses suggest the orb web may be a less successful or more limited foraging strategy compared to other foraging strategies (e.g. cursorial hunting). Orb webs are more limited in that they only encounter aerial prey whereas, in most cases, ground webs are able to capture aerial prey as well as prey on the ground. They not only refute the hypothesis proposed by (Bond & Opell 1998), but shed light on potential new mechanisms behind the success of the major groups that abandoned the orb web. We also found that it is likely that aerial web building clades, such as the araneoids, which predate the KTR diversified in response to the KTR insect pulse and a major increase in these ground-dwelling insect groups may have favored spiders that feed on cursorial prey and thus could help explain the concurrent increase in diversification in the RTA clade and non-orb weaving araneoids such as cobweb weavers.

## Summary and Future Directions

These results suggest that many of the behavioral traits measured are evolving independently of one another, and that they exhibit low repeatability across ontogeny (Chapter 1) and low phylogenetic signal (Chapter 2). It further provides evidence that traits associated with behavioral syndromes are readily uncoupled across ontogeny (Chapter 1) and macro-evolutionary time (Chapter 2) and are likely to easily escape evolutionary trade-offs. The results also suggest that web states (while not as labile as personality traits) are also able to change (Chapter 3). Hence, while behaviors and correlations are observed within species, it appears that they are mutable over macro-evolutionary time. Behavioral correlations likely arise when they are adaptive (Dall et al., 2004) rather than being the result of strict pleiotropy of one or a few genes or tight genetic linkage of a small number of loci (Sih et al., 2004) and it is likely that while spiders may take longer to evolve a new web type, web types do change over evolutionary time. These results make sense in light of research that suggests that thousands of genes influence single behaviors in organisms such as Drosophila. For example, aggression (Chen et al., 2002; Dierick and Greenspan, 2007; Edwards et al., 2006), locomotor behavior (Jordan et al., 2006) and odor-guided behavior (Lavagnino et al., 2008; Sambandan et al., 2008) have been extensively studied in this model organism and have been shown to be influenced by thousands of genes. Genetic mechanisms underlying such behaviors are thought to be extremely complex as evidenced by high levels of epistasis, plasticity and gene-by-environment interactions (Anholt, 2004; Bendesky and Bargmann, 2011; Dingemanse et al., 2010; Herczeg and Garamszegi, 2012; Mackay, 2013).

The results of this dissertation suggest a number of areas for future research. Comparative studies that infer selection from interspecific variation (e.g., Hansen 1997; Prum 1997) are

needed to determine whether behavioral syndromes measured in a single generation are sufficiently large to lead to long-term behavioral evolution. Over the past four decades, much research has centered upon the proposition that individual animals alter their behavior to cope with changing local environmental conditions (Piersma and Drent, 2003; Réale and Dingemanse, 2010). While an individual does not express the full range of behavioral trait values present in its population (Réale and Dingemanse, 2010), environmental effects have the potential to temporarily (Padilla and Adolph, 1996; Pigliucci, 2005) and permanently affect the phenotype produced by a particular genotype (Lynch and Walsh, 1998). One way to look at behavioral correlations is over deep evolutionary time and the other is to look at these behaviors and correlations across ontogeny and with experience. Behavioral and physiological traits show relatively low heritability, similar to life-history traits, and much lower than morphological traits (Mousseau and Roff, 1987; Stirling et al., 2002). It is worth noting that while low heritability suggests low additive genetic variation, there is a large body of work suggesting that pervasive epistatic interactions underlie many behaviors (Anholt, 2004; Anholt and Mackay, 2004; Mackay, 2001, 2004, 2013).

