DETERMINING THE PHYLOGEOGRAPHIC DYNAMICS OF THE EDGE RELATIONSHIPS BETWEEN *APHONOPELMA HENTZI* (GIRARD) AND ITS NEIGHBORS ALONG THE COLORADO RIVER BASIN (ARANEAE, MYGALOMORPHAE, THERAPHOSIDAE)

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ABSTRACT

DETERMINING THE PHYLOGEOGRAPHIC DYNAMICS OF THE EDGE RELATIONSHIPS BETWEEN *APHONOPELMA HENTZI* (GIRARD) AND ITS NEIGHBORS ALONG THE COLORADO RIVER BASIN (ARANEAE, MYGALOMORPHAE, THERAPHOSIDAE)

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Theraphosids, commonly known as “tarantulas”, are a group of large, hirsute spiders that have drawn relatively little interest in ecological, behavioral, or evolutionary research from biologists. The lack of research on theraphosids is problematic because of conservation concerns for this group and the need to compile good life history data, and determine taxonomic and phylogenetic relationships. I examined the phylogenetic relationships among populations of tarantulas of the genus *Aphonopelma* along the Colorado River basin in Texas to construct a framework within which to test ecological, evolutionary, and biogeographical hypotheses in an area of species transition and possible Pleistocene refugia.

In order to examine relationships among populations of the *A. hentzi* tarantulas and populations of neighboring species 890 bp were sequenced from two mitochondrial genes, *16S* and *ND1*, and their corresponding *tRNA* from 135 individuals. Ecological niche modeling was used to determine potential species distributions, both current and 21,000 ybp during the last glacial maximum of the Pleistocene.

Phylogenetic analysis suggests that there may be as many as eight species in the study area, seven south of the Colorado River and one (*A. hentzi*) to the north. The species found in the study include *A. hentzi*, *A. anax*, *A. armada*, *A. moderatum* and four potentially undescribed species (based on genetic distances and phylogenetic support). Population expansion analysis shows that the northern clade of *A. hentzi* (the Colorado River basin and north) split from the southern clade between 20,000-13,000 ybp, agreeing with the biogeographical hypothesis that the Colorado River basin was an area of Pleistocene refugia.

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INTRODUCTION

1.1 *Aphonopelma*

Theraphosids, commonly known as “tarantulas”, are a group of large, hirsute spiders well known to the public due to fear and curiosity yet they have drawn very little attention from biologists. Only three major efforts (Chamberlin & Ivie 1939; Chamberlin 1940; and Smith 1994) have been made to learn about the taxonomy, morphology, and relationships of *Aphonopelma*, the native tarantulas of the United States. The lack of research on theraphosids is problematic because of conservation concerns for this group and the need to compile good life history data, species distributions, and determine taxonomic and phylogenetic relationships.

The family Theraphosidae includes all species of tarantula and belongs to the infraorder Mygalomorphae, a lineage of spiders with a highly conserved morphology that is considered ancestral in the spider order Araneae. Morphology-based phylogenies of mygalomorph relationships have been shown to be problematic due to convergence and high levels of homoplasy (similar structures in different lineages, not inherited from a common ancestor) in the group, with many taxa retaining plesiomorphic (primitive character state) characters (Bond and Opell 2002; Ayoub *et al* 2007; Turner 2009). Until recently the few published phylogenies of mygalomorph relationships have been based on morphological data resulting in areas of conflict and congruence. Hedin and Bond (2006) show how molecular data can help resolve these problems of morphological homogeneity.

These phylogenetic conflicts are partly due to the age of the lineage of spiders, Devonian origin, with many extant families having fossil representatives found in the early to mid Cretaceous (Selden *et al* 1991; Penney *et al* 2003). Based upon fossil data, the mygalomorphs diverged from the rest of the spiders around 240 million years ago (mya) during

the middle Triassic (Selden and Gall 1992). Based on the rate of evolution of the nuclear gene elongation factor-1 gamma (*EF-1γ*), it is hypothesized that the mygalomorph lineages went through a rapid radiation during the Jurassic (Ayoub *et al* 2007). After being present at low diversity since the early Mesozoic, theraphosids replaced atypoid mygalomorphs as the dominant mygalomorph in the fossil record during the Palaeogene (65-23 mya) (Selden and Gall 1992). They are an ancient lineage having Gondwanan distribution (Turner 2009). In the New World, theraphosids have been present in southern Mexico during the Miocene, based on a specimen preserved in Chiapas amber, dating to around 16 mya (Dunlop *et al* 2007).

Aphonopelma is the only current genus of tarantula in North America, though some researchers believe there should be a resurrection of at least one or more genera for those species that range throughout the southwestern United States and Mexico (Prentice 2005; Hendrixson *pers comm*.; Longhorn *unpublished data*). The validity of the genus, as a North American group, is now being called into question based on molecular data (Turner 2009; Hendrixson *pers comm*.; Longhorn *unpublished data*). For now, the genus ranges throughout the southern third of the United States, west of the Mississippi river to California and down into Mexico and Central America (Prentice 2005). The genus holotype *A. seemanni* (Cambridge) is from Costa Rica. *Aphonopelma* is the sister taxa of the "red-legged" *Brachypelma* in Mexico (Turner 2009; Longhorn *unpublished data*). These groups of tarantulas are of Neotropical/Neartic origin (the boundary runs from the Rio Grande Valley around the central highlands of Mexico, including the Central Plateau and the Sierra Madre Oriental and Occidental, to the central Sinaloan Coast of Mexico.

The genus *Aphonopelma* was created by Reginald Pocock in 1901, but other generic names were used before *Aphonopelma* was recognized (Raven 1990; Smith 1994). Multiple genera have now been synonymized into *Aphonopelma*. They include *Apachepelma* Smith, *Chaunopelma* Chamberlin, *Clavopelma* Chamberlin, *Delopelma* Petrunkevitch, *Dugesiella* Pocock, *Gosipelma* Chamberlin, and *Rhechostica* Simon (Platnick 2008). The International

Commission on Zoological Nomenclature (ICZN) currently recognizes the name *Aphonopelma* to take precedence over all the other previous genera names (Opinion 1637, 1991). At present there are 91 described species of *Aphonopelma*, with 54 present in the United States (Platnick 2009).

Many current and past arachnologists are or were unsatisfied with the state of theraphosid taxonomy (Baerg 1958; Smith 1994; Pérez-Miles *et al* 1996; Prentice 1997 & 2005; Bond and Hedin 2006; Hendrixson *pers. comm.*; Longhorn *unpublished data*). Past descriptions have been based on a very small number of specimens and were frequently vague and very brief. During the ICZN deliberations, Raven (1990) claimed that Theraphosidae was a "nomenclatural and taxonomic nightmare", in part because it had been ignored for so long, because of the conserved nature of morphological features, as well as the fact that poor morphological characters have been chosen for diagnostic use in the past. Baerg (1958) also became so frustrated with *Aphonopelma* taxonomy that he stopped taxonomic research all together and switched to the study of the venom properties of arachnids. As a result, consideration of intraspecific variation within and among populations has been limited. Prentice (1997) shows that several of the characters used in the past for species-level distinctions have little value for morphological distinction due to considerable intraspecific variation.

Researchers have not taken into account the great variation that is possible within the populations of *Aphonopelma* studied, or even between males and females. Therefore, virtually nothing is known about the range of intraspecific morphological variation, how widely overlapping the characters are interspecifically, and how subjective the characters are that have been chosen in many *Aphonopelma* species. Normally only adult specimens can be used to distinguish between taxa. Males and females are sexually dimorphic as adults, but before this they can be very similar in appearance. Mygalomorph species are often determined by mature male palpal bulb morphology. Because of both phenotypic plasticity and genetic variability in the characters used for species recognition, this method can lead to incorrect identifications as

well as overlooking morphologically cryptic taxa (Hebert *et al* 2003). Due in part to this lack of good morphological characters for phylogenetic studies, DNA is essential as a way to investigate relationships and determine which morphological characters can be used in the future (Bond and Hedin 2006).

The first tarantula described from the United States was *A. hentzi* (Girard) in 1854. The type specimen is believed to have been collected in southwestern Oklahoma. The exact locality is unknown, but based on the writings of Girard (1854) during Randolph B. Marcy's exploration of the Red River, the type was collected somewhere in Cotton County, Oklahoma (Warriner 2008). The type material is now believed to be lost, although a male neotype and female paratype have been designated by Smith (1994) from specimens collected from Garfield County, Oklahoma (Warriner 2008). Theraphosid researchers Chamberlin and Ivie (1939), Chamberlin (1940) and Smith (1994) have described more US *Aphonopelma* than any other researchers, including all the tarantulas included in the *A. hentzi* complex (besides *A. hentzi* itself) though most were from small numbers of specimens. Smith (1994) coined the term "*Aphonopelma hentzi* complex" and included these species: *A. arnoldi* Smith, *A. baergi* Chamberlin, *A. clarki* Smith, *A. coloradanum* Chamberlin, *A. echinum* Smith, *A. gurleyi* Smith, *A. hentzi* (Girard), *A. hollyi* Smith, *A. odelli* Smith, *A. waconum* Chamberlin, and *A. wichitanum* Chamberlin. *A. hentzi*, is believed to have dispersed northwards around 4,000-8,000 years ago, based on paleoclimate data, following a desert-like ecosystem expansion (Janowski-Bell 2001). The *A. hentzi* range extends from southern and western Missouri, through southern Kansas, southeastern Colorado, northeastern New Mexico, encompassing all of Oklahoma, the northern half of Texas, western Arkansas, and northwestern Louisiana (Smith 1994; Murray 2006).

Recently, a revised study of the *A. hentzi* complex, using a combination of genetic and morphological data, redefined the intraspecific variation within the group (Murray 2006). This revision revealed that *A. hentzi* is a wide-ranging species, with extensive morphological variation, and theorized that it exhibits high ecological plasticity due to its wide distribution.

While looking at the taxonomic status of *A. baergi*, Warriner (2008) found the variation in metatarsal IV scopulation was as great for male and female specimens collected from the same localities as those from different localities. Adult female and male *Aphonopelma* from Arkansas, as well as adult males from Oklahoma, formed a relatively homogenous group in terms of carapace and leg lengths. He also found that spermathecal structure for all female specimens examined from Arkansas and Oklahoma exhibited little variation, as well as all male emboli examined displayed little variation (Warriner 2008). Murray (2006) also concluded that *A. hollyi* should be separated from the "*A. hentzi* complex", while *A. arnoldi* raises significant questions to be addressed in future research due to the molecular and morphological inconsistencies with *A. hentzi*. Hamilton (2008) found even more molecular evidence that *A. hollyi* should be recognized as its own species, separate from the *A. hentzi* complex, as well as showing a species transition from *A. hentzi* (east and north) to *A. hollyi* and an undescribed *A.* sp. as one moves west, up the mesas, and onto the High Plains. It is possible that this group is a recent colonizer of the central United States having diverged in the Pleistocene, which would explain why they have not experienced a high level of genetic divergence.

There is very little information on the overall genetic diversity and population structuring of tarantulas throughout their range (Murray 2006; Hamilton 2008). And except for basic information from specimens that very well could have been wrongly identified, the biology and ecology of *Aphonopelma* is poorly known. We do know that *Aphonopelma* possess life-history traits that differ markedly from other spiders, and from other arthropods in general. Their long life spans, indeterminate growth, delayed sexual maturity, and limited dispersal abilities may make them especially vulnerable to factors such as habitat destruction and collection for the pet trade (Baerg 1958; Foelix 1996; Janowski-Bell 2001). They are long-lived (15-30 years) and require 4-7 years to reach maturity (Baerg 1958; *pers. obs.*). They have a mortality rate before maturity of 99% (Baerg 1958). They do not "balloon", a common form of dispersal where spiderlings will release strands of silk and ride air currents away from their natal site. Although

some mygalomorphs (*Sphodros atlanticus* and *Ummidia* sp.) are known to disperse by ballooning, it is rare and theraphosid spiderlings are thought to be too large to balloon. It is generally thought that most spiders heavier than 1 mg are not likely to use ballooning to disperse (Suter 1999), though the mygalomorphs witnessed to balloon were between 1.25 mg and 3.45 mg (Coyle *et al* 1985).

It has been reported that burrowing mygalomorphs have very limited dispersal ability based on observed clustering of individuals within suitable habitat patches (Reichling 2000; Bond *et al* 2001; Woodman *et al* 2006). Most mygalomorphs exhibit extreme habitat fidelity, with low vagility, living in more moist microhabitats than theraphosids, and often exhibiting strong population divisions (Hendrixson and Bond 2005). Studies of several North American mygalomorph spiders have revealed deep phylogeographic structure in relatively restricted geographic areas, which has been interpreted as evidence for the existence of cryptic species (Bond *et al* 2001; Bond 2004; Hendrixson and Bond 2005).

Tarantulas in the genus *Brachypelma* are known to live in aggregations in Belize (Reichling 2000). The spiderlings are very small (~5 mm) and do not disperse far from the natal site. They are confined to over-land dispersal, which may contribute to the fact that many species have small, disjointed distributions, making them vulnerable to habitat destruction (Reichling 1997). It is believed that these low-vagility, long-lived spiders live in informal clusters that may be high in maternal relatedness because of this limited dispersal ability. As a consequence of limited dispersal, it is believed that mygalomorphs are prone to extreme population structuring (Bond *et al* 2001, 2006; Arnedo and Ferrández 2007). Tarantulas are large enough to counter this idea of limited dispersal when compared to other mygalomorphs (Janowski-Bell and Horner 1999; Janowski-Bell 2001). The larger sizes of tarantulas allowing for greater mobility, their less specialized habitat requirements, and higher resistance to desiccation due to their highly sclerotized exoskeleton could allow them to disperse over larger distances than other flightless, terrestrial arthropods (Woodman *et al* 2006). The ability to

colonize drier habitats required greater desiccation resistance, greater dispersal ability, and greater ability to find mates. So, the adaptation of tarantulas to drier conditions, like grasslands, could have allowed for them to radiate quickly given the correct habitat niche opening up as lineages evolved. Tarantulas provide a model for studying speciation and the delimitation of species boundaries, for male-biased sex dispersing organisms, due to the amount of intraspecific morphological variation.

Tarantula "colonies", non-randomly distributed clusters of individuals, have been witnessed by numerous researchers (Baerg 1958; Kotzman 1990; Smith 1994; Hamilton 2008; *pers. obs.*) to either be under-dispersed (Kotzman 1990; Reichling 2001) or over-dispersed (Janowski-Bell 2001). But according to Janowski-Bell (2001), *A. hentzi* did not occur in colonies in North Texas after quantitatively analyzing clustering observations. She did witness a clustered dispersal pattern of *A. hentzi* in Missouri glade habitat and stated that because *Aphonopelma* has been isolated in glades for thousands of years, the isolation may be why they are more tolerant of one another and less territorial than their Texas cousins. In North Texas, *A. hentzi* burrows were found to have densities from 0.0072 to 0.0128 per m^2 (Janowski-Bell 2001). In glade habitat in Missouri, *A. hentzi* were found to have densities from 0.0015 to 0.0039 per m^2 (Janowski-Bell 2001). *Brachypelma vagans* has been found to have population densities from 0.02 to 0.1 individuals per m^2 in Mexico, among the highest ever reported for theraphosids (Machkour M'Rabet *et al* 2005). *Brachypelma klaasi* reached a maximum density of 0.00006 individuals per m^2 in southwestern Mexico (Yáñez and Floater 2000) and *Acanthoscurria suina* and *Eupalaestrus weijenberghi* were found from 0.07 to 0.41 per m^2 in Uruguay (Pérez-Miles *et al* 2005). While studying the arboreal tarantula *Avicularia avicularia* in Trinidad, Stradling (1994) found 60 individuals in an area less than 100 m^2 had recolonized a tonka bean plantation. Reichling (1999) found 114 theraphosid burrows in a 40x60 m plot and 101 theraphosid burrows in a 94x71 m plot while looking at nearest neighbor relationships of tarantulas in Belize.

Human modification of the landscape in tropical Mexico has been shown to create favorable microhabitats for colonies of *B. vagans*, but only at moderate levels of disturbance (Machkour M'Rabet *et al* 2005). Assuming that colonies of tarantulas are not completely wiped out by a disturbance, they show an ability to recolonize these areas. I have witnessed this event in Texas as well, with *A. hentzi* and *A. anax* being able to recolonize, sometimes with great density, home subdivisions with large colonies happily living in the grass lawns. In LaGrange, Texas, I witnessed >150 *A. anax* adults per 3.23 individuals per m^2, an incredibly high density. This shows these organisms can, given the right conditions, live in remarkably dense populations.

Because plugged burrows are almost impossible to locate, these numbers should be considered a "snapshot in time" whereas the true population densities would generally be more than what has been documented (Janowski-Bell 2001). In the field, large burrows are found much more frequently than small burrows (Pérez-Miles et al 2005; *pers. obs.*). This suggests that there is a predominance of adults rather than juveniles in natural populations, and that juvenile survival is very low (~1%, Baerg 1958). From my experiences in the field, and in keeping juveniles of these species in the lab, many juveniles probably are being overlooked when searching. In the lab, spiderlings and small juveniles of *Aphonopelma* spp. often plug their burrow at all times, except when they need to eat, and not just when they are going to molt. When searching for *A. hentzi* in areas where they live in scrapes, a large number of juveniles and spiderlings have been encountered. If the different species in Texas are similar in their dispersal and population densities, as they seem to be, and similar in life history traits, it seems logical that more juveniles survive than previously thought. Leptokurtic dispersal, which involves having most individuals move only short distances but a few moving much further (Skalski and Gilliam 2003), could be a common pattern of dispersal seen in *Aphonopelma*.

Tarantula gene flow is male-biased with mature males leaving their burrows to search for potential mates in the spring and fall, depending on the species and ecology, with some

exhibiting two cycles of “wandering” males, like *A. hentzi* (Baerg 1958; Prentice 1997; Longhorn *et al* 2007; *pers. obs.*). Upon sexual maturity males undergo their ultimate molt, which causes a radical change in morphology by giving them their palpal bulbs, i.e. the male sex delivery organs. Their behavior also changes; they become more skittish and defensive and they build a sheet web to deposit sperm and then load their palpal bulbs before leaving their natal territory and disperse or “wander” large distances in search of a female. Most mature males generally do not survive more than a year after sexual maturity. Mating activity is seasonal, with some species mating in the spring and others mating in the fall, and some species experiencing two waves of maturing males (Baerg 1958; Shillington and Verrell 1997; Janowski-Bell and Horner 1999; Yáñez *et al* 1999; Pérez-Miles *et al* 2005; *pers. obs.*), which might provide an isolating mechanism among certain sympatric species.

Male tarantulas have been observed to travel through a variety of habitats, with apparent randomness, when searching for a mating partner. When locating a female burrow, local “cues” provided by females will cause males to exhibit a search behavior as they close in on the female burrow (Shillington and Verrell 1997; Janowski-Bell and Horner 1999). Using radio telemetry, mature male *A. hentzi* were found to randomly walk up to 1300 meters during a period of 18 days (Janowski-Bell and Horner 1999). When a male locates a female’s burrow, he rapidly shakes his body and drums his front two pairs of legs and pedipalps on the silk and substrate surrounding the mouth of the burrow to draw out the female in order to mate. It has been proposed that the silk laid down by female *Aphonopelma* aid males in finding potential mates, possibly through embedded pheromones in the silk (Minch 1978, 1979; Shillington and Verrell 1997). Courtship interactions involve chemical reception and vibratory communication between males and females (Minch 1979; Prentice 1997; Costa and Pérez-Miles 2002). Multiple matings have been witnessed with females mating with the same male multiple times, as well as mating with different males (Baerg 1958; Prentice 1997; Shillington and Verrell 1997; Janowski-Bell and Horner 1999; Janowski-Bell 2001). This behavior raises questions regarding

fertilization success, with the possibility of sperm competition, last-male precedence, and female mate choice playing important roles in the population demography of *Aphonopelma*. This behavior could be evidence of the "female-choice hypothesis", which may lead to speciation as a by-product of the female choosing males, based on some preference. Female-choice hypothesis predicts diverse intraspecific male genitalic morphology in species with promiscuous females (Fox *et al* 2001), which could be an answer as to why there is so much intraspecific morphological variation in palpal bulbs of *Aphonopelma* males. The mating system of *Aphonopelma* is described as a type of scramble-competition polygyny (males have more than one female partner) where the ability of males to locate spatially-scattered receptive females during a limited mating season is an important determinant of mating success in males (Shillington and Verrell 1997). Baerg (1958) suggested that males "wandering" long distances for mates could be an evolutionary reason to reduce inbreeding.

All species of the genus *Aphonopelma* live most of their lives secluded in subterranean burrows or scrapes under rocks or wood, in well-drained soil on inclined ground (Smith 1994; Reichling 1997). This sedentary lifestyle is expected to lead to limited genetic exchange and population substructure throughout the ranges of the genus *Aphonopelma*. Tarantulas are nocturnal generalist ambush predators that employ a sit-and-wait foraging strategy and have a very low basal metabolic rate (Wise 1993). They wait for prey at or near the entrances of their burrows or retreats, which are generally slightly wider than the spiders' prosoma, generally not venturing more than a few centimeters from the entrance (Prentice 1997). Prey items include common arthropods (mostly beetles, grasshoppers and crickets, and cockroaches) as well as the occasional small vertebrate (mice, reptiles) (Baerg 1958; Stradling 1994; Machkour M'Rabet *et al* 2007; Longhorn *pers. comm.*; *pers. obs*.). It is generally believed that prey availability in most localities exceeds the metabolic needs of spider populations (Wise 1993).

The burrow, or scrape, is critically important in survival and is thought to maintain relatively constant temperatures and humidity levels throughout the year (Smith 1994; Foelix

1996). Burrow shape, depth, and angle are influenced by the compactness of the soil and the position of obstacles such as rocks, wood, or other objects (Smith 1994) and burrow size is a good estimator of spider size (Pérez-Miles *et al* 2005). Burrows are lined and closed with silk to limit water loss (Baerg 1958). Burrow construction is highly variable in North American *Aphonopelma*, ranging from basic scrapes, tunnels under rocks with a short tunnel that opens into a chamber, to basic stand-alone burrows that travel at an angle perpendicular to the substrate with a tunnel opening into a basic chamber, to complex burrows that can be multiple feet deep, and have multiple specialized chambers for molting or brood care (Smith 1994). Tarantulas can actively regulate their body temperatures by movements inside or adjacent to burrows, to regions that are more favorable than the mean air temperature, for example retreating into the warmer burrow depths at the onset of cold weather and warming eggsacs in the sun at the edge of the burrow during the summer.

Burrow site preference, structure of the burrow and soil characteristics, have been shown to be related in the Mexican tarantula *B. vagans* (Machkour M'Rabet *et al* 2007). Other factors, fluctuation in daily temperature and humidity, have been shown to influence microhabitat selection in *B. klaasi* (Yáñez and Floater 2000). In Texas, vegetative cover appears to be a factor that predicts the location of burrows for *A. hentzi* (Janowski-Bell 2001). *A. hollyi*, a close relative of *A. hentzi* in NW Texas, displayed activity levels that were affected by date/time of the year and cloud cover (Hamilton 2008). *Aphonopelma* in NW Texas were shown to spend more than 96% of their time inside the burrow, only coming out to capture prey or mate (Hamilton 2008).

Based on a small sample size of females leaving burrows and establishing new ones (Hamilton 2008), as well as my personal observations and collecting of mature females and immature males "wandering" (*pers. obs.*), the evidence seems to suggest that tarantulas are not as sedentary (occupying one burrow during their adult life) as previously believed. In 2007, of 25 *A. hentzi* previously located in the spring from the Redbud Trail area of Austin, TX, only 13

were still in their original burrow/scrape in August. These observations show the ability of *Aphonopelma* to move after biotic disturbances or abiotic perturbations (flooding, drought, etc.) and recolonize areas or increase their opportunity for gene flow.

