NATURAL HYBRIDIZATION AND SPECIATION IN TOADS OF THE *ANAXYRUS AMERICANUS* GROUP

by

BRIAN E. FONTENOT

Presented to the Faculty of the Graduate School of The University of Texas at Arlington

in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS AT ARLINGTON

August 2009

ACKNOWLEDGMENTS

Several individuals, organizations, and institutions assisted me during my time at the University of Texas at Arlington (UTA) and this work would not have been possible without them. Financial support for this project was provided by my Ph.D. advisor, Paul Chippindale, my former Ph.D. advisor, Jeremy Marshall, and a Phi Sigma Biological Society Research Grant I received in 2006. Additionally, the Department of Biology at UTA supported me with a Graduate Teaching Assistantship for my entire tenure, allowing me to obtain valuable teaching experience, which I have greatly enjoyed.

I would like to thank the following individuals and academic institutions for donating tissue samples and allowing me to examine preserved specimens: Jonathan Campbell, Paul Ustach, and Carl Franklin at the University of Texas at Arlington Amphibian and Reptile Diversity Research Center, David Cannatella and Travis LaDuc at the Texas Natural History Collection, Gregory Watkins-Colwell at the Peabody Museum of Natural History, Kenneth Kozak, Benjamin Lowe, and Tony Gamble at the Bell Museum of Natural History, David Green and Michele Steigerwald at the Canadian Museum of Natural History, and Leslie Rissler at the University of Alabama Herpetological Collection.

I would also like to thank my committee members, Paul Chippindale, Jonathan Campbell, Dan Formanowicz, Pawel Michalak, Jeff Demuth, and Esther Betran for all of their guidance and help during my time at UTA. I have been a very independent student,

probably to a fault, but each of my committee members has helped me when I needed them and I am thankful.

I am extremely grateful to my fellow graduate students and friends: Jesse Meik, John Malone, Matt Watson, Michelle Lawing, Walter Schargel, Nate Bendik, Kerri Mitchell, Amie Treuer, Chad Larson, Matt Gifford, Roberto Brenes, Robert Makowsky, Corey Roelke, Christian Cox, John Morse, Charles Tracy, Chris Hamilton, Jeff Streicher, Thomas Eimermacher, Claudia Marquez, Sarah Schaak, Claudio Casola, Ben Anders, Rebecca Calisi, and Amy Carrillo. These individuals have all contributed to this work by providing tissue samples, helping out with laboratory work, consulting on statistical issues, and discussing my sometimes confusing results, but their most important contribution is their friendship. I would also like to thank Jennifer Pramuk, Gregory Pauly, Laura Vogel, David Green, and Norman Johnson for their invaluable advice and assistance with various aspects of this work.

The UTA staff, Linda Taylor, Gloria Burlingham, Peggy Fisher, Belinda Zollotuchen, and Morris Anthony, have helped me so many times in so many ways that it is impossible to measure. Without their help, I would not have been able to finish my dissertation and I am eternally grateful.

One individual who deserves special recognition is Carol Moulton. Carol helped me out in so many ways when I needed it most. I will always be grateful for our time together and look forward to a long friendship. Finally, I would like to thank my parents, John and Linda Fontenot, whose love and support allowed me to pursue my dreams.

July 17, 2009

ABSTRACT

NATURAL HYBRIDIZATION AND SPECIATION IN TOADS OF THE *ANAXYRUS AMERICANUS* GROUP

Brian E. Fontenot, Ph.D.

The University of Texas at Arlington, 2009

Supervising Professor: Paul T. Chippindale

The goal of speciation research is to elucidate the processes that lead to the formation of new species. Examining natural hybridization and the evolution of reproductive isolating barriers provides an opportunity to understand the dynamic processes that occur before, during, and after speciation events. Here, I use true toads of the *Anaxyrus americanus* group to examine the effects of natural hybridization on 1) the evolution of reproductive isolation using previously published laboratory hybridization data on multiple species within the family Bufonidae, 2) morphological variation using morphometric analysis of known *Anaxyrus americanus* group hybrids, and 3) molecular variation and genetic population structure in the *Anaxyrus americanus* group using AFLP markers.

First, postzygotic isolation shows a positive correlation with genetic divergence in bufonids, suggesting that diverging species will become increasingly isolated over time, as seen in other organisms. Toads also display some unusual patterns of reproductive isolation in that fertilization rate is not correlated with genetic divergence. Haldane's rule for inviability, strictly obeyed in most taxa, is followed in only 56% of crosses with females the affected sex in 70% of these cases. These results suggest that the homomorphic sex chromosomes seen in true toads and/or maternal effects may play a role in the unusual results regarding Haldane's rule for inviability. Examining offspring for adherence to Haldane's rule for sterility revealed that only males were affected and degree of ploidy in hybrid offspring does not appear to predict sterility.

Second, laboratory-produced hybrid offspring between members of the *Anaxyrus americanus* group can readily be distinguished from putative parental individuals raised under the same laboratory conditions as well as putative parentals collected from current natural populations. Using discriminant function analysis of morphometric characters, hybrid individuals were correctly classified in 84.3% of cases and putative parental individuals were correctly classified in 99.4% of cases. Hybrids deviated from parental species in aspects of cranium and forelimb morphology. Interestingly, hybrids did not possess morphological traits intermediate between that of their respective parental species; instead they display substantial morphological changes distinguishing them from either parental species. Transgressive segregation in morphological traits due to dominance effects at loci involved in complementary gene action could potentially

explain these results, although further research is needed before any firm conclusions can be made.

Finally, genetic analysis of 100 Amplified Fragment Length Polymorphism (AFLP) loci revealed discrete population structure within and among species, but species in close proximity sharing similar male advertisement calls displayed high levels of gene flow between populations. These results suggest that species in the *Anaxyrus americanus* group may exist as both distinct populations and as subdivided metapopulations connected by recent or ongoing gene flow, possibly due to natural hybridization. Isolation by distance and prezygotic isolating mechanisms appear to be the primary determinants of population structure within these species.

TABLE OF CONTENTS

CHAPTER PAGE

APPENDIX

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Evolutionary biologists long have been interested in speciation, the process by which multiple species are formed from one species (Johnson, 2006). However, because speciation events occur at evolutionary time scales, it is necessary to make predictions about historical evolutionary processes from observation of current patterns of genetic and morphological diversity (Coyne and Orr, 20004; Johnson, 2006). The goal of speciation research is to elucidate the mechanisms that lead to reproductive isolation between emerging species, and thus uncover the process of species formation. Species delimitation is important because species are the fundamental unit of all biological research. Understanding the patterns and processes governing speciation leads to a better understanding of how to identify and document biodiversity. Failure to accurately delimit species can result in spurious results for any type of biological research, and has had major impacts in areas such as conservation and management, where proper identification of species is crucial.

Some of the traditional methods used to examine the speciation process involve studying the evolution of reproductive isolating barriers. Reproductive isolating barriers are mechanisms that prevent gene exchange between species and can be placed in two main categories: prezygotic barriers that act before fertilization of the zygote and postzygotic barriers that act after fertilization of the zygote (Johnson, 2006).

Reproductive isolating barriers evolve over time and generally become stronger as species diverge (Arnold, 1997; Coyne and Orr, 1989, 1997, 2004). Because the evolution of reproductive isolation occurs over a period of time, it is possible to observe species at different stages of a timeline between one panmictic species to two or more reproductively isolated species (Johnson, 2006). Observing patterns of hybridization between related species at multiple levels of divergence allows researchers to identify both the strength of reproductive isolating barriers and the rate at which they evolve (Coyne and Orr, 1989, 1997).

Hybridization is defined as interbreeding between species (Arnold, 1997). Numerous studies have shown that laboratory hybridization is a useful technique for studying the evolution of both prezygotic and postzygotic reproductive isolation between species. A wide range of taxa have been examined using laboratory hybridization including angiosperms (Moyle et al., 2004), flies (Coyne and Orr, 1989, 1997), lepidopterans (Presgraves, 2002), frogs (Sasa et al., 1998), birds (Lijtmaer et al., 2003), fish (Bolnick and Near, 2005), and mammals (Fitzpatrick and Turelli, 2006). These studies have provided important information about the process of speciation such as: 1) the general time course of speciation, 2) the relationship between genetic divergence and reproductive isolation, 3) timing for the evolution of pre- and postzygotic isolation, and 4) the role of hybrid sterility and inviability in the formation of species.

Despite the wealth of knowledge gained from these studies, there is still much to learn about the process of speciation by examining other taxa with different characteristics, such as those with strong sexual selection or unusual chromosome morphology. Coyne and Orr (2004) as well as Johnson (2006) mention that hybridization studies in more taxa will be necessary before our knowledge of the speciation process is considered adequate. For example, faster male evolution in the frog genus *Xenopus* leads to contradictions to Haldane's Rule, one of the few general rules of speciation (Malone and Michalak, 2008). Haldane's Rule states that if one sex of hybrid offspring is negatively affected in terms of viability or sterility, it will be the heterogametic sex (Haldane, 1922). Additionally, little is known about the effect of homomorphic sex chromosomes, such as those seen in some crustaceans and most amphibians, on the evolution of reproductive isolation.

Answering these questions requires an organism that is both amenable to study and possesses life history characteristics distinguishing it from previously studied taxa. True toads of the genus *Anaxyrus* (referring to the former genus *Bufo* as recently revised by Frost et al., 2006) are an example of one such group. Toads possess homomorphic sex chromosomes, are presumed to be female heterogametic (although this has been experimentally confirmed for only one species; Ponse, 1941), and use male advertisement calls for reproduction, thus subjecting males to strong selective pressures as female choice has been shown to drive changes in male advertisement call and even cause speciation events via assortative mating (Cocroft and Ryan, 1995; Ryan et al., 1990; Boul et al., 2007). Additionally, they are easy to capture and rear in lab, and will readily hybridize both in the laboratory and in natural environments (Blair, 1972).

Several studies have provided evidence detailing the phylogenetic relationships within the family Bufonidae, a nearly cosmopolitan group with a likely origin in the

Upper Cretaceous (Blair, 1972; Frost et al., 2006; Graybeal, 1997; Masta et al., 2002; Pauly et al., 2004; Pramuk et al., 2008). While previous studies have resolved many basal relationships, the interspecific relationships among some species groups remain controversial. For example, species limits within the *Anaxyrus americanus* species group, widely distributed throughout North America (Figure A.1), have not been properly identified. The *A. americanus* group, thought to have arisen during the Pliocene epoch, includes *A. americanus, A. charlesmithi, A. houstonensis, A. terrestris, A. hemiophrys, A. microscaphus, A. woodhousii, A. fowleri*, and *A. velatus* (Blair, 1972; Dixon, 2000; Pramuk et al., 2008; Pauly et al., 2004). These species were traditionally identified on the basis of morphological traits and male advertisement call. Previous studies of mitochondrial DNA (mtDNA), morphological variation, karyotypes, and ecological evidence have not provided a strongly supported hypothesis of interspecific relationships in the *A. americanus* group (Blair, 1972; Bragg and Sanders, 1951; Masta et al., 2002; Pauly, et al., 2004). Figure A.2 shows a consensus hypothesis of relationships compiled from the results of two recent molecular studies using mtDNA (Masta et al., 2002 and Pauly et al., 2004). It is important to note that the phylogenetic relationships of *A. velatus, A. charlesmithi,* and *A. houstonensis* remain unresolved, and that molecular evidence points to the existence of three distinct lineages of *A. fowleri* (Masta et al., 2002), rendering it paraphyletic with respect to *A. terrestris*. The recent radiation of this group, combined with high levels of morphological and behavioral similarity, has resulted in a poorly defined taxonomy that may not accurately reflect evolutionary history (Frost et al., 2006; Masta et al., 2002; Pauly et al., 2004; Pramuk et al., 2008).

The occurrence of natural hybridization has further confounded our understanding of phylogenetic relationships in the *Anaxyrus americanus* group (A.P. Blair, 1941; W.F. Blair, 1956, 1959, 1961, 1963a, 1963b, 1964, 1966, 1972; Green and Parent, 2003; Jones, 1973; Masta et al., 2002; Vogel and Johnson, 2008; Volpe, 1952, 1959). The eight species of the *Anaxyrus americanus* group are characterized by very little genetic divergence and no strong postzygotic reproductive isolation, with hybrid offspring of both sexes viable and fertile through several generations, suggesting that admixture between species could potentially be frequent (A.P. Blair, 1941; W.F. Blair, 1956, 1959, 1961, 1963a, 1963b, 1964, 1966, 1972; Green and Parent, 2003; Jones, 1973; Masta et al., 2002; Vogel and Johnson, 2008; Volpe, 1952, 1959). Natural hybridization often results in a situation where traditional systematic methodology is unable to accurately resolve relationships (Arnold and Burke, 2006; Arnold, 1997). However, detailed analysis of natural hybridization and hybrid zones allows researchers to address many evolutionary questions, including the interspecific relationships of the species involved, the underlying processes leading to the generation of reproductive isolation, and the creation of novel evolutionary lineages (Arnold and Burke, 2006; Arnold, 1997; Coyne and Orr, 2004).

The *Anaxyrus americanus* group is useful for studying natural hybridization and speciation owing to its broad geographic range encompassing multiple habitat types, dense population sizes, homomorphic sex chromosomes, possible female heterogamety, conspicuous mating behavior, strong sexual selection, and a tendency to hybridize in both natural and laboratory environments (Blair, 1972; Green and Parent, 2003; Jones, 1973;

Volpe, 1952, 1959). Additionally, four members of the *A. americanus* group (*A. charlesmithi, A. fowleri, A. velatus,* and *A. woodhousii*) occur sympatrically and parapatrically in the deciduous, hardwood forests of eastern Texas, southeastern Oklahoma, western Louisiana, and southwestern Arkansas (Figure A.1; Conant and Collins, 1998; Dixon, 2000). Several researchers have hypothesized that this region contains populations of hybrid individuals, although this hypothesis has never been explicitly tested in a rigorous, quantitative fashion (A.P. Blair 1941, 1942; W.F. Blair, 1956, 1959, 1961, 1963a, 1963b, 1964, 1966,1972; Bragg and Sanders, 1951; Conant and Collins, 1998; Masta et al., 2002). Researchers have suggested that natural hybridization frequently occurs among members of the *A. americanus* group based on two types of evidence: 1) qualitative observations of putatively intermediate morphology in some populations (A.P. Blair, 1941, 1942; W.F. Blair, 1956, 1959, 1961, 1963a, 1963b, 1964, 1966, 1972; Green and Parent, 2003; Jones, 1973; Volpe, 1952, 1959), and 2) evidence of mtDNA introgression among lineages (Masta et al., 2002).

Although these lines of evidence suggest that natural hybridization may occur, they can be misleading. Overlap in morphological variation among and between members of this species group is considerable, making qualitative identification of intermediate (i.e. "hybrid") morphology difficult. Quantitative analysis of morphometric characters using multivariate statistics would allow for the identification of cryptic morphological variation that could be used to delimit species and potentially identify intermediate morphologies with statistical support. Also, maternally inherited mtDNA markers provide only indirect evidence of gene introgression and can fail to identify certain instances of hybridization. For example, if an individual is morphologically assigned to one species (in this case, the paternal species) but possesses the mtDNA of another (the maternal species), hybridization is assumed to have occurred between the two species. However, if the same two species hybridized in a reciprocal cross (where the maternal and paternal species are switched), this technique would fail to identify the hybridization event owing to the maternally inherited mtDNA. Therefore, the use of biparentally inherited molecular markers, such as nuclear DNA, is a more powerful approach for identifying gene introgression due to hybridization (Arnold, 1997). Natural hybridization in the *Anaxyrus americanus* group has never been evaluated using rigorous, quantitative analyses of morphological evidence and/or biparentally inherited molecular markers.

The combination of a recent radiation and multiple potential areas of natural contact make the *Anaxyrus americanus* group an ideal system for studying hybridization and speciation in a natural environment. Using multiple lines of evidence such as molecular markers and morphological variation has been shown to be effective in studies of natural hybridization in other taxa (Arnold, 1997). I will use analyses of laboratory hybridization data obtained from Blair (1972), molecular variation, and morphological variation to identify the effect of natural hybridization on the formation of species in the *A. americanus* group.

The objectives of this study include: 1) identifying the relationship between genetic divergence and reproductive isolation in the family Bufonidae 2) examining the patterns of sterility and inviability of hybrid offspring in the family Bufonidae 3) evaluating the efficacy of molecular techniques for identifying natural hybrids from wild populations in the *Anaxyrus americanus* group; 4) evaluating a putative natural hybrid zone involving multiple species and multiple potential contact zones; 5) further clarifying the phylogenetic relationships of the *Anaxyrus americanus* group; 6) assessing the effect of natural hybridization on morphological variation; and 7) furthering our understanding of the role natural hybridization plays in the speciation process.

CHAPTER 2

HYBRIDIZATION AND REPRODUCTIVE ISOLATION IN BUFONIDS

Introduction

Reproductive isolation is a defining characteristic of a biological species, and it is integral to creating and maintaining species boundaries (Coyne and Orr 2004). Breakdown in reproductive isolation can lead to hybridization between species, resulting in sterility or inviability of hybrid offspring, genetic assimilation of the rarer species, introduction of novel genetic variation, reinforcement of species boundaries, increased or decreased fitness of hybrids in natural environments, ploidy changes, and rapid speciation (Arnold 1997, 2006; Arnold and Burke 2006).

In order to evaluate the effects of hybridization on reproductive barriers and the speciation process, it is useful to observe the offspring of hybrid crosses. Studies of reproductive isolation using laboratory hybridization have been conducted on many different taxa including fungi (Le Gac et al. 2007), orchids (Scopege et al. 2007), angiosperms (Moyle et al. 2004), worms (Herrmann et al. 2006), flies (Coyne and Orr 1989a, 1997; Christianson et al. 2005), mosquitoes (Presgraves and Orr 1998; Slotman et al. 2005), butterflies and moths (Presgraves 2002), frogs (Sasa et al. 1998), birds (Price and Bouvier 2002; Tubaro and Litjamer 2002; Lijtmaer et al. 2003), fish (Mendelson 2003; Russell 2003; Mendelson et al. 2004; Bolnick and Near 2005), and mammals (Fitzpatrick 2004; Fitzpatrick and Turelli 2006). Laboratory hybridization studies often use measures of genetic distance between species as a proxy for divergence time between species to analyze the relationship between genetic divergence and reproductive isolation. These studies have contributed to our knowledge of evolutionary biology by providing information about the rate of evolution of prezygotic and postzygotic isolation among species, the rate of evolution of hybrid inviability and sterility, and the prevalence of Haldane's Rule among organisms regardless of sex determination system (Coyne and Orr 1989b; Coyne and Orr 2004; Johnson 2006; but see Demuth and Wade 2007).

While patterns of reproductive isolation among organisms are consistent for the most part, some organisms do exhibit interesting exceptions to the patterns and processes underlying the genetics of speciation (Coyne and Orr 2004). Understanding patterns of reproductive isolation (and exceptions to these patterns) requires examining data from several diverse groups of organisms (Coyne and Orr 2004). True toads of the genus *Anaxyrus* have never been used to examine patterns of reproductive isolation despite a wealth of knowledge regarding interspecific hybridization in this group (Blair, 1972). Toads are diverse with more than 280 species distributed on all continents except Australia and Antarctica. Divergence estimates place the origin of the family Bufonidae in the upper Cretaceous 78–99 mya, with a likely geographical origin in South America (San Mauro et al. 2005; Pramuk et al. 2008). All major lineages of extant bufonids are believed to have dispersed from South America during the Eocene approximately 34–56 mya (San Mauro et al. 2005; Pramuk et al. 2008). Several researchers, most notably W.F. Blair, have used toads as models of vertebrate speciation owing to several aspects of their life history including ease of capture, male advertisement call mating system, external fertilization and proclivity to hybridize in laboratory environments (Blair 1956, 1959, 1961, 1963a, 1963b, 1964, 1966, and 1972).

In a landmark book compiling the results of his studies of reproductive isolation and hybridization in toads, Blair (1972) provided measurements of postzygotic isolation, percentage of fertilized eggs, percentage of fertilized eggs that hatched, number of larvae, percentage of metamorphosed tadpoles, developmental stage reached, and survivorship of hybrid individuals for species crosses involving all major clades within the family Bufonidae. Blair's efforts resulted in the largest hybridization dataset for any vertebrate species, both in terms of the total number of crosses and the number of species used in interspecific crosses. These data were originally collected in an effort to reconstruct the phylogenetic relationships of the family Bufonidae, but they also provide a means to assess the possible role of hybridization in generating the patterns of variation seen in the *Anaxyrus americanus* group.

Recent research has produced robust estimates of phylogenetic relationships among toads as well as a wealth of molecular data allowing for inference of interspecific genetic divergences (Cunningham and Cherry 2004; Pauly et al. 2004; Frost et al. 2006; Pramuk 2006; Pramuk et al. 2008). Examining the relationship between genetic divergence and the various metrics of reproductive isolation provided by Blair (1972) can provide insight into speciation events in the family Bufonidae. Additionally, certain unique features of Blair's data allow for an examination of previously unaddressed evolutionary questions such as how biased sex ratios, development, and polyploidy in hybrid offspring are related to interspecific genetic divergence.

Blair (1972) documented the developmental stage reached by all hybrid offspring, thus allowing for an examination of how development occurs in hybrid offspring, an overlooked aspect of reproductive isolation research. The data compiled by Blair (1972) also allow for an examination of Haldane's Rule in an organism with homomorphic sex chromosomes. Biased sex ratios in hybrid offspring are often attributed to Haldane's Rule, one of the few general rules of speciation (Coyne and Orr, 2004). Haldane's Rule states that when one sex of hybrid offspring is inviable or sterile, that sex is the heterogametic sex (Haldane, 1922), and this is thought to be an important first step toward reproductive isolation between species (Coyne and Orr, 1989, 1997; Presgraves, 2002; Sasa et al., 1998). Sasa et al. (1998) previously examined patterns of reproductive isolation in frogs (not true toads) but they were unable to examine the operation of Haldane's Rule owing to a lack of information about the sex ratios of hybrid offspring. Blair preserved many toad specimens at the Texas Natural History Collection at the University of Texas, and the sex ratios of hybrid offspring were obtained by dissection in order to examine the operation of Haldane's Rule.

Finally, Blair's data provide an opportunity to examine changes in ploidy of hybrid offspring. The relationship between hybrid polyploidy and interspecific divergence has not previously been examined in animals. Most toads possess a diploid chromosome number of 22 with the exception of several African species of toads, which possess a diploid chromosome number of 20. These differences do not prevent 20 chromosome toads from producing viable, and, in some cases, fertile offspring when crossed with 22 chromosome toads. Data from karyotype analyses were examined to assess the relationship between ploidy and interspecific divergence.

We used Blair's data, in conjunction with molecular data obtained from previously published studies and sex ratio data obtained from dissecting Blair's preserved specimens, to 1) examine the relationship between postzygotic reproductive isolation and interspecific divergence; 2) examine the general features of development in hybrid offspring; 3) examine the operation of Haldane's Rule regarding biased inviability and sterility of hybrid offspring; and 4) examine the effects of hybrid polyploidy on reproductive isolation.

Methods

The data found in Appendix H of Blair (1972), consisting of 1,934 crosses between 92 species of *Bufo,* was compiled to generate a reproductive isolation dataset. Blair's species crosses were generally made by *in vitro* fertilization using squashes of testis from one species to fertilize the eggs of another species in a petri dish. Various measures of reproductive isolation were quantified based on these *in vitro* fertilizations including; 1) the percentage of fertilized eggs; 2) percentage of hatching embryos; 3) number of tadpoles produced; 4) percentage metamorphosed; 5) fertility in backcross analysis; and 6) the stage at which eggs ceased to develop. Typically, each interspecific cross was represented by multiple replicates, and in these cases, the mean for each measure of reproductive isolation was used in subsequent analyses.

Calculation of Postzygotic Isolation Indices

Indices of postzygotic isolation were calculated following the methods of Zouros (1973), Coyne and Orr (1989a), Sasa et al. (1998), and Presgraves (2002). For reciprocal crosses, the number of completely inviable or sterile sexes was counted, giving a value ranging from 0 (both sexes viable/fertile in reciprocal crosses) to 4 (both sexes inviable/sterile in reciprocal crosses). Dividing this number by 4 produces an index of postzygotic isolation (IPO) ranging from 0 (no isolation between species) to 1 (complete isolation between species). Unidirectional crosses were calculated in a similar manner with the exception of dividing by 2 instead of 4, again resulting in an IPO value ranging from 0 to 1 (following Sasa et al. 1998).

Examination of the data frequently showed one direction of the cross produced offspring, but the reciprocal cross for the same species pair failed to produce offspring. This pattern has been identified as Darwin's Corollary to Haldane's Rule (Turelli and Moyle, 2007). To account for this pattern, the above-mentioned method was modified to obtain postzygotic isolation indices for subsequent analyses. For crosses that were performed in both directions, an additional category was added to the traditional postzygotic isolation index (Zouros, 1973; and Coyne and Orr 1989a; 1997) in order to account for species-level parent of origin effects operating in toads (Table A.1). Additionally, it should be noted that the fitness of hybrid offspring in natural environments could not be examined using these data; therefore all discussion of postzygotic reproductive isolation is limited only to whether or not offspring are viable and reach the metamorphosis stage of development in laboratory conditions.

Genetic Distance

Sequence data for the mitochondrial DNA fragment 12S–16S were downloaded from Genbank for 69 of the 92 species included in Blair's original dataset (Pauly et al. 2004; Frost et al. 2006; and Pramuk 2006). Alignments were performed manually using the program Se-Al version 2.0a11 (Rambaut 2006), and corrected for secondary structure using models obtained from Pauly et al. (2004). Genetic distance was calculated for all pairwise comparisons using the uncorrected p and the Kimura 2-parameter functions in PAUP* 4.0 Beta (Swofford 2001). The Kimura 2-parameter distance estimate was used to correct for multiple substitutions while considering transition and transversion substitution rates (Kimura 1980). The use of either uncorrected p or Kimura 2-parameter did not affect the results, so only the uncorrected p divergence estimates were used. After averaging data from replicate crosses, and excluding crosses for which IPO value and/or genetic distance estimates were unavailable, the final dataset consisted of 680 crosses.

Phylogenetic Correction

To ensure that observations of reproductive isolation were phylogenetically and statistically independent, we employed Coyne and Orr's (1989a, 1997) modification of Felsenstein's (1985) Independent Contrasts method. First, a phylogeny for the full molecular dataset (69 species) was generated using maximum parsimony methods in order to examine the phylogenetic relationships among species in the data. We then averaged the genetic distances and other measures of postzygotic isolation for species pairs that were not independent to produce a single, independent comparison.

Implementation of this modification reduced the dataset from 680 nonindependent crosses to 101 independent crosses.

Additionally, we examined the phylogenetic tree for concordance with previously published phylogenies (Frost et al., 2006; Pramuk, 2006; Pauly, 2004) in order to look for any disagreements or unusual relationships that may be indicative of introgression of mitochondrial DNA (mtDNA) due to natural hybridization. The phylogeny (based on the same mtDNA fragment from multiple studies and, in some cases, multiple individuals of a given species) largely agreed with all recent bufonid phylogenies. No unusual relationships or indirect evidence of hybridization were found, suggesting that the mtDNA data are not affected by recent natural hybridization events.

Haldane's Rule

Blair preserved many samples of hybrid offspring from the experimental crosses and these specimens are currently housed in the Texas Natural History Collection at the University of Texas. We visually examined the sex of all preserved hybrid specimens ($N = 869$), and used these data to examine the sex ratios of hybrid offspring. Hybrid specimens were dissected, and the gonads were examined to determine the sex of each individual and to assess the frequency of malformed reproductive organs in hybrids. While these do not represent all of the hybrid offspring from Blair's experiments, they do represent a wide spectrum of interspecific crosses, and the majority produced more than one adult hybrid offspring. We confirmed and counted the number of males and females from each cross, combined data from replicate crosses, and used these data to examine the operation of Haldane's Rule for inviability in the family Bufonidae.

Each cross was evaluated for statistical significance following Presgraves (2002). Crosses were scored as cases of complete inviability (one sex is entirely absent) or quantitative inviability (a statistically significant bias towards one sex). Due to relatively low sample sizes for the numbers of each sex resulting from a given cross (ranging from $N = 0$ to 18), we used a binomial distribution test to evaluate statistical significance (Sokal and Rohlf, 1995). This test provided threshold values for significant differences in the numbers of males and females produced from each cross. For a complete case of inviability to be considered statistically significant, one sex had to have at least six surviving adults, while the other sex had zero survivors. Quantitative cases (cases where both sexes were present, but there was a significant bias in the numbers of one sex) were also analyzed for statistical significance using a binomial distribution test.

Many crosses had sample sizes too low (i.e. < 6) to be evaluated statistically using the aforementioned method. The crosses that did not meet the statistical criteria for sample size were counted and placed into three distinct categories: only female offspring present, only male offspring present, and both sexes present. Additionally, inferences regarding Haldane's Rule for sterility were produced by examining the results of F1 hybrid test crosses. This dataset was smaller compared to that for inviability, but still provides estimates for the operation of Haldane's Rule for sterility in toads.

Ploidy analyses

Bogart (1972) investigated chromosome content in 50 species and 175 hybrid combinations of *Bufo*, and reported these results in Appendix G of Blair (1972). Hybrid offspring from Blair's crossing experiments could be placed into one of three categories:

diploids, triploids, and pentaploids. We used these data to examine the relationship between ploidy of hybrid offspring and genetic divergence between parental species. The relationship between ploidy and asymmetrical inviability and sterility of hybrid offspring was also examined.

Statistical Analyses

For the phylogenetically corrected dataset, genetic distance, percentage of eggs fertilized, percentage of embryos hatched, and percentage of metamorphosed larvae were arcsine square-root transformed to adhere to normality assumptions. IPO values were not normally distributed: therefore, non-parametric Spearman rank correlations were employed for analyses including these values. We used two-tailed, independent samples *t*-tests, corrected for multiple comparisons using Scheffe's F test, and Mann-Whitney *U* tests (corrected for multiple comparisons using Bonferroni correction) in order to evaluate differences between means. All analyses were performed using the program SPSS (SPSS version 16.0.2, SPSS Inc.).

Results

Genetic divergence showed a significant positive correlation with reproductive isolation (for uncorrected data, Spearman rank correlation: $r_s = 0.406$, $N = 680$, $P < 0.001$; Figure A.3A; for corrected data, Spearman rank correlation: $r_s = 0.538$, $N = 101$, $P < 0.001$; Figure A.3B). Overall, the average genetic divergence among species pairs was 0.067 ± 0.002 and average postzygotic isolation index was 0.77 for the phylogenetically corrected dataset ($N = 101$). These results suggest that postzygotic isolation increases with genetic divergence between diverging species in the family Bufonidae.

The other metrics of reproductive isolation followed patterns similar to that of genetic divergence, with the exception of fertilization percentage. The percentage of eggs that were fertilized in hybrid crosses was not related to genetic distance ($r_s = 0.078$, $N = 101$, $P < 0.437$; Figure A.4A), but the percentage of fertilized eggs that hatched ($r_s = -0.246$, $N = 101$, $P < 0.013$; Figure A.4B), the number of tadpoles ($r_s = -0.378$, $N = 101$, $P < 0.001$; Figure A.4C), and the percentage of tadpoles that reached metamorphosis ($r_s = -0.499$, $N = 101$, $P < 0.001$; Figure A.4D) all decreased with increasing divergence between species in hybrid crosses.

Hybrid offspring reached different stages of development depending on their genetic divergence values ($F_{2,489} = 62.729$; $P < 0.0001$; Figure A.5). Pairwise comparisons reveal that when parental species had lower genetic divergence, hybrid offspring reach later stages of development. The average genetic divergence between parental species for crosses that reached both gastrula and/or larval stages was nearly identical (average distance for gastrula stage = 0.089 ± .001, $N = 257$; average distance for larval stage = 0.089 ± .002, $N = 145$), but the average genetic divergence for crosses that reached the metamorphosis stage decreased by 0.003 substitutions/site (average distance = 0.062 ± .003, $N = 90$; Bonferroni corrected P for multiple comparisons, $P < 0.0001$ for all comparisons).

The vast majority of crosses (92.1%) show some degree of postzygotic reproductive isolation (IPO > 0.00), and the average genetic divergence at which a given level of postzygotic isolation was reached can be seen in Table A.2. The average genetic divergence corresponding to IPO values between 0.0 and 0.50 were similar, but there was a significant increase in genetic divergence between IPO values 0.60 and 0.80 (Table A.2). Interestingly, genetic divergence between species pairs with no postzygotic isolation (IPO = 0) ranged from 0.004–0.123, while species pairs with full postzygotic isolation (IPO =1) ranged from 0.027–0.152, suggesting that postzygotic isolation can be both weak between distantly related species pairs, and strong between closely related species pairs.

Haldane's Rule

Blair's preserved hybrid specimens featured significantly more males than females (males: $N = 501$ females: $N = 368$; $G = 20.44$, df = 1, $P < 0.001$). Among the statistically confirmed cases of inviability, females were the affected sex in 71% (5/7) of crosses, while males were the affected sex in 29% (2/7) of crosses. Considering all crosses (including those for which there was evidence of asymmetry, but insufficient numbers of offspring to provide a statistical test of sex ratios), females were the affected sex in 70% (65/93) of crosses and males were the affected sex in 30% (28/93) of crosses (Table A.3).

Crosses resulting in inviable hybrid females had higher average genetic divergence than crosses that resulted in inviable males (Bonferroni corrected P for multiple comparisons, Mann-Whitney $U = 1362.5$, $z = -2.374$, 2-tailed $P < .018$; Figure A.6) and crosses that resulted in viable offspring of both sexes (Bonferroni corrected P for multiple comparisons, Mann-Whitney $U = 1564$, $z = -4.369$, 2-tailed $P < .001$; Figure A.6). The average genetic divergence for crosses resulting in inviable males was not significantly different from crosses resulting in offspring of both sexes (Bonferroni corrected *P* for multiple comparisons, Mann-Whitney *U* = 1082.5, *z* = -1.769, 2-tailed *P* < 0.07; Figure A.6). Additionally, crosses resulting in offspring of both sexes cover a wide range of genetic divergences, as do crosses resulting in only male or only female offspring (neither sex affected; average uncorrected p = 0.041 ± 0.004, range = 0.00–0.12; males affected; average uncorrected p = 0.053 ± 0.005, range = 0.00–0.11; females affected; average uncorrected p = 0.065 ± 0.003, range = 0.00–0.12; Figure A.6).

Patterns of Sterility

In 38 test crosses involving hybrid males, 53% (*N* = 20) of males were sterile, and there was no difference in the number of sterile or fertile males (20 sterile vs. 18 fertile; *G* = 0.104; df =1; *P* > 0.05). Despite nearly equivalent numbers of sterile and fertile hybrid males, the average genetic distance for crosses that produced sterile hybrid males (0.064 ± 0.006) was significantly higher than the average genetic distance for crosses that produced fertile hybrid males (0.039 ± 0.005; Mann-Whitney *U* = 77.5, *z* = -2.823, *N* = 38, *P* < 0.004), suggesting that increased genetic divergence causes increased sterility in hybrid male toads. Examination of 501 male hybrid specimens revealed that 1% (*N* = 5) of males had regressed or malformed testes. Interestingly, 1.6% (*N* = 8) of hybrid males contained eggs in association with the Bidder's organ. The Bidder's organ is a unique organ present in male bufonids composed of rudimentary ovarian tissue that normally does not produce functional eggs (Petrini and Zaccanti 1998).

Data were available for 10 crosses involving three types of hybrid females (*Anaxyrus woodhousii* x *A. hemiophrys*; *A. terrestris* x *A. hemiophrys*; and *A. terrestris* x *A. woodhousii*). These hybrid females produced offspring of both sexes, suggesting they were fertile. Hybrid males for these same species pairs were also completely fertile. These species are closely related (average uncorrected p = 0.01), and therefore contradict the notion that Haldane's Rule operates during the earliest stages of divergence/speciation (Coyne and Orr, 2004). Additionally, 1.4% ($N = 5$) of the 369 hybrid females examined possessed regressed or malformed ovaries.

Ploidy of Hybrid Offspring

The degree of ploidy in hybrid offspring is positively related to genetic divergence between parental species. Crosses that resulted in triploid and pentaploid karyotypes had an average increase of about 0.02 substitutions/site (diploid average uncorrected p = 0.0713 ± 0.028, $N = 90$; triploid average uncorrected p = 0.0881 ± 0.0150, $N = 20$; pentaploid average uncorrected p = 0.0816 ± 0.0137; $N = 5$; $F_{2,112} = 3.70$; $P = 0.02$). There was no relationship between the level of ploidy for species pairs in a given cross and the likelihood of producing viable hybrid offspring (Fisher's Exact Test, $P = 0.2250$). Regarding sterility, only six crosses that were scored for fertility were also karyotyped. Among these six crosses, one was a sterile triploid, two were sterile diploids, and three were fertile diploids. While increased genetic divergence between parental species increases the chance that allopolyploidization (polyploidy resulting from hybridization with another species) will occur, polyploidy does not appear to have a clear relationship to hybrid inviability or sterility.

Discussion

Examining patterns of reproductive isolation in diverse taxa is an excellent method for understanding the patterns and processes involved in speciation (Orr et al., 2007). Toads, and anurans in general, are a useful system for studying speciation owing to their unique patterns of reproductive isolation (Coyne and Orr 2004; Mendelson et al. 2004; Malone et al. 2007; Malone and Michalak 2008a; 2008b) and the interesting diversity of sex determination systems seen in anurans (reviewed in Schmid and Steinlein 2001).

Many of the results from these analyses agree with previous studies of reproductive isolation. First, intrinsic postzygotic reproductive isolation shows a positive correlation with genetic divergence. Second, as genetic divergence increases, other metrics of reproductive isolation such as the percentage of hatching embryos, the number of tadpole larvae produced, and the percentage of larvae that reach metamorphosis all decrease in interspecific crosses. These results are concordant with a previous study of anurans (which included seven genera of anurans, but only one cross involving toads) conducted by Sasa et al. (1998) and other studies of reproductive isolation (Coyne and Orr 1989, 1997; Sasa et al. 1998; Presgraves 2002; Price and Bouvier 2002; Tubaro and Litjamer 2002; Lijtmaer et al. 2003; Mendelson 2003; Russell 2003; Fitzpatrick 2004; Mendelson et al. 2004; Moyle et al. 2004; Christianson et al. 2005; Le Gac et al. 2007). Overall, these data agree with the notion that the evolution of endogenous, postzygotic reproductive isolation is likely to occur between diverging species lineages.

Certain aspects of reproductive isolation in bufonids contradict the patterns of reproductive isolation observed in previous studies of other taxa. For example, the percentage of eggs fertilized in hybrid crosses was not correlated with genetic divergence (Figure A.4A), suggesting that postzygotic reproductive barriers take effect after the fertilization stage in toads, contrary to the results of Sasa et al. (1998), who found that fertilization is negatively correlated with genetic divergence in anurans. These results suggest that sperm/egg interactions are compatible among interspecific parents over a relatively wide range of genetic divergences.

Additionally, bufonids appear to show a relatively high degree of asymmetric inviability in reciprocal crosses (crosses with an IPO value of 0.6). For reciprocal crosses, 8% (19/233) resulted in offspring in only one direction of the cross, suggesting species-level, parent-of-origin effects. The average genetic divergence for these crosses (uncorrected $p = 0.068 \pm 0.002$) was elevated compared to crosses that did not show asymmetric inviability, but this does not explain how asymmetric inviability may occur. Inviability asymmetries may actually be expected under a Dobzhansky-Muller model of incompatibility accumulation (Johnson, 2000) and differences in the evolutionary rate of mitochondrial genomes between parental species could also account for these patterns of inviability asymmetries (Bolnick et al., 2008). It is possible that mechanisms such as stochastic incompatibility accumulation and mitochondrial genome evolution rates could profoundly influence reproductive isolation in anurans as well as other species (Sawamura 1996; Turelli and Moyle 2007). Parent-of-origin effects similar to those in this dataset have also been observed in natural populations of toads and other anuran species (Thornton 1955; Pfennig and Simovich 2002).

Development of Hybrid Offspring

Relatively high levels of genetic divergence (average uncorrected $p = 0.089$) between parental species were required for hybrid offspring to die during early developmental stages, and smaller degrees of genetic divergence enabled more advanced stages of development (Figure A.5). Maternally loaded RNA transcripts regulate early development in amphibians and other organisms up to the point of zygotic activation during the maternal-zygotic transition. The maternal-zygotic transition generally occurs during gastrulation in amphibians, and these data suggest that high levels of genetic divergence are required for crosses to fail during the gastrula stage (Briggs and King 1959; Moore 1955; Schier 2007).

Haldane's Rule

Anuran sex determination systems are known to be labile among species, but Hillis and Green (1990) provide evidence that female heterogamety (ZW sex determination) is the likely ancestral state in amphibians. While the effects of multiple sex determination systems on the operation of Haldane's Rule is unclear, one group of frogs (*Xenopus*) contradict Haldane's Rule because hybrid males are sterile, even though females have been confirmed as the heterogametic sex (*Xenopus*; Kobel 1985; 1996; Orr and Presgraves 2000; Coyne and Orr 2004; Malone et al. 2007, Malone and Michalak 2008a). Because toads possess homomorphic sex chromosomes (Blair, 1972), karyotype analyses cannot provide information about the sex determination mechanism for all toad species. However, sex-reversal experiments confirmed a ZW sex determination system for one bufonid species (*Bufo bufo*), in which females are the heterogametic sex (Ponse 1941; Engel and Schmid 1981; reviewed in Schmid and Steinlein 2001). While it is possible that some species of toads may not possess ZW sex determination, there is no experimental confirmation of any alternative sex determination systems in the family Bufonidae.

Assuming that all bufonids possess a ZW sex determination system, then asymmetrical inviability or sterility of hybrid offspring should affect females according to Haldane's Rule. These results confirm Haldane's Rule for inviability in that females are underrepresented among the total number of preserved hybrid specimens, and hybrid females were the affected sex in about 70% of hybrid crosses showing asymmetrical inviability. However, males were the affected sex in 30% of crosses and these represent exceptions to Haldane's Rule. The data suggest that complex mechanisms may be operating in toads because the number of exceptions to Haldane's Rule for inviability is relatively high compared to most organisms. For example, in butterflies and moths, only 4% (3/84) of all species pairs contradict Haldane's Rule for inviability (Presgraves 2002). In contrast, fruit flies of the genus *Drosophila* contradict Haldane's Rule for inviability in approximately 24% (13/17) of cases, a percentage similar to that seen in toads (Coyne and Orr, 1997; Sawamura, 1996). The contradictions to Haldane's Rule seen in *Drosophila* have been attributed primarily to maternal effects (Sawamura, 1996). If all toads possess a ZW sex determination system, it is possible that maternal effects also play a significant role in causing exceptions to Haldane's Rule for inviability in this group as well.

Toads are also unusual with regard to Haldane's Rule for sterility. Within the subset of hybrids used in further test crosses, 53% of hybrid male toads were sterile and no hybrid females were sterile, seemingly contradicting the operation of Haldane's Rule for sterility (assuming a ZW sex determination system). Additionally, there was a significant difference in the mean level of genetic divergence for sterile hybrid male toads compared to those that were fertile, with crosses between more divergent species producing sterile males and crosses between more closely related species producing fertile males. While a small number of exceptions to Haldane's Rule for sterility have been documented in other organisms (Sawamura 1996), the sterility patterns seen in toads are unusual. For example, 97% (29/30) of butterflies and moths and 98% (112/114) of *Drosophila* fruit flies adhere to Haldane's Rule for sterility (Coyne and Orr, 2004; Presgraves 2002).

Interestingly, nearly half (43%) of the interspecific crosses resulted in offspring of both sexes, despite extensive divergence among taxa (average uncorrected $p = 0.051 \pm 0.004$, ranging from 0.00 to 0.12). Crosses that result in offspring of both sexes should have low genetic divergences over a relatively narrow range (Coyne and Orr 2004), but this is not the case in toads. In fact, the range of genetic distance over which crosses produce only males, only females, or offspring of both sexes is nearly identical (Figure A.6).

Polyploidy and Sterility

Abnormal ploidy levels in hybrid male toads could potentially cause sterility, explaining the unusual levels of male sterility in these data. Polyploidy resulting from interspecific hybridization could result in abnormal gamete production, suggesting that degree of ploidy could be related to inviability or sterility of hybrid offspring. These data reveal no clear relationship; while genetic divergence between parental species is positively correlated with degree of ploidy, the likelihood of producing inviable or sterile offspring does not appear to be related to ploidy level. Bogart (in Blair, 1972) mentions the occurrence of triploid hybrid males that were completely fertile, indicating that ploidy cannot explain patterns of sterility in toads. These data suggest that the relationship between ploidy and hybrid abnormalities is ambiguous, and cannot fully explain the unusual patterns seen with regard to Haldane's Rule in toads.

Additional Hypotheses

The discordance of the sex ratio data with the operation of Haldane's Rule in other taxa raises interesting questions. In many vertebrate groups, genetic sex determination is mostly fixed in the species, but anurans are remarkable in that genetic sex determination is labile across clades (Hillis and Green 1990; Schmid and Steinlein 2001). For example, among anurans there are species with XX/XY, ZZ/ZW, and OO/OW sex determination, including one well-studied example in which XY and ZW sex determination systems occur among different populations of a single species (*Glandirana rugosa*; Miura et al. 1998; Ohtani et al. 2000; Ogata et al. 2003). While there is currently no evidence for multiple sex determination systems across clades, or within species of bufonids, the lability of genetic sex determination in amphibians suggests that this could be a viable explanation for why these results differ from the expectations of Haldane's Rule.

Table A.3 shows that Haldane's Rule operates correctly under the assumptions of a ZW sex determination system in 70% of crosses with asymmetrical inviability (i.e. females are inviable and males are viable), and it operates correctly under the assumption of an XY sex determination system in 30% of crosses with asymmetrical inviability (i.e. males are inviable and females are viable). If both ZW and XY sex determination systems do occur in bufonids, these data could then contain crosses between ZW and XY species. While relatively little is known about the consequences of hybridization between species with different sex determination mechanisms, it has been shown that *Glandirana rugosa* (in which both ZW and XY sex determination occur within the same species) can produce viable WY genotypes in crosses between XY and ZW individuals (Nishioka and Hanada 1994; Ogata et al. 2003). Offspring with the WY genotype show a heavily female-biased (55 of 57) sex ratio (Nishioka and Hanada 1994). If species crosses within these data are between individuals possessing differing sex determination systems, it is reasonable to expect WY genotype females in the data, and this would be consistent with the elevated numbers of crosses in which males were the affected sex. However, no firm conclusions regarding this hypothesis can be put forth without first establishing the sex determination system for additional species of toads, which would require extensive sex reversal experiments.

Alternatively, exceptions to Haldane's Rule may be expected to occur in a system where homomorphic sex chromosomes and sexual selection are present (Turelli and Orr, 1995; 2000). Researchers have focused on two mechanisms to explain Haldane's Rule: dominance effects and faster-male evolution (reviewed in Coyne and Orr, 2004). The dominance theory asserts that hybrid incompatibility alleles are often recessive, and the hemizygous nature of the heterogametic sex exposes it to the full effects of these alleles, thus causing increased levels of hybrid incompatibility (Turelli and Orr, 1995). If females are the heterogametic sex in toads, then dominance effects could explain the elevated levels of hybrid female inviability. However, since toads possess homomorphic sex chromosomes, dominance effects are expected to be weak if present at all (Coyne and Orr, 2004). Inviability for both male and female hybrid toads may be better explained by stochastic accumulation of Dobzhansky-Muller incompatibilities and maternal effects.

The faster-male evolution hypothesis asserts that strong sexual selection on males causes increased divergence in male-specific genes between species and/or that the process of spermatogenesis can be easily disrupted in a hybrid genetic background. Both of these phenomena could result in hybrid male sterility, regardless of sex determination system (Wu and Davis 1993; Wu et al. 1996). Dominance effects cannot explain hybrid male sterility in a ZW sex determination system as females, not males, should be adversely affected. Frogs of the genus *Xenopus* have been shown to contradict Haldane's Rule for sterility, despite possessing a ZW sex determination system, due to faster-male evolution (Malone et al. 2007, Malone and Michalak 2008a). Faster-male evolution produces dysfunctional males, whether males or females are the heterogametic sex in hybrid crosses.

Heterogametic females should be expected to closely adhere to Haldane's Rule because they are exposed to both cytoplasmic/maternal effect incompatibilities and sex chromosome-autosome incompatibilities (Turelli and Moyle 2007). However, female toads have fewer hemizygous loci that can be affected in dominance interactions owing to their homomorphic sex chromosomes, leaving cytoplasmic/maternal effects and stochastic accumulation of Dobzhansky-Muller incompatibilities as potential causes of both female and male hybrid inviability.

Interestingly, several crosses in these data resulted in fertile female hybrid offspring and sterile male offspring. This pattern can be explained under the assumption of faster-male evolution, as exceptions to Haldane's Rule in organisms with ZW sex determination are expected to occur when faster-male evolution is operating (Wu and Davis 1993). Evidence suggests that faster-male evolution is more likely to contribute to hybrid sterility than to inviability (Wu 1992; Presgraves and Orr 1998); however, these data contain exceptions to Haldane's Rule involving both sterility and inviability. These exceptions could be due to asymmetric cytoplasmic/maternal effects leading to unusually low occurrences of hybrid female inviability, and faster-male evolution effects leading to increased levels of hybrid male sterility. These results suggest that faster-male evolution could explain cases of hybrid male sterility in toads as seen with other anurans (Malone et al. 2006; Malone et al. 2007; Malone and Michalak 2008a; 2008b).

One mechanism that may result in faster-male evolution within the genus *Bufo*, and frogs in general, is sexual selection on male advertisement vocalizations. Male toads, like most anurans, produce an advertisement vocalization used to attract conspecific females to breeding sites for mating. Male advertisement vocalizations therefore act as premating isolating mechanisms, and often exhibit character displacement in areas of species overlap (A.P. Blair 1941; 1942; W.F. Blair, 1955; Fouquette 1975; Gerhardt 1994; Leary 2001).

One of the best-documented examples of divergent advertisement call displacement occurs among the members of the *Anaxyrus americanus* group. Several authors have found that members of this group often occur in sympatry, and advertisement calls for each species are subtly different, a possible prezygotic isolating mechanism (A.P. Blair 1941, 1942; W.F. Blair, 1956, 1959, 1961. 1963a, 1963b, 1964; Volpe 1955; Leary 2001; Masta et al. 2002). There is little to no postzygotic reproductive isolation among any of the members of this group, and Blair (1972) used hybrid offspring from crosses within this group in several further test crosses resulting in viable and, in some cases, fertile F_2 offspring from hybrid crosses and parental backcrosses. This lack of reproductive isolation, coupled with sympatric distributions, should result in frequent natural hybridization among the members of this group; however, lineages appear to remain relatively distinct. This could possibly be the result of strong prezygotic isolating mechanisms, such as male advertisement call. If male advertisement call is important as prezygotic reproductive isolating barrier, then selection should be relatively strong on male toads, and this could lead to faster-male evolution. Additionally, certain aspects of anuran vocalizations are dependent on morphological parameters such as body size; increasing the likelihood that genome-wide selection could be accelerated in male toads (Blair 1964). Although evidence of the strength of prezygotic isolation between toad species cannot be extracted from the current dataset, abundant evidence from anuran vocalization studies suggests that prezygotic isolation could be quite important (A.P. Blair 1941; 1942; W.F. Blair 1955; Gerhardt 1994; Price and Bouvier 2002).

The fact that toads deviate from some of the broad features of speciation seen in other taxa is quite intriguing. There is no relationship between fertilization rate and genetic divergence, suggesting that relatively high levels of genetic divergence are required before pre-gastrulation isolating barriers develop between bufonid species. The sex ratio patterns among toads are unusual when compared to other organisms; hybrid females are fertile even in the earliest stages of speciation, there is relatively little differential inviability or sterility between males and females (compared to other taxa) despite substantial genetic divergence. Additionally, there is no clear relationship between ploidy and degree of sterility in males.

Unusual patterns with regard to Haldane's Rule may be expected in a system with homomorphic sex chromosomes and strong selection on males. Other taxa in which Haldane's Rule is obeyed more strongly possess heteromorphic sex chromosomes, resulting in increased dominance effects generating the patterns attributed to Haldane's Rule (Coyne and Orr, 2004). In a system where the sex chromosomes are virtually indistinguishable from the autosomes, Haldane's Rule may not operate in the same fashion as in other organisms where dominance effects appear to explain the pattern; dominance effects would be non-existent and parent-of-origin effects may be quite common. Parent-of-origin effects can manifest in both the heterogametic and homogametic sexes, causing apparent contradictions to Haldane's Rule. Additionally, strong selection on males is expected to lead to a large faster male evolution effect. Collectively, these aspects suggest that Haldane's Rule may be less important in toads than other factors such as parent-of-origin effects, and faster male evolution.

While researchers have studied Haldane's Rule in a diverse range of taxa (e.g. flies, birds, butterflies and moths, and mammals), it is possible that this relatively small taxonomic sample has lead to generalizations for a phenomenon that may not affect some organisms with unique characteristics (e.g. sex determination lability or homomorphic sex chromosomes [i.e. genic sex determination]) in a predictable fashion.

Note: This chapter was adapted with some changes from a published article (Malone, J.H. and B.E. Fontenot (equivalent authors). 2008. Patterns of reproductive isolation in toads. *PLoS ONE*. 3(12): e3900.doi:10.1371/journal.pone.0003900). John Malone (JHM) and I (BEF) contributed equally in conception and design of the study, dissection of hybrid offspring, and writing. JHM compiled the postzygotic isolation data, calculated postzygotic isolation indices, and performed statistical analyses of sterility and polyploidy data. BEF compiled the sex ratio data, aligned the molecular data, produced the phylogenetically corrected dataset, created figures and tables, and performed statistical analyses on all of the data except sterility and polyploidy.

CHAPTER 3

MORPHOMETRIC ANALYSIS OF HYBRIDIZATION IN THE *ANAXYRUS AMERICANUS* GROUP

Introduction

Species boundaries within the *Anaxyrus americanus* group have been difficult to identify owing to general confusion among researchers regarding the taxonomic and systematic history of the group (Blair, 1972; Conant and Collins, 1998). This confusion stems primarily from the difficulty in identifying species through traditional morphological characters. Toads have a conserved body plan that makes morphological diagnosis of discrete species problematic (Blair, 1972). Traditionally, toad species have been delimited using qualitative characteristics that can be highly variable throughout the range of a putative species. Most species descriptions for the *A. americanus* group were not based on statistical analysis of morphological variation among numerous populations, but rather on generalized descriptions of cranial crest morphology, spotting patterns on the belly and back, gross advertisement call features, and the number of warts in the dorsal spots (Blair, 1972; Bragg and Sanders, 1951; Conant and Collins, 1998; Dixon, 2000; Sanders, 1986). Overlap in these characters among wide-ranging populations is of such a magnitude that it makes morphological diagnosis difficult for some species without quantitative analysis of morphological variation among many individuals.

