AN ANALYSIS OF INDIVIDUAL VARIATION IN BEHAVIOR

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ABSTRACT

Emily Schmidt: An Analysis of Individual Variation in Behavior
(Under the direction of Karin Pfennig)

Individual animals within a population often show differences in their behavior. Recently, scientists have begun to study the causes and consequences of individual variation in behavior explicitly rather than attributing differences to observer error or noise. The study of behavioral syndromes, or correlated suites of behaviors, has become especially popular. Here I analyzed the fitness consequences of facultative hybridization - a behavior expressed by some individual female spadefoot toads - and evaluated the stability of a behavioral syndrome across development in the house cricket. First, I examined facultative hybridization in the spadefoot toad, *Spea bombifrons*. In Chapter 2, I used a split-clutch design to compare the development, morphology, and fitness of pure *S. bombifrons* tadpoles and their hybrid half-siblings. I found that hybrid tadpoles developed more quickly than pure *S. bombifrons* tadpoles, indicating that female *S. bombifrons* can benefit from hybridization. In Chapter 3, I then evaluated the mate preferences of female hybrid spadefoot toads. I found that hybrids preferred the calls of hybrid males over *S. multiplicata* males in deep water, but showed no preference in shallow water. Hybrids did not show a preference for *S. bombifrons* over hybrid or *S. multiplicata* calls in either context. These results indicate that female hybrid spadefoot toads show context-dependent mate choice, and express a maladaptive preference for the calls of sterile hybrid males in some environments. Finally, I measured boldness and exploration in juvenile, subadult, and adult European house crickets, *Acheta domesticus*, to

determine if these behaviors comprise a behavioral syndrome in this species and if this syndrome differs between developmental stages. I found that boldness and exploration were positively correlated in subadult and adult crickets, but not in juvenile crickets. These results indicate that a behavioral syndrome linking boldness and exploration emerges later in development in the house cricket. Finally, I provide evidence that studies of personality in crickets can be successfully conducted in undergraduate lab courses. In sum, it is critical to analyze individual variation in order to fully understand the evolution of particular behaviors.

To my mom. Thank you for always believing in me.

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CHAPTER 1: INTRODUCTION

Studies of animal behavior have often documented differences in behaviors between populations, and between individuals within populations. For a long time, individual differences in behaviors were thought to be the result of error or noise around an adaptive mean level of behavior (Slater 1981; Carere and Maestripieri 2013b). However, recently researchers have begun to focus more explicitly on the causes and consequences of stable individual differences in behaviors. Such differences are important because they suggest that behavioral plasticity in animals is not unlimited (Sih et al. 2004a; Sih et al. 2004b). Instead, evolution may select for and maintain different behaviors, or even particular combinations of correlated behaviors (Wolf et al. 2007).

Research on individual differences in behavior has taken different forms. For example, many studies have focused on why individual females express different mate preferences, or even change their mate preferences over the course of a lifetime (Jennions and Petrie 1997; Cotton et al. 2006). In some cases, certain mate preferences may be adaptive in some environments but not others, such as those with different predation regimes (Russell and Magurran 2006). Such context-dependent mate choice can lead to different mate preferences in different populations, and could also result in individual females choosing different mates in different environments. In addition, females may vary their mate preferences depending on their own body condition. For example, females in poor condition may be less choosy than females in good condition (Cotton et al. 2006). Both context- and condition-dependent mate choice will result in individual females displaying different mate

preferences. In some cases, individual females may even differ in how likely they are to hybridize (Rosenthal 2013).

However, individual differences are not restricted to behaviors involved in sexual selection. In fact, over the last decade the study of behavioral syndromes – also referred to as animal personality – has become one of the fastest-growing fields of research in animal behavior (Sih et al. 2004b; Carere and Maestripieri 2013b). Briefly, behavioral syndromes are correlated suites of behaviors that may have the same underlying proximate mechanisms and/or be favored by natural (Sih et al. 2004b) or sexual selection (Schuett et al. 2010). Individuals reared in different environments may develop different behavioral syndromes, or an individual may express different combinations of behaviors over the course of ontogeny (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b).

In this dissertation, I evaluated individual variation in behavior from different levels of analysis in two different systems. In Chapter 2, I examined the fitness consequences of an intriguing example of variation in female mate preferences: facultative hybridization in the spadefoot toad *Spea bombifrons*. Where *S. bombifrons* co-occurs with *S. multiplicata* in the deserts of the southwestern United States, hybridization between the two species is relatively common. In addition, hybridization tends to be between female *S. bombifrons* and male *S. multiplicata* and is more common in shallow ponds (Pfennig and Simovich 2002). Previous research found that when tested in deep pools, female *S. bombifrons* preferred the calls of their own species to those of *S. multiplicata* males. However, when tested in conditions mimicking shallow ponds, some individual females switched to preferring *S. multiplicata*; that is, they were more likely to hybridize. Specifically, females in poor condition were more likely to switch to preferring heterospecifics in shallow water than females in good condition

(Pfennig 2007). Pfennig hypothesized that it is adaptive for female *S. bombifrons* in poor condition to hybridize in shallow ponds because hybrid offspring tadpoles develop more quickly than pure *S. bombifrons*, and therefore are more likely to metamorphose and escape the pond before it dries (2007). However, the available data supporting this hypothesis were collected from separate hybrid and pure species families. In Chapter 2, I directly evaluated the fitness consequences of hybridization using a split clutch design in which I bred female *S. bombifrons* to both *S. bombifrons* and *S. multiplicata* males. I then compared the development, morphology, and fitness of pure *S. bombifrons* and hybrid tadpoles generated from the same mother. This allowed me to directly test the fitness consequences of facultative hybridization in *S. bombifrons* females, a behavior that is both context- and condition-dependent.

In order to fully understand the consequences of facultative hybridization in spadefoot toads, it is also necessary to evaluate the mate preferences of the female hybrid offspring themselves. Hybrid females may prefer other hybrids, prefer one of the parental species, or show no preference at all (Rosenthal 2013). In Chapter 3, I tested the preferences of hybrid *Spea* females for the calls of *S. bombifrons*, *S. multiplicata*, and hybrid males in pairwise tests in both deep and shallow water. This allowed me to test whether female hybrid spadefoot toads display mate preferences, and if those preferences are context-dependent as shown in *S. bombifrons*. Combined, Chapters 2 and 3 provide crucial insights into the fitness consequences of the facultative hybridization behavior expressed by some *S. bombifrons* females.

After conducting research on hybridization in spadefoot toads, I sought to investigate the causes of individual variation in behavior by studying the development of behavioral

syndromes. However, rather than continue my research in the spadefoot toad system, I decided to study behavioral syndromes in the European house cricket, *Acheta domesticus*. Invertebrates are excellent organisms for the study of behavioral syndromes and have many advantages over vertebrates. In particular, invertebrates are relatively easy to maintain in the lab, reproduce quickly and have relatively short lifespans, allowing researchers to test the large sample sizes required to accurately measure behavioral correlations. Further, it is often easier to manipulate environmental conditions and examine multiple generations in invertebrates than it is in vertebrates, allowing for detailed developmental studies of behavior (Mather and Logue 2013; Kralj-Fiser and Schuett 2014). Crickets have all of these advantages and are becoming a popular group in which to study behavioral syndromes (Wilson et al. 2009; Hedrick and Kortet 2012; Niemela et al. 2012a; Niemela et al. 2012b; Niemela et al. 2012c; Sweeney et al. 2013; Dochtermann and Nelson 2014), allowing me to compare my results to those of other studies. In Chapter 4, I measured boldness and exploration in house crickets at three different stages: juveniles, subadults, and adults. I sought to determine whether boldness and exploration form a behavioral syndrome in the house cricket, and if so, if the correlations between these behaviors are consistent across development. Overall, this dissertation provides insight into the causes and consequences of individual variation in behavior.

CHAPTER 2: DEVELOPMENTAL AND MORPHOLOGICAL DIFFERENCES BETWEEN SPEA BOMBIFRONS AND HYBRID TADPOLES

Introduction

Hybridization between closely related species occurs frequently in nature, yet such matings are often attributed to mistakes in mate choice or reduced choosiness by the female (Veen et al. 2001). In addition, sexual selection theory has generally considered female mating preferences to be static and stable (Andersson 1994). Recent work, however, has revealed that individual females may facultatively alter their mating preferences based on their own condition and/or their external environment (Jennions and Petrie 1997; Widemo and Saether 1999; Hunt et al. 2005; Cotton et al. 2006; Chaine and Lyon 2008; Milner et al. 2010). In some systems, individual females actually prefer to mate with heterospecifics in situations in which hybridizing provides some potential fitness benefit (Lesna and Sabelis 1999; Veen et al. 2001; Pfennig 2007). However, little work has focused on quantifying the fitness benefits of condition-dependent mate choice and facultative hybridization.

Spadefoot toads, *Spea bombifrons* and *Spea multiplicata*, provide an ideal system in which to study the fitness benefits of facultative hybridization. These two species occur in sympatry in the deserts of the American southwest and breed in ephemeral ponds during the summer monsoons. These ponds vary in their depth and, correspondingly, how long it takes for them to dry. Long-term studies have shown that hybridization occurs in these ponds, and hybridization is generally more frequent in shallower ponds (Pfennig and Simovich 2002). This pattern of hybridization is likely adaptive, as hybrid tadpoles develop more quickly than

pure *S. bombifrons* tadpoles; therefore, hybrid tadpoles are more likely to escape quick-drying ponds than are pure *S. bombifrons* tadpoles (Pfennig and Simovich 2002; Pfennig 2007). Additionally, although females of both species strongly prefer conspecific males when tested in conditions mimicking deep, slow-drying ponds (Pfennig 2000), some individual female *S. bombifrons* actually switched their preference and preferred heterospecific males in conditions mimicking shallow, quick-drying ponds (Pfennig 2007). Furthermore, female *S. bombifrons* were more likely to switch their preference in shallow water if they were in relatively poor condition (Pfennig 2007).

Hybrid spadefoot tadpoles are relatively frequent in natural ponds. Although the frequency of hybridization has declined recently, the frequency of hybridization can be as high as 40% in some ponds (Pfennig and Simovich 2002). Despite the apparent benefits of hybridization in terms of the development time of offspring (Pfennig 2007), there are also costs to this behavior. As adults, female hybrid spadefoot toads are less fecund than pure species females (Simovich et al. 1991), and hybrid males are sterile (Wünsch and Pfennig 2013). However, there seem to be few fitness costs for hybrid tadpoles in competition with pure species spadefoot toad tadpoles. In an experimental study, hybrid tadpoles reared with pure species competitors performed as well or better than the pure *S. bombifrons* and *S. multiplicata* tadpoles (Pfennig et al. 2007).

The evidence to date suggests that facultative hybridization is an adaptive condition-dependent behavior in female *S. bombifrons*. However, this hypothesis has not yet been directly tested. In this study, I used a split-clutch design to quantify the fitness benefits of facultative hybridization in *S. bombifrons* females and directly compare hybrid and pure species tadpoles while controlling for the identity of the mother. This is an important

distinction from previous work, which compared separate families rather than half-sibships. I predicted that hybrids would develop more quickly than pure *S. bombifrons* tadpoles, particularly for females in poor condition. In addition, I also compared size and morphology between the two tadpole types and determined whether parental characteristics such the condition or size of the mother and father affected these metrics.

Methods

Generation of Tadpoles for Experiment

I collected male and female *Spea bombifrons* and male *S. multiplicata* from natural breeding aggregations near Rodeo, New Mexico in July 2011. I collected five toads of each type (female *S. bombifrons*, male *S. bombifrons*, and male *S. multiplicata*) from one population and four of each type from a second population. The breeding at the second population occurred two days after the first, but I used the same methods for both populations. I measured the mass and snout-vent length (SVL) of all toads at the collection site.

I transported all individuals to the Southwestern Research Station in Portal, Arizona and bred pairs of spadefoot toads using standard methods (Pfennig 2007). I used a split-clutch design to generate half-sibships (families) of pure *S. bombifrons* and hybrid tadpoles from each female. Females were bred with the *S. bombifrons* male with whom she had originally been collected and with a randomly assigned *S. multiplicata* male from the same population. For each female, I also randomly selected whether she would be paired with the conspecific or heterospecific male first. After each female released approximately half of her

eggs with the first male, I removed her and rinsed her off to remove any residual sperm. I then paired her with her assigned male from the other species and removed all individuals after the breeding.

Approximately two days later, when the tadpoles were free-swimming, I randomly selected tadpoles for the experiment. Because the original breeding aggregations were two days apart, I housed the tadpoles from the two different populations in two different rooms at the research station. (Therefore, I included population as a random effect in all analyses – see below.) All other methods were the same. I placed four full-sibling tadpoles into a small box filled approximately halfway with dechlorinated water. I paired each of these boxes with a box containing four half-sibling tadpoles (either *S. bombifrons* or hybrids) and placed them on racks. I alternated boxes for each individual female and randomized the placement of the *S. bombifrons* and hybrid boxes. At the start of the experiment, I set up 410 boxes containing 1640 tadpoles. There were 48 boxes (24 pure *S. bombifrons* and 24 hybrid) containing 192 tadpoles for each female, except for one female from the second population who produced a small clutch and had 27 boxes containing 108 tadpoles. I placed fans in each room and allowed the water in the boxes to evaporate over the course of the experiment to simulate the conditions in rapidly drying, shallow ponds. I fed the tadpoles a standard amount of detritus from natural ponds every three days and approximately 10 field-collected anostracan fairy shrimp per individual per day. After ten days, I euthanized all 1384 surviving tadpoles (an 84.39% survival rate overall) by immersing them in a 0.1% aqueous solution of tricane methanesulfonate (MS-222) using standard methods (Pfennig and Murphy 2002). I then preserved the tadpoles in 95% ethanol in labeled specimen vials and returned them to the lab at UNC-Chapel Hill.