Building upon the existing framework of genetic and morphological variation in *A. hentzi* and "neighboring" species (Murray 2006), I am interested in determining the phylogenetic relationships between the tarantulas throughout the Colorado River basin, the edge of the range of *A. hentzi* and the "neighboring" species, and what ecological differences or biogeographical events have caused these divergences. One key question is if the isolation of *A. hentzi* and the neighboring species was the result of parapatric speciation or was due to allopatric speciation when ecological differences and historical biogeographic events allowed for speciation.

1.2 Evolution

Defining the concept of a "species" is one of the most hotly debated topics in evolutionary biology. When trying to delimit species boundaries, the concept of what is a "species" becomes significant (Sites and Marshall 2003; de Queiroz 2007; Petit and Excoffier 2009). The phylogenetic species concept (PSC) defines a species as a monophyletic genetic cluster of individuals. It is the smallest set of organisms that share a common ancestor and can be distinguished from other such sets or organisms. These diagnosable geographic forms have evolved separately and have unique evolutionary histories, with the parent species having gone extinct. Because gene flow and effective population size are important in the application of PSC criteria, small populations will tend to be biased toward species-level recognition more often than will large populations. The PSC can also be used to examine the historical and demographic aspects of the speciation process (Mishler and Brandon 1987; de Queiroz and Donoghue 1988; Avise 2000; Velasco 2009).

Mitochondrial genes have served as the markers of choice for phylogeographic (the geographical distribution of genealogical lineages) and species-level phylogenetic analyses of animals because they often evolve much more rapidly than nuclear genes. They may overwrite

the traces of ancient relationships, but can reveal divergences among closely related species. When interpreted in the context of geography, mtDNA can provide insights into the historical demography and biogeography of species (Avise 2000; Knowles and Maddison 2002; Hebert *et al* 2004a; Richards *et al* 2007). Mitochondrial data are ideal markers because they share a number of favorable properties such as matrilineal inheritance, a general lack of recombination, a high mutation rate, reduced effective population size, and availability of universal PCR primers (Brower 1994; Moore 1995; Avise 2000). The small effective population size of mitochondria, relative to nuclear loci, may make it more probable that mitochondrial loci have tracked population history accurately. It is also thought that mtDNA haplotypes will coalesce and haplotypes will reach fixation within populations four times more quickly than nuclear markers (Moore 1995). Because of this, analysis of mtDNA should allow resolution of species limits in many groups that are difficult to resolve with nuclear-based markers, such as morphology (Wiens and Penkrot 2002). Relationships based on a mtDNA gene tree may not always reflect the true species phylogeny, but because the mitochondrial genome is maternally inherited and haploid, the likelihood of recovering more recent population level history increases. Mitochondrial DNA is also more likely to convey a gene tree that is congruent with the species tree (Moore 1995). Due to the rapidly evolving nature of mtDNA, recent mutations can be found that distinguish local populations, family units, and even individuals (Avise 2000). If species have diverged too rapidly to allow for morphological characters to differentiate, mtDNA phylogenies are thought to be particularly useful to delimit species (Wiens and Penkrot 2002). Unfortunately, because mtDNA is more susceptible to introgression, which could cause issues at species-level phylogenetic resolution, this could lead to an over-interpretation of speciation in a group, if nuclear markers are not included in the analysis.

It is known that single loci may not accurately reflect the true species history due to the stochastic nature of the lineage sorting process (Knowles and Maddison 2002; Knowles and Richards 2005). Using a combination of a slow evolving gene and a fast evolving gene can help

uncover deeper phylogenetic relationships and recent population dynamics and divergence (Hewitt 2001). This combination of two types of sequences has been shown to be the best method to date for establishing relationships within the subfamily Theraphosinae, a group of New World tarantulas that include *Aphonopelma* (Longhorn *et al* 2007).

The morphological criteria used to delineate tarantula species boundaries are thought to underestimate actual species-level diversity. Therefore using multiple genes is considered a rigorous method to examine species boundaries (Hendrixson and Bond 2005; Hendrixson and Bond 2007; Starrett and Hedin 2007). Concordance across multiple lines of evidence (morphological, molecular, and ecological data) is routinely used to define species boundaries (Stockman and Bond 2007). Pons *et al* (2006) suggests that to avoid issues with incompatible gene histories and unrecognized cryptic species, a DNA-based taxonomic system should use the sequence information itself as the primary source for establishing species membership and defining boundaries, instead of fitting specimens into predefined taxonomic groups. Wilcox *et al* (1997) demonstrated that a neotropical pseudoscorpion that was previously described as a single species, with a range spanning Central America to northern Argentina, was actually a complex of cryptic species.

Measures of genetic divergence can be used to infer species boundaries because of the strong correlation between genetic divergence and reproductive isolation. Geographic distance is hypothesized to play a major role in speciation for organisms with limited dispersal ability. But there is no absolute “cut-off” for genetic divergence in species recognition. Mitochondrial DNA sequence divergences are much larger among species than within species and thus their genealogies can capture what taxonomists recognize as species (Hebert *et al* 2004a). Because of the general concordance of mtDNA gene trees with species trees, rather than analyzing DNA from morphologically identified specimens, the process can be switched to identify species based on their DNA sequences and then determine how the morphology coincides with the species determinations (Hebert *et al* 2004a).

It is assumed the populations that neighbor *A. hentzi* will show exclusive mitochondrial genome changes before there is an apparent division in phenotype. Most gene flow in these spiders is paternal, so I would expect female mtDNA markers to show subdivision and isolation more clearly because of the uniparental pattern of inheritance. Because mtDNA sequences are not highly conserved and have a rapid mutation rate, they are useful for comparisons of individuals within species and for comparisons of species that are closely related because the number of sequence differences can easily be counted. But this approach has limits that are imposed by the rate of mtDNA sequence change. As the species become more distantly related, the number of sequence differences becomes larger until an accurate count becomes impossible.

Using mtDNA variation to delimit species has shown good support when used in poorly known groups (Wiens and Penkrot 2002; Hebert *et al* 2003; Hebert *et al* 2004a; Hebert *et al* 2004b; Barrett and Hebert 2005; Pons *et al* 2006; Petersen *et al* 2007). Current taxonomic DNA sequencing has been able to identify species-level entities based on genotypes from a representative sample of known individuals (Hebert *et al* 2003). Pairwise sequence divergence is defined as the mean number of base-pair differences between all pairs of haplotypes in the sample. With a threshold of 3% pairwise sequence divergence for "known species diagnosis", 98% (196 out of 200) of the South American lepidopterans species were correctly identified prior to being identified through morphological analysis (Hebert *et al* 2003). In a survey of 203 arachnid species from temperate-zone regions in North America, 96% of the species previously identified morphologically would be considered species using a 4% threshold for genetic divergence. The only exceptions found were between highly divergent populations of *Latrodectus hesperus*, a black widow species complex, which has since been proposed to represent distinct species. In the study, all congeneric species (belonging to the same species) pairs showed at least 3% sequence divergence (Barrett and Hebert 2005). Hamilton (2008) found three species (*A. hentzi*, *A. hollyi*, and a potential new *A*. sp.) in NW Texas when

comparing pairwise divergence in the mtDNA gene region *16S* between species, with an average of 5.42%.

The diversity and population structuring of *Aphonopelma* species in Texas could be the result of incipient speciation, where a geologic or environmental factor prevented gene flow. Barriers to gene flow and geographic distance are important factors determining species lineages by restricting breeding opportunities. Speciation may be more likely to take place in peripheral populations because of the pronounced genetic differentiation and limited gene flow. There are no identifiable geographic barriers to gene flow in Texas, leading to the idea that ecological barriers have been sufficient in promoting speciation. Bond *et al* (2001) show that the overriding factor in speciation in some groups of mygalomorphs depends on constraints of gene flow, rather than ecological specialization.

The distributional history of many lineages in Texas has undoubtedly involved repeated vicariant, dispersal, and extinction events. There are several processes that influence patterns of population genetic structure (e.g. gene flow, genetic drift, fragmentation). It is believed that for the maintenance of closely related species in sympatry, some form of isolating mechanism must be in place. High rates of intraspecific gene flow have been shown to rapidly promote monophyly at the species level (Petit and Excoffier 2009). Prentice (1997) hypothesizes that premating mechanisms are more evolutionarily significant than postmating mechanisms in *Aphonopelma*. These mechanisms were witnessed during field observations, in Arizona and California, of species of similar size having distinct, non-coinciding breeding seasons as well as morphological dissimilarities. I also have anecdotal evidence of this occurring, after witnessing different species' males emerging at different times of the year in the sympatric areas in central, south, and west Texas.

Allopatric speciation, the classic model of speciation, is an important and powerful force by creating reproductively isolated populations due to physical barriers or through isolation-by-distance. In this traditional view, the processes of natural selection, and adaptation act to drive

divergence between lineages inhabiting different environments. Speciation via niche conservatism occurs when lineages maintain their ancestral ecological niche, and their failure to adapt to new environments isolates incipient species (species in the process of diverging but still having the potential to interbreed) (Wiens 2004a). When environmental factors (biotic and abiotic) reduce the fitness of a population outside of the ancestral niche, Wiens (2004a) suggests that natural selection should favor traits that keep individuals within the niche (habitat selection becomes an evolutionarily heritable trait). Therefore this leads to speciation by limiting dispersal, aka gene flow, across barriers at the periphery of ranges (Wiens 2004a). Peterson *et al* (1999) found a pattern that supported allopatric speciation via geographic isolation rather than dispersal from the edges of distributions when they carried out ecological niche modeling for 37 sister species pairs of mammals, birds and butterflies. Unfortunately, the ecological factors determining the presence or absence of *Aphonopelma* throughout their range are poorly understood.

In contrast to allopatry, parapatric speciation requires the evolution of reproductive isolating mechanisms to occur when a population enters a new niche or habitat within the range of the parent species. The definition of a niche is important. The niche represents not only the ecological relationships of a species, but also its evolutionary history. A species' niche is reflected by its range boundaries, which are bound by the abiotic environment. Biotic interactions, such as competition and predation, can be proximate factors that limit the distributions of many species. In parapatry, the parent species lives in a continuous habitat, where subpopulations become geographically or ecologically isolated. This structuring occurs due to variations in the mating frequency of a population within a continuous geographical area where differences in niches along an environmental gradient have limited gene flow and thus created a cline. Parapatry is thought to require local adaptation along that environmental gradient in order to overcome the homogenizing effects of gene flow between geographically adjacent populations. According to Kozak and Wiens (2006), in parapatric speciation the

deepest genetic breaks between populations should be concordant with sharp transitions in environmental variables. Thorpe *et al* (2008) has shown that reduced gene flow can occur in areas where populations, or ecomorphs, are limited to certain habitat types, or niches, with no physical barrier to gene flow. Ecology, more and more frequently, is being shown to carry more importance in speciation research than previously thought, with researchers using models that emphasize the role of ecology over allopatric speciation (summarized in Thorpe *et al* 2008).

Speciation occurred many times during the major climatic shifts associated with pluvial-interpluvial cycles of the Pleistocene, which were repeated perhaps 15-20 times (Lomolino *et al* 2006). When glacial cycles cause changes in distribution, two different genetic patterns typically emerge, regional genetic structuring and/or decreased genetic diversity. Both of these patterns can be seen throughout the *Aphonopelma* ranges in Texas, with genetic structuring in the south and decreased genetic diversity in the north.

1.3 Pleistocene

The Pleistocene period lasted from around 1.65 million ybp to 10,000 ybp (Lomolino *et al* 2006). The Pleistocene glaciations have been shown to be important determinants of historical migration and current genetic diversity within and among populations (Church *et al* 2003). During the last glacial maximum (LGM), the Wisconsin around 21,000 to 18,000 ybp, landscapes and climates in North America were dramatically different from the present day. Continental ice sheets covered much of the northern portion of the continent causing climatic conditions to be considerably colder and drier, along with lowered sea levels (Waltari *et al* 2007). Most species experienced a reduction and fragmentation of their ranges due to the expanding continental ice sheets, distributional shifts of biomes and the fragmentation of primary habitats, as well as the development of unfavorable climate conditions that were beyond species' physical tolerances. The Pleistocene was characterized by a general cooling trend in North America with the glacial advances resulting in the migration or extinction of numerous populations. The most recent glacial period, the Wisconsin, reached its southern

limit in North America between 18,000 – 14,000 ybp at about 41^{o}N latitude (Church *et al* 2003). At the end of the Pleistocene, the glacial retreat was accompanied by a considerable warming of the climate, which may have presented an opportunity for organisms in southern refugia, and those adapted to warmer climates, to extend their ranges as new niches became suitable (Church *et al* 2003).

Repeated glacial cycles are thought to have had a strong influence on species' ranges and population sizes because of the effect on habitats and niches (Knowles 2001; McGaughran *et al* 2008; Walker *et al* 2009). Because species respond differently to climate change, the Pleistocene is an important, but less understood, event in the present composition and distribution of extant species.

Historical biogeographers are driven to uncover the events that determined distributions and the subsequent diversification patterns of evolutionary lineages. Understanding Pleistocene refugial distributions has been a focus because current population structure, intraspecific and interspecific genetic diversity, and potential for adaptation to local conditions depend on historical population structure (Waltari *et al* 2007). To understand the current patterns of distribution we see, we need to understand the events that led to those patterns. Populations that become restricted to refugia will diverge through changes in population size, founder events, and genetic drift, especially if the numbers of individuals within sub-populations are small. Range expansion, including bottlenecks, founder effects, and then exponential population growth, allows for the replacement of ancestral haplotypes with novel, derived haplotypes. According to Rowe *et al* (2004), if ancestral and derived haplotypes do not overlap, and are located in different geographic areas, then ancestral haplotypes should be found close to the origin of range expansion, whereas derived haplotypes are more likely to be found at the leading edge of the range expansion.

Founder effects lead to a lower genetic diversity in the newly colonized areas, than in the parental populations remaining in the glacial refugium (Hewitt 1996). Southern Europe

exhibits considerable haplotype richness in comparison to the lower diversity in northern populations. The southern haplotype richness is thought to be the result of the persistence of refugia and the accumulation of variation over several pluvial-interpluvial cycles, while the northern diversity has been caused by rapid postglacial expansion and colonization (Hewitt 2001). A consequence of repeated colonization events is that frequent local bottlenecks can result in the maintenance of reduced genetic variation. Rapid range expansion out of refugia involving repeated population bottlenecks is expected to exhibit decreased genetic diversity as sampling moves away from the ancestral source, particularly in haplotype number (Rowe *et al* 2004).

The expansions and contractions of the continental ice sheets are thought to have played an important role in shaping the distribution of biodiversity among current populations in North America. In the central U.S., fossil and palynological data provide support the existence of southern refugia during the last glacial maximum. Many temperate organisms are thought to have shifted their distributions to the south and recolonized the north as the ice sheets receded. Reduced levels of genetic variation in northern populations have also been uncovered, supporting the idea of expansion from southern refugia (Rowe *et al* 2004).

Though the continental glaciers never reached as far south as Texas, the state's climate and sea level underwent major changes during this time period. The climate was both more humid and cooler than that of today. Sea level during glacial advances was 300 to 450 feet lower than present (Spearing 2001; Sansom 2008). Paleontological evidence, the dating of speleothems (cave formations like stalactites and stalagmites), shows the climate of central Texas was both cooler and more wet than present (Musgrove *et al* 2001). The shift in climate was the result of a southward deflection of the jet stream due to the presence of the continental ice sheet (Musgrove *et al* 2001).

Pleistocene divergence models show that shifting species distributions would have promoted species divergence. Drift-induced divergence, despite the lack of long-standing

geographic barriers, has significantly contributed to species divergence during the Pleistocene (Knowles and Richards 2005). This divergence was due to species' distributions shifting frequently in response to the glacial cycles, and the fact that nuclear loci require a greater amount of time for lineage sorting than does mtDNA. In general, it is believed that colonization of previously glaciated areas can result in reduced genetic variation in the northern populations as a consequence of repeated and global bottleneck events, whereas southern populations exhibit relatively high levels of variation (Boulton *et al* 1998; Pedersen & Loeschcke 2001; Walker *et al* 2009).

Temperatures during the LGM appear to have been several degrees lower in central Texas than present (Musgrove *et al* 2001). July temperatures during the LGM were as much as 5.5^{o}C cooler than present (Nordt *et al* 1994). Mean annual temperatures in the region were at least 7^{o}C lower than today (Axelrod 1985). But there was an absence of extreme cold or long periods of subfreezing temperatures (Dalquest 1965).

In central North America what is now prairie was steppe-tundra and open woodland (22,500-14,000 ybp). Coniferous woodland, especially boreal conifers, was present over much or most of the Great Plains. As far west as south-central Kansas, *Picea glauca* and *Pinus flexilis* macrofossils have been found dating back to 18,000-14,000 ybp and sites in eastern Kansas predominately show spruce pollen between 15,000-24,000 ybp. The woody cover thinned out as one moved west, with an increasing proportion of prairie herb species dominating the pollen records (Axelrod 1985). Pollen evidence indicates that during the LGM the northern and central Great Plains, as far south as northern Kansas, was dominated by an open spruce forest with an understory of *Artemisia* and sedges while the southern High Plains region of the Texas Panhandle was covered by an *Artemisia* grassland (Hoyt 2000). Central Texas was covered with open deciduous forest, with some conifers and an understory of mixed grasses and shrubs. During the LGM, the grassland prairie was restricted to local areas. The region is thought to have been a forest-grassland mix with patchy grassland areas in which certain organisms, like

grasshoppers, were able to persist (Axelrod 1985). The height of the Red River, along with its larger flood plain, was estimated to have been well above the present level, potentially setting up a dispersal barrier to the north around 17,000 ybp (Dalquest 1965). This also could represented a barrier to retreating taxa from the north, which if they had been trapped to the north would have gone extinct due to the climate shift or forced to retreat into pocket refugia. This could have allowed for *A. hentzi* to survive in northern isolated pockets of grassland, waiting for the habitat to open up again, allowing for the rapid expansion and gene flow.

The historical ancestors of grasslands appeared during the Miocene-Pliocene transition (7-5 mya) when increasing aridity favored the rapid evolution of grasses (C_4 plants) (Axelrod 1985; Nordt *et al* 1994). As cooler, moister conditions came about and dominated during the Pleistocene glacial periods, the grasslands (C_4) retreated and woody vegetation (C_3) expanded its range. Climate change led to a greater number of cold-adapted species in North Texas (Dalquest 1965). Paleontological data taken from Fort Hood, Texas, just northeast of the Colorado River and part of the Colorado River Basin (Figure 2), show that climate change in the past 15,000 years caused a significant shift in C_3 to C_4 plants in central Texas. Plants that utilize the C_3 carbon fixation photosynthetic pathway include nearly all trees, shrubs, and cool season temperate grasses. These plants dominate forest communities and most other temperate zone plant communities. Plants using the C_4 photosynthetic pathway, mostly warm season grasses, are most abundant in warm, semiarid environments with high light intensity, which includes grasslands, savannas, and deserts where they have a competitive advantage by reducing water loss. During the late Pleistocene, C_4 plants comprised only about 40% (40% grasses and 60% trees) of the vegetative biomass in central Texas, suggesting that conditions were cooler and wetter than at any time during the past 15,000 years. The composition and differentiation of the present-day grasslands may have developed in the past 14,000 years or less (Hoyt 2000). As the glaciers retreated, warmer and drier climates restricted forests, about 12,500 ybp, leaving parkland vegetation dominated by grasses, shrubs, herbs and protected

trees near areas of water, leading to the present post oak/grassland vegetation we see today (Axelrod 1985).

In southern Texas, late Wisconsin packrat middens include piñon , juniper, and oak between 15,000-12,000 ybp. The northern Chihuahuan desert was dominated by woodland into the late Pleistocene with the desert scrub vegetation that now typifies the area first appearing in the Holocene (Axelrod 1985). Information from woodrat middens suggests that Chihuahuan desert in southwest Texas was extremely restricted, and perhaps totally absent from Texas during the Pleistocene, due to evidence of juniper woodland extending to extremely low elevations (Harris 1985). Woodlands (*Pinus*, *Juniperus*, and *Quercus* pollen) have been documented from the low elevations in the Trans-Pecos region of Texas due to evidence coming from Williams Cave in Culberson County, Texas, at the southern end of the Guadalupe Mountains, as well as woodrat middens dating from around 12,500 ybp (Harris 1985).

The time period 11,000 to 8,000 ybp was a transitional period between the late Pleistocene and the warmer and drier Holocene. Around 6,000 to 5,000 ybp C_3 plant communities were almost completely replaced by C_4 plants (reaching an abundance of up to 95% of the biomass), indicating a prairie expansion with the warmer and drier climate conditions (Nordt *et al* 1994). Due to the relatively new age of the grassland biome, there are few endemics observed here than in other adjacent biomes. Of the 108 grasshopper species that occur in the prairie biome only 3 are prairie endemics (Axelrod 1985).

Both plant and arthropod species experienced a general downward shift in elevation and latitude than what they use today (Elias 1992). The low level of genetic diversity observed in the mygalomorph *Atypus affinis* is thought to be the historical explanation to the species' post-glacial colonization of northern Europe (Pedersen & Loeschcke 2001). In another European mygalomorph lineage, most of the haplotypes observed in *Macrothele calpeiana* were of Pleistocene origin (Arnedo and Ferrández 2007). Displacement into allopatric glacial refugia and recolonization of previously glaciated/uninhabitable area has been shown to be an

important driving force in the divergence of grasshoppers (Knowles 2001). Populations of the spider *Agelenopsis aperta*, within the Rocky Mountains, exhibited significantly lower genetic diversity than populations east of the Rocky Mountains suggesting a post-Pleistocene range expansion (Ayoub & Riechert 2004). Elias *et al* (1992) uncovered the presence of cold-adapted arthropods in southern Colorado around 18,000 ybp, suggesting that temperate arthropods might also have been restricted and pushed to more southerly regions during the LGM.

Texas and northern Mexico has a rich and complicated geologic history, particularly during the Pleistocene, which has provided ample opportunities for speciation. The Trans-Pecos region of Texas is the most topographically varied region in Texas (Hoyt 2000), providing for a diverse number of niches. The present day Texas coast and sea level have been relatively stable for 5,000 years. Seas covered the state approximately 400 million years ago. Around 100 million years ago a seaway reached from the Arctic Ocean to the Gulf of Mexico, covering all of Texas. At the end of the Cretaceous (~65 mya) the Gulf of Mexico reached west to the Trans-Pecos (the area west of the Pecos River, bounded by the Rio Grande on the south and west) and north to the High Plains (Spearing 2001; Sansom 2008). When looking at a physiographic map of Texas, there is a noticeable S-curve that runs from the Dallas-Fort Worth area of NE Texas down toward Big Bend National Park. This line was the edge of the North American continent during the Cretaceous, closely following the now-buried western portions of the Quachita Mountains (still seen in SE Oklahoma). The Quachitas are approximately 300 million years old and were formed when the North American continent collided with the African continent to form the supercontinent Pangaea (Spearing 2001; Sansom 2008).