Behavioral traits, like many other traits, have some degree of temporal and situational plasticity. For example, shifts associated with ontogeny/state within the life cycle is seen in female mice who exhibit increased aggressive responses that are targeted towards male intruders while nursing pups. The aggressive response to males is lost when the pups have been weaned.(Yu et al., 2014). Studies have also shown that innate temperament can influence the degree to which temporal and situational plasticity might be exhibited (Koolhaas et al., 1999; Sih and Bell, 2008). Thus, inherently less aggressive mice have been shown to adjust their levels of aggression according to social context, while aggressive individuals do not (Natarajan et al.,

2009). Measuring individual variation and plasticity through repeated trials of individuals will provide information about the degree to which particular traits are stable or plastic (Figure 4.1).

Another way think of plasticity is variation around a species mean. This comes from estimates of plasticity, measurement error, and heritable intraspecific variation. Phylogenetic methods can estimate this as tip uncertainty, which has been shown to contribute strongly to underestimating correlations and phylogenetic signal when not accounted for (Felsenstein, 2008; Ives et al., 2007). If measurement error is removed from the tip variance estimate, then the phylogenetic estimation of uncertainty should correlate fairly well with plasticity. This will allow estimation of plasticity in many species rather than one species at a time, and lead to hypotheses that can then be tested by experimentalists using the gold standard approaches for estimating plasticity.

A pitfall of methods that account for within-species variation is that while characters can covary across individuals in a sample, the covariances of characters are assumed to be the same within all species. This assumption is contentious, since genetic drift and natural selection can alter genetic covariances as gene frequencies change between populations  Thus, the problems associated with genetic and phenotypic covariances are expected to be amplified between widely-diverged species (Felsenstein, 2008). The degree to which estimates of phylogenetic trait correlations change when phenotypic covariances are allowed to vary between species should be examined in the future. The prediction is that phenotypic covariances will be overestimated when constrained to be the same within all species. This will allow estimation of phylogenetic signal and phylogenetic trait correlations using correlations obtained from real datasets, and complement approaches listed throughout the dissertation.

The results of this dissertation found that behavioral traits are generally not repeatable over ontogeny and have habitat specific optima across macro-evolutionary time while behavioral correlations associated with behavioral syndromes are non-existent across ontogeny in *Agelenopsis lisa* and not conserved macro-evolutionary time in 19 species. This dissertation also found that diversification in spiders is highest in cursorial species, the orb web is not a key innovation in spiders, and that transitions between different web types happens and is relatively common. Future work on this topic should incorporate repeatability estimates from all species included in a phylogeny, as well as estimates of heritable intraspecific variation, covariance matrices (genetic or phenotypic), and measurement error in order to more accurately estimate the exact ultimate mechanisms acting to shape behavioral trait evolution.

## Works Cited


Anholt RR, 2004. Genetic modules and networks for behavior: lessons from Drosophila. BioEssays 26:1299-1306.

Anholt RR, Dilda CL, Chang S, Fanara J-J, Kulkarni NH, Ganguly I, Rollmann SM, Kamdar KP, Mackay TF, 2003. The genetic architecture of odor-guided behavior in Drosophila: epistasis and the transcriptome. Nature genetics 35:180-184.

Anholt RR, Mackay TF, 2004. Quantitative genetic analyses of complex behaviours in Drosophila. Nature Reviews Genetics 5:838-849.

Bendesky A, Bargmann CI, 2011. Genetic contributions to behavioural diversity at the gene–environment interface. Nature Reviews Genetics 12:809-820.

Bond JE, Opell BD, 1998. Testing adaptive radiation and key innovation hypotheses in spiders. Evolution:403-414.

Chen S, Lee AY, Bowens NM, Huber R, Kravitz EA, 2002. Fighting fruit flies: a model system for the study of aggression. Proceedings of the National Academy of Sciences 99:5664-5668.

Dall SR, Houston AI, McNamara JM, 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecology letters 7:734-739.

Dierick HA, Greenspan RJ, 2007. Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. Nature genetics 39:678-682.

Dingemanse NJ, Kazem AJN, Réale D, Wright J, 2010. Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology & Evolution 25:81-89.

Dingemanse NJ, Wright J, Kazem AJN, Thomas DK, Hickling R, Dawnay N, 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. Journal of Animal Ecology 76:1128-1138.