Morphological Measurements

Prior to conducting measurements, I briefly blotted each tadpole to remove residual ethanol. I measured the mass of each tadpole using an electronic scale and the SVL using digital calipers. I then determined the Gosner stage of both the left and right limb bud using standard methods (Gosner 1960) and averaged both for analysis. I also recorded the mouthparts score of each tadpole from 1-5 as a proxy for trophic morphology such that higher scores indicated a more carnivorous tadpole (Martin and Pfennig 2011). All measurements were conducted by observers blind to the type of tadpole (hybrid or pure *S. bombifrons*) and the identity of the mother.

Statistical Analysis

I measured condition for females, males, and tadpoles by taking the residuals of the cubic regression of mass on SVL using JMP 9.0 (SAS Institute 2010). I used linear mixed-effects models with population, female ID, male ID, and box as nested random effects to determine whether hybrid and pure *S. bombifrons* tadpoles differed in developmental (Gosner) stage or fitness measures (mass, SVL, and condition) and whether these metrics were affected by maternal or paternal fitness measures. I used similar models to determine the effect of morphology on development and to compare morphology between hybrids and pure *S. bombifrons* tadpoles. I recorded the proportion of the four tadpoles in each box that survived and compared the survival of hybrids and *S. bombifrons* as above, but without box

as a random effect. I determined the best model for each of these analyses using AIC. I performed these analyses in R v. 3.0.3 (R Core Team 2014).

Results

Overall Results

When all tadpoles were included in the analysis, there was no effect of tadpole type – aka, whether the tadpole was a *S. bombifrons* or a hybrid – on tadpole mass (df = 8, t = -0.876, p = 0.41), condition (df = 8, t = 1.324, p = 0.222), or survival (df = 8, t = 0.246, p = 0.811). Tadpole SVL was best explained by an additive model combining tadpole type (coeff: -0.26, df = 8, t = -2.151, p = 0.064) and maternal mass (coeff: 0.108, df = 6, t = 2.345, p = 0.057), although these effects were marginally non-significant (Fig. 2.1).

Maternal condition did not affect tadpole development as measured by Gosner stage (df = 6, t = 0.53, p = 0.615). Instead, after accounting for a marginal additive effect of tadpole type (coef: 0.29, t = 1.93, df = 6, p = 0.101), tadpole developmental stage was affected by a significant interaction between maternal mass and paternal mass (coef: -0.04, t = -2.85, df = 6, p = 0.029).

There was a significant interaction effect between tadpole type and paternal mass on tadpole morphology (coef: -0.193, df = 6, t = -4.1, p = 0.006; Fig. 2.2). However, similar to other studies (Pfennig and Pfennig 2005), in my sample *S. bombifrons* males were significantly heavier than the *S. multiplicata* males used to generate the hybrids (*S. bombifrons*: 13.639 ± 0.437 g; *S. multiplicata*: 10.528 ± 0.896 g; t = -3.120, df = 11.605, p =

0.009), so I cannot tease apart the effects of paternal mass and species on tadpole morphology with these data.

Morphology

I was interested in examining how tadpole morphology, as measured by mouthparts score, affected tadpole development and fitness measures. However, hybrid and *S. bombifrons* tadpoles produced significantly different numbers of each morph (X-sq = 72.15, df = 2, $p < 0.0001$). I classified tadpoles with a mouthparts score of 1-2 as omnivores, 2.5-3.5 as intermediates, and 4-5 as carnivores. *S. bombifrons* tadpoles had 516 omnivores, 135 intermediates, and 35 carnivores. Hybrid tadpoles had 616 omnivores, 68 intermediates, and 1 carnivore. Further, the majority of the carnivores – 30 *S. bombifrons* and the single hybrid – were all produced by the same female. Because morphology can affect tadpole size and development, I repeated my analyses including only those tadpoles classified as omnivores (MP = 1-2). I did not include intermediates in these analyses because at this early stage (10 days old) intermediates were likely developing into carnivores.

Omnivores Only

There were no significant differences in the mouthparts scores of hybrid and *S. bombifrons* omnivores (df = 8, t = 0.693, p = 0.508). When I restricted my analyses to omnivores, I found that tadpole development as measured by Gosner stage was best explained by an additive model combining tadpole type (coef: 0.428, df = 8, t = 2.987, p = 0.017) and maternal mass (coef: 0.114, df = 6, t = 2.632, p = 0.039). Heavier females

produced more developed offspring, and overall hybrids were more developed than *S. bombifrons* tadpoles (Fig. 2.3), but the interaction between maternal mass and tadpole type was not significant (df = 7, t = 0.618, p = 0.556). There was no interaction between maternal condition and tadpole type on tadpole developmental stage (df = 7, t = 0.735, p = 0.486). There were no significant differences between *S. bombifrons* and hybrid tadpoles in SVL (df = 8, t = -1.245, p = 0.248), mass (df = 8, t = -0.879, p = 0.405), or condition (df = 8, t = -0.2, p = 0.847).

Discussion

Overall, my analysis of the full dataset comparing hybrid tadpoles to their *S. bombifrons* half-siblings suggested that while there were no differences in tadpole mass, survival, or condition, my measures of tadpole development (Gosner stage), size (SVL) and morphology (mouthparts score) were affected by tadpole type (*S. bombifrons* or hybrid) and parental mass in complicated ways. Tadpole development was affected by an interaction between maternal and paternal mass and a non-significant additive effect of tadpole type. *S. bombifrons* were larger than hybrid tadpoles and SVL increased with maternal mass, but these additive effects were marginally non-significant. Finally, tadpole morphology was not affected by type, but heavier fathers produced more carnivorous offspring. However, these effects are difficult to interpret given the limitations of my design. My tadpoles were generated by nine different females, and the *S. multiplicata* males used to generate the hybrid offspring were significantly less massive than the *S. bombifrons* males used to generate their pure *S. bombifrons* half-siblings. In addition, *S. bombifrons* tadpoles were significantly more likely to become carnivores, while hybrid tadpoles were almost exclusively omnivores and

intermediates. Therefore, I repeated my analyses focusing only on omnivores and focusing on tadpole type and maternal measurements rather than paternal measurements.

I found that omnivorous hybrid and *S. bombifrons* tadpoles did not differ in survival, mass, SVL, condition, or mouthparts score. Only developmental stage was affected by tadpole type, such that hybrids were more developed at the end of the 10 day experiment than pure *S. bombifrons*. Further, there was also an additive effect of maternal mass, such that heavier females produced more developed tadpoles. However, there was no significant interaction between tadpole type and maternal condition, indicating that females in poorer condition did not gain more of a benefit from hybridizing in terms of development time than females in better condition. This is contrary to the predictions I made due to the fact that adult female *S. bombifrons* in poor condition are most likely to switch from preferring conspecifics in deep water to preferring *S. multiplicata* – aka, are more likely to hybridize – in shallow water (Pfennig 2007). However, in this experiment I was only able to compare hybrid and pure species offspring from nine *S. bombifrons* females. A larger sample size is needed to determine if the magnitude of the benefit female *S. bombifrons* receive from hybridizing is related to their condition.

Although the data did not support my hypothesis about the importance of female condition for generating differences between *S. bombifrons* and hybrid tadpoles, my results make several important contributions to the data on hybridization in spadefoot toads. Overall, my results suggest that there are no intrinsic disadvantages for hybrid spadefoot tadpoles relative to pure *S. bombifrons*. Hybrid tadpoles did not suffer any costs in terms of survival, size, mass, or condition. Although my tadpoles were reared with siblings of the same type, my results combined with previous experiments that reared hybrids with pure species

competitors (Pfennig et al. 2007) suggest that hybrid spadefoot toads do not suffer fitness costs at the tadpole stage. In fact, in the only metric for which I found significant differences between hybrid and *S. bombifrons* tadpoles – developmental stage – hybrids actually had an advantage over pure species tadpoles. This result confirms previous measurements of hybrid and *S. bombifrons* tadpoles produced by separate females which found that hybrids developed more quickly (Pfennig 2007). Further, although I did not find a significant role of maternal condition on tadpole development, I did find an effect of mass such that heavier females produced tadpoles that were more developed. Although the interaction between maternal mass and tadpole type was not significant, this does suggest that maternal fitness can play a role in how quickly hybrid tadpoles develop.

My results and those of other studies (Pfennig et al. 2007) show that hybrid tadpoles are not at a competitive disadvantage relative to *S. bombifrons* tadpoles, and in fact have an advantage in that hybrids can develop and escape shallow ponds more quickly than pure *S. bombifrons*. The importance of rapid development time in this system is believed to have led to facultative hybridization behavior in adult female *S. bombifrons* (Pfennig 2007). However, there is also evidence that the fertility costs that adult hybrids suffer – that is, male sterility and reduced female fecundity (Simovich et al. 1991; Wünsch and Pfennig 2013) - are exerting strong selective pressure in this species. The frequency of hybridization in the populations from which my spadefoot toads were collected has decreased over the last several decades (Pfennig 2003). However, even a relatively low frequency of hybridization can lead to introgression between populations and increase the genetic variation in sympatric populations relative to those in allopatry (Abbott et al. 2013). In the spadefoot toad system, it is possible that the ability of hybrid tadpoles to survive and develop quickly enough to escape

rapidly drying ponds – and for at least some adult female hybrids to successfully reproduce with pure species males – has contributed to recent range expansion in *S. bombifrons* (Chunco et al. 2012). That is, hybridization with *S. mulitplicata* may be allowing *S. bombifrons* to expand into drier habitats in which they would not usually be able to survive due to the relatively slow development time of *S. bombifrons* tadpoles. Overall, my results suggest that the benefit *S. bombifrons* females receive from producing rapidly developing hybrid tadpoles is not offset by fitness costs at the larval stage, and may contribute to the maintenance of hybridization in this system.

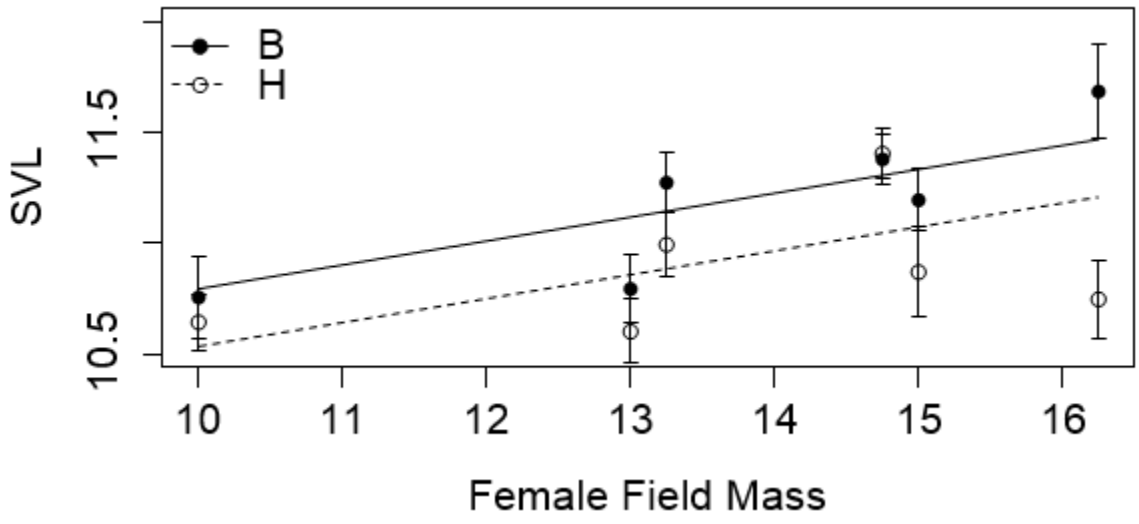


Figure 2.1. Relationship between maternal mass (g) and tadpole SVL (mm) for hybrid and pure *S. bombifrons* tadpoles. Both *S. bombifrons* and hybrid tadpoles were larger with increasing maternal mass, but *S. bombifrons* tadpoles were larger overall.

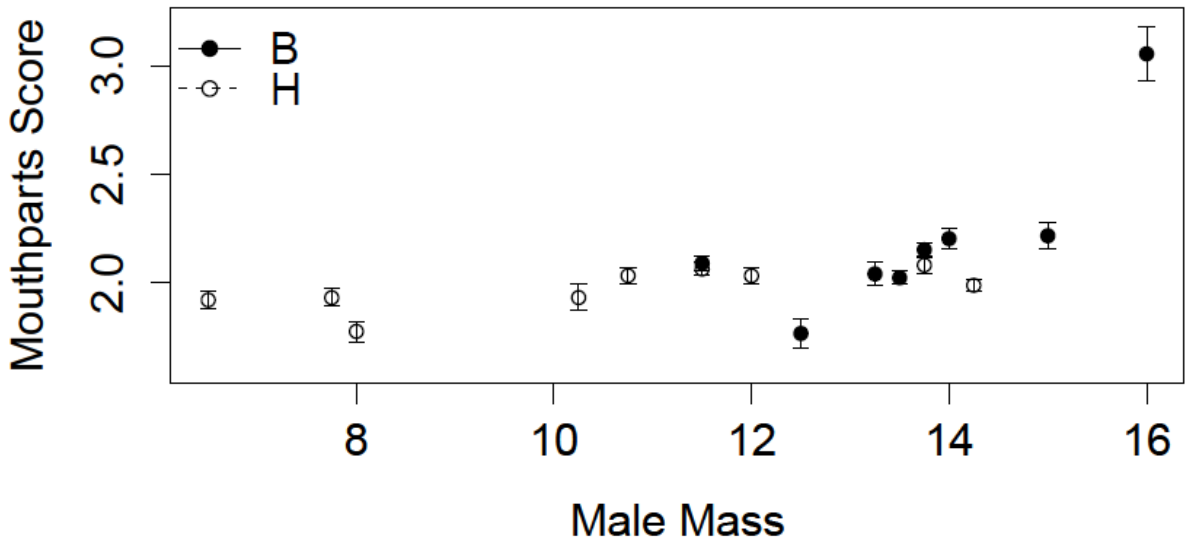


Figure 2.2. Tadpole morphology was affected by an interaction between tadpole type and paternal mass (g). However, *S. bombifrons* males were also heavier, so I cannot distinguish between the effects of paternal mass and paternal species on tadpole morphology. Higher mouthparts scores indicate more carnivorous tadpoles.