The Colorado River, the longest river entirely within the boundaries of Texas or any state in the United States, and has been shaping these regions of Texas for over ten million years (Figure 2). It carves a path diagonally through Texas from the northwest part of the state near Seminole, in the High Plains, towards Matagorda, in the Coastal Prairies, where it empties into the Gulf of Mexico. Because of its origins in the High Plains, the flow of the river is often

quite low in this region due to a lack of rainfall and a small number of significant springs (Spearing 2001; Sansom 2008). The consistency of the flow of the river changes significantly around the Colorado Bend State Park area, becoming a consistent, steady flow downstream to where it empties into the gulf. Upstream from Colorado Bend State Park, the flow can be quite low at times with patchy areas open for migration (gene flow) back and forth across the river. Near its origins in the Seminole area the river is nothing more than a creek-like formation being fed by underground springs.

Evolutionary theory predicts that small selective advantages in ecology, like foraging, fecundity, survival, etc., can lead to substantial morphological and molecular adaptations in populations, given enough time. It has been shown that the *A. hentzi* complex spiders are genetically and morphologically similar and that there are no obvious gene flow barriers found in the range (Murray 2006). Murray (2006) determined that most of these species should be synonymized under the senior name of *A. hentzi*, allowing for morphological variation across its geographic range. After sampling specimens from across the hypothesized *A. hentzi* complex range, and looking at variation in the cytochrome oxidase subunit 1 (*CO1*) mitochondrial gene, many specimens had 0-1 polymorphisms from *A. hentzi* (including the neotype from Smith 1994) inside a range centered on Oklahoma and Arkansas. Those outside of the hypothesized range, considered "neighboring tarantulas" had 4-7 polymorphisms from *A. hentzi* and those considered "peripheral tarantulas" had 52-90 polymorphisms from *A. hentzi* (Murray 2006). It was suggested that some uncertain "neighboring" species with distributions along the northwestern edge of the *A. hentzi* range including *A. coloradanum* and *A. echinum* (both from Colorado) are likely junior synonyms of *A. hentzi*. She also found that *A. arnoldi* and *A. hollyi* (both from West Texas) are likely candidates for removal from *A. hentzi*. Elsewhere, the uniqueness of other adjacent species is unknown, partly because a large region of central Texas has been undersampled. The Austin area appears to be the interface locale of *A. hentzi* and the "neighboring" and "peripheral" species of south-central Texas extending west. The

panhandle of Texas, northeastern New Mexico, and southeastern Colorado, are hypothesized to be the western extent of the *A. hentzi* complex, with a barrier projecting into Black Mesa, Oklahoma (Smith 1994; Murray 2006), with specimens from this locality clustering with specimens from southwest Texas. Murray (2006) believes that ecological barriers on the western and southern borders might be sufficient for separating the *A. hentzi* tarantulas from the peripheral species. It is proposed that *A. hentzi* should exhibit differences in habitat preference, from their neighboring species, yet the differences in Murray (2006) were not clearly defined or related to the ecological preferences that might provide the strongest evidence of isolation.

According to Murray (2006), based on both morphological and molecular data, there was a tight grouping of tarantulas in the geographical region of the *A. hentzi* complex when compared to the "neighboring" and "peripheral" tarantulas, with the *A. hentzi* complex tarantulas having more morphological similarity to each other than to the "peripheral" tarantulas. When Murray (2006) plotted geographic location versus genetic distance, a large gap or barrier between the *A. hentzi* complex tarantulas and the "neighboring" and "peripheral" tarantulas was shown. She found that *A. hentzi* covers a broad geographic range, with most samples sharing identical *CO1* sequences. When looking at the analysis of molecular data, the "peripheral" tarantulas always exhibited large genetic distance from *A. hentzi* and other likely candidates for synonymy (*A. baergi*, *A. clarki*, *A. gurleyi*, *A. odelli*, *A. waconum*, and *A. wichitanum*) though *A. coloradanum* and *A. echinum* were not originally included in the synonymy because of their slightly distinct morphology to the rest of *A. hentzi*, but based on the molecular data they should be included as well. In Murray (2006) the morphological data for *A. arnoldi* and *A. hollyi* was not conclusive, but the molecular data seemed to show they were different enough from *A. hentzi* to be their own species. Hamilton (2008) showed that *A. hollyi* is in fact different enough, due the molecular data, to be considered a valid species. In Murray (2006) the hypothesized mode of gene transfer among the *A. hentzi* tarantulas is a stepping-stone model, where neighboring populations exchange genetic material which is then transferred to more distant populations,

over and over again. Under this model, genetic homogenization would be predicted to occur within species, but likely over a long period of time. Due to this method requiring a long time period to work, an isolation-by-distance model could instead be working in the differences between the southern and northern populations of *A. hentzi* after a rapid range expansion. The isolation-by-distance model predicts that genetic similarity between populations will decrease as the geographic distance between them increases, due to the effect of geographic distance on gene flow. This process geographically restricts gene flow and generates a genetic structure, because random genetic drift is occurring locally (Hardy and Vekemans 1999).

I propose that the general tendency of *Aphonopelma* is to maintain their ecological niche, because ecology is an important factor driving divergence in the *Aphonopelma* tarantulas, and their failure to adapt to new environments, has enforced isolation, yet allowed for speciation in the neighboring populations along the Colorado River basin.

Due to a hypothesized rapid range expansion, I predicted that the northern populations of the *A. hentzi* range (north of the Colorado River basin) would express low levels of genetic diversity. This diversity is expected to be a subset of the genetic diversity seen in the southern portions of the range. Using molecular and ecological data, I examined the patterns and degree of genetic variation in *Aphonopelma* with the objective of assessing the relative importance of the Pleistocene in shaping distribution and diversification patterns. The objectives of this research are to use the phylogeny, in conjunction with molecular dating techniques, to infer the historical biogeographic events that may have played a role in the shaping of present-day distributions. The purpose of this research was to test the hypothesis that *A. hentzi* is a recent species with little intraspecific genetic variation throughout its range, having split from a southern lineage of *Aphonopelma*, and that differences in the niche and biogeographic history along the southern and western borders of the *A. hentzi* range created a barrier to further habitat expansion and promoted speciation for the tarantulas in those areas.

CHAPTER 2

METHODS

2.1 Taxon sampling

To gain an understanding of the natural variation in this group of spiders, 147 specimens were collected total, 89 by myself, 58 by others (researchers and the public), with GPS localities or detailed collecting information, from across Texas and the southwest United States (Figure 1). Spiders were collected from 15 sites haphazardly sampled from good potential habitats on either side of the Colorado River basin in central Texas (Figure 2). These were compared with specimens from 41 localities north, south, and west of the Colorado River basin.

I aimed to collect ≥3 individuals per location (Wiens and Penkrot 2002), though it was not always possible for specimens outside of the Colorado River basin. At each location, the series of individuals collected focused on adult females and mature males, but also included immature spiders if adults were not found and the number needed to gather to the required sampling total was not met. Each specimen was assigned a unique voucher name and haplotype designation. All specimens collected in this study will be preserved in 70% ethanol and deposited in collections in the United States.

2.2 Molecular Protocols

Tissue samples were collected, from both live and dead specimens, using the nonlethal technique of inducing limb autotomy of leg III on the right side of the spider (Longhorn *et al* 2007). In response to pressure, the limb will detach and the muscles will contract to prevent hemolymph loss. This response is an anti-predator response and the leg will regenerate with subsequent molts.

Legs were preserved in 100% ethanol and stored in a -80^{o}C freezer. Muscle tissue was extracted from the leg by making a small incision on the ventral face of the femur and then cutting out ~25mg of tissue. Genomic DNA was extracted using the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA). The concentration of the extracted DNA was quantified by using spectrophotometry (NanoDrop ND-1000, Thermo Scientific, Wilmington, DE).

A gene fragment of approximately 950 bp spanning two mitochondrial genes, *16S* (a slow evolving gene) and *ND1* (a fast evolving gene), including the *tRNA-Leu*, was amplified for sequencing using the polymerase chain reaction (PCR). Amplification was performed using a 10μL reaction of the PCR cocktail made up of reagents from a GoTaq kit (Promega): 2.0 μL GoTaq Buffer (5X); 1.0 μL dNTPs (2.5 μM); 0.1 μL BSA (bovine serum albumin); 0.8 μL $MgCl_2$; 4.3 μL ultra pure H_2O; 0.6 μL *16S* primer; 0.6 μL *ND1* primer; 0.1 μL GoTaq *Taq* polymerase; and 0.5 μL genomic DNA with 20 μL of wax on top. Primers used for amplification included the *16S* primer N1-J-12261 (TCRTAAGAAATTATTTGA) and the *ND1* primer LR-N-13398 (CGCCTGTTTAACAAAAACAT) (Longhorn *et al* 2007). Thermal cycle parameters were as follows: initial denaturation at 95^{o}C for 3 min; annealing of 52^{o}C for 45 sec; extension of 72^{o}C for 1 min; 39 cycles of denaturation at 95^{o}C for 45 sec, annealing at 52^{o}C for 45 sec; extension at 72^{o}C for 1 min; and final extension at 72^{o}C for 10 min. Following the PCR cycle, the products from each reaction were run on agarose gel to quantify the strength of each sample. Unincorporated dNTPs, primers, and other impurities were removed from the amplified PCR products using ExoSAP-IT (USB Corporation; Cleveland, OH).

Final PCR products were sequenced using an ABI 3130XL sequencer (Applied Biosystems, Foster City, CA) using the ABI Big Dye Terminator version 3.1 cycle sequencing kit. PCR primers served as sequencing primers for both genes; in addition, the internal reverse primer LR-N-12945 (CGACCTCGATGTTGAATTAA) was used to ensure overlap between genes.

2.3 DNA sequence alignment & Nucleotide diversity

Sequence editing was performed in Sequencher v4.1.2 (Genecodes, Madison, WI). Front and reverse primers were removed and editing was checked by eye and performed manually to resolve ambiguities. Contigs of the sequences were exported in FASTA file format.

Sequence alignment was conducted on 890 bp (*16S-tRNA-ND1*) of 135 specimens using the Clustal algorithm in *MEGA* v4 (Tamura *et al* 2007). Twelve specimens were not included in the analysis due to poor amplification, poor sequencing, or too many ambiguities to accurately call a base. NEXUS formatted files were then exported for phylogenetic usage.

DnaSP v5.00.05 (Librado and Rozas 2009) was used to count the number of haplotypes and nucleotide diversity (π) within the species. Haplotype number was determined by selecting sites with gaps/missing data "not considered", and invariable sites "removed". Nucleotide diversity was defined as the average number of nucleotide differences per site between two random sequences.

2.4 Phylogenetic analyses

To infer the phylogeny of the group, three different analyses were performed in order to check for concordance among topologies. Maximum Parsimony analysis was carried out using TNT v1.1 (Goloboff *et al* 2003). A traditional search of "Wagner trees" was performed using 100 random addition sequence replicates with tree bisection-reconnection (TBR) branch swapping, 10 trees saved per replication, quick collapsing during searches, replacing existing trees, all characters of equal weight, gaps treated as missing, and branches collapsed if maximum branch length is zero. Nonparametric bootstrapping (Felsenstein 1985) was used to evaluate the support of nodes using the above parameters based on 100 replicates. Bootstrapping parameters were set as a standard sample with replacement. *A. behlei* (Chamberlin, 1940), a species from northern Arizona, was used as the outgroup because it represented the most basal species in preliminary analyses. A 50% majority-rule consensus tree was produced.

For the Maximum Likelihood analysis and Bayesian analysis, the program ModelTest v3.7 (Posada and Crandall 1998) was used to determine the appropriate model of evolution that should be used. The model chosen for this data set was GTR+I+G (General time reversible + invariant site + gamma). The data set was considered as one continuous partition. Maximum Likelihood analysis was carried out in PAUP* v4.0b10 (Swofford 2002). A "fast" stepwise-addition search was performed using 10 bootstrap replicates, with random sequence addition, one tree held at each step, branch-swapping algorithm set to "none", trees with approximate likelihoods 5% or further from the target score are rejected without additional iteration, branches collapsed (creating polytomies) if branch length is less than or equal to 1e-08. Bayesian analysis was carried out in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). The analysis consisted of two simultaneous runs each consisting of four Markov Chain Monte Carlo (MCMC) chains run for 15,000,000 generations. Trees were sampled every 500 generations. The number of swaps per swapping cycle was set to two. The burn-in fraction was set to 0.25. The number of trees that were sampled was 58,000 with 57,420 found to be credible (99%). MCMC runs were summarized and investigated for convergence of parameters using the *sump* and *sumt* commands in MrBayes and the computer program Tracer v3.1 (Rambaut and Drummond 2005). Trees prior to burn-in and convergence were discarded. A 50% majority-rule consensus tree was produced. *A. behlei* (ABL001) (Chamberlin, 1940), *A. mojave* (AMJ001) (Prentice, 1997), *A. joshua* (AJO001) (Prentice, 1997), *A. eutylenum* (ACA001) (Chamberlin, 1940), *A.* sp. Hualapai (AHP001,002), *A.* sp. Huachuca Mtns. (AHU001,002), and *A.* sp. Hidalgo Co., NM (AHI002), species from the western United States, were used as the outgroup because they represented species that were highly divergent from the Texas species in preliminary analyses.

2.5 Estimating divergence times

The age of population (clade) expansions among lineages was estimated by using a general rate of divergence for mitochondrial genes in insects and arachnids (an arthropod molecular clock) of 2.3% per million years (Brower 1994) (others that have used this rate

include: Wilcox *et al* 1997; Masta 2000; Turgeon & McPeek 2002; Gantenbein *et al* 2003; Croucher *et al* 2004; Arnedo and Ferrández 2007; McGaughran *et al* 2008). A more conservative rate of 1.5%, an estimated rate for the salamander genus *Ambystoma* (Church *et al* 2003), was also used because of the differences in life history traits that *Aphonopelma* exhibit when compared to insects and most other arachnids, as well as their similarities to small vertebrates. This molecular clock technique was used for each clade (species) because it is widely agreed upon that different species evolve at different rates and because a uniform molecular clock in this analysis was rejected. Though a substitution rate of 4% per million years has been inferred for the mygalomorph spider *Aptostichus simus* based on biogeographic evidence (Bond *et al* 2001), this technique was used to give a more conservative estimate.

Variation in nucleotide substitution rates can arise due to differences between species' generation times, activity, and other life history factors. Species with high metabolic rates are predicted have high substitution rates, high levels of DNA repair, and less repair efficiency (Martin and Palumbi 1993). Factors that may affect, or are correlated with, rates of substitution include body size, metabolic rate, generation time, life span, age at first reproduction, population size, elevation (altitude) of habitat, and DNA replication and repair efficiency. Generation time and metabolic rate were seen to be the most important factors in substitution rates (Martin and Palumbi 1993; Murrell *et al* 2005). Life history traits among reptile species have been shown to cause a negative correlation, in their molecular evolution, between body size and substitution rate (Bromham 2002). Ectothermic vertebrates (an organism whose body temperature varies according to the temperature of the surroundings) have been documented to exhibit slower mtDNA substitution rates overall than do endotherms (an organism whose body temperature varies only within narrow limits and are maintained internally) of similar size (Martin and Palumbi 1993). Mite species in the order Acari with high substitution rates are known to have short generation times, while exhibiting more activity and having higher metabolic rates than other mites (Murrell *et al* 2005). With tarantulas being relative metabolic "rocks", their

substitution rates are predicted to be slower than other arachnids and insects, which have much faster generation times.

The equation used to estimate clade divergence: (mean genetic divergence for the clade ÷ rate of evolution per million years) × 1,000,000 = the expansion estimation (equation used in Wilcox *et al* 1997; Church *et al* 2003; Rowe *et al* 2004).

Multiple sources of data should be used to date vicariance events or calibrate molecular clocks and dating key evolutionary events precisely, such as lineage splits, from molecular data without fossil or biogeographical evidence can present significant issues, such as incorrect age estimates. Due to the absence of a fossil record for *Aphonopelma*, dating divergence times between species should be treated with caution because of the inability to calibrate a molecular clock. These potential issues are why I chose a more conservative approach by estimating intraspecific divergence. I also tested this method to roughly determine the ages that extant populations have been expanding/segregating from an ancestor, in order to determine how closely the dates match with biogeographic events.

To test the molecular data set for a universal molecular clock in the *Aphonopelma* studied, the programs BEAST v1.4.8 (Drummond and Rambaut 2003) and Tracer v1.4.1 (Rambaut and Drummond 2007) were used. According to Drummond and Rambaut (2003), for the data to be evolving in a clocklike manner the standard deviation of the Euclidean distances will exhibit a mean near 0.

2.6 Ecological Niche Modeling

Because comparing the phylogeny of a species studied with the geographic distribution of the species (Avise 2000) is thought to lead to an over-interpretation of the lineage history (Knowles and Maddison 2002), ecological niche modeling was used, in conjunction with phylogenetic tree topologies, to determine relationships and biogeographic history of the

lineages. Using an approach like ecological niche modeling for species delimitation, coupled with a genetic approach, has the potential to increase the rigor of phylogeographic research because of the close correlation between ecological divergence and reproductive isolation (Richards *et al* 2007). Graham *et al* (2004) has also shown that niche-based distribution models demonstrate speciation to be strongly correlated with ecological divergence. Ecological niche modeling, of both past and present environments, has been used by numerous researchers to answer questions regarding speciation mechanisms, extinction, hybrid zones, as well as potential Pleistocene refugia and dispersal corridors (Peterson *et al* 2004; Bond *et al* 2006; Carstens & Richards 2007; Knowles *et al* 2007).

Ecological niches and potential geographic distributions were modeled by using the maximum entropy method in Maxent v2.3 (Phillips *et al* 2006) to model current and past geographical distributions based on biologically meaningful climate variables. Niche modeling was performed as a way to assess ecological interchangeability, using 19 standard bioclimatic variables derived from modern monthly temperature and precipitation values (Worldclim 1.4, http://worldclim.org) at a high resolution of ~1 km^2 (30 arc-seconds). This method was chosen as a simple, direct method of determining whether the different *Aphonopelma* species throughout Texas could be distinguished by the ecological climate variables taken from their presence localities. These climatic variables include: Annual Mean Temperature, Mean Diurnal Range, Isothermality, Temperature Seasonality, Max Temperature of Warmest Month, Min Temperature of Coldest Month, Temperature Annual Range, Mean Temperature of Wettest Quarter, Mean Temperature of Driest Quarter, Mean Temperature of Warmest Quarter, Mean Temperature of Coldest Quarter, Annual Precipitation, Precipitation of Wettest, Precipitation of Driest Month, Precipitation Seasonality, Precipitation of Wettest Quarter, Precipitation of Driest Quarter, Precipitation of Warmest Quarter, Precipitation of Coldest Quarter. These have been shown to be particularly relevant in determining species distributions (Waltari *et al* 2007). The regularization parameter was set on the default of 1.0 so as to not overfit (fitting so close to the

training data that the model doesn't generalize well to independent test data) or underfit the data to the model. According to Phillips *et al* (2006), the potential for overfitting increases as the model complexity increases.

Maxent is a general-purpose machine-learning method that has been developed and specialized for predicting species' distributions, when only presence data are available for analysis (Reed *et al* 2008). Each algorithm is designed to extract the relationship between environmental variation and species occurrence. This relationship is then used to predict the species' distribution given the environmental conditions of the area and time period of interest (Richards *et al* 2007). The distribution models produced by Maxent are viewed as a prediction of the organism's realized niche (the environmental conditions that allow a species to persist in an area given the presence of competitors and predators) (Phillips *et al* 2006). Maxent has been shown to outperform other species presence modeling methods, like GARP, when comparing a number of species across geographic regions (Elith *et al* 2006). Maxent has also been demonstrated to be effective with small, representative sample sizes of presence-only data from as little as five sample points (collected from most or all of the important habitats) (Elith *et al* 2006; Pearson *et al* 2006; Reed *et al* 2008). Because of this, two species (*A. moderatum* with 5 presence localities, and *A.* sp. Amistad Brown with 4 presence localities) are borderline interpretable, but were left in the analysis to determine their efficacy when compared to the other species in the analysis. The rest of the species' presence localities used were as follows: *A. hentzi* used 52 presence localities, *A.* sp. Carlsbad Green used 9 presence localities, *A. anax* used 25 presence localities, *A. armada* used 17 presence localities, and *A.* sp. X used 13 presence localities.

This method can also be used to identify likely points of contact between species, in the present, and how the contact zones might have been influenced by past glacial cycles. To generate biogeographical hypotheses for the paleodistributions of *Aphonopelma* during the Last Glacial Maximum (21,000 ybp), Maxent was used to predict the potential distribution using the

paleoclimate layers created by Richards *et al* (2007) at ~18 km^2 (10 arc-minutes). This was performed by projecting the current distribution model from each species onto a reconstruction of the climate, when unfavorable climate conditions led to range contractions and fragmentation. The climate variables include: Annual Mean Temperature, Temperature Seasonality, Mean Temperature of Wettest Quarter, Mean Temperature of Driest Quarter, Mean Temperature of Warmest Quarter, Mean Temperature of Coldest Quarter, Annual Precipitation, Precipitation of Wettest Month, Precipitation of Driest Month, Precipitation Seasonality, Precipitation of Wettest Quarter, Precipitation of Driest Quarter, Precipitation of Warmest Quarter, Precipitation of Coldest Quarter. These layers were created from the Community Climate Model (CCM1 model) (Kutzbach & Guetter 1986), which incorporates atmospheric dynamics, including radiative and convective processes, condensation, and evaporation into the model.

Paleodistribution modeling can estimate historical species' distributions and allow biogeographical hypotheses to be tested for taxa without a detailed fossil record (Carstens & Richards 2007; Knowles *et al* 2007), like tarantulas. This method can also add a historical perspective to past population associations that might have influenced the patterns of genetic variation we see today. A combination of phylogenetics and ecological niche modeling has been used to test historical biogeographical hypotheses for species of conservation concern (Weaver *et al* 2006) as well as detect signatures of population extinction (Bond *et al* 2006). Species prediction maps for both the past and present, based off of the Maxent distribution outputs, were created in ArcGIS v9.2 (ESRI, Redland, CA), a geographic information system environment.

In order to determine the habitat characteristics that allow or prevent *Aphonopelma* species' existence in Texas and if the species were significantly separating themselves due to the ecological factors in their habitat, statistical values of the ecological variables stated above were extracted from the presence localities using DIVA-GIS v5.4 (Hijmans *et al* 2005), a GIS

program specifically created for mapping and analyzing biodiversity data and testing biogeographical hypotheses.

Discriminant Function Analysis (DFA) was chosen to statistically analyze the extracted ecological variables. DFA is a procedure for classifying objects (specimens) into groups (species groups) based on information on various predictor or classification variables, like ecological variables (Kachigan 1991; McCune and Grace 2002; Dytham 2003). The analysis was carried out in SYSTAT 12 (Systat Software, Inc., Chicago, IL.).

DFA takes objects (specimens) and allows the user to group them *a priori* (into species groups), based on known background information. The purpose is to maximize the among-group variation relative to the within-group variation, with independent variables used as predictors of group membership. The idea of the method is to provide a set of weightings that allow the groups to be distinguished. The weightings are given to each of the measured variables to maximize the differences between groups (Kachigan 1991; McCune and Grace 2002; Dytham 2003).

DFA has been used in ecological research for purposes such as summarizing the differences between groups, multivariate testing of whether or not two or more groups differ significantly from each other, predicting group membership, and comparing occupied versus unoccupied habitat to determine the habitat characteristics that allow or prevent a species' existence (McCune and Grace 2002).

CHAPTER 3

RESULTS

3.1 Nucleotide Diversity

Forty-three haplotypes were found among the 126 ingroup specimens. With ten sites taken out of this analysis due to alignment gaps and missing data the number of variable polymorphic sites in the molecular data set was 213. There were 260 mutations with 202 parsimony informative sites. Base frequencies were calculated in PAUP* v4.0b10 (Swofford 2002): A = 0.34044; C = 0.17293; G = 0.14223; T = 0.34439.