Natural hybridization further complicates the issue of species delimitation. Postzygotic reproductive isolation is incomplete for several species of toads (Blair, 1972;

Malone and Fontenot, 2008), including all members of the *Anaxyrus americanus* group, and the confusing morphological variation has often been attributed to past and present hybridization events (Blair, 1972; Conant and Collins, 1998; Green and Parent, 2003; Masta et al., 2002). Several members of the *A. americanus* group occur in sympatry or parapatry, and the potential for homogenizing, interspecific gene exchange is high in many cases. However, lineages appear to remain distinct according to mtDNA data, implying that prezygotic isolation may have played a significant role in speciation within the *A. americanus* group as suggested by previous researchers (A.P. Blair, 1941, 1942; W.F. Blair, 1955, 1956, 1959, 1961, 1963a, 1963b, 1964, 1966, 1972; Leary, 2001).

Quantitative analyses of morphological variation can provide important clues not only about the evolutionary history of the *Anaxyrus americanus* group, but also about the effect of hybridization on broad patterns of morphological variation within the *A. americanus* group. Morphometric analyses have proven useful in identifying cryptic species in the family Bufonidae by uncovering fine scale variation previously unrecognized by taxonomists (Mendelson, 1994, 1997a, 1997b). Traditional classifications of the *A. americanus* group were based primarily on identifying qualitative characteristics that could be misleading owing to considerable overlap in these characters among and between species (Blair, 1972; Conant and Collins, 1998; Dixon, 2000). Additionally, hybridization has rarely been evaluated as a possible source of morphological variation in the *A. americanus* group (but see Green and Pustowka, 1997 and Green and Parent, 2003).

W.F. Blair conducted laboratory hybridization studies of many species within the family Bufonidae over several decades, and the hybrid offspring from these experiments are currently housed at the Texas Natural History Collection at the University of Texas in Austin, Texas (Blair, 1955, 1956, 1959, 1961, 1963a, 1963b, 1964, 1966, 1972). Blair performed many interspecific crosses between various members of the *Anaxyrus americanus* group (summarized in Blair, 1972), and the hybrid offspring from these crosses provide valuable information about the impact of hybridization on the *A. americanus* group.

Here, I evaluate patterns of morphological variation among known hybrid offspring resulting from interspecific crosses within the *Anaxyrus americanus* group. In order to assess the impact of hybridization on morphological variation in the hybrid offspring, morphometric data were evaluated using multivariate statistical techniques. Discriminant function analysis (DFA) was used to reveal cryptic morphological variation, identify the characters that most heavily influenced morphological variation, and determine whether or not natural hybridization can be detected using morphometric analysis. The results of these analyses can inform hypotheses regarding the interspecific relationships among the members of the *Anaxyrus americanus* group.

Methods

I examined 546 preserved museum specimens of *Anaxyrus americanus* (*n* = 40), *A. charlesmithi* (*n* = 25), *A. fowleri* (*n* = 65), *A. hemiophrys* (*n* = 14), *A. houstonensis* (*n* = 22), *A. terrestris* (*n* = 46), *A. velatus* (*n* = 85), *A. woodhousii* (*n* = 60), and various known hybrids between the above species preserved by W.F. Blair. The morphological data consist of measurements taken from individuals obtained both in the field from various localities, as well as from the Canadian Museum of Nature (CMN) in Guelph, Ontario, University of Texas at Arlington Amphibian and Reptile Diversity Research Center (ARDRC) in Arlington, Texas, and the Texas Natural History Collection (TNHC) in Austin, TX (see Appendix C). All specimens collected in the field for the molecular analyses (see Appendix B) were included in the morphological dataset, and will be deposited as voucher specimens at the ARDRC.

Morphometric characters

I evaluated a series of mensural characters (following the methods of Duellman, 1970; as modified by Mendelson 1994, 1997a, and 1997b), for each individual including: snout-vent length (SVL), tibia length (TIB), foot length (FT), head width (HW), head length (HL), tympanum diameter (TYMP), diameter of the orbit (ORB; internal distance between bony margins of orbit), posterior crest width (PCW; distance between lateral edges of supraorbital crest, taken from anterior side of junction with preorbital and canthal crests), anterior crest width (ACW; distance between lateral edges of supraorbital crest, taken from posterior side of junction with preorbital and canthal crests), eye-nostril distance (END; distance from posterior edge of bony margin of orbit to posterior margin of nostril), supratympanic spur length (SPTYMP; straight distance between anterior and posterior margins of supratympanic crest; including some measure of the thickness of the postorbital crest), finger one length (F1; distance from medial edge of pollical tubercle to tip of digit), finger three length (F3; distance from base between second and third fingers to tip of digit), parotoid gland length (PARL; straight distance from most anterior point to most posterior point of gland), and parotoid gland width (PARW; straight distance across parotoid gland, taken near the midpoint along its length and oriented perpendicular to the longitudinal axis of the gland).

Adult specimens of both sexes were included in the analyses and juveniles were excluded from the analyses to account for ontogenetic variation. All specimens were fixed in 10%, buffered formalin and preserved in 70% ethanol for at least one year prior to measurement to account for shrinkage artifacts resulting from the preservation process (Hayek et al., 2001; Lee, 1982). All measurements were taken using digital calipers to the nearest .01 mm.

Statistical analyses

Species pairs for which both parents and hybrid offspring were available were analyzed using discriminant function analysis (DFA) in the SPSS 16.0® software package (SPSS version 16.0.2, SPSS Inc.). All measurements were log transformed to satisfy the assumption of normality. Additionally, all measurements were regressed against SVL to assess the relationship between each variable and a proxy for body size. Measurements with a significant regression against SVL were then size-adjusted by using the residuals from the regression in place of the original values to remove the influence of body size.

DFA requires an *a priori* grouping classification of individuals in order to assess the variation within and between groups, and to determine whether or not individuals can be correctly classified according to their given groups. Individuals were grouped according to the following classifications: parent 1, parent 2, F1 hybrid, F2 hybrid, or backcross to a parent species. Individuals of unknown parentage collected from the field were assigned to either parent 1 or parent 2 and individuals of known parentage from Blair's (1972) hybridization studies were grouped according to their known status. Prior probabilities of group membership were calculated using the compute from group sizes option and leave-one-out classification was used to generate jackknifed, cross-validated classification matrices to determine how accurately species could be grouped according to *a priori* classifications.

Results

Group comparisons

Discriminant function analysis shows that the members of the *Anaxyrus americanus* group do not occupy unique regions in morphospace with respect to one another when analyzed collectively (Figure A.7). Only 56.6% of cases resulted in correct classifications after the jackknifed, cross-validated classification matrix was calculated. *A. hemiophrys* was correctly classified most often (85.7%), while *A. charlesmithi* was correctly classified only 28% of the time. Aspects of cranial morphology such as SPTYMP, PCW, HL, TYMP, END, and PARL appear to be the primary contributors to morphological variation among the members of the *A. americanus* group. Table A.4 contains the summary statistics for the DFA.

DFA results also indicate that known hybrids between members of the *Anaxyrus americanus* group can be distinguished from putative parental individuals sampled in the field and from museum collections, including the parental individuals Blair (1972) used to create the hybrid offspring examined in this study (Figure A.8). While 54% of cases were correctly classified into their *a priori* groupings overall, only 0.6% (2/356) of parental species were misclassified as hybrids and 15.7% (11/70) of F1 hybrids were misclassified as parentals after jackknifed cross-validation. Hybrids and putative parentals group together in cohesive clusters, indicating relatively discrete separation between parentals and hybrids, but not necessarily among parentals or among hybrids. A suite of morphological characters including ORB, F1, SPTYMP, HL, PCW, FT, and TIB appear to be the primary contributors to separation between hybrids and parentals, suggesting that deviations from the general morphology of the cranium and limbs are common in hybrid individuals. Summary statistics for this analysis are given in Table A.4.

Pairwise comparisons

Anaxyrus americanus x Anaxyrus fowleri

Overall, 67.9% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus americanus* and *A. fowleri* occupied relatively distinct positions in morphospace with some overlap, while F1 hybrids were interspersed between the two parental species in an intermediate region with high overlap between parentals and hybrids (Figure A.9). The jacknifed, cross-validated classification matrix for this comparison are givenin Table A.5. The variables PARW, ORB, SVL, F3, SPTYMP, END, and FT contributed most to separation on the first discriminant factor. The variables ACW, SPTYMP, END, ORB, HW, TYMP, TIB, and FT contributed most to separation on the second discriminant factor. Summary statistics for this analysis are given in Table A.4.

Anaxyrus americanus x Anaxyrus houstonensis

Overall, 80% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus americanus* and *A. houstonensis* occupied relatively distinct positions in morphospace with a small degree of overlap, while F1 hybrids occupied a separate region from either parental species with no overlap between parentals and hybrids (Figure A.10). The jacknifed, cross-validated classification matrix for this comparison are given in Table A.6. The variables FT, F1, ORB, TIB, and PCW contributed most to separation on the first discriminant factor. The variables TYMP, FT, TIB, PARL, ACW contributed most to separation on the second discriminant factor. Summary statistics for this analysis are given in Table A.4.

Anaxyrus americanus x Anaxyrus terrestris

Overall, 89% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus americanus* and *A. terrestris* occupied relatively distinct positions in morphospace with a small degree of overlap, while F1 hybrids and backcrosses occupied a region separate from either parental species with overlap between the two hybrid classes, but not between parentals and hybrids (Figure A.11). The jacknifed, cross-validated classification matrix for this comparison are given in Table A.7. The variables F1, FT, ORB, and TYMP contributed most to separation on the first discriminant factor. The variables SPTYMP, F1, ACW, HW, PCW, and PARW contributed most to separation on the second discriminant factor. Summary statistics for this analysis are given in Table A.4.

Anaxyrus americanus x Anaxyrus woodhousii

Overall, 86.2% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus americanus* and *A. woodhousii* occupied relatively distinct positions in morphospace with a small degree of overlap, while F1 hybrids occupied a separate region from either parental species with no overlap between parentals and hybrids (Figure A.12). The jacknifed, cross-validated classification matrix for this comparison are given in Table A.8. The variables F1, ORB, PARW, SVL, TYMP, and FT contributed most to separation on the first discriminant factor. The variables SPTYMP, ORB, SVL, TYMP, HW, FT, and PCW contributed most to separation on the second discriminant factor. Summary statistics for this analysis are given in Table A.4.

Anaxyrus hemiophrys x Anaxyrus houstonensis

Overall, 94.7% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus hemiophrys* and *A. houstonensis* occupied totally distinct positions in morphospace with no overlap, while F1 hybrids occupied a separate region from either parental species with no overlap between parentals and hybrids (Figure A.13). The jacknifed, cross-validated classification matrix for this comparison are given in Table A.9. The variables PCW, FT, TYMP, SPTYMP, END, ACW, and ORB contributed most to separation on the first discriminant factor. The variables TYMP, SVL, ORB, PARL, and HW contributed most to separation on the second discriminant factor. Summary statistics for this analysis are given in Table A.4.

Anaxyrus houstonensis x Anaxyrus terrestris

Overall, 92.1% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus houstonensis* and *A. terrestris* occupied relatively distinct positions in morphospace with slight overlap, while F1 hybrids occupied a separate region from either parental species with no overlap between parentals and hybrids (Figure A.14). The jacknifed, cross-validated classification matrix for this comparison are given in Table A.10. The variables F1, ACW, FT, and ORB contributed most to separation on the first discriminant factor. The variables SPTYMP, TYMP, HW, ORB, PARL, PCW, and SVL contributed most to separation on the second discriminant factor. Summary statistics for this analysis are given in Table A.4.

Anaxyrus terrestris x Anaxyrus hemiophrys

Overall, 85.7% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus terrestris* and *A. hemiophrys* occupied distinct positions in morphospace with overlap of only one individual (Figure A.15). F1 hybrids occupied a separate region from either parental species with no overlap between parentals and hybrids or between F1 hybrids, backcrosses, or F2 hybrids (Figure A.15). Backcross individuals occupied an intermediate position in morphospace between the two parental species with a high degree of overlap with parentals, and F2 hybrids occupied a separate region of morphospace closer to the parental species than F1 hybrids with no overlap between parentals, backcrosses, or F1 hybrids (Figure A.15). The jacknifed, cross-validated classification matrix for this comparison are given in Table A.11. The variables PCW, SPTYMP, ACW, TYMP, HW, and END contributed most to separation on the first discriminant factor. The variables TYMP, SPTYMP, FT, PARW, and PARL contributed most to separation on the second discriminant factor. Summary statistics for this analysis are given in Table A.4.

Anaxyrus terrestris x Anaxyrus woodhousii

Overall, 81.6% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus terrestris* and *A. woodhousii* occupied relatively distinct positions in morphospace with a small amount of overlap (Figure A.16). F1 hybrids, backcrosses, and F2 hybrids occupied a separate region from either parental species with slight overlap between parentals and strong overlap among hybrid categories (Figure A.16). The jacknifed, cross-validated classification matrix for this comparison are given in Table A.12. The variables F1, ORB, TYMP, and SVL contributed most to separation on the first discriminant factor. The variables SPTYMP, ORB, PCW, TYMP, SVL, and PARW contributed most to separation on the second discriminant factor. Summary statistics for this analysis are given in Table A.4.

Anaxyrus woodhousii x Anaxyrus hemiophrys

Overall, 82.9% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus woodhousii* and *A. hemiophrys* occupied distinct positions in morphospace with overlap of only one individual, while F1 hybrids occupied a position intermediate between the two parental species with some overlap between parentals and hybrids (Figure A.17). The jacknifed, cross-validated classification matrix for this comparison are given in Table A.13. The variables ORB, TYMP, END, SVL, HW, PARL, and PCW contributed most to separation on the first discriminant factor. The second discriminant factor was not significant so it was not considered for further analysis. Summary statistics for this analysis are given in Table A.4.

Anaxyrus woodhousii x Anaxyrus houstonensis

Overall, 77.9% of individuals were correctly classified into their *a priori* groupings. *Anaxyrus woodhousii* and *A. houstonensis* occupied slightly overlapping areas in morphospace, while F1 hybrids occupied a separate region from either parental species with no overlap between parentals and hybrids (Figure A.18). The jacknifed, cross-validated classification matrix for this comparison are given in Table A.14. The variables ORB, PARW, F1, SVL, SPTYMP, TIB contributed most to separation on the first discriminant factor. The variables SPTYMP, TIB, TYMP, and HL contributed most to separation on the second discriminant factor. Summary statistics for this analysis are given in Table A.4.

Discussion

Analysis of the *Anaxyrus americanus* group reveals that despite a high degree of overlap in morphological variation among species (Figure A.7), hybridization can be detected using morphometric analyses. However, the impact of hybridization on members of the *A. americanus* group is interesting. Natural hybridization in animals has traditionally been thought to result in hybrid offspring with morphological characteristics intermediate between those of the respective parent species (Arnold, 1997; Seehausen, 2004). Most of the hybrid crosses analyzed in this study suggest that hybrid offspring differ from either of their parents, displaying substantial changes in morphology and occupying unique positions in morphospace. For example, in all pairwise species comparisons (except those between *Anaxyrus americanus* x *A. fowleri* and *A. woodhousii* x *A. hemiophrys* where hybrids appeared intermediate between parental species) hybrid individuals occupied a discrete region of morphospace separate from either parental species rather than an intermediate position between the parental species.

The characteristics that contributed most to variation among species were mainly aspects of the cranium, cranial crests (ORB, TYMP, SPTYMP, PCW, and ACW), and limbs (F1, TIB, FT). Gross cranial crest morphology (e.g. presence or absence of a supratympanic spur) has traditionally been used as a diagnostic character to delimit species in the *Anaxyrus americanus* group (Conant and Collins, 1998); however, fine scale analysis of quantitative variation in this trait has not previously been examined in this fashion. These results suggest that some of the traditional characters used to diagnose species are useful when examined using quantitative methods. Additionally, hybrids appear to deviate from parental species in aspects of limb morphology, with hybrids often showing differences in limb length from parental species.

Because all of the hybrids used in this study were generated by laboratory hybridization experiments (Blair, 1972), it would be prudent to consider unknown laboratory effects as a potential cause of the anomalous morphological variation in hybrids. Blair's preserved specimens included 190 hybrid offspring (including all generations of hybrids) between members of the *Anaxyrus americanus* group and 49 individuals that were used as the parental species to generate these hybrid offspring. Only 2 out of 356 (0.6%) putative parental individuals (including both Blair's parentals and field-collected specimens) were misclassified as hybrids using DFA. Blair's parent species were raised and housed under the same laboratory conditions as the hybrid offspring (Blair, 1972) so these results suggest that laboratory effects are unlikely to have contributed to hybrid morphological variation. Additionally, the paucity of field-collected individuals misclassified as hybrids suggests that hybridization in nature may be relatively infrequent, despite incomplete postzygotic reproductive isolation (Green and Parent, 2003; Malone and Fontenot, 2008).

The fact that hybrids appear to possess morphological variation beyond that of either parental species could be the result of epistatic genetic interactions interacting to produce novel allelic combinations (Arnold, 1997; Rieseberg et al., 1999a and 1999b). In this scenario, hybridization could serve to break up gene complexes and reshuffle the variation into novel combinations. These novel allelic combinations could result in radical changes between hybrid and parental morphologies (Erickson and Fenster, 2006; Rieseberg et al., 1999a,b and 2003a,b). Rieseberg et al. (1999a,b and 2003a,b) found that hybrids in *Helianthus* sunflowers displayed different morphological traits from their parental species, including many that lead to increased fitness in novel environments, although epistatic interactions could not account for the novel variation in most instances.

The occurrence of hybrid individuals displaying morphological, physiological, or behavioral characteristics beyond the range of both parental species can also be attributed to transgressive segregation, a phenomenon where hybrids display traits beyond the range of their respective parental species (Rieseberg et al., 1999a and 1999b; Seehausen, 2004; Stelkens and Seehausen, 2009). This phenomenon may play a role in providing hybrids with the novel variation required to colonize new adaptive niches and ultimately diverge into new species as seen in some plants (Rieseberg et al., 2003a and 2003b; Stelkens and Seehausen, 2004). Rieseberg et al. (1999a) found evidence for transgressive segregation in 97% (110/113) of plant species and 78% (45/58) of animal species examined, and they suggest that transgressive segregation is likely to be more common than epistasis or dominance effects in generating unusual hybrid morphologies. Transgressive segregation appears to be a common and widespread phenomenon, especially among plants and domesticated animals, but evidence for wild animals exists as well (Goldberg et al., 1996; Jackson and Tinsley, 2003; Rieseberg et al., 1999a).

Several explanations for transgressive segregation have been put forth, but the most widely accepted hypotheses involve complementary gene action between parental species fixed for alleles of opposite sign at quantitative trait loci (QTL) resulting in extreme hybrid trait values (Seehausen, 2004; Stelkens and Seehausen, 2009). Transgressive segregation resulting from complementary gene action can only be expressed in F2 hybrids or later generations (Stelkens and Seehausen, 2009); however, transgressive phenotypes occur in all hybrid generations (including F1 hybrids) in the *Anaxyrus americanus* group. Stelkens and Seehausen (2009) outline a scenario in which transgressive phenotypes can occur in F1 hybrids if parental species express dominant alleles and recessive alleles at different loci involved in complementary gene action. For example, if one parent has the genotype A_bb and the other has the genotype aaB_, then hybrids bearing the transgressive genotypes A_B_ or aabb can be produced (Stelkens and Seehausen, 2009). If these loci influence the expression of morphological characteristics, then hybrids could possess character traits that transgress beyond that of either parental species.

Stelkens and Seehausen (2009) also found that transgressive segregation in animals is more likely to occur between species that are phenotypically similar and have low levels of genetic divergence. The *Anaxyrus americanus* group fits these criteria and it is possible that the extreme variation between hybrid and parental morphologies is a result of transgressive segregation, as this phenomenon has previously been documented for the physiological trait of parasite resistance in two frog species. Goldberg et al. (1996) found that hybrids between *A. microscaphus* and *A. woodhousii* harbored far fewer helminth parasites than either parental species, possibly providing hybrids a release from parasite loads that could be advantageous in certain environments. Jackson and Tinsley (2003) found a similar result for transgressive parasite resistance traits in hybridizing members of the frog genus *Xenopus*.

Overall, hybridization between members of the *Anaxyrus americanus* group appears to have a substantial impact on morphological variation, and is detectable using morphometric analysis of multiple phenotypic characters. It is impossible to comment on the functional significance or fitness effects of the transgressive phenotypes observed in hybrid offspring of the *A. americanus* group with these data. Indeed, it is equally likely that transgressive phenotypes could result in positive, neutral, or negative consequences for hybrids in natural situations (Arnold, 1997). However, when hybrids occur in novel or disturbed environments, transgressive segregation can potentially provide the necessary genotypic and/or phenotypic variation to exploit previously unutilized niches, thus facilitating speciation (Seehausen, 2004).

CHAPTER 4

AFLP ANALYSIS OF THE *ANAXYRUS AMERICANUS* GROUP

Introduction

Molecular data allow for genome-wide analysis of current and historical hybridization events. Mitochondrial DNA can provide evidence of uniparental mitochondrial introgression between species, while nuclear DNA can be used to directly examine biparental genetic exchange between species. This information would be useful to determine the extent to which natural hybridization affects species in the *Anaxyrus americanus* group. If molecular analyses indicate that natural hybridization is uncommon in current populations, then patterns of morphological and molecular variation could be attributed to a number of possible factors such as: 1) historical hybridization events leading to rapid speciation, 2) incomplete lineage sorting among recently diverged species, 3) conserved morphology resulting in widespread morphological homoplasy, or a combination of these factors. Conversely, if molecular analyses indicate that natural hybridization is common in current populations, then high overlap in morphological variation and low levels of genetic differentiation between species would be expected to occur as admixture would have a homogenizing effect on the phenotype and genotype of hybridizing populations.

The use of molecular evidence allows for evaluation of the above hypotheses regarding the radiation of the *Anaxyrus americanus* group. Mitochondrial DNA has previously been used to examine the phylogenetic relationships in this group, however a consensus of the exact interspecific relationships and species boundaries has been elusive as the *A. americanus* group appears to be the most recently evolved group of toads in North America with the split between *A. americanus* and *A. fowleri* estimated to have occurred as recently as 2 million years ago (Masta et al., 2002; Pauly et al., 2004). Additionally, there appears to be relatively little interspecific variation in the mitochondrial markers previously used (Masta et al., 2002; Pauly et al., 2004). The mitochondrial markers used (cytochrome *b*, 12S, 16S, and intervening and adjacent tRNAs) appear to evolve slowly in this group, resulting in satisfactory resolution of deep relationships and uncertainty in the relationships of more recently evolved species (Fontenot, 2003; Frost et al., 2006; Masta et al., 2002; Pauly et al., 2004). Owing to the recent radiation of the *A. americanus* group, rapidly evolving nuclear genes will likely provide molecular markers more suitable to uncovering cryptic molecular variation and a better understanding of the evolutionary history of these toads.

Masta et al. (2002) and Vogel and Johnson (2008) found a small number of *Anaxyrus woodhousii, A. fowleri* and *A. americanus* that did not group with putative conspecifics in phylogenetic analyses using mtDNA, and they attributed these discrepancies to mitochondrial introgression from natural hybridization. However, this hypothesis was not tested using a biparentally inherited marker such as nuclear DNA. One method of generating large numbers of nuclear DNA markers is the Amplified Fragment Length Polymorphism (AFLP) technique developed by Vos et al. (1995). The AFLP technique involves using restriction endonuclease digestion of genomic DNA along with selective Polymerase Chain Reaction (PCR) amplifications to rapidly generate a large number of markers, distributed throughout the nuclear genome (Bensch and Akesson, 2005; Meudt and Clarke, 2006; Vos et al., 1995). This provides a unique, anonymous multilocus DNA fingerprint for each individual, and the alleles can then be visualized and scored for presence/absence in individuals and frequency in populations (Bensch and Akesson, 2005; Bonin et al., 2007; Meudt and Clarke, 2006). This technique is useful in studying species in which hybridization occurs frequently as it generates markers that are biparentally inherited, highly variable, and possibly species-specific (Meudt and Clarke, 2006; Ruegg, 2008). AFLP techniques have been used to examine genetic variation, population structure, and hybridization in birds (Roussot et al., 2003; Ruegg, 2008), fish (Poompuang and Na-Nakorn, 2004), insects (Tan et al., 2001; Zhong et al., 2003, 2004; Demuth and Wade, 2007), and plants (Rieseberg et al., 1999; Lotti et al., 2000; Oliver et al., 2001). Additionally, AFLP methods do not require *a priori* knowledge of sequences and can be used as an exploratory technique for species such as toads, that do not have well-documented genomes or established molecular markers (Bonin et al., 2007; Meudt and Clarke, 2006; Ruegg, 2008).

The AFLP method generates data that are amenable to several types of statistical analyses shown to be useful in studies of natural hybridization (Bensch and Akesson, 2005; Bonin et al., 2007; Ruegg, 2008). Principal coordinates analysis (PCoA) is a multivariate technique suitable for use with presence/absence data such as that in an AFLP DNA profile. Analyzing the binary DNA profile matrices using PCoA will allow for coarse visualization of the genetic affinities of individual species and populations

(Meudt and Clarke, 2006). PCoA can also be used to identify putative hybrids, as hybrids should occupy a position intermediate to that of the putative parental species in multivariate space.

AFLP data can also be used to assess population structure from genetic data. Traditionally, metrics such as Wright's F_{ST} and techniques such as analysis of molecular variance (AMOVA) have been used to characterize population structure, but these techniques require the use of *a priori*, subjective information about predefined populations (Bonin et al., 2007; Reeves and Richards, 2009). AFLP data are amenable to Bayesian and non-Bayesian clustering techniques that identify population structure directly from the polymorphic genetic profiles, thus removing the subjectivity associated with identifying intraspecific population structure (Bonin et al., 2007).

Additional information obtained from AFLP analyses include estimates of population-level genetic diversity using Bayesian approaches to estimate allelic frequencies from dominant AFLP markers (Krauss, 2000; Holsinger et al., 2002; Zhivotovsky, 1999), and tree-based estimates of phylogenetic relationships using AFLP data to uncover cryptic variation from throughout the nuclear genome (Barluenga et al., 2006; Bonin et al., 2007; Koopman, 2005; Pelser et al., 2003). Furthermore, AFLP markers can potentially uncover species-specific genetic markers that can be used to identify natural hybrids from wild populations.

The objectives of this study are to use AFLP data to 1) examine genetic diversity within some members of the *Anaxyrus americanus* group, 2) evaluate the efficacy of AFLP markers to identify current and historical hybridization events, 3) document genetic subdivisions and intraspecific population structure, and 4) provide further clarification of the phylogenetic relationships in the *A. americanus* group.

Methods

Specimens in the molecular analyses were collected from Texas, Louisiana, Alabama, Arkansas, Connecticut, Minnesota, Delaware, Maine, New York, New Hampshire, Mississippi, Michigan, Florida, South Carolina, Kansas, and Oklahoma, including a transect from western Texas to eastern Louisiana traversing a suspected region of genetic exchange involving *Anaxyrus woodhousii, A. velatus,* and *A. fowleri*. Individuals collected from the field (*n* = 183) were combined with tissue samples (*n* = 48) obtained from the Peabody Museum of Natural History (HERA) at Yale University, the University of Alabama Herpetological Collection (UAHC), and the Bell Museum of Natural History (JFBM) at the University of Minnesota. Collection localities for all individuals included in the molecular analyses are given in Figure A.19. All members of the *Anaxyrus americanus* group are represented except the endangered *A. houstonensis*. Species that occur in the putative hybrid zone (*A. fowleri, A. velatus,* and *A. woodhousii*) have the largest representation. AFLP data were also obtained for the outgroup *Anaxyrus cognatus,* as this species' phylogenetic position is sister to the *A. americanus* group and provides a reference against which to evaluate genetic diversity in the *A. americanus* group (Masta et al., 2002; Pauly et al., 2004). Data obtained from each individual includes (1) tissue samples for genetic analyses, (2) morphometric measurements, and (3) GPS locality. All field-collected specimens will be deposited in the University of Texas at Arlington Amphibian and Reptile Diversity Research Center.

AFLP data were obtained following the methods of Makowsky et al. (2009). Genomic DNA was fragmented using *EcoR* I and *Mse* I restriction enzymes at 37°C for 6 hours and ligated using a T4 ligase enzyme at 16°C for 12 hours. After restriction digestion and ligation, 2 µl of the product were used in a preselective amplification with the following PCR conditions: 1) initial touchdown procedure with 1 minute at 72°C, 50 seconds at 94°C, 1 minute at 50°C, and 1 minute at 72°C, 2) 24 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 1 minute and extension at 72°C for 1 minute, and 3) a final extension at 72°C for 7 minutes. For preselective PCR, I used *EcoR* I + G and an *Mse* I + GC primers. After preselective amplification, 4 µl of preselective product underwent selective amplification using more specific, selective primers (*EcoR* I + GA primer and *Mse* I + GC primer) and the following PCR conditions: 1) initial touchdown procedure with 50 seconds at 94°C, 1 minute at 57°C, and 2 minutes at 72°C, and 2) 19 cycles of denaturation at 94°C for 15 seconds, annealing at 56°C for 1 minute and extension at 72°C for 1 minute. *EcoR* I primers were labeled with fluorescent dye (FAM) to enable visualization of fragments by the sequencing machine. Selective PCR products were purified using a standard ethanol cleanup protocol and sequenced using an ABI 3130 capillary sequencer with a Rox 400 HD size standard. Fragments were scored using the Genemarker® program and fragments less than 100 base pairs in length were removed from the data to avoid complications from homoplasy (Vekemans et al., 2002). The remaining scorable fragments were assembled into a presence/absence matrix for statistical analysis. Genemarker® software was then used to verify the fluorescence peaks for each loci in each individual with peaks that received a threshold value less than 100 being identified as absent and peaks receiving a threshold value greater than 100 and showing a distinct peak being identified as present.

Statistical analyses

Analyses were first carried out on all species (except *Anaxyrus hemiophrys* because only one individual could be genotyped for AFLP markers) to document genetic diversity and intraspecific population structure. Analyses were also carried out for all pairwise interspecific comparisons to evaluate genetic diversity, interspecific population structure, and possible hybridization among species. The program GENALEX 6.2 (Peakall and Smouse, 2006) was used to create a matrix of genetic distances using Nei's D (Nei, 1972), conduct Principal Coordinate analyses, perform Mantel tests for Isolation-by-Distance, and perform AMOVA analyses both within species at the subpopulation level and for interspecific pairwise comparisons.

Measures of genetic diversity and population structure were obtained using the program HICKORY 1.0 (Holsinger et al., 2002) using the *f*-free, full model option. AFLP markers are scored as dominant characters thus making direct observation of heterozygosity impossible. To counter this, HICKORY 1.0 (Holsinger et al., 2002) implements a Bayesian estimation for diploid species assuming Hardy-Weinberg equilibrium and a non-uniform prior distribution of allelic frequencies following the methods of Zhivotovsky (1999). This approach uses the observed fragment frequencies to produce robust Bayesian estimates of allelic frequencies, heterozygosity (H), and genetic differentiation (F_{ST}). These estimates have been shown to be accurate, despite the fact that they are estimated from dominant markers (Krauss, 2000). Estimated heterozygosity (H_T) is the Bayesian analog of average heterozygosity (H) among individuals (Holsinger et al, 2002). Genetic differentiation of populations was estimated using the F_{ST} analog θ^B described in Holsinger et al. (2002). The Shannon Information Index (I), a measure of genetic diversity, Nei's G_{ST} (a measure of genetic differentiation between populations analogous to F_{ST} and θ^B), and N_M (the estimated number of migrants between populations as a measure of gene flow) were calculated using the program POPGENE (Yeh and Boyle, 1997). The program TFPGA (Miller, 1997) was used to create a UPGMA tree of relationships among species with 10,000 bootstrap replicates to measure nodal support.

The Principal Coordinate-Modal Clustering (PCO-MC) technique of Reeves and Richards (2009) was used to objectively identify clusters of distinct population structure within species. The identification of cryptic population structure using dominant AFLP markers has traditionally been achieved using Bayesian techniques that objectively identify population structure from genetic polymorphism data (Pritchard et al., 2000; Reeves and Richards, 2009). However, these methods can be limiting because they require *a priori* knowledge of the number of subpopulations in the data and determining this number often requires multiple, time-intensive analyses before reliable results can be produced (Reeves and Richards, 2009).

Reeves and Richards (2009) describe a technique that utilizes Principal Coordinates Analysis coupled with a "modal clustering" density estimation procedure to analyze population structure. PCO-MC is an accurate, Bayesian model-free method requiring far less computational time. Additionally, the PCO-MC technique does not require *a priori* knowledge of the number of subpopulations as the valley-seeking density estimation algorithm employed in the modal clustering technique is capable of identifying population structure without prior knowledge of the number of populations. This technique thus provides an objective method by which to evaluate not only the number of subpopulations, but also the assignment of individuals to subpopulations. Reeves and Richards (2009) show that this technique actually outperforms the Bayesian methods of Pritchard et al. (2000) when the number of loci examined is high (e.g. ≥ 100). Following the methods of Reeves and Richards (2009), the program NTSYS 2.2 (Exeter Software) was used to generate principal coordinate values and the modal clustering procedure was performed using the MODECLUS package in SAS 9.1 (SAS Institute, Cary, NC).

Clusters were identified as significant if they returned a stability value (a statistic calculated by the MODECLUS package as a metric that approximates the percentage of informative multivariate space where clusters are found) greater than 15 as values higher than this indicate true population structure in both simulation studies and real data (Reeves and Richards, 2009). The clusters identified using the PCO-MC analysis were used to divide each species into subpopulations. It should be noted that clusters can include individuals assigned to multiple clusters, possibly indicating hybridization or species misidentification. The resulting subpopulations were then used in an AMOVA to evaluate the levels of genetic diversity within species and between subpopulations.

Various authors have long considered the deciduous hardwood forests of southern Oklahoma, eastern Texas and western Louisiana a region of possible genetic interchange between the species *Anaxyrus woodhousii, A. velatus, A. fowleri,* and *A. charlesmithi*

(A.P. Blair, 1941; W.F. Blair, 1956, 1959, 1961, 1963a, 1963b, 1964, 1966, 1972; Masta et al., 2002; Vogel and Johnson, 2008; Volpe, 1952, 1959). In order to evaluate levels of introgression due to past or present hybridization, it is necessary to obtain estimates of genetic differentiation and gene flow among and within populations occurring parapatrically or sympatrically (Lampert et al., 2003; McDermott and McDonald, 1993). I estimated the pairwise genetic differentiation (G_{ST}) and gene flow (N_M) for all pairwise species comparisons, and further subdivided the species into groups comprised of sympatric species from the putative hybrid zone region (*A. woodhousii, A. velatus, A. fowleri,* and *A. charlesmithi*), sympatric species with similar male advertisement calls from the putative hybrid zone region (*A. woodhousii, A. velatus,* and *A. fowleri*), and allopatric species with similar male advertisement calls (*A. americanus, A. charlesmithi,* and *A. terrestris*) in order to evaluate the amount of gene flow and population structure between populations while considering both geographic location and reproductive behavior.

Results

The AFLP technique was applied to 231 individuals with 178 of these individuals showing sufficient amplification to allow genotype analysis (Appendix B). One additional specimen of *Anaxyrus hemiophrys* was also successfully amplified, but was excluded from the analyses, as it was the only individual of that species for which data was obtained, and could not be used in subsequent analyses. After editing of the multilocus profiles, 100 scorable loci were obtained for 178 individuals and 100% of these loci were polymorphic (Table A.15).

An overall summary of genetic diversity as well as the genetic diversity for each individual species are given in Table A.15. The Shannon Information Index (*I*), a measure of gene diversity, ranged from 0.31 to 0.55, the Bayesian-estimated average heterozygosity (H_T) ranged from 0.30 to 0.41, the Bayesian-estimated genetic differentiation (θ^B) ranged from 0.09 to 0.23, and the percentage of polymorphic loci ranged from 59% to 93%. Pairwise genetic distances (Table A.16) between species ranged from 0.02 (*Anaxyrus fowleri* to *A. velatus*) to 0.46 (*A. fowleri* to *A. cognatus*). Pairwise differences in θ^B are given in Table A.16 with the only significant difference occurring between *A. americanus* and *A. cognatus*. Pairwise G_{ST} ranged from 0.02 to 0.33 and pairwise N_M ranged from 1.0 to 21.1 (Table A.17). Figure A.20 depicts the results of the UPGMA cluster analysis of the relationships among species, as calculated by pairwise genetic distance (Nei's *D*) with 10,000 bootstrap replicates as a measure of nodal support.

Intraspecific analyses

Anaxyrus americanus

Table A.15 summarizes the genetic diversity in *Anaxyrus americanus*. Principal Coordinates Analysis (PCoA) revealed that 66.4% of the variance in the AFLP data could be explained by the first three eigenvalues (Table A.18). A scatterplot of each individual's position according to its loadings on the first three axes are given in Figure A.21. Principal Coordinate-Modal Clustering (PCO-MC) analysis revealed distinct population structure with three clusters, one unique cluster (stability value = 90) and two clusters (stability values = 38 and 17) with overlapping membership (Table A.19). An

AMOVA performed on these three clusters was significant, and revealed that variation among subpopulations accounted for 47% of the variance and variation within subpopulations accounted for 53% of the variance (Table A.20). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.43$, $P < 0.01$) indicating isolation-by-distance among subpopulations.

Anaxyrus charlesmithi

Table A.15 summarizes the genetic diversity in *Anaxyrus charlesmithi*. PCoA revealed that 72.9% of the variance in the AFLP data could be explained by the first three eigenvalues (Table A.18). A scatterplot of each individual's position according to its loadings on the first three axes are given in Figure A.22. PCO-MC analysis revealed distinct population structure with four clusters, one unique cluster (stability value = 36) and three clusters (stability values = 33, 29, and 17) with overlapping membership (Table A.19). An AMOVA performed on these four clusters was significant, and revealed that variation among subpopulations accounted for 24% of the variance and variation within subpopulations accounted for 76% of the variance (Table A.20). A Mantel test between genetic and geographic distance matrices was not significant ($r = -0.01$, $P < 0.53$) indicating that there is no isolation-by-distance among subpopulations.

Anaxyrus cognatus

Table A.15 summarizes the genetic diversity in *Anaxyrus cognatus*. PCoA revealed that 68% of the variance in the AFLP data could be explained by the first three eigenvalues (Table A.18). A scatterplot of each individual's position according to its loadings on the first three axes are given in Figure A.23. PCO-MC analysis could not detect significant population structure, so the Mantel test and AMOVA were not performed on *A. cognatus* (Table A.19).

Anaxyrus fowleri

Table A.15 summarizes the genetic diversity in *Anaxyrus fowleri*. PCoA revealed that 70.9% of the variance in the AFLP data could be explained by the first three eigenvalues (Table A.18). A scatterplot of each individual's position according to its loadings on the first three axes are given in Figure A.24. PCO-MC analysis revealed distinct population structure with three clusters reaching a significant stability value, one unique cluster (stability value = 30) and two clusters (stability values = 26 and 24) with overlapping membership (Table A.19). An AMOVA performed on these three clusters was significant, and revealed that variation among subpopulations accounted for 44% of the variance and variation within subpopulations accounted for 56% of the variance (Table A.20). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.39$, $P < 0.01$) indicating isolation-by-distance among subpopulations.

Anaxyrus terrestris

Table A.15 summarizes the genetic diversity in *Anaxyrus terrestris*. PCoA revealed that 68.1% of the variance in the AFLP data could be explained by the first three eigenvalues (Table A.18). A scatterplot of each individual's position according to its loadings on the first three axes are given in Figure A.25. PCO-MC analysis could not detect significant population structure, so the Mantel test and AMOVA were not performed on *A. terrestris* (Table A.19).

Anaxyrus velatus

Table A.15 summarizes the genetic diversity in *Anaxyrus velatus*. PCoA revealed that 65.6% of the variance in the AFLP data could be explained by the first three eigenvalues (Table A.18). A scatterplot of each individual's position according to its loadings on the first three axes are given in Figure A.26. PCO-MC analysis could not detect significant population structure, so the Mantel test and AMOVA were not performed on *A. velatus* (Table A.19).

Anaxyrus woodhousii

Table A.15 summarizes the genetic diversity in *Anaxyrus woodhousii*. PCoA revealed that 66.5% of the variance in the AFLP data could be explained by the first three eigenvalues (Table A.18). A scatterplot of each individual's position according to its loadings on the first three axes are given in Figure A.27. PCO-MC analysis revealed distinct population structure with two clusters reaching a significant stability value, no unique clusters and two clusters (stability values = 29 and 20) with overlapping membership (Table A.19). An AMOVA performed on these two clusters was significant, and revealed that variation among subpopulations accounted for 30% of the variance and variation within subpopulations accounted for 70% of the variance (Table A.20). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.36$, $P < 0.01$) indicating isolation-by-distance among subpopulations.

Interspecific analyses

Anaxyrus cognatus x *Anaxyrus americanus* group

Pairwise genetic distances and difference in θ^B between *Anaxyrus cognatus* and the 6 members of the *A. americanus* group are given in Table A.16. Overall, the individual members of the *A. americanus* group were most divergent in genetic distance when compared to *A. cognatus*. Additionally, the only significant difference in θ^B was between *A. americanus* and *A. cognatus*. PCoA was performed on all pairwise comparisons of *Anaxyrus cognatus* and the 6 members of the *A. americanus* group individually, and the results of these analyses are given in Table A.21. Scatterplots of each individual's position according to its loadings on the first three coordinate axes are given in Figures A.28, A.29, A.30, A.31, A.32, and A.33. Pairwise comparisons between *A. cognatus* and all members of the *A. americanus* group for G_{ST} and N_M showed high genetic differentiation and low gene flow. These results are given in Table A.17. *A. cognatus* was distinct from all other members of the *A. americanus* group according to PCoA, genetic distance, G_{ST} and N_M. These results confirm the sister group relationship between *A. cognatus* and the *A. americanus* group, and provide a baseline with which to evaluate the magnitude of differences in genetic diversity and admixture in interspecific comparisons within the *A. americanus* group.

Anaxyrus americanus x Anaxyrus charlesmithi

Pairwise genetic distances and difference in θ^B between *Anaxyrus americanus* and *A. charlesmithi* are given in Table A.16. Overall, *A. americanus* and *A. charlesmithi* are moderately distinct from one another with approximately 8% pairwise divergence and a high, but non-significant, difference in θ^B. Pairwise G_{ST} and N_M between *A. americanus* and *A. charlesmithi* show little genetic differentiation and moderately high gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.34. The two species occupy relatively distinct positions in coordinate space with overlap of one *A. americanus* and two *A. charlesmithi*. An AMOVA test was significant, with 12% of variation attributed to among population variation and 88% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.22$, $P < 0.004$) indicating isolation-by-distance among species.

Anaxyrus americanus x Anaxyrus fowleri

Pairwise genetic distances and difference in θ^B between *Anaxyrus americanus* and *A. fowleri* are given in Table A.16. Overall, *A. americanus* and *A. fowleri* are distinct from one another with approximately 20% pairwise divergence, but a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. americanus* and *A. fowleri* show moderate genetic differentiation and moderate gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.35. The two species occupy distinct positions in coordinate space with overlap of only one *A. americanus* individual. An AMOVA test was significant, with 11% of variation attributed to among population variation and 89% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.66$, $P < 0.001$) indicating isolation-by-distance among species.

Anaxyrus americanus x Anaxyrus terrestris

Pairwise genetic distances and difference in θ^B between *Anaxyrus americanus* and *A. terrestris* are given in Table A.16. Overall, *A. americanus* and *A. terrestris* are distinct from one another with approximately 10% pairwise divergence, and a relatively high, but non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. americanus* and *A. terrestris* show little genetic differentiation and moderately high gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.36. The two species occupy relatively distinct positions in coordinate space with overlap of two *A. terrestris* and two *A. americanus*. An AMOVA test was significant, with 16% of variation attributed to among population variation and 84% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.39$, $P < 0.001$) indicating isolation-by-distance among species.

Anaxyrus americanus x Anaxyrus velatus

Pairwise genetic distances and difference in θ^B between *Anaxyrus americanus* and *A. velatus* are given in Table A.16. Overall, *A. americanus* and *A. velatus* are distinct from one another with approximately 20% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. americanus* and *A. velatus* show moderate genetic differentiation and moderate gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.37. The two species occupy distinct positions in coordinate space with overlap of only two *A. velatus*. An AMOVA test was significant, with 6% of variation attributed to among population variation and 94% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.65$, $P < 0.001$) indicating isolation-by-distance among species.

Anaxyrus americanus x Anaxyrus woodhousii

Pairwise genetic distances and difference in θ^B between *Anaxyrus americanus* and *A. woodhousii* are given in Table A.16. Overall, *A. americanus* and *A. woodhousii* are distinct from one another with approximately 18% pairwise divergence, and a relatively high, but non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. americanus* and *A. woodhousii* show moderate genetic differentiation and moderate gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.38. The two species occupy distinct positions in coordinate space with overlap of two *A. woodhousii* and one *A. americanus*. An AMOVA test was significant, with 14% of variation attributed to among population variation and 86% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.60$, $P < 0.001$) indicating isolation-by-distance among species.

Anaxyrus charlesmithi x Anaxyrus fowleri

Pairwise genetic distances and difference in θ^B between *Anaxyrus charlesmithi* and *A. fowleri* are given in Table A.16. Overall, *A. charlesmithi* and *A. fowleri* are distinct from one another with approximately 11% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. charlesmithi* and *A. fowleri* show little genetic differentiation and moderately high gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.39. Several individuals occupy the same region in coordinate space with large areas of overlap. Both *A. charlesmithi* and *A. fowleri* also displayed distinct groups of non-overlapping individuals sampled from more geographically distant localities. An AMOVA test was significant, with 14% of variation attributed to among population variation and 86% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.21$, $P < 0.03$) indicating isolation-by-distance among species.

Anaxyrus charlesmithi x Anaxyrus terrestris

Pairwise genetic distances and difference in θ^B between *Anaxyrus charlesmithi* and *A. terrestris* are given in Table A.16. Overall, *A. charlesmithi* and *A. terrestris* are moderately distinct from one another with approximately 9% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. charlesmithi* and *A. terrestris* show little genetic differentiation and moderately high gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.40. Several individuals occupy the same region in coordinate space with large areas of overlap. Both *A. charlesmithi* and *A. terrestris* also displayed distinct groups of non-overlapping individuals sampled from more geographically distant localities. An AMOVA test was significant, with 11% of variation attributed to among population variation and 89% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.14$, $P < 0.04$) indicating isolation-by-distance among species.

Anaxyrus charlesmithi x Anaxyrus velatus

Pairwise genetic distances and difference in θ^B between *Anaxyrus charlesmithi* and *A. velatus* are given in Table A.16. Overall, *A. charlesmithi* and *A. velatus* are moderately distinct from one another with approximately 9% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. charlesmithi* and *A. velatus* show little genetic differentiation and moderately high gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.41. Several individuals occupy the same region in coordinate space with large areas of overlap. Both *A. charlesmithi* and *A. velatus* also displayed distinct groups of non-overlapping individuals sampled from more geographically distant localities. An AMOVA test was not significant (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.22$, $P < 0.005$) indicating isolation-by-distance among species.

Anaxyrus charlesmithi x Anaxyrus woodhousii

Pairwise genetic distances and difference in θ^B between *Anaxyrus charlesmithi* and *A. woodhousii* are given in Table A.16. Overall, *A. charlesmithi* and *A. woodhousii* are moderately distinct from one another with approximately 8% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. charlesmithi* and *A.*

woodhousii show little genetic differentiation and moderately high gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.42. Several individuals occupy the same region in coordinate space with large areas of overlap. Both *A. charlesmithi* and *A. woodhousii* also displayed distinct groups of non-overlapping individuals sampled from more geographically distant localities. An AMOVA test was significant, with 9% of variation attributed to among population variation and 91% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.23$, $P < 0.014$) indicating isolation-by-distance among species.

Anaxyrus fowleri x Anaxyrus terrestris

Pairwise genetic distances and difference in θ^B between *Anaxyrus fowleri* and *A. terrestris* are given in Table A.16. Overall, *A. fowleri* and *A. terrestris* are moderately distinct from one another with approximately 8% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. fowleri* and *A. terrestris* show little genetic differentiation and moderately high gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.43. Several individuals occupy the same region in coordinate space with large areas of overlap. Both *A. fowleri* and *A. terrestris* also displayed distinct groups of non-overlapping individuals sampled from multiple localities. An AMOVA test was significant, with 15% of variation attributed to among population variation and 85% attributed to within population variation (Table A.23). A

Mantel test between genetic and geographic distance matrices was significant ($r = 0.27$, $P < 0.004$) indicating isolation-by-distance among species.

Anaxyrus fowleri x Anaxyrus velatus

Pairwise genetic distances and difference in θ^B between *Anaxyrus fowleri* and *A. velatus* are given in Table A.16. Overall, *A. fowleri* and *A. velatus* are genetically similar to one another with approximately 2% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. fowleri* and *A. velatus* show virtually no genetic differentiation and the highest rate of gene flow for any species comparison (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.44. Most individuals occupy the same region in coordinate space with large areas of overlap. The overlapping individuals were sampled from geographically proximate locations. *A. fowleri* also displayed a small, but distinct group of non-overlapping individuals sampled from more geographically distant localities. An AMOVA test was significant, with 3% of variation attributed to among population variation and 97% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.31$, $P < 0.001$) indicating isolation-by-distance among species.

Anaxyrus fowleri x Anaxyrus woodhousii

Pairwise genetic distances and difference in θ^B between *Anaxyrus fowleri* and *A. woodhousii* are given in Table A.16. Overall, *A. fowleri* and *A. woodhousii* are genetically similar to one another with approximately 4% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. fowleri* and *A.*

woodhousii show virtually no genetic differentiation and a very high rate of gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.45. Several individuals occupy the same region in coordinate space with large areas of overlap. The overlapping individuals were comprised of five *A. woodhousii* and four *A. fowleri* sampled from geographically proximate locations. Both *A. fowleri* and *A. woodhousii* also displayed distinct groups of non-overlapping individuals sampled from more geographically distant localities. An AMOVA test was significant, with 5% of variation attributed to among population variation and 95% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.38$, $P < 0.001$) indicating isolation-by-distance among species.

Anaxyrus terrestris x Anaxyrus velatus

Pairwise genetic distances and difference in θ^B between *Anaxyrus terrestris* and *A. velatus* are given in Table A.16. Overall, *A. terrestris* and *A. velatus* are distinct from one another with approximately 11% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. terrestris* and *A. velatus* show little genetic differentiation and moderately high gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.46. Several individuals occupy the same region in coordinate space with small areas of overlap. The overlapping individuals were comprised of two *A. terrestris* and four *A. velatus* sampled from geographically proximate locations. Both *A. terrestris* and *A. velatus* also displayed distinct groups of non-overlapping individuals sampled from more geographically distant localities. An AMOVA test was not significant (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.42$, $P < 0.001$) indicating isolation-by-distance among species.

Anaxyrus terrestris x Anaxyrus woodhousii

Pairwise genetic distances and difference in θ^B between *Anaxyrus terrestris* and *A. woodhousii* are given in Table A.16. Overall, *A. terrestris* and *A. woodhousii* are moderately distinct from one another with approximately 9% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. terrestris* and *A. woodhousii* show little genetic differentiation and moderately high gene flow (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.47. Several individuals occupy the same region in coordinate space with small areas of overlap. The overlapping individuals were comprised of two *A. woodhousii* and four *A. terrestris* sampled from geographically proximate locations. Both *A. terrestris* and *A. woodhousii* also displayed distinct groups of non-overlapping individuals sampled from more geographically distant localities. An AMOVA test was significant, with 6% of variation attributed to among population variation and 94% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.39$, $P < 0.001$) indicating isolation-by-distance among species.

Anaxyrus velatus x Anaxyrus woodhousii

Pairwise genetic distances and difference in θ^B between *Anaxyrus velatus* and *A. woodhousii* are given in Table A.16. Overall, *A. velatus* and *A. woodhousii* are genetically similar to one another with approximately 4% pairwise divergence, and a non-significant difference in θ^B. Pairwise G_{ST} and N_M between *A. velatus* and *A. woodhousii* show very little genetic differentiation and the second highest rate of gene flow of any species comparison (Table A.16). The results of the PCoA are given in Table A.22 and scatterplots of each individual's loadings on the first three axes are given in Figure A.48. Most individuals occupy the same region in coordinate space with large areas of overlap. The overlapping individuals were comprised of individuals sampled from geographically proximate locations. Both *A. velatus* and *A. woodhousii* also displayed distinct groups of non-overlapping individuals sampled from more geographically distant localities. An AMOVA test was significant, with 7% of variation attributed to among population variation and 93% attributed to within population variation (Table A.23). A Mantel test between genetic and geographic distance matrices was significant ($r = 0.23$, $P < 0.009$) indicating isolation-by-distance among species.

Additional comparisons

Analysis of the species that occur sympatrically and/or parapatrically in the putative hybrid zone (*Anaxyrus woodhousii, A. fowleri, A. velatus,* and *A. charlesmithi*) reveals a G_{ST} value of 0.08 indicating little genetic differentiation and an N_M value of 5.4 indicating moderately high gene flow among populations. Analysis of the species that possess similar male advertisement calls and occur sympatrically and/or parapatrically (*A.*

woodhousii, A. fowleri, A. velatus) reveals a G_{ST} value of 0.04 indicating very little genetic differentiation and an N_M value of 10.8 indicating high levels of gene flow among populations. Analysis of the species that possess similar male advertisement calls and occur allopatrically (*A. americanus, A. charlesmithi,* and *A. terrestris*) reveals a G_{ST} value of 0.11 indicating moderate genetic differentiation and an N_M value of 4.0 indicating moderate levels of gene flow among populations.

Discussion

AFLP analysis of genetic variation at 100 nuclear loci reveals many interesting patterns with regard to genetic diversity in the *Anaxyrus americanus* group. Patterns of intraspecific genetic diversity as well as interspecific genetic diversity, including the outgroup species (*A. cognatus*), are summarized below.

Anaxyrus cognatus

PCO-MC analysis could not detect significant population structure in this species, suggesting that *Anaxyrus cognatus* is a cohesive species. This is likely due to the limited geographic range from which individuals were sampled. However, *A. cognatus* is included in these analyses to provide a baseline against which to evaluate the magnitude of differences in intra- and interspecific genetic diversity among the closely related members of the *A. americanus* group. *A. cognatus* was genetically differentiated from the *A. americanus* group with an average N_M value of 1.1 and pairwise genetic distance ranging from 31–48%. Additionally, 41% of the 100 loci were fixed in *A. cognatus*, while the average percentage of fixed loci for the *A. americanus* group was approximately 12%, providing clear distinction of *A. cognatus* from the *A. americanus* group.

Anaxyrus americanus

This species has significant intraspecific population structure with three clusters identified by PCO-MC analysis, a significant AMOVA test when divided into subpopulations, and the highest θ^B of all the species, suggesting that *Anaxyrus americanus* is comprised of genetically discrete subpopulations. Additionally, the Mantel test of geographic versus genetic distances indicates significant isolation by distance among subpopulations. These subpopulations are geographically cohesive, with one cluster occurring in the northeastern United States and the other two clusters occurring in the northern Midwest region of the United States. *A. americanus* appears to be most closely allied with *A. charlesmithi* and *A. terrestris* with an average N_M value of 5.4 and pairwise genetic distance ranging from 8–10% (Table A.16). All three species were collected from allopatric regions; however, these species do share a similar male advertisement call. *A. americanus* is differentiated from the remaining species, *A. woodhousii, A. fowleri,* and *A. velatus* with an average N_M value of 2.8 and pairwise genetic distance ranging from 18–20% (Table A.16). *A. americanus* and *A. fowleri* occur sympatrically over most of their respective distributions, but their differing male advertisement calls, and the relatively long time since they shared a common ancestor are likely reasons for their differentiation in these analyses. Green (1984) documented natural hybridization between *A. americanus* and *A. fowleri* using allozyme data, and concluded that introgression occurred so infrequently that it likely did not interfere with the maintenance of discrete gene pools. Green and Parent (2003) also document evidence of past hybridization between these two species in a "localized sympatric" hybrid zone similar to that described by Woodruff (1973). This suggests that hybridization between these two species is sporadic in nature, and variable in both temporal and spatial occurrence agreeing with the results of Green and Parent (2003).