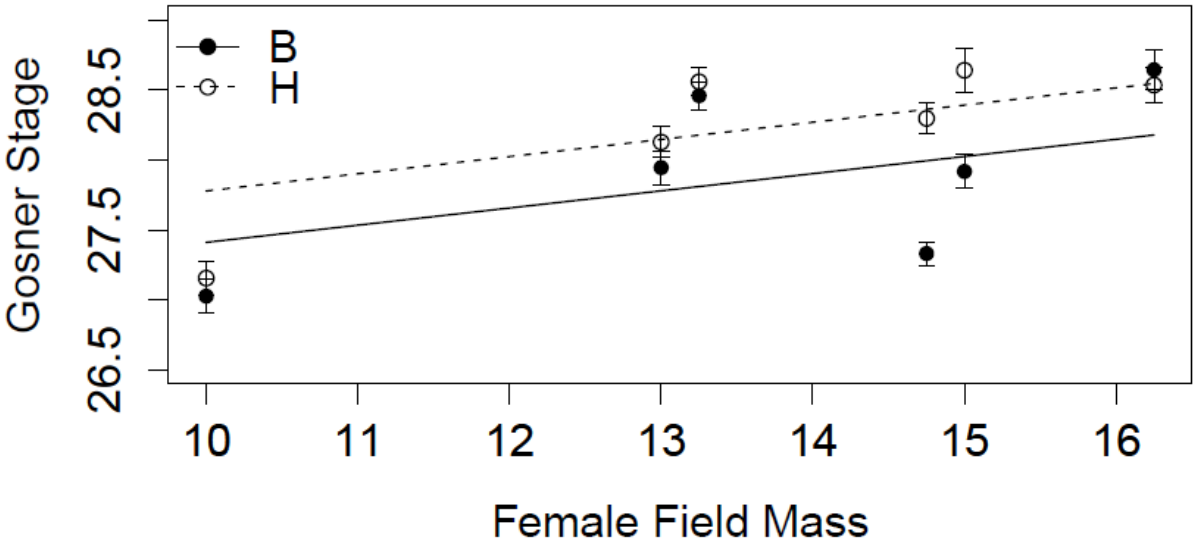


Figure 2.3. Effect of tadpole type and maternal mass (g) on tadpole developmental stage. Hybrids were more developed than *S. bombifrons* tadpoles. Within both hybrids and *S. bombifrons*, tadpole developmental stage increased with increasing maternal mass.

CHAPTER 3: HYBRID MATE CHOICE AS A SPECIES ISOLATING MECHANISM: ENVIRONMENT MATTERS

Introduction

A central goal of biology is to understand how new species arise and remain distinct (Coyne and Orr 2004; Grant & Grant 2008; Price 2008; Pfennig & Pfennig 2012). Under the biological species concept, species are defined as evolutionarily distinct groups that do not exchange genes because they have evolved traits—'isolating mechanisms'—that prevent gene flow between them (Mayr 1963; Coyne and Orr 2004). A major class of these isolating mechanisms consists of maladaptive traits in hybrids that prevent them from backcrossing to either parent population (Barton and Hewitt 1985; Coyne and Orr 2004). Hybrid maladaptation therefore plays a key role in speciation. Consequently, identifying the causes of such maladaptation is crucial to understanding the origins and maintenance of biodiversity (Coyne and Orr 2004; Abbott et al. 2013).

Historically, speciation research has concentrated on three main sources of hybrid maladaptation: decreased survival, reduced fertility, and decreased likelihood of succeeding in either parental niche (Arnold 1997; Coyne and Orr 2004; Nosil 2012; Abbott et al. 2013). A further possibility that has received relatively less attention is that hybrids might express maladaptive reproductive traits that contribute to reproductive isolation between species (Noor 1997; Russell and Magurran 2006; Svedin et al. 2008; Clark et al. 2010; Lemmon and Lemmon 2010; Rosenthal 2013; Latour et al. 2014). Specifically, viable, fertile hybrids might fail to appropriately produce or respond to courtship signals (Russell and Magurran

2006; Svedin et al. 2008; Clark et al. 2010; Lemmon and Lemmon 2010; Rosenthal 2013). Alternatively, hybrids might express mate preferences that reduce their likelihood of mating with fitness-enhancing mates; for example, hybrids might possess intermediate preferences for hybrid males (Hoy et al. 1997, Doherty & Gerhardt 1983, Ritchie 2000, Selz et al. 2014) that are sterile or otherwise poor quality mates. In extreme cases, such dysfunctional reproductive behavior could render viable, fertile hybrids 'behaviorally sterile', thereby acting as a key isolating mechanism between species (Noor 1997, Russell and Magurran 2006).

In systems where hybrids reproduce with parental species, mate preferences that influence to which parental species they mate will determine patterns of gene flow between species, including whether such gene flow is directional (Christophe and Baudoin 1998; den Hartog et al. 2010; Charpentier et al. 2012; Veen et al. 2012; Rosenthal 2013; Culumber et al. 2014; Latour et al. 2014; Paczolt et al. 2015). Moreover, because female mate choice can depend on the environment or female condition (Cotton et al. 2006), the impact of hybrid mate choice on the extent and pattern of introgression between species could vary in space or time. Thus, evaluating hybrid mate choice and whether it varies across different contexts is critical for explaining reproductive isolation and patterns of gene exchange, if any, between species (Svedin et al. 2008; Rosenthal 2013).

I addressed these issues using hybrid female spadefoot toads. As described below, this system is well suited for evaluating hybrid female preferences for sterile hybrid males versus pure-species males across different environments.

Methods

Study System

I studied first-generation (F1) hybrid females of two spadefoot toads: *Spea bombifrons* and *S. multiplicata*. These species hybridize in the southwestern United States and northern Mexico (Pfennig et al. 2012). Hybrids are viable; however, F1 hybrid females produce half as many eggs as pure-species females whereas F1 hybrid males are sterile (Simovich et al. 1991; Wünsch and Pfennig 2013). Hybrid males attempt to attract mates, but their calls are intermediate between those of pure-species males (Pfennig 2000). If hybrid females possess intermediate preferences for sterile males, this could lead to selection disfavoring hybrid females.

Because of the costs of hybridization in *Spea*, female *S. multiplicata* avoid hybridizing where the two species co-occur (Pfennig 2000; Pfennig and Rice 2014). Likewise, *S. bombifrons* females also avoid hybridization (Pfennig 2007). However, in certain environments – specifically shallow, rapidly drying ponds – *S. bombifrons* females benefit by hybridizing (Pfennig 2007). Hybrid tadpoles develop faster than pure *S. bombifrons* tadpoles, so hybrids are more likely to reach metamorphosis and therefore survive in shallow, highly ephemeral pools (Pfennig and Simovich 2002; Pfennig 2007). By contrast, in deep, long-lasting ponds, *S. bombifrons* females receive no such benefit because *S. bombifrons* tadpoles can escape the ponds (Pfennig and Simovich 2002; Pfennig 2007). Consequently, *S. bombifrons* females have evolved facultative preferences for conspecifics: they prefer conspecific males in deep, long lasting pools, but switch their preferences and prefer *S. multiplicata* males in shallow, ephemeral pools (Pfennig 2007). Such context-

dependent preferences could be inherited by hybrid females and impact their mate choice decisions.

Phonotaxis Tests

I tested the mate preferences of 20 gravid, lab-bred F1 hybrid females. The females were derived from 12 families (i.e., some females were siblings). For 10 females *S. bombifrons* was maternal, and for 10 females *S. multiplicata* was maternal.

Each female was presented with the following pair-wise choices of male call stimuli: F1 hybrid calls versus *S. multiplicata* calls; F1 hybrid calls versus *S. bombifrons* calls; and *S. bombifrons* calls versus *S. multiplicata* calls. For each pairwise combination, each female was tested four times in deep water and four times in shallow water. The order in which females were presented the call pairings and water level was random. The call stimuli were synthesized and consisted of average parameters for each call type (Pfennig 2000, 2007).

I measured female mate preferences using previously published methods (Pfennig 2007). Specifically, I placed each toad in the center of a circular wading pool 1.8 m in diameter filled approximately to 30 cm (deep water) or 6 cm (shallow water). Each toad was initially placed on a central platform 2 cm above water level, equidistant between two platforms set 180° apart at the edges of the pool. I placed a speaker on each of these two platforms. Two additional platforms were set at 90° from the speakers to serve as neutral areas. I scored females as preferring a call stimulus when they approached and touched a speaker. I recorded the time taken for females to touch the speaker as a female's latency to choose; for each female I averaged this value for each pair type in each water level across the

four trials per water level. If a female did not touch a speaker within 30 minutes, she was considered non-responsive in that test.

Statistical Analysis

I determined whether females differed in their responsiveness to male call pairings or to the different water levels by contrasting latency time to choose for these variables using Wilcoxon tests. I also contrasted the overall average time hybrid females took to choose a stimulus with the mean time measured previously for pure species females. To do so, I used a Wilcoxon test to determine if the overall mean for hybrids differed from the hypothesized mean of 439 seconds, which is a previously measured combined mean to choose between conspecific and heterospecific calls across different water levels for *S. multiplicata* and *S. bombifrons* females (Pfennig 2007).

To contrast female preferences for the three types of males across the different water levels, I used a Cox proportional hazards regression analysis and clustered the data by individual (J. Weiss, unpublished MS). This analysis allowed me to combine the information from multiple testing of each female for multiple call stimuli. I could thereby contrast the overall probabilities of a female choosing the hybrid, *S. multiplicata*, or *S. bombifrons* calls in deep versus shallow water across all tests to determine whether water level had a significant effect on these combined probabilities. I used R v. 3.0.3 with the *mlogit* (Croissant 2013), *survival* (Therneau 2014), *mvtnorm* (Genz et al. 2014), and *compositions* (van den Boogaart et al. 2014) packages.

Finally, to visualize variation in female mate choice behavior I calculated the percentage of four trials within a test that each female chose a hybrid call, or the percentage of trials she chose the *S. bombifrons* call for the *S. bombifrons* vs *S. multiplicata* trials. I also tested to see whether these preferences were correlated with one another by performing Spearman correlations in JMP v 9.0.

Results

I found that hybrid females did not differ in the time taken to choose a stimulus depending on either the call-pair stimuli ($X^2 = 1.22$, df = 2, p = 0.545) or on water level ($X^2 = 0.21$, df = 1, p = 0.646; Table 3.1). However, hybrid females overall responded more quickly to the stimuli (mean time to choose (+/- SD) = 385.2 (98.33) sec) than did pure-species females from a previous study that had been presented conspecific versus heterospecific calls across the two water levels (Wilcoxon signed rank = -57; df = 19, p = 0.033).

Generally, hybrid females did not express strong preferences in any call pairings or water level, except in deep water when presented hybrid calls versus *S. multiplicata* calls (Fig. 3.1; Table 3.2). Specifically, in deep water, females were equally likely to choose hybrid calls or *S. bombifrons* calls (Z = -0.866, p = 0.386) and equally likely to choose either of the parental calls (Z = -1.521, p = 0.128). However, females were significantly more likely to choose hybrid calls over *S. multiplicata* calls (Z = -2.007, p = 0.045; Fig. 3.2, Table 3.3). In shallow water, females showed no preferences: they were equally likely to choose hybrid calls versus *S. bombifrons* calls (Z = -0.215, p = 0.83) or *S. multiplicata* calls (Z = 0.958, p = 0.338), and did not prefer calls of either parental species (Z = 1.231, p = 0.219).

I found a significant interaction between water level and the probability of choosing *S. multiplicata* calls: in shallow water the probability that a female would choose *S. multiplicata* over hybrid calls increased relative to deep water ($Z = 2.122$, $p = 0.034$). By contrast, I found no interaction between depth and the probability of choosing *S. bombifrons* calls versus hybrid calls ($Z = 0.475$, $p = 0.635$). Thus, water level affected the probability that a female chose hybrid calls over *S. multiplicata* calls, but not the probability that a female chose hybrid calls over *S. bombifrons* calls. Finally, I found that females became more likely to choose *S. multiplicata* over *S. bombifrons* in shallow water relative to deep water ($Z = 2.087$, $p = 0.037$). Female preferences were not correlated across tests or environments (Table 3.4).

Discussion

I used pair-wise choice tests in deep and shallow water to evaluate the preferences of hybrid females for the calls of pure-species males and sterile hybrid males. Hybrid females did not express a significant preference for any particular male type except in deep water. In the deep-water environment, spadefoot toad hybrid females preferred the calls of sterile hybrid males versus those of *S. multiplicata*, indicating that hybrid female mate preferences could be maladaptive in at least some circumstances. Spadefoot females breed no more than once per year, so choosing a sterile mate carries severe lifetime fitness costs. Critically, such behavior would lower the incidence of backcrossing to either parent species, and therefore reduce gene flow between the two species.

Generally, the role of hybrid reproductive behavior as a reproductive isolating mechanism has been underappreciated relative to studies of hybrid sterility, inviability, or

ecological performance (Rosenthal 2013). Nevertheless, my results comport with an emerging body of evidence (Noor 1997, Russel & Magurran 2006, Svedin et al. 2008, Clark et al. 2010, Lemmon & Lemmon 2010, Latour et al. 2014), which reveals that maladaptive hybrid mating behaviors could contribute to reproductive isolation between species.

Despite finding that hybrid mate preferences can potentially serve as an isolating barrier in at least some conditions, my results also reveal that hybrid mate choice depends on a female's environment. Female mate choice is often context- or condition-dependent (Cotton et al. 2006). In the case of spadefoots, hybrid females did not switch their mate preferences from one male type to another (as occurs in pure-species *S. bombifrons* females). Instead, hybrid females as a group appear to become less choosy depending on habitat type or males that are encountered.