In order to measure the degree of polymorphism (genetic variation) within the sampled populations, nucleotide diversity (π) was determined according to Nei and Li (1979). The average number of nucleotide differences per site between two random sequences (π) within the *A. hentzi* clade is 0.00361. The average number of nucleotide differences per site between two random sequences (π) within the *A. anax* clade is 0.01633. The average number of nucleotide differences per site between two random sequences (π) within the *A. armada* clade is 0.00080. The average number of nucleotide differences per site between two random sequences (π) within the *A. moderatum* clade is 0.00045. The average number of nucleotide differences per site between two random sequences (π) within the *A.* sp. Carlsbad Green clade is 0.00080. The average number of nucleotide differences per site between two random sequences (π) within the *A.* sp. Amistad Brown clade is 0. The average number of nucleotide differences per site between two random sequences (π) within the *A.* sp. X clade is 0.01094 (species clades from Figure 3).

One major clade of spiders includes *A. hentzi*, *A. moderatum*, *A.* sp. Carlsbad Green, *A.* sp. Amistad Brown. The average sequence divergence (uncorrected p-distance) within *A.*

hentzi (AB001-005, AIR001, ACB001-007, ARP001-004, ARB001-005, AC001-004, ACO001, ATO001-002, AOK001-003, AD001-005, AGP001, AWA001, ALE001, ARR001, AAU001-002, ACP001-002, ATR001, ADR001, ADR009, AT001, ABU001, AHY001, ABG001) is 0.387%, with a minimum divergence of 0% and a maximum of 2.756% (Table 1). The average sequence divergence for *A. moderatum* (ADR002, ADR004, ADR005, ADR006, ADR011) is 0.045%, with a minimum divergence of 0% and a maximum of 0.113%. The average sequence divergence for *A.* sp. Carlsbad Green (ASE001-006, ASR001-002, 005) is 0.109%, with a minimum divergence of 0% and a maximum of 0.339%. The average sequence divergence for *A.* sp. Amistad Brown (ADR007-008, 010, ASV001) is 0.038%, with a minimum divergence of 0% and a maximum of 0.113%. The closest relative to the *A. hentzi* clade, a specimen from near Big Bend National Park in southwest Texas, *A.* sp. Big Bend (ABB001), is 5.65% divergent from the rest of *A. hentzi*. The next closest relative to the *A. hentzi* clade, *A. moderatum*, has an average sequence divergence of 5.84% from the Big Bend spider (ABB001), and 6.96% divergent from *A. hentzi* (Table 1).

The second major clade of spiders includes *A. anax*, *A. armada*, and *A.* sp. X. The average sequence divergence for *A. anax* (ALG001-015, ASP001, AHA001, ACC001-002, ADW001-004, AEC001-004) is 1.66%, with a minimum divergence of 0% and a maximum of 3.23%. The average sequence divergence for *A. armada* (ABS001-005, ASN001-006, AIR002-003, AMD001, ADW005-006) is 0.083%, with a minimum divergence of 0% and a maximum of 0.452%. The average sequence divergence for *A.* sp. X (ALA001, AKC001-004, ABG002-003, AHI001) is 1.07%, with a minimum divergence of 0% and a maximum of 2.41% (Table 1). ARC001, a spider from Rucker Canyon in the Chiricahua Mountains of Arizona, was taken out of the *A.* sp. X analyses because even though the mtDNA says it belongs in this species group, morphologically it is a very different spider, and a possible case of mitochondrial introgression. The closest relatives to *A. anax* are *A. armada* and *A.* sp. X. *A. anax* has an average sequence

divergence of 6.75% from *A. armada* and 7.71% from *A.* sp. X. *A. armada* has an average sequence divergence of 5.51% from *A.* sp. X (Table 1).

A. hentzi has an average sequence divergence of 8.82% from *A. anax*, 8.84% from *A. armada*, 9.92% from *A.* sp. X., 11.39% from *A.* sp. Carlsbad Green, and 12.21% from *A.* sp. Amistad Brown. The *A.* sp. Big Bend spider is 8.52% divergent from *A. armada*, 8.94% divergent from *A. anax*, and 9.41% from *A.* sp. X. *A. moderatum* has an average sequence divergence of 7.96% from *A. armada*, 9.09% from *A. anax*, 9.53% from *A.* sp. X, 11.56% from *A.* sp. Carlsbad Green and 12.68% from *A.* sp. Amistad Brown. *A.* sp. Carlsbad Green has an average sequence divergence from *A.* sp. Amistad Brown of 9.16%, 10.82% from *A. armada*, 12.37% from *A.* sp. X, 12.76% from *A. anax*, and 12.79% from *A.* sp. Big Bend. *A.* sp. Amistad Brown has an average sequence divergence of 11.38% from *A. armada*, 11.92% from *A.* sp. Big Bend, 12.86% from *A.* sp. X, and 13.06% from *A. anax* (Table 1).

3.2 Phylogeny

Many different criteria can be used to delimit species, depending on the species concept you are using. I used the tree-based methods for species delineation with DNA data, which recognize species as historical lineages outlined in Wiens and Penkrot (2002) and used by Hendrixson and Bond (2005) and Starrett and Hedin (2007); in conjunction with the percentage of pairwise sequence divergence outlined in Hebert et al (2003) and used in Barrett and Hebert (2005), Hebert *et al* (2003), Hebert *et al* (2004a), Hebert *et al* (2004b), Petersen *et al* (2007), Pons *et al* (2006), and Wiens and Penkrot (2002). In all three analyses, each species clade was recovered as a strongly supported monophyletic group (Figure 3).

3.2.1 A. hentzi

Results from the Bayesian analysis are congruent with both the parsimony and the likelihood analyses, and will therefore be used to present the relationships. The *A. hentzi* clade is strongly supported (100% posterior probability value), with a northern clade and three southern clades making up a southern group. The northern clade consistently includes the

same specimens (AB001, 002, 003, 004, 005; AIR001; ACB001, 002, 003, 004, 005, 006, 007; ARP001, 003, 004; ARB001, 002; AC001, 002, 003, 004; ACO001; ATO001, 002; AOK001, 002, 003; AD001, 002, 003, 004, 005; AGP001; AWA001; ALE001; ARR001; AAU002) in all three topologies. The southern group also consistently includes the same 14 specimens in all three topologies (ARB003, 004, 005; ACP001, 002; ATR001, ADR001, 009, ARP002; AT001; AAU001; ABUOO1; AHY001; ABG001). In the parsimony analysis, the *A. hentzi* clade is strongly supported (100% bootstrap value), with a northern clade and three southern clades. In the likelihood analysis, the *A. hentzi* clade is also strongly supported (100% bootstrap value), with a northern clade and three southern clades. In all three analyses the topologies within the northern and southern groups are the same. These phylogenies show unequivocal support for a monophyletic *A. hentzi* species group. *A. hentzi* is consistently placed as the sister group to the *A.* sp. Big Bend spider and *A. moderatum* where it is strongly supported in the Bayesian analysis (100% posterior probability) and the parsimony analysis (94% bootstrap value), though weekly supported in the likelihood analysis (70% bootstrap value) (Figure 3).

3.2.2 A. sp. Big Bend & *A. moderatum*

In the Bayesian analysis, the Big Bend spider (ABB001) is placed as the sister spider to the *A. hentzi* lineage, though weakly supported (58% posterior probability value). In the likelihood analysis, *A.* sp. Big Bend is weakly considered to be the sister group to *A. moderatum* (60% bootstrap value). And in the parsimony analysis, the Big Bend haplotype is considered the sister group to both *A. hentzi* and *A. moderatum*. *A. moderatum* (ADR002, 004, 005, 006, 011) is strongly supported in the Bayesian analysis (100% posterior probability value), as well as the parsimony analysis (100% bootstrap value) and the likelihood analysis (100% bootstrap value) (Figure 3).

3.2.3 A. sp. Carlsbad Green & *A.* sp. Amistad Brown

The *A.* sp. Carlsbad Green species clade (ASE001, 002, 003, 004, 005, 006; ASR001, 002, 005) and the *A.* sp. Amistad Brown species clade (ADR007, 008, 010; ASV001) are

consistently placed as sister taxa in all three analyses with a Bayesian posterior probability value of 100%, a parsimony bootstrap value of 100%, and a likelihood bootstrap value of 100%. The only discordance in the topologies is that in the parsimony and likelihood analyses *A.* sp. Carlsbad Green and *A.* sp. Amistad Brown represent their own lineage as sister taxa, whereas in the Bayesian analysis they share a common ancestor with *A. hentzi*, *A.* sp. Big Bend, and *A. moderatum* clade. The Bayesian analysis strongly supports the *A.* sp. Carlsbad Green species clade (100% posterior probability value), as does the parsimony analysis (100% bootstrap value) and the likelihood analysis (100% bootstrap value). The Bayesian analysis also strongly supports the *A.* sp. Amistad Brown species clade (100% posterior probability value), as does the parsimony analysis (100% bootstrap value) and the likelihood analysis (100% bootstrap value) (Figure 3).

3.2.4 A. anax

A. anax species (ALG001, 002, 003, 005, 006, 008, 009, 010, 011, 012, 013, 014, 015; AEC001, 002, 003, 004; ACC001, 002; AHA001; ADW001, 002, 003, 004; ASP001), the dominant species in south Texas, is consistently recovered in all three analyses with a 100% Bayesian posterior probability value, 100% MP bootstrap value, and 100% ML bootstrap value. The topologies also recover exactly the same groupings within the species. *A. anax* is strongly supported to be the sister taxon to both *A. armada* and *A.* sp. X in the Bayesian analysis (posterior probability value of 100%), and weekly supported in both the likelihood analysis (60% bootstrap value) and parsimony analysis (72% bootstrap value) (Figure 3).

3.2.5 A. armada & *A.* sp. X

A. armada (ABS001, 002, 003, 004, 005; ASN001, 002, 003, 004, 006; AIR002, 003; ASR003, 004; AMD001; ADW005, 006) and the *A.* sp. X species clade (ALA001; AKC001, 002, 003, 004; ADV001, 002, 003, 004; ABG002, 003; AHI001; ARC001) are consistently placed as sister taxa in all three analyses with a Bayesian posterior probability value of 99%, a parsimony bootstrap value of 59%, and a likelihood bootstrap value of 60%. The Bayesian analysis

strongly supports the *A. armada* species clade (100% posterior probability value), as does the parsimony analysis (100% bootstrap value) and the likelihood analysis (100% bootstrap value). The Bayesian analysis also strongly supports the *A.* sp. X species clade (100% posterior probability value), as does the parsimony analysis (100% bootstrap value) and the likelihood analysis (100% bootstrap value) (Figure 3).

3.3 Divergence Estimates

The molecular data set was shown to be evolving in a significantly non-clocklike manner. The standard deviation of the Euclidean distances, after 10,000,000 states and throwing out the first 1,000,000 for burn-in, exhibited a mean of 1.343. Due to species lineages evolving at different rates, the ages of population expansion were analyzed independently.

The age of population (clade) expansion among lineages equation estimated that the northern clade of *A. hentzi* (the Colorado River basin and everything to the north) split from southern clade(s) (the Colorado River basin and everything to the south) between ~12,630 ybp (at 2.3% per my) and ~19,367 ybp (at 1.5% per my).

While the equation was designed to examine intraspecific population expansion, I used this method to roughly determine the ages that extant populations have been expanding/segregating from an ancestor. The age of the southern clade of *A. hentzi* is between ~421, 516 ybp (at 2.3% per my) and ~646,325 ybp (at 1.5% per my). The *A. moderatum* species clade split from an ancestor between ~19,621 ybp (at 2.3% per my) and ~30,086 ybp (at 1.5% per my). The *A.* sp. Carlsbad Green species clade split from an ancestor between ~47,440 ybp (at 2.3% per my) and ~72,742 ybp (at 1.5% per my). The *A.* sp. Amistad Brown species clade split from an ancestor between ~16,351 ybp (at 2.3% per my) and 25,072 ybp (at 1.5% per my).

The *A. anax* species clade split from an ancestor between ~721,459 ybp (at 2.3% per my) and ~1,106,237 ybp (at 1.5% per my). The *A. armada* species clade split from an ancestor between ~36,258 ybp (at 2.3% per my) and ~55,596 ybp (at 1.5% per my). The *A.* sp. X

species clade split from an ancestor between ~464,126 ybp (at 2.3% per my) and ~711,660 ybp (at 1.5% per my).

3.4 Ecological Niche Modeling

After measuring the goodness of fit of the models, Maxent generated probability distributions for the individual species and from their presence localities and 19 ecological variables (Figure 4). The probability distribution maps indicate the probability that conditions are suitable for the presence of the species being examined (Phillips *et al* 2006).

3.4.1 Present distribution

According to Maxent, the environmental variables that contributed the most to the *A. hentzi* model (Figure 5) were bio11 (mean temperature of coldest quarter) at 25%, bio9 (mean temperature of driest quarter) at 22.2%, bio14 (precipitation of driest month) at 14.8%, bio1 (annual mean temperature) at 9.5%, bio15 (precipitation seasonality (coefficient of variation)) at 9.4%, and bio10 (mean temperature of warmest quarter) at 8.4%. When performing the jackknife test of variable importance, the variable bio9 (mean temperature of driest quarter) was the most useful variable when used by itself in determining the distribution. The AUC (area under the curve) value was 0.992.

The variables that contributed the most to the *A. moderatum* model (Figure 8) were bio1 (annual mean temperature) at 20%, bio10 (mean temperature of warmest quarter) at 19.3%, bio9 (mean temperature of driest quarter) at 18.9%, and bio18 (precipitation of warmest quarter) at 14.2%. When performing the jackknife test of variable importance, the variable bio10 (mean temperature of warmest quarter) was the most useful variable when used by itself in determining the distribution. The AUC value was 1.000.

The variables that contributed the most to the *A.* sp. Carlsbad Green model (Figure 11) were bio9 (mean temperature of driest quarter) at 30.2%, bio8 (mean temperature of wettest quarter) at 25.9%, bio11 (mean temperature of coldest quarter) at 10.8%, and bio2 (mean diurnal range (mean of monthly (max temp - min temp))) at 8%. When performing the jackknife

test of variable importance, the variables bio8 (mean temperature of wettest quarter) and bio19 (mean temperature of warmest quarter) were the most useful variable when used by themselves in determining the distribution. The AUC value was 1.000.

The variables that contributed the most to the *A*. sp. Amistad Brown model (Figure 10) were bio1 (annual mean temperature) at 25.9%, bio17 (precipitation of driest quarter) at 18.1%, bio8 (mean temperature of wettest quarter) at 13.7%, and bio19 (precipitation of coldest quarter) at 9.8%. When performing the jackknife test of variable importance, the variable bio19 (precipitation of coldest quarter) was the most useful variable when used by itself in determining the distribution. The AUC value was 0.970.

The variables that contributed the most to the *A. anax* model (Figure 6) were bio11 (mean temperature of coldest quarter) at 29.5%, bio17 (precipitation of driest quarter) at 25.2%, bio10 (mean temperature of warmest quarter) at 12.1%, and bio18 (precipitation of warmest quarter) at 9.8%. When performing the jackknife test of variable importance, the variables bio1 (annual mean temperature) and bio10 (mean temperature of warmest quarter) were the most useful variable when used by themselves in determining the distribution. The AUC value was 0.999.

The variables that contributed the most to the *A. armada* model (Figure 7) were bio11 (mean temperature of coldest quarter) at 32.5%, bio9 (mean temperature of driest quarter) at 24.6%, bio8 (mean temperature of wettest quarter) at 11.2%, and bio14 (precipitation of driest month) at 8.3%. When performing the jackknife test of variable importance, the variable bio9 (mean temperature of driest quarter) was the most useful variable when used by itself in determining the distribution. The AUC value was 0.991.

The variables that contributed the most to the *A*. sp. X model (Figure 9) were bio6 (minimum temperature of coldest month) at 48.5%, bio8 (mean temperature of wettest quarter) at 20.7%, and bio14 (precipitation of driest month) at 13.9%. When performing the jackknife test of variable importance, the variable bio6 (minimum temperature of coldest month) was the

most useful variable when used by itself in determining the distribution. The AUC value was 0.970.

3.4.2 Pleistocene distribution (21,000 ybp)

In order to identify likely points of contact between the *Aphonopelma* species and how the contact zones might have been influenced by past glacial cycles, paleodistribution niche modeling was used to estimate historical species distributions (Figure 12). The environmental variables that contributed the most to the *A. hentzi* model (Figure 13) were bio11 (mean temperature of coldest quarter) at 34.4%, bio9 (mean temperature of driest quarter) at 19.3%, bio14 (precipitation of driest month) at 15.9%, and bio15 (precipitation seasonality (coefficient of variation)) at 10.4%. When performing the jackknife test of variable importance, the variables bio9 (mean temperature of driest quarter) and bio10 (mean temperature of warmest quarter) were the most useful variables when used by themselves in determining the distribution. The AUC (area under the curve) value was 0.991.

The variables that contributed the most to the *A. moderatum* model (Figure 16) were bio1 (annual mean temperature) at 28.9%, bio9 (mean temperature of driest quarter) at 21.7%, bio18 (precipitation of warmest quarter) at 16%, and bio10 (mean temperature of warmest quarter) at 15.8%. When performing the jackknife test of variable importance, the variable bio10 (mean temperature of warmest quarter) was the most useful variable when used by itself in determining the distribution. The AUC value was 1.000.

The variables that contributed the most to the *A.* sp. Carlsbad Green model (Figure 19) were bio9 (mean temperature of driest quarter) at 32%, bio8 (mean temperature of wettest quarter) at 24.7%, bio11 (mean temperature of coldest quarter) at 19.4%, and bio13 (precipitation of wettest month) at 9.4%. When performing the jackknife test of variable importance, the variables bio8 (mean temperature of wettest quarter) and bio19 (mean temperature of warmest quarter) were the most useful variable when used by themselves in determining the distribution. The AUC value was 1.000.

The variables that contributed the most to the *A.* sp. Amistad Brown model (Figure 18) were bio1 (annual mean temperature) at 28.9%, bio17 (precipitation of driest quarter) at 21.4%, bio8 (mean temperature of wettest quarter) at 17.4%, bio18 (precipitation of warmest quarter) at 13.6%, and bio9 (mean temperature of driest quarter) at 11.6%. When performing the jackknife test of variable importance, the variables bio19 (precipitation of coldest quarter) and bio8 (mean temperature of wettest quarter) were the most useful variables when used by themselves in determining the distribution. The AUC value was 0.962.

The variables that contributed the most to the *A. anax* model (Figure 14) were bio11 (mean temperature of coldest quarter) at 34.6%, bio17 (precipitation of driest quarter) at 25.3%, bio10 (mean temperature of warmest quarter) at 9.4%, and bio18 (precipitation of warmest quarter) at 8.9%. When performing the jackknife test of variable importance, the variables bio1 (annual mean temperature) and bio10 (mean temperature of warmest quarter) were the most useful variables when used by themselves in determining the distribution. The AUC value was 0.999.

The variables that contributed the most to the *A. armada* model (Figure 15) were bio11 (mean temperature of coldest quarter) at 31.9%, bio9 (mean temperature of driest quarter) at 28.8%, bio8 (mean temperature of wettest quarter) at 13.8%, and bio14 (precipitation of driest month) at 9%. When performing the jackknife test of variable importance, the variable bio9 (mean temperature of driest quarter) was the most useful variable when used by itself in determining the distribution. The AUC value was 0.991.

The variables that contributed the most to the *A*. sp. X model (Figure 17) were bio8 (mean temperature of wettest quarter) at 41.6%, bio9 (mean temperature of driest quarter) at 16.2%, bio4 (temperature seasonality (standard deviation *100)) at 14.1%, bio18 (precipitation of warmest quarter) at 8.2%, and bio14 (precipitation of driest month) at 6.8%. When performing the jackknife test of variable importance, the variables bio8 (mean temperature of

wettest quarter) and bio11 (mean temperature of coldest quarter) were the most useful variables when used by themselves in determining the distribution. The AUC value was 0.964.

3.5 Discriminant Function Analysis

A discriminant function derived from the climate variables for *Aphonopelma* was correct 87% of the time in classifying individuals (Wilks' Lambda = 0.007; P < 0.000). Forty-eight out of 52 (92%) individuals of *A. hentzi* were classified correctly as being present in their predicted distribution due to the climate variables. Two *A. hentzi* were incorrectly classified as *A. moderatum*, and two were classified incorrectly as *A.* sp. X. Twenty-five out of 25 (100%) individuals of *A. anax* were classified correctly as being present in their predicted distribution due to the climate variables. Eleven out of 17 (65%) individuals of *A. armada* were classified correctly as being present in their predicted distribution due to the climate variables. Two *A. armada* were incorrectly classified as *A. hentzi*, two were incorrectly classified as *A.* sp. Carlsbad Green, and two were incorrectly classified as *A. anax*. Ten out of 14 (71%) individuals of *A*. sp. X were classified correctly as being present in their predicted distribution due to the climate variables. Two *A.* sp. X were incorrectly classified as *A. anax*, one was incorrectly classified as *A.* sp. Amistad Brown, and one was incorrectly classified as *A.* sp. Carlsbad Green. Nine out of 9 (100%) individuals of *A.* sp. Carlsbad Green were classified correctly as being present in their predicted distribution due to the climate variables. Five out of 5 (100%) individuals of *A. moderatum* were classified correctly as being present in their predicted distribution due to the climate variables. One out of 4 (25%) individuals of *A.* sp. Amistad Brown were classified correctly as being present in their predicted distribution due to the climate variables. One *A.* sp. Amistad Brown was incorrectly classified as *A.* sp. X, and two were incorrectly classified as *A. moderatum*.

3.6 Qualitative

3.6.1 Fecundity data

Five females laid eggsacs in the lab after being collected (ACB002, ACB003, AC003, AC004, ALG013), one eggsac was collected with the female (AB005), and one eggsac was collected after it was found abandoned in the scrape (ACB004). The number of spiderlings from each spp. eggsac are as follows: *A. hentzi*: ACB002 = 648; ACB003 = 609; ACB004 = 181; AB005 = 470; AC003 = 626; AC004 = 785, *A. anax*: ALG013 = 393.

3.6.2 Wasp predation event

The main predators of juvenile and adult *Aphonopelma* are small insectivorous mammals and parasitoid wasps of the family Pompilidae. During my fieldwork, a *Pepsis* sp. predation event was witnessed on a juvenile *A. moderatum*. This specimen was being dragged in the open, in the midst of typical Chihuahuan desert scrub, back to the wasps' burrow. I rescued the spider, checked it for any eggs that may have been laid on it, and stored it in a travel container. At the time of the attack, the spider was paralyzed but with a faint ability to move its metatarsi. Anecdotal evidence about the effects of the venom on the spider and advice on how to take care of the spider until the venom effects have worn off (Breene *et al* 1996; Moellendorf *pers. comm.*) allowed me to document the effects of the venom on the spider. The spider was set up in a small plastic container with ~1 inch of humid shredded coconut coir substrate, and stored in a warm, dark area. Every week the spider was checked on and while laying on its back (dorsal side) a small drop of water was placed on the chelicerae area. The spider was completely paralyzed for the first month, slowly regaining the ability to move its legs, but only in a waving manner. The spider could not walk or move itself, and if put on its dorsal side it did not have the ability to right itself. At the three month mark, the spider was finally able to erratically walk and right itself. Soon after that, the spider ate its first meal, a cricket. After eating a few meals, the spider acted as if it was fully over the effects of the venom.