Anaxyrus charlesmithi

This species has significant intraspecific population structure with four clusters identified by PCO-MC analysis, a significant AMOVA test when divided into subpopulations, and a θ^B value of 0.13, suggesting that *Anaxyrus charlesmithi* is comprised of genetically discrete subpopulations connected by relatively high levels of intraspecific and interspecific gene flow. The Mantel test was not significant, indicating no isolation by distance among subpopulations, which is likely due to the fact that the putative geographic range of *A. charlesmithi* is relatively restricted (Figure A.1). *A. charlesmithi* appears to share genetic material with all of the other species in the *A. americanus* group with an average N_M value of 5.8 (ranging from 5.6 to 6.5) and pairwise genetic distances ranging from only 8–11% (Table A.16). One possible explanation for this low level of differentiation with other species is that the range of *A. charlesmithi* is surrounded on all sides by at least one member of the *A. americanus* group (Figure A.1), and it is possible that this species is acting as a "genetic sink", acquiring genetic material from its sympatric and parapatric congeners. Another possible explanation is that the traditional descriptions of *A. charlesmithi* and its range are incorrect and some of the individuals referred to *A. charlesmithi* in this study are, in fact, a different species within the *A. americanus* group.

Anaxyrus fowleri

This species has significant intraspecific population structure with three clusters identified by PCO-MC analysis, a significant AMOVA test when divided into subpopulations, and a θ^B value of 0.16, suggesting that *Anaxyrus fowleri* is comprised of genetically discrete subpopulations. Additionally, the Mantel test of geographic versus genetic distances indicates significant isolation by distance among subpopulations. The subpopulations are relatively geographically cohesive with one unique cluster of northeastern individuals and two clusters of southern and southeastern individuals. *A. fowleri* is most closely allied with species that share a similar male advertisement call (*A. woodhousii* and *A. velatus*), while it appears to be more genetically differentiated from sympatric *A. americanus* and *A. terrestris,* and parapatric *A. charlesmithi*. The average N_M value between *A. fowleri*, *A. woodhousii,* and *A. velatus* is 15.3, while the average N_M value between *A. fowleri*, *A. americanus, A. charlesmithi* and *A. terrestris* is 4.8, suggesting that *A. fowleri* has a higher rate of gene flow with species that possess similar advertisement calls and/or exist in close geographic proximity. There are virtually no morphological differences between *A. fowleri* from Louisiana and *A. velatus* from eastern Texas (Fontenot, 2003), and it is possible that some individuals referred to either *A. velatus* or *A. fowleri* in this study could belong to the other species or all individuals in this geographic region could belong to one previously unrecognized species. This could explain the unusually high rate of gene flow between the two groups ($N_M = 21.1$).

Anaxyrus terrestris

This species does not possess significant intraspecific population structure with only one cluster identified by PCO-MC analysis, despite a θ^B value of 0.14, which does suggest moderate genetic structure. It is possible that genetic diversity within *Anaxyrus terrestris* is not great enough to be detected by the PCO-MC technique; however, PCoA scatterplots suggest at least two populations may be identified (Figure A.25). *A. terrestris* appears to be related equally to the other members of the *A. americanus* group in a manner similar to that of *A. charlesmithi*, with an average N_M value of 5.6 (ranging from 4.7 to 6.5) and pairwise genetic distances ranging from only 8–11% (Table A.16). *A. terrestris* may also play the role of "genetic sink" for other members of the *A. americanus* group. It is unlikely that *A. terrestris* could be misidentified, as this species possesses hypertrophied postorbital crests, a diagnostic morphological feature that readily separates it from other members of the *A. americanus* group. Of course, this morphological distinctiveness does not preclude the possibility of historic or ongoing introgression from other species.

Anaxyrus velatus

This species does not possess significant intraspecific population structure with only one cluster identified by PCO-MC analysis, despite a relatively high θ^B value of 0.19, which does suggest moderate genetic structure. It is likely that the putative range of this species is too small (Figure A.1) to result in sufficient differentiation among populations to be detected by PCO-MC analysis. *Anaxyrus velatus* is most closely allied with species that possess a similar male advertisement call and occur in close geographic proximity (*A. woodhousii* and *A. fowleri*) with an average N_M value of 12.4 and pairwise genetic distances ranging from only 2–3%. *A. terrestris* and *A. charlesmithi* are slightly more genetically differentiated with an average N_M value of 5.7 and pairwise genetic distances ranging from 9–10%. *A. velatus* is most genetically differentiated from *A. americanus*, an allopatric species with a different male advertisement call, with an N_M value of 2.8 and a pairwise genetic distance of 20%. A potential confounding factor that could explain the high rate of gene flow between *A. velatus* and *A. fowleri* is the aforementioned uncertainty regarding the identity of populations referred to *A. fowleri* in western Louisiana. Like other species in the *A. americanus* group, interspecific gene flow in *A. velatus* appears to be most common with species that occur in close geographic proximity and possess similar male advertisement calls.

Anaxyrus woodhousii

This species has significant intraspecific population structure with two clusters identified by PCO-MC analysis, a significant AMOVA test when divided into subpopulations, and a θ^B value of 0.14, suggesting that *Anaxyrus woodhousii* is composed of genetically discrete subpopulations. These populations are geographically cohesive with one cluster comprised of a small number of individuals from western Texas and the other containing individuals from north-central Texas, Oklahoma, and Missouri. Additionally, the Mantel test indicated significant isolation by distance among subpopulations. This subdivision agrees with that found in a previous analysis of the population genetic structure of *A. woodhousii* conducted by Masta et al. (2003), as well as the traditional recognition of a southwestern subspecies of *A. woodhousii* (*A.*

woodhousii australis). *A. woodhousii* is also most closely related to *A. fowleri* and *A. velatus*; species with similar male advertisement calls. These species share an average N_M value of 12.4 and pairwise genetic distances of approximately 4%. *A. woodhousii* is more differentiated from *A. americanus, A. charlesmithi*, and *A. terrestris* with an average N_M value of 4.9 and pairwise genetic distances ranging from 8–18%. Again, similar male advertisement call and geographic proximity appear to be the primary determinant of interspecific gene flow in *A. woodhousii*.

Genetic diversity in the *Anaxyrus americanus* group

The patterns of genetic diversity seen in the *Anaxyrus americanus* group are not surprising given the relatively young age of the group. Pauly et al. (2004) found this group to be the most recent radiation of Nearctic bufonids, and Masta et al. (2002) estimate the split between *A. americanus* and *A. fowleri* to have occurred as recently as two million years ago. Additionally, the *A. americanus* group was affected by Pleistocene glaciation, and likely inhabited refugia in the southern United States as recently as a few thousand years ago (Blair, 1972 and Masta et al., 2003). Postzygotic reproductive isolation between these species is incomplete (Blair, 1972; Malone and Fontenot, 2008), and isolation in allopatric areas and/or isolation through prezygotic barriers (such as differing male advertisement calls) appear to be the primary determinants of genetic differentiation between members of the *A. americanus* group.

Evolutionary relationships in the *Anaxyrus americanus* group

AFLP analysis of the *Anaxyrus americanus* group does not support the same evolutionary relationships among species supported by other types of data. For example, UPGMA analysis of these data (Figure A.20) results in an evolutionary tree that differs from that generated using mtDNA (Figure A.2). The conflict between these two sources likely arises from the rapid radiation of the *A. americanus* group coupled with historic and possibly ongoing gene flow between populations.

Discordance between mtDNA and nuclear DNA could also be attributed to sex-biased dispersal within species (Petit and Excoffier, 2009). If dispersal were sex-biased, discordance between biparental and uniparental genetic markers is expected. For example, if dispersal were female-biased, then biparentally inherited nuclear markers will introgress more frequently than uniparentally inherited mtDNA marker, and the opposite would be true if dispersal were male-biased (Petit and Excoffier, 2009). In either case, examining genetic markers in the farthest-dispersing sex provides more accurate delimitation of species (Petit and Excoffier, 2009). Owing to their male polygynous reproductive system, where female choice determines mating success rather than male-male resource competition, toads are expected to have male-biased dispersal (Smith and Green, 2006). If dispersal in toads is male-biased, then nuclear markers should provide more accurate delimitation of species boundaries. However, Smith and Green (2006) found no sex-biased dispersal among *Anaxyrus fowleri* from Canada. More research is required to determine whether or not sex-biased dispersal exists in other members of the *A. americanus* group before any conclusions can be made regarding the possible relationship between asymmetric dispersal and discordance between nuclear and mtDNA evidence.

The lack of support for previously identified evolutionary relationships among species coupled with clear subpopulation structure within species may indicate a recent division of one or more populations into several genetically differentiated subpopulations that persist despite recent and possibly ongoing gene flow between subpopulations. Incomplete lineage sorting among recently diverged species should result in retention of a large number of ancestral polymorphisms among species, further complicating species delimitation (Petit and Excoffier, 2009; Stelkens and Seehausen, 2009). Species in the *Anaxyrus americanus* group appear to be structured in a metapopulation framework with weakly differentiated subpopulations connected by at least some gene flow. This pattern would be consistent with one or more species recently expanding their range from Pleistocene refugia after glacial retreats and coming into secondary contact before postzygotic reproductive isolation could be achieved. Seehausen (2004) hypothesized that hybridization between closely related species preceding and during a radiation would result in a pattern of discordance between uniparentally and biparentally inherited markers and it is possible that these data reflect that pattern (see Chapter 5).

AFLP analysis of hybridization

Hybridization between species is often examined using AFLP markers (Bonin et al., 2007), and one of the objectives of this study was to evaluate the efficacy of AFLP markers in identifying current and historical hybridization events. In order to observe hybridization among species, AFLP markers must be differentially fixed among species (e.g. fixed for presence in one species and fixed for absence in the other). Hybrids can thus be identified as individuals with intermediate genotypes between two parental species with fixed diagnostic differences (Bonin et al., 2007; Meudt and Clarke, 2005). The high degree of polymorphic loci seen in the *Anaxyrus americanus* group provided little information about rates of hybridization because there were no fixed, diagnostic loci for any of the species. However, some information could still be obtained regarding hybridization in the *A. americanus* group. High levels of gene flow and genetic similarity between species that occur in close geographic proximity indicate that hybridization has likely facilitated genetic exchange between species in the past and may still continue in the present. Because members of the *A. americanus* group are all capable of producing viable and fertile hybrid offspring in interspecific crosses (Blair, 1972; Malone and Fontenot, 2008), the impact of hybridization is likely to be important. These results, coupled with the findings of other researchers (Green, 1984; Green and Parent, 2003; Masta et al., 2002; and Vogel and Johnson; 2008) suggest that historic and current hybridization may have played a role in speciation within the *A. americanus* group.

Conclusions

Many studies of genetic diversity in amphibians have revealed substantial genetic differentiation among populations across a wide range of taxonomic and geographic scales (Barber, 1999; Lampert et al., 2003; Masta et al., 2003; Phillips, 1994; Rowe et al., 1998; Rowe et al., 2000; and Schaffer et al., 2000). Overall, species in the *Anaxyrus americanus* group do cluster into discrete populations, although differentiation among populations decreases when two or more species occur in close geographic proximity, especially among species possessing similar male advertisement calls. Species in the *A. americanus* group can exist as both discrete populations with genetic structure, and subdivided populations that persist despite apparently high levels of gene flow with sympatric or parapatric species. Isolation by distance appears to be the primary determinant of genetic differentiation among and within species, particularly for those species distributed across a wide geographic range, such as *A. americanus* and *A. fowleri*.

CHAPTER 5

CONCLUSIONS AND IMPLICATIONS

The results presented here suggest that natural hybridization could have played an important role in the formation of species within the *Anaxyrus americanus* group. Determining whether or not natural hybridization among these species has lead to an increase in genetic variation and functional novelty or to reduced fitness and reinforcement of existing species boundaries is impossible with these data alone. However, some conclusions can be made regarding the evolutionary history of the *Anaxyrus americanus* group by examining laboratory hybridization data, morphological variation in hybrids, and AFLP markers.

First, postzygotic reproductive isolation is very weak or absent between all members of the *Anaxyrus americanus* group as it is for other toad species separated by similar levels of genetic divergence. While postzygotic reproductive isolation in toads is positively correlated with genetic distance, species within the *A. americanus* group have not achieved a level of divergence that would facilitate the evolution of postzygotic reproductive isolating barriers. Thus, the production of viable and fertile hybrids resulting from natural hybridization between members of this group is quite possible if prezygotic isolating barriers are not present.

Second, natural hybridization can be detected using morphometric analyses as it has a discernable impact on the phenotype of hybrid offspring and clearly distinguishes them from either parent. Only two of ten hybrid crosses resulted in hybrid offspring with morphological characteristics intermediate between that of their respective parents. This number is probably conservative, as one of the two crosses (*Anaxyrus woodhousii x A. hemiophrys*) appeared to show some separation of hybrids on the second discriminant factor (Figure A.17), but that factor was not statistically significant likely due to insufficient sample size. Hybrids differ from their parental species primarily in aspects of cranium and limb morphology. Distinctive hybrid morphologies could be the result of transgressive segregation. Stelkens and Seehausen (2009) find that transgressive segregation increases in animal taxa that are phenotypically similar and exhibit lower levels of genetic divergence. Additionally, if dominance effects occur at loci involved in complementary gene action, then transgressive phenotypes are given in F1 hybrids as well as later generation hybrids and backcrosses (Stelkens and Seehausen, 2009).

Finally, examining genetic variation using AFLP markers reveals that species within the *Anaxyrus americanus* group can persist as relatively discrete species or in a metapopulation framework where genetically differentiated subpopulations are connected by varying levels of gene flow. Several species show evidence of distinct subpopulation structure and/or very recent or ongoing gene flow, suggesting that one or more panmictic populations may have recently been divided into several weakly differentiated subpopulations lacking postzygotic reproductive isolation. However, it is difficult to determine whether or not high levels of gene flow are due to ongoing hybridization or retention of ancestral polymorphisms as the *A. americanus* group is a young radiation (Pauly et al., 2004). Additionally, there appears to be discordance between the evolutionary relationships supported by mtDNA and nuclear AFLP data. Species sharing similar male advertisement calls are most closely related according to the AFLP data, while mtDNA shows that species do not group together according to male advertisement call and suggests convergence and/or divergence between members of the *A. americanus* group (Masta et al., 2002; Pauly et al., 2004).

Toads, and indeed almost all frog species, use male advertisement calls as a prezygotic method of identifying conspecifics and avoiding interspecific hybridization (Cocroft and Ryan, 1995; Ryan et al., 1990). Additionally, female frogs have been shown be quite sensitive to subtle changes in male advertisement call, and males of some species have been shown to exploit sensory biases in female call preference (Ryan et al., 1990). The advertisement calls of male frogs are often labile and can potentially be altered fairly rapidly with only minor physiological changes (Cocroft and Ryan, 1995; Ryan et al., 1990). This lability in call production could result in rapid responses by male frogs to differences in female preference.

Prezygotic isolation can rapidly isolate species that are not isolated by intrinsic postzygotic barriers, and this could have played a role in speciation within the *Anaxyrus americanus* group in a manner similar to that seen in the frog *Physalaeumus pustulosus* (Boul et al., 2007). Boul et al. (2007) found that sexual selection drove speciation in the *Physalaeumus pustulosus* species group with females selecting males that had divergent advertisement calls. Differential female preferences among populations lead to behavioral isolation and divergence in male advertisement call as a response to female choice. Boul et al. (2007) also found that the divergence in male call was not due to drift and that gene flow was higher between species that shared similar male advertisement calls as seen in the *Anaxyrus americanus* group.

Virtually nothing is known about the fitness, survivorship, or mating success of hybrids (i.e. extrinsic postzygotic isolation) between members of the *A. americanus* group in natural conditions (but see Green, 1984 and Green and Parent, 2003). If survivorship or mating success of hybrids were low, then there would likely be a selective pressure to evolve prezygotic isolating barriers to avoid hybridization. Toads in the *A. americanus* group have two major types of male advertisement calls: a musical trill (*A. americanus, A. charlesmithi, A. hemiophrys, A. houstonensis,* and *A. terrestris*) and a bleating trill (*A. woodhousii, A. fowleri,* and *A. velatus*). These calls differ primarily in pulse rate with the musical trill having a higher pulse rate than the bleating trill (Sullivan et al., 1996). Female amphibians are highly attuned to differences in these calls, and can choose between heterospecifics and conspecifics using differences in pitch, dominant frequency, pulse rate, and duration (Cocroft and Ryan, 1995; Ryan et al., 1990).

Female choice to select males based on various properties of the male advertisement call has been demonstrated for *A. cognatus, A. americanus,* and *A. woodhousii* (Sullivan, 1983a, 1983b, 1992; Gatz, 1981; Howard, 1988; Licht, 1976; Waldman, 2001). Females in the *A. americanus* group appear to select mates on the basis of dominant frequency, an indicator of male body size, or high effort calls (i.e. longer calls or faster pulse rates), an indicator of stamina and potentially fitness (Sullivan, 1983a, 1983b, 1992; Gatz, 1981; Howard, 1988; Licht, 1976). Additionally, Waldman (2001) found that *A. americanus* females can discriminate between closely related males on the basis of their advertisement calls, thus avoiding inbreeding and indicating the fine scale variation that female frogs are capable of detecting.

AFLP results show that species sharing similar male advertisement calls have higher rates of gene flow and genetic similarity, suggesting that similar male advertisement calls may not be effective as prezygotic isolating barriers (assuming these genetic similarities are not due to the retention of ancestral polymorphisms). However, species with different male advertisement calls have lower rates of gene flow and genetic similarity. In sympatric species, differing male advertisement calls or staggered breeding seasons could be an example of reproductive character displacement to avoid hybridization, also known as reinforcement. Currently, there are no rigorous studies of reproductive character displacement in the *Anaxyrus americanus* group, but previous research has suggested that differences in male advertisement call and staggered breeding seasons could play an important role in preventing heterospecific mating events (A.P. Blair, 1941, 1942 and W.F. Blair, 1956). Male toads also use species-specific release vocalizations to prevent amplexus by conspecific or heterospecific males. Leary (2001) documented evidence of convergent character displacement in sympatric populations of *A. fowleri* and *A. terrestris* in release vocalizations. These species already possess divergent advertisement calls in sympatry, but also appear to have converged on a similar release vocalization to facilitate communication between heterospecific males, further strengthening potential prezygotic isolation between the species.

Collectively, the results presented here suggest that the evolutionary history of toads in the *Anaxyrus americanus* group may be similar to that of a syngameon complex.

A syngameon is defined as a complex of species that can be relatively discrete with regard to morphology and/or ecology, but only weakly differentiated genetically and capable of frequent or occasional hybridization (Seehausen, 2004). The syngameon hypothesis of Seehausen (2004) provides a plausible scenario whereby natural hybridization can facilitate the creation and radiation of new species. If environments are disturbed (such as during a glacial advance or retreat), then conditions within those environments can become heterogeneous and certain combinations of alleles may be more favorable in certain environments. Natural hybridization can facilitate the shuffling of existing variation and creation of novel allelic combinations allowing hybrids to take advantage of these novel conditions (Seehausen, 2004). As new environments or resources are made available following a disturbance, continued natural hybridization can fuel the creation of novel lineages and foster a radiation of species. Recent evidence suggests that radiations of *Heliconius* butterflies (Gilbert, 2003) and Hawaiian silversword plants (Barrier et al., 1999) were facilitated by natural hybridization in such a manner. Seehausen (2004) also predicted that as the rate of exploitation of underutilized niches and/or resources began to decrease, the benefits of hybridization would decrease and genomic incompatibilities between existing species would begin to accumulate, thus slowing the radiation.

One of the predicted signatures of radiations facilitated by hybridization is discordance between nuclear and cytonuclear (e.g. mtDNA or chloroplast DNA) genetic markers (Seehausen, 2004). In hybridizing populations, uniparental genetic markers will remain distinct, with fixation of the marker from one species and loss of the marker from the other species (Petit and Excoffier, 2009; Seehausen, 2004). However, nuclear markers will remain intermediate between species, leading to genealogical discordance between the two types of genetic markers. Several studies have documented genetic discordances among species affected by natural hybridization, and the syngameon hypothesis has been evoked to explain radiations in Darwin's finches (Freeland and Boag, 1999; Grant and Grant, 1996), Hawaiian crickets (Shaw, 1996; Shaw, 2002), Hawaiian silversword plants (Barrier et al., 1999), *Heliconius* butterflies (Gilbert, 2003) and cichlid fish (Seehausen et al., 1997). The *Anaxyrus americanus* group is capable of interspecific hybridization and was recently affected by Pleistocene glaciation as recently as 15,000 years ago (Blair, 1972; Masta et al., 2003; Pauly et al., 2004). It is possible that the discordance between the evolutionary relationships supported by mtDNA (Masta et al., 2002; Pauly et al., 2004) and those found in this study could be the signature of past (and possibly ongoing) hybridization events that allowed hybrid species to take advantage of newly available habitats or resources following the retreat of glaciers from North America. However, evaluation of this hypothesis requires additional data and continued research.

Future research should expand on the preliminary results found in this study and evaluate the role of prezygotic isolation in maintaining species boundaries within the *Anaxyrus americanus* group. It would be useful to obtain mtDNA data for the individuals in this study in order to quantitatively examine whether or not discordance between nuclear and cytonuclear markers is real or spurious. Additionally, sampling of the species in this study from additional geographic localities could provide further resolution of intraspecific population structure, interspecific gene flow, and geographic boundaries between groups. Analyses of morphometric variation in hybrids could be expanded to include species from other clades in the family Bufonidae in order to determine whether or not the results of this study can be extended to toads outside the *A. americanus* group. Finally, quantitative analysis of male advertisement calls from areas of allopatry and sympatry coupled with mate choice experiments could provide evidence for the strength of prezygotic isolation in the *A. americanus* group.

APPENDIX A

TABLES AND FIGURES

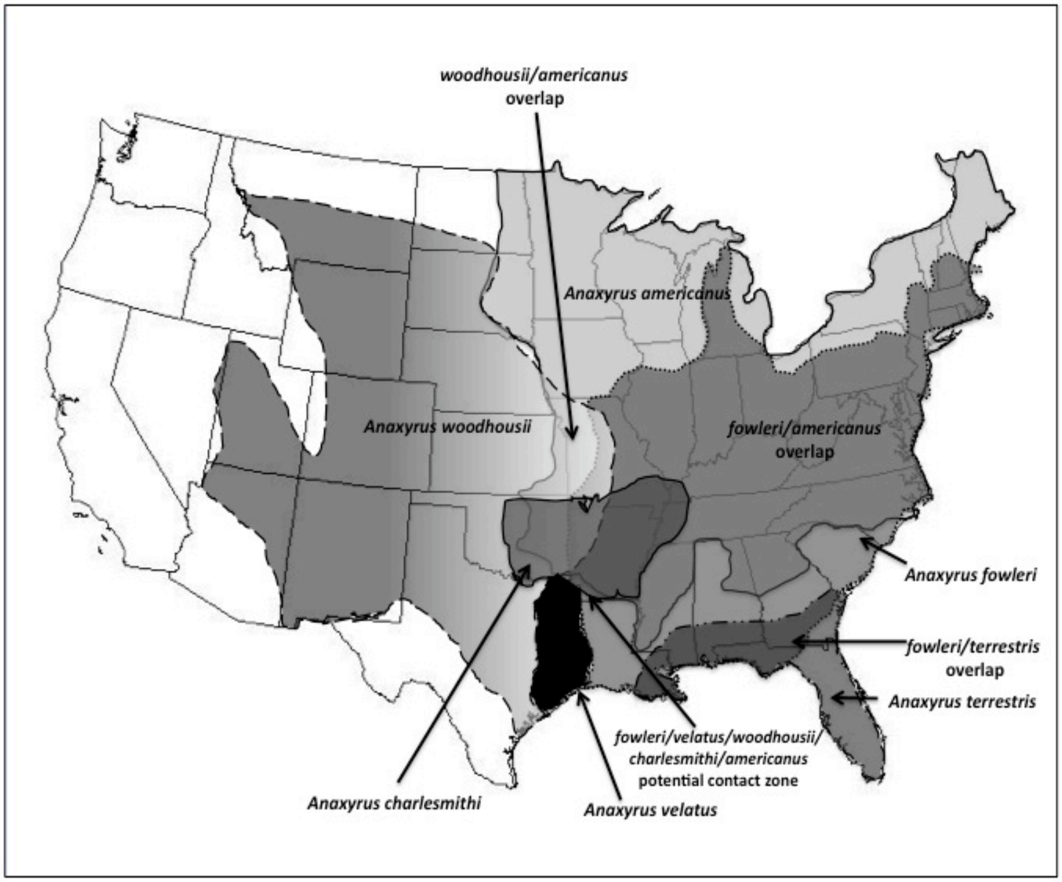

Figure A.1. Geographic distribution of the *Anaxyrus americanus* group in the United States of America (*Anaxyrus houstonensis, Anaxyrus microscaphus* and *Anaxyrus hemiophrys* not shown).

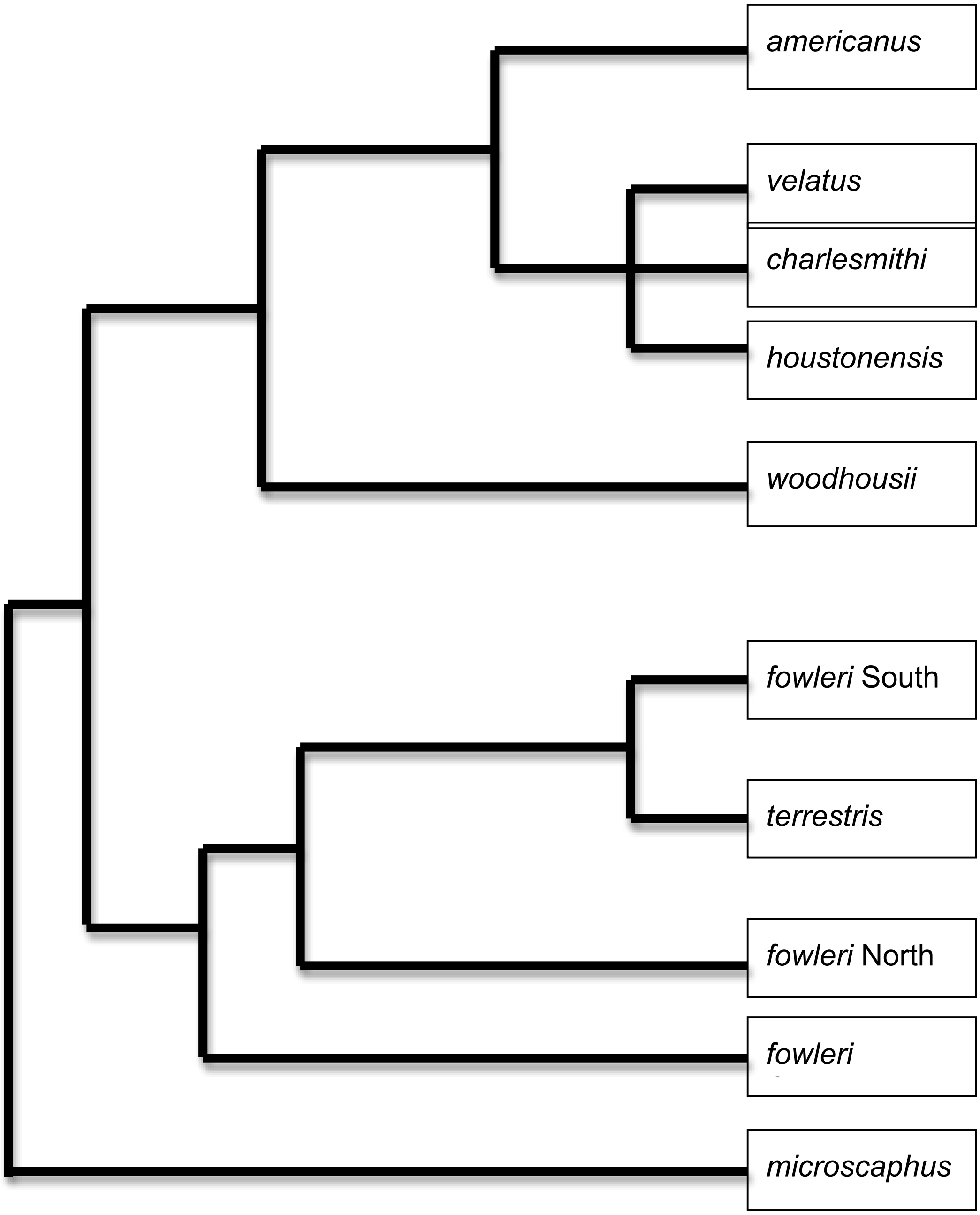

Figure A.2. Hypothesis of interspecific relationships within the *Anaxyrus americanus* group (using combined results of Masta et al., 2002 and Pauly et al., 2004).

Table A.1. Index of postzygotic reproductive isolation for reciprocal cross data (IPO). V/F, viable and/or fertile; I, inviable or sterile. (A) Both sexes viable or fertile in both reciprocal crosses; (B) one sex sterile or inviable in one cross only; (C) one sex inviable or sterile in both directions of cross; (D) both sexes inviable or sterile in only one direction of cross; (E) one sex viable or fertile in both directions of cross; (F) both sexes inviable or sterile in both directions of cross; M = males; F = females.

	A		B		C		D		E		F	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Cross 1	V/F	V/F	V/F	V/F	V/F	I	I	I	V/F	I	I	I
Cross 2	V/F	V/F	V/F	1	V/F	I	V/F	V/F	I	I	I	I
Code	0		1		2		3		4		5	
IPO	0.00		0.20		0.40		0.60		0.80		1.00	

Table A.2. Average genetic distance for each reproductive isolation index level. Levels spanned by a vertical bracket are not significantly different by Scheffe's F test.

Reproductive Isolation Index	Mean genetic distance ± SE (N)
0.00	0.045 ± 0.035 (2)
0.20	0.043 ± 0.007 (3)
0.40	0.043 ± 0.003 (9)
0.50	0.046 ± 0.007 (7)
0.60	0.058 ± 0.002 (11)
0.80	0.073 ± 0.002 (24)
1.00	0.076 ± 0.003 (45)
Mean	0.067 ± 0.002 (101)

Table A.3. Cases of Haldane's Rule for inviability in toads. "Unconfirmed cases" are those crosses in which one sex is present while the other is absent, but sample sizes are too low to evaluate statistically. "Quantitative cases" are crosses that showed a statistical bias in the number of individuals for a particular sex. "Complete cases" are those crosses that resulted in zero offspring of one sex and more than six offspring of the opposite sex. The total number of crosses used to analyze the operation of Haldane's Rule was 93 (65 cases in which females are the affected sex and 28 in which males are the affected sex).

	Unconfirmed cases	Quantitative cases	Complete cases	Total
Females affected	60	1	4	65
Males affected	26	1	1	28
Neither affected	70	–	–	70

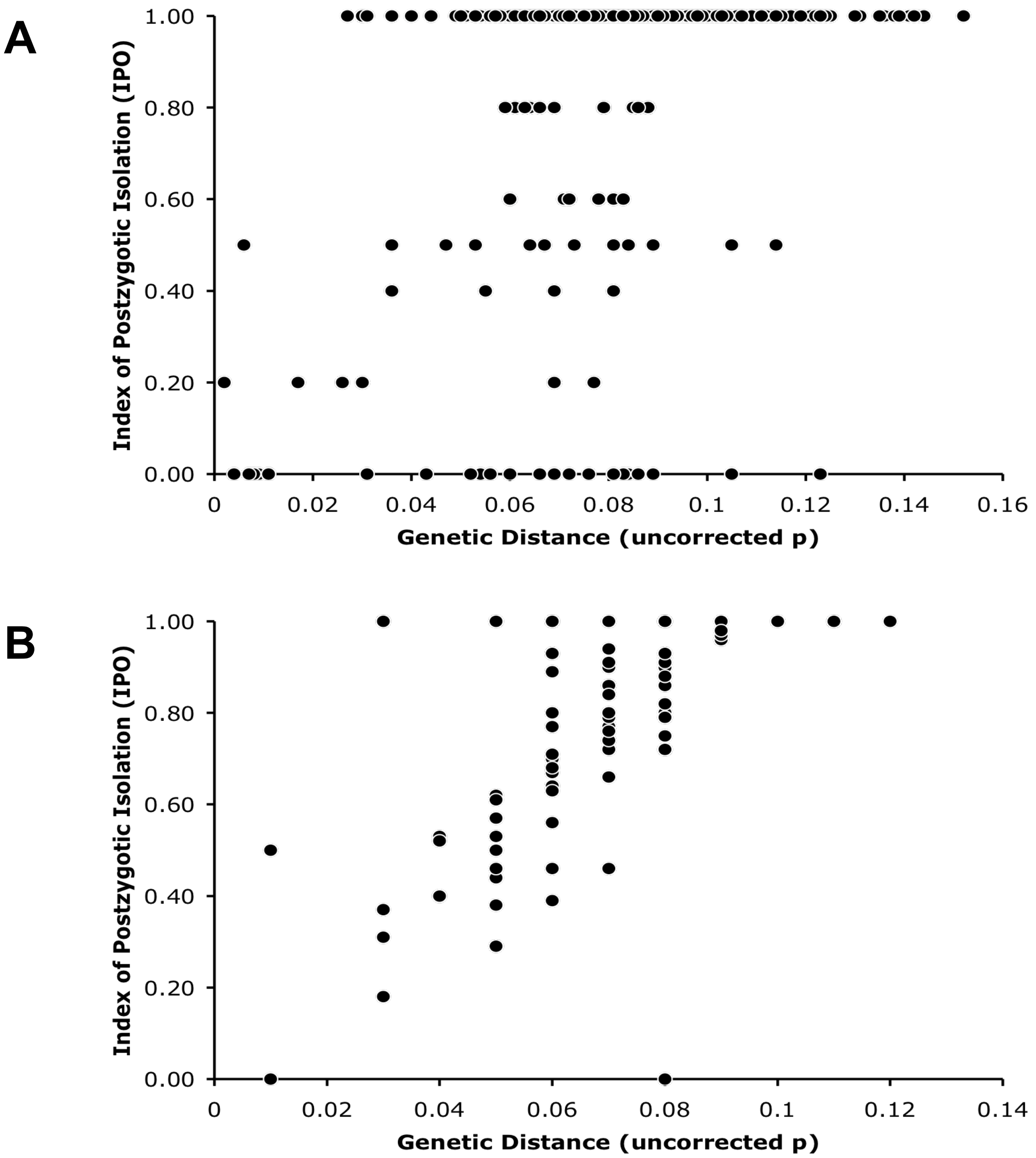

Figure A.3. Scatterplot of postzygotic isolation indices and genetic divergence (uncorrected p) of (A) the entire dataset ($r_s = 0.538$, $N = 101$, $P < 0.001$), and (B) corrected for phylogenetic independence ($r_s = 0.406$, $N = 680$, $P < 0.001$). Both show that postzygotic reproductive isolation increases with increasing genetic divergence.

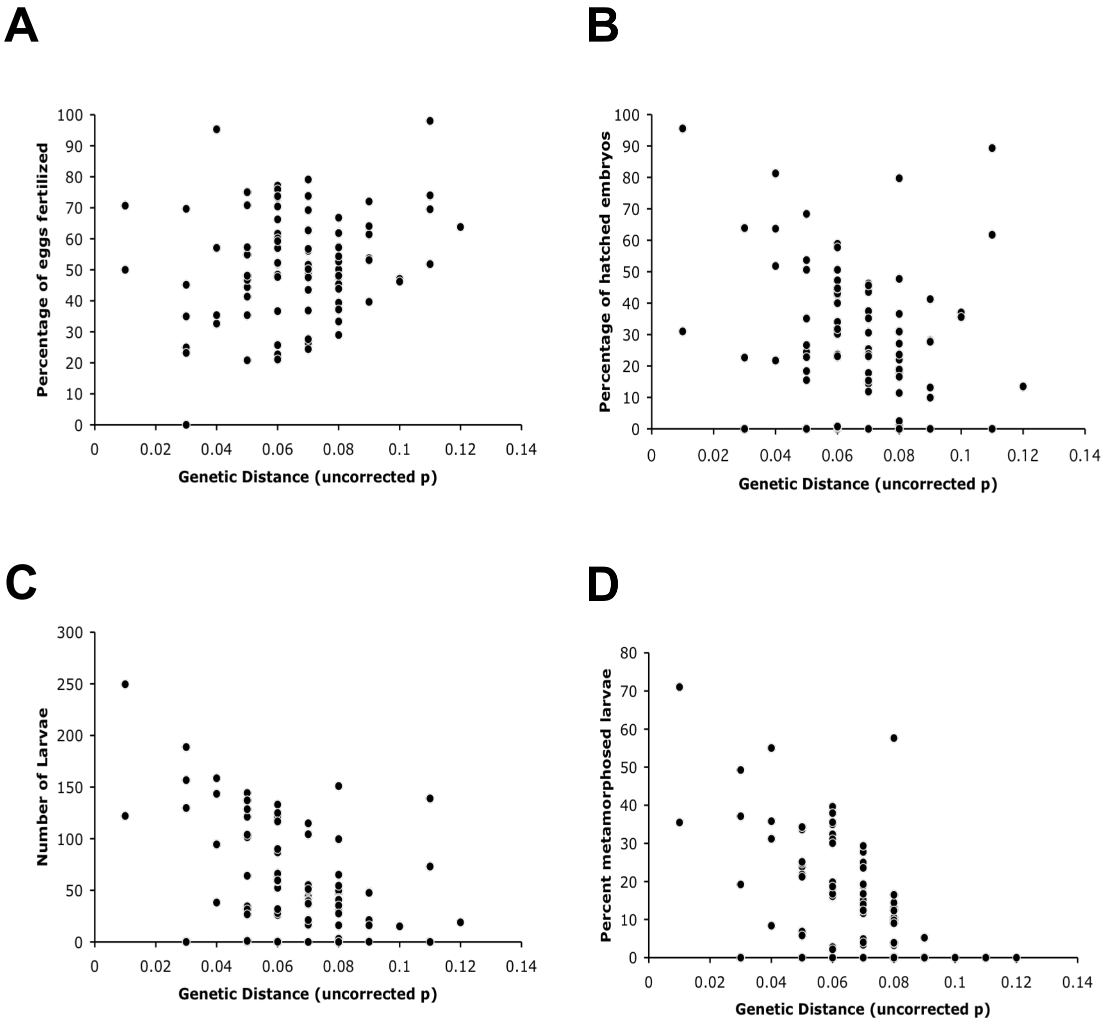

Figure A.4. Scatterplot of (A) percentage of eggs fertilized ($r_s = 0.078$, $N = 101$, $P < 0.437$), (B) percentage of embryos hatched ($r_s = -0.246$, $N = 101$, $P < 0.013$), (C) the number of larvae ($r_s = -0.378$, $N = 101$, $P < 0.001$), and (D) the percentage of larvae that metamorphosed into froglets ($r_s = -0.499$, $N = 101$, $P < 0.001$) compared to genetic divergence between species in interspecific hybrid crosses for the phylogenetically corrected dataset.

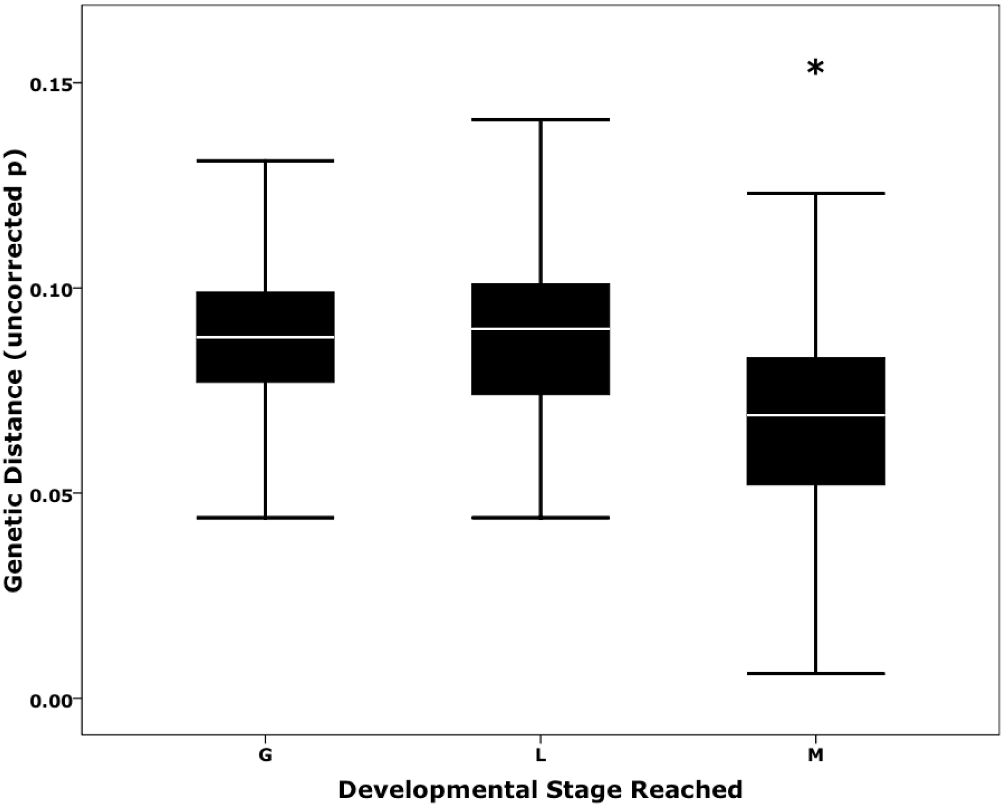

Figure A.5. Boxplots of genetic divergence (uncorrected p) compared to the latest developmental stage reached in hybrid crosses. Upper and lower whiskers represent 75th and 25th quartiles and white line represents the median genetic divergence. G = gastrula; L = larvae/tadpoles; and M = metamorphosis into adults. Sample sizes are shown above each category of developmental stage. * represents results of multiple comparison tests for each pairwise comparison.

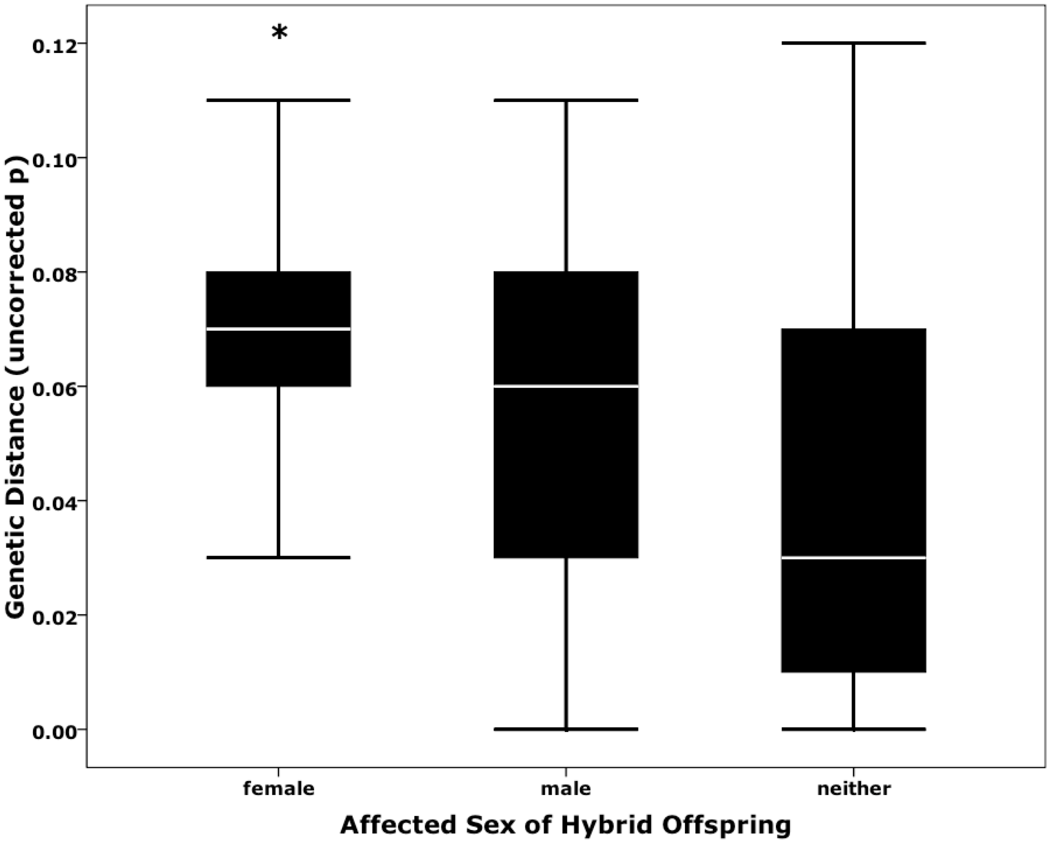

Figure A.6. Boxplots of genetic divergence compared to negatively affected sex in hybrid crossings. Upper and lower whiskers represent 75th and 25th quartiles respectively. White bars represent median genetic divergence for each group. * represents results of multiple comparison tests for each pairwise comparison.

Table A.4 Summary statistics for discriminant function analysis of multiple comparisons within the *Anaxyrus americanus* group.

Species Comparison	Factor	Eigenvalue	Percent Variance	Wilk's Lambda	DF	*P*-value
Anaxyrus americanus group	Factor 1	0.98	31.6	0.09	105	< 0.001
	Factor 2	0.85	27.3	0.18	84	< 0.001
Parental species x FI hybrids	Factor 1	2.94	51.8	0.03	180	< 0.001
	Factor 2	0.87	15.4	0.11	154	< 0.001
americanus x fowleri	Factor 1	1.62	84.9	0.30	30	< 0.001
	Factor 2	0.29	15.1	0.78	14	< 0.061
americanus x houstonensis	Factor 1	3.16	62.8	0.08	30	< 0.001
	Factor 2	1.88	37.2	0.35	14	< 0.001
americanus x terrestris	Factor 1	2.88	50.4	0.05	45	< 0.001
	Factor 2	2.38	41.7	0.20	28	< 0.001
americanus x woodhousii	Factor 1	3.72	72	0.09	30	< 0.001
	Factor 2	1.45	28	0.41	14	< 0.001
hemiophrys x houstonensis	Factor 1	9.16	82.9	0.03	30	< 0.001
	Factor 2	1.88	17.1	0.35	14	< 0.009
houstonensis x terrestris	Factor 1	2.98	58.5	0.08	30	< 0.001
	Factor 2	2.12	41.5	0.32	14	< 0.001
terrestris x hemiophrys	Factor 1	5.66	63.4	0.02	60	< 0.001
	Factor 2	2.17	24.3	0.13	42	< 0.001
terrestris x woodhousii	Factor 1	4.65	68.5	0.04	60	< 0.001
	Factor 2	1.46	21.5	0.23	42	< 0.001
woodhousii x hemiophrys	Factor 1	3.61	94.8	0.18	30	< 0.001
	Factor 2	0.20	5.2	0.83	14	< 0.517
woodhousii x houstonensis	Factor 1	1.25	61.3	0.25	30	< 0.001
	Factor 2	0.79	38.7	0.56	14	< 0.001

Table A.5. Discriminant function classification matrix for *Anaxyrus americanus, A. fowleri* and hybrids between these species.

Species	*americanus*	*fowleri*	F1 hybrid
americanus	74.4%	10.3%	15.4%
fowleri	9.2%	69.2%	21.5%
F1 hybrid	18.5%	25.9%	55.6%

Table A.6. Discriminant function classification matrix for *Anaxyrus americanus, A. houstonensis* and hybrids between these species.

Species	*americanus*	*houstonensis*	F1 hybrid
americanus	79.5%	15.4%	5.1%
houstonensis	18.2%	77.3%	4.5%
F1 hybrid	0%	11.1%	88.9%

Table A.7. Discriminant function classification matrix for *Anaxyrus americanus, A. terrestris* and hybrids between these species.

Species	*americanus*	*terrestris*	F1 hybrid	Backcross
americanus	82.1%	17.9%	0%	0%
terrestris	2.2%	95.6%	2.2%	0%
F1 hybrid	0%	0%	100%	0%
Backcross	0%	0%	100%	0%

Table A.8. Discriminant function classification matrix for *Anaxyrus americanus, A. woodhousii* and hybrids between these species.

Species	*americanus*	*woodhousii*	F1 hybrid
americanus	76.9%	20.5%	2.6%
woodhousii	11.9%	88.1%	0%
F1 hybrid	0%	0%	100%

Table A.9. Discriminant function classification matrix for *Anaxyrus hemiophrys, A. houstonensis* and hybrids between these species.

Species	*hemiophrys*	*houstonensis*	F1 hybrid
hemiophrys	85.7%	14.3%	0%
houstonensis	0%	100%	0%
F1 hybrid	0%	0%	100%

Table A.10. Discriminant function classification matrix for *Anaxyrus houstonensis, A. terrestris* and hybrids between these species.

Species	*houstonensis*	*terrestris*	F1 hybrid
houstonensis	90.9%	9.1%	0%
terrestris	8.9%	91.1%	0%
F1 hybrid	0%	0%	100%

Table A.11. Discriminant function classification matrix for *Anaxyrus terrestris, A. hemiophrys* and hybrids between these species.

Species	*terrestris*	*hemiophrys*	F1 hybrid	Backcross	F2 hybrid
terrestris	93.3%	2.2%	2.2%	2.2%	0%
hemiophrys	7.1%	78.6%	0%	7.1%	7.1%
F1 hybrid	0%	0%	100%	0%	0%
Backcross	16.7%	16.7%	0%	66.7%	0%
F2 hybrid	0%	0%	0%	66.7%	33.3%

Table A.12. Discriminant function classification matrix for *Anaxyrus terrestris, A. woodhousii* and hybrids between these species.

Species	*terrestris*	*woodhousii*	F1 hybrid	Backcross	F2 hybrid
terrestris	95.6%	2.2%	2.2%	0%	0%
woodhousii	16.9%	74.6%	6.8%	0%	1.7%
F1 hybrid	0%	0%	87%	0%	13%
Backcross	0%	0%	9.4%	79.2%	11.3%
F2 hybrid	0%	0%	10%	30%	60%

Table A.13. Discriminant function classification matrix for *Anaxyrus woodhousii, A. hemiophrys* and hybrids between these species.

Species	*woodhousii*	*hemiophrys*	F1 hybrid
woodhousii	91.5%	1.7%	6.8%
hemiophrys	7.1%	71.4%	21.4%
F1 hybrid	22.2%	33.3%	44.4%

Table A.14 Discriminant function classification matrix for *Anaxyrus woodhousii, A. houstonensis* and hybrids between these species.

Species	*woodhousii*	*houstonensis*	F1 hybrid
woodhousii	74.6%	23.7%	1.7%
houstonensis	13.6%	81.8%	4.5%
F1 hybrid	0%	0%	100%

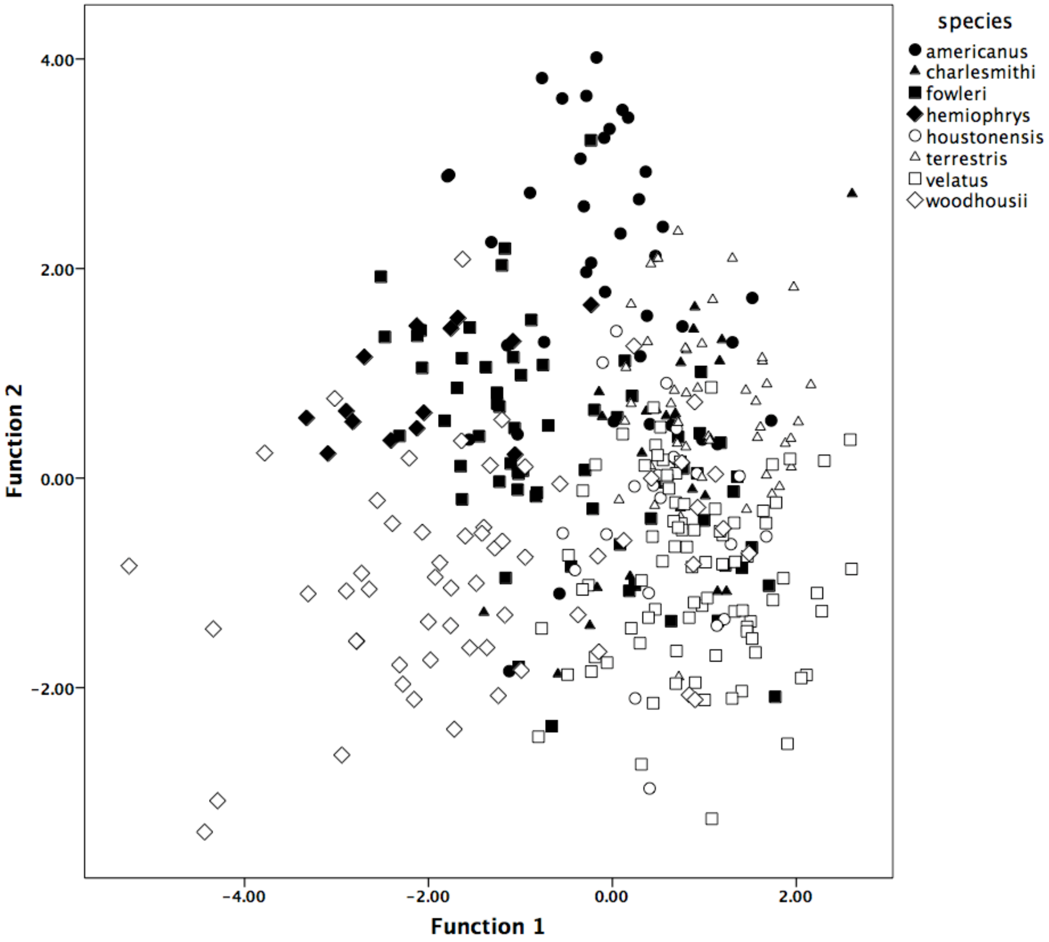

Figure A.7 Discriminant function analysis plot of the first two discriminant functions for individuals in the *Anaxyrus americanus* group.