Generally, the possibility that female hybrids might vary their mate choice behavior in this way has two key implications. First, whether hybrid mate choice is an effective isolating mechanism will depend on the environment. Second, patterns of hybrid mate choice (and how they vary with the environment) can impact the directionality, if any, of introgression between species (Christophe & Baudoin 1998, den Hartog et al. 2010, Charpentier et al. 2012, Veen et al. 2012, Culumber et al. 2014, Latour et al. 2014, Paczolt et al. 2015). The expression of alternative preferences by hybrid females across different habitats could generate habitat-dependent patterns of introgression that are linked to female mate preferences. In the absence of understanding how hybrid mate choice varies across habitats, the ultimate cause of environmental variation in introgression could be missed (Rosenthal 2013).

Moreover, if hybrid females mate randomly, then the relative frequencies of male types in a population can also contribute to mating patterns (Malmos et al. 2001, Culumber et al. 2014). Thus, the extent to which relative male abundance dictates patterns of introgression will likely depend on the strength of female preferences. When hybrid mate preferences are weakly expressed (e.g., as in the spadefoots in shallow water habitats; Table 3.2), the relative frequencies of different male types might be more important to reproductive isolation – or lack thereof – than when mate preferences are stronger (e.g., as in the spadefoots in deep water habitats; Table 3.2), especially if females reject non-preferred males.

From evolutionary and ecological perspectives, understanding speciation requires determining under what environmental circumstances reproductive isolation evolves and is either maintained or breaks down. Hybrid mate preferences will potentially play a key role in this process depending on how those preferences vary with the environment and the relative abundance of pure-species and hybrid males. Thus, evaluating how these different factors combine is a critical next step to ascertaining the role of hybrid reproductive behavior in the origins and maintenance of species.

Table 3.1. Latency for hybrid females to choose a stimulus in each trial. There were no significant differences in latency across the different trials.

Stimuli	Water Level	Average Latency (s) ± SE
S. bombifrons vs hybrid	Deep	322.35 ± 38.42
	Shallow	400.7 ± 58.84
S. multiplicata vs hybrid	Deep	347.3 ± 38.36
	Shallow	448.16 ± 53.52
S. bombifrons vs *S. multiplicata*	Deep	428.88 ± 41.36
	Shallow	363.55 ± 47.00

Table 3.2. Mean hybrid female preferences for alternative call stimuli in either deep (D) or shallow (S) water. Females were presented pairwise call stimuli of *S. bombifrons* (B), *S. multiplicata* (M) or F1 hybrid (H) males. Preferences are presented as percent *S. bombifrons* (in B v M trials) or percent hybrids (in B v H and M v H) chosen across repeated presentation of a given stimulus set. Random mating is 50%.

Call Stimuli	Mean preference (SD), %
B v M (D)	57.1 (23.49)
B v M (S)	41.62 (31.20)
B v H (D)	48.35 (23.86)
B v H (S)	50.85 (27.17)
M v H (D)	64.19 (23.78)
M v H (S)	48.34 (25.78)

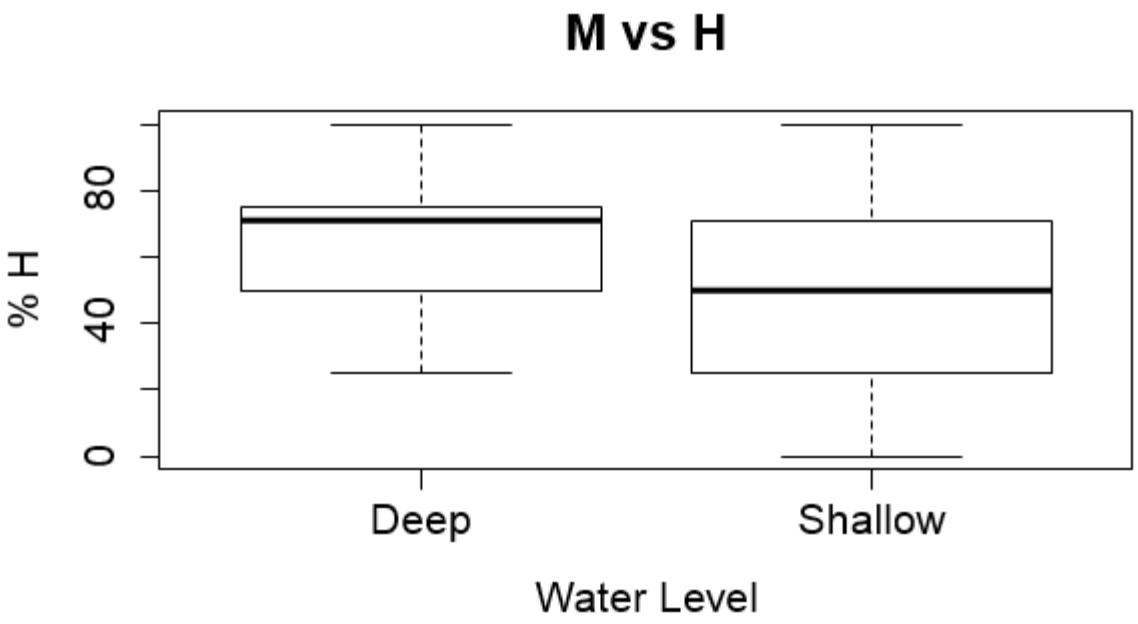
Table 3.3. Probabilities that female hybrid spadefoot toads chose hybrid, *S. bombifrons* or *S. multiplicata* calls in deep versus shallow water. Different letters signify differences within environment; bold face indicates significant difference between environments.

Call Type	Deep Water	Shallow Water
Hybrid	0.388 [a]	0.318[a]
S. bombifrons	0.339 [a,b]	0.306 [a]
S. multiplicata	**0.273** [b]	**0.376** [a]

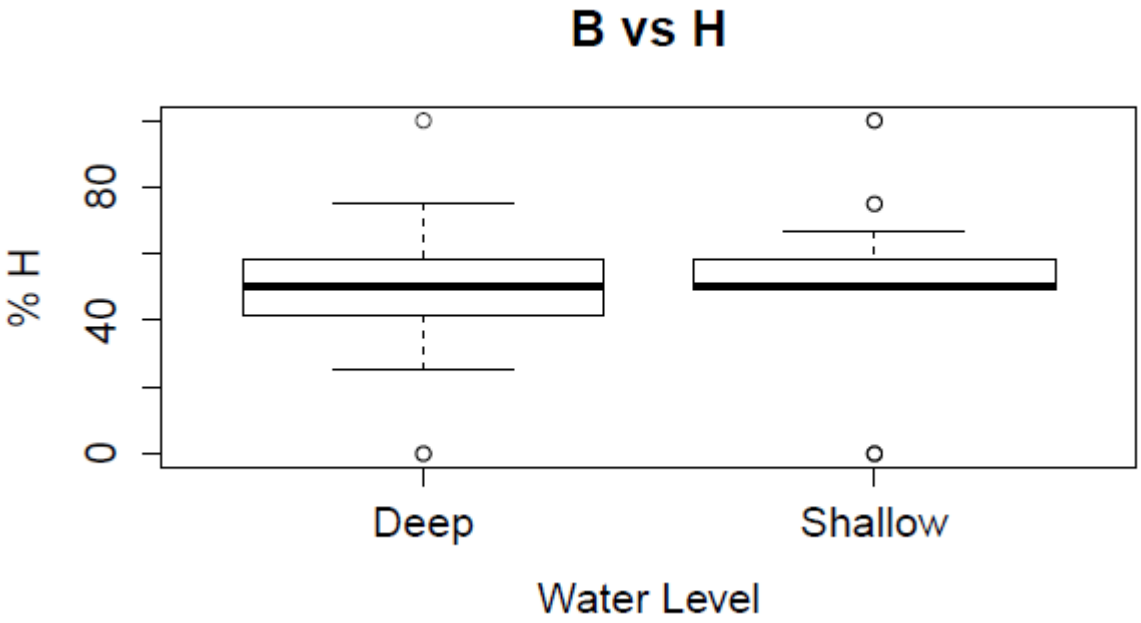
Table 3.4. Spearman correlations (ρ) of preferences across different stimuli pairings. All are $p > 0.15$. B = *S. bombifrons*; M = *S. multiplicata*; H = F1 hybrids; D = deep water environment; S = shallow water environment. $N = 20$ for all.

	B v M (D)	B v M (S)	B v H (D)	B v H (S)	M v H (D)	M v H (S)
B v M (D)	---					
B v M (S)	-0.164	---				
B v H (D)	0.298	-0.168	---			
B v H (S)	-0.155	0.186	0.247	---		
M v H (D)	0.234	-0.025	0.310	-0.028	---	
M v H (S)	0.313	-0.227	0.041	0.115	0.008	---

A.


M vs H
% H
0
40
80
Deep
Shallow
Water Level

B.


B vs H
% H
0
40
80
Deep
Shallow
Water Level

C.

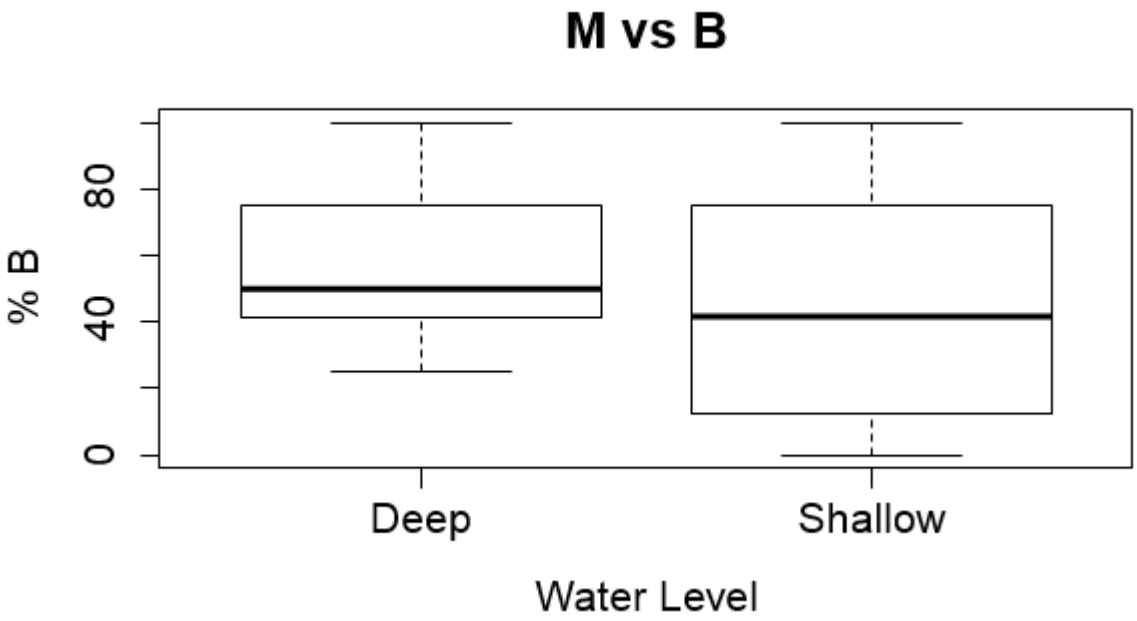


Figure 3.1. Variation in hybrid female preferences. The boxplots show average preferences of 20 hybrid *Spea* females for the hybrid or *S. bombifrons* stimulus across all four trials by water level, with 50% indicating preference is not different from random. A. *S. multiplicata* vs hybrid. Females preferred hybrid calls in deep water, but showed no preference in shallow water. B. *S. bombifrons* vs hybrid. Females showed no preference for either call in deep or shallow water. C. *S. bombifrons* vs *S. multiplicata*. Females showed no preference for either call in deep or shallow water.

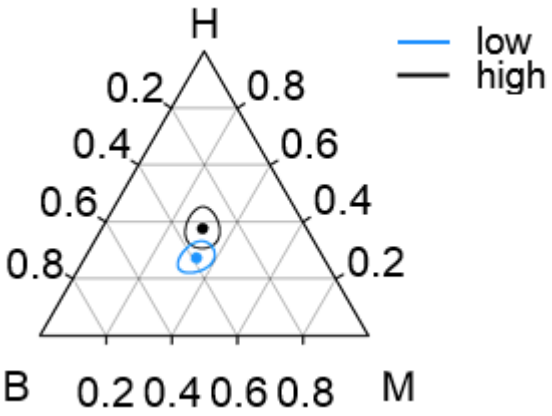


Figure 3.2. Ternary diagram showing combined probabilities (with 95% simultaneous joint confidence regions) of hybrid females choosing hybrid (H), *S. bombifrons* (B) or *S. multiplicata* (M) calls in deep versus shallow water.

CHAPTER 4: A BEHAVIORAL SYNDROME VARIES ACROSS ONTOGENY IN THE HOUSE CRICKET, ACHETA DOMESTICUS

Introduction

Over the past decade, there has been a surge of interest in the relatively new field of animal personality. While various researchers have identified this field by different names (e.g. animal personality (Dall et al. 2004; Gosling 2008; Carere and Maestripieri 2013a); coping style (Koolhaas et al. 1999); temperament (Reale et al. 2007); behavioral syndromes (Sih et al. 2004a; Sih et al. 2004b)), they all describe the same idea: individual behaviors do not vary independently from one another, but instead are often correlated across time and/or contexts. Here I will use the term behavioral syndromes and identify the individual behaviors involved in these syndromes as personality traits. Reale et al.(2007) identified five major personality traits: (1) boldness, or behavior under risky situations; (2) exploration, defined as activity in a novel environment; (3) general activity level in a familiar environment; (4) aggression toward conspecifics; and (5) sociability, or the tendency to associate with conspecifics. Correlations between any or all of these traits may comprise a behavioral syndrome in a particular species or population.

Within a syndrome, individuals showing different combinations of the behaviors involved are said to have different behavioral types (Sih et al. 2004a; Sih et al. 2004b). For example, in many populations boldness and aggression are positively correlated (Sih et al. 2004b; Bell 2005; Sih et al. 2012); however, individuals within these populations have different behavioral types, such as bold-aggressive or shy-nonaggressive. These individuals may display behavioral plasticity and become less bold and aggressive in certain contexts,

such as under the threat of predation, but the rank-order differences between individuals will be maintained (Sih et al. 2004b). Although some studies focus only on variation in individual personality traits (Smith and Blumstein 2008), it is the study of the correlations between these traits that represents a relatively new way of thinking in behavioral ecology (Bell 2007).