CHAPTER 4

DISCUSSION

With hopes of resolving the species boundaries for a group of tarantulas that were thought to have recently diverged, molecular and ecological data were used to examine the patterns and degree of genetic variation of the *Aphonopelma* in Texas, with the objective of assessing the relative importance of the Pleistocene in shaping distribution and diversification patterns. It was proposed that the general tendency of *Aphonopelma* would be to maintain their ecological niche and that the failure to adapt to new environments enforced isolation and allowed for speciation in the group.

4.1 Phylogenetics

Because many different criteria can be used to delimit species and due to the many different views on which one to use, I chose a conservative method by applying DNA tree-based methods, in conjunction with percentage of pairwise sequence divergence compared between species groups/clades, in order to recognize the historical lineages. This method was shown to be highly effective in this group of spiders. In all three phylogenetic analyses, each species clade was recovered as a strongly supported monophyletic group (Figure 3). When analyzing the uncorrected p-distances, all "species" were separated by ≥ 5% divergence (Table 1). I propose that eight species can be delimited in this analysis, with 4 being new/undescribed, throughout Texas.

According to the Bayesian analysis, two major branches of *Aphonopelma* were found in Texas: one including *A. anax*, *A. armada*, and *A.* sp. X (100% posterior probability), and a second group including *A. hentzi*, *A.* sp. Big Bend, *A. moderatum*, *A.* sp. Amistad Brown, and *A.* sp. Carlsbad Green. This first branch represents a group of two older species in south Texas, *A. anax* and *A.* sp. X, with a relatively new lineage that spread north, *A. armada*. The second

branch represents a group of five species, three of which are relatively new to the biological scene in Texas and one which has a northern clade that very recently began expanding away from the southern clade. These species include: *A. hentzi*, *A.* sp. Big Bend, *A. moderatum*, *A.* sp. Carlsbad Green, and *A.* sp. Amistad Brown (Figure 3).

The *A. anax* clade, a large, bulky spider with short uniform setae, was collected throughout the Coastal Plain in Texas (Figure 21). *A. anax* shares morphological similarities, both in spermathecal structure and palpal bulb morphology, with the North American *Aphonopelma* sister group, the red-legged *Brachypelma* from Mexico, indicating that the ancestor of this species most likely originated in Mexico before dispersing north. The Bayesian topology shows that *A. armada* and *A.* sp. X originated from a common ancestor with *A. anax* (99% posterior probability). Both of those species are significantly divergent both visually and molecularly. Prior to this analysis, *A. anax* would have easily been considered the dominant species in south Texas. And while that is true along the coastal Plain, another spider, one that seems to have been frequently overlooked in the past, is the dominant species in south Texas (west of the coastal Plain and into Mexico and New Mexico). *A.* sp. X, a potential new species, is a large-bodied spider as well, though instead of looking like *A. anax*, it closely resembles *A. hentzi*, though quite a bit larger and frequently referred to as an *A. hentzi* on steroids by myself (Figure 24). It has an average sequence divergence from *A. anax* of 7.71% (Table 1). The specimens collected ranged over a large distribution that extends from southeast of San Antonio, across Texas, west towards the “sky islands” in southwest New Mexico. *A.* sp. X has often been referred to as *A. echinum* by collectors. This is probably due to the incredibly close visual resemblance it has to *A. hentzi*, the spider with which *A. echinum* needs to be synonymized, information based off of morphological and molecular data (Murray 2006; Hendrixson *pers. comm.*). *A. armada* is a spider smaller than either *A. anax* or *A.* sp. X, from central and west Texas, and differentiated from other species by having much longer, thinner legs, much shorter overall setae without the “hairy” appearance, and possessing a distinct black

patch of urticating hairs on the dorsal side of its abdomen (Figure 22). It has an average sequence divergence from *A. anax* of 6.75% and 5.51% from *A.* sp. X (Table 1).

The second clade includes *A. hentzi*, *A.* sp. Big Bend, *A. moderatum* with strong support (100% posterior probability) as a group, as well as *A.* sp. Carlsbad Green and *A.* sp. Amistad Brown with less than strong support (67% posterior probability). In both the likelihood and parsimony analyses, these two species represented their own third species clade. Though weakly supported in the Bayesian analysis, they are both highly divergent not only from each other but from all other species in the analysis as well. *A.* sp. Carlsbad Green (Figure 26) has an average sequence divergence of 9.16% from *A.* sp. Amistad Brown and ≥ 10% divergent from every other species (Table 1). *A.* sp. Amistad Brown has an average sequence divergence of ≥ 11% from every other species (Table 1).

A. hentzi's closest sister taxa in this analysis are *A.* sp. Big Bend and *A. moderatum*. *A.* sp. Big Bend, while only represented by one specimen, is a fascinating spider in a number of ways. This spider, basal to the *A. hentzi* clade, visually represents what would be considered *A. hentzi*. It also looks identical to the *A.* sp. X, which is found sympatrically. The average sequence divergence between *A. hentzi* and *A.* sp. Big Bend is 5.65%, and *A.* sp. Big Bend is 9.41% divergent from *A.* sp. X (Table 1). Based on the 3% divergence cutoff, this spider, *A.* sp. Big Bend should be considered a separate species from *A. hentzi*. Is this incomplete lineage sorting from the *A. hentzi* ancestor? The southern lineage of *A. hentzi* is a very old tarantula lineage in this region, so probably not. Could it be that this is not an undescribed species, instead just representing an old lineage of *A. hentzi*? It could, but that would be contrary to the 3% divergence cutoff. The next closest taxa to *A. hentzi*, *A. moderatum* has an average of 6.96% divergence to *A. hentzi* and 5.84% to *A.* sp. Big Bend (Table 1). Increasing the % divergence cutoff to include *A.* sp. Big Bend in the *A. hentzi* clade, representing the basal most lineage, would be problematic in terms of the relationship to *A. moderatum*, where one spider is considered a part of the *A. hentzi* species at 5.65% divergence and another is not at 5.84%

(Table 1). The support (58% posterior probability) for *A.* sp. Big Bend being included in *A. hentzi* is also problematic due to the low support, though this could potentially increase with more specimens included in the future. Visually *A. moderatum* (Figure 23) is a very distinct spider from *A. hentzi* and *A.* sp. Big Bend. It also exhibits an ecological shift in distribution south along the border. While it can be found sympatrically with *A. hentzi* in Del Rio, this area is close the western border of its range and looks to be the southeastern border of *A. hentzi*.

A. hentzi, is the dominant species found throughout central and north Texas, ranging into Oklahoma, Kansas, southeastern Colorado, as well as western Missouri, Arkansas and parts of northwestern Louisiana (Figure 20). Often referred to as "The Texas Brown tarantula", that boring non-descript name belies the truly impressive biology and evolution of this spider. The humble beginnings of *A. hentzi* seem to have started in south Texas. The specimens collected for this study range from Del Rio, a border town southwest of San Antonio, the Black Gap Wildlife Management Area near Big Bend, and Terlingua, all the way up to the Austin area, most of central Texas near Brady, the Ivie Reservoir, Colorado Bend State Park, Waco, to north Texas sites in Dallas, Grand Prairie, and into Oklahoma and Colorado. This entire distribution has an average sequence divergence of only 2.756%. When looking at the Bayesian topology, two main groups of *A. hentzi* emerge; one large polytomic clade of specimens within the Colorado River Basin and everything to the north, and a group of related specimens encompassing the Colorado River basin and everything to the south. When these two groups are broken down, the evolutionary history of this species begins to take shape. The average sequence divergence for the specimens in the southern group is 0.97%, but the average sequence divergence for the specimens in the northern group is 0.03% over that entire range. This is a significant difference and one that can be explained by both ecological and biogeographical evidence.

The population expansion dates estimated in this analysis show how the dominant southern lineages of *Aphonopelma* (*A. anax*, *A.* sp. X, and the southern group of *A. hentzi*) have

been living in the region since early in the Pleistocene. *A. anax*, the oldest lineage in Texas, split from an ancestor between 1,106,000 and 721,000 ybp, would have had a much larger potential distribution due to the fluctuating sea levels increasing the coastal Plain in Texas and northern Mexico. *A.* sp. X (from 711,000 to 460,000 ybp) would have had shared large distribution throughout northern Mexico and southwest Texas with the ancestral lineage of *A. hentzi*, which split from an ancestor between 646,000 ybp and 421,000 ybp. *A.* sp. Big Bend could have also been sympatric in this region, but due to only having one specimen I could not use the divergence dating technique. These lineages could have made up an ancestral group of cryptic species that dominated these niches until the numerous vicariance events of Pleistocene drove the divergence of the tarantulas in the region, resulting in the lineages *A. armada*, *A. moderatum*, *A.* sp. Amistad Brown, and *A.* sp. Carlsbad Green seen today. The shifting of habitat locale and composition caused splits of ancestral populations in the *A.* sp. Carlsbad Green lineage between 72,000 and 47,000 ybp; *A. armada* between 55,000 and 36,000 ybp; *A. moderatum* between 30,000 ybp and 19,000 ybp; *A.* sp. Amistad Brown between 25,000 and 16,000 ybp; and the northern clade of *A. hentzi* between 19,000 and 12,000 ybp.

With the Trans-Pecos region of Texas considered the most topographically varied region in Texas (Hoyt 2000), it has provided a diverse number of niches. These dates suggest that central to southern Texas appears to have been an area of Pleistocene refugia for *Aphonopelma*. One possible explanation to what is driving the differences between the two clades of *A. hentzi* and the speciation of the other species is vicariance driven by the late Pleistocene-Holocene warming, drying, and vegetation change that separated once contiguous populations. These dates and sequence divergences confirm the hypothesized rapid range expansion for *A. hentzi*, as well as the predicted low levels of genetic diversity that the northern populations (north of the Colorado River basin), a subset of the genetic diversity seen in the southern portions of the range, would express if they were impacted by the Pleistocene glacial cycles. This data roughly confirms the dates used in Janowski-Bell (2001), which believed *A.*

hentzi dispersed into Missouri approximately 8,000-4,000 ybp, based on dates of when glades were created by invading forests, thus isolating groups of organisms.

The lack of phylogenetic resolution in the *A. hentzi* clade and the short branch lengths indicate a very recent divergence (Figure 3). The star-like pattern combined with the widely distributed haplotypes support a rapid expansion from southern refugia, with the Colorado River basin (Figure 2) representing the main area. The large genetic diversity between the *A. hentzi* southern and northern populations suggests that the ancestral population had a high genetic diversity due to a long history where divergent haplotypes accumulated, as seen in similar findings by Mulcahy and Mendelson (2000). After a rapid expansion, it is difficult for populations and genomes behind the expansion to advance, forcing them to diffuse at a much slower rate unless they possess some distinct selective advantage that suits a significant change in environment (summarized in Hewitt 2001). Because *A. hentzi* seems to be highly ecologically "plastic", perhaps there are no ecological barriers to gene flow in the northern populations until the furthermost northern distribution is reached.

4.2 Ecological Niche Modeling

Integrating phylogeographic and ecological niche modeling has been shown to provide new insights into the processes driving speciation, particularly the role of local adaptation to environmental variables in allopatric divergence (summarized in Wiens 2004b). These variables have been shown to cause a variety of mygalomorph spiders (*Aptostichus* spp., *Atypus* spp., *Macrothele calpeiana*, *Promyrmekiaphila* spp.) to exhibit differences in habitat preference, when variables like temperature, precipitation, soil characteristics, soil pH, and vegetation cover were analyzed (Jiménez-Valverde and Lobo 2006; Řezáč *et al* 2007; Stockman and Bond 2007; Bond and Stockman 2008). Based on Stockman and Bond (2007), both ecological and genetic divergence should be used to determine species status.

Unfortunately, the niche modeling fails to reasonably predict accurate distributions for the species with which at least some distribution information was known. The probability

distribution modeled by Maxent for *A. hentzi*, represents a small distribution that is not indicative of the known *A. hentzi* distribution, by failing to predict most of its northern and northwestern range even with sample points in Colorado and northern Oklahoma (Figure 5). The environmental variables that were shown to be contributing the most to predicting the distribution of *A. hentzi*, mean temperature of the coldest quarter and mean temperature of the driest quarter, predictably answer why the northern distribution is severely restricted. Throughout most of the northern range, the long-cold winters, coupled with winter being the driest time period of the year in these habitats, are what presumably withhold a further northern dispersal of *A. hentzi*. *A. hentzi* can be found sympatrically with *A. moderatum*, *A.* sp. Amistad Brown, *A. armada*, *A.* sp. Big Bend, and *A.* sp. X. This result suggests that different environmental factors may be important at the extremes of the study area. This is particularly relevant because the analysis tried but did not cover the full geographic distribution of the northern group and may not have summarized the full ecological range.

The predicted distribution for *A. anax* predicts a small distribution, encompassing south Texas, failing to predict known distribution areas of *A. anax* in south Texas (Figure 6). The environmental variables that were shown to be contributing the most to predicting the distribution of *A. anax*, mean temperature of the coldest quarter, precipitation of the driest quarter, annual mean temperature, and mean temperature of the warmest quarter, explain the predicted and known distribution of *A. anax* by suggesting that these variables limit this spiders' dispersal away from the coastal Plain where it would potentially be too dry for it to survive, and limiting its dispersal to the north where it would be too cold for it to survive the winters. It is known to occur sympatrically with *A.* sp. X and *A. armada*.

The probability distribution for *A. armada* predicts a distribution that encompasses most of the sampled areas and covers most of central Texas and drifting into west Texas (Figure 7). This distribution seems to fit with what I have seen and heard about the distribution of this spider. The predicted distribution does drift into north Texas, which does not exist on the

ground. This spider is limited by the Colorado River basin and cannot be found to the north. A problem with the predicted distribution is that the model does not predict *A. armada* to occur in a region where it is known to exist, as seen by the DeWitt County sample points. The environmental variables that were shown to be contributing the most to predicting the distribution of *A. armada*, mean temperature of the coldest quarter and mean temperature of the driest quarter, could explain why this species is limited in dispersing to the east and southeast of Texas. This explanation could represent why the furthermost southeastern samples, in DeWitt County, were not predicted to be suitable habitat and could represent eastern edge of the *A. armada* distribution. It is known to occur sympatrically with *A. anax*, *A. hentzi*, and *A.* sp. Carlsbad Green.

The probability distribution for *A.* sp. X predicts a very large distribution that spans from southeast Texas to western New Mexico (Figure 9). Because not much is known about this spider, there is no known distribution to compare to the one predicted by the model. What seems fairly evident, based upon my knowledge and experience, is that this spider does not range as far north as it is predicted. This spider seems to be restricted to southwest Texas, with a finger-like projection east to just southeast of San Antonio. Obviously much more sampling is needed before fully understanding the dynamics of this species' distribution. The environmental variables that were shown to be contributing the most to predicting the distribution of *A.* sp. X, minimum temperature of the coldest month and mean temperature of the wettest quarter, should explain what would limit the dispersal of this species to the north and the southeast. It is known to occur sympatrically with *A. anax*, *A. hentzi*, and *A.* sp. Big Bend.

The *A. moderatum* and *A.* sp. Carlsbad Green predicted distributions are problematic due to their incredibly small distribution size and localized predicted niche. The probability distribution for *A. moderatum* predicts a very small, localized distribution, from west of Del Rio southeast along the border (Figure 8). *A. moderatum* is known to be found in a swath down the border of Texas and into northern Mexico, though the species is thought to have smaller

distribution relative to the other known sympatric species (*A. hentzi*, *A.* sp. Amistad Brown, and potentially *A. anax*). The environmental variables that were shown to be contributing the most to predicting the distribution of *A. moderatum*, annual mean temperature, mean temperature of the warmest quarter, and mean temperature of the driest quarter, explain the limited Chihuahuan desert range that this species seems to have.

The probability distribution for *A.* sp. Carlsbad Green shows a very small, patchy distribution along the border of Texas and New Mexico (Figure 11). *A.* sp. Carlsbad Green is known to range further southwest and west, both in Texas and in New Mexico. It is known to occur sympatrically with *A. armada*. The environmental variables that were shown to be contributing the most to predicting the distribution of *A.* sp. Carlsbad Green, mean temperature of the driest quarter, mean temperature of the wettest quarter, mean temperature of the warmest quarter, exhibit why this species is limited to far west Texas and southeastern New Mexico.

The sister taxa *A.* sp. Carlsbad Green and *A.* sp. Amistad Brown are not known to have an overlapping distribution. The probability distribution for *A.* sp. Amistad Brown predicts a very large distribution, encompassing southwest Texas, along the border, parts of New Mexico and northern Mexico (Figure 10). From my collecting and information from others who have collected this species, the distribution is concentrated along the border with Mexico, not dispersing very far north into Texas. The problems associated with this distribution could be attributed to only four sample points being used in the modeling. The environmental variables that were shown to be contributing the most to predicting the distribution of *A.* sp. Amistad Brown, annual mean temperature, precipitation of the driest quarter, and precipitation of the coldest quarter, show a spider that is restricted to Chihuahuan desert habitat and should not disperse very far north into Texas due to winter extremes.

The *A. anax* and *A. hentzi* distributions are problematic because they are centered on areas of high-density sampling. Maxent is supposedly able to accurately predict a species

distribution from as small as five representative samples (Elith *et al* 2006; Pearson *et al* 2006; Reed *et al* 2008). Some of this data does not seem to agree with that assumption. It could be possible that both of these species exhibit such wide latitude in their habitat preference, that Maxent does a poor job of recognizing the true distribution. *A. anax* is known to range into east Texas, having been found both at the edge of and inside pine forest habitat, though rare. This suggests that because this spider would not have to worry about the effects of a dry environment, it may allow the spider to survive in cooler winter extremes. This failure of the Maxent model to accurately predict the *A. hentzi* distribution is evidence that this species is quite "ecologically plastic" with an ability to survive in a wide-range of habitats, and that given the opportunity at the end of the Pleistocene, this spider rapidly spread into new niches, dominating the landscape and outcompeting other *Aphonopelma* species.

According to Phillips *et al* (2006), the AUC (area under the curve) values tend to be higher for species with narrow ranges, relative to the study area described by the environmental data. The species with narrow ranges that fall into this cautionary group are *A. moderatum* with an AUC of 1.000 and *A.* sp. Carlsbad Green with an AUC of 1.000. *A. hentzi* (0.992) and *A. anax* (0.999) fall into the group of species with high AUC values, which could be due to the model underpredicting their actual ranges.

According to Carstens and Richards (2007), better representative sampling is needed for the ecological niche modeling because areas may be overpredicted or underpredicted due to inadequate or biased sampling. Future research on this group of tarantulas is going to be needed, making sure to sample from the best representative areas of these species' distributions as possible in order to limit prediction errors.

Paleodistribution niche modeling was used to estimate the historical species distributions, for the *Aphonopelma* in this region, in order to determine how they were influenced by the Pleistocene glacial cycles (Figure 12). The paleoclimate probability distributions for *A. hentzi* and *A. moderatum* consistently shifted their ranges to the south. It was predicted that *A.*

hentzi would essentially occupy the same small distribution, with a slight shift to the south of the southernmost range border (Figure 13). The probability distribution for *A. moderatum* predicts a shift in distribution south and west into northern Mexico (Figure 16). To get a more accurate reading of the *A. hentzi* shift, modeling should be carried out to include only the southern clade specimens, because the northern specimens were obviously not occupying the distributions that they are today. The same technique could be carried out in an attempt to model more accurately the present day southern and northern niches, by splitting them into their own separate analyses.

According to the paleoclimate modeling *A.* sp. Amistad Brown, *A. anax*, *A. armada*, and *A.* sp. X all incurred a favorable shift in their distributions to the north, contrary to what would have been expected. The probability distribution for *A.* sp. Carlsbad Green predicts essentially the same restricted distribution (Figure 19). The probability distribution for *A.* sp. Amistad Brown predicts a slightly more favorable distribution throughout its range and a slight shift to the north (Figure 18). The probability distribution for *A. anax* predicts a slightly more favorable distribution, with a very small expansion to the south and west (Figure 14). The probability distribution for *A. armada* predicts an expansion of the niche to encompass most of central, south, and west, as well as a favorable habitat expansion north into the panhandle of Texas (Figure 15). The probability distribution for *A.* sp. X predicts a large expansion of the distribution in all directions (Figure 17). This suggests that these species could have experienced range expansions during the Pleistocene when a number of speciation events were occurring in this group of *Aphonopelma*, based on the expansion dating, and only recently have been experiencing range contractions. This would seem to be contrary to what is happening in the *A. hentzi* lineage and a possible explanation as to why *A. hentzi* is such a dominant species in the Colorado River basin and areas to the north.

The paleoclimate modeling seems to fail at predicting correct distributions . The efficacy of these modeled distributions cannot be determined, but certainly seem to disagree

with the known dynamics of habitat shifts during the Pleistocene. I suggest that on numerous times during this period, highly favorable refugia existed throughout this region where competition and other factors drove speciation in this group.

There has been considerable debate regarding whether the differences seen between the predicted distributions of ecological niche modeling and phylogeographic results are due to the effects of biotic interactions between species (realized niche) and to what degree the models capture these interactions (summarized in Stockman and Bond 2007; summarized in Waltari *et al* 2007). The difficulty of Maxent to correctly predict the known *A. hentzi* and *A. anax* distributions could be due to the question of whether the modeling is actually determining the fundamental or the realized niche. The realized niche has been proposed to be what Maxent is predicting in these analyses (Phillips *et al* 2006). But, if the analysis were able to predict the realized niche, it should have no problem predicting the northern range of *A. hentzi*, where there are no other tarantula species to compete with. Thus the Maxent models may be underestimating the true environmental range of the *Aphonopelma* species in Texas.

In order to determine, with statistical significance, whether the environmental variables used in Maxent were limiting/preventing where the members of the *a priori* species groups are found/could be potentially found, a DFA was performed which showed that the species were correctly classified 87% of the time as being present in their predicted distribution due to the climate variables (Wilks' Lambda = 0.007; $P < 0.000$).

A number of surprising results were found, including that for most of the species' misclassifications, they occurred with species that were found sympatrically at some point in their distribution. Forty-eight out of 52 (92%) *A. hentzi* individuals were classified correctly as being present in their predicted distribution due to the climate variables. Two *A. hentzi* were incorrectly classified as *A. moderatum*, and two were classified incorrectly as *A.* sp. X, which is not surprising due to these species being sympatric in part of their ranges. Ten out of 14 (71%) individuals of *A*. sp. X were classified correctly as being present in their predicted distribution

due to the climate variables. Two *A.* sp. X were incorrectly classified as *A. anax*, one was incorrectly classified as *A.* sp. Amistad Brown, and one was incorrectly classified as *A.* sp. Carlsbad Green. The *A. anax* misclassification is not surprising due to the two species being sympatric in a part of their range. Eleven out of 17 (65%) individuals of *A. armada* were classified correctly as being present in their predicted distribution due to the climate variables. Two *A. armada* were incorrectly classified as *A. hentzi*, two were incorrectly classified as *A.* sp. Carlsbad Green, and two were incorrectly classified as *A. anax*.

Three finding are interesting because they were classified correctly 100% of the time, *A. anax*, *A.* sp. Carlsbad Green, and *A. moderatum*. This is interesting because all three of these species can be found in relatively unique habitats and nowhere else, as confirmed by the niche modeling. *A. anax* individuals were classified correctly 25 out of 25 (100%) times as being present in their predicted distribution due to the climate variables. This is surprising due to *A.* sp. X being found sympatrically with *A. anax*. Nine out of 9 (100%) individuals of *A.* sp. Carlsbad Green and 5 out of 5 (100%) individuals of *A. moderatum* were classified correctly as being present in their predicted distribution due to the climate variables.