Edwards AC, Rollmann SM, Morgan TJ, Mackay TF, 2006. Quantitative genomics of aggressive behavior in Drosophila melanogaster. PLoS Genetics 2:e154.

Felsenstein J, 2008. Comparative methods with sampling error and within-species variation: contrasts revisited and revised. The American Naturalist 171:713-725.

Ives AR, Midford PE, Garland T, 2007. Within-species variation and measurement error in phylogenetic comparative methods. Systematic Biology 56:252-270.

Hansen TF, 1997. Stabilizing selection and the comparative analysis of adaptation. Evolution:1341-1351.

Herczeg G, Garamszegi LZ, 2012. Individual deviation from behavioural correlations: a simple approach to study the evolution of behavioural syndromes. Behavioral Ecology and Sociobiology 66:161-169.

Johnson JC, Sih A, 2007. Fear, food, sex and parental care: a syndrome of boldness in the fishing spider, Dolomedes triton. Animal Behaviour 74:1131-1138.

Jordan KW, Morgan TJ, Mackay TF, 2006. Quantitative trait loci for locomotor behavior in Drosophila melanogaster. Genetics 174:271-284.

Koolhaas J, Korte S, De Boer S, Van Der Vegt B, Van Reenen C, Hopster H, De Jong I, Ruis M, Blokhuis H, 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience & Biobehavioral Reviews 23:925-935.

Lavagnino N, Anholt R, Fanara J, 2008. Variation in genetic architecture of olfactory behaviour among wild-derived populations of Drosophila melanogaster. Journal of evolutionary biology 21:988-996.

Lynch M, Walsh B, 1998. Sinauer Associates; Sunderland, MA: 1998. Genetics and analysis of quantitative traits.

Mackay TF, 2001. Quantitative trait loci in Drosophila. Nature Reviews Genetics 2:11-20.

Mackay TF, 2004. The genetic architecture of quantitative traits: lessons from< i> Drosophila</i>. Current opinion in genetics & development 14:253-257.

Mackay TF, 2013. Epistasis and quantitative traits: using model organisms to study gene-gene interactions. Nature Reviews Genetics.

Mousseau TA, Roff DA, 1987. Natural selection and the heritability of fitness components. Heredity 59:181-197.

Natarajan D, de Vries H, Saaltink D-J, De Boer SF, Koolhaas JM, 2009. Delineation of violence from functional aggression in mice: an ethological approach. Behavior genetics 39:73-90.

Padilla DK, Adolph SC, 1996. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. Evolutionary Ecology 10:105-117.

Piersma T, Drent J, 2003. Phenotypic flexibility and the evolution of organismal design. Trends in Ecology & Evolution 18:228-233.

Pigliucci M, 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution 20:481-486.

Prum RO, 1997. Phylogenetic tests of alternative intersexual selection mechanisms: trait macroevolution in a polygynous clade (Aves: Pipridae). American Naturalist:668-692.

Réale D, Dingemanse NJ, 2010. Personality and individual social specialisation. Social behaviour: genes, ecology and evolution:562.

Sambandan D, Carbone MA, Anholt RR, Mackay TF, 2008. Phenotypic plasticity and genotype by environment interaction for olfactory behavior in Drosophila melanogaster. Genetics 179:1079-1088.

Sih A, Bell A, Johnson JC, 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19:372-378.

Sih A, Bell AM, 2008. Insights for behavioral ecology from behavioral syndromes. Advances in the Study of Behavior 38:227-281.

Stirling D, Réale D, Roff D, 2002. Selection, structure and the heritability of behaviour. Journal of Evolutionary Biology 15:277-289.

Yu Q, Teixeira C, Mahadevia D, Huang Y, Balsam D, Mann J, Gingrich J, Ansorge M, 2014. Dopamine and serotonin signaling during two sensitive developmental periods differentially impact adult aggressive and affective behaviors in mice. Molecular psychiatry 19:688-698.