The existence of behavioral syndromes suggests that the behaviors involved do not evolve independently, and that organisms do not show unlimited behavioral plasticity (Sih et al. 2004b). One potential explanation for the existence of behavioral syndromes is that correlations between behaviors due to similar proximate mechanisms prevent individuals from reaching independent evolutionary optima for each behavior (Sih et al. 2004b). This may explain the persistence of suboptimal behaviors, as selection for one behavior may promote or restrict the expression of another (Sih et al. 2004a; Sih et al. 2004b; Reale et al. 2007; Sih et al. 2012). Evidence for this hypothesis has been found in a species of Amazonian social spider (Pruitt et al. 2010), in which the same behavioral syndrome is found in geographically distant populations that vary in their ecological conditions and selective environments.

However, another major explanation for the existence of behavioral syndromes suggests that particular behavioral correlations can be favored by natural selection (Dingemanse and Reale 2005). This hypothesis suggests that correlations between behaviors can be favored or decoupled by selection in different environments (Sih et al. 2004b). Theoretical work suggests that ecological factors such as predation regime and resource availability can play an important role in the evolution of behavioral syndromes (Luttbeg and Sih 2010; Wolf and Weissing 2010), and empirical studies in several species have supported

this claim (Bell and Stamps 2004; Dingemanse et al. 2004; Bell 2005; Bell and Sih 2007; Dingemanse et al. 2007; Herczeg et al. 2009).

Despite the wealth of recent studies focusing on behavioral syndromes and their implications for evolution, the stability of behavioral syndromes across development has been characterized for relatively few species (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b). Developmental studies of behavioral syndromes are critical for understanding the proximate mechanisms that dictate correlations between behaviors (Stamps and Groothuis 2010a). Behavioral syndromes may remain stable throughout development if the proximate mechanisms underlying linked behaviors do not change across ontogeny. Alternatively, behavioral correlations may break down over time if organisms undergo a major physical reorganization during development, such as during metamorphosis (Wilson and Krause 2012a; Wilson and Krause 2012b). Behavioral syndromes can also be decoupled across development, or even emerge later in ontogeny, if individuals face different selective pressures at different life history stages (Sinn et al. 2008; Groothuis and Trillmich 2011; Adriaenssens and Johnsson 2013). Such ontogenetic changes would suggest that correlations between behaviors are not constrained. However, the few studies that have tested if behavioral syndromes are decoupled across development thus far have found mixed results (Brodin 2009; Brodin et al. 2012; Wilson and Krause 2012a), and even males and females can differ in the stability of personality traits across development (Hedrick and Kortet 2012). These conflicting results demonstrate the need for further study across taxa in order to identify the factors that lead to the stability or breakdown of behavioral syndromes across developmental shifts.

In this study, I sought to determine whether behavioral syndromes are stable across development in the house cricket, *Acheta domesticus*. Crickets are an attractive system for studying behavioral syndromes due to their wide array of well-characterized behaviors and the relative ease with which they can be reared in the lab. Although a number of studies have looked at field crickets in the genus *Gryllus* (Niemela et al. 2012a; Niemela et al. 2012b; Niemela et al. 2012c), to date there has been only one study on behavioral syndromes in the European house cricket *Acheta domesticus* (Wilson et al. 2009). They found that exploratory males and females were also bolder following a simulated predation event, but their study did not incorporate a developmental component. Another study showed that exploratory behavior is repeatable in the house cricket (Dochtermann and Nelson 2014), but the authors did not examine any other personality traits. A developmental study found that in the cricket *G. integer,* juveniles were bolder than adults (Niemela et al. 2012b), but that study did not measure other behavioral traits and therefore did not examine the development of a behavioral syndrome.

Here, I measured boldness and exploration in juvenile, subadult, and adult European house crickets. I focused on boldness and exploration because these personality traits have been found to be positively correlated in many species, including *A. domesticus* (Groothuis and Carere 2005; Bourne and Sammons 2008; Wilson et al. 2009; Mazué et al. 2015). I predicted that across all individuals, boldness and exploration would be positively correlated and form a behavioral syndrome as found by Wilson and colleagues (2009). I also predicted that crickets of different ages would show significant differences in boldness and exploration, and that the correlation between these behaviors would differ between age groups. Finally, I led a lab on behavioral syndromes in crickets in an undergraduate animal behavior course

using the same methods and compared the results to those found in the developmental experiment.

Methods

I obtained fresh supplies of ¼", ½", and adult house crickets from a commercial vendor at the start of each week. I designated ¼" crickets as juveniles and ½" crickets as subadults. Upon arrival, I transferred crickets into ten-gallon aquaria and provided them with egg carton shelters and plenty of food and water to reduce competition. Each week, the order in which crickets were tested was randomized with respect to age and sex. The experiment lasted a total of three weeks and I tested a total of 480 crickets.

I measured boldness and exploration in the same arena (Fig. 4.1). I placed the cricket into a small covered plastic refuge and allowed it to habituate for 2 minutes. After the habituation period, I lifted the cover so that the exit was available. I recorded the latency for each cricket's entire body to emerge from the refuge. This time served as a measure of boldness. If the cricket did not emerge after 5 min, I recorded its latency as 300 seconds and gently coaxed the cricket out of the refuge. After each cricket left the refuge, I replaced the plastic cover so that the cricket could not return to the refuge.

Once the cricket left the refuge, I began a 5 min exploration trial. Over the course of the trial, I recorded the following: the total number of grids the cricket entered; the number of times it crossed either of the center lines; the number of objects it touched with any body part; and the time at which it first touched each object. Arenas were rinsed and dried in between each test.

After each cricket was tested, I placed it into a small glass vial and then placed it into the freezer for several hours before immersing the cricket in ethanol. Later, I measured the wet mass of each cricket and used electronic calipers to measure its head width. Measurements were taken under a microscope, and if a juvenile had a small ovipositor its sex was recorded as female. All other juvenile crickets were designated as unknown sex. Four trained undergraduate research assistants assisted with all the behavioral and morphological measurements for this experiment.

In a separate experiment, I tested the behavior of twenty subadult crickets two days apart to see if individuals were consistent in their behavior. These crickets also served as the control animals for an undergraduate's experiment, and were placed in a freezer for approximately five minutes after the first behavioral tests. All crickets resumed normal behavior after freezing.

Statistical Analyses

The boldness score for each individual was 300 s (the maximum time in the refuge) minus the time it took the cricket's full body to emerge from the refuge. All measures of exploration were significantly correlated with one another, so I used the number of grids a cricket entered across the 5 min exploration trial as my measure of exploration when testing for correlations. I chose to use this measure as opposed to performing a principal components analysis in order to compare my results to other studies using the same methods. Similarly, head width and mass were highly correlated ($p<0.0001$) so I focused on mass in my analyses. When testing whether sexes differed in personality traits or behavioral syndromes, I restricted my analyses to subadults and adults as I could not determine the sex of most

juveniles. I used t-tests and ANOVAs with post-hoc Tukey's HSD tests to test whether age groups and/or sexes differed in mass or behavioral traits. I used Spearman's correlations to determine whether boldness and exploration were correlated in the same individuals two days apart. I also performed Spearman's correlations to determine whether there were significant correlations between mass, boldness and exploration both across individuals and within sexes or age groups. Finally, I used a MANOVA to determine whether the relationship between boldness and exploration differed across age groups or sexes and linear discriminant analysis to group this relationship by age group. All analyses were performed in JMP v. 9.0 (SAS Institute 2010) except the MANOVA and linear discriminant analysis, which were performed in R v. 3.0.3 (R Core Team 2014). All measurements are reported as means ± standard errors.

Undergraduate Lab Experiment

In the fall semester of 2014, I performed a cricket personality lab in the Animal Behavior Lab course at UNC-Chapel Hill (see Appendix A). The lab had 8 sections taught by four different Teaching Assistants, including the author, who trained the other TAs in the methodology for the experiment. Students carried out the experiment in groups of 3-5 and were responsible for testing 5 crickets each. Using the same methods as above, they measured boldness and exploration for 148 *A. domesticus* crickets over the course of one week and I tested to see whether these behaviors were significantly correlated with one another. Each table had testing arenas that were the same except for the novel objects used and their locations on the grid. I obtained these crickets from the Pfennig lab's supply of *A. domesticus* and they varied in size from ¼" to ½". However, a preliminary experiment had

shown that it was difficult for students to reliably measure crickets so I did not measure mass or length in this experiment.

In the spring semester of 2015, I performed this lab again. This time, I ordered the crickets from a different company and received adult tropical house crickets, *Gryllodes sigillatus*. All other methods were the same, and over the course of one week the students tested 135 crickets. I tested to see if boldness and exploration were correlated with one another in this species. For the lab experiments, ~200 crickets were housed together in an aquarium and provided with plenty of shelters and *ad libitum* food and water. Therefore, densities throughout the week varied similarly to the main experiment described above. Each cricket was tested once and returned to a separate aquarium.

Results

Personality Traits

The three different age groups showed significant differences in mass ($df = 467,2$, $F = 416.124$, $p < 0.0001$), with Tukey's HSD post-hoc tests revealing all three groups to be significantly different from one another ($p < 0.0001$; Table 4.1). Boldness did not vary by age group ($df = 468,2$, $F = 0.548$, $p = 0.578$), but there were significant differences across age groups in all measures of exploration (Table 4.2). All measures of exploration were significantly correlated with one another ($p < 0.05$), so I used the number of grids a cricket entered over the course of the trial as my measure of exploration for the remainder of the analyses. Females were larger than males ($df = 310,1$, $t = -3.93$, $p = 0.0001$), but males and females did not differ in any behavioral measurements (Table 4.3).

The subadult crickets that I tested twice, two days apart, showed consistent boldness and exploration behaviors (Fig. 4.2). The average boldness score was 231.55 for the first test and 217.30 for the second test, and these scores were significantly correlated with one another ($\rho = 0.622$, $p = 0.003$). The average exploration score was 58.00 for the first test and 59.20 for the second test, and these scores were also significantly correlated ($\rho = 0.791$, $p < 0.0001$).

Behavioral Syndromes

When all individuals were included in the analysis, I found that exploration (as measured by the number of grids entered during the 5 min exploration trial) was significantly positively correlated with boldness ($\rho = 0.14$, $p = 0.002$). Further, exploration was also significantly positively correlated with mass ($\rho = 0.214$, $p < 0.0001$). There was no correlation between mass and boldness ($\rho = -0.052$, $p = 0.262$).

I found that the relationship between boldness and exploration differed significantly between age groups (Pillai's trace = 0.023, df = 934,4, F = 2.740, p = 0.028). Specifically, this relationship was different between adults and juveniles (Pillai's trace = 0.028, df = 308,1, F = 4.446, p = 0.012). There was no difference in the relationship between boldness and exploration for adults and subadults (Pillai's trace = 0.004, df = 312,1, F = 0.551, p = 0.577) and the difference for subadults and juveniles was marginally non-significant (Pillai's trace = 0.019, df = 311,1, F = 2.946, p = 0.054). When I restricted my analyses to adults and subadults, males and females did not show different relationships between boldness and exploration (Pillai's trace = 0.006, df = 307,1, F = 0.984, p = 0.375). A linear discriminant analysis examining this relationship by age group showed that the first linear discriminant

explained 94.1% of the variation in the data (eigenvalue = 2.282, boldness coefficient = -0.008, exploration coefficient = 0.07; Fig. 4.3).

Based on the results of the MANOVA, I looked at the correlations between boldness, exploration, and mass for juveniles, subadults, and adults separately. I found that while boldness and exploration were significantly correlated in adults and subadults, these behaviors were not correlated in juveniles. Further, juveniles showed a significant positive correlation between mass and exploration (Table 4.4).

Undergraduate Lab Experiment

In the fall 2014 experiment, 54 crickets were identified as females and 94 were designated of unknown sex. Females were significantly bolder (262.111 ± 9.741) than crickets of unknown sex (226.766 ± 11.005, df = 0146, t = -2.168, p = 0.032), but unknown crickets could be males or immature females. Females and unknown crickets showed no significant difference in exploration as measured by the number of grids they entered (Females 44.907 ± 1.988, Unknown 44.394 ± 1.720, df = 146, t = -0.189, p = 0.851). There was not a significant correlation between boldness and exploration in the *A. domesticus* crickets, but the correlation did trend toward significance ($\rho = 0.1364$, $p = 0.0982$).

In the spring 2015 experiment, the students tested 73 female and 62 male *G. sigillatus*. Females and males did not show significant differences in boldness (Females 193.534 ± 14.335, Males 198.548 ± 15.554, df = 133, t = 0.237, p = 0.813) or exploration (Females 41.164 ± 1.982, Males 39.339 ± 2.150, df = 133, t = -0.624, p=0.534). Overall, *G.*

sigillatus crickets showed a highly significant positive correlation between boldness and exploration ($\rho = 0.4271$, $p < 0.0001$).

Discussion

Developmental Experiment

I found that juvenile, subadult and adult European house crickets did not show differences in boldness, unlike in the cricket *G. integer*, where juveniles were bolder than adults (Niemela et al. 2012b). However, crickets of different age groups did show differences in exploratory behavior: specifically, juveniles were less exploratory than adults. I also did not find any differences in the behavior of male and female crickets when juveniles were excluded from the analysis. Further, individual subadult crickets showed consistent behavior when measured twice, two days apart, which suggests that boldness and exploration are personality traits in this species.

Overall, I found that there was a significant positive correlation between boldness and exploration across all the crickets tested. This suggests that there is a behavioral syndrome linking these traits in *A. domesticus*, as was found in a previous study using different behavioral measures (Wilson et al. 2009). However, the relationship between these two variables was different for the different age groups. Boldness and exploration were positively correlated in adults and subadults, but there was no significant association between these behaviors in juveniles. However, juvenile crickets did show a significant positive correlation between mass and exploration that was not present in the other age groups (but was present when all crickets were included in the analysis). These results suggest that a behavioral

syndrome linking boldness and exploration emerges later in development in *A. domesticus*, and this may be due to an increase in exploratory behavior in older, larger crickets.