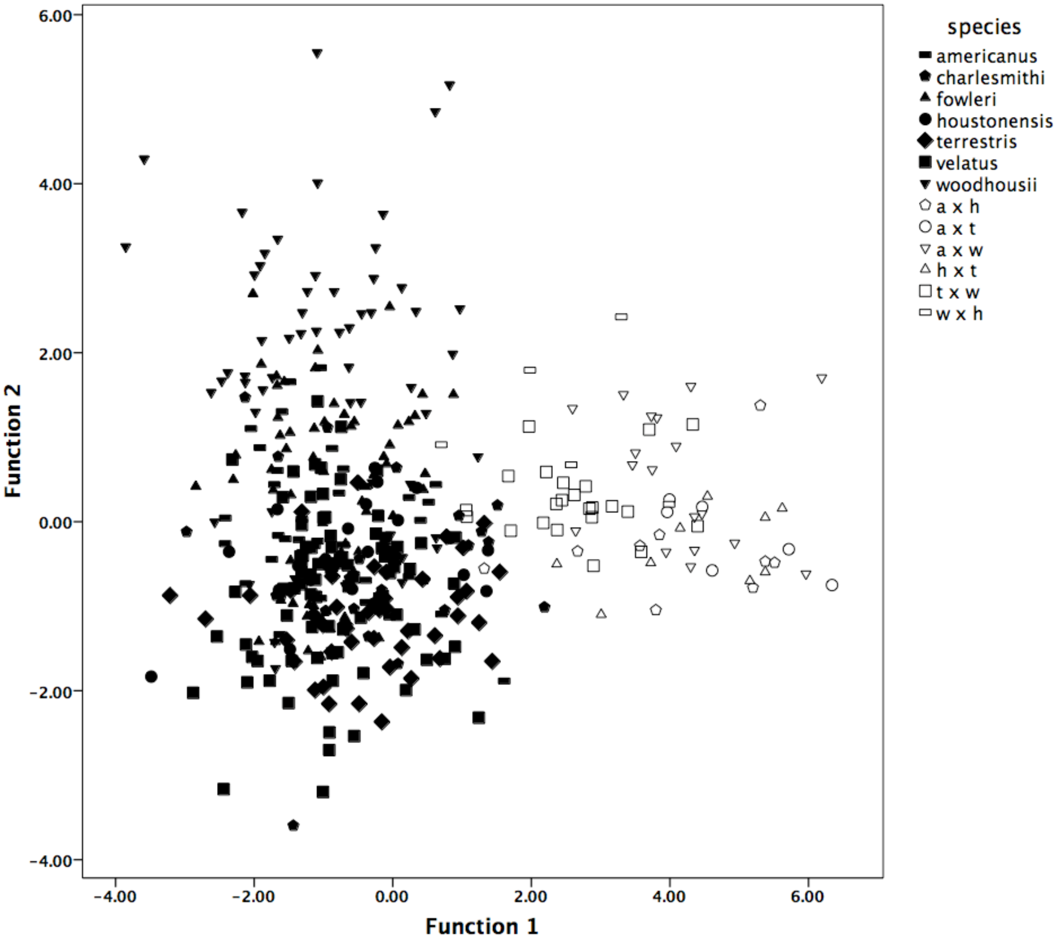

Figure A.8. Discriminant function analysis plot of the first two discriminant functions for individuals in the *Anaxyrus americanus* group and F1 hybrids between these species. Parental species are indicated by solid symbols and hybrids are indicated by open symbols.

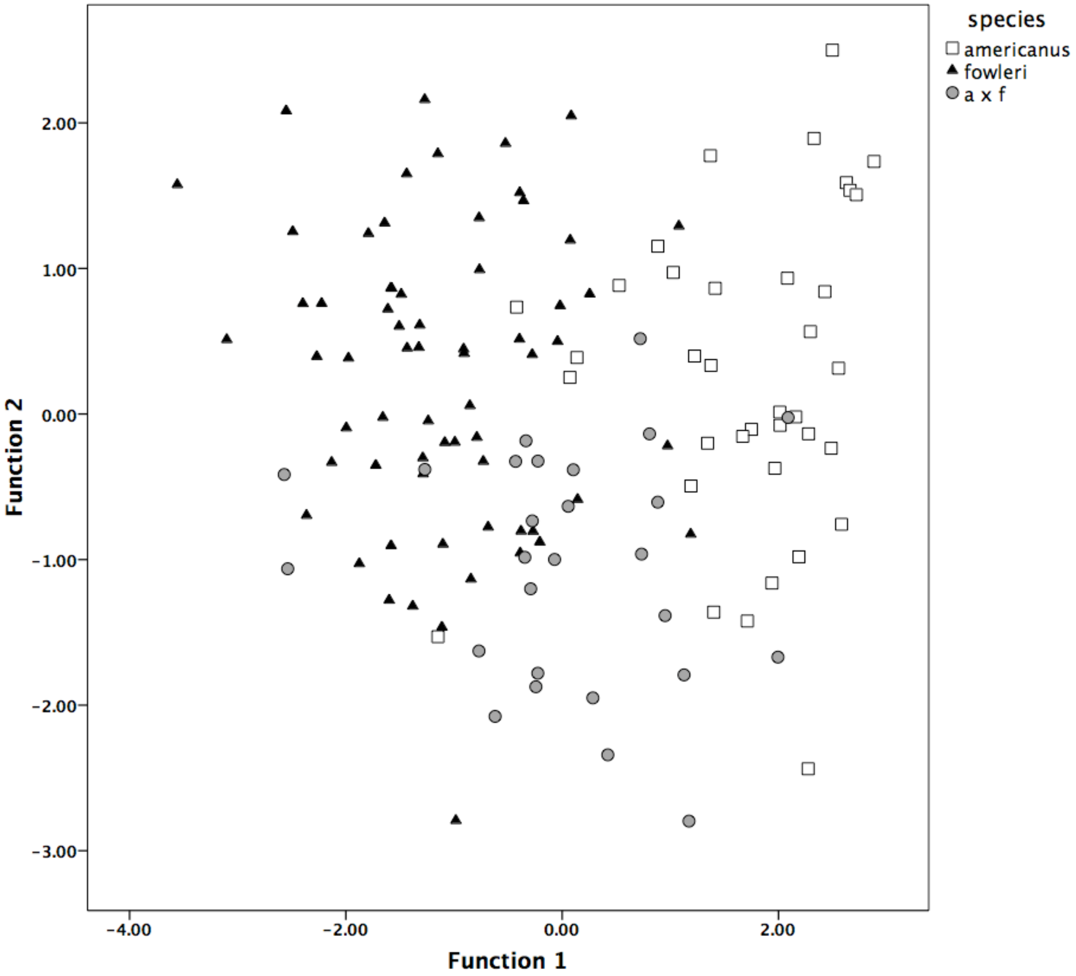

Figure A.9. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus americanus, A. fowleri,* and hybrids between these species. Hybrids are indicated by gray symbols.

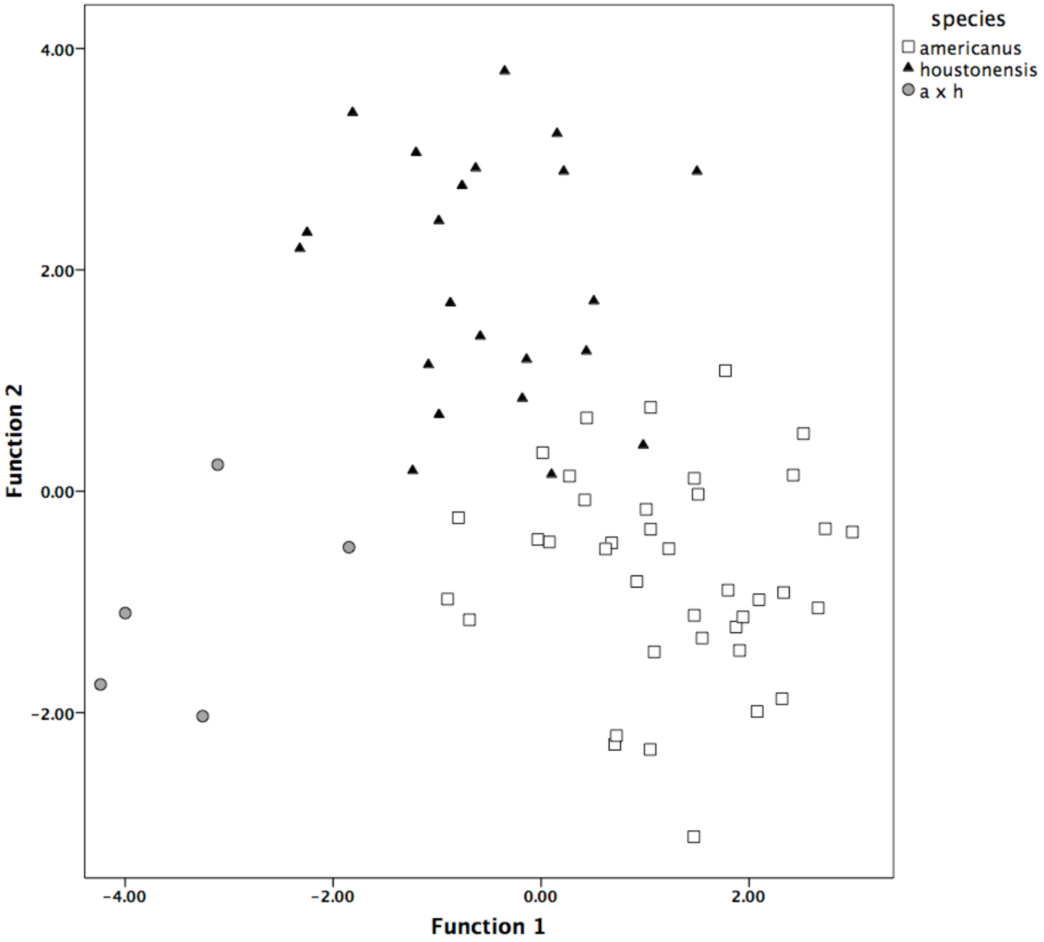

Figure A.10. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus americanus, A. houstonensis,* and hybrids between these species. Hybrids are indicated by gray symbols.

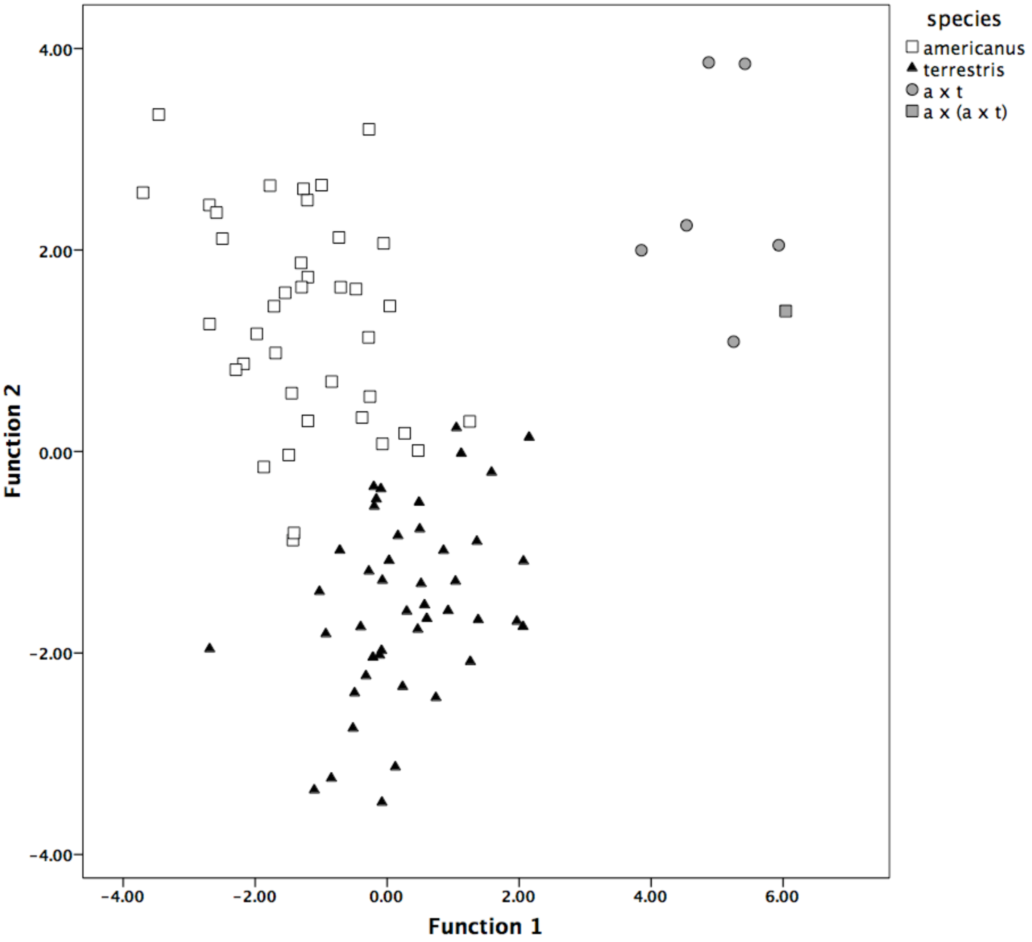

Figure A.11. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus americanus, A. terrestris,* and hybrids between these species. Hybrids are indicated by gray symbols.

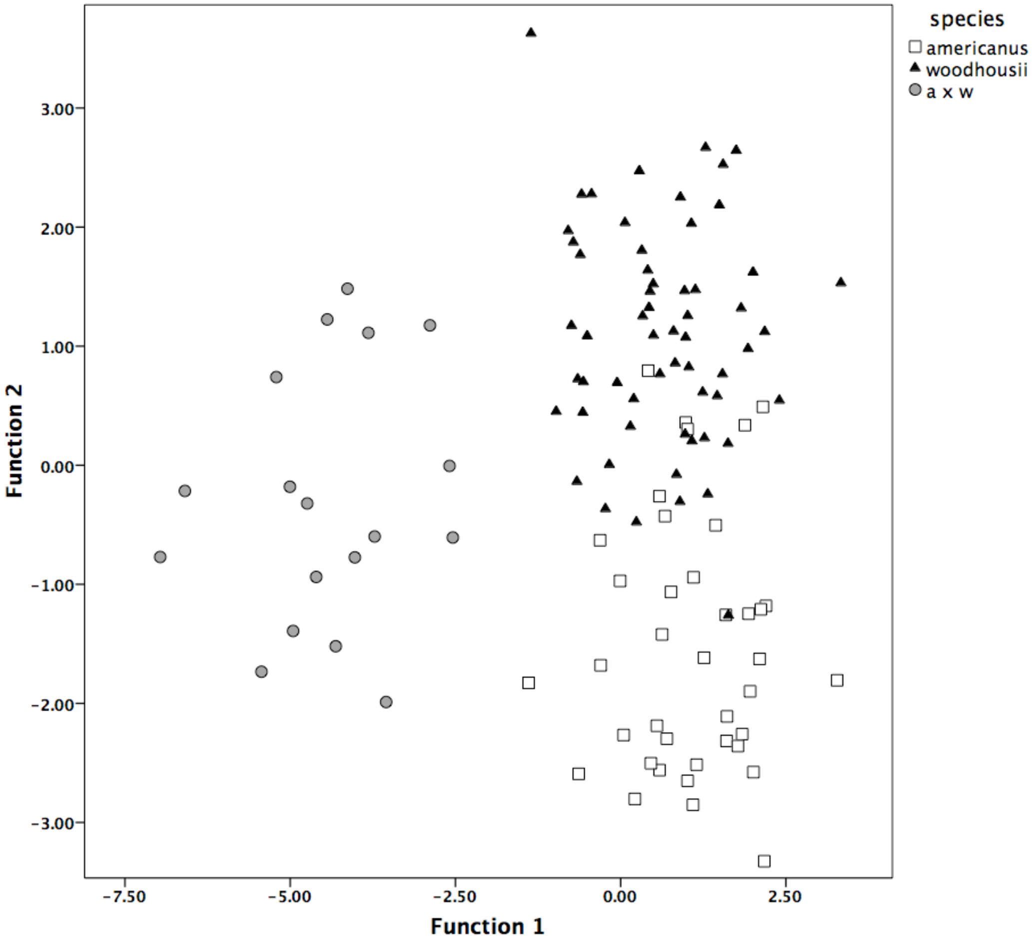

Figure A.12. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus americanus, A. woodhousii,* and hybrids between these species. Hybrids are indicated by gray symbols.

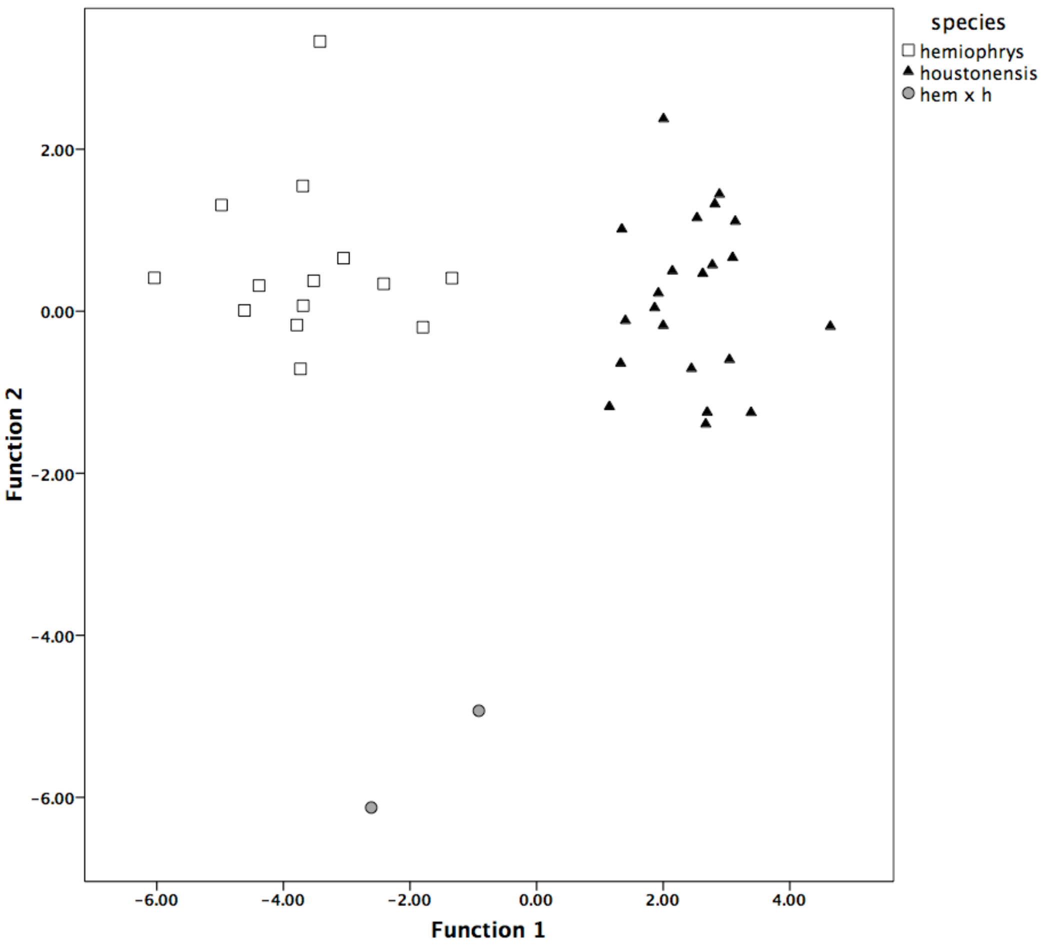

Figure A.13. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus hemiophrys, A. houstonensis,* and hybrids between these species. Hybrids are indicated by gray symbols.

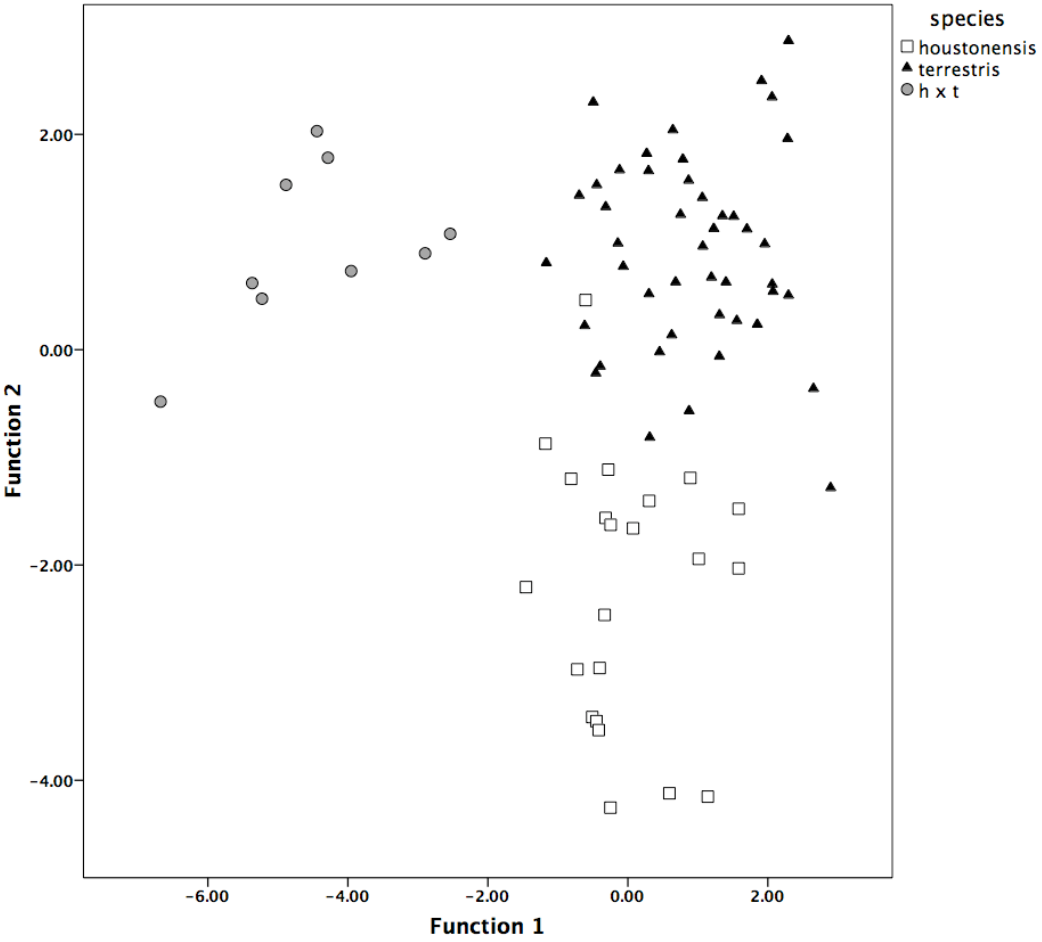

Figure A.14. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus houstonensis, A. terrestris,* and hybrids between these species. Hybrids are indicated by gray symbols.

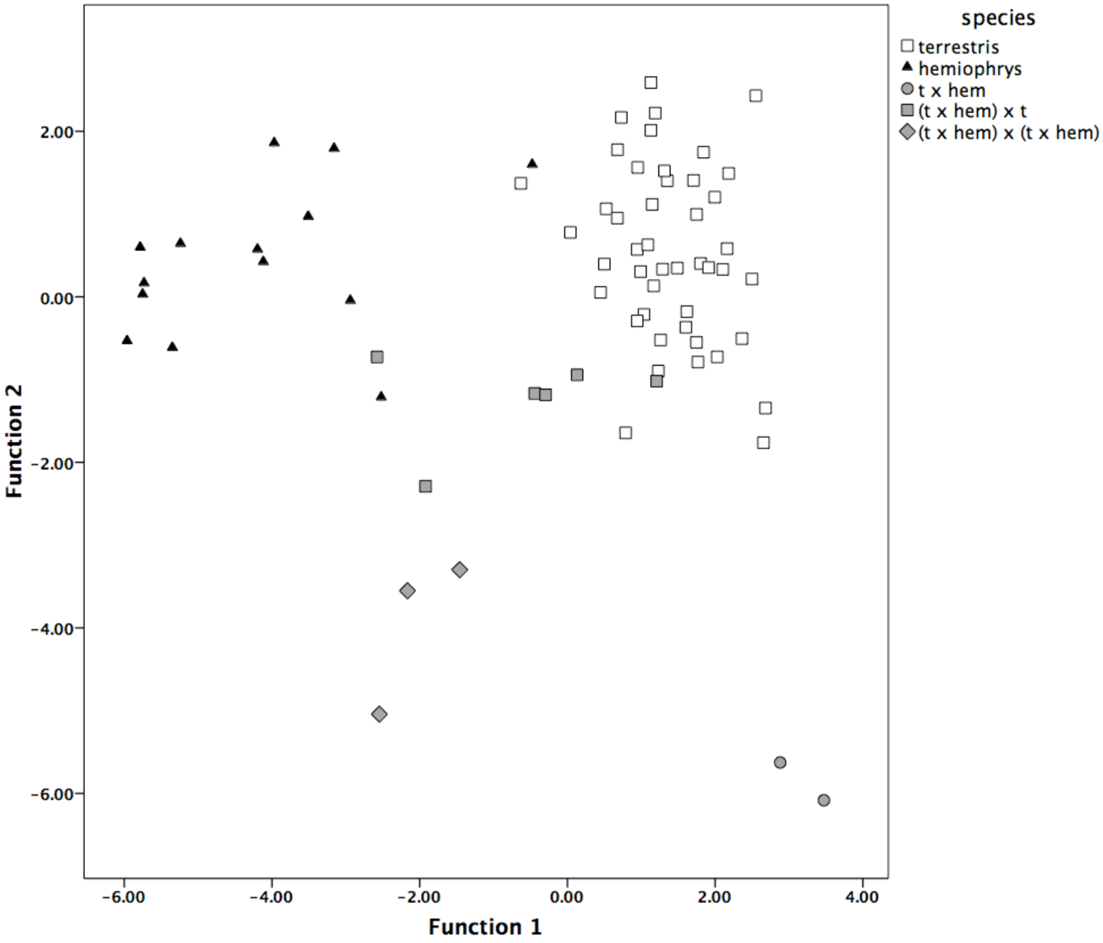

Figure A.15. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus terrestris, A. hemiophrys,* and hybrids between these species. Hybrids are indicated by gray symbols.

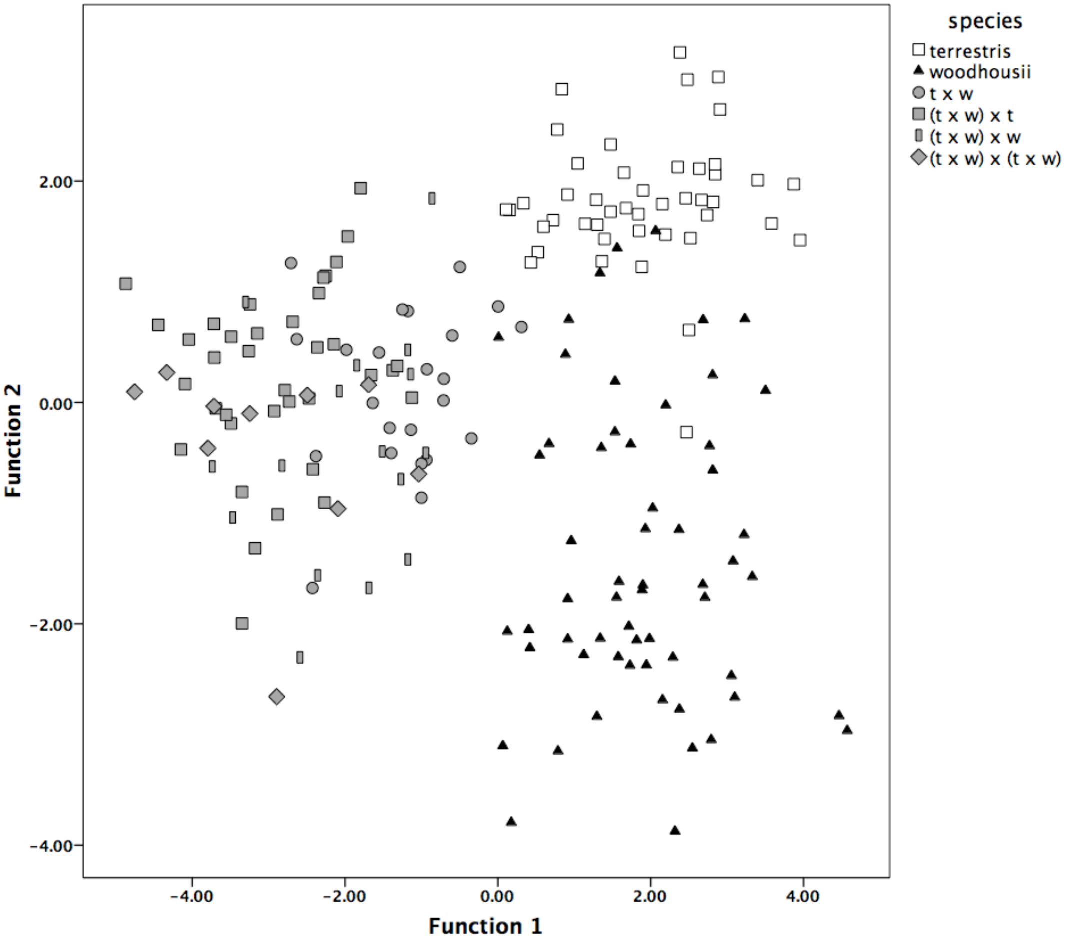

Figure A.16. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus terrestris, A. woodhousii,* and hybrids between these species. Hybrids are indicated by gray symbols.

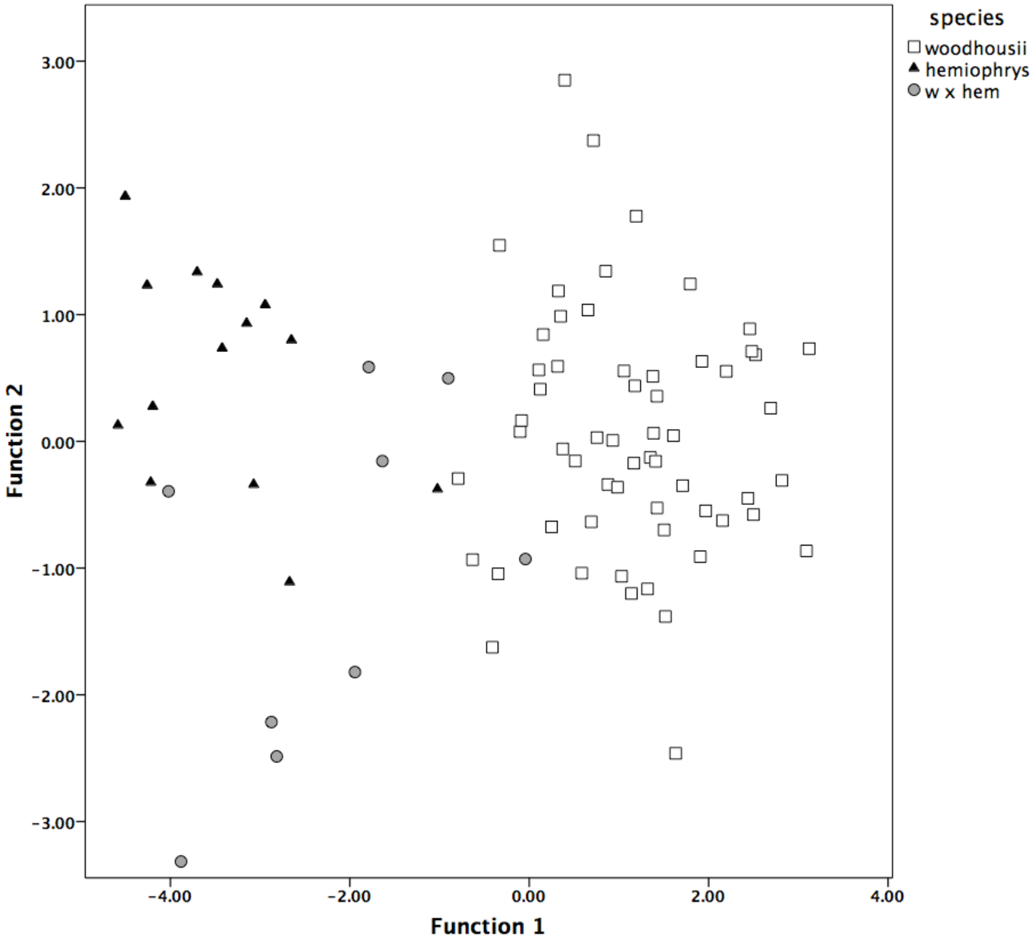

Figure A.17. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus woodhousii, A. hemiophrys,* and hybrids between these species. Hybrids are indicated by gray symbols.

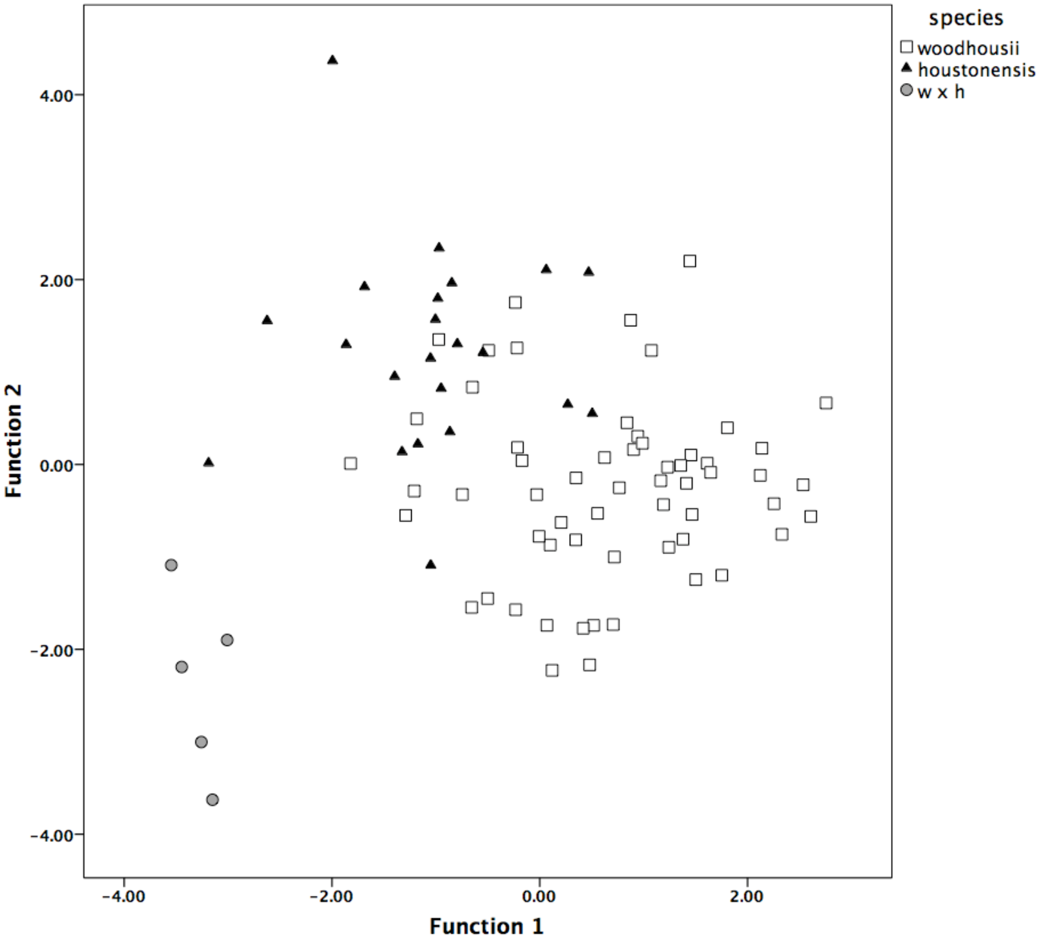

Figure A.18. Discriminant function analysis plot of the first two discriminant functions for *Anaxyrus woodhousii, A. houstonensis,* and hybrids between these species. Hybrids are indicated by gray symbols.

Table A.15. Descriptive statistics of 100 AFLP loci from the *Anaxyrus americanus* group.

Species	*n*	Number of Loci	N*a* (S.D.)	N*e* (S.D.)	I (S.D.)	H_T (S.D.)	θ^B (S.D.)	Percent Polymorphic Loci
americanus	18	100	1.87 (0.34)	1.56 (0.34)	0.48 (0.23)	0.36 (0.01)	0.23 (0.05)	87
charlesmithi	19	100	1.86 (0.35)	1.68 (0.33)	0.54 (0.23)	0.41 (0.01)	0.13 (0.03)	86
fowleri	44	100	1.89 (0.31)	1.64 (0.32)	0.53 (0.21)	0.33 (0.04)	0.16 (0.04)	89
terrestris	17	100	1.88 (0.33)	1.71 (0.33)	0.55 (0.22)	0.41 (0.02)	0.14 (0.04)	88
velatus	50	100	1.93 (0.26)	1.65 (0.30)	0.54 (0.18)	0.30 (0.04)	0.19 (0.04)	93
woodhousii	23	100	1.82 (0.39)	1.63 (0.34)	0.50 (0.25)	0.36 (0.03)	0.14 (0.04)	82
cognatus	7	100	1.59 (0.49)	1.34 (0.36)	0.31 (0.28)	0.41 (0.02)	0.09 (0.04)	59
All Populations	178	100	2.00 (0.00)	1.76 (0.25)	0.61 (0.12)	0.41 (0.04)	0.27 (0.02)	100

n: number of individuals
N*a*: number of observed alleles
N*e*: numer of expected alleles
I: Shannon Index (a measure of gene diversity)
H_T: expected heterozygosity estimate calculated under Hardy-Weinberg genotypic proportions
θ^B: Bayesian estimated genetic differentiation (comparable to Wright's *Fst*)

Table A.16. Bayesian estimated pairwise θ^B (Holsinger et al., 2002) with 95% credibility intervals below diagonal and pairwise genetic distances (Nei's D) above diagonal. Values in bold indicate significant differences in θ^B between species.

	americanus	*charlesmithi*	*cognatus*	*fowleri*	*terrestris*	*velatus*	*woodhousii*
americanus		0.0836	0.3314	0.2001	0.1047	0.2007	0.1810
charlesmithi	0.10 -0.01–0.21		0.3808	0.1075	0.0938	0.0947	0.0820
cognatus	**0.14** **0.01–0.26**	0.04 -0.07–0.15		0.4620	0.3150	0.4773	0.4569
fowleri	0.06 -0.05–0.17	0.04 -0.14–0.06	0.07 -0.19–0.04		0.0834	0.0215	0.0373
terrestris	0.09 -0.03–0.19	0.02 -0.11–0.08	0.05 -0.16–0.06	0.02 -0.07–0.13		0.1053	0.0939
velatus	0.04 -0.09–0.15	0.07 -0.18–0.04	0.10 -0.22–0.01	0.03 -0.14–0.08	0.05 -0.16–0.06		0.0365
woodhousii	0.09 -0.03–0.20	0.01 -0.10–0.09	0.05 -0.15–0.06	0.03 -0.07–0.13	0.01 -0.10–0.10	0.05 -0.05–0.17	

Table A.17. Nei's G_{ST} below the diagonal and N_M above the diagonal for all pairwise species comparisons.

	americanus	*charlesmithi*	*cognatus*	*fowleri*	*terrestris*	*velatus*	*woodhousii*
americanus		5.6	1.2	2.7	4.7	2.8	2.9
charlesmithi	0.08		1.2	5.2	5.8	5.9	6.2
cognatus	0.29	0.29		1.0	1.4	1.0	1.0
fowleri	0.15	0.09	0.32		6.5	21.1	12.1
terrestris	0.09	0.08	0.26	0.07		5.4	5.6
velatus	0.15	0.08	0.33	0.02	0.08		12.6
woodhousii	0.15	0.08	0.33	0.04	0.08	0.04	

Table A.18. Summary statistics for Principal Coordinates Analysis of individual species in the *Anaxyrus americanus* group.

Species	1st Eigenvalue (% variance)	2nd Eigenvalue (% variance)	3rd Eigenvalue (% variance)	Cumulative % variance
americanus	11.59	7.38	6.41	66.43
	(30.34)	(19.31)	(16.78)	
charlesmithi	21.97	6.22	4.98	72.85
	(48.26)	(13.66)	(10.93)	
fowleri	17.24	6.18	4.21	70.94
	(44.26)	(15.88)	(10.80)	
terrestris	13.09	8.36	6.75	68.06
	(31.59)	(20.18)	(16.29)	
velatus	11.42	5.61	5.00	65.58
	(34.00)	(16.69)	(14.89)	
woodhousii	9.66	7.34	5.47	66.46
	(28.52)	(21.81)	(16.13)	
cognatus	8.53	7.38	5.83	68.00
	(26.68)	(23.09)	(18.23)	
All Individuals	38.32	13.34	12.16	74.82
	(44.93)	(15.64)	(14.25)	

Table A.19. Population structure of the *Anaxyrus americanus* group using Principal Coordinate-Modal Clustering (PCO-MC) analysis.

	americanus	*charlesmithi*	*cognatus*	*fowleri*	*terrestris*	*velatus*	*woodhousii*
Number of unique clusters	1	1	1	1	1	1	0
Number of overlapping clusters	2	3	1	2	1	1	2
Total number of clusters	3	4	1	3	1	1	2

Table A.20. Results of AMOVA performed on species divided into clusters identified through PCO-MC analysis.

Species	Source of variation	df	Variance component	% Variance	ϕ statistic	*P*-value
americanus	Among	2	0.52	47	0.47	< 0.01
	Within	15	0.59	53		
charlesmithi	Among	3	0.25	24	0.24	< 0.01
	Within	15	0.79	76		
fowleri	Among	2	0.51	44	0.44	< 0.01
	Within	41	0.64	56		
woodhousii	Among	1	0.39	30	0.30	< 0.01
	Within	21	0.90	70		

Table A.21. Summary statistics for Principal Coordinates Analysis of all pairwise comparisons between *Anaxyrus cognatus* and the *Anaxyrus americanus* group.

Species Comparison	1^{st} Eigenvalue (% variance)	2nd Eigenvalue (% variance)	3rd Eigenvalue (% variance)	Cumulative % variance
cognatus x americanus	25.93	9.70	6.65	74.02
	(45.39)	(16.98)	(11.65)	
cognatus x charlesmithi	30.55	16.04	5.53	79.85
	(46.81)	(24.58)	(8.47)	
cognatus x fowleri	31.32	13.85	5.72	81.41
	(50.10)	(22.15)	(9.16)	
cognatus x terrestris	28.93	17.34	6.86	75.46
	(46.98)	(10.68)	(11.14)	
cognatus x velatus	32.06	7.47	5.01	78.82
	(56.73)	(13.22)	(8.86)	
cognatus x woodhousii	32.72	8.22	6.15	78.54
	(54.58)	(13.7)	(10.25)	

Table A.22. Summary statistics for Principal Coordinates Analysis for all pairwise comparisons within the *Anaxyrus americanus* group.

Species Comparison	1st Eigenvalue (% variance)	2nd Eigenvalue (% variance)	3rd Eigenvalue (% variance)	Cumulative % variance
americanus x charlesmithi	19.33	9.32	7.70	71.22
	(37.88)	(18.25)	(15.09)	
americanus x fowleri	26.27	13.11	6.16	76.65
	(44.22)	(22.06)	(10.37)	
americanus x terrestris	15.43	9.55	8.20	66.96
	(31.13)	(19.28)	(16.54)	
americanus x velatus	49.04	13.75	12.75	75.54
	(27.28)	(7.65)	(7.09)	
americanus x woodhousii	23.51	8.09	7.46	73.03
	(43.97)	(15.12)	(13.94)	
charlesmithi x fowleri	21.12	13.05	6.25	76.17
	(39.79)	(24.59)	(11.78)	
charlesmithi x terrestris	19.27	12.29	7.21	73.60
	(36.58)	(23.33)	(13.69)	
charlesmithi x velatus	21.40	7.54	5.37	71.68
	(44.72)	(15.75)	(11.21)	
charlesmithi x woodhousii	21.52	8.30	5.52	72.81
	(44.33)	(17.10)	(11.38)	
fowleri x terrestris	20.95	11.05	6.26	74.40
	(40.74)	(21.49)	(12.17)	
fowleri x velatus	17.42	8.06	5.35	70.81
	(40.01)	(18.51)	(12.29)	
fowleri x woodhousii	16.61	7.74	6.35	69.10
	(37.39)	(17.42)	(14.29)	
terrestris x velatus	19.76	7.73	6.16	70.85
	(41.61)	(16.27)	(12.97)	
terrestris x woodhousii	17.26	8.67	5.79	67.85
	(36.92)	(18.54)	(12.40)	
velatus x woodhousii	12.74	7.92	5.86	66.85
	(32.11)	(19.96)	(14.78)	

Table A.23. AMOVA results for all pairwise comparisons within the *Anaxyrus americanus* group (*P*-values in bold indicate non-significant differences).

Species comparison	Source of variation	df	Variance component	% variance	ϕ statistic	*P*-value
americanus x charlesmithi	Among	1	2.09	12	0.12	<
	Within	35	15.27	88		0.01
americanus x fowleri	Among	1	1.78	11	0.11	<
	Within	60	13.77	89		0.01
americanus x terrestris	Among	1	2.89	16	0.16	<
	Within	33	14.83	84		0.01
americanus x velatus	Among	1	0.81	6	0.06	<
	Within	66	13.61	94		0.01
americanus x woodhousii	Among	1	2.35	14	0.14	<
	Within	39	14.11	86		0.01
charlesmithi x fowleri	Among	1	2.08	14	0.14	<
	Within	61	12.48	86		0.01
charlesmithi x terrestris	Among	1	1.96	11	0.11	<
	Within	34	15.53	89		0.01
charlesmithi x velatus	Among	1	0.24	2	0.02	**<**
	Within	67	12.49	98		**0.06**
charlesmithi x woodhousii	Among	1	1.27	9	0.09	<
	Within	40	13.38	91		0.01
fowleri x terrestris	Among	1	2.19	15	0.15	<
	Within	59	12.20	85		0.01
fowleri x velatus	Among	1	0.35	3	0.03	<
	Within	92	10.7	97		0.01
fowleri x woodhousii	Among	1	0.59	5	0.05	<
	Within	65	11.18	95		0.01
terrestris x velatus	Among	1	0.13	1	0.01	**<**
	Within	65	12.76	99		**0.17**
terrestris x woodhousii	Among	1	0.84	6	0.06	<
	Within	38	13.69	94		0.02
velatus x woodhousii	Among	1	0.78	7	0.07	<
	Within	71	10.69	93		0.01

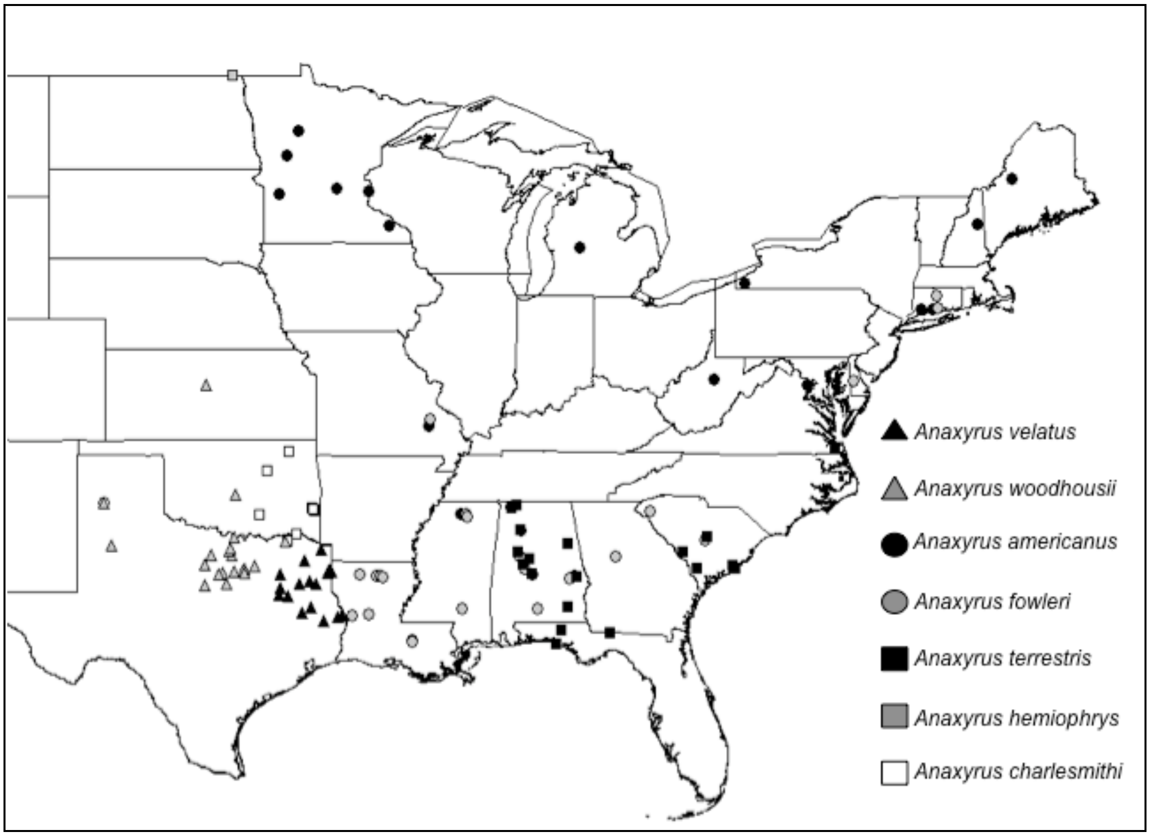

Figure A.19. Collection localities for specimens examined in AFLP analysis.

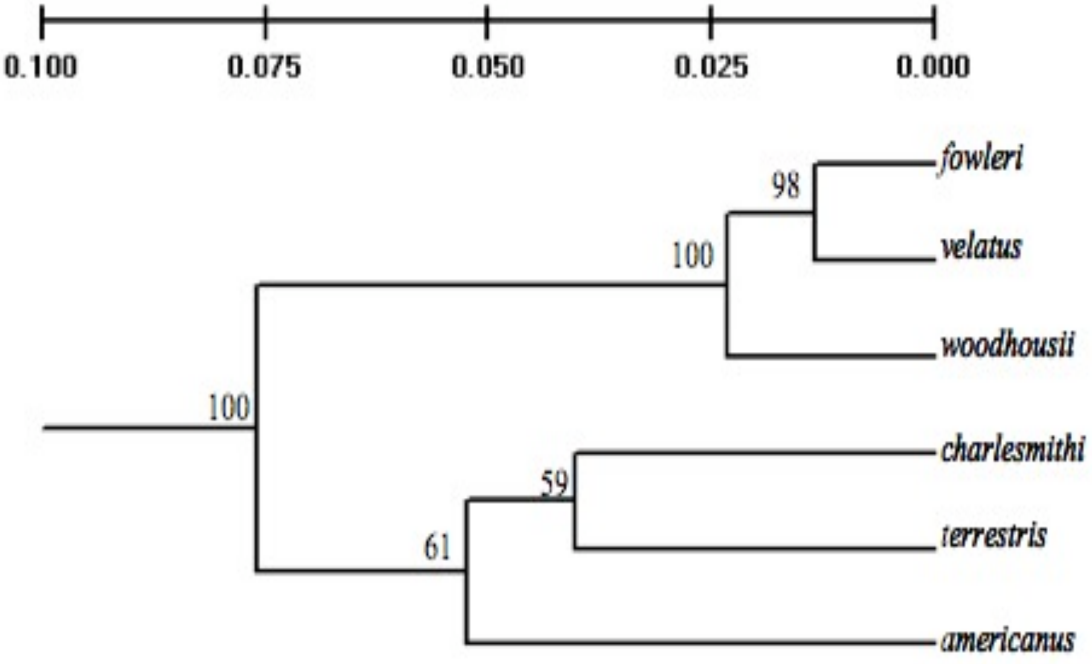

Figure A.20. UPGMA dendrogram of species relationships based on Nei's genetic distances from 100 AFLP loci. Nodal support was assessed using a bootstrap analysis with 10,000 replicates. The number at each node equals percentage of bootstrap support.

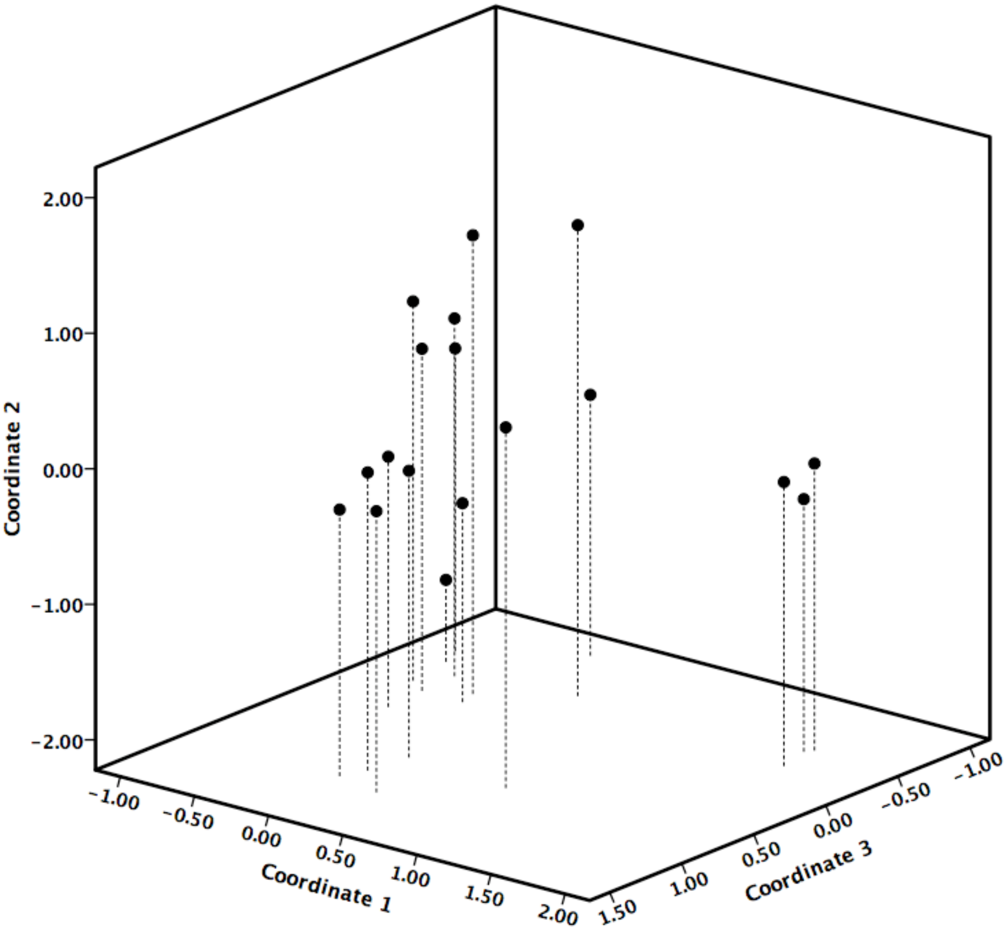

Figure A.21. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus americanus*.