One out of 4 (25%) individuals of *A.* sp. Amistad Brown were classified correctly as being present in their predicted distribution due to the climate variables. One *A.* sp. Amistad Brown was incorrectly classified as *A.* sp. X, and two were incorrectly classified as *A. moderatum*. One reason for this could be the small sample size of individuals used, four instead of the recommended ≥ five. Both of the species that *A.* sp. Amistad Brown was incorrectly classified as are species that can be found along the border in south Texas. It's known that *A. moderatum* and *A.* sp. Amistad Brown are sympatric in the Del Rio area, but whether *A.* sp. Amistad Brown is sympatric with *A.* sp. X is unknown as of now, though the probability is high based on the areas they have been collected from and the niche modeling results.

Unfortunately, an issue with ecological niche modeling is that it assumes a species is in equilibrium with its environmental requirements and that its distribution is mainly determined by the environment and not by other factors such as competition or dispersal limitations. This problem can lead to incorrect assumptions based on a DFA analysis.

Scrutinizing the phylogenetic, ecological niche modeling, and DFA data allows us an attempt at determining the mode of speciation that has been driving divergence in the *Aphonopelma* throughout Texas. I propose that both allopatric and parapatric speciation can been witnessed in this group of spiders. The species living sympatrically could be segregating themselves ecologically by preferring burrow sites based on differences in microhabitats along a cline in a larger habitat. Differences that I have witnessed include choosing free-standing burrows versus scrapes and shifts in soil type/structure and vegetation, which end up creating pockets (metapopulations) of species throughout the same area. Burrows of adult and juvenile *A. hentzi, A. anax, A. armada, A. moderatum, A.* sp. Carlsbad Green, as well as *A.* sp. X were witnessed to be closely aggregated suggesting that offspring settle near their maternal burrow in a preferred habitat, though potentially not as close as the *Atypoides riversi* seen by Ramirez and Chi (2004) due to tarantulas' larger size and ability to cover more ground.

South Texas, an area consistently populated by these *Aphonopelma* lineages throughout the Pleistocene, provided refugia through climatic contractions and expansions, allowing genomes to survive and diverge without large geographical displacement. The shifts in habitat during the Pleistocene provided numerous chances for vicariant events to occur, thereby driving speciation and setting up the ecological niches that we see today.

As Bond and Stockman (2008) state, an overlap of the predicted species' distributions could suggest evidence of niche conservatism among sister species or lineages. Because of the high overlap of predicted distributions, I propose that allopatric speciation with niche conservatism occurred in the lineages of *A.* sp. X, *A.* sp. Big Bend, and *A. anax*. These species represent "older" lineages in south Texas that were not able to tolerate the climatic conditions of

central and north Texas as the habitats changed during the Holocene. If the climate across a barrier became unsuitable, thereby limiting gene flow between populations, niche conservatism would play a significant role in speciation. According to Wiens (2004a) allopatric speciation through geographic isolation is often associated with niche conservatism.

A. hentzi, while having a southern group representing one of the "older" lineages, violates allopatric speciation due to its incredible ecological plasticity. I propose that the *A. hentzi* lineage split from its ancestral population due to parapatry. Assuming the interactive role of biotic and abiotic factors, the data also points to parapatric speciation in the lineages of *A. armada*, *A.* sp. Carlsbad Green, *A.* sp. Amistad Brown, and *A. moderatum*, as populations were fragmented and isolated the during contractions and expansions and adapted to different niches.

4.3 Qualitative findings

The new/undescribed species that were found in this analysis (*A.* sp. X, *A.* sp. Carlsbad Green, *A.* sp. Big Bend, and *A.* sp Amistad Brown) cannot be *A. steindachneri* (Ausserer, 1875) (valid California species), *A. helluo* (Simon, 1891) (valid Baja California species), or *A. pseudoroseum* (*nomina dubium*), all of which had previously been thought to possibly range into Texas, because of the analysis of Prentice (1997). I predict upon further analysis that there will be an *A. anax* complex in Texas, which will include the species *A. breenei* (Smith, 1995) and *A. harlingenum* (Chamberlin, 1940) needing to by synonymized.

A. heterops (Chamberlin, 1940), also described from Texas could very well end up being the same spider as *A. moderatum*, which has three color forms; a dark-banded form near Del Rio, a blonde form near where *A. heterops* is described from, and a transitional form in between near Uvalde that has dark bands on the dorsal side of the femurs but no bands on the ventral side of the femurs.

Two species previously thought to occur in Texas are both mystery spiders unfortunately, *A. texense* (Simon, 1891) and *A. mordax* (Ausserer, 1871) (Breene *et al* 1996).

A. texense is presumed to not occur in Texas (Longhorn *pers. comm.*). Based on preliminary morphology and molecular analysis of AHY001 (Hayes County, Texas, south of Austin), *A. mordax* could represent another species needing to be synonymized with *A. hentzi*, with this spider consistently grouping with the rest of the *A. hentzi* clade.

Based on interesting observations through discussions with other arachnologists, hobbyists collecting in the field, pictures of specimens, and one spider from Terlingua, Texas in this analysis (ATR001), there is a thought that a dwarf *Aphonopelma* species occurs in the Davis Mountains and other areas of southwest Texas. This spider, collected by Dave Moellendorf, had been kept in captivity as a pet for six years prior to being used in this study, molting every year and never growing larger than when it was collected. Visually, it looks like a very small *A. hentzi*, with the same body proportions and setae structure. According to the molecular data, this spider is one of the *A. hentzi* in the southern species group. The interesting point of these observations is that this spider lives sympatrically in the Big Bend area of Texas with the much larger *A.* sp. Big Bend. If this spider is an old lineage of *A. hentzi*, and has been isolated in this habitat due to shifting habitats and climate change, this spider could be a case of insular dwarfism, in order to survive limited resources in an unfavorable habitat or to reduce the interspecific competition with the larger *A.* sp. Big Bend.

4.4 Conclusions

One of the purposes of this research was to test the hypotheses that *A. hentzi* is a recent species with little intraspecific genetic variation throughout its range, having split from a southern lineage of *Aphonopelma*, and that differences in the niche and biogeographic history along the southern and western borders of the *A. hentzi* range created a barrier to further habitat expansion and promoted speciation for the tarantulas in those areas. All three of these hypotheses, combined with *A. hentzi*'s high ecological plasticity explain the pattern we see in the *Aphonopelma* throughout Texas.

As discussed in Stockman and Bond (2007), both ecological and genetic divergence should be demonstrated for elevation to species status. With the DFA data significantly stating that these species groups are separating themselves based on the environmental variables analyzed from their habitats, and the strong support both in the phylogenetic tree topologies and the amount of genetic divergence between species groups, these groups of related tarantulas should be viewed as "good" species. There is an important distinction between "describing" and "delimiting" species via molecular data. In this work, I attempted to delimit the species in this study area of Texas (Figure 1 & 2). Before describing new species, we need to fully understand the distribution, morphological variation, behavioral differences, genetic divergence and population structuring, niches, and ecological differences to truly determine whether these are new species.

The question that needs to be asked should address what adaptations have allowed *A. hentzi* to exploit the habitat and outcompete other tarantula species. Adaptive radiation is occurs when species derived from a common ancestor diversify into different ecological niches. This frequently leads to a morphological change that coincides with being adapted to the new niche (Thorpe *et al* 2008; Turgeon & McPeek 2002). Future research should attempt to uncover adaptive radiation in the morphology between species lineages. Differences in habitat preferences (e.g. the environmental variables evaluated in this study, elevation, soil type and structure, etc…) could be ultimate reasons why different morphology is found in different niches (big, robust bodies versus small and thin; "hairy", stocky legs versus thin, longer legs).

A lack of morphological differentiation may indicate sibling species, two populations that have recently diverged genetically, but not enough time has passed for diagnosable morphological characters to evolve. Alternatively, morphological stasis could be indicative of niche conservatism (Wiens 2004a). If *A.* sp. X looks like a large *A. hentzi* and *A.* sp. Big Bend, but it's not the sister group, then niche conservatism seems the most plausible answer as to why these species look similar morphologically and occupy similar niches.

Analyses based on mitochondrial data provide robust phylogenetic and phylogeographic estimates, but under certain circumstances a mitochondrial gene tree will reflect evolutionary processes other than phylogenetic descent, like introgressive hybridization and incomplete lineage sorting. Undetected mitochondrial introgression and incomplete lineage sorting can confuse phylogenetic inference. But if these are recognized, they can provide insights into lineage history and the evolutionary process that would otherwise remain undetected if relying upon other data (Croucher *et al* 2004; Hebert and Gregory 2005; McGuire *et al* 2007; Turgeon & McPeek 2002). Because of this, future work should use nuclear DNA markers in combination with the mtDNA for phylogenetic inference. Partitioning the data and comparing those topologies with the combined data set will help to add evidence of past and present relationships.

During the analysis a case of possible mitochondrial introgression was found between one specimen, ARC001 from Rucker Canyon (Figure 25) in the Chiricahua Mountains in the sky islands of Cochise County, Arizona, and the *A.* sp. X species clade, whose farthest western specimen AHI001, from the Animas Mountains in Hidalgo County, New Mexico. Both of these spiders are found in an area along the border between the two states, an area that is known to be species-rich in tarantula species, with a unique geologic and biogeographic history. These two specimens are only 0.45% divergent from each other yet incredibly divergent from each other morphologically. If these two species are ecologically distinct, this low divergence result could come from incomplete lineage sorting. If so, then the speciation events that gave rise to their distinct morphologies and ecologies must have occurred recently enough that the mitochondrial genomes retained in the descendent species have yet to accumulate independent mutations (McGuire *et al* 2007).

To determine if this is a case introgression, which would be the first documented in tarantulas, a wide range of samples need to be collected across the geographic ranges of the two, analyzing both morphological and molecular data, in order to see if the low divergent

haplotypes are found nearest to the zone of potential contact. If shared haplotypes are not randomly distributed throughout the ranges of these species but rather are concentrated near their geographic points of contact, then it is concluded that introgression rather than incomplete lineage sorting has resulted (McGuire *et al* 2007). If haplotypes are shared across multiple sites, it suggests that there is still ongoing gene flow among sites (Avise 2000), though if not it could represent the signature of past hybridization events or hybridization zones. I propose that these tarantulas have come into secondary contact with neighboring species during the interglacials, thus promoting periodic hybridization, especially in the species rich refugia of the southwestern United States and Mexico.

If species have diverged too rapidly for morphological characters to differentiate them, mtDNA phylogenies are thought to be particularly useful to delimit species, though male-biased dispersal and female philopatry can bias estimates of gene flow when they are based on mitochondrial markers (Wiens and Penkrot 2002). When selecting the genetic markers for delimiting species, researchers have neglected to look at the amount of gene flow that the markers are experiencing (Petit and Excoffier 2009). As a consequence of sex-biased dispersal, different DNA regions are subject to different levels of gene flow, due to their mode of inheritance (maternal, parental, or biparental).

Mitochondrial DNA differences in tarantulas, a male sex-biased dispersal organism, as compared to Lepidoptera which are female-biased, could account for problems in delimiting tarantula species in the future due to these differences in gene flow. Petit and Excoffier (2009) found that molecular markers associated with the least-dispersing sex should be less helpful in determining species identification. Because female tarantulas are the least-dispersing sex and because mtDNA is maternally inherited, future work on delimiting *Aphonopelma* species should include nuclear markers (Hendrixson and Bond 2007; summarized in Starrett and Hedin 2007).

Because only a limited set of molecular markers are available for arachnids (Ayoub *et al* 2007), more independent nucleotide data is needed, both mitochondrial and nuclear loci, to

resolve the issues facing *Aphonopelma*. Future work will resolve the issues of the genus *Aphonopelma* being polyphyletic (Turner 2009), with three distinct clades that are quite divergent from each other (a North American clade, and two Central American clades) by using a total-evidence approach, including both nuclear and mtDNA, to determine exactly how many genera need to be resurrected or described.

The information presented here, as well as future work, will help us to understand the evolution of theraphosid dispersal, which is poorly known but perhaps critical to understanding how species colonize new areas. This research represents data that shows *Aphonopelma*, and tarantulas in general, have a greater ability to disperse into habitats than previously thought, as seen by how quickly *A. hentzi* recolonized the northern part of their range. Because the nuclear markers that we presently have for tarantulas are relatively invariant for determining population structure, microsatellite markers (for which there are no present markers) or ISSR (Inter Simple Sequence Repeats) would be able to sufficiently characterize population structure and potential hybridization events (Machkour M'Rabet *et al* 2009). The use of hypervariable microsatellites will reveal the population genetic structure and dispersal patterns of recent times (Late Pleistocene and Holocene) that are needed to answer these questions.

In order to quantify if *Aphonopelma* live in genetically related "colonies" or metapopulations, microsatellites can be used to track relatedness away from females by sampling spiderlings found in the immediate area. And in order to answer if mature male "wandering" evolved first, as a response to the need to reduce the chances for inbreeding (Baerg 1958), or if a quicker maturation time evolved first in order for males to limit inbreeding with a larger dispersal ability to increase gene flow (Janowski-Bell 2001), nuclear data can help potentially answer the ultimate reason to this dilemma. Nuclear markers can also be used to help unravel the fertilization success of males, after multiple matings by different males, and whether there is sperm competition as well as female mate choice in *Aphonopelma*.

Because tarantulas are long-lived, low-vagility ectotherms with a unique life-history and the potential to easily become isolated, I believe they can be used as a model organism to understand dispersal and speciation in certain arthropod and herpetological lineages. This research is important because as habitats become more fragmented in the future, a leading cause of extinction, organisms with low vagility and limited dispersal, such as tarantulas, are vulnerable to habitat destruction and will need conservation efforts, such as those already in place to protect endangered Mexican tarantulas of the genus *Brachypelma*. An understanding of the ecology and evolutionary relationships of the North American tarantulas will help mitigate possible future population extinction events.

APPENDIX A

FIGURES

Figure 1. Map of the collection sites for the *Aphonopelma* used in this study. Colors denote species group (blue = *A. hentzi*, red = *A. anax*, yellow = *A. armada*, orange = *A. moderatum*, brown = *A.* sp. Amistad Brown, pink = *A.* sp. X, green = *A.* sp. Carlsbad Green, aqua = *A.* sp. Big Bend, white = outgroup specimens).

Figure 2. Map of the Colorado River basin as defined by the USGS.

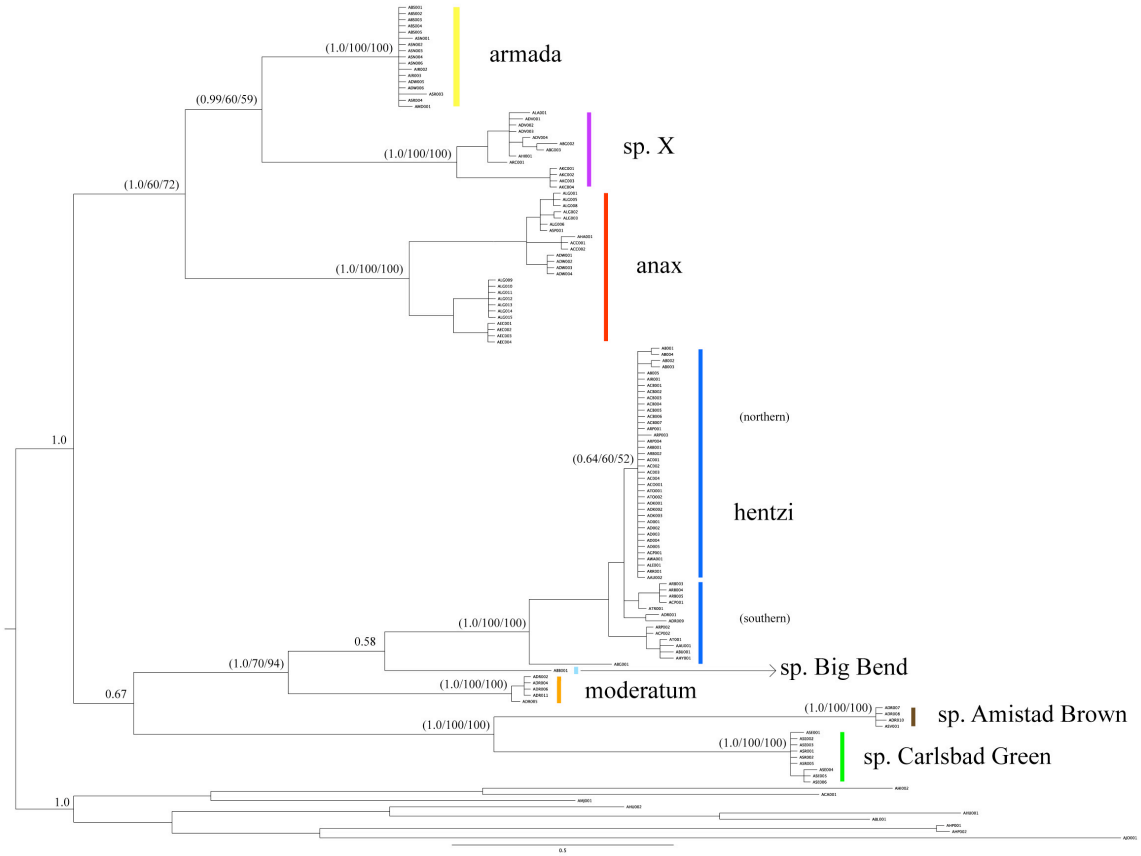

Figure 3. Bayesian inference for the combined *16S-ND1* mtDNA region. Posterior probabilities are listed with Maximum Likelihood bootstrap values and Maximum Parsimony bootstrap values above the branches (Bayesian/ML/MP). The species groups are designated with the corresponding colors from the collection site map, as well as both the present and paleoclimate modeling maps.

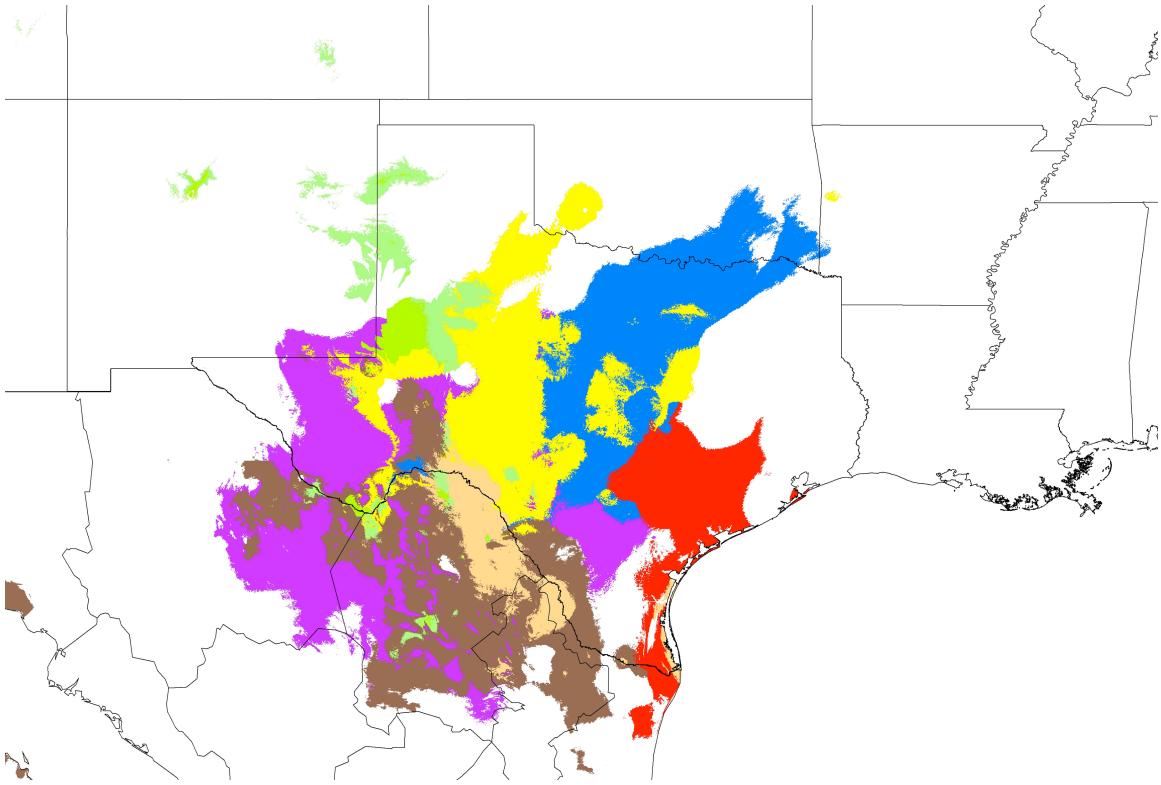

Figure 4. The predicted distributions from Maxent of the *Aphonopelma* species in Texas. Colors denote the species groups and correspond to the collection site map (blue = *A. hentzi*, red = *A. anax*, yellow = *A. armada*, orange = *A. moderatum*, brown = *A.* sp. Amistad Brown, pink = *A.* sp. X, green = *A.* sp. Carlsbad Green). *A.* sp. Big Bend, found in southwest Texas, was not included in the Maxent analyses due to only having one specimen.

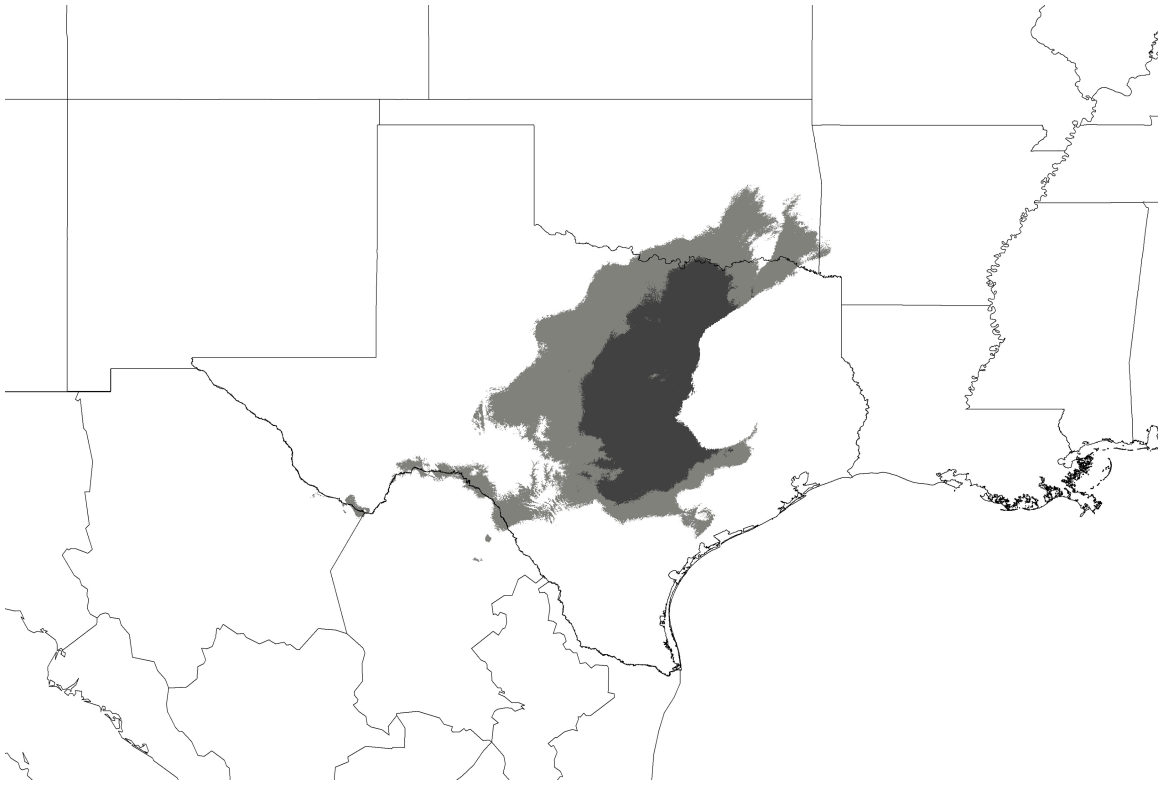

Figure 5. The predicted distributions from Maxent of *A. hentzi*. The top two prediction classes were used for the map (light grey = 39% - 61%; dark grey = 61% - 92%).