Other studies have found that behavioral syndromes can emerge later in development, and have generally linked this emergence to selection by predation events occurring at the juvenile stage (Bell and Sih 2007; Adriaenssens and Johnsson 2013). However, it is unlikely that such an event occurred in the facility in which my crickets were raised. Another study on firebugs found that while correlations between behaviors remained stable across development, adults became less bold and exploratory than juveniles after their final ecdysis (Gyuris et al. 2012). Therefore, it is possible that physiological changes leading up to the crickets' final molt are correlated with the developmental changes in behavior found in this study.

Unfortunately, due to experimental constraints I was not able to measure the behavior of individual crickets at multiple points across development. Further, because I ordered my crickets from a commercial supplier, I cannot tell whether my results are due to changes within the organisms or responses to changes in diet, density, or other environmental factors that may have been altered before I received the crickets. Longitudinal studies of individual crickets reared in different environments will be needed to distinguish between these possibilities. Stamps and Groothuis (2010a) noted that even in the most controlled studies of development of behavioral syndromes, it is impossible to make sure the environments of all individual animals are identical prior to starting the experiment. Maternal effects such as differential allocation can affect the behavior of animals raised in the same environment, and may play an important role in the development of behavioral syndromes (Reddon 2012). However, carefully controlled manipulation of diet, density, and other factors in experiments

that follow individual crickets over ontogeny will allow me to determine what factors dictate the emergence of a behavioral syndrome in *A. domesticus*.

Undergraduate Lab Experiment

In two different semesters of Animal Behavior Lab at UNC-Chapel Hill, I examined behavioral syndromes in two different species: the European house cricket *A. domesticus* and the tropical house cricket *G. sigillatus*. The undergraduate students did not find a significant correlation between boldness and exploration in the 148 *A. domesticus* they tested in Fall 2014, although the p-value was less than 0.1. In that particular semester, I did not have access to adult *A. domesticus* and the students tested both juvenile and subadult crickets. As a result, I also could not reliably distinguish between males and females. As my results above show, juvenile house crickets do not appear to have a behavioral syndrome linking boldness and exploration. Therefore, it is likely that the correlation in this experiment was not significant because the students tested a mix of age groups but did not distinguish between them on their data sheets. In addition, in my developmental study the significant correlation between boldness and exploration was still relatively weak, with a Spearman's correlation of 0.14. Therefore, it is also possible that having many different students test a comparatively low number of crickets in the chaotic undergraduate lab environment made the behavioral syndrome more difficult to detect.

In contrast, in Spring 2015 there was a highly significant correlation between boldness and exploration in the 135 *G. sigillatus* crickets the undergraduate students tested. All of the crickets I tested that week were adults: females had long ovipositors and males called to attract females while held in the aquarium. Due to the spread of densovirus in

commercial supplies of *A. domesticus* in the United States and elsewhere (Szelei et al. 2011), the tropical house cricket has emerged as one of several alternative species in the market. The fact that I found such a strong correlation between boldness and exploration in *G. sigillatus* tested in a chaotic lab environment suggests that this species could be an excellent system for further studies of behavioral syndromes. Controlled studies conducted by a small number of observers will be needed to confirm my results from the undergraduate lab experiment, but it is promising that such a strong relationship emerged even in a relatively uncontrolled environment. Finally, my study shows that undergraduate animal behavior labs are an excellent way to gather a large amount of behavioral data in a short amount of time while still getting results comparable to those gathered by trained research students.

Table 4.1. Cricket mass by age group. All age groups had significantly different masses (Tukey's HSD, $p < 0.0001$).

Age (N)	**Mass (g)**
Adult (161)	0.321 ± 0.008
Subadult (157)	0.164 ± 0.005
Juvenile (152)	0.074 ± 0.005

Table 4.2. Behavioral traits by age group. Sample sizes are given in parentheses for each measurement. Crickets of different ages did not differ in boldness, but showed significant differences for all measurements of exploration. Tukey's HSD post-hoc tests revealed that: adults entered more grids than juveniles ($p = 0.007$); adults took less time to touch an object than both subadults ($p = 0.0003$) and juveniles ($p < 0.0001$); all three groups differed in the number of objects touched ($p < 0.05$); and juveniles crossed the center lines fewer times than both adults ($p < 0.0001$) and subadults ($p = 0.028$).

	Boldness	**# Grids Entered**	**Time to Touch First Object (s)**	**# Objects Touched**	**# Times Crossed Center Lines**
Adults	255.197±5.182 (157)	56.932±1.088 (161)	42.307±3.841 (150)	2.255±0.071 (161)	32.398±1.383 (161)
Subadults	252.761±4.943 (159)	55.241±1.122 (162)	73.278±6.266 (144)	1.975±0.082 (162)	28.525±1.363 (162)
Juveniles	259.800±4.277 (155)	52.000±1.206 (156)	80.703±7.13 (118)	1.577±0.091 (156)	23.814±1.098 (156)
df	468, 2	476,2	409,2	476,2	476,2
F	0.548	4.797	12.882	17.247	10.973
p	0.578	0.009	<0.0001	<0.0001	<0.0001

Table 4.3. Sex differences in mass and personality traits. Only adults and subadults were included in these analyses, as we could not distinguish the sexes in juveniles. Sample sizes are given in parentheses for each measurement. Females were larger than males, but there were no sex differences in boldness or exploration as measured by the number of grids entered.

	Mass (g)	**Boldness**	**Exploration (# Grids)**
Female	0.269±0.01 (166)	249.588±5.294 (160)	56.323±1.109 (167)
Male	0.219±0.008 (146)	257.020±4.972 (150)	55.92±1.14 (150)
df	310,1	308,1	315,1
t	-3.93	1.020	-0.253
p	0.0001	0.308	0.800

Table 4.4. Correlations between boldness, exploration, and mass by age group.

	Exploration-Boldness		**Exploration-Mass**	
	Spearman's ρ	p	Spearman's ρ	p
Adults	0.1575	**0.0496***	0.045	0.569
Subadults	0.2366	**0.0027***	0.1425	0.0751
Juveniles	0.0354	0.662	0.1750	**0.0311***

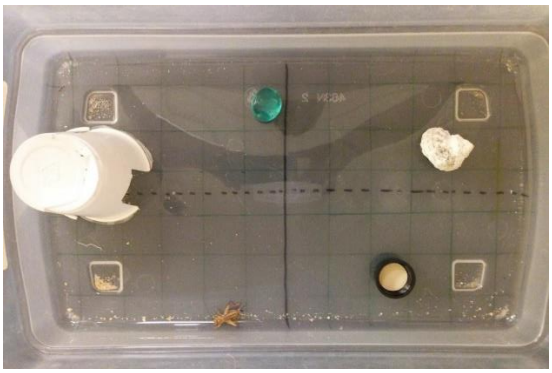


Figure 4.1. Arena used to measure boldness and exploration. Small plastic boxes were divided into a grid pattern with center lines in bold and contained a plastic refuge and three novel objects of similar size (shells, caps, glass beads, etc). Multiple arenas were used with the same general layout. See text of methods for more details.

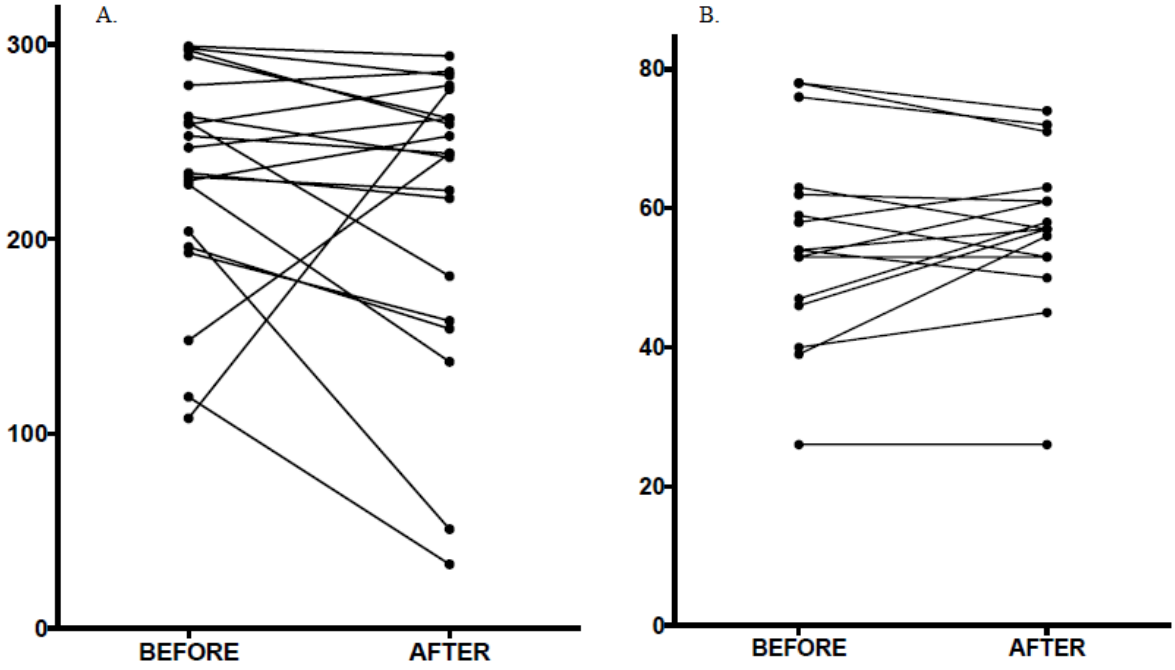


Figure 4.2. Individual consistency in boldness (A) and exploration (B) in subadult house crickets measured twice, two days apart. Both tests were significantly correlated with one another for both behavioral traits, indicating that boldness and exploration are personality traits in subadult house crickets.

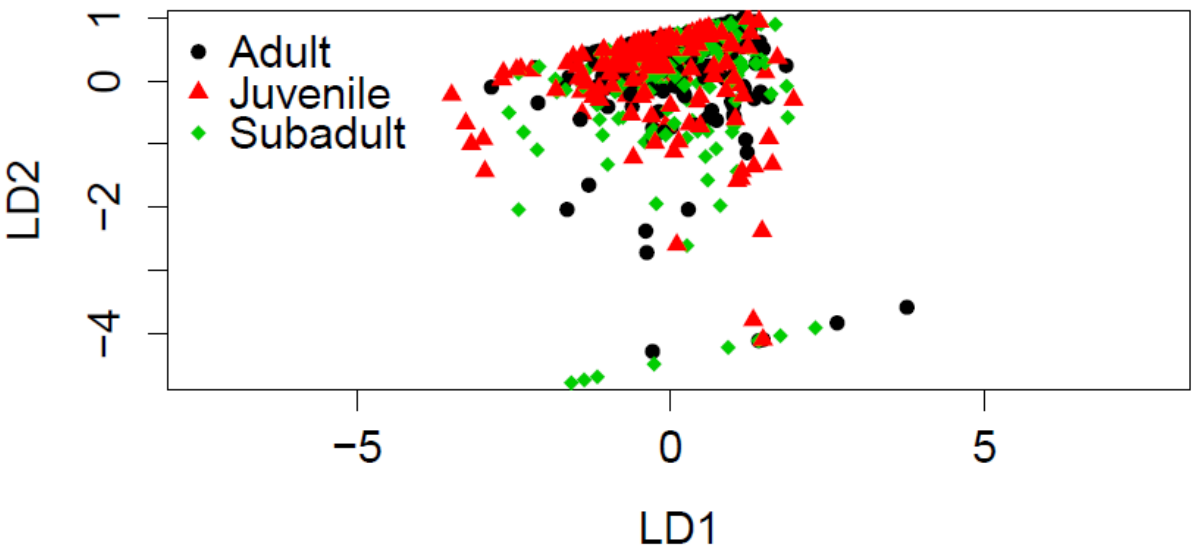


Figure 4.3. Linear discriminant analysis grouping the relationship between boldness and exploration by age. The first linear discriminant (LD1) explained 94.1% of the variation in the data, and the second linear discriminant explained 5.9% of the variation. A MANOVA confirmed that the relationship between boldness and exploration was significantly different for adult, subadult and juvenile crickets ($p = 0.028$).

CHAPTER 5: CONCLUSIONS

In my dissertation research, I evaluated the causes and consequences of individual differences in behavior in spadefoot toads and European house crickets. First, I measured the fitness consequences of hybridization in the spadefoot toad *S. bombifrons*. Previous studies have shown that individual female *S. bombifrons* hybridize with male *S. multiplicata* in certain environments. Specifically, hybridization occurs most often in shallow ponds that are likely to dry out quickly (Pfennig and Simovich 2002), and females in poor condition are most likely to hybridize (Pfennig 2007). In Chapter 2, I evaluated the fitness consequences of hybridization by performing a split-clutch experiment in which I bred female *S. bombifrons* with both *S. bombifrons* and *S. multiplicata* males, creating half-sibships of pure species and hybrid tadpoles that shared the same mother. After ten days, I preserved the tadpoles and later measured their development (as measured by Gosner stage), morphology (as measured by mouthparts score), and other measures of fitness (snout-vent-length, mass, condition, and survival). I found no differences between hybrid and *S. bombifrons* tadpoles for most of my measurements. However, in accordance with previous studies (Pfennig 2007), I found that hybrid tadpoles were more developed than *S. bombifrons* tadpoles. Although the benefit of hybridization did not depend on maternal condition as originally predicted, increased maternal mass was associated with more developed tadpoles of both types. Overall, my results in Chapter 2 confirm that when the identity of the mother is controlled for experimentally, hybrid tadpoles are more developed than their pure species siblings but do not suffer any disadvantage in fitness measures such as SVL, mass, and survival. This

suggests that although hybridization is still costly at the adult stage (Simovich et al. 1991; Wünsch and Pfennig 2013), hybridizing *S. bombifrons* females do not suffer immediate consequences in terms of the fitness of their offspring at the tadpole stage, and in fact can benefit from the increased ability of their offspring to develop quickly and escape rapidly drying ponds.