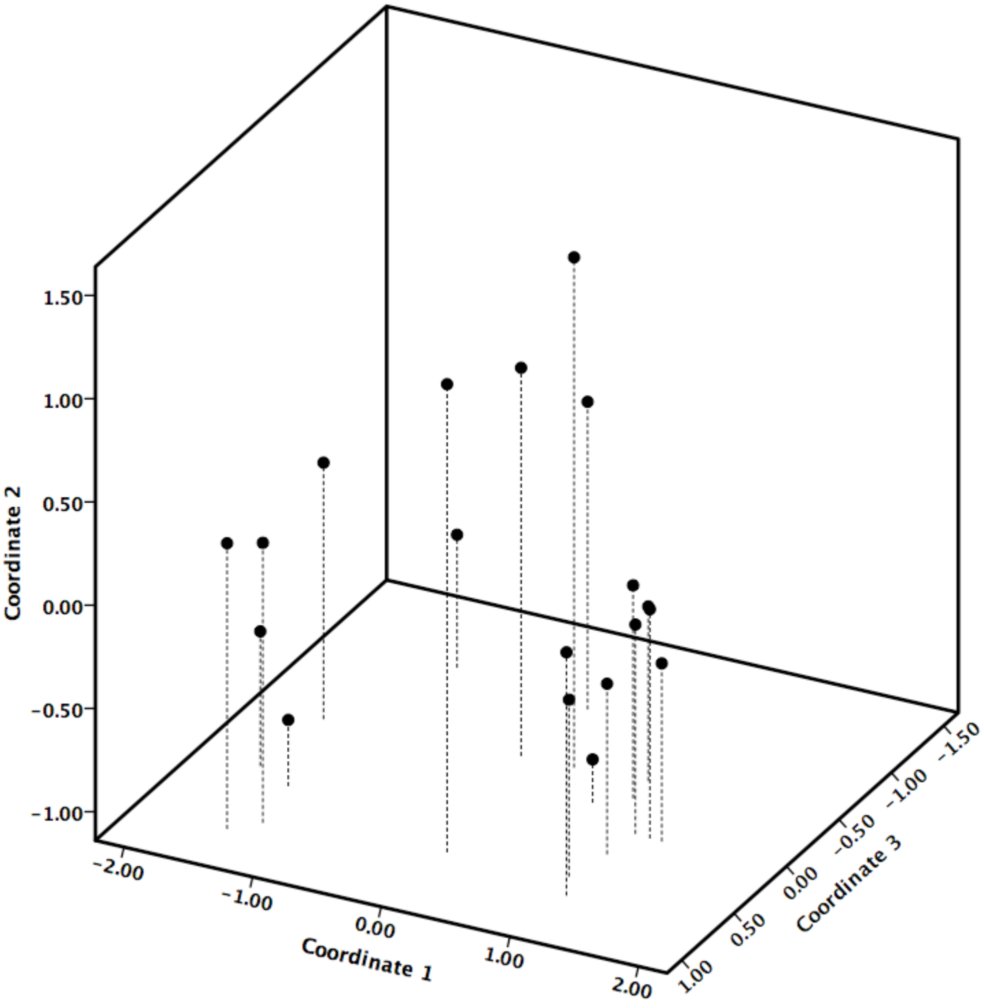

Figure A.22. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus charlesmithi*.

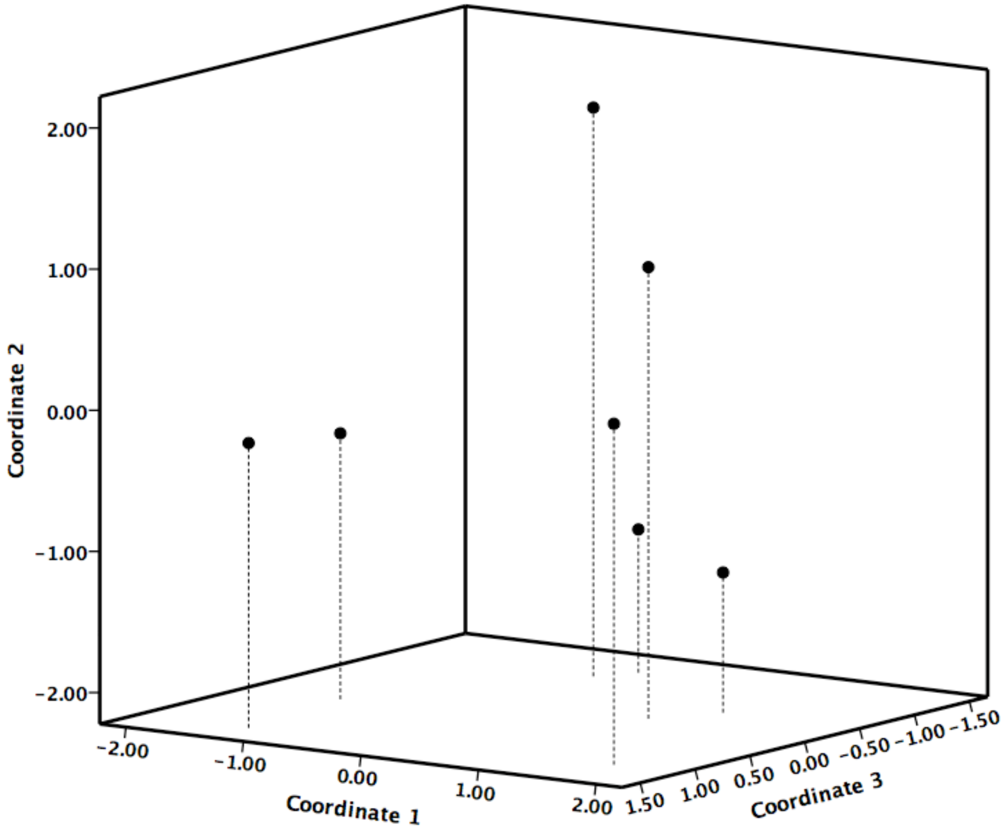

Figure A.23. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus cognatus*.

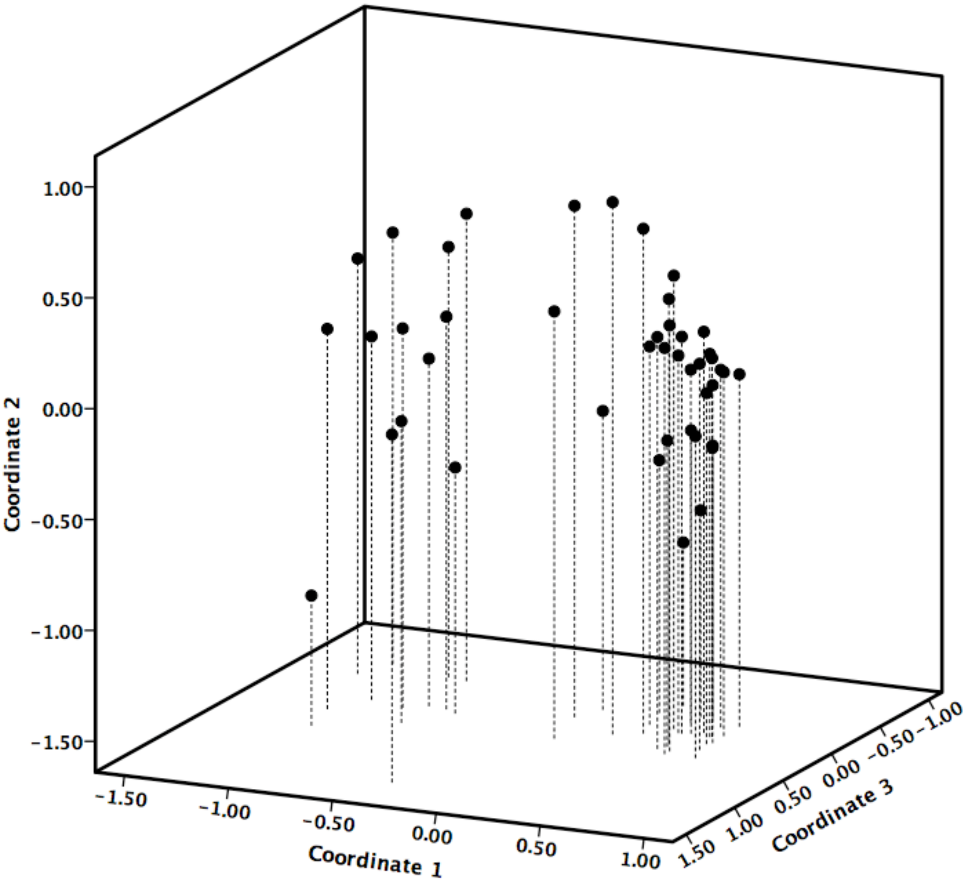

Figure A.24. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus fowleri*.

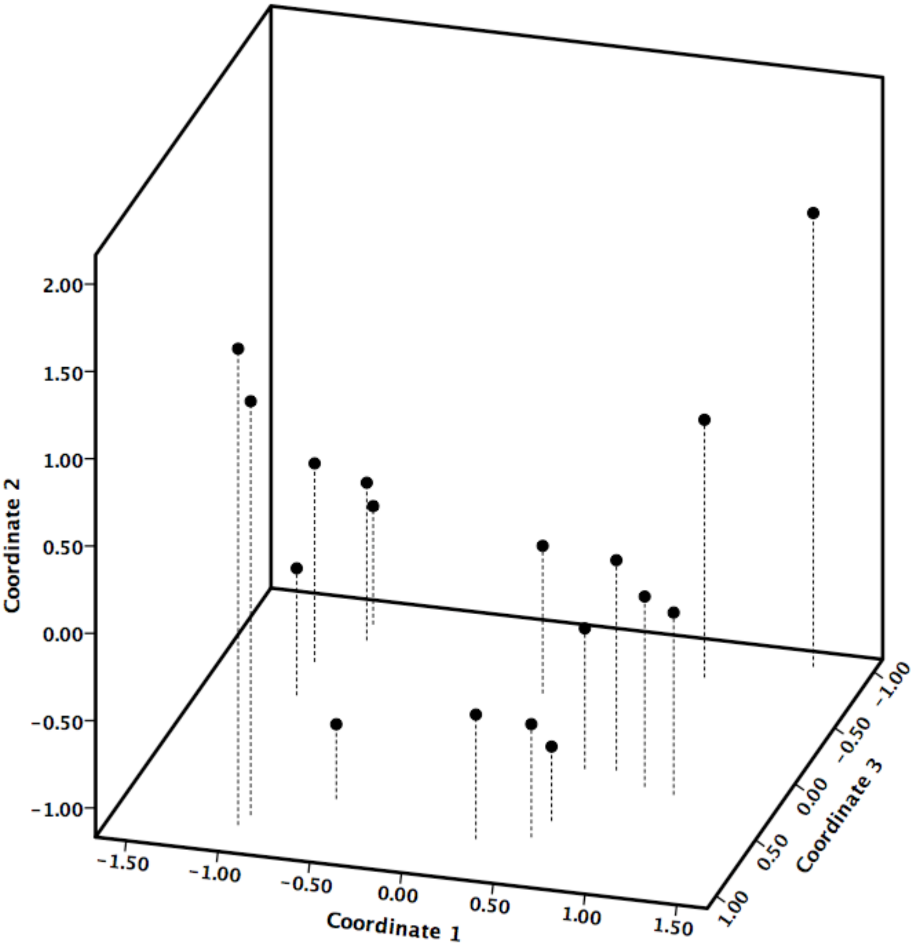

Figure A.25. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus terrestris*.

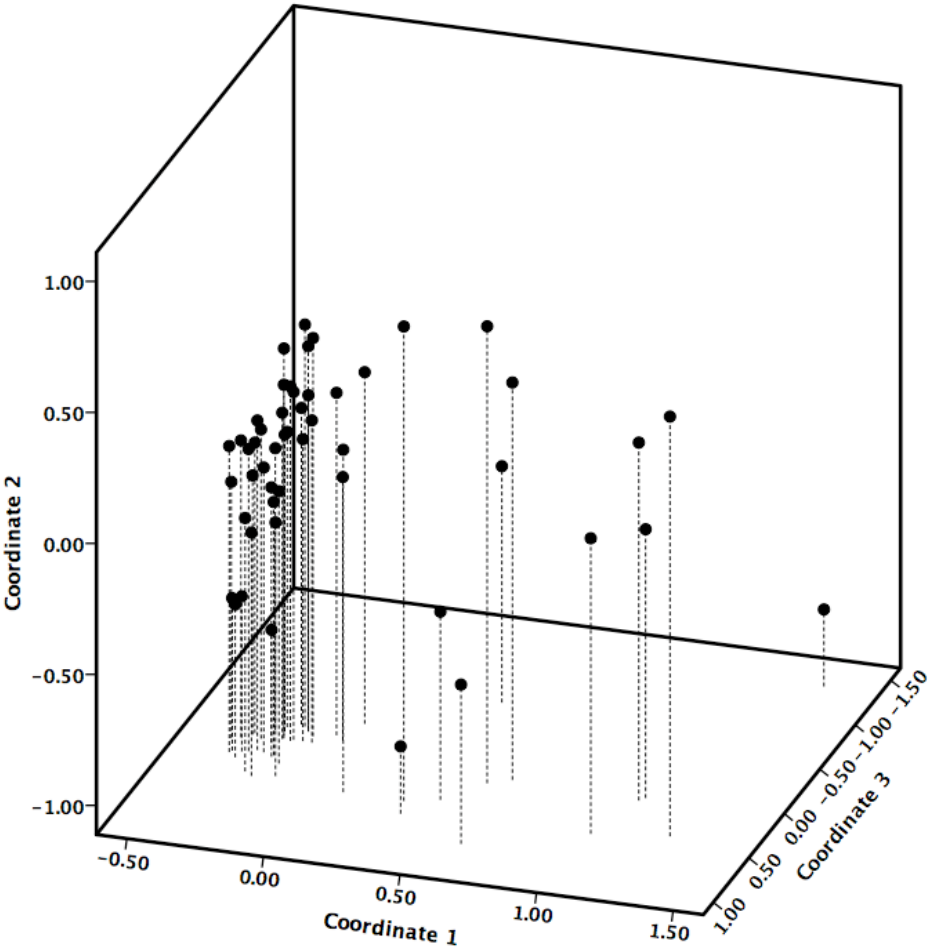

Figure A.26. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus velatus*.

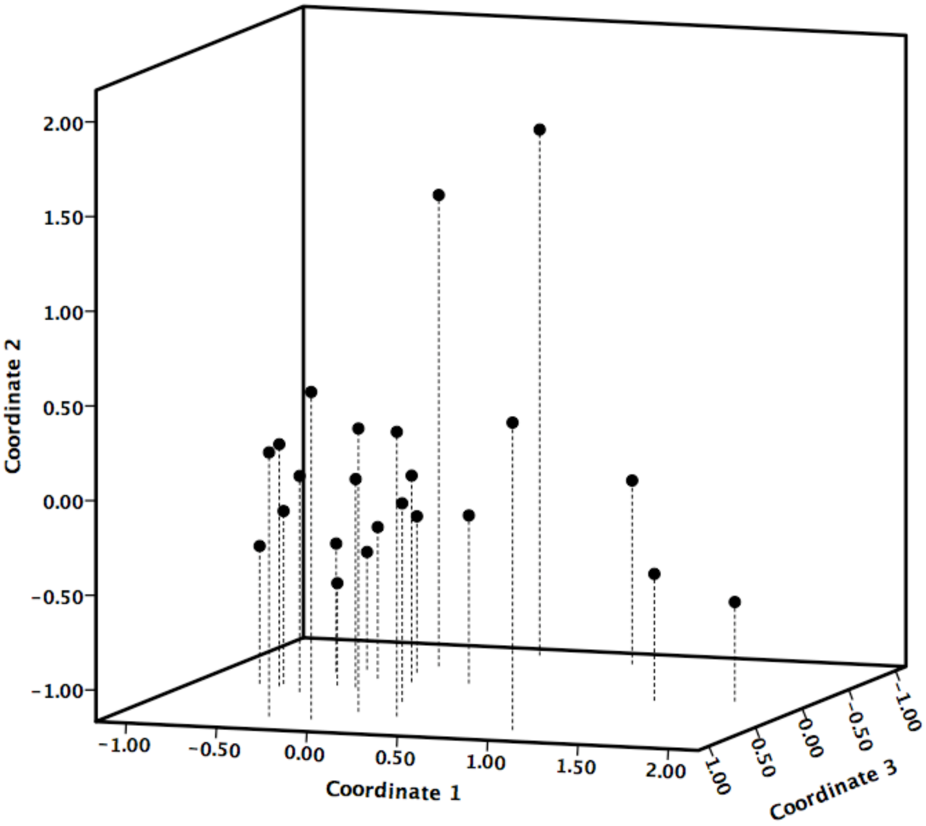

Figure A.27. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus woodhousii*.

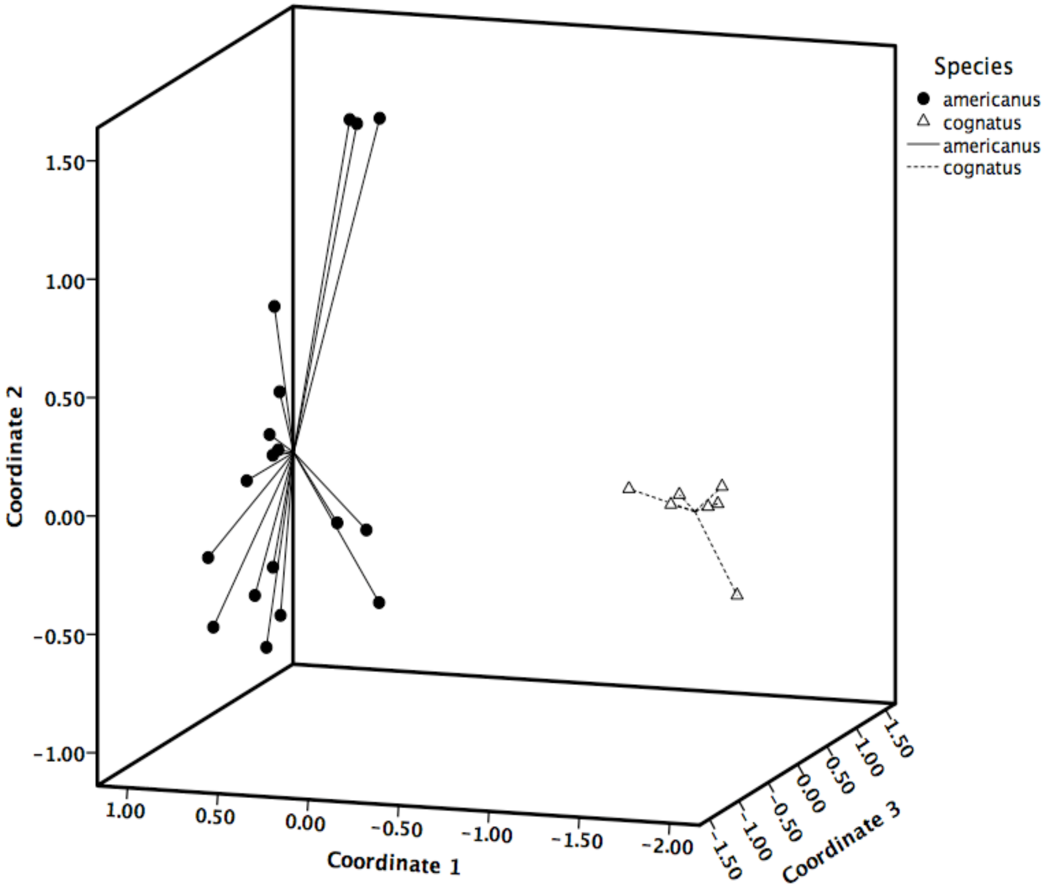

Figure A.28. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus americanus* and *A. cognatus*. Lines originate from centroid of each group.

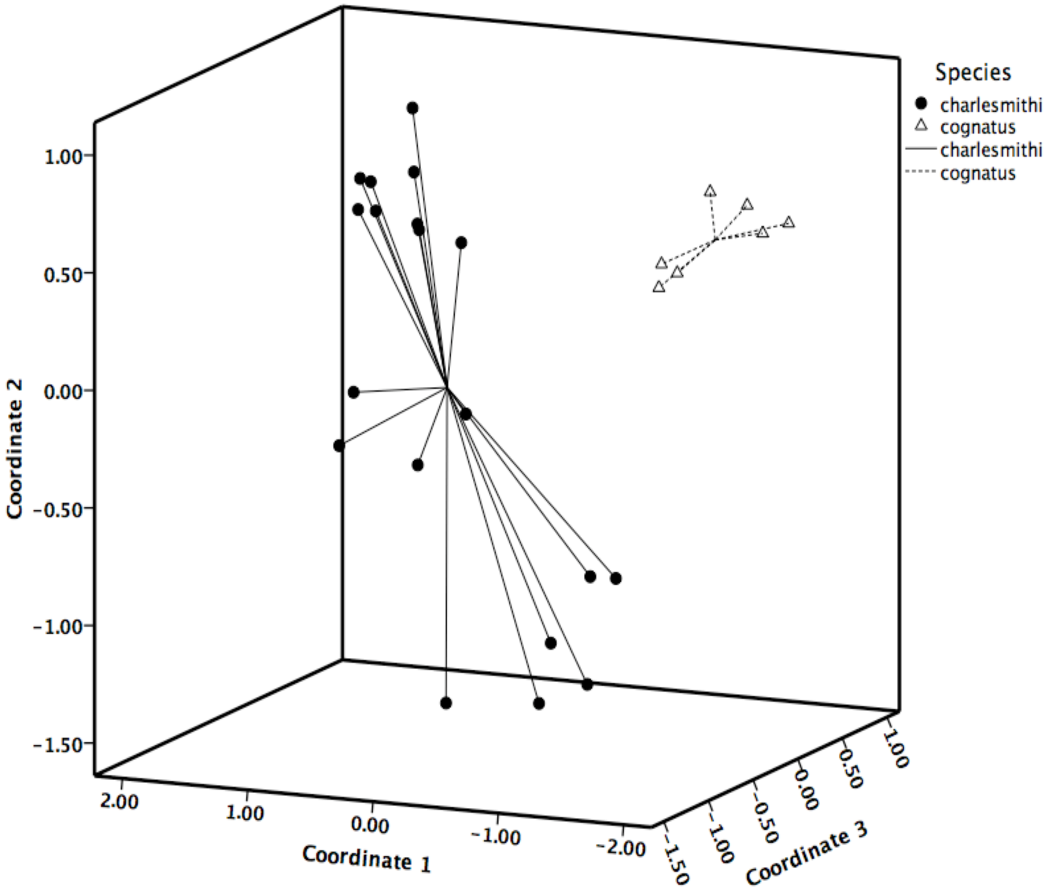

Figure A.29. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus charlesmithi* and *A. cognatus*. Lines originate from centroid of each group.

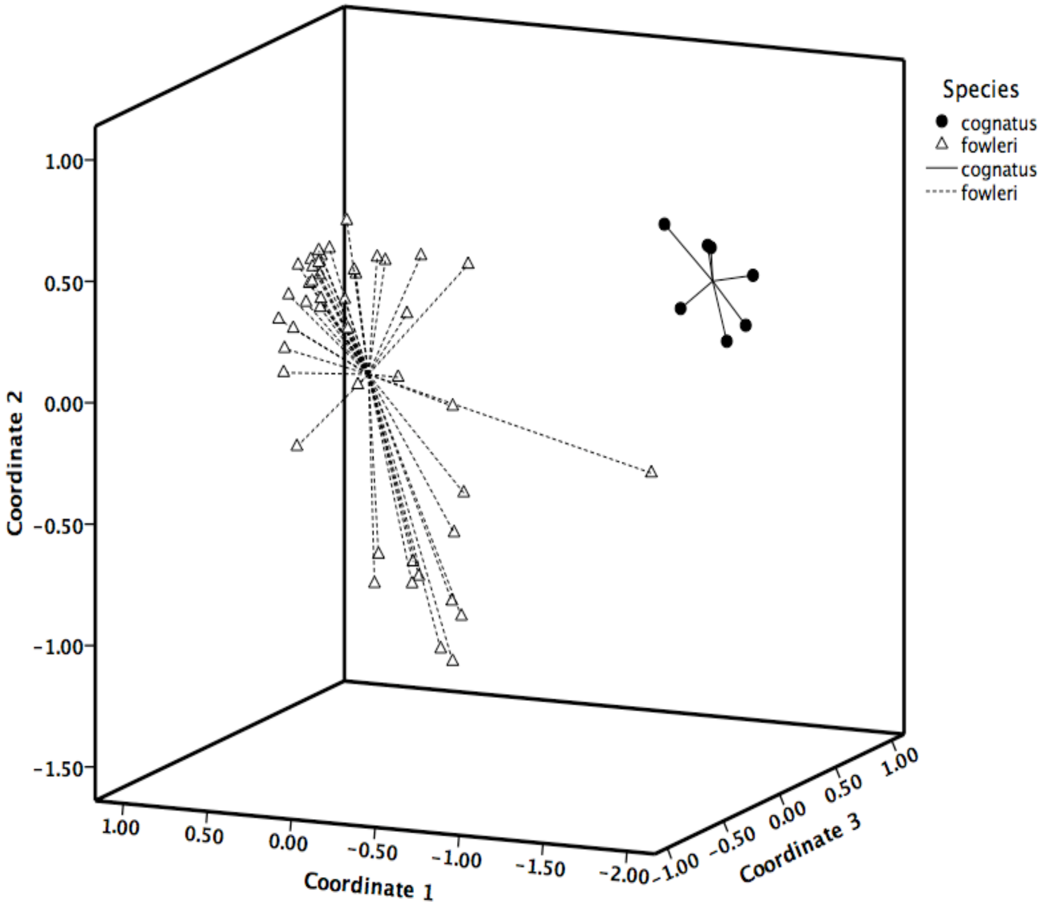

Figure A.30. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus fowleri* and *A. cognatus*. Lines originate from centroid of each group.

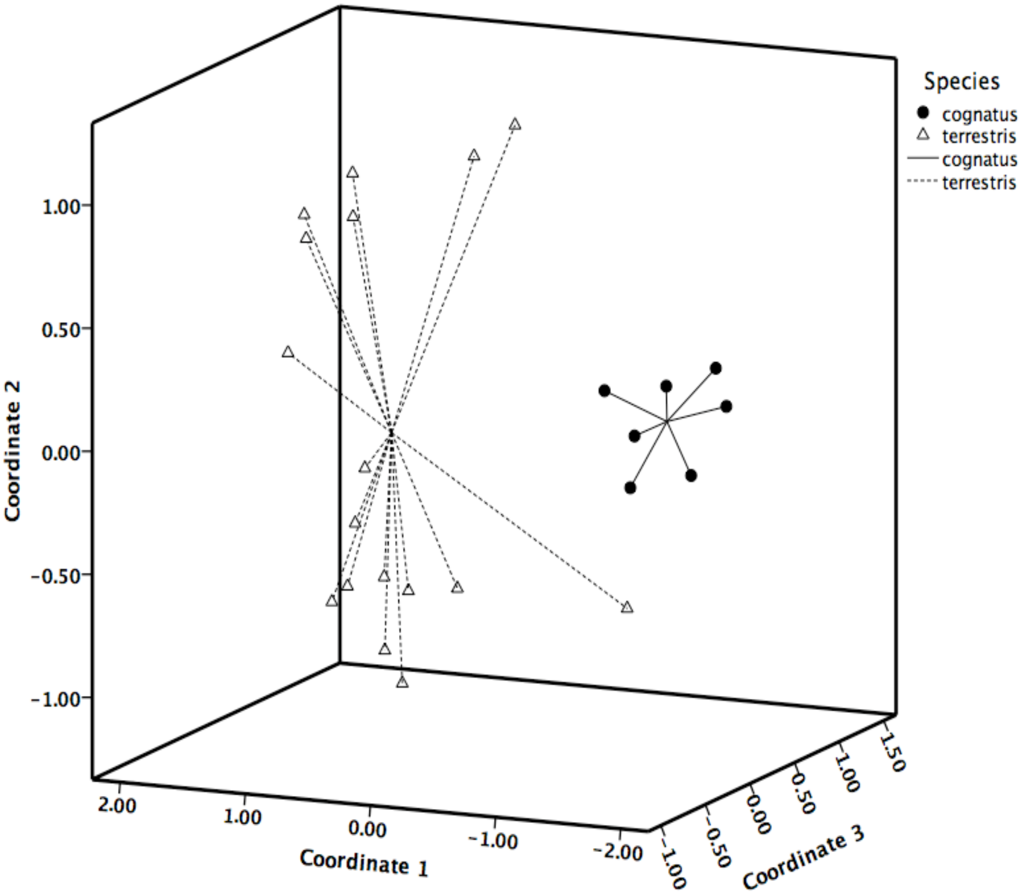

Figure A.31. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus terrestris* and *A. cognatus*. Lines originate from centroid of each group.

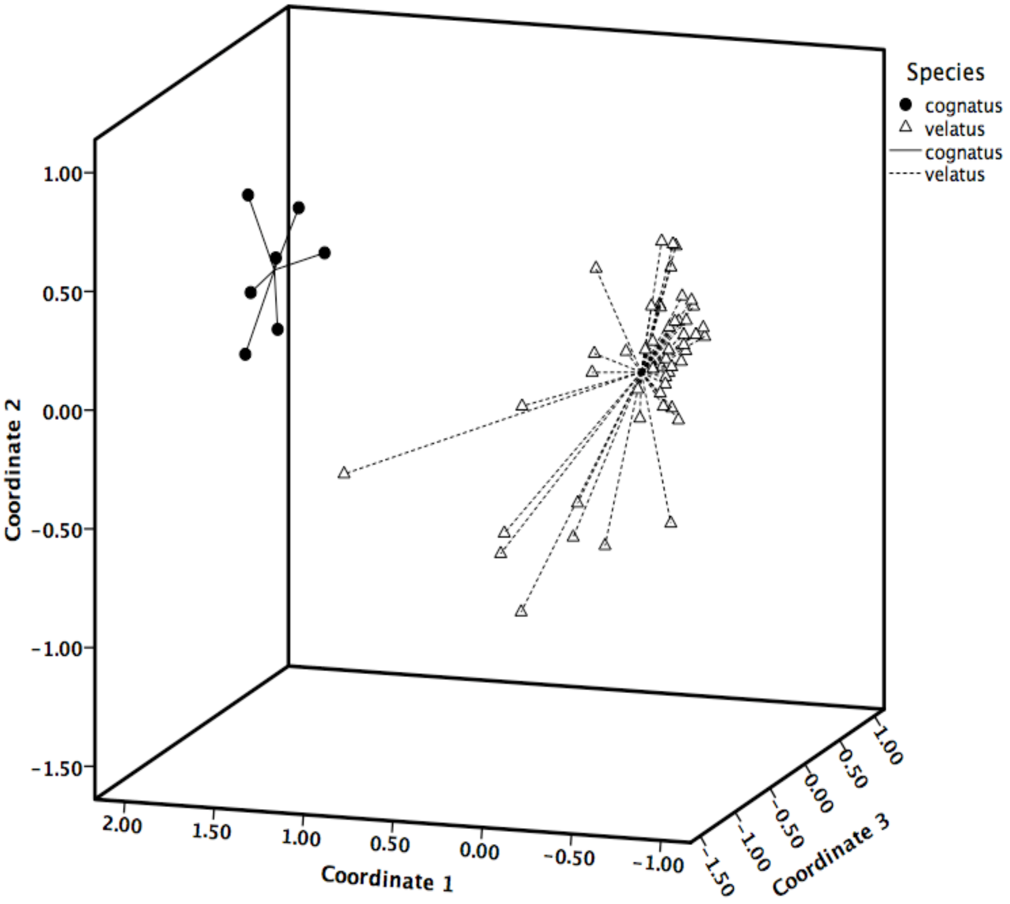

Figure A.32. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus velatus* and *A. cognatus*. Lines originate from centroid of each group.

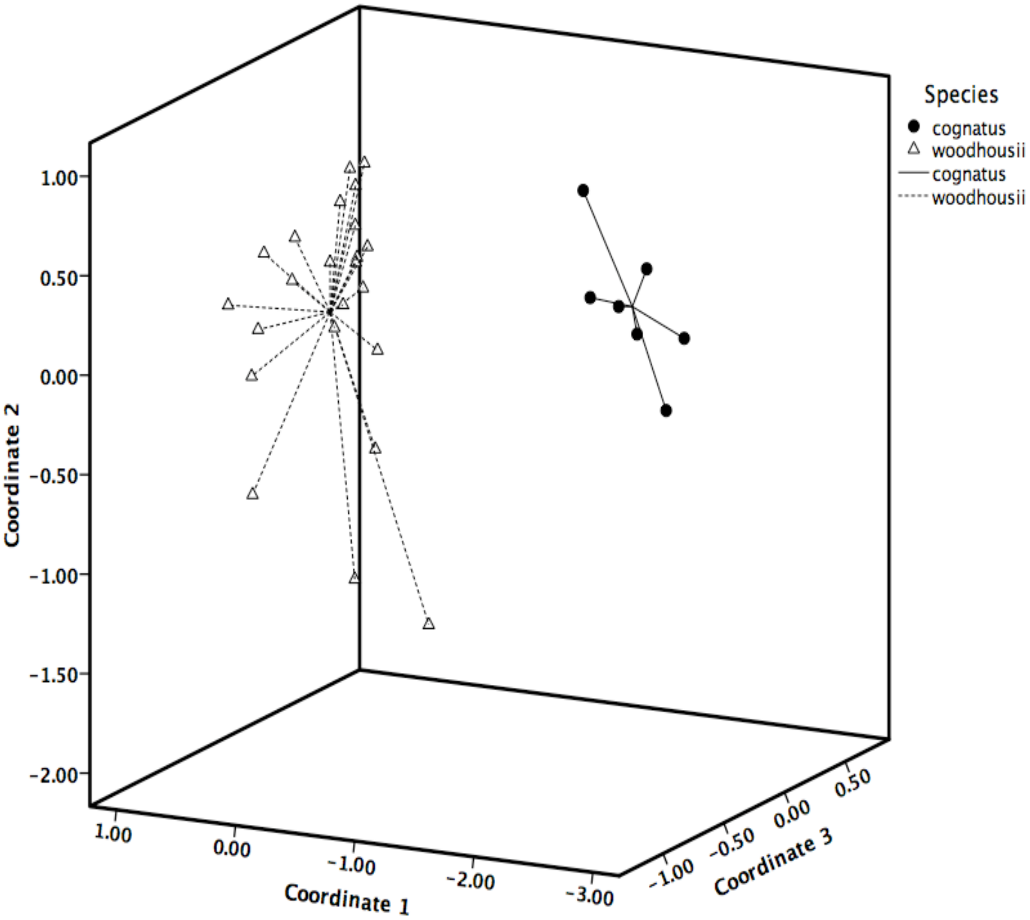

Figure A.33. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus woodhousii* and *A. cognatus*. Lines originate from centroid of each group.

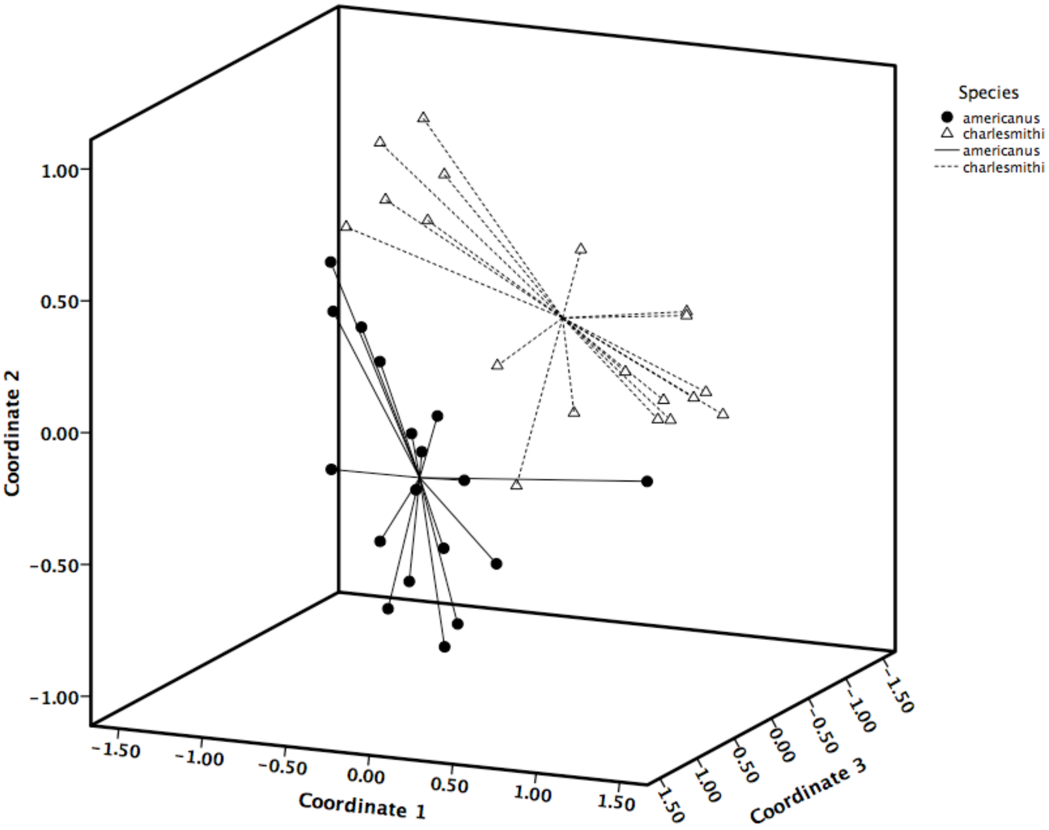

Figure A.34. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus americanus* and *A. charlesmithi*. Lines originate from centroid of each group.

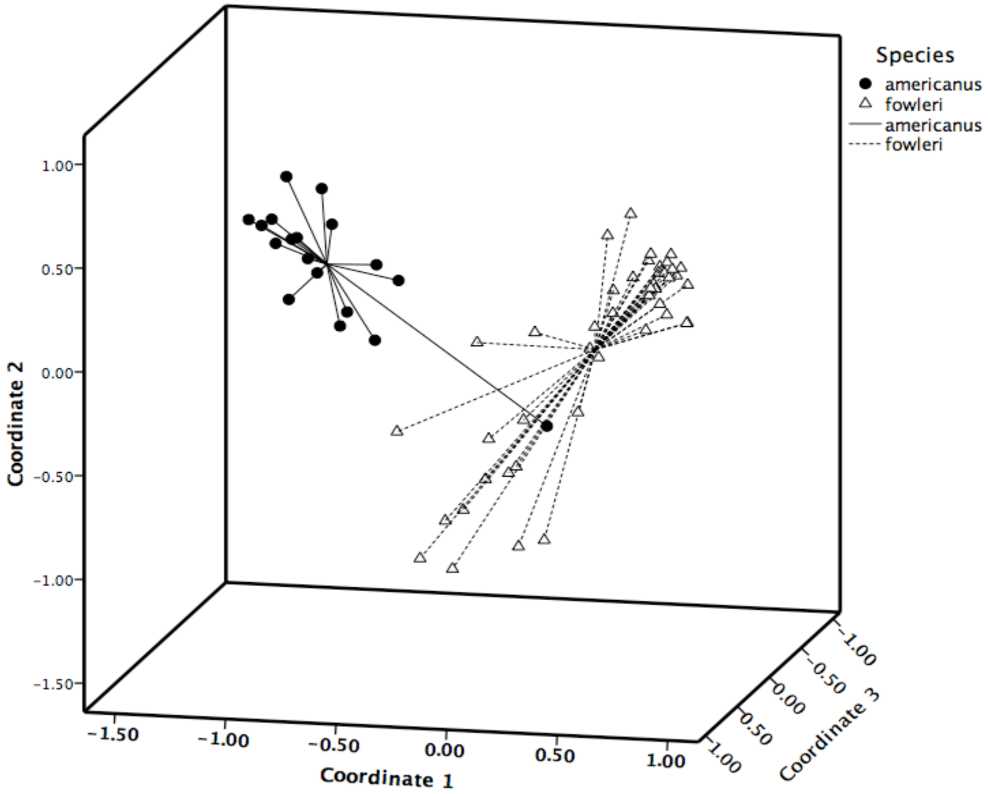

Figure A.35. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus americanus* and *A. fowleri*. Lines originate from centroid of each group.

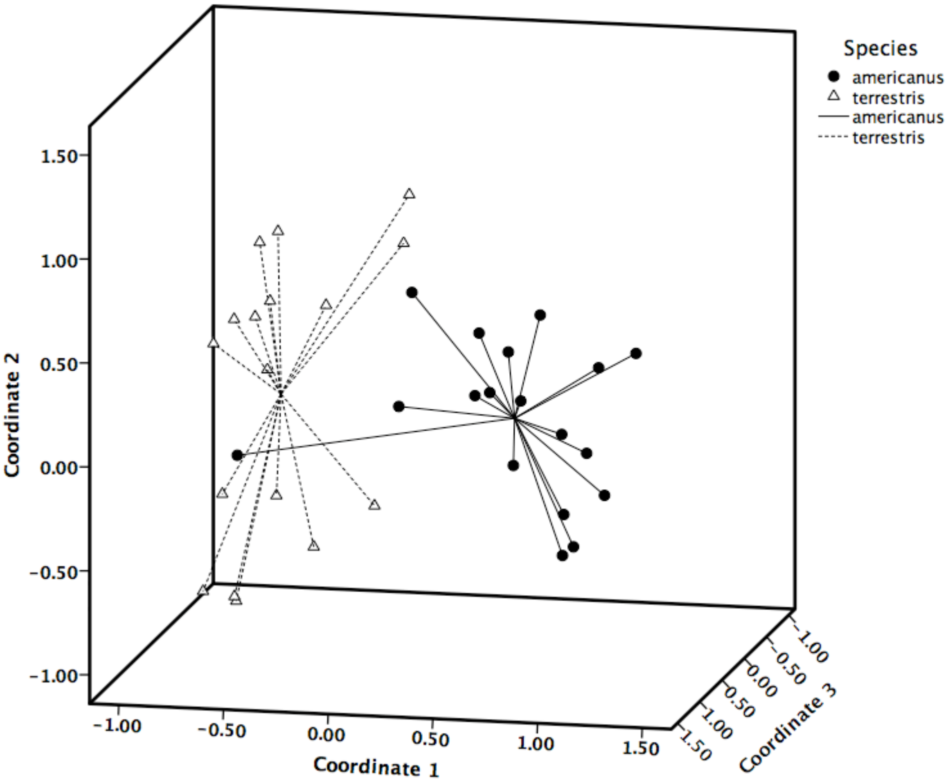

Figure A.36. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus americanus* and *A. terrestris*. Lines originate from centroid of each group.

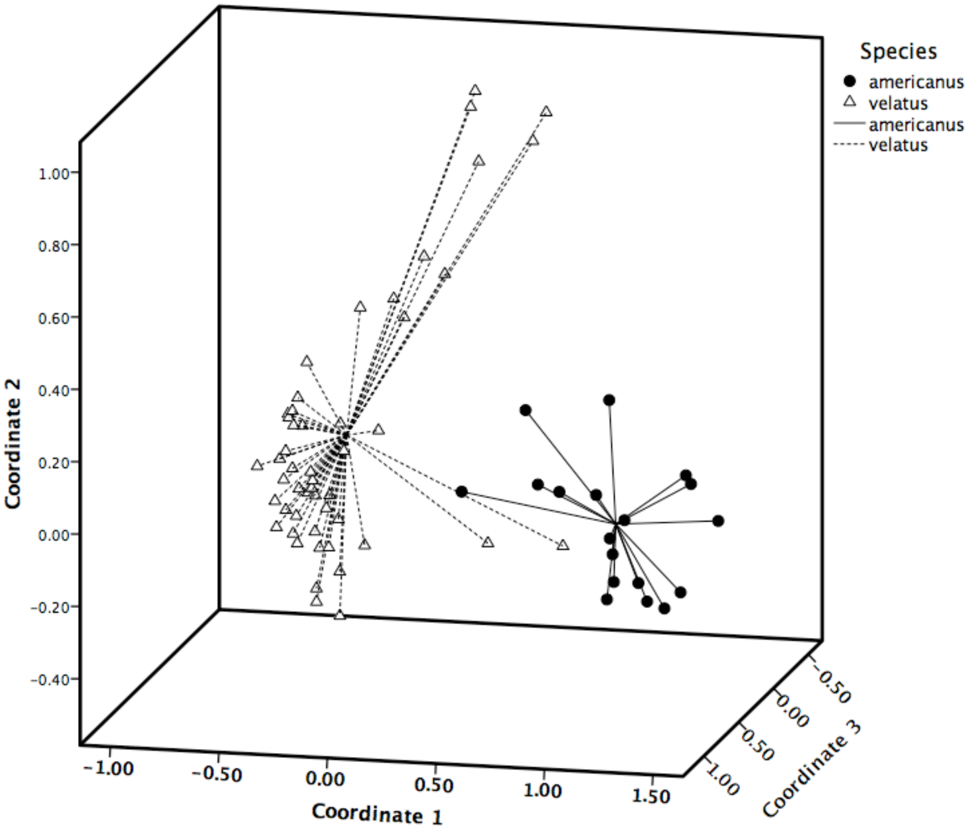

Figure A.37. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus americanus* and *A. velatus*. Lines originate from centroid of each group.

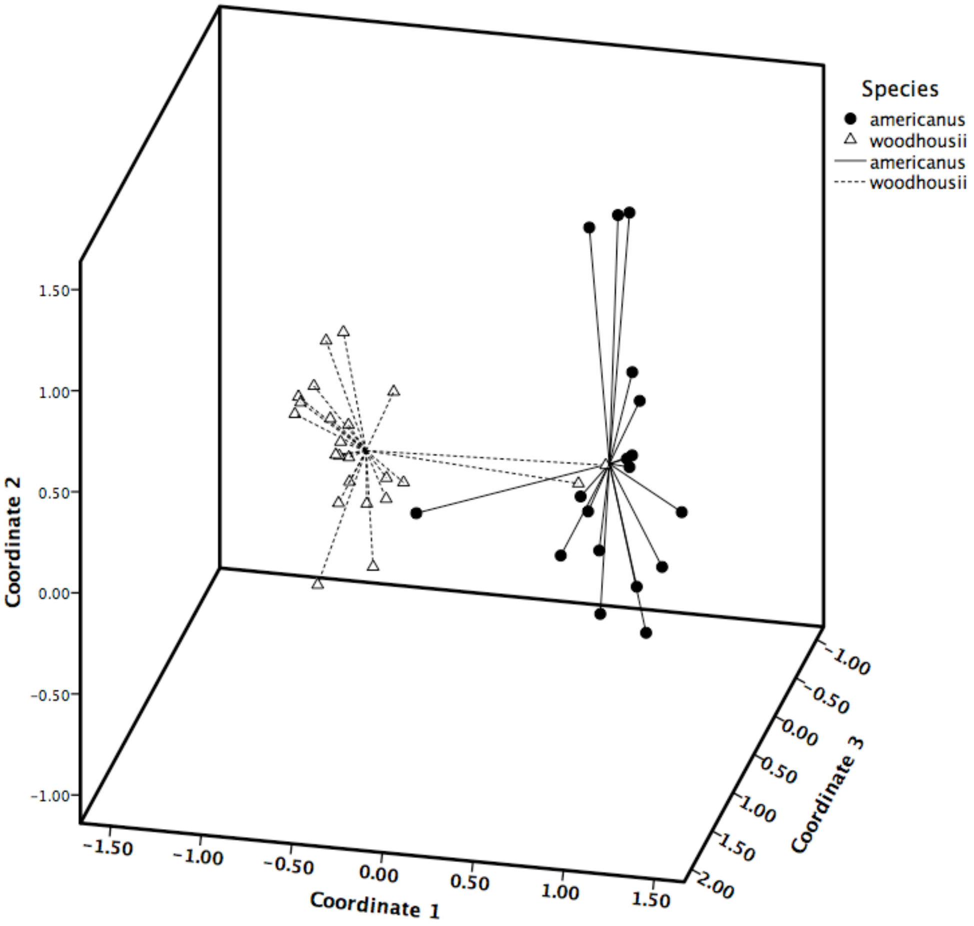

Figure A.38. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus americanus* and *A. woodhousii*. Lines originate from centroid of each group.

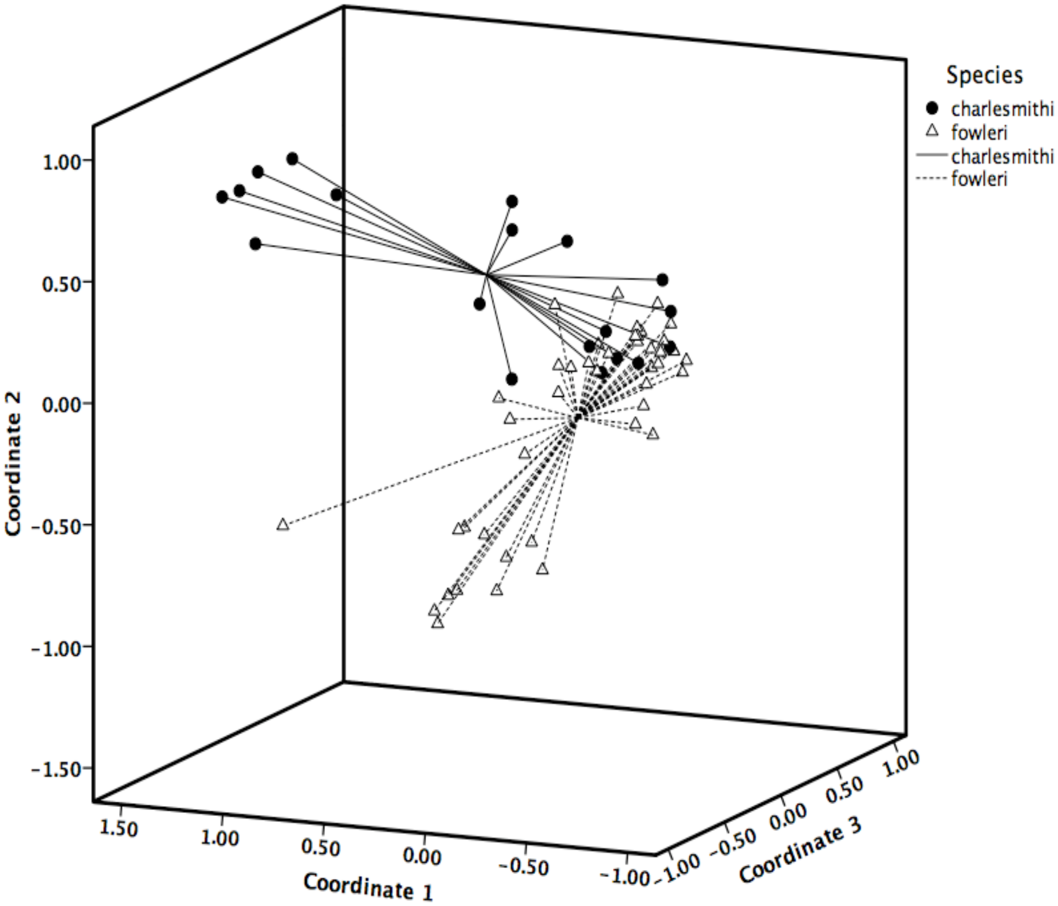

Figure A.39. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus charlesmithi* and *A. fowleri*. Lines originate from centroid of each group.

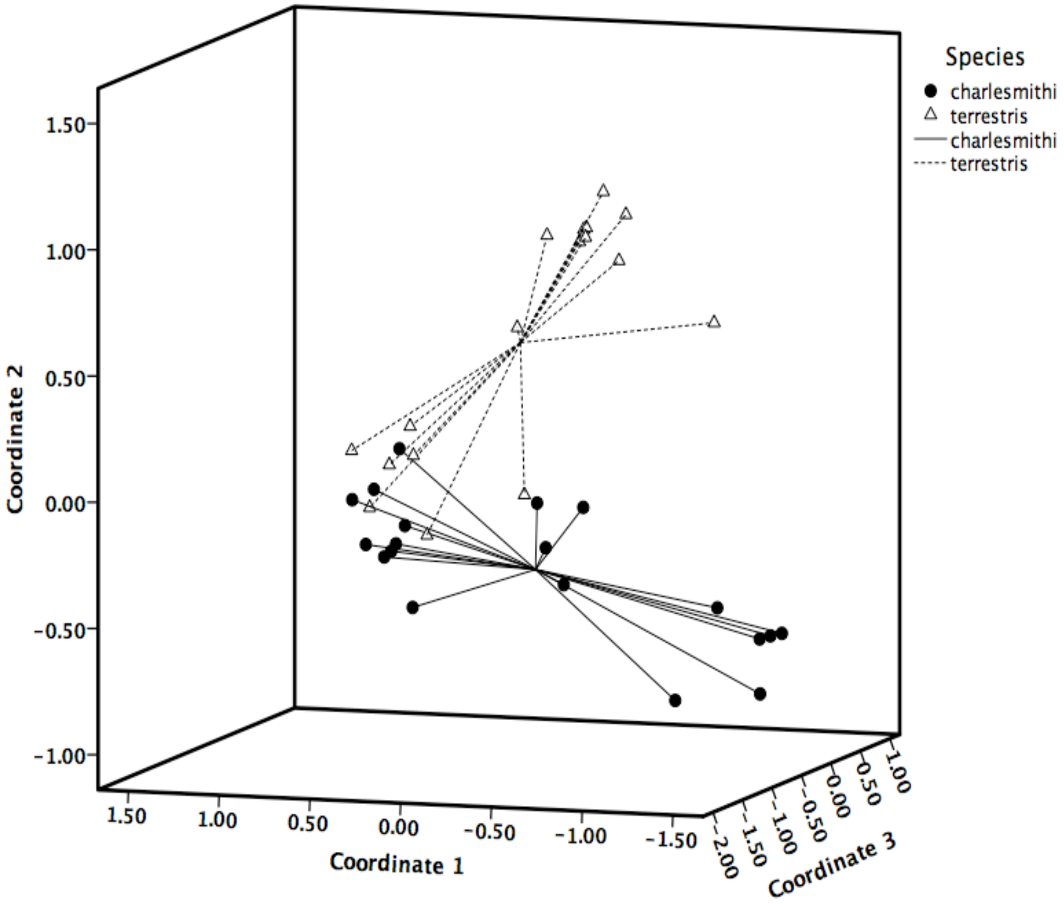

Figure A.40. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus charlesmithi* and *A. terrestris*. Lines originate from centroid of each group.

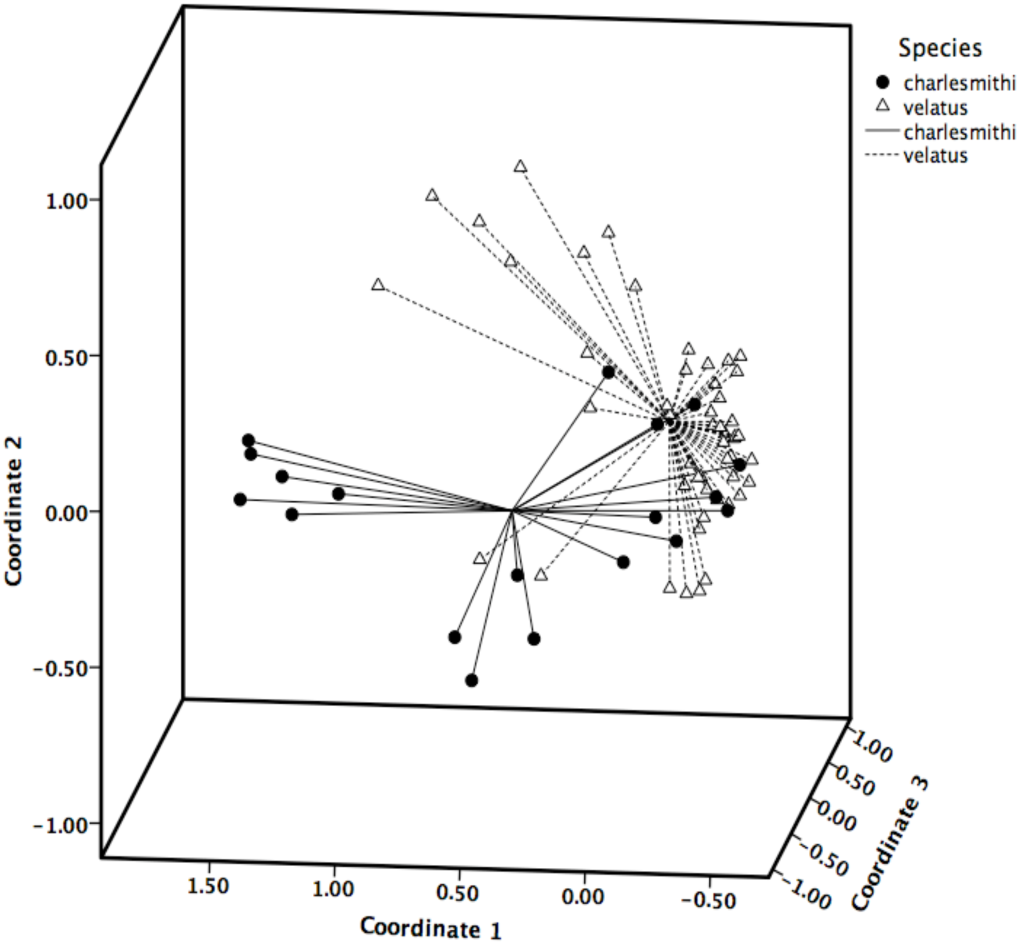

Figure A.41. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus charlesmithi* and *A. velatus*. Lines originate from centroid of each group.