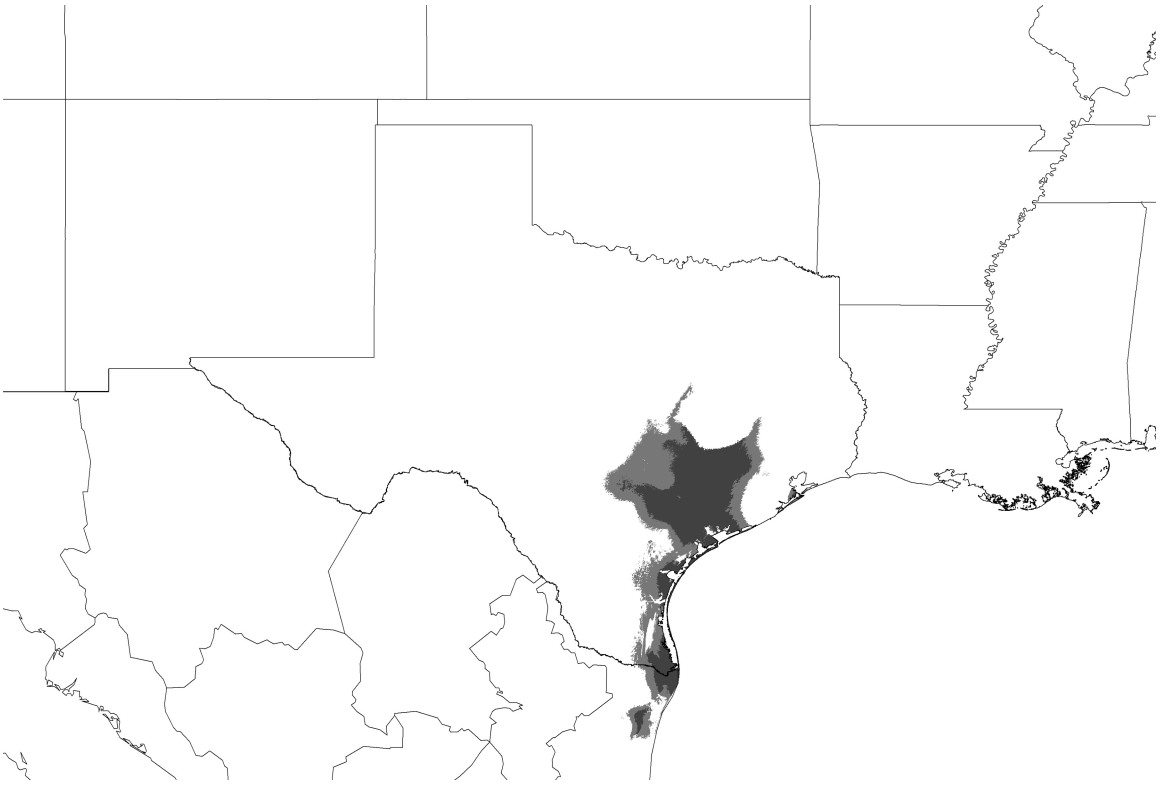

Figure 6. The predicted distributions from Maxent of *A. anax*. The top two prediction classes were used for the map (light grey = 40% - 60%; dark grey = 60% - 92%).

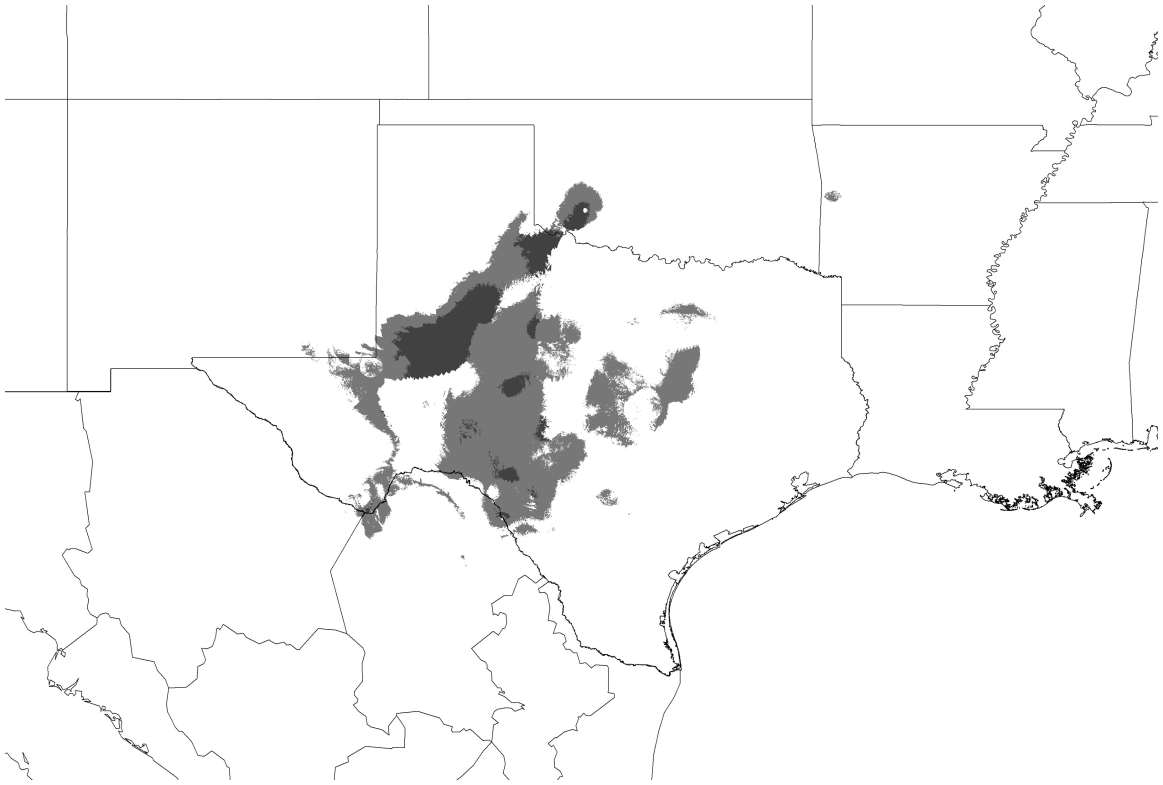

Figure 7. The predicted distributions from Maxent of *A. armada*. The top two prediction classes were used for the map (light grey = 36% - 59%; dark grey = 59% - 94%).

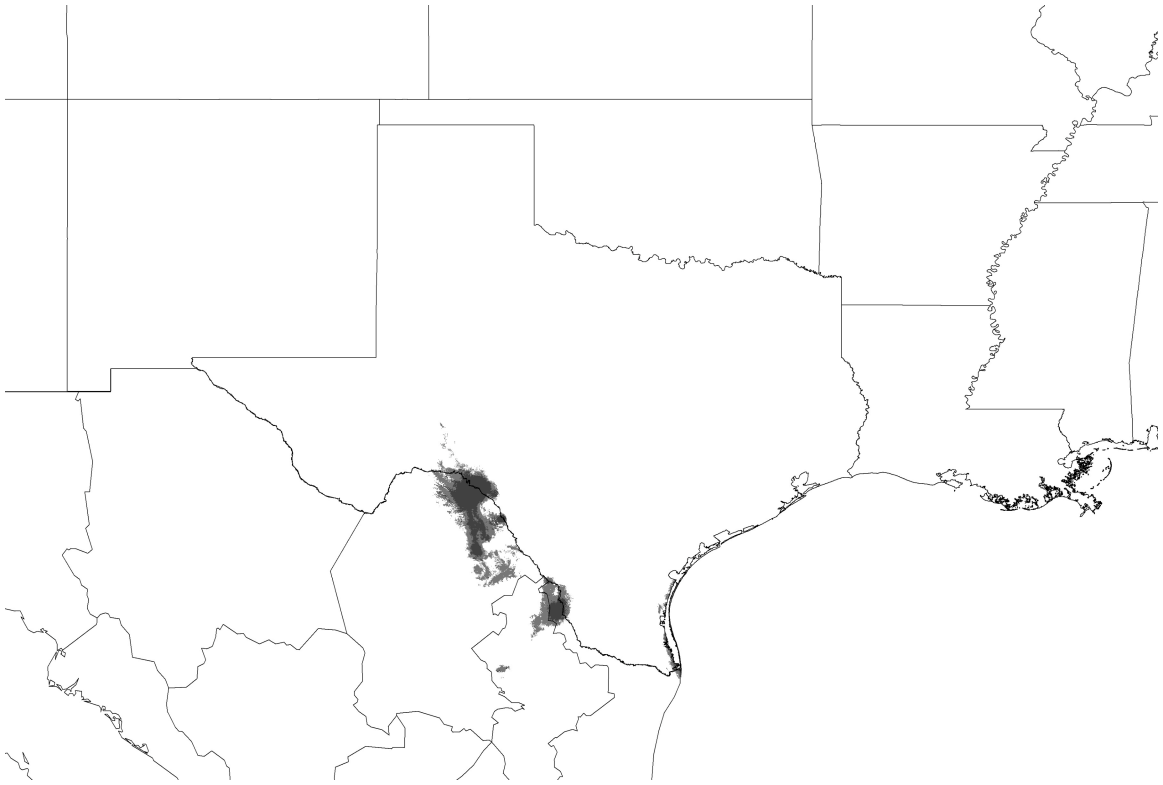

Figure 8. The predicted distributions from Maxent of *A. moderatum*. The top two prediction classes were used for the map (light grey = 38% - 66%; dark grey = 66% - 99%).

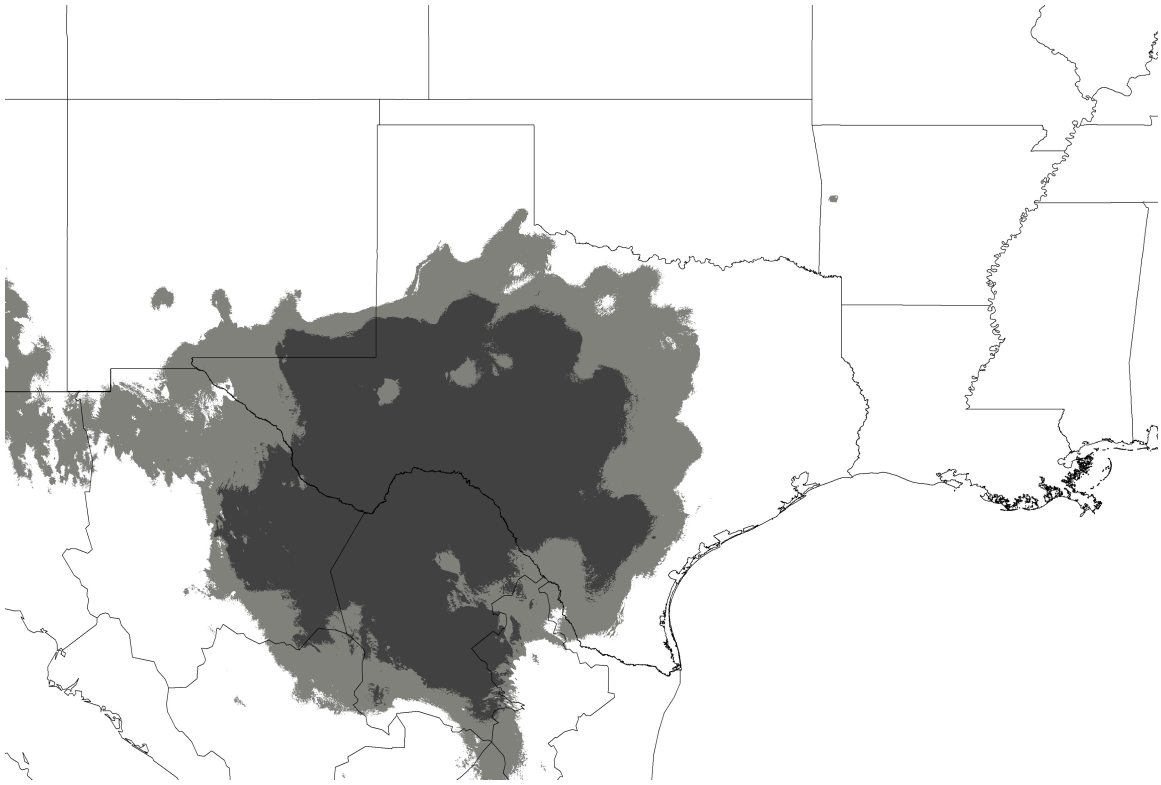

Figure 9. The predicted distributions from Maxent of *A.* sp. X. The top two prediction classes were used for the map (light grey = 33% - 54%; dark grey = 54% - 86%).

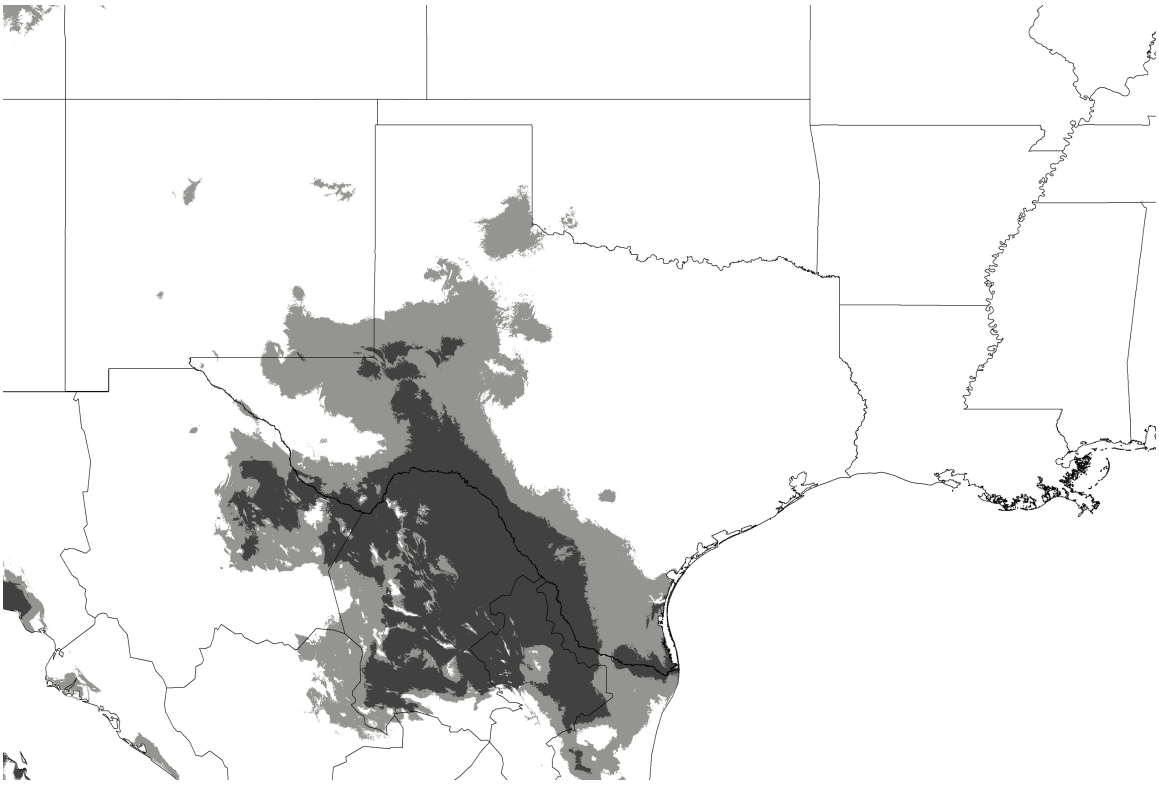

Figure 10. The predicted distributions from Maxent of *A.* sp. Amistad Brown. The top two prediction classes were used for the map (light grey = 37% - 61%; dark grey = 61% - 98%).

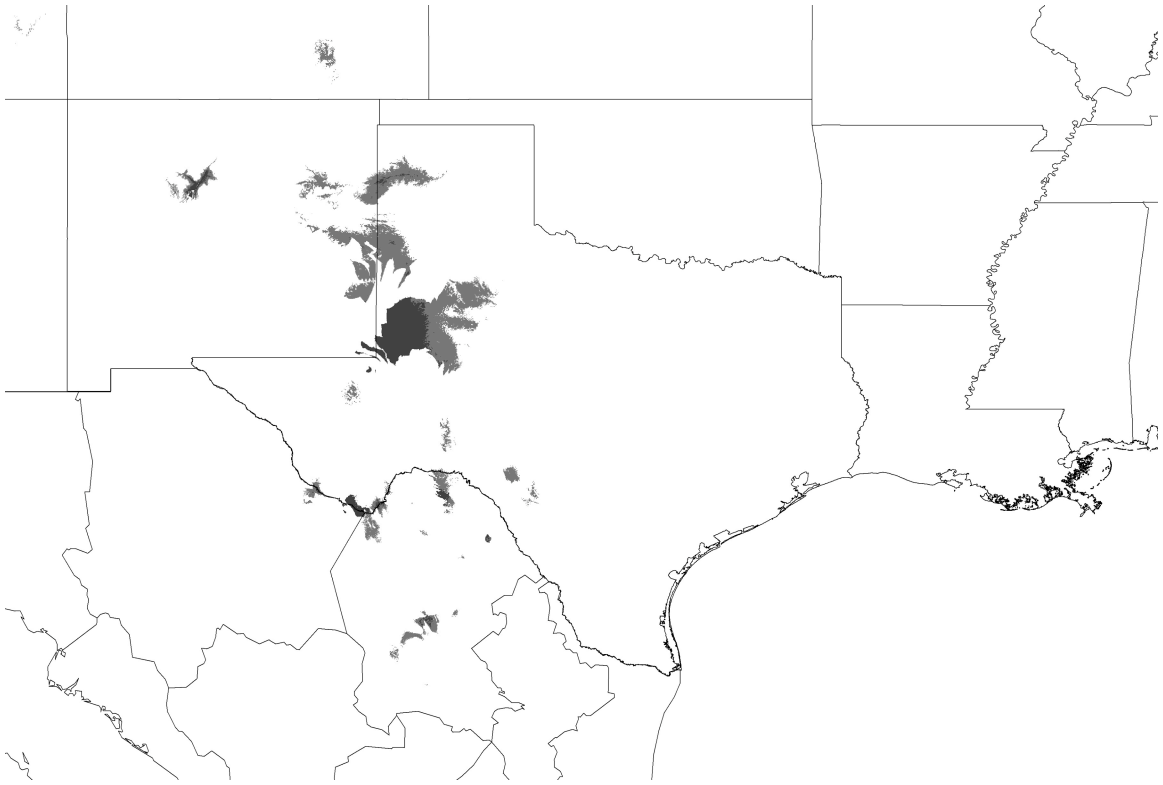

Figure 11. The predicted distributions from Maxent of *A.* sp. Carlsbad Green. The top two prediction classes were used for the map (light grey = 35% - 63%; dark grey = 63% - 99%).

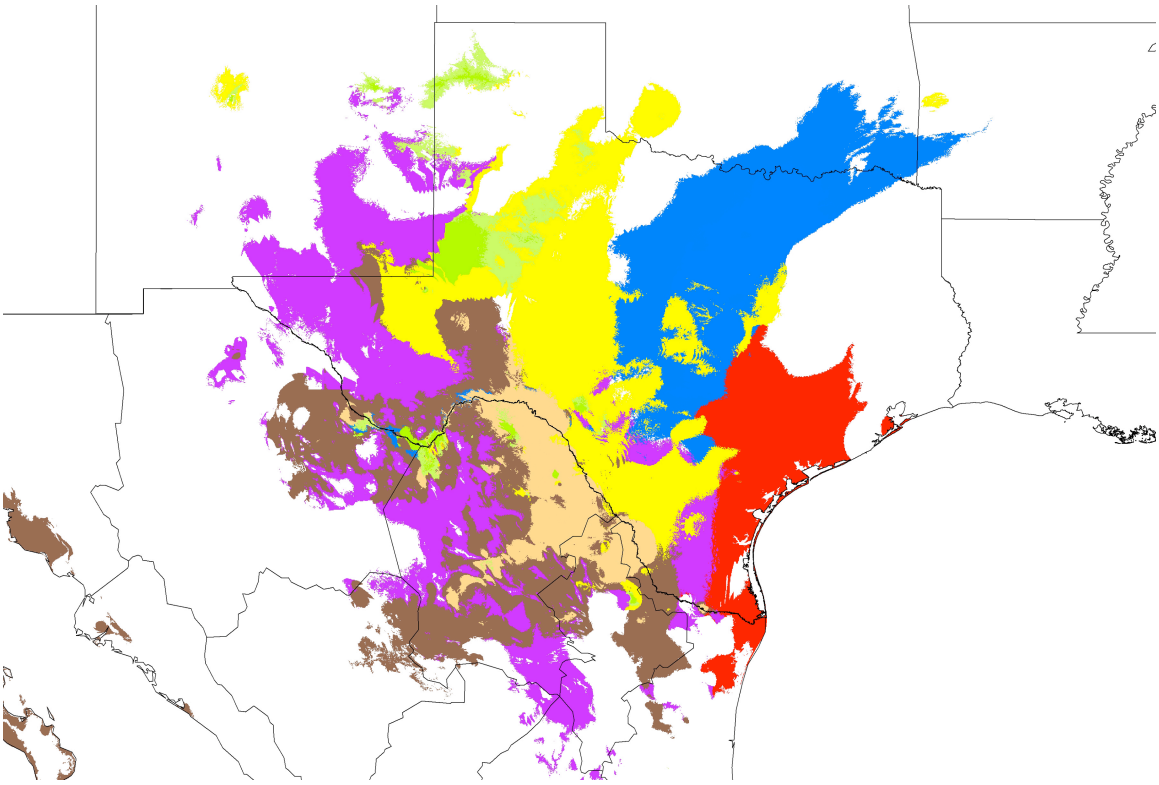

Figure 12. The predicted paleoclimate (21,000 ybp) distributions from Maxent of the *Aphonopelma* species in Texas. Colors denote the species groups and correspond to the collection site map (blue = *A. hentzi*, red = *A. anax*, yellow = *A. armada*, orange = *A. moderatum*, brown = *A.* sp. Amistad Brown, pink = *A.* sp. X, green = *A.* sp. Carlsbad Green). *A.* sp. Big Bend, found in southwest Texas, was not included in the Maxent analyses due to only having one specimen.