I next looked at the behavior of the hybrid offspring themselves. In Chapter 3, I measured the mate preferences of female hybrid *Spea* for three different pairs of male call stimuli – *S. bombifrons* vs hybrid, *S. multiplicata* vs hybrid, and *S. bombifrons* vs *S. multiplicata* – in conditions mimicking both deep and shallow ponds. Although there was variation in the preferences of individual females, in most cases hybrid females did not show significant preferences for any stimuli. However, in deep water, females significantly preferred the calls of hybrid males over the calls of *S. multiplicata*. When tested with the same stimuli in shallow water, however, the same females exhibited no preference. These results demonstrate that hybrid females display mate preferences in at least some situations, and importantly, that these preferences depend in part on the environment. In the spadefoot toad system, this suggests that in deep ponds hybrid females may express maladaptive preferences for hybrid males, as hybrid males are sterile (Wünsch and Pfennig 2013). However, in shallow ponds hybrid females would likely mate with pure species as well as hybrid males and produce backcrossed offspring. As a result, sexual selection against hybrid females can depend on the environment. In sum, my results demonstrate that it is important to consider mate preferences of hybrids – and hybrid behavior in general – when examining the stability of species boundaries.

Finally, I decided to examine behavioral variation in individuals in more detail and conducted a study of animal personality, or behavioral syndromes, in the European house cricket, *Acheta domesticus*. In Chapter 4, I tested if house crickets display a behavioral syndrome linking boldness and exploration, and if that behavioral syndrome varies across developmental stages. By measuring the behaviors of three different groups of crickets – juveniles, subadults, and adults – I found that overall, boldness and exploration were significantly positively correlated and therefore comprise a behavioral syndrome in this species. However, when I looked at the different age groups I found that there was no correlation between boldness and exploration in juvenile crickets. Instead, the behavioral syndrome linking these behaviors emerged later in development and was found only in subadults and adults. Developmental studies of personality, particularly in invertebrates, are rare (Stamps and Groothuis 2010a; Stamps and Groothuis 2010b; Mather and Logue 2013; Kralj-Fiser and Schuett 2014), and these results lay the groundwork for additional studies of the proximate mechanisms leading to the emergence of a behavioral syndrome in house crickets.

In addition, in Chapter 4 I present evidence that crickets are a tractable system for undergraduate laboratory exercises examining individual variation in behavior. Data collected by approximately 100 animal behavior students produced results that were comparable to my own study. Further, a second group of students found evidence that boldness and exploration are also significantly correlated in the tropical house cricket, *Gryllodes sigillatus*. I hope that these results will convince other educators to include studies of behavioral variation in their own courses. To facilitate this, I have developed lesson plans

for undergraduate (Appendix A) and high school level (Appendix B) lab exercises focusing on cricket personality.

In conclusion, in this dissertation I have evaluated individual variation in behavior from different perspectives in two different species. In my studies of spadefoot toads, I showed that a particularly interesting behavior expressed by some individual female *S. bombifrons* in certain environments – facultative hybridization – does not lead to reduced fitness in terms of their larval offspring, and in fact can provide a fitness benefit in that hybrid offspring develop more quickly that pure *S. bombifrons* tadpoles. I then looked at the behavior of hybrids themselves, and showed that female hybrid *Spea* prefer the calls of hybrid males only in certain contexts. This result demonstrates that it is important to examine the behavior of hybrids themselves when conducting studies of hybridization and how it varies across environments. Finally, I examined a behavioral syndrome in different developmental stages of the house cricket. I found that a behavioral syndrome linking boldness and exploration is not present in juvenile crickets, but emerges later in development. Overall, my dissertation provides insight into the causes and consequences of individual variation in behavior in two systems: spadefoot toads and house crickets.

APPENDIX A: PERSONALITY IN CRICKETS: AN UNDERGRADUATE ANIMAL BEHAVIOR LAB EXERCISE

I. Intro: Animal Personality

Anyone who has owned a dog or a cat will attest to the fact that animals, like people, have different personalities. Some dogs are more active and social than others, just as some people are more outgoing than others. However, it is only recently that researchers in animal behavior have begun to seriously study the topic of animal personality.

"Personalities" in animals are referred to by many different terms, but the most commonly used is *behavioral syndromes*. Basically, the idea is that individual behaviors do not vary independently from one another, but instead may be correlated with one another. Many behaviors may be part of a behavioral syndrome, and each individual behavior can be called a *personality trait*. Researchers have focused on five major personality traits in animals: (1) **boldness**, or behavior under risky situations; (2) **exploration**, activity in a novel environment; (3) **activity** in a familiar environment; (4) **aggression** toward conspecifics; and (5) **sociability**, or the tendency to associate with conspecifics (Reale et al. 2007). Any or all of these personality traits may be part of a behavioral syndrome in a particular species or population.

For any of these behaviors, individuals can still show variation depending on the context in which the behavior is expressed. For example, a cricket may be less bold if there is a predator present than if there is not. However, if boldness is a personality trait in crickets, then we would expect "bold" crickets to always be bolder than crickets that are relatively shy. In other words, personality looks at the relative differences, or ranks, between individuals.

So why are behavioral syndromes important? Scientists who study animal behavior are interested in this idea because correlated traits, including personality traits, can't easily evolve independently from one another. This means that organisms may not be able to express the optimal behavior in every situation. Therefore, the question is why behavioral syndromes evolved at all. There are two major hypotheses:

The **constraint hypothesis** suggests that behaviors are correlated because the different behaviors have the same underlying proximate mechanisms. For example, aggression is commonly associated with testosterone levels. If boldness is also affected by testosterone, increases in testosterone will affect both aggression and boldness. Similarly, behaviors may be controlled by the same genes.

The **adaptive hypothesis** suggests that natural selection may actually favor behavioral syndromes such that certain combinations of behaviors are more favorable in certain environments.

Although these hypotheses are not mutually exclusive, they do lead to different predictions. For example, let's say you are studying behavioral syndromes involving boldness and aggression in a species of fish. You have individuals from two different populations: one that has heavy predation and one that has no predators at all. If you are interested in comparing behavioral syndromes in these two populations, what types of predictions might you make for the two different hypotheses described above?

Researchers are currently investigating the evolution of behavioral syndromes. However, the field is new enough that studies are still being conducted to determine whether or not a particular species displays behavioral syndromes at all. In this lab, we will determine whether domestic crickets have behavioral syndromes.

II. Study System: The House Cricket, *Gryllodes sigillatus*

In this lab, we will determine whether the tropical house cricket, *Gryllodes sigillatus*, displays behavioral syndromes. Specifically, we will measure three different behaviors – boldness, exploration, and sociability – and determine whether or not they are correlated in our population in the lab.

A. Identifying your crickets

Males and females may show different behaviors. Therefore, it is important to be able to determine the sex of your crickets. Adult male and female crickets can be easily identified by sight. Females have a long ovipositor – this is not a stinger, but rather a structure the female uses to deposit her eggs. Males do not have an ovipositor (Figure A.1).

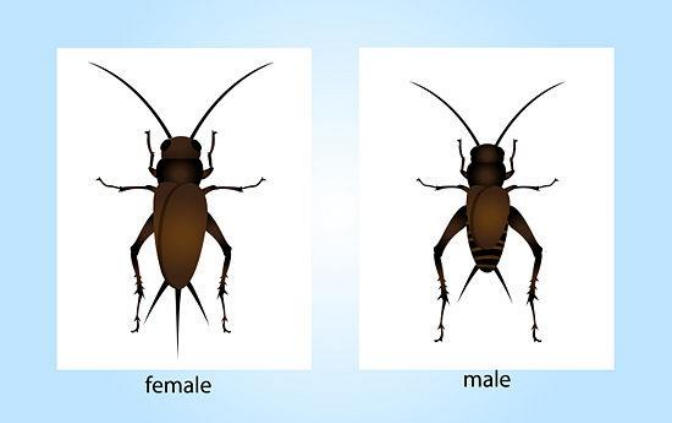


Figure A.1. Female versus male cricket anatomy. Females can be identified by the presence of an ovipositor.

Domestic crickets are more social than many wild species. However, they will still display aggressive behavior, particularly when they have been isolated from other crickets. Aggressive behavior in crickets follows a set pattern of behaviors that increase in intensity

over time, similar to paradise fish. Female crickets may engage in less stereotyped fights, but will still exhibit similar behaviors. If we have time, we will see if our male crickets engage in aggressive behaviors.

III. Procedure

Each lab group will be given 5 crickets to use as focal individuals for this lab. You should label each container with a number to keep track of individuals, and record the sex of each individual.

The easiest way to transfer your crickets from their home container to the experimental setups is to coax your cricket into the vial provided. Take great care not to squish your cricket or allow it to escape into the lab.

We will use standard methods to measure the boldness, exploration, and sociability of each individual cricket in that order. (As an exercise, think about ways we could measure aggression and activity if we had time.)

A. Boldness and Exploration

Boldness is defined as behavior under risky situations, while exploration is defined as activity in a novel environment (Reale et al. 2007). In this experiment, we will use a novel experimental arena to measure these two behaviors (Figure A.2).

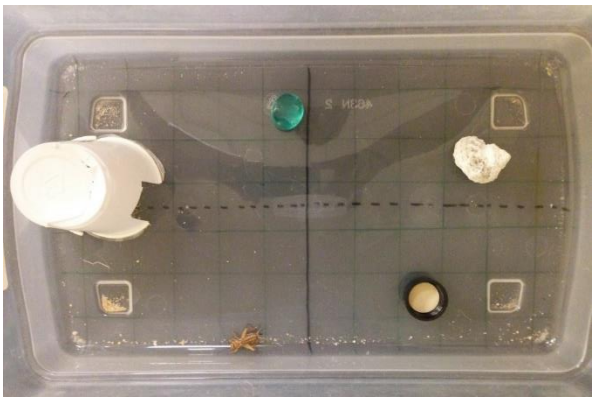


Figure A.2. Experimental arena for measuring boldness and exploration.

1. At the start of each trial, place your cricket into the plastic refuge and cover with the plastic cup. Let your cricket habituate for two minutes.

2. After two minutes, lift the cup so that the door in the refuge is exposed. Time how long (in seconds) it takes your cricket to emerge from the refuge. You should record both the time at which its antennae first emerge and the time at which the entire body emerges. These latencies represent your boldness measures – bolder crickets will have shorter latencies to emerge. Once your cricket has fully emerged, gently cover the opening of the refuge again so the cricket doesn't go back inside to hide. If your cricket does not emerge in 5 mins, record the latency as 300 seconds and coax the cricket out of the refuge using a pen or other object. For data analysis, boldness = (300-time to emerge), such that higher values represent bolder crickets.

3. Once the cricket has emerged, we will conduct a 5 min exploration trial. The experimental arena has a grid and 3 novel objects. Over the course of 5 mins record:

-how many grids the cricket enters

-how many objects the cricket touches

-how long it takes the cricket to touch each object the FIRST time only

To simplify data analysis for your lab report, exploration = the number of grids your cricket enters. Higher values represent more exploratory crickets. If you want to create a formula for exploration that incorporates more variables, get approval from your TA and make sure you clearly define how you calculated exploration in your methods section.

B. Sociability

Sociability is defined as interactions with conspecifics. We will use a second experimental arena to measure sociability (Fig. A.3).

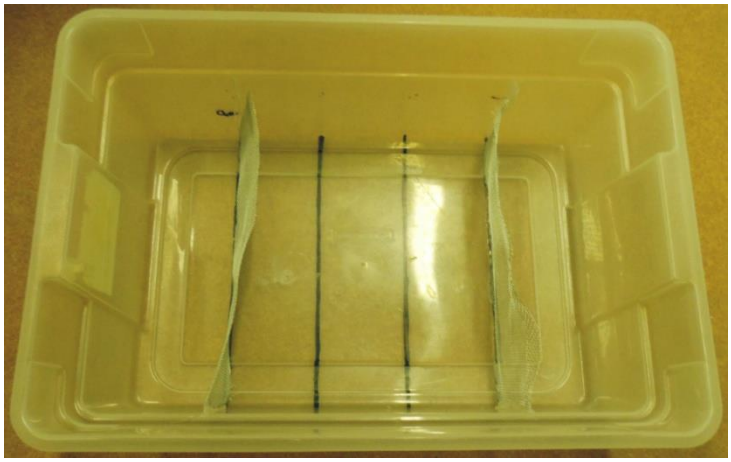


Figure A.3. Sociability test arena.

1. Obtain three extra crickets from the bucket and place them into one of the small sections of the arena. These will be your stimulus individuals.

2. Place your focal individual into the large center compartment. Every 5 sec for 5 min, record which of the three smaller sections the focal cricket is in: the association zone closest to the stimulus individuals, the neutral zone in the center, or the avoidance zone close to the empty compartment.

3. To calculate your sociability score, calculate the percentage of scans the cricket is in the association zone, NOT INCLUDING times in the neutral zone. For data analysis, sociability = #As/(#As+#Av). Higher values represent more sociable crickets.