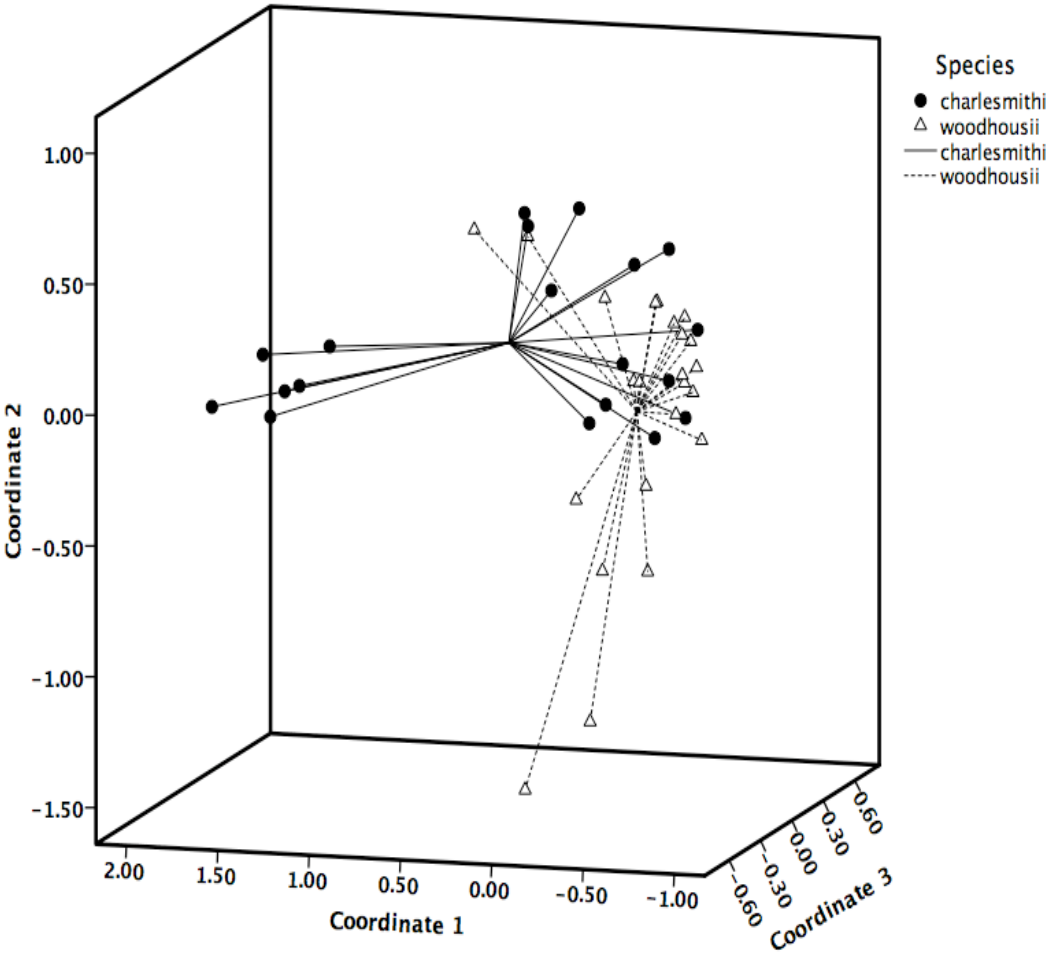

Figure A.42. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus charlesmithi* and *A. woodhousii*. Lines originate from centroid of each group.

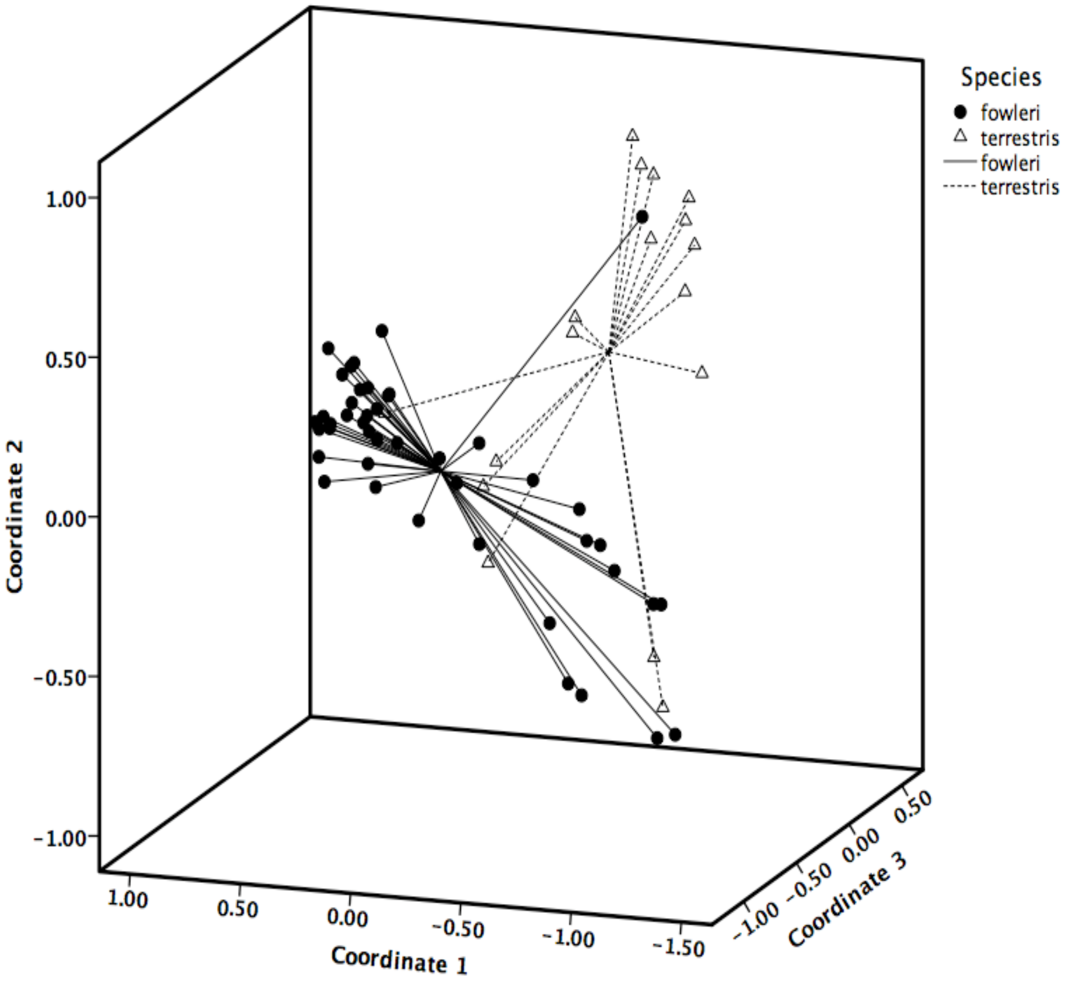

Figure A.43. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus fowleri* and *A. terrestris*. Lines originate from centroid of each group.

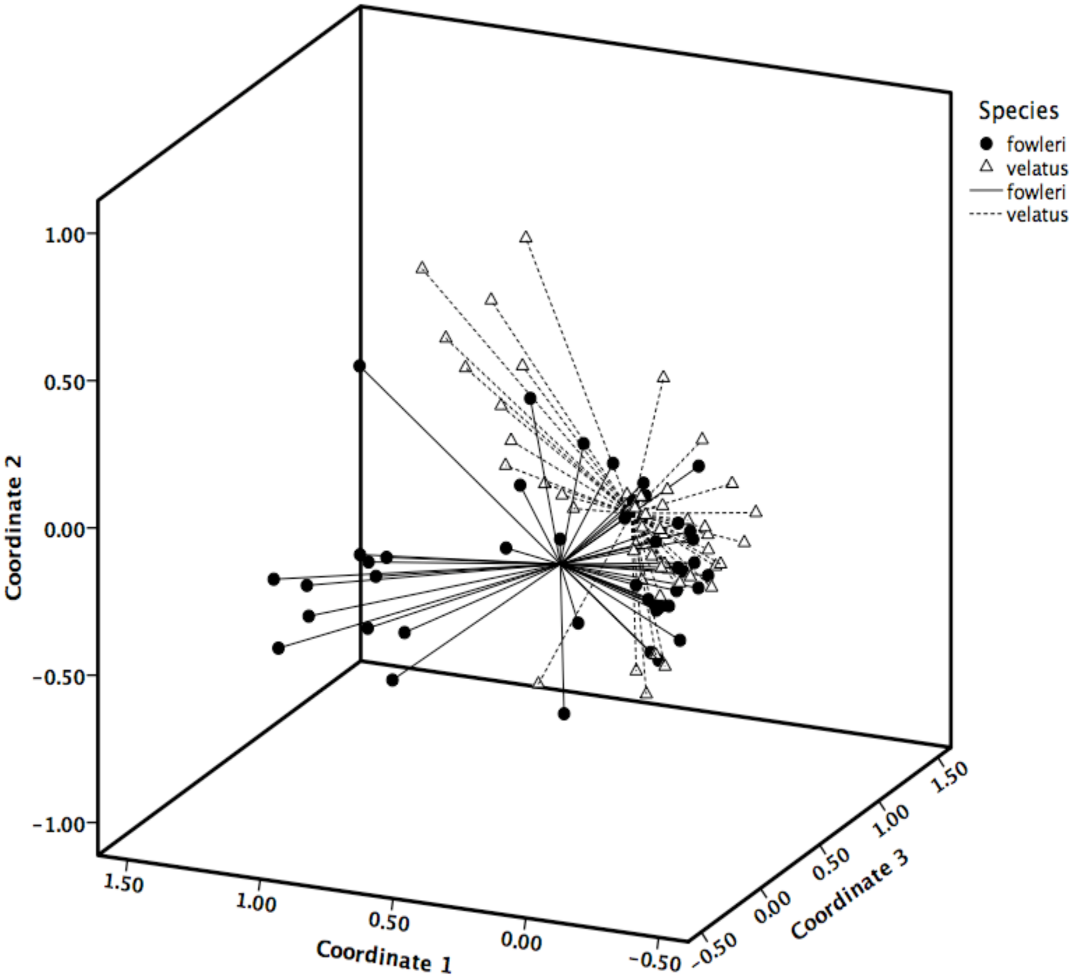

Figure A.44. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus fowleri* and *A. velatus*. Lines originate from centroid of each group.

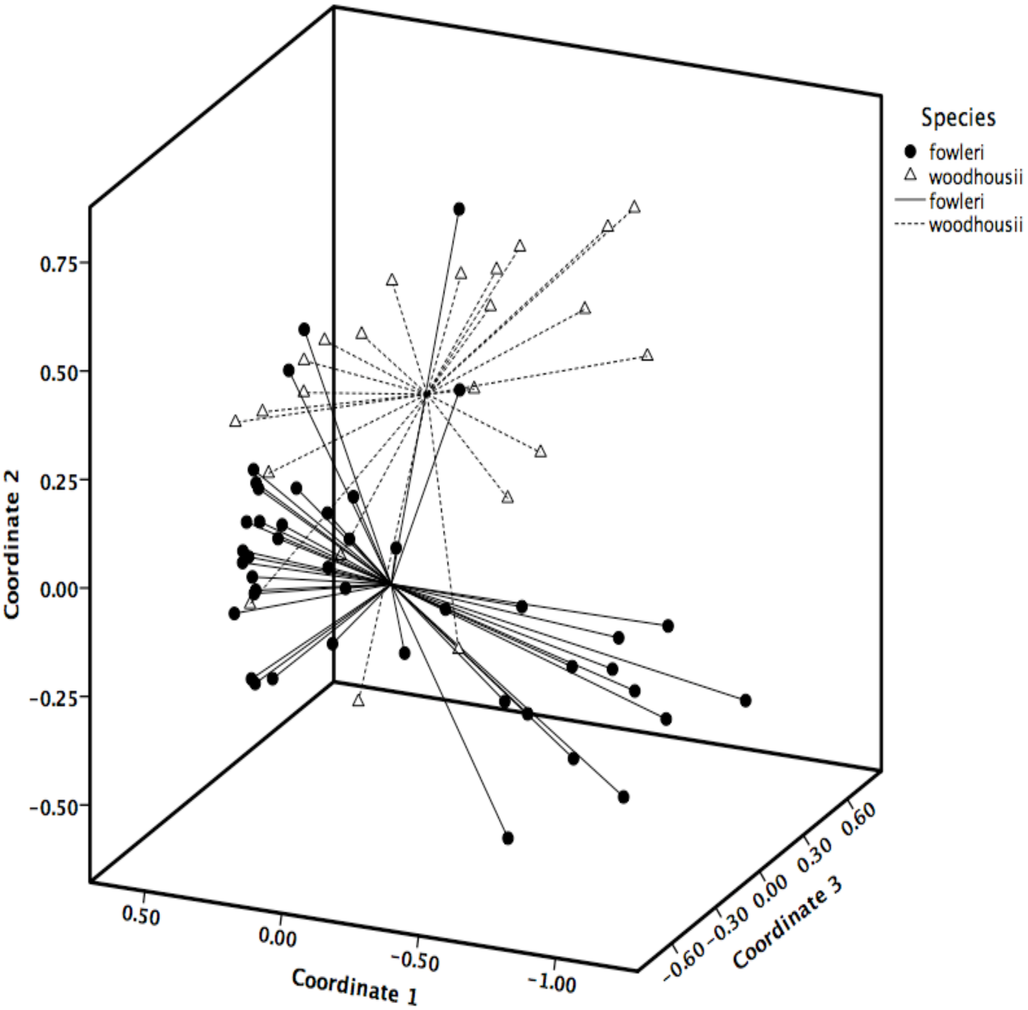

Figure A.45. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus fowleri* and *A. woodhousii*. Lines originate from centroid of each group.

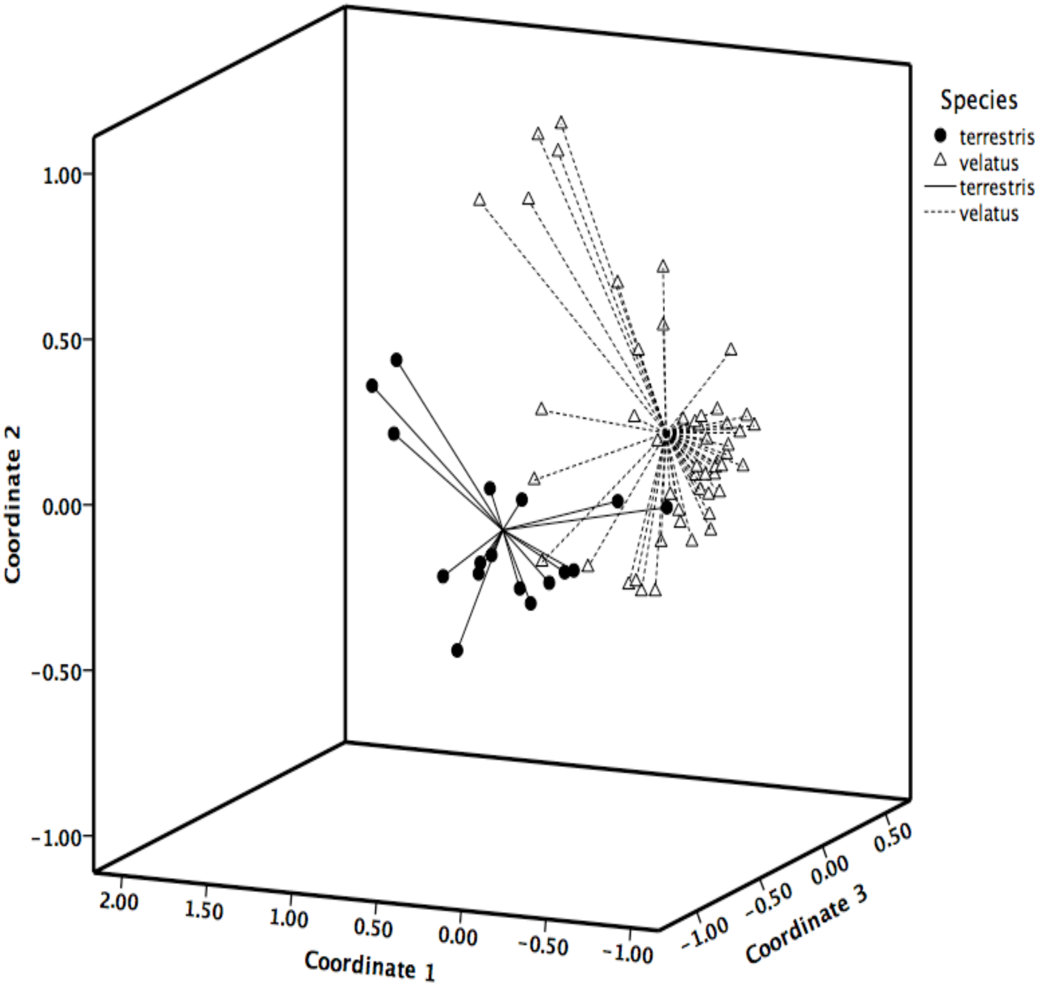

Figure A.46. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus terrestris* and *A. velatus*. Lines originate from centroid of each group.

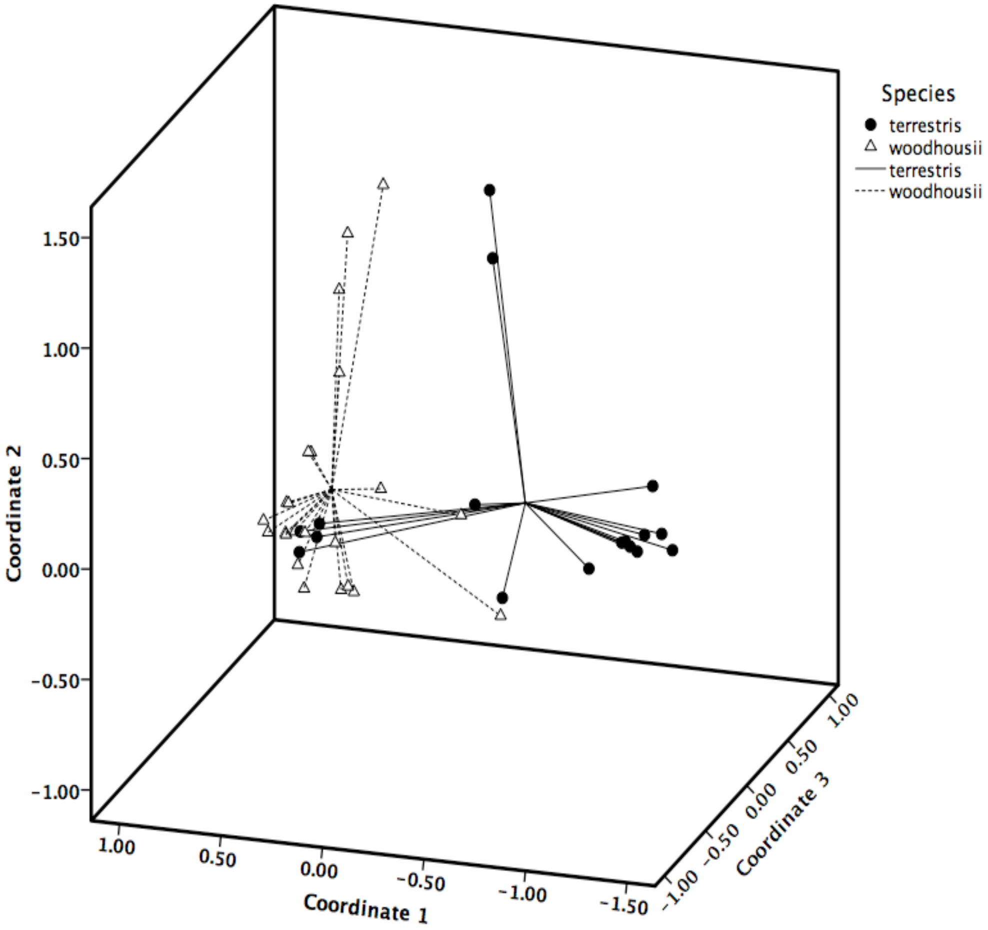

Figure A.47. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus terrestris* and *A. woodhousii*. Lines originate from centroid of each group.

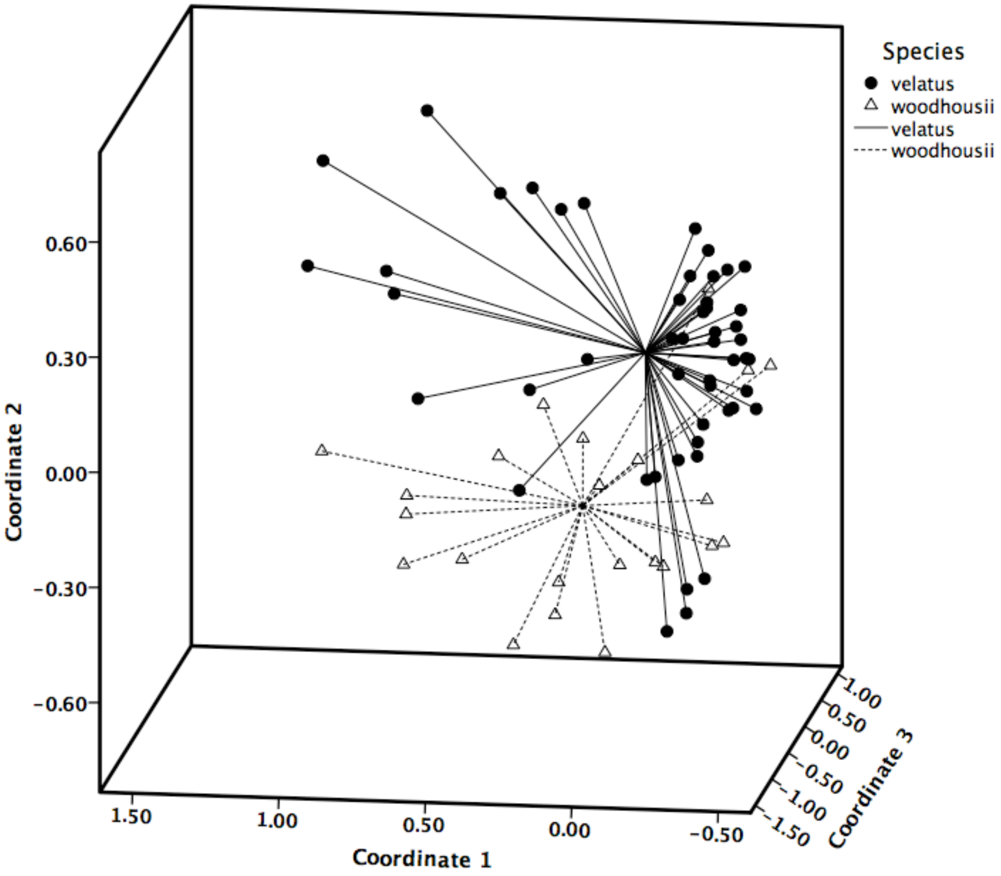

Figure A.48. Principal coordinates analysis plot of AFLP pairwise individual genetic distances for *Anaxyrus velatus* and *A. woodhousii*. Lines originate from centroid of each group.

APPENDIX B

SPECIMENS EXAMINED IN AFLP ANALYSES

Species	ID#	Latitude	Longitude	County	State
Bufo americanus	BF089	43.3250	-85.3103	Montcalm	MI
Bufo americanus	BF090	43.3250	-85.3103	Montcalm	MI
Bufo americanus	HERA010214	41.332	-73.2064	Fairfield	CT
Bufo americanus	HERA010347	45.5913	-69.9999	Somerset	ME
Bufo americanus	HERA010372	42.1795	-79.4495	Chautauqua	NY
Bufo americanus	HERA010512	44.1105	-71.2177	Carroll	NH
Bufo americanus	HERA 9853	41.3267	-72.8043	New Haven	CT
Bufo americanus	JFBM14318	45.1084	-95.9051	Chippewa	MN
Bufo americanus	JFBM14319	45.1085	-95.9052	Chippewa	MN
Bufo americanus	JFBM14320	45.1086	-95.9053	Chippewa	MN
Bufo americanus	JFBM14326	45.3082	-93.8993	Wright	MN
Bufo americanus	JFBM14331	46.3595	-95.6243	Otter Tail	MN
Bufo americanus	JFBM14332	46.3595	-95.6243	Otter Tail	MN
Bufo americanus	JFBM14347	45.2212	-92.7724	Washington	MN
Bufo americanus	JFBM14366	44.0876	-92.0189	Winona	MN
Bufo americanus	JWS001	38.8462	-77.2469	Fairfax	VA
Bufo americanus	TG0019	34.5761	-89.4778	Marshall	MS
Bufo americanus	UAHC14993	39.0208	-80.5435	Lewis	WV
Bufo charlesmithi	BF008	34.6833	-94.6352	Leflore	OK
Bufo charlesmithi	BF009	34.6833	-94.6352	Leflore	OK
Bufo charlesmithi	BF010	34.6833	-94.6352	Leflore	OK
Bufo charlesmithi	BF038	33.6835	-95.5981	Lamar	TX
Bufo charlesmithi	BF039	33.6835	-95.5981	Lamar	TX
Bufo charlesmithi	BF064	33.9008	-95.2888	Choctaw	OK
Bufo charlesmithi	BF065	33.9008	-95.2888	Choctaw	OK
Bufo charlesmithi	BF066	33.9008	-95.2888	Choctaw	OK
Bufo charlesmithi	BF067	33.9008	-95.2888	Choctaw	OK
Bufo charlesmithi	BF069	34.5452	-96.5918	Pontotoc	OK
Bufo charlesmithi	BF166	35.9962	-96.3334	Creek	OK
Bufo charlesmithi	BF167	35.9962	-96.3334	Creek	OK

Bufo charlesmithi	BF170	36.5979	-95.5325	Nowata	OK
Bufo charlesmithi	BF186	34.7423	-94.7238	LeFlore	OK
Bufo charlesmithi	BF187	34.7122	-94.6648	LeFlore	OK
Bufo charlesmithi	BF188	34.7122	-94.6648	LeFlore	OK
Bufo charlesmithi	BF189	34.7147	-94.6768	LeFlore	OK
Bufo charlesmithi	BF190	34.7147	-94.6768	LeFlore	OK
Bufo charlesmithi	BF191	34.7147	-94.6768	LeFlore	OK
Bufo cognatus	BF141(Cog)	34.9270	-102.1061	Randall	TX
Bufo cognatus	BF180	34.9270	-102.1061	Randall	TX
Bufo cognatus	BF181	34.9270	-102.1061	Randall	TX
Bufo cognatus	BF182	34.9270	-102.1061	Randall	TX
Bufo cognatus	BF183	34.9270	-102.1061	Randall	TX
Bufo cognatus	BF184	34.9270	-102.1061	Randall	TX
Bufo cognatus	BF185	34.9270	-102.1061	Randall	TX
Bufo fowleri	BF011	30.4095	-91.1974	E. Baton Rouge	LA
Bufo fowleri	BF013	30.3886	-91.2116	E. Baton Rouge	LA
Bufo fowleri	BF014	30.3886	-91.2116	E. Baton Rouge	LA
Bufo fowleri	BF015	30.3886	-91.2116	E. Baton Rouge	LA
Bufo fowleri	BF017	30.3848	-91.2149	E. Baton Rouge	LA
Bufo fowleri	BF018	30.3848	-91.2149	E. Baton Rouge	LA
Bufo fowleri	BF019	30.3848	-91.2149	E. Baton Rouge	LA
Bufo fowleri	BF020	30.3859	-91.2149	E. Baton Rouge	LA
Bufo fowleri	BF021	30.3859	-91.2149	E. Baton Rouge	LA
Bufo fowleri	BF109	32.5313	-92.4734	Lincoln	LA
Bufo fowleri	BF110	32.5313	-92.4734	Lincoln	LA
Bufo fowleri	BF111	32.5363	-92.3592	Ouachita	LA
Bufo fowleri	BF112	32.5363	-92.3592	Ouachita	LA
Bufo fowleri	BF113	32.5036	-92.2524	Ouachita	LA
Bufo fowleri	BF114	32.5036	-92.2524	Ouachita	LA
Bufo fowleri	BF115	32.5036	-92.2524	Ouachita	LA
Bufo fowleri	BF116	32.6129	-93.0558	Claiborne	LA
Bufo fowleri	BF117	32.6129	-93.0558	Claiborne	LA
Bufo fowleri	BF118	32.6129	-93.0558	Claiborne	LA

Bufo fowleri	BF119	32.6129	-93.0558	Claiborne	LA
Bufo fowleri	BF125	31.2458	-93.3511	Vernon	LA
Bufo fowleri	BF126	31.2458	-93.3511	Vernon	LA
Bufo fowleri	BF127	31.2458	-93.3511	Vernon	LA
Bufo fowleri	BF128	31.2458	-93.3511	Vernon	LA
Bufo fowleri	BF129	31.2458	-93.3511	Vernon	LA
Bufo fowleri	BF130	31.2598	-92.7601	Rapides	LA
Bufo fowleri	BF131	31.2598	-92.7601	Rapides	LA
Bufo fowleri	BF145	34.6868	-82.8141	Pickens	SC
Bufo fowleri	BF146	34.6868	-82.8141	Pickens	SC
Bufo fowleri	BF154	31.4394	-89.4301	Covington	MS
Bufo fowleri	CMW1003	32.5930	-86.9871	Dallas	AL
Bufo fowleri	CMW1027	33.7398	-80.8562	Calhoun	SC
Bufo fowleri	HERA010313	41.7665	-72.6732	Hartford	CT
Bufo fowleri	HERA010491	38.9687	-75.6174	Kent	DE
Bufo fowleri	HERA010493	38.9688	-75.6175	Kent	DE
Bufo fowleri	TG0020	34.578	-89.3316	Marshall	MS
Bufo fowleri	TG0024	34.48	-89.2689	Lafayette	MS
Bufo fowleri	TG0025	34.49	-89.2690	Lafayette	MS
Bufo fowleri	TG0146	37.715	-90.5968	St. Francois	MO
Bufo fowleri	UAHC14625	34.0173	-87.3593	Winston	AL
Bufo fowleri	UAHC14629	34.0172	-87.3592	Winston	AL
Bufo fowleri	UAHC15113	33.1985	-87.4045	Tuscaloosa	AL
Bufo fowleri	UAHC15115	33.1986	-87.4046	Tuscaloosa	AL
Bufo fowleri	UAHC15193	31.4541	-86.7871	Conecuh	AL
Bufo hemiophrys	JFBM14328	48.9803	-97.5559	Pembina	ND
Bufo terrestris	BF134	30.7180	-85.9342	Holmes	FL
Bufo terrestris	BF135	30.7180	-85.9342	Holmes	FL
Bufo terrestris	BF141 (Terr)	32.7888	-81.1269	Hampton	SC
Bufo terrestris	BF155	30.6426	-84.2029	Leon	FL
Bufo terrestris	BF156	30.6426	-84.2029	Leon	FL
Bufo terrestris	BF163	32.7863	-79.7872	Charleston	SC
Bufo terrestris	BF165	33.3642	-97.6839		

Bufo terrestris	BF169	32.4775	-85.3902	Lee	AL
Bufo terrestris	BF171	30.3073	-86.0960	Walton	FL
Bufo terrestris	BF172	30.3073	-86.0960	Walton	FL
Bufo terrestris	BF173	30.3073	-86.0960	Walton	FL
Bufo terrestris	BF174	30.3073	-86.0960	Walton	FL
Bufo terrestris	CMW1028	33.2974	-81.6545	Barnwell	GA
Bufo terrestris	JWS008	36.7114	-76.2396	Chesapeake	VA
Bufo terrestris	UAHC14513	34.7786	-87.6992	Lauderdale	AL
Bufo terrestris	UAHC14621A	34.0172	-87.3592	Winston	AL
Bufo terrestris	UAHC14621B	34.0173	-87.3593	Winston	AL
Bufo velatus	BF001	32.2561	-95.1847	Smith	TX
Bufo velatus	BF002	32.2561	-95.1847	Smith	TX
Bufo velatus	BF003	32.2561	-95.1847	Smith	TX
Bufo velatus	BF004	32.2561	-95.1847	Smith	TX
Bufo velatus	BF005	32.2561	-95.1847	Smith	TX
Bufo velatus	BF006	32.2561	-95.1847	Smith	TX
Bufo velatus	BF007	32.2561	-95.1847	Smith	TX
Bufo velatus	BF022	31.3231	-95.1009	Trinity	TX
Bufo velatus	BF023	31.3231	-95.1009	Trinity	TX
Bufo velatus	BF026	31.8770	-95.6051	Anderson	TX
Bufo velatus	BF027	31.8770	-95.6051	Anderson	TX
Bufo velatus	BF028	31.8770	-95.6051	Anderson	TX
Bufo velatus	BF029	31.9113	-95.8972	Anderson	TX
Bufo velatus	BF030	31.9113	-95.8972	Anderson	TX
Bufo velatus	BF031	31.9113	-95.8972	Anderson	TX
Bufo velatus	BF032	32.1424	-95.8569	Henderson	TX
Bufo velatus	BF034	32.1424	-95.8569	Henderson	TX
Bufo velatus	BF035	32.2713	-94.5882	Rusk	TX
Bufo velatus	BF036	32.2713	-94.5882	Rusk	TX
Bufo velatus	BF037	32.2713	-94.5882	Rusk	TX
Bufo velatus	BF040	33.0618	-95.0248	Camp	TX
Bufo velatus	BF041	33.0618	-95.0248	Camp	TX
Bufo velatus	BF042	33.0618	-95.0248	Camp	TX

Bufo velatus	BF045	31.2236	-93.6750	Sabine	TX
Bufo velatus	BF047	31.2236	-93.6750	Sabine	TX
Bufo velatus	BF048	31.2236	-93.6750	Sabine	TX
Bufo velatus	BF049	32.7100	-94.1203	Harrison	TX
Bufo velatus	BF050	32.7038	-94.1157	Harrison	TX
Bufo velatus	BF051	32.7181	-94.1357	Harrison	TX
Bufo velatus	BF052	32.6378	-94.2015	Harrison	TX
Bufo velatus	BF053	32.6378	-94.2015	Harrison	TX
Bufo velatus	BF054	32.7032	-94.0708	Marion	TX
Bufo velatus	BF061	33.4019	-94.4254	Bowie	TX
Bufo velatus	BF062	33.4019	-94.4254	Bowie	TX
Bufo velatus	BF063	33.4019	-94.4254	Bowie	TX
Bufo velatus	BF068	31.2236	-93.6750	Sabine	TX
Bufo velatus	BF085	31.0424	-94.3269	Jasper	TX
Bufo velatus	BF088	31.1836	-93.8125	Sabine	TX
Bufo velatus	BF105	31.5212	-94.7672	Nacogdoches	TX
Bufo velatus	BF121	31.2212	-93.6796	Sabine	TX
Bufo velatus	BF122	31.2212	-93.6796	Sabine	TX
Bufo velatus	BF123	31.2212	-93.6796	Sabine	TX
Bufo velatus	BF124	31.2212	-93.6796	Sabine	TX
Bufo velatus	MG0001	32.2561	-95.1847	Smith	TX
Bufo velatus	MG0002	32.2561	-95.1847	Smith	TX
Bufo velatus	RLG2634	32.5883	-95.8567	Van Zandt	TX
Bufo velatus	RLG2637	32.5883	-95.8567	Van Zandt	TX
Bufo velatus	RLG2638	32.5883	-95.8567	Van Zandt	TX
Bufo velatus	RLG2639	32.5883	-95.8567	Van Zandt	TX
Bufo velatus	SCB006	32.3757	-94.8491	Gregg	TX
Bufo woodhousii	BF024	33.5220	-101.8460	Lubbock	TX
Bufo woodhousii	BF043	32.8729	-96.7561	Dallas	TX
Bufo woodhousii	BF044	32.8729	-96.7561	Dallas	TX
Bufo woodhousii	BF095	32.6825	-97.5084	Tarrant	TX
Bufo woodhousii	BF096	32.6825	-97.5084	Tarrant	TX
Bufo woodhousii	BF097	32.6825	-97.5084	Tarrant	TX

Bufo woodhousii	BF098	32.6825	-97.5084	Tarrant	TX
Bufo woodhousii	BF099	32.7428	-97.1998	Tarrant	TX
Bufo woodhousii	BF101	32.6182	-97.1526	Tarrant	TX
Bufo woodhousii	BF102	32.6182	-97.1526	Tarrant	TX
Bufo woodhousii	BF107	32.9010	-98.5560	Palo Pinto	TX
Bufo woodhousii	BF108	32.8950	-98.5310	Palo Pinto	TX
Bufo woodhousii	BF132	33.2446	-98.3290	Jack	TX
Bufo woodhousii	BF133	38.8265	-98.4747	Russell	KA
Bufo woodhousii	BF162	33.6969	-95.6854	Lamar	TX
Bufo woodhousii	BF168	32.6051	-98.0339	Parker	TX
Bufo woodhousii	BF175	33.8330	-97.4950	Montague	TX
Bufo woodhousii	BF176	33.4580	-97.6120	Montague	TX
Bufo woodhousii	BF177	34.9270	-102.1061	Randall	TX
Bufo woodhousii	BF178	34.9270	-102.1061	Randall	TX
Bufo woodhousii	BF179	34.9270	-102.1061	Randall	TX
Bufo woodhousii	CJF3182	32.6051	-98.0339	Hood	TX
Bufo woodhousii	RMC00001	32.7834	-97.1400	Tarrant	TX

APPENDIX C

SPECIMENS EXAMINED IN MORPHOLOGICAL ANALYSES

SPECIES	ID#	DESIGNATION	SVL	TIB	FT	HW	HL	TYMP	ORB	PCW	ACW	END	SPTYMP	F1	F3	PARL	PARW
(t x he) x (t x he)	TNHC240	Blair hybrid	43.1	16.8	27.8	17.4	14.2	3.4	6.8	4.2	3.0	10.4	0.1	8.0	7.1	6.7	3.8
(t x he) x (t x he)	TNHC242	Blair hybrid	47.2	17.4	30.6	19.2	14.8	2.7	8.5	3.0	3.0	11.2	0.8	8.5	7.4	7.5	3.1
(t x he) x (t x he)	TNHC249	Blair hybrid	41.1	18.2	28.8	17.9	13.8	1.9	7.1	2.9	2.4	9.6	0.1	7.3	5.9	7.3	4.5
(t x hem) x t	TNHC83	Blair hybrid	44.0	16.5	28.2	18.2	14.5	3.2	7.3	4.8	3.0	10.4	0.6	7.8	6.5	9.6	5.4
(t x hem) x t	TNHC84	Blair hybrid	52.8	19.8	33.1	21.7	15.1	3.0	8.1	3.1	3.3	10.9	1.0	8.7	6.4	9.8	6.2
(t x hem) x t	TNHC85	Blair hybrid	53.6	18.4	31.1	21.9	14.7	2.5	7.4	3.8	2.9	10.6	0.9	8.6	6.7	9.9	6.2
(t x hem) x t	TNHC86	Blair hybrid	47.0	18.4	32.7	18.9	13.0	2.5	7.0	3.8	2.8	9.1	0.1	8.0	7.1	9.3	5.5
(t x hem) x t	TNHC87	Blair hybrid	46.4	18.7	30.5	18.5	15.0	2.6	7.9	3.4	2.5	9.6	0.1	9.3	6.8	9.1	5.9
(t x hem) x t	TNHC88	Blair hybrid	67.1	25.3	42.3	28.6	20.3	3.6	8.7	4.6	4.0	12.7	0.7	10.4	8.5	12.3	6.6
(t x w) x (t x w)	TNHC65	Blair hybrid	52.4	23.0	35.6	23.2	15.8	3.1	8.5	4.1	3.2	11.3	0.9	8.3	7.1	12.0	7.3
(t x w) x (t x w)	TNHC66	Blair hybrid	66.3	30.7	42.8	30.2	21.8	3.8	10.2	4.1	3.6	13.5	0.1	9.4	8.6	12.5	7.7
(t x w) x (t x w)	TNHC67	Blair hybrid	53.8	21.1	34.2	22.0	16.5	3.5	8.3	4.6	3.0	11.6	0.1	8.3	7.2	10.0	5.7
(t x w) x (t x w)	TNHC68	Blair hybrid	50.1	21.2	33.1	21.3	14.9	2.6	8.8	3.2	2.4	11.0	0.1	8.1	6.8	10.3	7.9
(t x w) x t	TNHC436	Blair hybrid	51.3	19.3	29.3	20.5	16.1	3.2	7.1	5.4	2.8	11.2	1.0	7.0	7.0	10.3	6.8
(t x w) x t	TNHC437	Blair hybrid	44.6	16.4	25.9	17.9	13.5	2.6	6.8	4.9	2.3	10.1	1.1	5.6	6.2	10.0	5.7
(t x w) x t	TNHC438	Blair hybrid	41.4	16.0	24.6	18.7	13.9	2.2	6.3	4.2	2.5	9.6	0.8	6.3	5.8	11.1	5.0
(t x w) x t	TNHC439	Blair hybrid	40.4	13.9	22.1	15.8	12.6	2.0	6.1	4.2	1.9	9.5	0.6	5.1	5.7	8.2	5.1
(t x w) x t	TNHC440	Blair hybrid	47.4	18.0	28.2	19.7	13.8	3.3	7.1	5.8	2.6	10.6	1.4	5.8	6.5	10.2	6.3
(t x w) x t	TNHC441	Blair hybrid	54.0	20.1	31.3	21.8	17.2	3.1	7.4	6.0	2.8	11.6	1.9	7.9	7.3	11.4	5.4
(t x w) x t	TNHC442	Blair hybrid	47.6	18.6	30.9	20.1	15.7	3.1	6.8	4.7	3.4	10.6	1.7	7.0	6.5	10.2	5.4
(t x w) x t	TNHC443	Blair hybrid	54.4	22.6	30.8	22.5	18.6	3.1	8.2	6.3	3.2	11.7	1.8	7.1	7.3	11.8	6.1
(t x w) x t	TNHC444	Blair hybrid	46.2	16.6	26.2	19.8	14.3	3.0	5.9	4.2	2.4	10.4	1.3	6.3	6.4	9.9	5.3
(t x w) x t	TNHC445	Blair hybrid	42.1	15.6	23.9	16.6	11.1	2.9	5.6	3.5	1.7	9.8	0.7	5.6	5.9	10.1	4.5
(t x w) x t	TNHC446	Blair hybrid	44.6	16.8	27.8	19.0	14.7	3.1	6.7	4.8	2.5	10.1	1.4	6.5	6.2	10.6	4.7
(t x w) x t	TNHC447	Blair hybrid	46.1	16.8	28.1	18.3	15.6	2.6	6.9	4.2	2.1	10.4	0.9	6.9	6.4	9.1	4.2
(t x w) x t	TNHC449	Blair hybrid	51.4	18.4	29.3	20.2	14.6	3.1	7.4	4.2	1.9	11.2	0.1	7.6	7.0	11.2	7.3
(t x w) x t	TNHC450	Blair hybrid	57.0	23.2	34.2	22.9	18.9	3.5	8.5	5.2	2.3	12.1	0.6	7.9	7.6	11.3	7.1
(t x w) x t	TNHC451	Blair hybrid	48.7	19.0	26.7	20.2	15.5	3.1	7.4	5.2	2.7	10.8	1.2	6.9	6.7	11.2	5.2
(t x w) x t	TNHC452	Blair hybrid	42.2	16.7	26.0	18.8	13.4	3.4	6.7	3.9	2.2	9.8	0.6	6.1	5.9	10.0	5.9

(t x w) x t	TNHC453	Blair hybrid	60.9	23.8	37.2	26.0	18.5	3.7	8.7	4.9	2.3	12.7	1.6	10.2	8.0	14.7	7.6
(t x w) x t	TNHC454	Blair hybrid	53.7	17.8	29.0	20.8	14.7	3.2	8.0	4.0	2.4	11.5	0.1	7.4	7.2	13.5	6.9
(t x w) x t	TNHC455	Blair hybrid	48.4	18.8	26.3	20.2	15.1	2.5	7.4	4.1	2.0	10.7	1.1	7.4	6.6	11.5	5.6
(t x w) x t	TNHC456	Blair hybrid	44.7	16.6	27.6	18.7	14.3	2.9	7.3	3.6	2.2	10.2	0.8	6.8	6.2	9.9	6.3
(t x w) x t	TNHC457	Blair hybrid	43.6	18.1	26.0	19.1	14.1	2.4	7.7	4.2	2.3	10.0	0.6	5.7	6.1	9.4	4.9
(t x w) x t	TNHC458	Blair hybrid	56.8	20.5	32.6	23.0	18.4	3.8	8.4	4.7	3.0	12.0	1.6	7.5	7.6	13.5	8.2
(t x w) x t	TNHC459	Blair hybrid	51.9	18.7	30.7	21.3	16.2	3.0	7.7	3.8	1.9	11.3	0.7	7.1	7.0	11.9	6.2
(t x w) x t	TNHC460	Blair hybrid	56.6	21.0	33.7	23.1	17.9	2.3	9.0	4.5	2.0	12.0	1.0	8.3	7.6	12.2	6.5
(t x w) x t	TNHC461	Blair hybrid	47.7	17.8	27.0	18.9	14.9	2.6	6.8	4.0	2.1	10.6	0.6	6.6	6.5	10.6	5.1
(t x w) x t	TNHC462	Blair hybrid	49.7	19.1	29.9	21.1	13.2	3.2	6.8	4.2	2.5	10.9	1.4	7.8	6.8	11.7	6.2
(t x w) x t	TNHC463	Blair hybrid	46.3	17.2	26.6	19.4	14.2	3.1	7.1	4.2	2.1	10.4	1.3	6.9	6.4	10.6	5.2
(t x w) x t	TNHC466	Blair hybrid	52.5	20.0	28.7	21.2	15.7	3.3	8.0	4.7	2.2	11.4	1.1	7.9	7.1	12.4	6.7
(t x w) x t	TNHC468	Blair hybrid	50.0	18.2	29.3	20.2	15.3	2.7	6.7	4.4	1.9	11.0	0.8	7.1	6.8	11.3	6.5
(t x w) x t	TNHC469	Blair hybrid	53.2	17.6	31.9	22.0	16.6	2.9	8.9	4.4	2.4	11.5	0.8	8.1	7.2	10.8	6.9
(t x w) x w	TNHC229	Blair hybrid	61.0	24.7	39.6	26.7	18.7	3.4	8.7	4.2	3.1	12.7	1.5	9.2	8.0	12.5	7.0
(t x w) x w	TNHC230	Blair hybrid	49.8	20.5	31.2	20.8	15.8	3.5	7.0	4.4	2.6	10.9	1.6	7.4	6.8	9.5	4.0
(t x w) x w	TNHC232	Blair hybrid	47.9	19.6	30.9	21.7	16.5	3.0	7.9	3.5	2.6	10.6	0.1	7.1	6.6	10.3	4.7
(t x w) x w	TNHC234	Blair hybrid	54.2	20.7	35.7	23.8	17.3	4.3	8.7	4.6	3.0	11.6	0.9	7.9	7.3	11.6	5.5
(t x w) x w	TNHC235	Blair hybrid	40.9	14.7	26.1	17.7	13.2	2.6	6.3	3.5	2.5	9.6	0.9	6.1	5.8	6.9	3.5
(t x w) x w	TNHC79	Blair hybrid	61.1	21.4	32.9	13.6	17.0	3.1	8.9	4.6	3.0	12.7	0.1	8.3	8.1	10.5	5.1
(t x w) x w	TNHC80	Blair hybrid	59.1	24.5	38.5	27.1	17.9	3.1	9.4	4.7	3.1	12.4	0.1	8.9	7.8	11.5	5.6
(t x w) x w	TNHC81	Blair hybrid	49.9	20.6	25.6	10.5	15.5	2.8	6.9	3.8	2.2	11.0	0.1	7.7	6.8	10.1	5.9
(t xw) x (t x w)	TNHC193	Blair hybrid	41.3	16.9	26.0	18.2	14.1	3.2	5.6	4.0	2.2	9.6	1.0	5.2	5.8	8.6	3.7
(t xw) x (t x w)	TNHC194	Blair hybrid	44.7	17.6	25.7	18.1	13.5	2.9	7.0	4.9	2.3	10.2	0.7	5.6	6.2	8.6	4.8
(t xw) x (t x w)	TNHC195	Blair hybrid	40.1	14.7	24.0	17.4	12.1	2.5	6.4	3.7	1.9	9.4	0.5	4.6	5.7	7.3	4.5
(t xw) x (t x w)	TNHC196	Blair hybrid	40.9	16.7	24.7	17.5	14.1	3.6	6.4	4.4	3.7	9.6	1.0	4.6	5.8	8.1	4.8
(t xw) x (t x w)	TNHC197	Blair hybrid	54.8	23.4	34.9	26.4	19.1	3.9	7.7	5.3	3.6	11.7	1.1	7.2	7.3	10.6	6.4
(t xw) x (t x w)	TNHC198	Blair hybrid	51.5	21.6	30.6	21.1	16.1	3.3	7.8	4.9	2.8	11.2	1.2	7.0	7.0	9.3	5.1
a x (a x t)	TNHC540	Blair hybrid	37.2	16.2	26.0	14.1	11.1	3.1	6.1	3.4	2.4	9.0	1.0	4.4	5.4	8.0	4.3
a x (t x w)	TNHC250	Blair hybrid	59.9	26.0	38.8	27.7	19.9	3.2	9.2	4.5	2.8	12.5	0.8	10.8	7.9	12.7	6.6
a x (t x w)	TNHC251	Blair hybrid	73.3	29.2	37.9	31.0	24.2	3.3	10.4	4.1	2.8	14.6	1.9	11.2	9.4	13.3	7.5

a x (t x w)	TNHC252	Blair hybrid	57.4	23.3	25.3	24.3	16.7	3.2	8.3	4.2	2.5	12.1	0.1	9.6	7.6	11.0	6.4
a x (t x w)	TNHC254	Blair hybrid	56.4	19.4	28.2	23.3	18.7	2.5	7.9	4.8	3.2	12.0	0.1	9.1	7.5	10.6	4.8
a x (t x w)	TNHC255	Blair hybrid	49.8	16.0	30.5	21.9	16.1	3.0	8.1	3.0	2.3	10.9	0.5	7.1	6.8	11.3	4.9
a x (t x w)	TNHC256	Blair hybrid	65.5	25.8	31.8	26.6	18.9	3.8	7.9	3.7	2.7	13.4	0.8	8.1	8.6	12.1	6.5
a x (t x w)	TNHC258	Blair hybrid	59.9	23.7	29.4	25.9	16.9	4.1	9.3	4.1	2.6	12.5	0.3	9.7	7.9	10.7	6.0
a x f	CMN21795a	Field hybrid	56.1	24.9	40.4	23.3	16.8	4.1	9.2	4.9	2.6	11.7	0.1	10.7	7.9	12.6	5.5
a x f	CMN21795b	Field hybrid	50.3	24.1	38.6	20.5	14.1	3.5	9.4	4.3	2.9	11.6	0.1	11.0	7.8	10.2	5.6
a x f	CMN21795c	Field hybrid	51.9	22.9	36.0	19.7	15.0	3.4	8.0	4.4	3.3	10.2	0.1	12.1	7.7	10.8	4.2
a x f	CMN21812	Field hybrid	58.3	27.4	42.4	24.5	20.1	4.3	9.0	4.6	2.9	12.2	0.1	11.4	7.9	12.7	6.1
a x f	CMN21899	Field hybrid	56.0	20.8	35.8	20.8	16.5	3.3	7.9	3.6	1.6	11.3	0.1	10.6	8.2	11.8	6.9
a x f	CMN21905	Field hybrid	52.7	18.9	30.8	19.6	14.9	2.9	7.4	4.3	2.8	10.2	0.1	10.9	6.5	11.1	5.6
a x f	CMN21906a	Field hybrid	57.1	22.9	38.7	20.9	15.6	3.5	8.7	4.6	2.1	11.1	0.5	10.8	8.3	11.2	5.6
a x f	CMN21906b	Field hybrid	49.6	19.4	31.1	18.1	13.2	2.6	7.5	3.9	2.4	9.9	0.9	8.1	5.1	9.2	5.7
a x f	CMN21911a	Field hybrid	53.7	22.2	33.2	18.7	14.0	3.3	8.5	4.4	2.4	10.8	0.1	9.7	6.7	10.9	5.3
a x f	CMN21911b	Field hybrid	56.3	24.0	35.3	20.3	12.3	3.0	8.4	4.9	2.7	10.7	0.1	10.5	8.0	11.2	5.6
a x f	CMN21911c	Field hybrid	54.5	22.9	36.9	20.2	15.5	2.8	8.0	4.9	2.6	10.3	0.1	10.7	7.4	10.2	6.3
a x f	CMN21911d	Field hybrid	58.2	24.3	37.7	22.0	15.9	4.0	7.9	4.5	2.7	10.5	1.6	10.9	8.1	11.1	5.6
a x f	CMN21958	Field hybrid	48.8	21.7	37.5	20.3	13.3	3.9	8.3	4.1	2.2	10.8	1.0	10.1	7.3	10.3	5.8
a x f	CMN21963a	Field hybrid	52.6	21.1	39.6	19.7	15.7	3.1	7.6	4.1	2.3	10.2	0.1	10.4	7.1	11.4	5.3
a x f	CMN21963b	Field hybrid	58.2	22.7	36.7	20.9	14.4	3.1	8.0	3.3	2.1	10.7	0.1	10.4	7.2	11.1	6.0
a x f	CMN21963c	Field hybrid	53.7	22.4	35.9	20.4	15.4	3.1	8.2	3.6	2.3	11.2	0.1	10.1	6.4	10.1	5.4
a x f	CMN21963d	Field hybrid	51.5	19.0	34.3	19.0	14.2	3.5	7.8	3.0	1.8	10.7	0.1	9.9	7.7	9.8	5.3
a x f	CMN21963e	Field hybrid	57.6	23.1	38.2	19.4	15.4	3.1	8.4	4.5	2.5	10.5	0.1	9.7	6.9	10.5	5.5
a x f	CMN21963f	Field hybrid	53.8	20.6	34.8	19.6	13.8	2.9	6.9	3.7	1.9	9.9	0.1	9.7	6.3	10.0	5.4
a x f	CMN21963g	Field hybrid	50.8	20.1	34.6	19.0	14.7	3.9	7.6	4.3	1.5	10.3	0.1	9.7	6.8	11.4	6.1
a x f	CMN21963h	Field hybrid	50.7	22.9	34.1	19.2	14.1	2.8	7.8	3.8	1.9	10.2	0.1	9.5	7.0	10.9	5.1
a x f	CMN21963i	Field hybrid	54.2	22.3	37.8	20.8	16.4	3.9	7.9	3.7	2.2	10.7	0.1	11.5	8.3	10.5	6.3
a x f	CMN21963j	Field hybrid	56.1	23.0	36.0	19.3	14.7	3.3	6.7	4.3	2.5	9.5	1.5	10.6	7.5	9.6	5.8
a x f	CMN21963k	Field hybrid	53.6	22.6	35.7	18.7	14.1	3.1	8.6	3.7	2.0	10.6	0.1	10.4	7.3	11.5	5.1
a x f	CMN21963l	Field hybrid	59.8	24.4	39.2	22.0	15.9	2.8	8.0	4.4	2.4	10.6	0.1	10.6	8.4	12.6	5.2
a x f	CMN29447a	Field hybrid	64.2	26.8	43.1	23.2	15.7	3.9	8.5	3.8	2.5	12.3	0.1	12.9	9.3	11.8	5.2

a x f	CMN29447b	Field hybrid	65.7	24.1	39.8	25.0	19.5	4.6	9.5	4.5	2.7	12.3	1.1	13.4	10.4	13.1	6.9
a x h	TNHC15	Blair hybrid	55.7	20.2	31.8	21.9	13.6	3.3	6.9	4.0	2.7	11.9	1.2	8.4	7.5	12.2	6.9
a x h	TNHC16	Blair hybrid	42.6	15.2	23.6	16.9	10.6	2.8	6.1	3.5	2.1	9.8	1.0	6.6	6.0	8.4	6.1
a x h	TNHC17	Blair hybrid	49.3	16.9	26.7	17.6	12.4	3.0	6.8	3.8	2.0	10.9	1.0	6.4	6.7	9.3	6.4
a x h	TNHC18	Blair hybrid	55.0	19.0	32.7	22.9	16.3	4.0	7.3	4.0	1.9	11.7	2.2	9.1	7.4	9.0	5.7
a x h	TNHC19	Blair hybrid	49.2	17.9	27.4	19.8	15.6	2.8	6.8	4.3	2.7	10.8	1.1	6.4	6.7	9.8	6.1
a x h	TNHC20	Blair hybrid	53.2	19.1	28.3	20.2	14.7	2.1	6.9	3.9	2.6	11.5	0.1	6.8	7.2	9.8	6.3
a x h	TNHC21	Blair hybrid	44.7	16.7	26.0	16.9	13.2	3.2	6.6	3.9	2.3	10.2	1.2	5.2	6.2	7.4	4.1
a x h	TNHC22	Blair hybrid	47.5	18.6	29.8	19.4	15.4	3.6	7.4	3.6	2.4	10.6	0.7	9.0	6.5	9.8	6.4
a x t	TNHC161	Blair hybrid	50.0	18.4	27.1	19.8	13.7	2.3	6.7	3.8	2.3	11.0	0.7	7.7	6.8	8.5	5.9
a x t	TNHC162	Blair hybrid	51.2	15.8	26.9	21.0	13.6	3.2	6.0	4.2	2.2	11.2	1.8	6.8	6.9	9.8	5.7
a x t	TNHC163	Blair hybrid	46.4	16.9	27.1	20.2	15.3	2.4	6.5	4.0	2.5	10.4	1.7	7.0	6.4	10.6	6.0
a x t	TNHC164	Blair hybrid	56.3	22.2	35.7	25.9	17.9	3.9	8.3	4.8	2.5	11.9	1.5	8.1	7.5	13.4	6.9
a x t	TNHC165	Blair hybrid	46.2	19.7	31.2	18.2	15.1	2.6	7.1	3.6	2.0	10.4	1.1	6.4	6.4	10.8	5.8
a x t	TNHC166	Blair hybrid	54.0	18.4	32.2	20.6	13.9	3.4	7.2	4.2	2.2	11.6	1.2	6.4	7.3	10.5	6.1
a x w	TNHC136	Blair hybrid	56.0	19.9	30.1	22.1	17.0	3.6	7.5	3.8	2.7	11.9	0.1	8.2	7.5	10.2	5.0
a x w	TNHC137	Blair hybrid	49.2	17.6	27.9	18.2	13.6	3.1	6.4	3.5	2.7	10.8	0.1	6.7	6.7	10.5	5.0
a x w	TNHC138	Blair hybrid	51.3	18.0	26.7	20.7	17.0	3.0	7.3	3.9	2.9	11.2	0.1	7.0	7.0	9.8	4.5
a x w	TNHC139	Blair hybrid	53.0	22.3	32.9	24.0	20.9	3.7	8.5	4.0	2.5	11.4	0.9	8.0	7.1	11.2	5.9
a x w	TNHC141	Blair hybrid	44.1	17.9	27.5	18.4	14.1	3.0	6.8	3.9	2.4	10.1	0.6	7.0	6.1	8.7	4.6
a x w	TNHC142	Blair hybrid	52.0	20.4	29.1	21.9	18.2	4.1	7.0	3.7	2.5	11.3	0.1	7.9	7.0	10.4	5.9
a x w	TNHC143	Blair hybrid	56.2	20.6	31.8	22.5	16.6	3.6	7.2	3.9	2.7	11.9	0.1	9.4	7.5	11.7	5.8
a x w	TNHC544	Blair hybrid	47.3	18.8	30.1	17.8	13.3	2.8	6.8	3.1	1.8	10.6	1.0	6.8	6.5	7.2	3.8
a x w	TNHC546	Blair hybrid	52.1	19.6	31.0	18.4	15.1	3.6	6.4	3.9	2.7	11.3	0.1	7.1	7.0	10.0	3.8
a x w	TNHC???	Blair hybrid	50.4	17.7	28.7	18.8	15.3	3.1	7.1	3.9	1.9	11.0	0.9	7.5	6.9	9.9	5.2
a x w	TNHC182	Blair hybrid	41.2	16.1	23.8	16.7	12.8	3.7	6.0	2.6	1.7	9.6	0.8	6.3	5.8	7.5	3.3
a x w	TNHC184	Blair hybrid	43.1	16.3	26.1	16.8	13.8	3.7	6.1	2.6	1.5	9.9	0.1	5.4	6.0	9.3	4.6
a x w	TNHC185	Blair hybrid	50.1	17.1	29.3	20.6	16.3	2.9	7.1	3.7	2.2	11.0	0.5	7.2	6.8	9.9	5.6
a x w	TNHC186	Blair hybrid	46.8	18.9	30.6	19.5	15.4	3.5	7.3	4.3	2.7	10.5	1.2	5.6	6.4	9.8	5.1
a x w	TNHC521	Blair hybrid	44.7	18.1	26.2	17.2	13.7	3.2	6.1	3.6	3.0	10.2	1.3	5.6	6.2	9.4	6.4
A. americanus	BF089	Field parental	81.2	31.6	58.0	32.2	20.2	5.8	12.1	5.8	3.0	16.1	1.8	17.3	12.3	14.0	8.1