# APPENDIX D

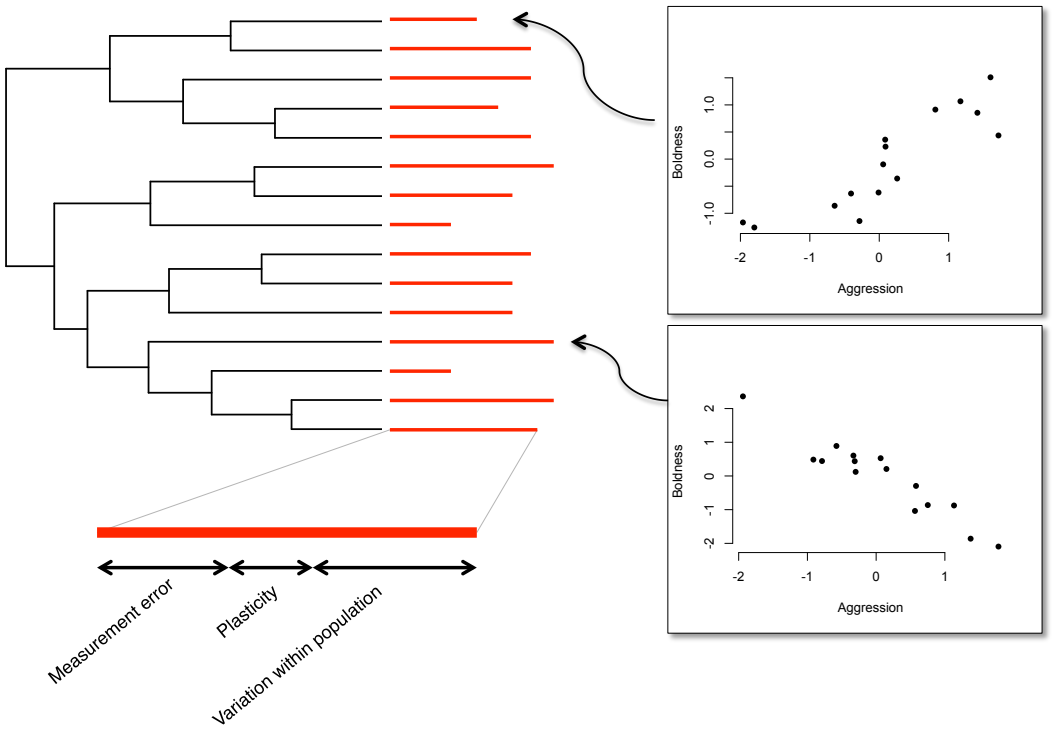


**Figure 4.1.** Schematic diagram of evolution of behavior. Scatterplots show hypothetical correlation between boldness and aggression in two different species. Within a species, the correlation seems robust, but the direction differs between two species and so there must have been a change in this correlation somewhere on the phylogeny. Traits evolve on a phylogeny according to some model (OU, BM, etc.) on the black lines, but the actual observed values have that plus variation not explained by the phylogeny (red lines). This variation is a combination of measurement error, plasticity, and variation within a population. Measurement error (1) can best be quantified using the requested video system, while 2) population variation (2) can be assessed from measuring multiple individuals and 3) plasticity will be evaluated through testing of individuals through their lives. Plasticity may also, in theory, be evaluated using phylogenetic information once the other factors contributing to tip variance are accounted for.

# VITA

Jennifer Bosco is the oldest of three children born in Waterbury, CT. She attended high school at Chase Collegiate School in Waterbury, CT and graduated in 2008. After graduation she attended the College of the Holy Cross in Worcester, MA where she received a Bachelor of Arts in Biology in 2012. She accepted a graduate teaching assistantship at the University of Tennessee, Knoxville, in the Ecology and Evolutionary Biology Department. She graduated with a Master of Science degree in Statistics and a Doctorate of Philosophy in Spring 2017.