IV. Analysis for the lab report

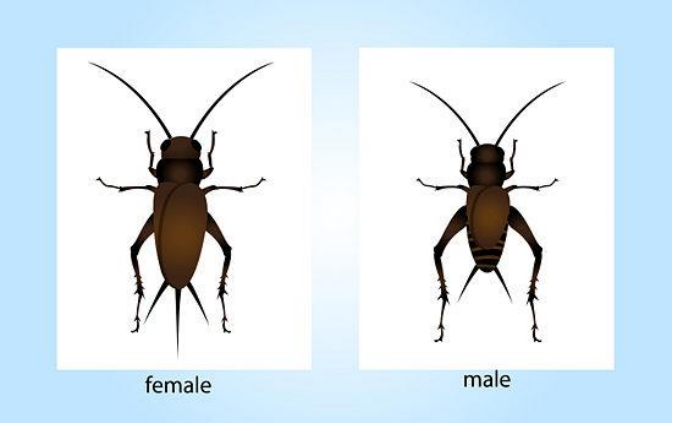
At the end of week, all data from the 8 labs will be combined into one data file and posted to Sakai. For your lab report, you will see if any of the pairs of traits are correlated with one another (boldness and exploration, boldness and sociability, and exploration and sociability) to determine if there are behavioral syndromes in house crickets. The following website explains how to do correlations in excel (and how to download the Data Analysis Toolpak if you haven't already): http://www.excel-easy.com/examples/correlation.html

Excel will not provide a p-value for your correlations, but you can obtain one by plugging in your r (correlation statistic) and sample size (N=number of crickets in the analysis) into this calculator: http://www.danielsoper.com/statcalc3/calc.aspx?id=44 Make sure you use the two-tailed p-value, NOT the one-tailed. Remember that if you have a large sample size, correlations that may appear weak can actually be statistically significant.

For your lab report, you are only required to calculate and show the correlations for the three pairs of behaviors. However, there are many other things you could do with the data given the statistical tests we have already covered in lab. Feel free to include additional tests, or suggest them in the discussion section of your lab report.

APPENDIX B: DO CRICKETS HAVE PERSONALITY? EXPLORING INDIVIDUAL VARIATION IN CRICKET BEHAVIOR: A LESSON PLAN FOR HIGH SCHOOL BIOLOGY

Introduction: Students will use live crickets to explore individual variation in behavior and investigate whether crickets have personality. Students will examine boldness and exploration using live crickets and simple arenas that can be constructed quickly with inexpensive materials. Students will then plot their data to visualize individual variation in the behaviors they study. Advanced students can also plot their data in a scatterplot to show how these behaviors vary with one another and formulate hypotheses about how factors such as sex and age might affect their results.



Adult female crickets have an **ovipositor** to deposit eggs. Small juvenile crickets can't be distinguished.

Standards Addressed: NC Essential Science Standards Bio 2.1.2, Common Core Math Standards S-ID.1, S-ID.6, Next Generation Science Standards HS-LS2-8

Note: Used to demonstrate variation as required for evolution by natural selection, but doesn't perfectly match those standards

Learning Objectives:

-Recognize that there is variation in behavior – aka "animal personality" - and discuss why this variation is important.

-Measure boldness and exploration behaviors in crickets.

-Graph data collected by the class and interpret what these data suggest about personality in crickets.

Appropriate Grade Level: High school, but can be modified for middle school. I have also written (and implemented) a more technical lab for a college animal behavior course that might be helpful for some advanced IB/AP classes. Please contact me if you are interested in either of these!

Group Size: Groups of 3-4 students each, maximum ~20-30 students

Setting: Indoor

Approximate Time of Lesson: ~2 hours; may split activity and results into separate class periods

Resources Needed for Students:

-Data sheets, either premade by instructor or designed themselves

-One experimental arena (see activity) per group and animals to test (see resources needed for educators)

-1 stopwatch or clock with second hand per group

-Covered tube to transport crickets. I use plastic centrifuge tubes covered in colored tape, but you could easily make these out of toilet paper rolls or other materials. The idea is

to use something that is dark and "safe" that the cricket will want to crawl into, providing for safer transport than picking up the crickets by hand.

Resources Needed for Educators:

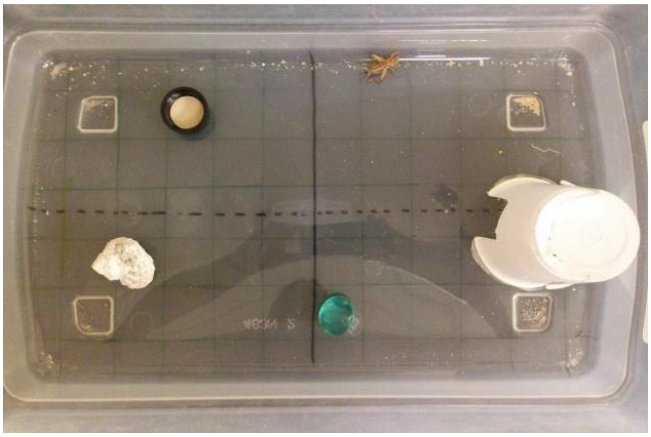
-Crickets. These can be bought cheaply at a pet store, and kept in a glass or plastic bin. Pet stores sell "cricket keepers" which include plastic tubes for transport, but these are not necessary. Most suppliers will include egg carton pieces which should be kept in the container for shelters. You don't need to buy the cricket food at the store, as these are designed to give crickets extra nutrients before they are fed to pet reptiles. You can just provide pieces of apple or potatoes, which have the extra benefit of providing water at the same time.

-Square plastic boxes for arenas

-Plastic cups, Sharpie, random small objects, hot glue for creating the arena (avoid smelly/toxic glues). Sample objects include aquarium beads, shells, pen caps, etc – anything that isn't food and fits in a single square on the grid. The arena should be fairly deep such that crickets can't jump out.

-A projector and computer program for graphing such as excel would be helpful, but if not available you can use graph paper or a board to visualize the data.

Sample experimental arena:



The arena should be divided into a grid pattern and have 3-4 objects randomly placed in the grid. On one end of the arena glue a refuge with a door and place a removable cup over it.

Lesson Activity:

Engagement

-Begin by asking students to describe their pet's behavior. Usually they will use terms such as playful, shy, fearful, hyper, etc – guide them toward the idea that just as humans have different personalities, anyone who has spent time with an animal is intuitively aware that individual dogs/cats/etc are different from one another.

-Although this is something most people understand, personality in animals has only been studied formally for the last 10-15 years. How could you define a personality? In animals, we define it as **consistent individual differences in behavior**. We also study 5 main behaviors in personality research: boldness, exploration, sociability, aggression, and activity (see background reading page for definitions).

-Most animals will change their behavior based on the situation – for example, they will be less active if they smell a predator. But if activity is a personality trait in that species, some

individuals will always be more active than others, even if there is a predator present! (Aka, a cost of an active personality is high mortality if predators are present.) Why then are there still active individuals? (Benefits like being able to get more food when there isn't a predator, whereas in that situation less active animals won't be able to compete as well.)

-There are many examples like this – basically you want to convey the idea that different personality traits can coexist because of different costs and benefits in different environments. Variation in the environment can lead to different behaviors being favored by natural selection.

-In this lesson, we will actually measure behaviors in different individual crickets to see how much variation there is. We will focus specifically on boldness and exploration.

Exploration

-Students will measure two behaviors: **boldness** and **exploration**. Boldness is behavior under risky situations. **Exploration** is activity in a novel environment. Ask students how they would define these behaviors in humans before providing these definitions. Show students the arenas and ask how we can measure these behaviors given this setup.

-Each group will get 1 arena and a small number of crickets (3-5, depending on time). Students will carefully move crickets from their container into the refuge: aka, a small plastic cup with a doorway that is covered with another cup such that the door is blocked.

-Have the cricket stay in the refuge for 1 minute. This is called the **habituation** phase, and allows the cricket to feel safe in the enclosed space. (I usually have a 2 min period to give the crickets enough time, but this could make students bored so 1 should be fine.)

-After 1 minute, lift the top cup so the door is open. **Record how many seconds it takes the cricket to fully emerge** from the refuge into the arena, with a maximum time of 2 minutes (can shorten for time/attention spans – most crickets come out within 2 minutes). Bolder crickets will come out more quickly than shy crickets. (For graphing the data, have students subtract how many seconds it took from the max of 120s – this way higher numbers represent bolder animals.) If the cricket does not come out within the 2 mins, record the time as 120s and coax the cricket out with a pen.

Note: If you use adult crickets, you may be able to see some of them feeling around with their antenna before fully coming out of the refuge.

-Once the cricket comes out or is coaxed out, begin a 3-5 minute exploration trial (depending again on time and how patient your students are).

-**Record how many squares the cricket enters** throughout the trial. The easiest way to do this is have a paper grid with as many squares as the arena and the positions of the objects and refuge marked. Students can then trace the path of the cricket with a pen and count how many squares are marked after the trial is over. By having 2 students do this at the same time and compare their results, you can demonstrate how important it is for observers to be accurate and consistent.

Note: It is common for crickets to circle around the edge of the arena. The most exploratory crickets are the ones who venture into the center of the arena.

-Also **record how many objects the cricket touches**. You can also have students record how long it takes them to touch each object.

-After the exploration trial, return crickets to their home container. Make sure that groups do not accidentally measure the same cricket twice.

Explanation

-Ask students to summarize their experiences at the end of the activity. What did they observe? Some groups had crickets that came out right away, others took longer; some crickets went all over the arena and climbed on the objects, while others went around the edge a few times and didn't touch any objects.

-Ask students what kind of graph we could create to show the variation in our crickets' behavior.

Elaboration

-Help the students to create a histogram graphing variation in boldness, the number of grids the crickets entered, and the number of objects they touched (see below for an example). Ask the students to interpret the graph. For boldness, there will likely be many crickets that come out right away, several that don't come out for the full 2 minutes, and some with intermediate values.

-You can also create a scatterplot to see how behaviors relate to each other. Make a plot with boldness on the x axis and # squares on the y axis, have the students plot the values for the crickets they tested, and have them describe the relationship between the two. Make another plot with # objects on the x axis and squares on the y axis. With a small number of crickets tested, the relationship between boldness and exploration will likely be pretty scattered; however, the relationship between the number of objects touched and the number of squares

entered should be a strong positive correlation. You can use this to show that the squares and objects are both testing the same personality trait.

Note: For really strong evidence of boldness and exploration being personality traits, you would need to measure the same individuals multiple times (see background).

Evaluation

-To connect this lesson with knowledge about the importance of variation in natural selection, pose the following scenario. Imagine that all the crickets we tested lived in an area together, and a predator such as a bird or a frog were introduced. Looking at your data, which crickets would likely be hunted? How would this change the graph? (The predators would likely target the boldest/most exploratory individuals, making the population less bold/exploratory. If all the individuals had the same behaviors, we would not see this response to selection by a predator.)

-As an extension, you could also ask the class to come up with ideas about what makes individual crickets have different behaviors. Some simple possibilities include: different genes; the environment (maybe some crickets grew up in an area with a lot of predators and learned to be shy); sex (males may explore more because they need to find mates); how hungry they are; size (maybe large crickets have fewer predators and are bolder); etc. See extensions below for more ideas.

-See assessment/evaluation for ideas about projects.

Possible Extensions of Activity

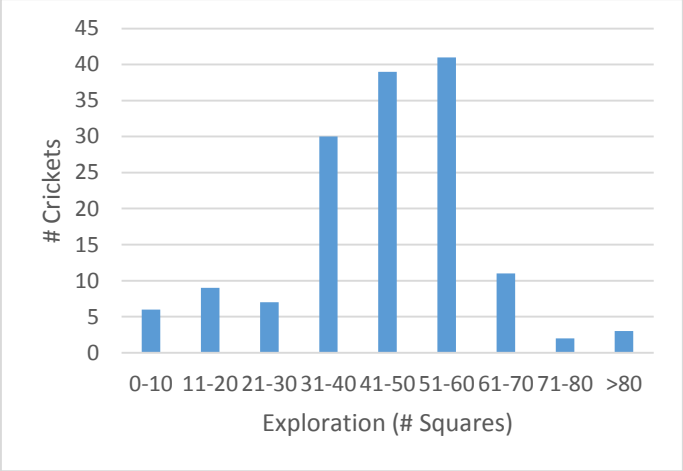
-Other behaviors commonly examined in personality research are sociability, aggression, and activity. I have included some information on these at the end of the lesson plan if you would like to incorporate them.

-To extend this lesson into a broader lesson on behavior, you may want to start by having your students create an **ethogram** of cricket behaviors. Break students into groups and have them observe a cricket in its home cage with 1-2 other crickets. Have them name and define all distinct behaviors they see and perhaps compare their lists of behaviors to published cricket ethograms. This could then easily lead into having the class compare the behaviors they saw and a discussion of individual differences. There are labs for making cricket ethograms that are freely available online. I'd also be happy to discuss ideas if you would like to make this a major part of the lesson plan.

-After graphing the data, you may have groups discuss what factors may have led to the differences they observed and formulate hypotheses about these effects. For example, crickets of different sexes or sizes may have different behaviors. Crickets may also modify their behavior in the presence of a simulated predator. If you have time, you may have students design and even carry out experiments to evaluate their hypotheses.

-Advanced classes: rather than just graphing levels of boldness etc for each individual, have students make a plot such that boldness is on one axis and exploration is on the other and each point is an individual cricket. Is there a clear relationship between how these behaviors are related to one another? Ex: in data collected by my undergraduate students, boldness and exploration are positively correlated with one another such that bolder crickets also explore more.

Final Product: Students can produce bar graphs of the variation in their data and use this to calculate simple statistics such as average exploration and standard deviations. Advanced students may use a scatterplot to see how boldness and exploration correlate with one another, or how different measures of exploration (ex. # of squares vs # of objects touched) are correlated. I have provided an example below from data collected by my Spring 2014 undergraduate class that shows variation in exploration behavior as measured by how many squares the cricket entered in a 5 minute period.



Assessment/Evaluation: A lab report (or sections such as an abstract or results) would help to ensure students can interpret the pattern they graphed and understand the importance of the study. This would also allow teachers to incorporate literacy standards into this lesson plan. Exact content/assignment will vary based on the level of the students.

Full Standards Addressed:

NC Essential Science Standards Bio 2.1.2: Analyze the survival and reproductive success of organisms in terms of behavioral, structural, and reproductive adaptations.

Common Core Math Standards S-ID.1: Represent data with plots on the real number line (dot plots, histograms, and box plots); S-ID.6: Represent data on two quantitative variables on a scatter plot, and describe how the variables are related.

Next Generation Science Standards HS-LS2-8: Evaluate the evidence for the role of group behavior on individual and species' chances to survive and reproduce.

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