A. americanus	BF090	Field parental	70.4	23.8	43.6	25.7	17.2	5.6	10.5	4.7	2.5	12.9	0.1	13.3	9.3	13.8	8.3
A. americanus	CMN21700	Field parental	63.2	24.9	44.9	24.1	17.2	4.6	8.2	5.2	3.0	11.8	1.3	11.2	8.2	12.6	7.6
A. americanus	CMN21705	Field parental	69.0	27.9	50.4	25.8	18.5	4.3	9.0	4.8	2.5	12.6	1.4	13.3	9.7	12.9	6.0
A. americanus	CMN21714	Field parental	61.3	23.7	43.8	25.6	19.9	4.9	9.4	4.8	2.8	12.8	0.4	11.2	8.3	12.5	5.9
A. americanus	CMN21732	Field parental	57.3	22.2	40.2	20.1	14.6	3.6	8.6	3.4	2.3	11.2	0.6	11.8	7.9	10.4	5.1
A. americanus	CMN21737	Field parental	81.0	30.2	56.3	31.5	19.2	4.9	10.7	4.1	2.7	14.1	0.8	15.3	10.6	13.9	6.9
A. americanus	CMN21743	Field parental	50.2	18.3	31.6	18.5	14.8	3.1	7.0	3.4	2.0	8.6	0.1	8.7	6.8	9.8	5.7
A. americanus	CMN21752	Field parental	74.6	27.3	48.7	27.8	19.0	4.7	9.9	5.3	3.0	13.4	2.2	14.1	8.8	12.2	7.6
A. americanus	CMN21763	Field parental	31.5	11.2	18.8	12.1	10.4	1.4	5.3	3.4	1.6	7.7	0.7	5.6	4.1	5.9	3.6
A. americanus	CMN21770	Field parental	55.7	22.3	38.6	21.4	16.7	4.3	8.5	5.5	2.6	12.2	0.1	9.7	7.5	10.6	5.5
A. americanus	CMN21772	Field parental															
A. americanus	CMN21778	Field parental	51.6	19.8	32.6	18.4	14.1	3.1	7.6	4.6	2.0	10.5	0.6	10.2	7.6	10.5	5.8
A. americanus	CMN21781	Field parental	72.6	30.8	50.6	28.6	19.5	4.7	10.9	6.5	3.2	14.3	1.6	14.7	10.0	15.3	8.3
A. americanus	CMN21788	Field parental	52.0	21.5	34.6	17.4	14.1	3.4	7.3	4.0	2.6	10.2	1.4	9.5	7.4	10.0	5.5
A. americanus	CMN21791	Field parental	57.0	22.7	40.8	22.6	16.7	3.9	8.1	4.0	1.9	11.4	0.9	10.6	7.3	12.1	6.2
A. americanus	CMN21803	Field parental	59.4	20.6	39.4	23.0	17.2	4.3	8.8	5.0	2.9	12.0	0.9	11.8	8.9	10.2	5.0
A. americanus	CMN21821	Field parental	61.9	23.9	41.8	22.1	17.1	3.9	8.9	4.0	2.5	11.7	1.1	12.3	8.4	11.6	5.9
A. americanus	CMN21823	Field parental	62.9	26.9	44.8	24.3	16.8	3.8	9.2	5.4	3.1	12.0	1.4	11.6	8.4	12.0	6.6
A. americanus	CMN21824	Field parental	65.6	27.5	46.3	24.1	16.7	3.7	9.1	4.8	2.9	11.2	1.1	12.9	8.4	13.0	8.1
A. americanus	CMN21826	Field parental	64.4	24.8	46.3	24.8	16.2	4.7	10.5	4.5	3.3	13.7	0.9	12.6	9.9	12.9	5.6
A. americanus	CMN21831	Field parental	57.4	24.7	39.5	22.3	17.0	3.8	8.8	4.5	2.4	12.0	1.6	12.6	8.4	10.5	5.7
A. americanus	CMN21832	Field parental	65.9	25.4	44.7	21.1	16.0	3.9	8.8	3.7	2.5	11.9	0.1	13.1	8.9	12.5	7.6
A. americanus	CMN21848	Field parental	52.2	20.4	35.7	18.7	14.1	3.0	7.1	4.0	2.1	10.1	1.0	9.7	6.2	8.8	6.0
A. americanus	CMN21888	Field parental	57.0	23.8	41.6	22.6	17.4	3.2	8.1	4.7	3.2	11.5	0.1	11.6	8.3	10.9	6.3
A. americanus	CMN21928	Field parental	52.9	21.8	39.2	20.6	15.5	3.4	8.5	4.8	1.8	10.8	0.9	11.7	8.1	11.5	5.0
A. americanus	UTAA1229	Field parental	76.2	29.2	47.0	30.2	14.9	5.2	13.0	5.1	3.0	16.8	3.3	13.8	9.7	15.2	8.9
A. americanus	UTAA1230	Field parental	76.6	29.6	48.6	33.5	17.7	5.8	13.4	5.5	3.8	16.5	2.6	15.3	11.0	13.0	8.8
A. americanus	UTAA1231	Field parental	58.7	22.7	36.9	23.9	16.2	4.1	10.8	5.6	3.0	13.5	2.6	11.4	6.6	13.7	6.3
A. americanus	UTAA18261	Field parental	41.2	16.3	25.7	15.7	9.9	3.2	7.5	3.6	1.9	10.7	1.3	7.5	5.2	9.0	3.9
A. americanus	UTAA18262	Field parental	61.0	23.7	39.6	26.4	14.6	4.8	10.7	6.1	2.9	14.2	2.1	11.7	8.9	13.7	7.3
A. americanus	UTAA20827	Field parental	58.1	25.5	35.6	23.1	12.2	5.0	11.1	3.8	2.4	14.3	0.1	10.4	7.6	12.8	5.5

A. americanus	UTAA20829	Field parental	51.3	22.5	34.2	21.7	9.4	5.5	9.7	4.7	2.8	13.1	0.1	9.9	7.5	12.2	5.7
A. americanus	UTAA39071	Field parental	68.6	25.5	44.0	31.0	13.3	4.8	12.6	5.7	3.7	16.6	2.8	14.7	9.6	14.6	7.4
A. americanus	UTAA39072	Field parental	71.0	28.0	44.6	31.1	16.3	6.4	12.9	5.9	3.4	16.3	3.3	11.6	8.6	14.0	6.8
A. americanus	UTAA39073	Field parental	51.9	18.0	31.7	21.6	13.3	4.0	8.2	4.3	2.4	11.4	1.3	9.5	7.7	8.4	5.9
A. americanus	UTAA39074	Field parental	72.9	29.9	46.4	30.2	15.3	5.6	13.0	5.2	3.3	16.7	1.2	12.9	10.2	15.0	7.7
A. americanus	UTAA39075	Field parental	66.0	29.0	40.7	24.4	11.9	4.1	11.4	4.8	2.9	14.6	0.1	11.3	8.8	12.1	6.5
A. americanus	UTAA39076	Field parental	67.2	29.0	44.9	24.2	14.5	5.6	12.0	4.6	3.2	14.9	0.1	12.3	8.8	14.4	7.4
A. americanus	UTAA46526	Field parental	77.8	27.3	45.4	29.2	18.6	5.7	13.0	4.3	3.1	16.5	1.8	13.0	10.4	15.7	8.5
A. charlesmithi	BF0008	Field parental	71.2	22.9	41.3	32.4	17.6	4.0	10.5	5.3	3.1	14.7	2.7	11.6	9.1	13.3	6.8
A. charlesmithi	BF0009	Field parental	80.0	25.6	42.6	32.5	19.5	5.1	11.5	6.2	3.9	15.4	2.9	12.0	8.2	13.3	7.7
A. charlesmithi	BF0010	Field parental	64.7	21.2	35.7	26.8	15.7	3.9	9.4	5.8	3.3	13.5	2.0	11.2	7.7	12.0	5.2
A. charlesmithi	BF038	Field parental	71.7	26.0	40.8	28.3	15.1	5.8	12.1	5.1	3.8	15.8	0.1	11.4	8.3	14.9	6.7
A. charlesmithi	BF039	Field parental	56.5	25.0	36.8	23.8	13.4	4.5	11.3	5.5	3.3	14.7	0.1	11.2	7.6	12.5	5.7
A. charlesmithi	BF064	Field parental	47.5	18.6	27.5	19.5	11.4	3.1	8.6	4.6	2.8	11.5	0.8	9.1	6.6	11.6	5.6
A. charlesmithi	BF065	Field parental	50.9	19.5	29.9	20.7	12.0	3.6	8.4	4.3	2.5	11.7	0.1	8.3	5.6	12.3	5.7
A. charlesmithi	BF066	Field parental	36.7	14.8	21.6	14.5	9.3	2.0	6.9	3.6	1.9	9.8	0.1	6.4	5.0	8.6	4.3
A. charlesmithi	BF067	Field parental	44.0	16.1	24.9	17.7	10.2	3.0	7.6	4.4	3.4	10.5	0.6	7.2	5.6	8.4	4.3
A. charlesmithi	BF069	Field parental	47.5	19.1	30.5	20.5	10.8	3.0	9.1	3.0	2.0	11.6	0.1	10.1	6.5	10.8	4.7
A. charlesmithi	BF167	Field parental	58.7	22.5	36.0	22.9	15.8	4.1	7.6	4.2	2.6	11.5	0.9	9.7	7.8	11.4	5.8
A. charlesmithi	BF167	Field parental	67.9	24.6	42.0	28.3	17.7	5.1	10.0	5.1	4.0	13.4	3.2	13.5	8.8	11.8	6.6
A. charlesmithi	BF170	Field parental	41.7	13.3	23.4	15.1	12.2	1.6	6.1	3.5	2.3	8.1	1.1	7.1	5.9	7.6	4.2
A. charlesmithi	BF186	Field parental	56.7	20.1	40.9	25.8	16.7	5.4	11.1	5.6	3.3	10.4	1.4	11.9	7.6	12.4	5.7
A. charlesmithi	BF187	Field parental	65.5	19.9	42.5	28.0	18.0	3.8	10.9	3.1	4.3	14.3	2.5	13.3	8.6	15.4	9.8
A. charlesmithi	BF188	Field parental	73.3	24.3	41.3	27.9	19.8	4.5	10.1	4.0	2.3	10.6	2.4	13.8	9.4	14.9	7.1
A. charlesmithi	BF189	Field parental	52.8	19.8	32.9	21.4	15.0	3.9	8.0	4.4	3.0	11.0	1.4	8.1	7.1	9.9	7.5
A. charlesmithi	BF190	Field parental	59.9	22.1	35.0	24.4	16.8	4.8	9.4	4.0	2.6	12.2	1.8	9.9	7.9	13.1	7.0
A. charlesmithi	BF191	Field parental	64.3	19.4	35.5	24.3	16.3	3.8	9.8	4.5	2.3	12.1	2.8	9.8	8.4	13.6	6.4
A. charlesmithi	UTAA37236	Field parental	55.5	21.5	34.2	25.1	12.5	3.5	10.6	5.1	3.3	13.6	1.9	10.5	8.4	11.5	5.8
A. charlesmithi	UTAA38079	Field parental	48.4	18.2	29.5	12.0	9.3	3.7	9.4	4.2	2.8	12.7	1.7	9.1	7.2	11.8	6.6
A. charlesmithi	UTAA39481	Field parental	72.3	25.1	40.8	28.2	12.8	5.2	11.6	4.8	3.4	15.3	2.0	11.7	9.2	13.8	7.7
A. charlesmithi	UTAA39551	Field parental	71.9	23.8	37.8	26.3	15.4	5.0	11.7	4.3	2.4	14.6	2.1	10.1	8.5	12.8	7.7

A. charlesmithi	UTAA39557	Field parental	69.0	24.2	38.9	26.2	12.3	4.5	11.9	5.1	3.0	15.1	1.3	12.9	8.6	14.6	8.7
A. charlesmithi	UTAA39559	Field parental	76.3	25.9	43.5	29.3	14.9	5.0	12.7	5.4	2.9	16.2	2.0	13.0	8.7	15.4	8.7
A. fowleri	BF011	Field parental	53.9	18.4	32.0	21.8	15.2	4.1	9.0	4.9	3.1	11.5	1.3	9.7	5.7	10.0	4.9
A. fowleri	BF012	Field parental	54.5	19.1	32.2	23.5	12.9	4.6	9.1	5.6	3.1	12.3	1.4	10.9	7.6	12.1	5.6
A. fowleri	BF013	Field parental	53.2	19.2	33.9	22.2	13.5	5.1	8.2	5.0	3.0	11.7	1.3	10.1	7.6	11.6	5.6
A. fowleri	BF014	Field parental	59.4	20.1	31.2	24.8	14.5	4.7	10.1	5.7	3.3	12.9	1.4	11.5	7.4	12.8	5.7
A. fowleri	BF015	Field parental	63.5	22.8	36.1	26.6	15.2	4.7	9.9	6.9	3.8	13.5	0.8	12.1	8.8	12.9	5.8
A. fowleri	BF016	Field parental	61.5	20.1	35.8	24.9	13.9	5.7	10.4	5.9	3.3	13.9	0.1	11.4	7.7	14.6	6.6
A. fowleri	BF017	Field parental	67.3	21.6	35.8	26.6	17.8	5.7	10.1	5.8	3.8	13.4	1.4	12.8	8.5	13.4	7.5
A. fowleri	BF018	Field parental	52.7	19.7	33.2	21.3	12.9	4.0	8.5	5.0	2.7	11.9	0.9	10.8	7.0	12.0	5.6
A. fowleri	BF019	Field parental	52.1	19.9	34.9	21.0	13.2	4.6	8.5	4.9	2.7	12.0	0.9	10.6	7.5	11.8	5.7
A. fowleri	BF020	Field parental	54.5	20.8	32.4	23.5	14.0	4.3	9.2	5.3	3.1	12.3	0.9	12.1	8.7	12.7	5.5
A. fowleri	BF021	Field parental	52.7	21.0	32.6	22.9	12.7	4.2	8.2	5.3	3.2	12.0	1.7	11.1	7.4	11.4	5.5
A. fowleri	BF109	Field parental	54.9	20.2	32.0	20.8	14.9	3.6	8.1	4.6	3.0	10.6	0.6	9.9	6.2	11.1	6.8
A. fowleri	BF110	Field parental	67.3	24.4	38.0	26.7	18.6	5.2	8.8	4.0	2.9	11.8	2.2	11.4	7.6	14.8	6.3
A. fowleri	BF112	Field parental	55.1	19.6	30.2	20.4	15.8	4.0	8.4	5.0	3.1	11.2	0.9	9.0	5.8	11.0	4.9
A. fowleri	BF113	Field parental	52.8	22.0	31.4	21.2	14.2	3.6	8.6	4.3	2.9	11.4	0.8	8.6	6.2	10.4	4.2
A. fowleri	BF114	Field parental	55.8	21.9	33.9	21.7	18.0	3.7	8.7	5.1	2.7	11.9	0.1	11.1	7.1	10.1	4.9
A. fowleri	BF115	Field parental	51.9	19.8	32.4	19.6	15.8	3.6	8.0	4.7	3.1	10.6	0.8	9.7	6.7	11.3	4.5
A. fowleri	BF116	Field parental	54.9	21.2	33.4	23.7	17.0	5.3	8.4	3.8	3.0	11.3	0.1	9.2	6.9	12.2	5.6
A. fowleri	BF117	Field parental	55.4	20.4	32.2	21.3	16.5	4.2	8.1	5.0	3.1	11.0	1.7	10.1	6.5	10.8	5.2
A. fowleri	BF118	Field parental	55.8	21.3	34.2	24.2	17.2	4.3	7.9	4.5	3.3	11.1	1.0	10.2	6.1	10.6	5.8
A. fowleri	BF119	Field parental	68.2	24.3	38.7	26.1	19.6	4.7	9.3	4.6	3.3	12.8	0.6	10.5	7.7	13.4	5.8
A. fowleri	BF125	Field parental	69.7	24.5	40.1	27.7	19.0	4.1	8.8	4.6	3.1	11.6	1.7	10.1	8.8	13.1	5.0
A. fowleri	BF126	Field parental	71.8	25.7	39.4	27.2	19.3	4.2	9.5	4.7	3.1	12.9	0.1	13.2	9.3	13.1	6.8
A. fowleri	BF127	Field parental	64.9	23.9	36.4	24.7	16.4	3.9	9.0	4.6	3.5	11.9	0.1	10.7	7.2	13.6	5.4
A. fowleri	BF128	Field parental	65.8	24.2	38.7	25.2	16.8	4.9	8.4	5.2	2.5	11.2	0.8	11.2	7.4	13.1	5.8
A. fowleri	BF129	Field parental	55.4	19.6	32.6	22.6	15.6	3.7	8.1	4.7	2.9	11.0	0.1	8.9	6.6	11.0	5.0
A. fowleri	BF130	Field parental	54.3	19.7	32.3	19.9	15.6	4.2	8.2	4.2	3.2	11.1	0.9	9.1	7.0	9.9	4.9
A. fowleri	BF131	Field parental	57.8	21.5	34.6	23.1	14.8	4.1	8.3	4.9	3.5	11.4	1.5	9.4	6.5	10.4	4.6
A. fowleri	BF146	Field parental	47.4	19.4	31.1	18.5	13.5	3.0	7.2	3.9	2.0	9.8	0.1	9.5	6.1	11.4	5.6

A. fowleri	BF154	Field parental	63.4	23.9	37.9	24.6	17.0	4.3	9.7	4.6	2.4	12.7	0.8	11.4	8.3	11.2	6.5
A. fowleri	CMN21699	Field parental	49.9	19.7	31.6	19.8	13.8	3.6	7.8	3.9	2.5	10.5	0.1	9.9	6.1	9.2	5.0
A. fowleri	CMN21701	Field parental	48.9	19.7	31.6	18.2	14.5	2.8	7.0	4.2	2.4	10.2	0.1	9.1	6.2	9.3	4.9
A. fowleri	CMN21704	Field parental	49.0	19.0	30.9	17.5	13.5	3.0	6.9	3.6	2.4	10.3	0.1	9.4	8.0	10.0	4.1
A. fowleri	CMN21736	Field parental	50.3	19.3	32.5	18.5	13.6	2.6	7.4	4.1	2.4	9.7	0.1	9.4	6.5	10.2	5.6
A. fowleri	CMN21740	Field parental	45.3	18.0	31.8	17.7	14.7	2.1	7.1	3.3	1.8	9.8	0.1	9.0	6.1	9.6	5.2
A. fowleri	CMN21744	Field parental	32.9	12.2	21.1	12.5	10.0	2.0	5.3	2.8	1.8	7.2	0.1	6.0	4.0	6.7	4.0
A. fowleri	CMN21750	Field parental	47.4	19.9	32.7	18.1	14.1	2.8	6.4	3.3	2.4	9.0	0.1	8.3	5.8	9.8	4.5
A. fowleri	CMN21756	Field parental	44.4	19.0	30.0	16.7	12.9	2.3	6.6	3.5	2.2	8.8	0.1	9.3	5.6	8.1	6.1
A. fowleri	CMN21779	Field parental	53.8	23.2	37.2	19.9	13.5	2.9	7.8	2.9	2.5	10.5	0.1	10.2	6.9	12.6	5.3
A. fowleri	CMN21794	Field parental	62.3	27.4	42.1	24.5	18.1	4.3	9.8	3.8	3.0	12.3	0.1	11.8	8.8	13.0	6.9
A. fowleri	CMN21798	Field parental	65.4	30.2	47.1	24.8	19.1	3.8	8.7	4.3	2.0	12.1	0.1	14.4	9.7	13.4	4.4
A. fowleri	CMN21801	Field parental	40.0	19.1	27.4	15.4	12.2	2.3	7.1	2.8	2.0	9.2	0.1	7.6	5.9	8.0	5.3
A. fowleri	CMN21814	Field parental	46.0	21.7	33.2	17.7	13.2	3.0	7.3	4.2	3.0	9.7	0.1	9.5	6.4	9.5	5.0
A. fowleri	CMN21846	Field parental	58.3	26.8	43.1	23.8	17.1	3.5	9.4	4.3	2.9	11.9	0.1	12.4	9.6	13.2	6.3
A. fowleri	CMN21877	Field parental	48.7	22.5	35.3	19.6	14.3	2.7	7.3	3.2	2.3	10.0	0.1	10.6	6.9	9.3	5.4
A. fowleri	CMN21882	Field parental	54.8	22.9	35.2	20.0	15.0	3.2	7.3	3.7	2.0	10.6	0.1	9.5	7.2	10.9	6.2
A. fowleri	CMN21883	Field parental	56.8	26.5	42.0	21.6	17.3	3.6	8.4	3.7	2.9	11.2	0.1	10.5	8.3	11.5	4.8
A. fowleri	CMN21894	Field parental	52.4	21.1	34.1	20.6	14.5	2.7	7.5	3.9	2.8	10.1	0.1	10.5	7.6	10.6	5.3
A. fowleri	CMN21898	Field parental	52.8	21.6	35.3	20.4	15.2	3.9	7.7	4.1	3.0	10.9	0.1	10.2	7.1	10.7	5.1
A. fowleri	CMN21901	Field parental	48.6	21.6	32.6	18.3	13.5	2.8	7.6	3.8	2.7	10.4	0.1	8.3	5.9	9.5	4.8
A. fowleri	CMN21916	Field parental	67.4	26.1	43.5	22.9	18.1	3.5	8.2	4.0	2.6	11.6	0.1	12.4	8.2	12.5	5.2
A. fowleri	CMN21925	Field parental	54.9	19.8	35.7	21.3	14.8	2.7	8.7	3.8	2.8	11.2	0.1	10.7	7.1	10.6	5.0
A. fowleri	CMN21930	Field parental	69.2	24.3	37.7	20.9	14.8	3.7	8.4	3.5	2.9	11.9	0.1	11.0	8.0	11.0	6.3
A. fowleri	CMN21935	Field parental	49.3	18.7	30.6	17.8	12.8	3.1	7.6	3.8	2.5	10.0	0.1	8.5	6.5	9.9	4.0
A. fowleri	CMN21942	Field parental	50.7	20.9	33.1	18.1	14.0	3.5	7.4	3.8	2.6	9.6	0.1	10.8	7.2	9.9	5.1
A. fowleri	CMN21959	Field parental	55.3	26.8	41.9	21.2	17.0	3.5	7.8	3.2	2.0	11.0	0.1	10.7	7.9	11.3	5.4
A. fowleri	CMW1003	Field parental	50.1	20.9	33.6	19.6	13.1	4.0	8.1	4.2	2.8	10.7	0.9	8.6	6.8	10.0	4.4
A. fowleri	CMW1004	Field parental	49.8	20.6	33.2	19.3	13.6	3.4	7.5	3.6	2.3	10.2	0.1	9.8	6.7	11.2	4.8
A. fowleri	CMW1005	Field parental	49.5	21.2	34.9	18.8	14.8	3.0	7.5	4.1	2.2	9.7	0.6	9.2	6.4	8.9	5.7
A. fowleri	CMW1007	Field parental	47.5	20.4	31.9	18.7	13.1	3.7	7.4	3.8	2.3	9.6	0.1	9.4	7.2	10.4	4.7

A. fowleri	CMW1013	Field parental	55.5	21.8	35.2	22.0	14.7	4.4	8.8	3.9	2.5	11.5	0.1	9.1	7.4	10.6	5.8
A. fowleri	CMW1022	Field parental	45.0	19.6	30.3	17.8	13.1	3.1	6.6	2.7	2.1	9.3	0.1	8.2	6.2	10.6	5.5
A. fowleri	CMW1024	Field parental	43.3	17.6	26.7	15.9	11.8	2.2	5.6	3.7	2.3	7.7	0.1	7.1	6.1	8.5	5.4
A. fowleri	CMW1025	Field parental	48.1	20.2	29.6	18.5	12.4	2.8	8.0	3.8	2.2	9.3	0.1	7.4	6.6	9.4	4.8
A. fowleri	CMW1026	Field parental	40.1	17.3	29.2	16.7	12.1	2.5	6.7	3.2	1.8	9.3	0.1	8.4	5.7	7.7	3.5
A. hemiophrys	TNHC26844	Blair parental	56.2	21.0	36.7	21.0	13.3	3.1	8.7	1.9	2.4	10.7	0.1	11.1	8.0	10.0	6.3
A. hemiophrys	TNHC26890	Blair parental	59.9	21.4	39.5	21.9	13.9	3.5	8.8	2.7	2.8	10.5	0.1	10.2	7.5	12.4	5.5
A. hemiophrys	TNHC26891	Blair parental	53.8	19.3	34.0	21.1	12.7	3.2	8.4	2.2	2.0	10.3	0.1	8.9	7.9	8.2	5.0
A. hemiophrys	TNHC26892	Blair parental	46.7	18.1	33.0	18.0	12.0	3.1	6.9	2.9	2.5	9.7	0.1	8.0	6.3	9.1	5.4
A. hemiophrys	TNHC26893	Blair parental	56.0	19.9	36.2	20.1	14.3	3.1	7.2	1.9	2.4	10.5	0.1	9.6	8.5	11.1	6.8
A. hemiophrys	TNHC26895	Blair parental	54.7	20.1	37.1	20.1	14.5	3.2	7.8	2.2	2.4	10.3	0.1	9.0	9.1	10.1	5.6
A. hemiophrys	TNHC26896	Blair parental	43.0	17.9	30.6	16.3	10.7	2.2	6.1	1.6	1.9	9.0	0.1	8.7	6.3	8.9	4.7
A. hemiophrys	TNHC33216	Blair parental	56.1	22.1	36.2	21.7	14.6	3.5	8.0	4.2	2.6	10.5	0.9	11.3	6.1	10.2	5.9
A. hemiophrys	TNHC33217	Blair parental	49.0	18.3	32.2	18.7	11.6	3.4	7.3	2.2	2.2	9.5	0.1	7.2	6.4	10.7	6.6
A. hemiophrys	TNHC33218	Blair parental	53.1	19.8	34.1	20.4	13.7	2.8	7.8	2.9	2.9	11.1	0.7	8.8	8.5	10.5	4.0
A. hemiophrys	TNHC33219	Blair parental	55.8	20.5	36.9	23.3	14.2	3.0	7.9	2.0	2.1	9.9	0.9	9.2	7.5	10.9	7.3
A. hemiophrys	TNHC33220	Blair parental	48.8	17.6	31.2	17.9	12.3	2.6	7.5	3.6	2.8	9.2	0.1	10.0	6.1	8.3	5.4
A. hemiophrys	TNHC33221	Blair parental	50.6	19.3	33.4	18.1	12.3	3.0	6.9	2.0	1.6	9.5	0.7	8.3	6.5	11.3	5.1
A. hemiophrys	TNHC35540	Blair parental	51.4	20.7	35.7	20.2	14.4	3.6	6.6	2.0	2.5	10.5	0.7	9.9	8.0	9.5	5.1
A. houstonensis	TNHC25629	Blair parental	46.8	19.1	32.0	21.9	18.2	3.1	7.2	3.8	2.3	10.5	1.6	10.3	7.3	10.2	6.0
A. houstonensis	TNHC28860	Blair parental	57.5	22.7	37.7	22.1	15.4	3.4	8.0	4.3	2.9	10.4	2.2	11.3	9.3	12.7	5.5
A. houstonensis	TNHC34740	Blair parental	57.1	20.2	36.2	20.7	14.4	16.4	8.0	4.6	2.5	10.3	0.9	11.2	6.3	10.3	4.7
A. houstonensis	TNHC35536	Blair parental	63.0	21.4	36.1	23.8	16.8	4.1	8.5	3.4	3.0	11.4	2.0	10.0	8.1	13.1	5.3
A. houstonensis	TNHC35537	Blair parental	67.4	23.6	39.3	26.9	16.9	5.1	9.2	3.0	2.8	12.7	2.0	12.2	9.3	15.7	7.7
A. houstonensis	TNHC49381	Blair parental	56.9	19.2	34.3	22.3	16.7	3.9	10.8	3.5	2.7	11.4	1.2	8.9	7.3	11.7	5.7
A. houstonensis	TNHC49385	Blair parental	60.1	21.1	36.3	23.0	17.6	3.8	10.7	3.9	2.6	11.6	0.8	10.9	7.3	13.5	5.8
A. houstonensis	TNHC49388	Blair parental	59.1	20.7	33.9	21.8	16.3	4.6	7.7	3.7	2.6	10.1	1.3	9.5	5.8	11.8	5.7
A. houstonensis	TNHC49392	Blair parental	51.5	19.2	34.6	22.1	15.8	3.5	7.0	3.4	2.3	10.7	1.6	10.9	8.2	11.1	5.6
A. houstonensis	TNHC50117	Blair parental	68.0	22.7	41.2	27.7	22.1	5.4	8.4	4.8	3.5	12.4	2.4	11.9	9.1	13.2	6.4
A. houstonensis	UTAA40636	Field parental	44.7	19.0	25.7	18.9	10.7	4.9	9.1	4.3	2.3	11.3	0.1	9.2	6.4	9.7	5.1
A. houstonensis	UTAA40637	Field parental	73.8	26.5	41.5	30.6	16.5	6.6	12.0	4.4	3.8	16.0	1.8	11.3	8.4	14.9	5.6

A. houstonensis	UTAA40638	Field parental	55.1	22.2	33.4	20.8	12.0	4.8	10.1	4.4	2.5	13.0	0.8	10.1	7.5	10.9	5.9
A. houstonensis	UTAA41609	Field parental	55.3	20.0	31.2	21.8	13.2	4.3	10.2	4.4	3.1	13.1	1.8	9.1	7.0	10.9	4.4
A. houstonensis	UTAA41633	Field parental	55.7	22.4	35.8	22.2	14.0	5.0	10.2	5.0	2.9	14.0	0.6	11.5	7.4	11.9	5.7
A. houstonensis	UTAA41634	Field parental	69.3	22.1	38.7	27.3	17.3	5.5	11.9	6.2	3.8	16.5	1.2	9.6	8.1	13.5	5.1
A. houstonensis	UTAA42209	Field parental	56.8	21.0	34.1	22.5	12.8	4.2	9.0	4.8	2.9	12.0	0.1	9.1	7.8	11.6	5.5
A. houstonensis	UTAA42210	Field parental	59.2	24.6	33.9	24.7	12.1	4.7	10.9	4.8	2.8	13.8	1.2	10.0	8.1	11.4	5.4
A. houstonensis	UTAA42211	Field parental	56.6	19.9	32.6	21.1	12.0	4.9	10.2	4.6	2.8	12.4	1.5	9.6	6.9	11.8	6.0
A. houstonensis	UTAA42212	Field parental	70.1	26.3	41.4	28.6	14.1	6.1	12.1	5.3	2.8	16.4	1.7	13.4	10.2	13.4	6.0
A. houstonensis	UTAA42439	Field parental	53.2	21.2	31.8	20.6	11.2	4.2	9.1	4.4	2.6	12.0	1.2	8.9	7.6	10.1	4.5
A. houstonensis	UTAA42439	Field parental	68.4	23.9	39.4	27.8	16.4	5.7	12.6	5.2	4.5	15.8	1.6	12.3	9.0	12.9	5.7
A. terrestris	TNHC24690	Blair parental	49.7	20.0	33.2	21.4	15.1	3.7	8.1	4.5	3.3	8.6	1.9	8.2	6.9	8.3	4.8
A. terrestris	TNHC24697	Blair parental	59.3	22.7	38.9	24.3	19.3	5.2	8.7	6.8	3.4	11.5	3.0	10.6	8.1	13.0	7.1
A. terrestris	TNHC24703	Blair parental	48.6	20.9	35.6	22.3	15.5	4.0	7.7	4.8	3.4	11.0	1.8	10.5	7.9	10.7	6.0
A. terrestris	TNHC33647	Blair parental	55.0	24.1	37.9	25.2	18.5	4.6	9.0	5.3	4.0	12.5	2.8	10.7	8.3	11.0	5.9
A. terrestris	TNHC34259	Blair parental	59.2	25.2	42.3	25.8	17.3	4.8	9.4	5.0	3.4	12.5	1.7	11.3	7.8	11.7	7.8
A. terrestris	TNHC34263	Blair parental	56.7	21.6	35.2	22.9	16.7	4.1	8.1	5.1	3.7	11.7	2.3	9.0	7.1	11.6	5.3
A. terrestris	TNHC34311	Blair parental	107	38.8	63.1	48.6	32.4	6.8	13.5	6.9	6.6	19.2	5.1	21.3	14.4	17.5	9.6
A. terrestris	TNHC4675	Blair parental	60.2	21.7	35.1	26.7	20.1	4.1	10.0	6.2	3.7	13.5	2.7	10.7	8.3	10.7	5.7
A. terrestris	TNHC55355	Blair parental	62.8	24.1	40.1	26.3	19.2	4.2	8.8	3.7	3.3	12.4	2.8	11.0	8.5	14.1	7.9
A. terrestris	TNHC55363	Blair parental	65.9	23.3	38.3	26.2	19.4	4.0	8.8	5.0	3.5	13.1	2.3	10.7	7.7	12.3	7.1
A. terrestris	BF134	Field parental	61.4	21.4	36.7	24.9	16.8	3.7	7.7	4.6	2.7	11.7	2.3	9.6	6.0	9.9	5.6
A. terrestris	BF135	Field parental	62.3	20.3	34.0	24.1	16.4	4.0	8.0	4.4	2.7	11.2	2.7	10.9	7.4	11.4	6.1
A. terrestris	BF142	Field parental	58.8	22.1	36.0	25.3	17.3	4.6	9.0	6.3	4.1	12.8	2.3	11.3	6.6	10.3	6.1
A. terrestris	BF155	Field parental	36.4	12.8	21.2	14.9	10.5	2.4	5.5	3.2	2.2	8.0	0.5	6.3	5.3	7.1	4.1
A. terrestris	BF156	Field parental	54.8	18.6	32.8	21.7	15.3	3.0	7.9	5.8	3.2	10.8	1.9	8.8	7.3	9.4	5.5
A. terrestris	BF163	Field parental	57.9	22.0	36.9	23.6	15.5	4.2	7.5	4.7	3.4	10.9	1.3	10.4	7.7	11.0	6.2
A. terrestris	BF164	Field parental	45.4	17.1	27.0	16.8	12.5	2.8	7.0	4.1	2.7	9.2	1.0	7.4	6.3	8.7	4.9
A. terrestris	BF169	Field parental	46.4	18.7	31.9	20.1	14.1	3.3	7.3	4.5	3.1	9.4	1.1	7.1	6.4	8.2	5.5
A. terrestris	BF171	Field parental	58.9	21.5	36.5	23.6	15.6	4.1	8.5	5.2	3.5	10.0	2.2	10.6	7.8	11.4	5.2
A. terrestris	BF172	Field parental	54.2	19.9	33.4	21.8	14.9	3.8	7.4	5.0	3.5	11.1	1.7	9.8	7.3	10.4	4.6
A. terrestris	BF173	Field parental	55.0	20.8	34.3	21.0	14.9	3.2	8.1	5.4	3.6	11.1	1.5	8.8	7.4	11.3	6.4

A. terrestris	BF174	Field parental	55.4	21.1	36.1	22.1	16.9	3.2	8.0	5.0	3.5	10.8	2.4	10.4	7.4	9.1	5.9
A. terrestris	CMW1006	Field parental	52.9	21.3	33.8	21.6	16.3	3.5	7.4	3.8	2.5	10.9	1.6	10.3	7.4	11.2	5.9
A. terrestris	CMW1011	Field parental	46.5	16.3	26.5	17.9	14.0	3.6	6.8	4.6	2.7	10.1	1.5	7.8	6.4	8.6	4.4
A. terrestris	CMW1012	Field parental	57.8	23.6	38.4	25.5	18.2	4.4	8.7	4.9	4.5	12.3	1.3	11.6	7.7	11.5	5.8
A. terrestris	CMW1028	Field parental	50.8	18.9	33.1	20.6	16.3	3.6	7.9	4.2	3.3	10.4	1.7	9.5	6.9	8.8	5.2
A. terrestris	UTAA1472	Field parental	48.8	20.2	31.6	21.5	10.1	2.8	8.8	5.7	2.8	11.9	3.2	9.0	6.4	9.5	4.4
A. terrestris	UTAA20833	Field parental	57.5	22.7	38.0	22.1	14.4	4.9	10.2	4.3	3.0	13.1	2.2	11.4	5.7	11.2	4.8
A. terrestris	UTAA3619	Field parental	53.6	19.4	31.4	22.8	11.4	3.2	9.7	4.6	2.7	12.9	2.6	10.4	7.9	10.7	5.2
A. terrestris	UTAA3620	Field parental	51.7	18.6	28.7	19.8	11.4	3.7	9.4	5.4	2.7	12.3	1.9	9.6	6.8	10.1	5.5
A. terrestris	UTAA3623	Field parental	43.8	18.3	28.1	19.0	9.7	3.0	8.4	3.4	2.3	11.5	2.1	10.4	7.2	8.7	5.1
A. terrestris	UTAA38075	Field parental	66.0	25.8	42.7	29.7	12.1	4.8	12.0	6.4	3.9	16.0	3.3	12.1	10.7	12.7	5.9
A. terrestris	UTAA38076	Field parental	50.9	20.3	32.4	22.2	12.8	3.8	10.5	4.9	3.7	13.5	2.9	10.7	8.1	9.6	4.8
A. terrestris	UTAA39081	Field parental	58.4	22.0	37.8	24.5	14.3	4.8	11.3	5.3	3.3	13.9	2.5	10.8	8.8	13.6	7.0
A. terrestris	UTAA39082	Field parental	68.6	29.3	39.6	28.9	17.6	5.6	11.7	5.8	4.0	15.0	1.6	13.2	9.7	13.9	5.9
A. terrestris	UTAA39083	Field parental	62.7	23.3	38.9	25.1	14.6	4.5	11.7	6.3	3.6	15.3	3.0	11.1	9.2	12.0	5.8
A. terrestris	UTAA414	Field parental	51.3	20.8	33.9	22.3	12.0	4.2	10.8	5.9	4.1	14.8	2.0	9.9	8.5	10.8	5.7
A. terrestris	UTAA415	Field parental	38.6	14.5	26.3	19.0	9.7	3.8	8.2	5.3	2.5	11.0	2.2	8.3	6.9	7.4	4.3
A. terrestris	UTAA48278	Field parental	61.1	22.3	35.8	26.5	15.7	5.0	10.8	6.4	3.1	14.2	3.4	11.5	7.6	10.9	5.0
A. terrestris	UTAA700	Field parental	42.3	16.4	26.2	19.0	11.3	3.2	8.7	4.8	2.0	11.1	2.3	7.5	5.2	8.8	4.5
A. terrestris	UTAA701	Field parental	44.9	18.4	31.0	20.3	12.1	4.0	8.3	5.0	2.8	11.0	2.7	9.2	5.8	10.6	4.8
A. terrestris	UTAA702	Field parental	41.2	17.8	29.4	19.4	12.2	3.4	8.3	4.8	2.1	11.6	2.0	8.9	5.9	8.8	3.9
A. terrestris	UTAA703	Field parental	41.3	17.1	28.6	18.5	9.9	3.2	8.2	4.9	2.9	10.8	2.4	8.2	6.0	9.7	5.0
A. terrestris	UTAA8064	Field parental	43.8	17.1	28.2	19.0	10.1	3.7	9.4	4.5	2.8	11.7	3.6	8.5	7.2	9.6	5.6
A. terrestris	UTAA8065	Field parental	43.0	16.0	27.0	17.2	8.9	3.2	8.2	4.5	2.5	10.7	1.6	7.8	5.7	9.5	5.4
A. velatus	BF0001	Field parental	45.0	14.5	22.8	17.1	10.8	3.2	6.5	5.1	2.9	8.7	0.1	7.4	5.2	9.3	5.0
A. velatus	BF0002	Field parental	50.0	18.9	25.8	20.6	12.0	4.3	8.1	5.6	3.5	10.6	1.8	8.2	6.0	10.1	4.6
A. velatus	BF0003	Field parental	45.1	15.0	23.5	18.6	11.5	3.3	7.2	5.3	3.0	9.5	1.7	8.3	5.9	9.1	5.0
A. velatus	BF0004	Field parental	40.1	12.4	22.1	15.8	10.8	3.1	6.9	4.9	2.6	8.3	0.1	6.8	5.0	9.2	4.6
A. velatus	BF0005	Field parental	33.0	10.0	18.3	13.2	8.3	2.5	5.4	3.8	2.6	7.3	0.1	6.4	4.3	6.5	3.0
A. velatus	BF0006	Field parental	50.7	17.0	25.3	21.0	12.1	4.2	7.9	5.9	3.3	10.8	0.1	9.5	6.6	11.2	5.8
A. velatus	BF0007	Field parental	54.5	18.9	32.4	22.3	14.0	4.4	8.5	5.8	3.4	12.1	1.6	10.2	6.5	11.0	5.7

A. velatus	BF022	Field parental	57.8	20.3	34.4	23.5	15.2	5.0	9.7	5.7	3.4	12.9	1.0	11.3	8.2	13.0	6.4
A. velatus	BF023	Field parental	51.5	17.7	30.7	22.3	11.9	4.4	8.4	4.7	3.2	11.7	0.9	9.6	6.9	10.7	5.1
A. velatus	BF026	Field parental	56.3	22.6	35.6	22.1	12.3	4.1	9.2	4.2	2.9	12.5	0.8	10.3	7.4	12.1	6.2
A. velatus	BF027	Field parental	61.6	22.8	37.0	25.2	12.6	5.5	10.6	5.4	3.2	13.7	1.0	11.3	8.2	11.3	6.0
A. velatus	BF028	Field parental	58.1	22.2	35.9	22.4	12.6	4.9	9.5	4.8	3.5	13.4	0.9	11.4	8.5	11.6	5.8
A. velatus	BF029	Field parental	64.2	24.0	36.6	24.9	14.0	5.2	10.2	5.8	3.2	14.5	0.1	12.0	9.1	13.6	5.3
A. velatus	BF030	Field parental	58.3	22.1	35.8	21.6	13.2	4.2	9.6	5.9	3.4	13.2	0.9	10.4	7.7	11.6	5.3
A. velatus	BF031	Field parental	54.5	21.6	30.7	21.6	12.1	4.4	9.4	5.6	3.4	12.3	0.8	9.8	6.4	12.0	5.9
A. velatus	BF032	Field parental	65.4	24.8	39.3	25.6	14.3	5.2	11.1	5.1	3.7	14.6	0.1	12.6	8.9	15.1	5.9
A. velatus	BF033	Field parental	58.5	22.1	34.3	22.2	12.6	3.8	9.6	5.2	2.7	13.4	0.9	10.2	6.3	10.8	4.5
A. velatus	BF034	Field parental	51.6	20.1	31.1	21.1	12.8	3.9	8.9	4.1	2.9	12.7	0.8	8.9	6.5	10.4	5.0
A. velatus	BF035	Field parental	60.9	21.1	33.9	23.5	15.1	5.2	10.4	4.6	3.2	13.5	0.6	9.8	8.3	11.8	5.0
A. velatus	BF036	Field parental	66.8	24.1	37.0	27.4	14.5	5.3	10.9	5.7	3.7	14.4	1.2	10.4	8.2	14.3	6.3
A. velatus	BF037	Field parental	52.2	21.5	32.5	20.8	10.1	5.0	9.9	4.6	2.6	12.2	0.7	9.1	7.5	10.9	5.6
A. velatus	BF040	Field parental	42.4	15.9	25.2	16.5	9.4	3.7	7.6	3.8	2.6	10.6	0.1	7.5	6.1	9.9	5.0
A. velatus	BF041	Field parental	56.7	20.3	31.2	22.9	13.9	4.5	10.3	4.8	3.1	12.6	0.1	9.5	6.3	13.4	5.7
A. velatus	BF042	Field parental	70.6	25.9	37.7	27.1	15.6	5.5	12.1	4.2	3.2	15.8	0.1	12.5	8.2	13.3	6.9
A. velatus	BF049	Field parental	68.4	25.3	40.2	27.2	15.2	5.0	12.3	5.4	3.8	15.8	0.1	11.8	8.3	14.2	7.5
A. velatus	BF050	Field parental	65.6	24.7	38.3	24.7	14.5	5.0	11.3	4.9	3.4	14.6	0.1	12.2	9.1	12.4	7.2
A. velatus	BF051	Field parental	53.4	19.9	28.5	20.8	12.6	4.5	8.9	3.9	2.3	12.3	0.8	9.0	7.5	10.2	5.1
A. velatus	BF052	Field parental	64.5	24.3	35.7	25.9	15.4	4.5	11.2	5.0	3.5	14.8	0.9	11.7	7.3	13.4	7.5
A. velatus	BF053	Field parental	65.6	25.2	38.0	24.6	17.0	4.9	10.7	5.0	3.1	14.2	1.7	11.5	9.5	11.5	5.6
A. velatus	BF054	Field parental	54.5	21.5	31.9	23.5	13.2	5.0	10.7	5.0	3.3	12.9	0.8	9.7	7.0	11.4	5.6
A. velatus	BF061	Field parental	66.6	23.8	36.1	25.3	15.7	4.9	10.7	4.1	2.7	13.4	0.1	11.5	8.5	12.9	7.4
A. velatus	BF062	Field parental	56.3	20.4	31.6	23.3	13.4	4.0	9.6	4.2	2.7	13.0	0.7	10.3	6.5	10.8	5.2
A. velatus	BF063	Field parental	51.4	18.9	28.7	19.8	12.6	3.8	9.1	3.7	2.6	12.3	0.8	8.3	5.5	9.4	5.4
A. velatus	BF068	Field parental	44.9	17.8	25.7	17.9	11.2	3.6	8.2	3.7	2.1	10.4	0.7	7.8	5.1	8.9	5.1
A. velatus	BF082	Field parental	56.2	21.8	35.1	24.2	14.6	4.6	8.7	4.8	2.7	11.5	1.3	11.0	8.1	10.9	5.8
A. velatus	BF083	Field parental	53.1	21.5	33.4	21.2	15.1	4.1	9.0	4.4	2.9	11.6	0.9	10.1	7.2	12.1	5.2
A. velatus	BF084	Field parental	66.6	23.8	38.1	25.5	18.8	4.7	9.6	4.9	3.6	12.9	1.5	11.5	7.9	12.2	5.9
A. velatus	BF085	Field parental	64.7	23.1	35.1	25.2	15.0	4.4	9.3	4.7	3.2	12.5	0.9	11.4	8.1	12.8	5.2