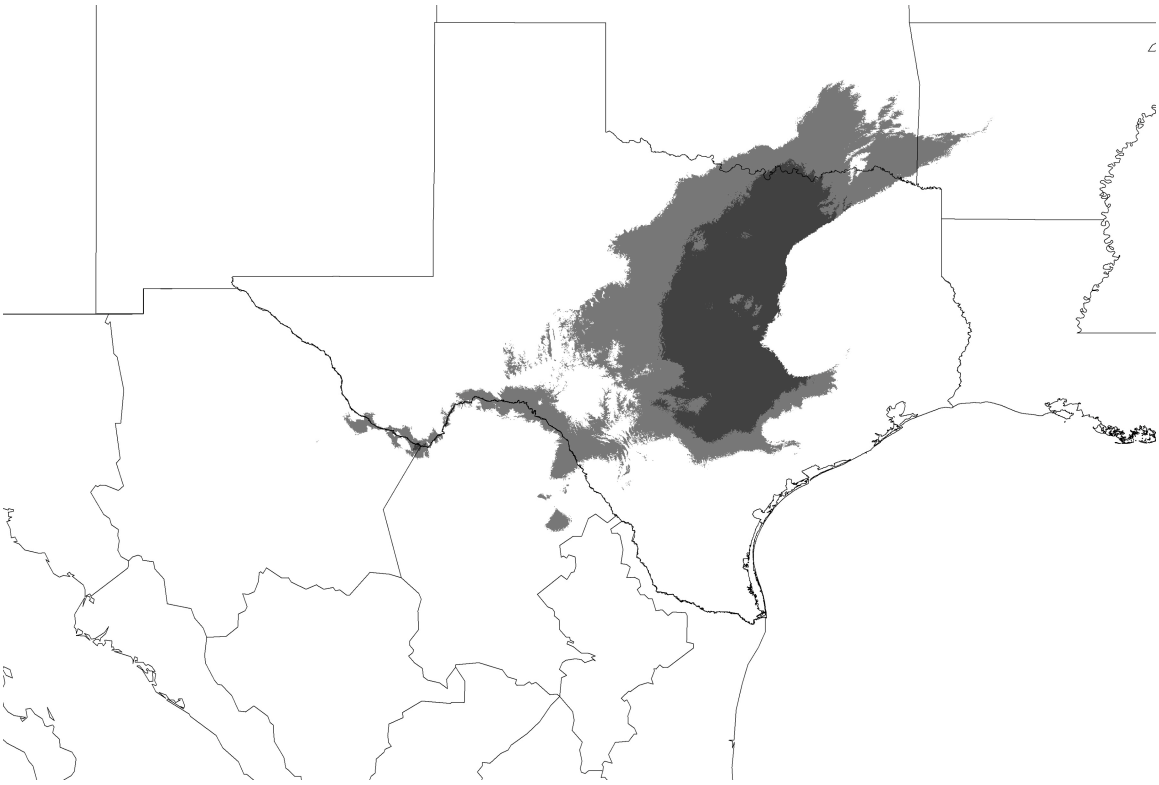

Figure 13. The predicted paleoclimate (21,000 ybp) distributions from Maxent of *A. hentzi*. The top two prediction classes were used for the map (light grey = 35% - 57%; dark grey = 57% - 91%).

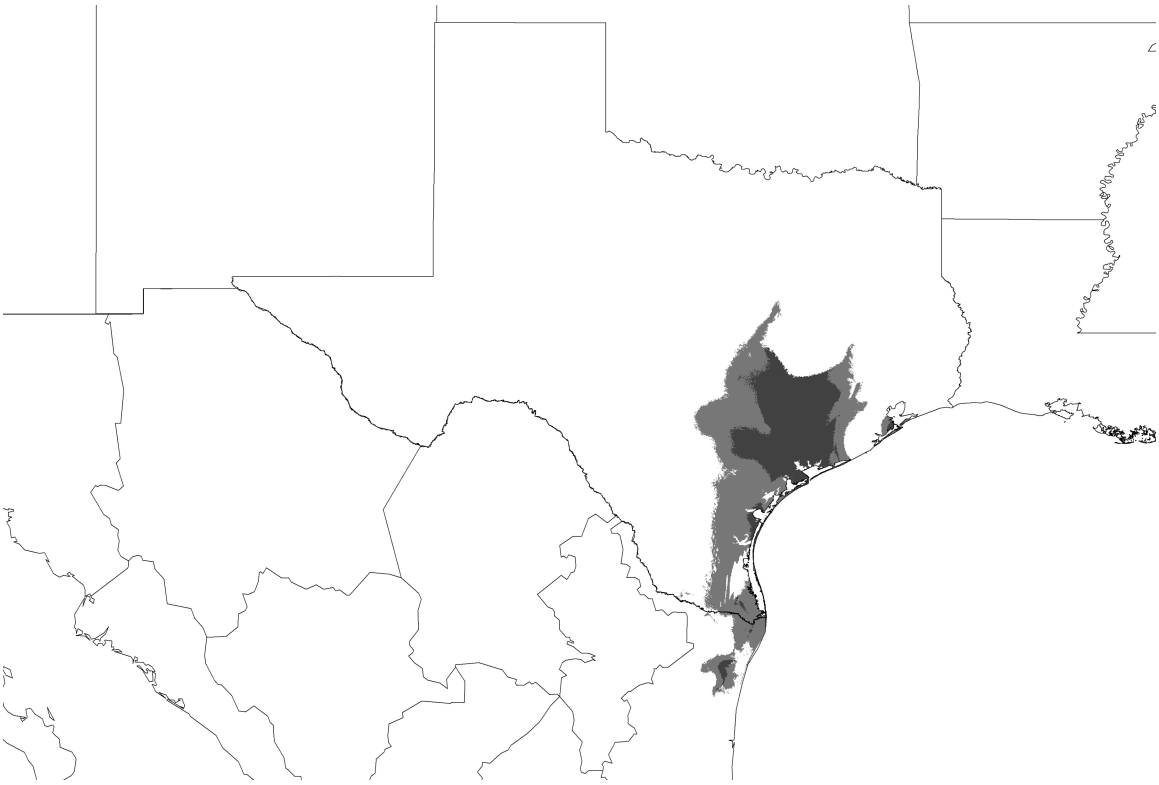

Figure 14. The predicted paleoclimate (21,000 ybp) distributions from Maxent of *A. anax*. The top two prediction classes were used for the map (light grey = 40% - 60%; dark grey = 60% - 89%).

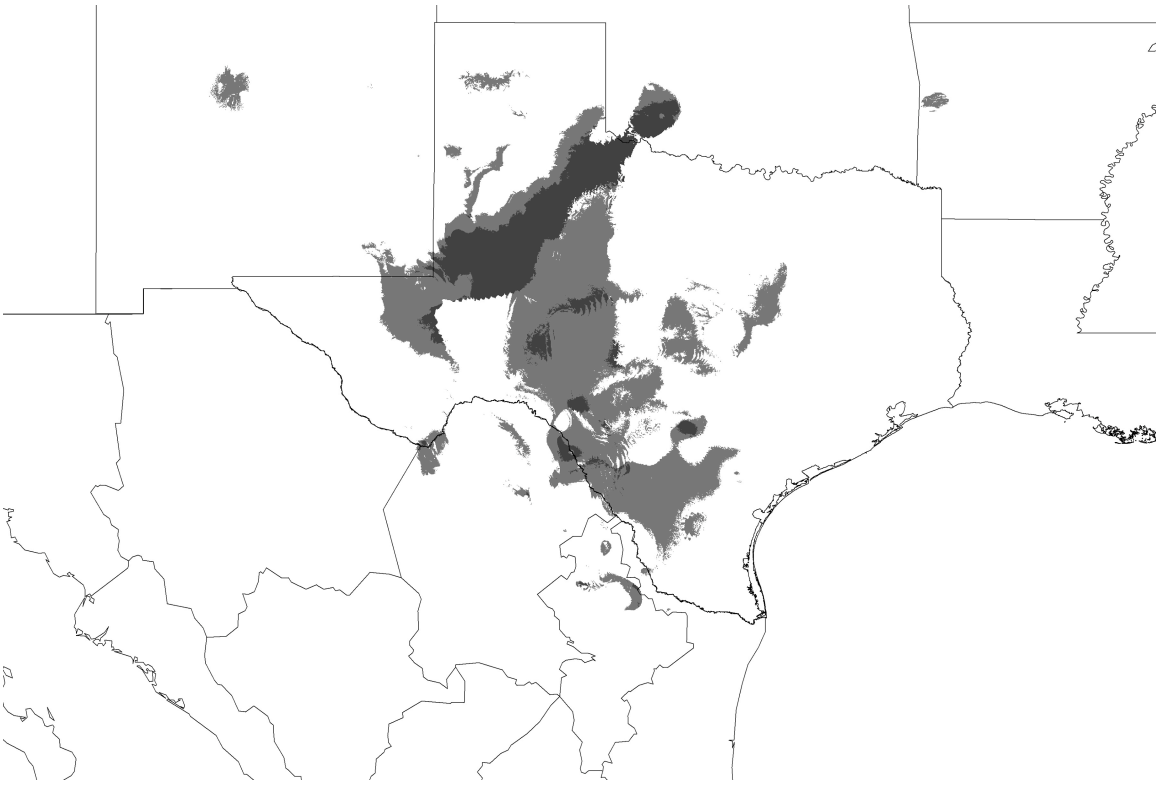

Figure 15. The predicted paleoclimate (21,000 ybp) distributions from Maxent of *A. armada*. The top two prediction classes were used for the map (light grey = 34% - 60%; dark grey = 60% - 96%).

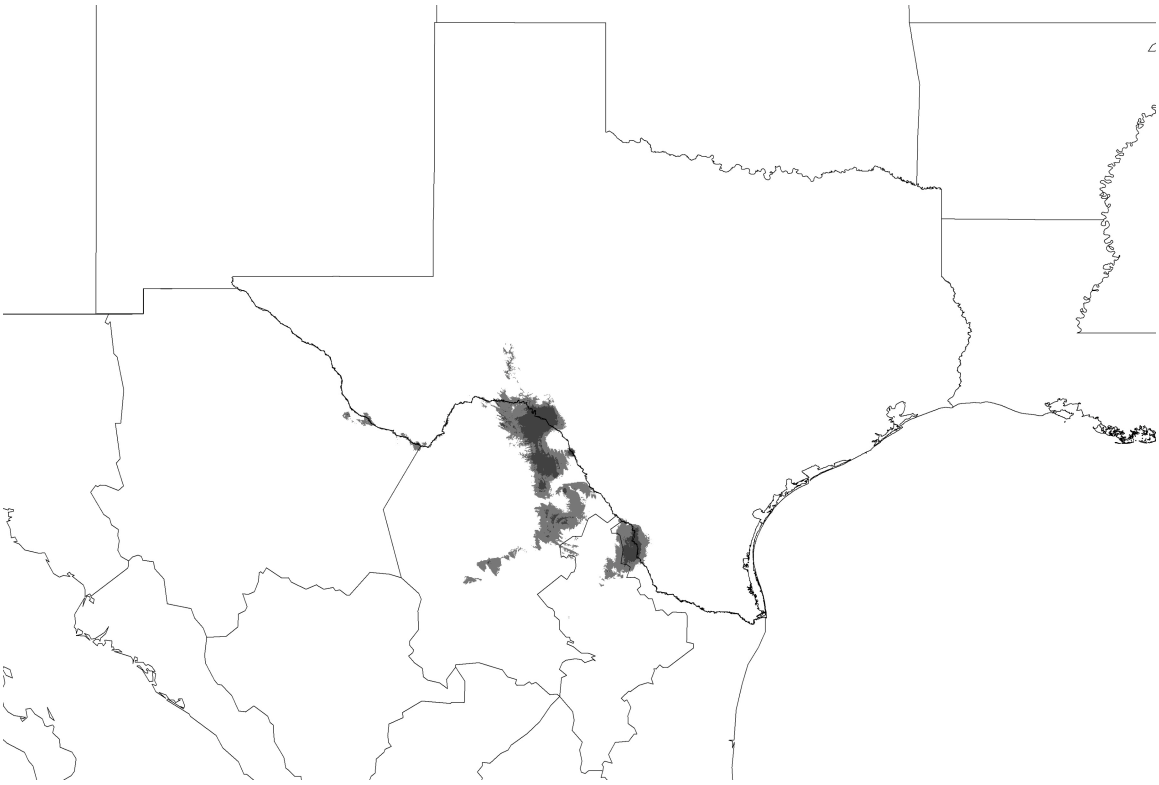

Figure 16. The predicted paleoclimate (21,000 ybp) distributions from Maxent of *A. moderatum*. The top two prediction classes were used for the map (light grey = 37% - 60%; dark grey = 60% - 98%).

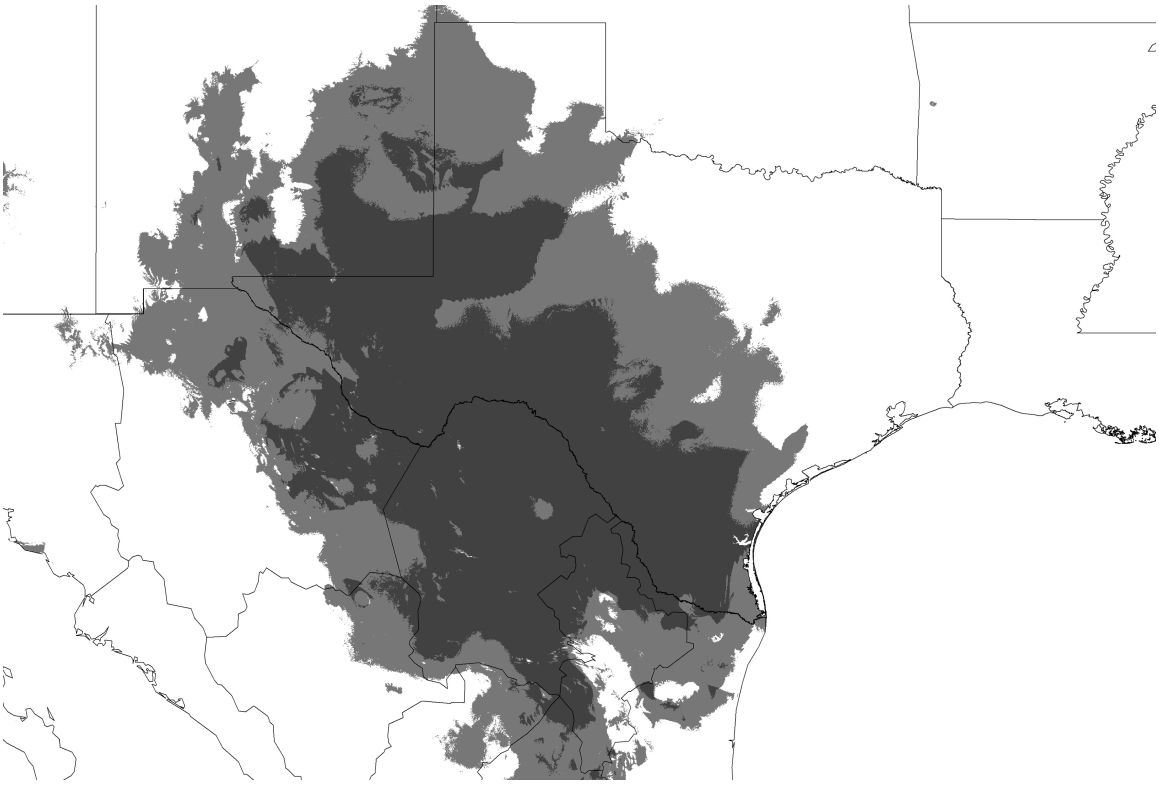

Figure 17. The predicted paleoclimate (21,000 ybp) distributions from Maxent of *A.* sp. X. The top two prediction classes were used for the map (light grey = 39% - 57%; dark grey = 57% - 88%).

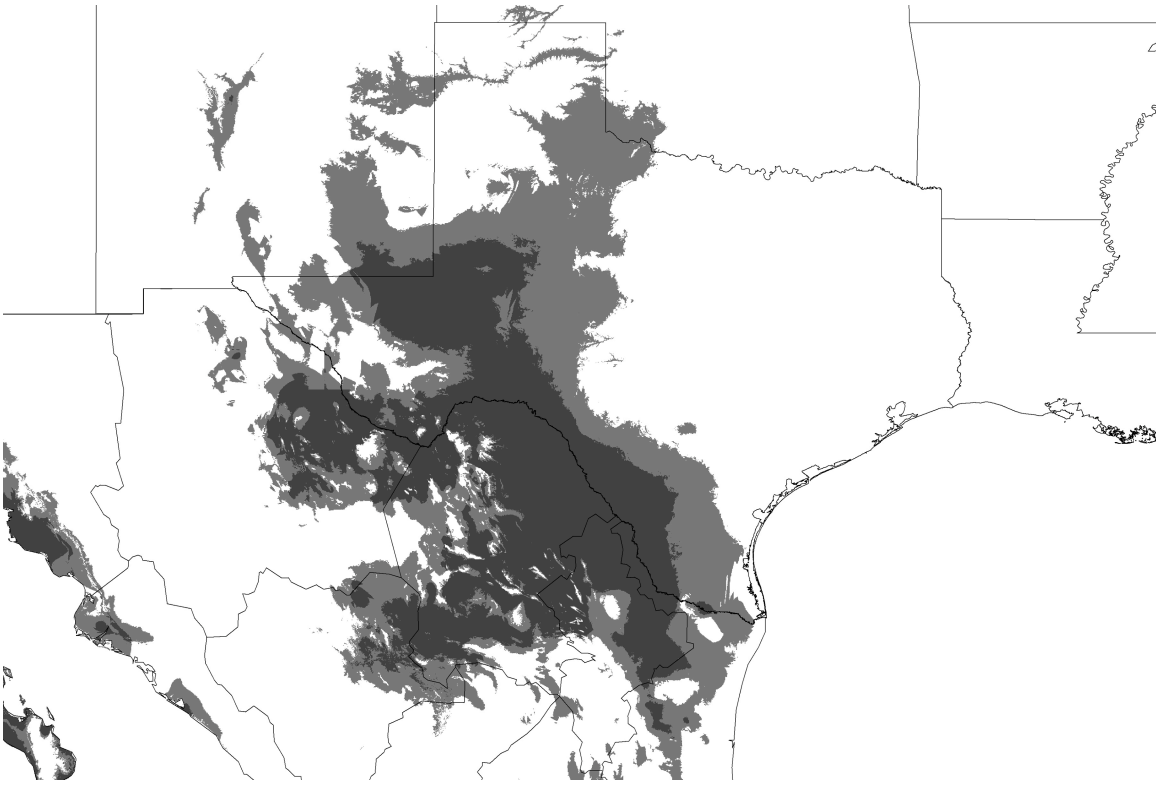

Figure 18. The predicted paleoclimate (21,000 ybp) distributions from Maxent of *A.* sp. Amistad Brown. The top two prediction classes were used for the map (light grey = 38% - 59%; dark grey = 59% - 92%).

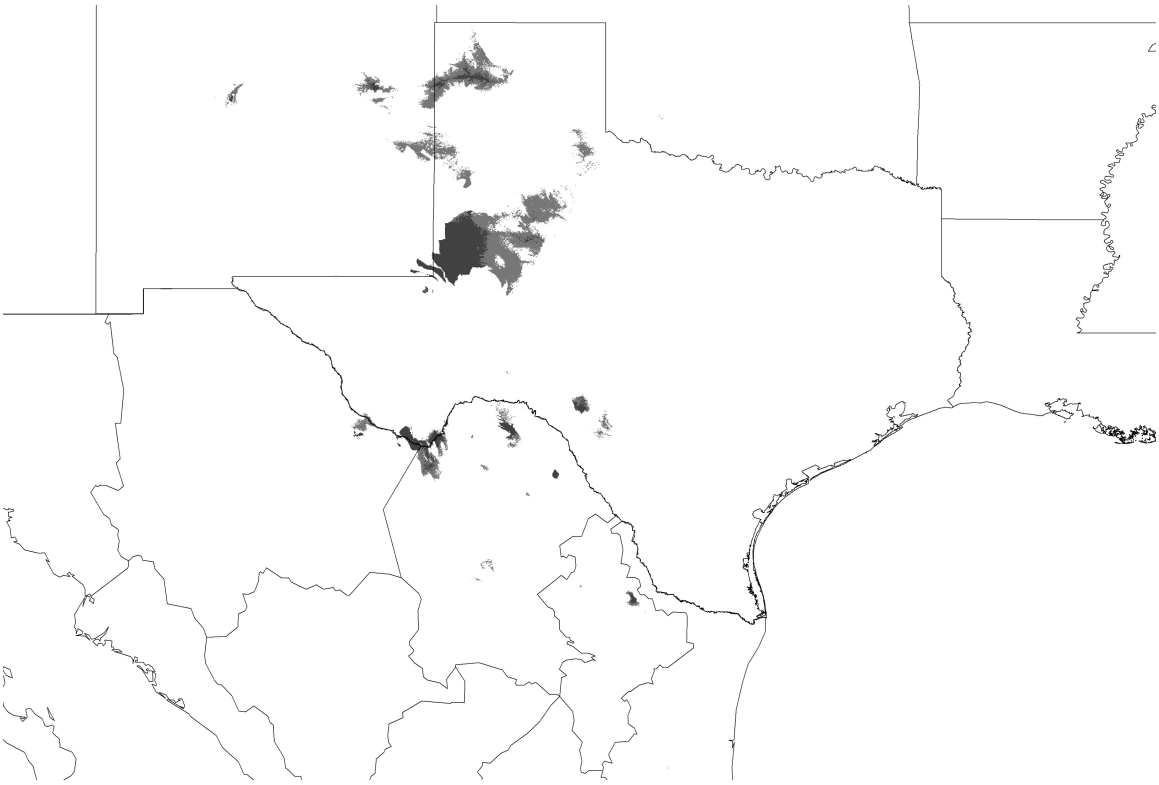

Figure 19. The predicted paleoclimate (21,000 ybp) distributions from Maxent of *A.* sp. Carlsbad Green. The top two prediction classes were used for the map (light grey = 38% - 64%; dark grey = 64% - 99%).

Figure 20. *Aphonopelma hentzi*.

Figure 21. *Aphonopelma anax*.

Figure 22. *Aphonopelma armada*.

Figure 23. *Aphonopelma moderatum*.

Figure 24. *Aphonopelma* sp. X. This species group has potentially experienced a mitochondrial introgression event occur in the past with *A.* sp. Rucker Canyon (Figure 25).

Figure 25. *Aphonopelma* sp. Rucker Canyon. This species has potentially experienced a mitochondrial introgression event in the past with *A.* sp. X (Figure 24).

Figure 26. *Aphonopelma* sp. Carlsbad Green.

APPENDIX B

TABLES

Table 1. Average percent genetic p-distances for the *Aphonopelma* species within Texas.

	A. hentzi	*A.* sp. Big Bend	*A. moderatum*	*A. anax*	*A.* sp. X	*A. armada*	*A.* sp. Carlsbad Green	*A.* sp. Amistad Brown
A. hentzi	0.39	-	-	-	-	-	-	-
A. sp. Big Bend	5.65	0	-	-	-	-	-	-
A. moderatum	6.96	5.84	0.04	-	-	-	-	-
A. anax	8.83	8.94	9.09	1.66	-	-	-	-
A. sp. X	9.92	9.41	9.53	7.71	1.07	-	-	-
A. armada	8.84	8.52	7.96	6.75	5.51	0.08	-	-
A. sp. Carlsbad Green	11.39	12.79	11.56	12.76	12.37	10.82	0.11	-
A. sp. Amistad Brown	12.21	11.92	12.68	13.06	12.86	13.38	9.16	0.04

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BIOGRAPHICAL INFORMATION

Chris A. Hamilton was born 2 July 1976 in Little Rock, Arkansas. He was raised for most of his life in Wichita and Overland Park, Kansas where he captured, studied, and raised reptiles, his first love. After graduating from Blue Valley North High School in Overland Park, Kansas, he received the following degrees: B.A. in Photojournalism from Western Kentucky University (1999); M.S. in Biology from the University of Texas at Arlington under Dr. Dan Formanowicz (2009). The large skip between the B.A. and Masters included a number of years working as a staff photographer for newspapers and as a freelance photographer for numerous national and international magazines. The final major assignments of his career included working on two stories, each with a science focus, for National Geographic Magazine. These experiences drove him back to science, back to school at UTA, and to a new love, tarantulas. Chris will be pursuing his PhD in evolutionary biology at East Carolina University, where with Dr. Jason Bond, he will be working to understand the evolutionary history, relationships, and biogeography of the tarantulas of North America.