A. velatus	BF088	Field parental	57.7	22.1	34.7	23.2	14.7	4.5	9.3	4.7	3.2	12.6	1.4	9.7	7.4	11.6	5.5
A. velatus	BF105	Field parental	46.1	18.3	28.4	19.4	15.6	3.4	8.3	4.7	2.8	10.3	0.1	8.9	6.4	8.9	4.8
A. velatus	BF121	Field parental	56.6	22.1	35.9	22.3	15.9	4.3	9.0	4.8	3.2	11.9	0.1	10.8	7.8	11.7	6.5
A. velatus	BF122	Field parental	54.9	21.1	35.5	21.8	15.1	4.1	8.9	4.3	3.1	11.5	0.7	11.2	6.4	11.0	6.3
A. velatus	BF124	Field parental	53.7	22.0	33.1	21.9	14.3	3.7	8.7	4.8	2.8	11.0	1.0	10.5	8.6	12.2	5.7
A. velatus	MG0001	Field parental	32.3	10.1	18.2	13.7	8.8	2.9	5.7	3.7	2.2	7.4	0.1	4.8	4.2	8.1	3.8
A. velatus	MG0002	Field parental	37.3	12.6	21.0	15.6	10.4	2.8	6.9	4.3	2.8	9.0	1.3	6.9	5.8	7.3	3.7
A. velatus	RLG2634	Field parental	55.4	21.5	31.3	23.6	12.6	4.3	9.7	4.7	3.8	13.2	1.0	10.4	8.1	10.4	5.1
A. velatus	RLG2637	Field parental	37.3	15.2	21.5	15.9	10.0	3.0	6.8	3.6	2.3	9.1	0.9	7.2	5.3	8.3	3.9
A. velatus	RLG2638	Field parental	47.6	18.5	29.3	20.9	11.9	4.1	8.4	4.3	2.6	11.2	0.8	8.4	6.3	9.5	5.0
A. velatus	RLG2639	Field parental	71.5	26.0	40.4	27.9	15.7	5.4	11.8	5.3	3.8	15.4	1.4	11.0	8.8	14.1	6.2
A. velatus	SCB006	Field parental	67.3	26.5	43.5	28.4	18.7	5.5	9.2	4.4	3.0	11.9	1.9	12.4	8.4	15.2	6.5
A. velatus	UTAA1228	Field parental	74.9	23.6	39.0	29.8	15.2	5.2	10.7	6.3	3.8	14.5	1.2	11.6	8.4	16.5	6.9
A. velatus	UTAA17454	Field parental	62.9	18.9	37.5	25.8	14.8	4.7	10.4	6.1	3.5	14.1	0.1	11.7	7.7	12.2	7.0
A. velatus	UTAA17917	Field parental	80.5	29.0	45.6	32.9	16.9	5.8	10.2	5.2	3.9	15.5	2.3	14.7	10.6	15.2	5.6
A. velatus	UTAA18051	Field parental	55.8	17.6	28.2	20.3	14.5	3.9	8.6	4.6	3.1	12.0	0.8	9.5	7.1	11.4	6.3
A. velatus	UTAA18052	Field parental	54.7	19.0	31.1	21.7	13.0	4.5	9.3	5.6	3.7	12.2	0.8	10.1	7.3	11.0	4.3
A. velatus	UTAA18053	Field parental	51.0	15.1	32.0	20.9	13.3	4.5	9.7	5.6	3.8	12.7	1.4	10.9	7.0	10.9	5.2
A. velatus	UTAA18054	Field parental	61.1	20.2	34.4	25.4	13.2	6.0	9.5	5.4	3.5	13.1	1.4	12.0	7.1	13.2	6.4
A. velatus	UTAA27446	Field parental	41.2	13.4	24.0	18.0	10.1	3.1	7.2	4.0	2.1	9.7	0.1	9.0	6.4	9.1	4.9
A. velatus	UTAA27446	Field parental	58.0	19.1	32.4	23.4	12.5	4.5	9.0	5.0	3.3	12.2	0.1	9.8	7.6	12.6	5.6
A. velatus	UTAA40204	Field parental	43.8	14.6	26.9	18.4	11.0	3.3	8.0	4.3	2.9	10.9	1.0	9.3	6.3	9.7	4.8
A. velatus	UTAA40205	Field parental	54.6	18.9	32.4	23.5	12.8	5.6	9.6	5.0	4.1	13.2	1.2	10.7	7.4	11.7	5.6
A. velatus	UTAA40265	Field parental	61.1	18.5	33.1	22.9	12.1	5.1	9.5	5.2	3.3	13.0	1.0	11.0	7.5	11.0	5.1
A. velatus	UTAA40266	Field parental	45.0	14.2	27.8	20.9	10.3	3.3	7.1	4.6	3.2	10.5	0.8	9.1	4.9	10.6	4.7
A. velatus	UTAA40268	Field parental	55.8	18.0	32.6	23.4	13.1	4.6	9.5	5.3	3.7	13.3	1.0	10.4	7.1	11.0	5.2
A. velatus	UTAA40269	Field parental	65.3	24.4	35.5	25.9	14.1	5.3	10.9	3.1	2.7	14.5	1.9	11.5	8.3	12.0	6.2
A. velatus	UTAA40627	Field parental	41.4	14.6	23.8	17.8	10.2	3.7	8.0	4.5	3.0	11.0	0.7	7.5	5.8	10.2	5.1
A. velatus	UTAA40905	Field parental	20.9	7.4	13.7	8.8	6.3	1.9	4.8	2.8	1.7	5.6	0.1	3.8	3.5	5.4	2.7
A. velatus	UTAA40922	Field parental	24.7	8.8	15.5	9.7	6.2	1.7	5.1	3.1	1.7	6.6	0.6	4.4	3.1	6.6	3.2
A. velatus	UTAA41534	Field parental	59.6	22.4	34.5	24.3	14.5	6.0	10.9	5.0	3.5	14.8	1.9	9.7	8.8	12.1	5.1

A. velatus	UTAA41538	Field parental	46.6	19.1	28.6	19.1	10.9	4.4	9.7	4.3	2.8	12.6	1.5	8.3	7.2	11.3	4.4
A. velatus	UTAA41630	Field parental	43.8	19.7	31.5	20.0	11.5	3.8	9.0	3.9	2.5	11.6	1.2	8.3	6.5	9.5	4.7
A. velatus	UTAA41651	Field parental	47.0	19.6	28.9	19.4	12.8	3.8	12.1	4.6	2.6	11.8	1.0	9.2	6.9	9.3	5.6
A. velatus	UTAA41652	Field parental	53.6	20.9	30.7	20.8	12.5	4.3	9.4	4.4	3.2	12.4	1.5	9.5	7.7	11.4	7.5
A. velatus	UTAA41677	Field parental	51.9	22.4	33.8	21.5	11.5	4.4	10.3	4.2	2.7	12.9	1.2	9.7	6.8	12.5	5.7
A. velatus	UTAA42229	Field parental	52.2	20.8	32.5	21.5	11.3	4.1	9.8	4.9	2.9	13.2	0.7	9.6	6.6	11.9	5.2
A. velatus	UTAA42235	Field parental	48.2	18.7	29.8	18.8	12.0	4.3	9.6	3.9	3.2	12.3	1.0	8.8	7.1	10.7	5.2
A. velatus	UTAA42236	Field parental	48.6	19.4	29.4	19.5	12.0	3.8	9.3	5.3	2.9	11.9	2.0	7.6	6.8	10.8	5.1
A. velatus	UTAA42237	Field parental	56.9	22.8	34.4	23.3	12.5	4.4	9.9	4.3	3.0	12.7	2.2	10.2	7.7	11.2	4.9
A. velatus	UTAA45004	Field parental	36.7	14.5	22.0	15.7	9.6	3.6	7.6	4.3	2.5	10.1	0.8	7.1	5.7	8.6	3.8
A. velatus	UTAA45005	Field parental	34.8	13.5	22.6	15.0	9.0	3.4	7.7	3.3	1.8	9.7	0.8	6.5	5.9	7.0	3.1
A. velatus	UTAA45006	Field parental	32.0	12.1	20.9	14.2	8.9	3.2	8.0	3.7	2.5	10.3	1.1	5.2	4.6	7.6	3.6
A. velatus	UTAA45007	Field parental	34.3	13.9	21.2	15.8	8.7	2.9	7.5	3.7	1.8	9.7	0.1	6.5	5.0	8.0	3.6
A. velatus	UTAA45008	Field parental	62.1	23.5	38.6	24.9	14.4	5.7	10.3	5.0	3.5	14.1	1.8	10.6	8.2	14.9	6.6
A. velatus	UTAA45009	Field parental	44.6	14.9	25.0	15.0	10.0	3.1	8.5	3.7	2.5	11.0	0.1	7.2	6.2	9.4	5.0
A. velatus	UTAA45010	Field parental	55.8	24.5	37.6	23.1	13.3	5.0	9.4	4.5	3.1	13.6	1.3	10.4	7.3	12.3	5.7
A. woodhousii	TNHC1327	Blair parental	43.5	15.6	24.7	18.1	13.7	3.3	7.4	4.6	3.0	10.0	0.1	7.9	6.1	9.2	4.7
A. woodhousii	TNHC15825	Blair parental	80.4	32.7	51.0	34.9	21.5	4.7	11.3	3.8	3.5	15.1	0.1	14.4	11.1	15.5	7.6
A. woodhousii	TNHC15829	Blair parental	72.7	32.5	46.9	32.3	21.5	4.5	10.0	4.5	4.3	13.7	0.1	13.7	10.1	15.0	5.9
A. woodhousii	TNHC15832	Blair parental	69.7	31.1	47.9	31.8	21.4	4.6	11.4	5.2	3.3	15.4	0.1	14.2	9.5	15.3	6.9
A. woodhousii	TNHC15833	Blair parental	71.1	29.4	42.7	29.6	19.9	3.9	9.3	4.3	3.0	13.9	0.1	12.9	9.3	14.8	7.7
A. woodhousii	TNHC15834	Blair parental	64.9	25.7	43.5	27.8	18.7	4.1	10.1	4.4	3.7	12.9	0.1	13.3	9.9	12.9	5.6
A. woodhousii	TNHC15839	Blair parental	75.2	30.7	46.1	30.2	19.7	4.4	10.4	4.8	3.6	13.9	0.1	13.9	9.5	16.2	6.8
A. woodhousii	TNHC15839	Blair parental	75.2	35.3	50.1	33.5	21.2	5.1	11.9	4.4	3.5	15.4	0.1	16.1	11.5	16.1	6.2
A. woodhousii	TNHC15840	Blair parental	72.6	32.6	46.7	32.9	20.8	4.7	11.1	4.5	4.0	14.7	0.1	14.2	8.8	14.1	5.7
A. woodhousii	TNHC15841	Blair parental	42.8	30.1	45.3	33.0	20.1	4.4	12.6	2.7	2.9	14.9	1.4	13.3	9.4	11.9	7.2
A. woodhousii	TNHC15842	Blair parental	74.5	29.5	45.4	32.6	20.4	4.0	11.1	1.6	3.1	14.7	0.1	12.4	9.7	14.4	7.0
A. woodhousii	TNHC15843	Blair parental	78.4	33.4	50.0	30.2	21.0	5.2	11.4	3.2	3.0	14.4	0.1	14.4	10.2	14.9	6.2
A. woodhousii	TNHC15844	Blair parental	64.3	26.5	43.1	27.9	16.9	5.2	10.3	3.6	3.9	13.0	0.1	13.3	8.7	14.1	7.5
A. woodhousii	TNHC15845	Blair parental	67.3	29.5	45.9	29.3	19.3	4.8	11.1	3.4	3.1	13.8	0.1	13.4	10.8	12.3	7.0
A. woodhousii	TNHC18842	Blair parental	62.9	23.8	37.1	26.2	16.1	4.5	4.7	4.6	3.7	13.1	0.1	10.4	8.9	12.6	5.4

A. woodhousii	BF024	Field parental	85.4	29.7	53.2	34.3	17.2	6.2	12.8	5.7	4.3	17.6	0.1	14.7	10.3	16.6	6.4
A. woodhousii	BF043	Field parental	103	32.9	51.4	34.8	21.6	6.2	13.2	3.7	3.1	18.5	0.1	17.3	11.3	20.3	10.0
A. woodhousii	BF044	Field parental	91.2	33.4	47.6	32.8	18.9	6.6	12.9	5.6	3.4	17.7	0.1	15.6	11.3	16.0	8.4
A. woodhousii	BF095	Field parental	74.6	31.4	48.3	30.9	20.8	4.8	11.2	4.4	3.8	15.1	0.9	13.2	9.6	13.4	6.1
A. woodhousii	BF096	Field parental	83.4	33.8	48.5	32.3	19.8	4.8	12.5	3.4	2.9	17.1	0.1	14.2	10.6	14.6	6.2
A. woodhousii	BF097	Field parental	81.5	33.1	48.8	32.2	20.9	5.4	11.0	6.7	3.2	15.8	0.1	13.3	10.4	13.8	7.4
A. woodhousii	BF098	Field parental	93.4	36.5	58.5	37.2	22.2	5.5	10.8	5.3	3.9	15.3	1.3	14.8	11.7	13.3	7.8
A. woodhousii	BF099	Field parental	82.8	34.1	50.1	31.8	22.6	4.2	12.3	6.0	4.1	15.7	0.1	16.4	10.5	14.8	8.1
A. woodhousii	BF101	Field parental	87.1	31.8	45.5	32.0	23.4	5.5	10.7	4.5	3.6	15.3	0.1	15.1	11.0	15.9	6.6
A. woodhousii	BF102	Field parental	52.8	20.6	31.3	20.6	14.4	3.1	7.8	3.3	2.9	11.4	0.6	9.9	7.1	11.2	4.5
A. woodhousii	BF107	Field parental	88.9	35.1	55.2	34.9	20.7	5.2	11.6	4.5	4.0	16.0	0.1	15.5	10.5	16.3	8.1
A. woodhousii	BF108	Field parental	78.9	30.7	46.7	31.6	21.6	4.6	10.3	4.4	3.8	14.7	0.8	13.7	8.6	15.2	6.3
A. woodhousii	BF132	Field parental	59.1	21.9	34.8	23.8	16.9	4.0	9.8	5.2	3.0	12.7	1.2	10.8	7.2	11.7	5.8
A. woodhousii	BF132	Field parental	67.9	29.9	42.2	28.4	21.0	4.8	10.1	4.7	3.2	13.5	0.1	13.0	9.0	14.3	6.1
A. woodhousii	BF133	Field parental	70.7	30.0	48.3	28.4	19.5	4.2	10.2	3.9	3.0	14.2	0.1	13.7	8.3	15.0	6.6
A. woodhousii	BF140	Field parental	46.2	18.6	27.2	19.5	13.6	3.7	6.6	3.0	2.7	8.9	0.1	7.5	5.9	8.9	5.3
A. woodhousii	BF162	Field parental	66.4	23.9	39.6	25.6	19.3	4.1	8.3	4.7	2.6	11.6	1.5	11.7	8.7	13.9	7.2
A. woodhousii	BF165	Field parental	66.2	27.4	42.7	28.2	19.1	4.8	9.2	3.9	2.9	13.1	0.1	11.7	8.6	13.7	6.1
A. woodhousii	BF168	Field parental	70.6	27.2	43.0	27.7	19.3	4.0	9.8	3.7	2.8	13.3	0.1	14.0	9.1	13.3	6.5
A. woodhousii	BF175	Field parental	58.4	21.5	35.4	24.3	18.1	3.4	8.5	3.4	2.9	11.5	0.5	9.6	7.8	11.3	6.3
A. woodhousii	BF176	Field parental	75.6	29.0	48.2	30.6	18.3	4.1	9.2	3.7	2.8	13.0	0.1	13.0	9.7	12.8	6.8
A. woodhousii	UTAA13577	Field parental	45.5	20.3	29.2	18.9	11.4	2.9	9.4	3.2	2.4	11.6	0.1	9.9	7.5	10.8	4.6
A. woodhousii	UTAA1700	Field parental	55.0	24.7	37.6	22.4	13.0	4.6	11.4	5.1	3.2	14.5	1.4	11.6	7.7	11.0	5.6
A. woodhousii	UTAA18982	Field parental	64.1	24.4	38.4	26.3	13.0	5.4	12.1	4.2	2.9	14.1	1.6	11.6	7.7	11.7	5.5
A. woodhousii	UTAA20173	Field parental	45.2	15.5	25.6	17.8	9.8	3.1	8.6	4.0	2.5	10.5	1.6	7.9	5.7	9.8	4.4
A. woodhousii	UTAA3025	Field parental	58.9	22.2	35.5	24.7	14.9	4.7	10.7	5.1	3.4	14.3	1.4	10.3	7.8	12.4	6.0
A. woodhousii	UTAA3034	Field parental	50.5	20.5	32.5	23.4	9.3	5.1	10.4	5.0	2.8	13.4	0.1	8.3	7.3	11.3	6.8
A. woodhousii	UTAA3036	Field parental	58.3	25.9	37.6	25.7	12.1	5.3	11.0	5.7	3.6	13.7	2.0	11.2	8.9	11.3	6.7
A. woodhousii	UTAA3038	Field parental	62.3	27.4	40.3	25.8	13.8	6.2	9.6	5.3	3.1	14.3	1.8	12.7	9.9	13.7	6.4
A. woodhousii	UTAA34028	Field parental	64.6	21.7	32.3	24.8	12.1	5.3	10.9	4.7	3.2	14.4	1.5	9.3	8.0	11.8	5.0
A. woodhousii	UTAA34029	Field parental	57.1	21.5	33.0	23.4	12.5	5.3	10.4	4.7	2.8	13.5	1.0	8.6	7.3	11.2	5.3

A. woodhousii	UTAA34030	Field parental	50.2	18.2	30.0	20.8	8.3	4.4	9.9	4.3	2.9	12.5	1.6	9.0	7.2	9.9	5.4
A. woodhousii	UTAA34031	Field parental	46.2	17.3	26.9	18.7	8.0	4.7	9.4	3.9	1.9	12.3	0.1	9.2	6.8	9.4	4.7
A. woodhousii	UTAA34333	Field parental	89.1	35.4	60.2	36.8	16.5	7.9	15.0	3.0	3.3	17.9	0.1	17.9	10.6	18.0	8.2
A. woodhousii	UTAA38080	Field parental	67.1	26.2	44.4	27.7	14.1	5.1	11.6	4.0	2.3	15.7	0.1	14.5	9.2	13.3	7.1
A. woodhousii	UTAA38081	Field parental	65.0	29.6	42.0	27.1	11.5	5.2	12.8	5.1	2.7	16.4	0.1	14.4	9.8	13.8	7.1
A. woodhousii	UTAA53937	Field parental	72.9	30.3	49.3	29.4	15.9	5.8	13.5	1.3	3.2	16.8	0.1	16.6	10.2	14.8	6.4
A. woodhousii	UTAA53938	Field parental	81.8	32.7	46.8	31.6	18.3	5.5	14.2	2.4	2.3	17.5	0.1	16.8	9.7	16.5	7.8
A. woodhousii	UTAA53939	Field parental	76.8	29.1	48.2	27.3	16.0	4.9	11.9	1.9	1.2	15.5	0.1	16.1	11.2	14.8	6.3
A. woodhousii	UTAA53940	Field parental	33.1	12.8	21.0	15.1	7.5	1.8	6.8	1.2	0.8	8.3	0.1	7.4	4.6	7.3	4.3
A. woodhousii	UTAA54480	Field parental	88.4	32.7	58.6	34.6	17.7	5.6	14.6	1.2	1.4	18.5	0.1	17.7	11.5	18.3	7.6
A. woodhousii	UTAA54640	Field parental	70.4	27.9	45.4	30.7	16.4	4.6	12.5	1.4	1.4	16.3	0.1	12.2	8.2	13.1	6.3
A. woodhousii	UTAA8327	Field parental	92.9	36.5	55.0	35.7	19.4	6.2	13.6	3.8	4.3	18.4	0.1	17.2	11.7	15.5	7.8
A. woodhousii	UTAA8328	Field parental	84.4	33.0	48.2	31.5	19.2	5.9	13.1	4.5	3.6	15.4	0.1	13.8	9.7	18.4	8.4
A. woodhousii	UTAA8338	Field parental	83.8	29.3	48.7	31.9	16.8	5.2	13.3	4.9	3.5	17.5	0.1	15.5	10.2	16.5	6.5
h x a	TNHC1123	Blair hybrid	55.9	18.9	31.5	22.7	15.8	4.1	8.0	4.5	2.3	11.9	1.1	8.6	7.5	9.9	5.1
h x t	TNHC1134	Blair hybrid	57.3	19.2	31.4	23.4	15.6	3.2	8.1	4.6	2.0	12.1	0.8	7.3	7.6	10.8	7.1
h x t	TNHC1137	Blair hybrid	49.0	16.7	27.8	20.0	11.4	3.1	6.7	3.9	1.9	10.8	0.8	6.8	6.7	7.8	4.9
h x t	TNHC1138	Blair hybrid	52.4	17.3	27.2	19.6	15.6	3.2	6.7	4.1	2.5	11.3	1.4	6.9	7.1	8.4	5.1
h x t	TNHC1139	Blair hybrid	48.5	18.3	27.9	18.4	15.6	3.2	6.6	3.7	1.5	10.7	1.1	6.8	6.6	10.2	5.1
h x t	TNHC1140	Blair hybrid	49.6	17.5	28.1	20.0	16.2	2.7	7.0	4.5	2.3	10.9	1.5	6.8	6.8	9.8	5.9
h x t	TNHC1144	Blair hybrid	45.8	16.8	27.9	19.7	16.1	3.3	7.3	4.8	2.3	10.3	1.1	7.3	6.3	9.9	5.4
h x t	TNHC1297	Blair hybrid	56.8	22.0	34.1	22.7	18.2	4.0	8.2	4.2	2.2	12.0	1.0	7.9	7.6	10.6	6.8
hem x h	TNHC1372	Blair hybrid	44.9	16.4	27.8	18.4	13.8	2.5	6.8	3.3	2.3	10.2	1.0	8.9	6.2	7.8	5.6
hem x h	TNHC1373	Blair hybrid	42.2	15.7	26.0	17.7	14.0	2.1	5.8	2.6	2.2	9.8	0.9	6.2	4.7	7.9	4.6
hem x wood	TNHC555	Blair hybrid	48.0	15.4	26.6	18.7	13.7	2.4	6.9	3.0	2.3	9.2	0.6	6.5	4.6	9.1	5.0
hem x wood	TNHC556	Blair hybrid	35.7	11.9	21.0	13.6	10.3	2.2	5.3	3.2	2.1	6.7	0.5	5.7	4.9	4.6	3.6
hem x wood	TNHC557	Blair hybrid	41.0	16.4	25.4	17.5	13.0	1.9	6.2	4.1	2.9	8.7	1.3	5.6	5.1	7.8	4.1
hem x wood	TNHC956A	Blair hybrid	58.6	19.8	34.5	21.5	12.8	3.9	7.5	4.5	2.6	10.3	0.7	7.3	6.4	9.5	5.5
hem x wood	TNHC956B	Blair hybrid	53.6	20.2	32.7	20.2	13.9	3.9	7.8	4.3	3.4	10.4	0.6	9.1	7.4	10.5	5.6
t x (t x w)	TNHC302	Blair hybrid	54.3	21.3	33.2	22.9	17.6	3.2	7.9	4.2	2.6	11.6	1.1	8.2	7.3	11.6	6.7
t x (t x w)	TNHC303	Blair hybrid	53.8	20.4	32.5	22.5	16.0	2.2	7.4	5.4	2.4	11.6	1.0	8.3	7.2	11.9	5.8

t x (t x w)	TNHC304	Blair hybrid	48.9	18.3	29.6	20.9	15.0	3.0	7.5	4.6	2.6	10.8	0.1	7.1	6.7	9.7	6.0
t x (t x w)	TNHC305	Blair hybrid	65.7	22.9	27.7	27.5	18.7	3.5	8.4	5.1	3.4	13.4	1.9	10.0	8.6	12.5	8.0
t x (t x w)	TNHC306	Blair hybrid	65.5	24.5	38.3	29.5	16.8	3.7	8.8	4.9	3.5	13.4	1.2	10.0	8.6	14.0	8.1
t x (t x w)	TNHC89	Blair hybrid	65.0	23.6	33.4	28.6	17.9	3.8	9.3	6.1	3.5	13.3	0.1	8.7	8.5	14.5	8.9
t x (t x w)	TNHC90	Blair hybrid	53.1	22.1	27.7	23.1	16.7	4.0	7.9	4.6	2.3	11.5	1.1	8.2	7.2	12.5	7.1
t x h	TNHC23348	Blair hybrid	55.6	21.5	34.4	23.8	18.5	4.1	8.7	5.0	3.3	11.8	2.5	8.1	7.4	10.7	5.2
t x h	TNHC57	Blair hybrid	41.2	15.7	19.8	17.5	12.9	2.7	6.1	3.6	2.1	9.6	1.3	6.8	5.8	8.9	4.6
t x hem	TNHC37	Blair hybrid	49.2	17.7	24.8	19.3	16.2	3.0	7.5	4.2	3.3	9.8	1.3	8.0	6.2	8.9	3.3
t x hem	TNHC38	Blair hybrid	44.5	18.2	23.3	18.1	15.3	2.7	7.0	4.2	2.9	10.0	0.7	7.8	5.9	7.2	4.2
t x w	TNHC28328	Blair hybrid	66.6	26.1	42.2	28.1	20.1	4.8	9.8	4.0	2.9	13.5	1.5	8.7	8.7	12.9	6.7
t x w	TNHC28329	Blair hybrid	74.9	27.5	39.8	29.5	18.3	4.0	10.3	5.8	3.3	14.8	2.0	10.5	9.6	14.3	7.1
t x w	TNHC28330	Blair hybrid	60.1	24.4	38.8	26.4	17.3	4.6	9.8	4.4	2.8	12.5	1.7	10.1	7.9	11.9	7.6
t x w	TNHC28331	Blair hybrid	56.0	23.8	35.9	24.5	15.4	4.5	9.5	3.8	3.0	11.9	1.7	8.0	7.5	11.5	6.0
t x w	TNHC28332	Blair hybrid	75.3	25.8	42.2	29.9	21.5	4.7	10.6	5.8	3.4	14.9	1.4	10.4	9.7	14.5	7.2
t x w	TNHC28333	Blair hybrid	66.8	23.3	36.9	26.0	14.3	4.0	9.4	5.8	2.9	13.6	0.9	9.1	8.7	13.6	6.0
t x w	TNHC28334	Blair hybrid	66.0	26.5	41.8	26.5	20.6	4.1	10.2	4.7	3.4	13.4	2.1	10.6	8.6	14.2	6.6
t x w	TNHC28335	Blair hybrid	65.4	25.2	39.6	26.5	15.8	4.8	9.2	4.7	3.2	13.4	1.7	9.0	8.5	12.9	7.4
t x w	TNHC28336	Blair hybrid	66.7	25.3	37.1	27.5	18.5	4.5	9.6	4.7	3.6	13.6	1.9	9.8	8.7	12.5	6.9
t x w	TNHC28337	Blair hybrid	62.7	24.7	39.0	27.8	15.5	3.8	9.4	4.2	2.8	12.9	0.8	9.3	8.2	13.2	6.6
t x w	TNHC28338	Blair hybrid	73.8	26.6	43.1	29.3	17.4	4.4	9.6	5.5	3.1	14.7	1.5	10.4	9.5	15.2	9.1
t x w	TNHC28339	Blair hybrid	56.7	23.4	38.3	24.7	16.3	3.6	8.5	4.7	3.1	12.0	1.9	8.7	7.6	11.5	7.1
t x w	TNHC28340	Blair hybrid	63.1	25.3	40.2	27.0	16.1	4.2	8.8	5.0	3.0	13.0	2.2	9.3	8.3	12.0	7.4
t x w	TNHC28341	Blair hybrid	67.0	27.0	41.5	28.5	15.5	4.9	10.0	4.6	2.7	13.6	1.4	10.0	8.7	12.0	8.7
t x w	TNHC28342	Blair hybrid	66.7	25.6	43.2	28.9	21.4	4.9	10.3	4.7	3.9	13.6	2.1	10.4	8.7	13.4	8.1
t x w	TNHC45	Blair hybrid	61.2	25.0	35.8	26.1	17.0	4.2	9.3	4.7	3.3	12.7	1.7	8.6	8.1	12.1	6.1
t x w	TNHC60	Blair hybrid	38.1	19.3	30.5	19.7	14.8	3.1	7.4	4.5	3.3	9.1	2.2	5.4	5.5	7.4	5.2
w x (t x w)	TNHC221	Blair hybrid	35.6	13.9	21.6	16.7	12.9	2.3	6.9	2.9	1.8	8.7	1.0	5.1	5.2	7.4	4.4
w x (t x w)	TNHC222	Blair hybrid	35.1	15.1	21.1	15.1	12.9	2.1	6.8	3.1	1.8	8.7	0.1	5.0	5.1	6.3	3.6
w x (t x w)	TNHC223	Blair hybrid	46.8	16.8	29.4	20.0	16.3	2.5	8.2	3.5	2.0	10.5	0.1	6.4	6.4	9.7	5.1
w x (t x w)	TNHC224	Blair hybrid	43.2	18.6	25.5	20.1	16.0	3.2	7.2	3.7	2.9	9.9	0.1	7.5	6.0	10.4	5.0
w x (t x w)	TNHC225	Blair hybrid	46.3	18.8	27.4	19.6	15.5	3.5	7.7	3.1	2.1	10.4	0.1	6.4	6.4	9.4	4.1

w x (t x w)	TNHC226	Blair hybrid	50.3	19.3	32.1	23.3	18.1	3.2	9.0	4.8	2.5	11.0	0.1	8.2	6.8	10.0	4.6
w x (t x w)	TNHC227	Blair hybrid	50.2	20.2	31.6	21.7	16.7	2.5	8.5	3.3	2.9	11.0	0.1	8.0	6.8	9.0	4.8
w x (t x w)	TNHC228	Blair hybrid	54.4	20.3	32.7	22.8	18.9	3.2	8.7	4.7	2.9	11.7	0.1	8.6	7.3	11.0	5.5
w x a	TNHC534	Blair hybrid	54.5	18.5	30.6	19.5	14.3	3.4	7.0	3.7	2.4	11.7	1.2	7.4	7.3	10.4	5.4
w x a	TNHC535	Blair hybrid	51.9	19.8	31.6	21.3	15.3	3.5	6.6	4.2	2.3	11.3	1.0	7.9	7.0	9.7	5.7
w x a	TNHC536	Blair hybrid	42.6	15.5	25.0	18.4	14.4	3.3	7.2	3.6	2.6	9.8	1.0	5.4	6.0	7.7	4.5
w x h	TNHC1124	Blair hybrid	43.3	17.3	26.6	17.3	15.0	3.1	7.4	2.9	2.5	9.9	0.1	7.6	6.1	9.8	4.5
w x h	TNHC1127	Blair hybrid	64.4	25.7	38.9	26.7	18.8	4.5	9.3	3.8	2.8	13.2	0.1	8.8	8.4	12.2	6.9
w x h	TNHC1128	Blair hybrid	63.0	24.1	38.0	25.5	19.9	3.7	9.0	4.5	2.8	13.0	1.4	9.3	8.3	13.2	5.1
w x h	TNHC1129	Blair hybrid	57.6	20.6	35.2	23.8	16.7	4.3	8.5	3.3	2.4	12.1	0.1	8.9	7.7	12.4	5.5
w x h	TNHC1322	Blair hybrid	48.3	19.3	29.7	20.7	16.2	2.3	7.2	3.9	2.7	10.7	1.4	6.9	6.6	8.9	5.2
w x hem	TNHC10	Blair hybrid	60.9	24.6	39.9	25.7	17.1	4.0	8.4	3.3	3.4	11.6	0.1	11.1	9.4	11.9	5.7
w x hem	TNHC11	Blair hybrid	62.1	24.0	40.5	27.9	18.4	3.8	9.1	3.1	2.4	12.1	0.1	10.8	8.2	11.7	6.4
w x hem	TNHC58	Blair hybrid	72.6	30.0	50.7	30.4	17.6	3.8	10.4	2.9	2.8	14.0	0.1	13.4	10.9	13.9	8.0
w x hem	TNHC7	Blair hybrid	68.7	28.6	47.4	19.6	16.9	4.8	9.2	4.1	3.0	12.5	0.1	14.9	9.9	11.6	7.3
w x t	TNHC103	Blair hybrid	51.7	19.7	31.0	21.6	17.0	3.4	7.2	4.8	3.3	11.2	1.8	6.4	7.0	9.0	3.8
w x t	TNHC104	Blair hybrid	57.5	21.4	35.2	23.3	16.0	3.5	7.9	4.8	2.4	12.1	1.2	8.8	7.7	8.9	4.4
w x t	TNHC105	Blair hybrid	59.3	18.9	32.8	22.1	13.8	4.1	7.8	4.1	2.5	12.4	0.1	7.0	7.9	10.6	5.2
w x t	TNHC106	Blair hybrid	47.8	18.4	30.5	20.6	14.8	3.0	7.4	3.8	2.5	10.6	1.2	6.7	6.6	9.5	4.3
w x t	TNHC107	Blair hybrid	57.7	19.1	31.3	23.6	15.6	3.8	7.9	4.7	3.2	12.2	1.2	7.7	7.7	10.1	5.2
w x t	TNHC820	Blair hybrid	54.8	21.5	33.1	23.5	15.6	4.4	8.5	3.8	2.3	11.7	1.0	8.3	7.3	10.1	6.4

REFERENCES

Arnold, M. L., and J. M. Burke. 2006. Natural hybridization. Pp. 399-413 in C. W. Fox, and J. A. Wolf, eds. *Evolutionary genetics: concepts and case studies*. Oxford Univ. Press, New York.

Arnold, M. L. 1997. *Natural hybridization and evolution*. Oxford University Press, NY.

___________. 2006. Evolution through genetic exchange. Oxford University Press, New York.

Barber, P.H. 1999. Patterns of gene flow and population genetic structure in the canyon treefrog, *Hyla arenicolor*. *Molecular Ecology*. 8, pp. 563–576.

Barluenga, M. *et al.* 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature*. 439, pp. 719–723.

Barrier, M., B.G. Baldwin, R.H. Robichaux, and M.D. Purugganan. 1999. Interspecific hybrid ancestry of a plant adaptive radiation: allopolyploidy of the Hawaiian silversword alliance (Asteraceae) inferred from floral homeotic gene duplications. *Molecular Biology and Evolution*. 16, pp. 1105–1113.

Beltran, M., C.D. Jiggins, V. Bull, M. Linares, J. Mallet, W.O. McMillan, and E. Bermingham. 2002. Phylogenetic discordance at the species boundary: comparative gene genealogies among rapidly radiating *Heliconius* butterflies. *Molecular Biology and Evolution*. 19, pp. 2176–2190.

Bensch, S. and M. Akesson. 2005. Ten years of AFLP in ecology and evolution: why so few animals? *Molecular Ecology.* 14, pp. 2899–2914.

Blair, A. P. 1941. Variation, isolation mechanisms, and hybridization in certain toads. *Genetics*. 26, pp. 398–417.

_________. 1942. Isolating mechanisms in a complex of four species of toads. *Biological Symposia.* 6, pp. 235–249.

Blair, W. F. 1955. Mating call and stage of speciation in the *Microhyla olivacea-M. carolinensis* complex. *Evolution* . 9, 469–480.

_________. 1956. Call difference as an isolation mechanism in southwestern toads (genus

Bufo). *Texas Journal of Science*. 8, 87–106.

_________. 1959. Genetic compatibility and species groups in U. S. toads (*Bufo*). *Texas Journal of Science*. 11, pp. 427–453.

_________. 1961. Further evidence bearing on intergroup and intragroup genetic compatibility in toads (genus *Bufo*). *Texas Journal of Science.* 13, pp. 163–175.

_________. 1963a. Evolutionary relationships of North American toads of the genus *Anaxyrus*:

a progress report. *Evolution*. 17, pp. 1–16.

_________. 1963A. Intragroup genetic compatibility in the *Bufo americanus* species group

of toads. *Texas Journal of Science.* 15, pp. 15–34.

_________.1964. Isolating mechanisms and interspecies interactions in anuran amphibians.

Quarterly Review of Biology. 39, pp. 334–344.

_________. 1966. Genetic compatibility in the *Bufo valliceps* and closely related groups of

toads. *Texas Journal of Science*. 18, pp. 333–351.

_________.1972. Evidence from Hybridization. In *Evolution in the genus* Bufo (W.F. Blair, ed.). pp. 93–101. Univ. of Texas Press, Austin.

Bogart, J.P. 1972. Karyotypes. *In Evolution in the genus* Bufo (W.F. Blair, ed.) pp. 171–195. Univ. of Texas Press, Austin.

Bolnick, D.I. and T. Near. 2005. Tempo of hybrid inviability in centrarchid fishes (Teleostei: Centrarchidae). *Evolution*. 59, pp. 1754–1767.

Bonin, A., D. Ehrich, and S. Manel. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology*. 16, pp. 3737–3758.

Boul, K.E., W.C. Funk, C.R. Darst, D.C. Cannatella, and M.J. Ryan. 2007. Sexual selection drives speciation in an Amazonian frog. *Proc. R. Soc. B.* 274, pp. 399–406.

Bragg, A.N. and Sanders, O. 1951. *Bufo woodhousii velatus*. *Wassman Journal of Biology.* 9, pp. 366–378.

Cocroft, R.B. and M.J. Ryan. 1995. Patterns of advertisement call evolution in toads and chorus frogs. *Animal Behavior*. 49, pp. 283–303.

Conant, R. and J.T. Collins. 1998. *A field guide to reptiles and amphibians of eastern and central North America. 4th ed*. Houghton Mifflin Company, New York.

Coyne, J. A. and Orr, H.A. 1989. Patterns of speciation in Drosophila. *Evolution*. 43(2), pp. 362–381.

______________________. 1997. "Patterns of speciation in *Drosophila*" revisited. *Evolution*. 51, pp. 295–303.

______________________. 2004. *Speciation*. Sinauer, Sunderland, Massachusetts.

Demuth, J.P. and M.J. Wade. 2007. Population differentiation in the beetle *Tribolium castaneum*. I. Genetic architecture. *Evolution*. 61(3), pp. 494–509.

Dixon, J.R. 2000. *Amphibians and Reptiles of Texas. 2nd ed*. Texas A&M University Press, College Station, TX.

Duellman, W.E. 1970. The hylid frogs of Middle America. *University of Kansas Museum of Natural History Monographs*. 1, pp. 1–753.

Erickson, D.L. and C. Fenster. 2006. Intraspecific hybridization and the recovery of fitness in the native legume *Chamaecrista fasciculate*. *Evolution*. 60(2), pp. 225–233.

Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist*. 125, pp. 1–15.

Fitzpatrick, A.M. and M. Turelli. 2006. The geography of mammalian speciation: mixed signals from phylogenies and range maps. *Evolution*. 60, pp. 601–615.

Fontenot, A.E. 2003. Molecular and morphological analysis of the taxonomic status of the East Texas Toad, *Bufo velatus*. University of Texas at Tyler. Unpublished thesis.

Freeland, J.R. and P.T. Boag. 1999. The mitochondrial and nuclear genetic homogeneity of the phenotypically diverse Darwin's ground finches. *Evolution*. 53, 1553–1563.

Frost, D.R., T. Grant, J. Faivovich, R.H. Bain, A. Haas, C. F.A. Haddad, R.O. de Sa, A. Channing, M. Wilkinson, S.C. Donnellan, C.J. Raxworthy, J.A. Campbell, A.L. Blotto, P. Moler, R.C. Drewes, R.A. Nussbaum, J.D. Lynch, D.M. Green, and W.C. Wheeler. 2006. The Amphibian tree of life. *Bulletin of the American Museum of Natural History*. No. 297, pp. 1–370.

Gatz, A.J. 1981. Non-random mating by size in American toads, *Bufo americanus*. *Animal Behavior*. 29, pp. 1004–1012.

Gilbert, L.E. 2003. Adaptive novelty through introgression in *Heliconius* wing patterns: evidence for shared genetic "tool box" from synthetic hybrid zones and a theory of diversification. In *Ecology and Evolution Taking Flight: Butterflies as Model Systems* (Boggs, C.L. et al., eds.), pp. 281–318. University of Chicago Press, Chicago, IL, USA.

Goldberg, S.R., C.R. Bursey, K.B. Malmos, B.K. Sullivan, and H. Cheam. 1996. Helminths of the southwestern toad *Bufo microcscaphus*, woodhouse's toad *Bufo woodhousii* (Bufonidae), and their hybrids from central Arizona. *Great Basin Naturalist*. 56(4), pp. 369–374.

Grant, B.R. and P.R. Grant. 1996. High survival of Darwin's finch hybrids: effects of beak morphology and diets. *Ecology*. 77, pp. 500–509.

Graybeal A. 1997. Phylogenetic relationships of Bufonid frogs and tests of alternate macroevolutionary hypotheses characterizing their radiation. *Zoological Journal of the Linnaean Society.* 119, pp. 297–338.

Green, D.M. 1984. Sympatric hybridization and allozyme variation in the toads *Bufo americanus* and *B. fowleri* in southern Ontario. *Copeia*. 1984(1), pp. 18–26.

Green, D.M. and Parent, C. 2003. Variable and asymmetric introgression in a hybrid zone in the toads, *Bufo americanus* and *Bufo fowleri. Copeia.* 1, pp. 34–43.

Green, D.M. and C. Pustowka. 1997. Correlated morphological and allozyme variation in the hybridizing toads *Bufo americanus* and *Bufo hemiophrys*. *Herpetologica*. 53(2), pp. 218–228.

Haldane, J.A.S. 1922. Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics.* 12, pp. 101–109.

Haldane, J.A.S. 1930. A mathematical theory of natural and artificial selection. Part IV. Isolation. *Proceedings of the Cambridge Philosophical Society*. 26, pp. 220–230.

Hayek, L.C., W.R. Heyer, and C. Gascon. 2001. Frog morphometrics: a cautionary tale. *Alytes*. 18(3–4), pp. 153–177.

Hillis, D. M. and D. M. Green. 1990. Evolutionary changes of heterogametic sex and sex-determination systems in the phylogenetic history of amphibians. *Journal of Evolutionary Biology*. 3, pp. 49–64.

Holsinger, K.E., P.O. Lewis, and D.K. Dey. 2002. A Bayesian approach to inferring population structure from dominant markers. *Molecular Ecology*. 11, pp. 1157–1164.

Howard, R.D. 1988. Sexual selection on male body size and mating behavior in American toads. *Animal Behavior*. 36, pp. 1796–1808.

Jackson, J.A. and R.C. Tinsley. 2003. Parasite infectivity to hybridising host species: a link between hybrid resistance and allopolyploid speciation? *International Journal of Parasitology*. 33, 137–144.

Johnson, N.A. 2006. The evolution of reproductive isolating barriers. pp. 374–386 In *Evolutionary Genetics: Concepts and Case Studies*. (C.W. Fox and J.A. Wolf eds.). Oxford University Press, New York, New York.

Jones, J.M. 1973. Effects of thirty years hybridization on the toads *Bufo americanus* and *Bufo woodhousii fowleri* at Bloomington, Indiana. *Evolution.* 27, pp. 435–448.

Koopman, W.J.M. 2005. Phylogenetic signal in AFLP data sets. *Systematic Biology.* 54, pp. 197–217.

Krauss, S.L. 2000. Accurate gene diversity estimates from amplified fragment length polymorphism (AFLP) markers. *Molecular Ecology*. 9, pp. 1241–1245.

Lampert, K.P., A.S. Rand, U.G. Mueller, and M.J. Ryan. 2003. Fine-scale genetic pattern and evidence for sex-biased dispersal in the tungara frog, *Physalaemus pustulosus*. *Molecular Ecology*. 12(12), pp. 3325–3334.

Leary, C.J. 2001. Evidence of convergent character displacement in release vocalizations of *Bufo fowleri* and *Bufo terrestris* (Anura: Bufonidae). *Animal Behavior*. 61, pp. 431–438.

Lee, J.C. 1982. Accuracy and precision in anuran morphometrics: artifacts of preservation. *Systematic Zoology*. 31, pp. 266–281.

Licht, L.E. 1976. Sexual selection in toads (*Bufo americanus*). *Canadian Journal of Zoology*. 54, pp. 1277–1284.

Lijtmaer, D.A., A. Mahler, and P.L. Tubario. 2003. Hybridization and postzygotic isolation patterns in pigeons and doves. *Evolution.* 57, pp. 1411–1418.

Lotti, C., S. Salvi, A. Pasqualone, R. Tuberosa, A. Blanco. 2000. Integration of AFLP markers into an RFLP-based map of durum wheat. *Plant Breeding*. 119(5), pp. 393–401.

Malone, J.H. and B.E. Fontenot (equivalent authors). 2008. Patterns of reproductive isolation in toads. *PLoS ONE*. 3(12): e3900.doi:10.1371/journal.pone.0003900.

Malone, J.H. and P. Michalak. 2008. Physiological sex predicts hybrid sterility regardless of genotype. *Science*. 319, p. 59.

Makowsky, R., J. Chesser, and L.J. Rissler. 2009. A striking lack of genetic diversity across the wide-ranging amphibian *Gastrophryne carolinensis* (Anura: Microhylidae). *Genetica*. 135, pp. 169–183.

Masta, S.E., A.K. Sullivan, T. Lamb, and E.J. Routman. 2002. Molecular systematics, hybridization, and phylogeography of the *Bufo americanus* complex in Eastern North America. *Molecular Phylogenetics and Evolution.* 24(2), pp. 302–314.

Masta, S.E., N.M. Laurent, and E.J. Routman. 2003. Population genetic structure of the toad *Bufo woodhousii*: an empirical assessment of the effects of haplotype extinction on nested cladistic analysis. *Molecular Ecology*. 12, pp. 1541–1554.

McDermott, J.M. and B.A. McDonald. 1993. Gene flow in plant pathosystems. *Annual Review of Phytopathology*. 31, pp. 353–373.

Mendelson, III, J.R. 1994. A new species of toad (Anura: Bufonidae) from the lowlands of eastern Guatemala. *Occasional Paper of the Museum of Natural History.* 166, pp. 1–21.

________________. 1997a. A new species of toad (Anura: Bufonidae) from the Pacific highlands of Guatemala and southern Mexico, with comments on the status of *Anaxyrus valliceps macrocristatus*. *Herpetologica*. 53(1), pp. 14–30.

________________. 1997A. A new species of toad (Anura: Bufonidae) from Oaxaca, Mexico with comments on the status of *Bufo cavifrons* and *Bufo cristatus*. *Herpetologica*. 53(2), pp. 268–285.

Meudt, H.M. and A.C. Clarke. 2006. Almost forgotten or latest practice? AFLP applications, analyses and advances. *TRENDS in Plant Science.* 12(3), pp. 106–117.

Miller, M.P. 1997. Tools for Population Genetic Analyses (TFPGA): a Windows® program for analysis of allozyme and molecular population genetic data. Version 1.3 Free program distributed by the author over the Internet from the website http://herb.bio.nau.edu/miller

Moyle, L.C., M.S. Olson, and P. Tiffin. 2004. Patterns of reproductive isolation in three angiosperm genera. *Evolution*. 58, pp. 1195–1208.

Nishioka, M., and H. Hanada. 1994. Sex of reciprocal hybrids between the Hamakita (XX-XY type) population and the Murakami (ZW-ZZ type) population of *Rana* *rugosa*. Sci. Rep. LaA. Amphibian Biol. Hiroshima Univ. 13, pp. 35-50.

Nei, M. 1972. Genetic distance between populations. *American Naturalist*. 106, pp. 283–292.

Ogata, M., H. Ohtani, T. Igarashi, Y. Hasegawa, Y. Ichikawa, and I. Miura. 2003. Change of the heterogametic sex from male to female in the frog. *Genetics*. 164, pp. 613-620.

Oliver, M., J. Garcia-Mas, M. Cardus, N. Pueyo, A. Lopez-Sese, M. Arroyo, H. Gomez-Paniaua, P. Arus, M.C. de Vicente. 2001. Construction of a reference linkage map for melon. *Genome*. 44(5), pp. 836–845.

Pauly, G.A., D.M. Hillis, and D.C. Cannatella. 2004. The history of a nearctic colonization: molecular phylogenetics and biogeography of the nearctic toads (*Bufo*). *Evolution.* 58(11), pp. 2517–2535.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. 6, pp. 288–295.

Pelser, P.A. *et al.* 2003. Phylogeny reconstruction in the gap between too little and too much divergence: the closest relatives of *Senecio jacobaea* (Asteraceae) according to DNA sequences and AFLPs. *Molecular Phylogenetics and Evolution*. 29, pp. 613–628.

Petit, R.J. and L. Excoffier. 2009. Gene flow and species delimitation. *Trends in Ecology and Evolution*. 24(7), pp. 386–393.

Pfennig, K.S. and M.A. Simovich. 2002. Differential selection to avoid hybridization in two toad species. *Evolution*. 56, pp. 1840–1848.

Phillips, C.A. 1994. Geographic variation of mitochondrial DNA variants and the historical biogeography of the spotted salamander, *Ambystoma maculatum*. *Evolution*. 48, pp. 567–707.

Poompuang, S., and U. Na-Nakorn. 2004. A preliminary genetic map of walking catfish (Clarias macrocephalus). *Aquaculture*. 232(1-4), pp. 195–203.

Ponse, K. 1941. La proportion sexuelle dans la descendanceissue des oeufs produits par l'rgane de Bidder des crapauds femelles. *Rev. Suisse. Zool*. 48, pp. 541–544.

Pramuk, J. A., T. Robertson, J.W. Sites Jr., and A.P. Noonan. 2008. Around the world in 10 million years: biogeography of the nearly cosmopolitan true toads (Anura: Bufonidae). *Global Ecology and Biogeography.* 17(1), pp. 72–83.

Presgraves, D.C. 2002. Patterns of postzygotic isolation in Lepidoptera. *Evolution*. 56(6), pp. 1168–1183.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155, pp. 945–959.

Rieseberg, L.H., M.A. Archer, and R.K. Wayne. 1999a. Transgressive segregation, adaptation, and speciation. *Heredity*. 83, pp. 363–372.

Rieseberg, L.H., M.J. Kim, G.J. Seiler. 1999b. Introgression between the cultivated sunflower and a sympatric wild relative, Helianthus petiolaris (Asteraceae). *International Journal of Plant Sciences*. 160(1), pp. 102–108.

Rieseberg, L.H., A. Widmer, A.M. Arntz, and J.M. Burke. 2003a. The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. *Philosophical Transactions of the Royal Society of London*. 358, pp. 1141–1147.

Rieseberg, L.H., O. Raymond, D.M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J.L. Durphy, A.E. Schwarzbach, L.A. Donovan, and C. Lexer. 2003b. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science*. 301, pp. 1211–1216.

Roussot, O., K. Feve, F. Plisson-Petit, F. Pitel, J.M. Faure, C. Beaumont, A. Vignal. 2003. AFLP linkage map of the Japanese quail Coturnix japonica. *Genetics Selection Evolution* 35(5), pp. 559–572.

Rowe, G. T.J.C. Beebe, and T. Burke. 1998. Phylogeography of the natterjack toad *Bufo calamita* in Britain: genetic differentiation of native and translocated populations. *Molecular Ecology*. 7, pp. 751–760.

Rowe, G. T.J.C. Beebe, and T. Burke. 2000. A microsatellite analysis of natterjack toad, *Bufo calamita*, metapopulations. *Oikos*. 88, pp. 641–651.

Ryan, M.J. J.H. Fox, W. Wilczynski, and A.S. Rand. 1990. Sexual selection for sensory exploitation in the frog *Physalaeumus pustulosus*. *Nature*. 343, pp. 66–67.

Ruegg, K. 2008. Genetic, morphological, and ecological characterization of a hybrid zone that spans a migratory divide. *Evolution*. 62(2), pp. 452–466.

Sasa, M.M., P.T. Chippindale, and N.A. Johnson. 1998. Patterns of postzygotic isolation in frogs. *Evolution*. 52(6), pp. 1811–1820.

Sanders, O. 1986. The heritage of *Bufo woodhousei* Girard in Texas (Salientia: Bufonidae). *Occasional Papers of the Strecker Museum*. Baylor University. 1, 1–28.

Sawamura, K. 1996. Maternal effect as a cause of exceptions for Haldane's Rule. *Genetics*. 143, pp. 609–611.

Schaffer, H.B., G.M. Fellers, A. Magee, and S.R. Voss. 2000. The genetics of amphibian declines: population substructure and molecular differentiation in the Yosemite toad, *Bufo canorus* (Anura, Bufonidae) based on single-strand conformation polymorphism analysis (SSCP) and mitochondrial DNA sequence data. *Molecular Ecology*. 9, pp. 245–257.

Seehausen, O., J.J.M. van Alphen, and F. Witte. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*. 277, pp. 1808–1811.

Seehausen, O. 2004. Hybridization and adaptive radiation. *Trends in Ecology and Evolution*. 19(4), pp. 198–207.

Shaw, K.L. 1996. Sequential radiations and patterns of speciation in the Hawaiian cricket genus *Laupala* inferred from DNA sequences. *Evolution*. 50, pp. 237–255.

Shaw, K.L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proceedings of the National Academy of Science*. 99, 16122–16127.

Smith, M.A. and D.M. Green. 2006. Sex, isolation and fidelity: unbiased long-distance in a terrestrial amphibian. *Ecography*. 29, pp. 649–658.

Stelkens, R. and O. Seehausen. 2009. Genetic distance between species predicts novel trait expression in their hybrids. *Evolution*. 63(4), pp. 884–897.

Sullivan, B.K. 1983a. Sexual selection in the Great Plains toad (*Bufo cognatus*). *Behavior*. 84(314), pp. 258–264.

Sullivan, B.K. 1983b. Sexual selection in Woodhouse's toad (*Bufo woodhousei*) II: Female choice. *Animal Behavior*. 31(4), pp. 1011–1017.

Sullivan, B.K. 1992. Sexual selection and calling behavior in the American toad (*Bufo americanus*). *Copeia*. 1992(1), pp. 1–7.

Sullivan, B.K., K.B. Malmos, and M.F. Given. 1996. Systematics of the *Bufo woodhousii* complex (Anura: Bufonidae): advertisement call variation. *Copeia*. 2, pp. 174–280.

Tan, Y.D., C.L. Wan, Y.F. Zhu, C. Lu, Z.H. Xiang, H.W. Deng. 2001. An amplified fragment length polymorphism map of the silkworm. *Genetics* 157(3), pp. 1277–1284.

Thornton, W.A. 1955. Morphological and mating call comparisons in the *Bufo valliceps* complex. *American Midland Naturalist*. 71, pp. 232–245.

Turelli, M. and L.C. Moyle. 2007. Asymmetric postmating isolation: Darwin's corollary to Haldane's rule. *Genetics*. 176, pp. 1059–1088.

Turelli, M. and H.A. Orr. 1995. The dominance theory of Haldane's rule. *Genetics.* 140, pp. 3809–402.

Turelli, M. and H.A. Orr. 2000. Dominance, epistasis, and the genetics of postzygotic isolation. *Genetics*. 154, pp. 1663–1679.

Vekemans X., T. Beauwens, M. Lemaire, I. Roldan-Ruiz. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology*. 11, pp. 139–151.

Vogel L. S. and S.G. Johnson. 2008. Estimation of hybridization and introgression frequency in toads (genus: *Bufo*) using DNA sequence variation at mitochondrial and nuclear loci. *Journal of Herpetology*. 42(1), pp. 61–75.

Volpe, E. P. 1952. Physiological evidence for natural hybridization of *Bufo americanus* and *Bufo fowleri. Evolution*. 6(4), pp. 393–406.

_________. 1959. Experimental and natural hybridization between *Bufo terrestris* and *Bufo fowleri*. *American Midland Naturalist*. 61(2), pp. 295–312.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Vandelee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP- a new technique for DNA-Fingerprinting. *Nucleic Acids Research*. 23(21), pp. 4407–4414.

Waldman, B. 2001. Kin recognition, sexual selection, and mate choice in toads. In: *Anuran Communication*. M.J. Ryan, editor. Smithsonian Institution.

Woodruff, D.S. 1973. Natural hybridization and hybrid zones. *Systematic Zoology*. 22, pp. 213–218.

Wu, C.I. and A.W. Davis. 1993. Evolution of postmating reproductive isolation: the composite nature of Haldane’s Rule and its genetic basis. *American Naturalist*. 142, pp. 187–212.

Yeh, F.C. and T.J.B. Boyle. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian Journal of Botany*. 129, p. 157.

Zhivotovsky, L.A. 1999. Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology*. 8, pp. 907–913.

Zhong, D., A. Pai, G.Y. Yan. 2003. Quantitative trait loci for susceptibility to tapeworm infection in the red flour beetle. *Genetics* 165(3), pp. 1307–1315.

_______________________. 2004. AFLP-based genetic linkage map for the red flour beetle (Tribolium castaneum). *Journal of Heredity* 95(1), pp. 53–61.

BIOGRAPHICAL INFORMATION

Brian E. Fontenot was born in Nederland, Texas to John and Linda Fontenot. He received his Bachelor's degree in Wildlife and Fisheries Sciences from Texas A&M University in 2000, his Master's degree in Biology from the University of Texas at Tyler in 2003, and his Doctoral degree from the University of Texas at Arlington in 2009. He has worked on a variety of projects related to the conservation, ecology, systematics, biogeography, and speciation of various reptiles and amphibians. He has authored or co-authored four peer-reviewed scientific articles, six non peer-reviewed articles, and served as a manuscript reviewer for two scientific journals. He received the Outstanding Graduate Research Award from the Department of Biology at the University of Texas at Arlington in 2009. His current research is focused on elucidating the effects of natural hybridization on speciation events in the family Bufonidae. His future plans include pursuing a career in biology that will allow him to continue his development as a teacher and contribute to amphibian speciation research.